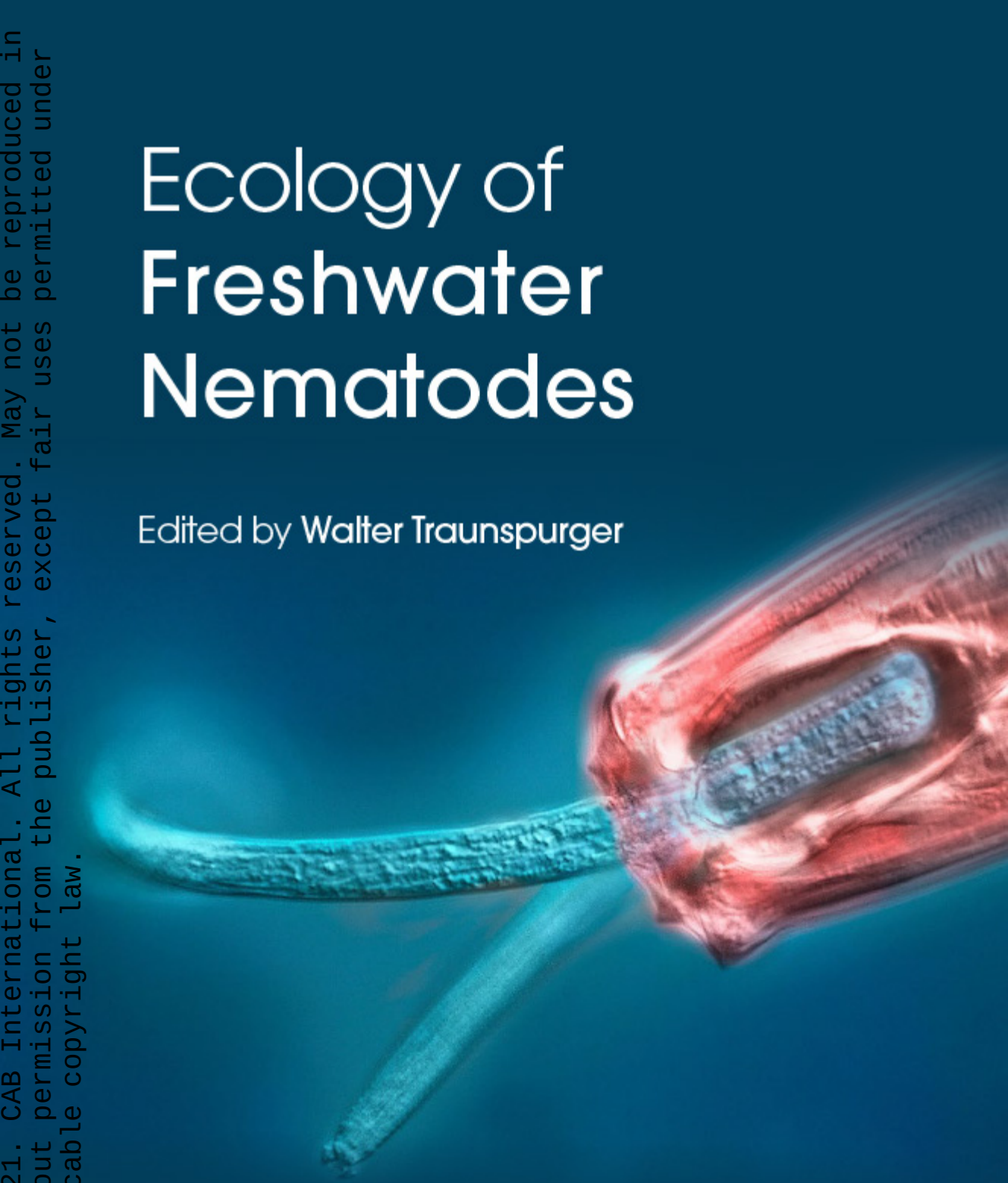
Ecology of Freshwater Nematodes

Edited by Walter Traunspurger



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To my family Gerti, Julia, and Janik

Contents

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Preface

Nematodes have conquered almost all habitats on Earth and have adapted to almost all of its living conditions. Indeed, in lakes, rivers, ponds, or other aquatic habitats it is not uncommon to find up to 1 million nematode individuals per square meter and over 100 species. It is therefore astonishing that the most common metazoans on Earth – about 70% of all metazoans are nematodes – receive so little research attention. Then again, why should they? At first impression, nematodes are unremarkable, but under the microscope their unique characteristics are revealed. These include a transparent body in which the inner organization can be seen, the many variations in the anatomy of their oral cavity, and a body structure that is surrounded by a cuticle of high diversity. Moreover, the short generation time (a few days to months) of many species makes nematodes ideal for laboratory studies, including life cycle, predator–prey, and competition experiments. Nematodes are also well suited for metacommunity analyses and for investigating the mechanisms of dispersal. Recent mesocosm-based studies of nematodes have demonstrated that these 'artificial ecosystems' offer a very convenient scale for evaluating the effects of nutrients, predators, pollutants, etc., on important ecological parameters, such as the abundance, species richness, and secondary production of nematodes, over a period of several months.

It is therefore my hope that this book, with its 12 chapters, will encourage all ecologists to consider free-living nematodes as a model organism in their investigations, but also show how important it is to study the fundamentals of ecology, for example, the distribution and diversity of a group of organisms as well as the interactions of those organisms with others. Detailed studies of this type will ultimately provide a better understanding of food webs, their role in the respective habitat, and the changes therein caused by human activities. In this context, research during the

past 20 years has determined that, in addition to aquatic environments, nematodes are good indicators of sediment and soil quality.

The book by Eyualem-Abebe, Andrássy, and Traunspurger, published in 2006, was a first milestone in the ecology of freshwater nematodes. Since then, there have been several papers and books focusing on the ecology and ecotoxicology of these organisms. This book takes into account much of the recent research on the ecology of freshwater nematodes. It contains many new chapters as well as revisions and updates of the chapters of the 2006 book. My intention was to write a comprehensive yet readable guide for interested biologists, from students to career scientists.

This book was only possible with the help of many students at the bachelor's, Master's, and PhD levels, assistant professors, and many nematologists in Europe and worldwide. Through many years of teaching at Bielefeld University I have found that while students often initially assume that nematodes are 'boring', they become fascinated with these organisms once they have explored them in depth.

I would like to thank Bielefeld University and its Faculty of Biology for providing the excellent conditions enabling research on the taxonomy and ecology of free-living nematodes as well as the sharing of this knowledge with students. I would also like to thank my 'Animal Ecology' working group, especially Hendrik Fueser, Birgit Gansfort, Sebastian Höss, Nabil Majdi, Christoph Ptatscheck, and Janina Schenk, all of whom carried out many new and interesting experiments that contributed to the chapters of the book. Finally, during the past 20 years, the most important person in my laboratory in Bielefeld has been my technical assistant, Steffi Gehner.

I would like to thank Wendy Ran, for copy-editing earlier versions of the chapters, and CABI, for its support of research into the ecology of freshwater nematodes.

Walter Traunspurger
Bielefeld
January 2021

1 Introduction to Freshwater Nematodes in Ecology: Current Knowledge and Research

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Highlights

- Nematodes are tiny roundworms that abound in most parts of the biosphere.
- They show remarkably diverse life strategies and occupy important positions in food webs.
- Their role in ecosystems has nonetheless been largely ignored by ecologists and nematologists.
- This book offers guidelines for studying the ecology of free-living nematodes, with the aim of increasing interest in this topic in current and future generations of scientists.

1.1 A Short Summary of Nematode Morphology and Reproduction

Nematodes are tiny roundworms, usually elongated, bilaterally symmetrical, and rod- or thread-like in shape. They comprise the 'phylum'

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Nematoda, the name of which derives from a Latinized form of the Greek words *nema-* (meaning thread) and *-eidos* (meaning form or resemblance) (Andrássy, 2005). Most species are microscopic and translucent, with the body lengths of most freshwater species ranging between 0.3 and 5 mm. Parasitic species may be much larger depending on the size of their host. For example, the body length of the largest nematode described so far, *Placentonema gigantissima*, a parasite of sperm whales, may exceed 6–8 m (Gubanov, 1951).

The nematode body wall is composed of an outer non-cellular sheath (the cuticle), an inner syncytial layer (the hypodermis), and the somatic musculature. The body wall determines the shape of the nematode, serves as a barrier to external physico-chemical obstacles, biotic agents, and pathogens, enables direct contact between the worm and its environment, and allows the exchange of fluids and gases into and out of the nematode's body (Andrássy, 2005). The surface of the cuticle may be entirely smooth (as observed by light microscopy) or marked by various transverse or longitudinal structures (Fig. 1.1a,b). During nematode ontogenesis, from egg to adult, the cuticle normally is shed four times (molting or ecdysis).

The general cavity contains an alimentary tract made up of a mouth or oral aperture (Fig. 1.1c–g) and amphid (Fig. 1.1h), followed by an esophagus (Fig. 1.1i,k) and an intestine that opens to the outside via an anus. The excretory system of nematodes is unparalleled among invertebrates because it does not rely on cilia, flame cells, or protonephridia. The nervous system of Nematoda is rather complex. It mainly consists of a central part. The nerve ring ('brain'), and a number of (predominantly six) nerve chords extending anteriorly or posteriorly through the entire body. The longitudinal nerves are then provided with several ganglia.

The female genital system differs substantially from that of the male. In the female, the genital tube or gonad consists of the ovaries, oviducts, uterus (with or without eggs), spermatheca, and vagina (Fig. 1.1l,m). It opens through a separate pore, the vulva, on the ventral side of the body. The male genital system consists of a larger number of sexual characters or structures and is made up of primary and secondary organs. The former includes the testis, seminal vesicle, ejaculatory duct, cloacal chamber, and associated glands, and the latter the copulatory muscles, spicula, gubernaculum, guiding pieces, genital papillae, supplementary organs, and bursa (Fig. 1.1n–p). Most nematode species are bisexual (especially marine nematode species). Sex ratios are variable, but for most free-living nematode species females and males occur in near equal abundance. In other nematode genera and species males are much fewer in number (e.g. *Eumonhystera* spp., *Plectus* spp.). For example, in *Rhabdolaimus* spp. the male:female ratio is typically 1:1000. Moreover, there are several species, such as *Bunonema*, in which only females are found, with males either thus far unobserved because of their rarity or their complete absence. Some of these species are capable of parthenogenesis, a process of monosexual reproduction in which progeny develop from unfertilized ova (eggs), without the participation of male genital cells (spermatozoa). Monosexual reproduction is also exhibited by the few species that are hermaphroditic,

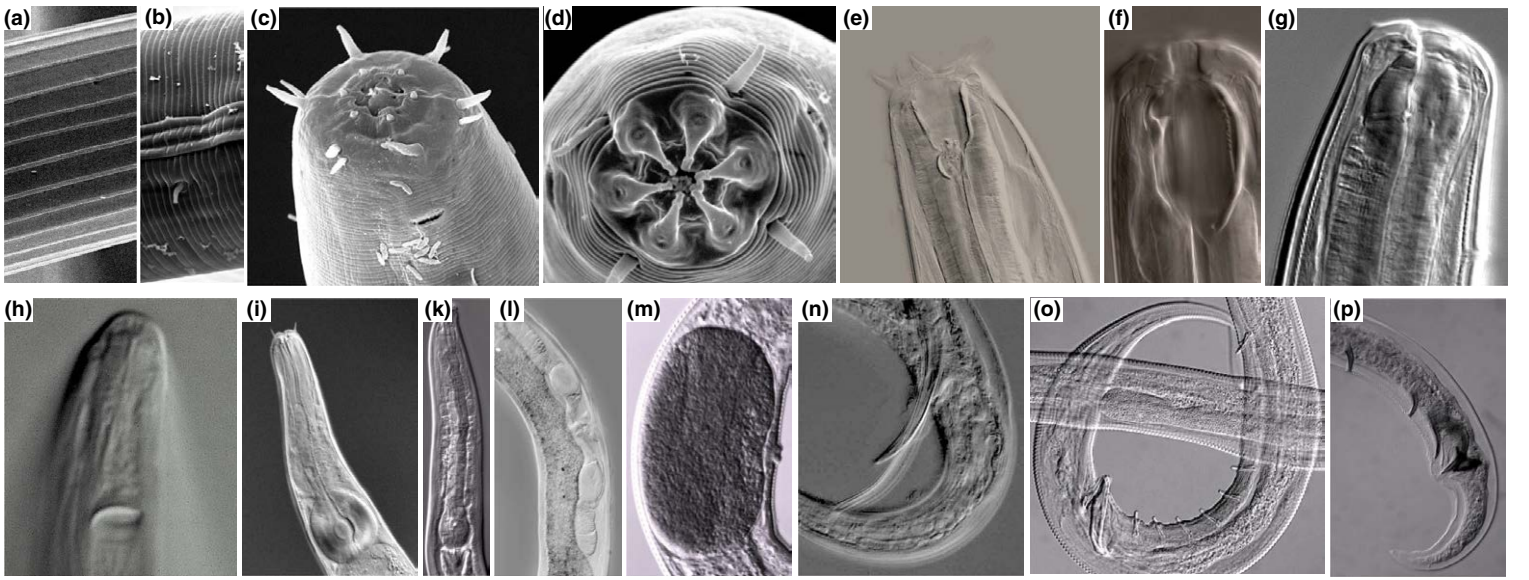

Fig. 1.1. Examples of representative morphological characteristics of freshwater nematodes: (**a**) and (**b**) cuticle ornamentation; (**c**) and (**d**) anterior part showing papillae, lips, and other cephalic setae; (**e**), (**f**), and (**g**) head with inner mouth structures; (**h**) amphid; (**i**) and (**k**) examples of esophagus shape; (**l**) and (**m**) female genital system; (**n**), (**o**), and (**p**) male genital system.

especially those belonging to the Secernentia (Andrássy, 2005). In this case, the hermaphrodite parent, a female-like individual with the usual external (and internal) female characters, produces both eggs and sperm and reproduction occurs through self-fertilization.

1.2 What Is the Role of Nematodes in Freshwater Ecosystems?

1.2.1 A brief history and definition of ecology

Free-living nematodes are widespread in inland waters. Their diverse morphologies and life strategies, as briefly discussed above, reflect their many functions in freshwater ecosystems, the main subject of this book. Although ecology emerged with the Industrial Revolution and the changes in human society that accompanied it, ecological questions were already being posed two millennia earlier. Both Aristotle and Pliny the Elder contemplated the relationship between living beings and their environment as well as the role of humans in the balances of nature. The natural histories developed by these philosophers remained unchallenged until the emergence of the classification system of Linnaeus, the publication of Malthus' *An Essay on the Principle of Population*, the biogeographical reports of Humboldt, the economics of Liebig, and, especially, Darwin's theory of evolution. Together, these works gave rise to the definition of ecology proposed in 1866 by Haeckel: 'Ecology is the science of the relations of living beings, plants and animals, between them and with their environment'. But it was not until the beginning of the 20th century that a more rigorous approach to ecology emerged. Important ecologists, among others, during the past 100 years are: Alfred J. Lotka and Vito Volterra, who, working independently, developed predator–prey models; Vladimir Vernadski, who introduced the biogeochemical concept of 'biosphere'; Arthur G. Tansley, who introduced the concept of ecosystem; and Raymond L. Lindemann, who was among the first to implement the ecosystem concept of Tansley, further defining the key concepts of 'food webs' and 'energy loops'. Indeed, the second half of the 20th century can be considered as the beginning of 'the age of ecology', as later proposed by Donald Worster (1994), as it marked the beginning of an awareness that pollution and its potentially irreversible damage to the environment pose major threats to a sustainable existence. As the list of anthropogenic pressures has grown, ecologists have sought ways to monitor and even protect both ecosystems and the vital services they provide to humankind. Thus, modern ecology is not a self-contained discipline but draws upon genetics, mathematics, modern observational techniques, and computer science in its broad areas of research. Through synergies among these different research fields, ecology is able to consider biological mechanisms at various scales, as summarized schematically in Fig. 1.2. The response of an organism (such as a single nematode) to its global environment (biosphere) can be deconstructed according to the individual ecosystems that compose its biome (e.g. desertic, tropical, Arctic). The temporal and spatial limitations of each ecosystem are reflected in the

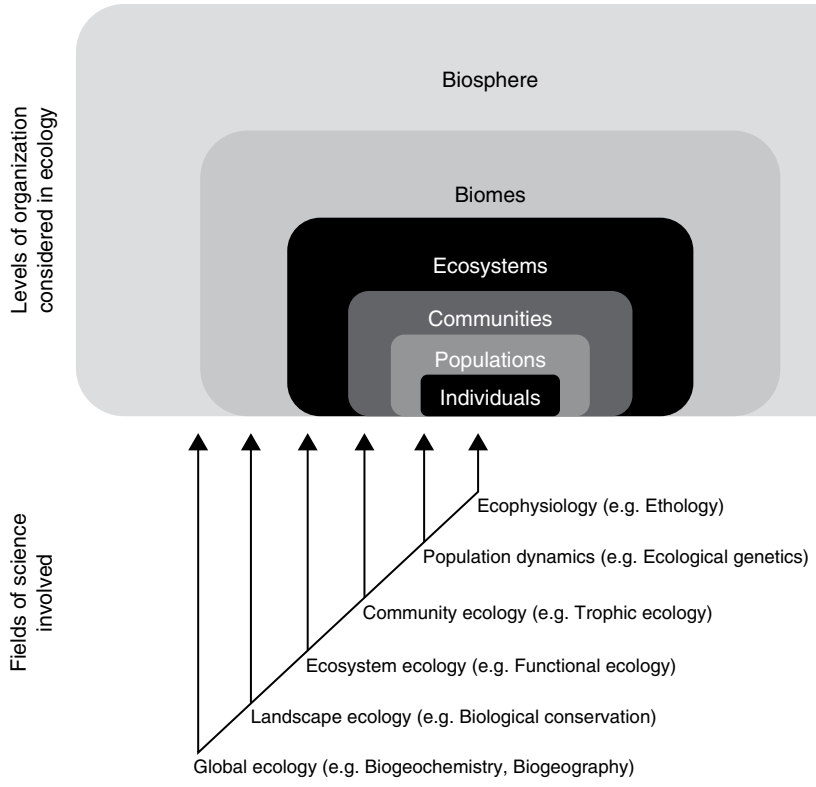

Fig. 1.2. Conceptual scheme of scaling in ecology and the subdisciplines involved in ecology studies. (Author's own figure.)

interactions of coherent assemblages (e.g. algal, nematode, and fish communities may interact within a defined lake ecosystem), each of which is made up of populations of different species made up of individuals of the same species. Because biological interactions may decouple systems and thus hinder direct physico-chemically based determinations of patterns, different observational levels are necessary in ecology to reveal the controls that act on patterns and processes at different scales.

The pioneering ecological concepts developed during the 20th century led to many new questions with respect to species diversity, abundance and biomass patterns, cycles of species co-existence, and the stability and regulation of species numbers. Efforts to answer those questions have given rise to the emergence of unifying research frameworks. One example is the metabolic theory of ecology (Brown *et al.*, 2004), which seeks to explain organismal relationships through coherent correlations between the metabolic rate, body size, and temperature. Sutherland *et al.* (2013) pointed out that, despite growing research activity, many questions and large knowledge gaps remain, as advances have come more slowly than in other scientific disciplines such as chemistry and even astrophysics. Furthermore, the spatiotemporal scale needed to study ecological mechanisms adequately varies widely, depending on the size of

the ecosystem, the communities considered, the number of interacting species and individuals, and the nature of their interactions. Problems related to complexity and scaling are the main obstacles hindering the emergence of a unified theory of ecology (Allen and Hoekstra, 2015). In response, ecologists have adopted the approach of first reducing the size and complexity of the ecosystem of interest, by studying community interactions and their mechanisms in laboratory experiments using microcosms as 'micro-ecosystems' before attempting to validate the results in field studies.

1.2.2 Distribution and dispersal of free-living nematodes

Only arthropods have a range of habitats and variety of lifestyles comparable in extent to that of Nematoda. Nematodes outnumber other multicellular animals in the ocean floor, inland waters, and soils, making them essential components of nearly all ecosystems on Earth (Traunspurger, 2002; Danovaro *et al.*, 2008; Van Den Hoogen *et al.*, 2019). An important proportion of nematode species are found globally (Andrássy, 2005; Zullini, 2014), with some being stenotypic (occurring in a few specific habitats) and others eurytopic (occurring in multiple habitats). The reasons for these differences in habitat specificity are poorly understood, but cosmopolitanism seems less common than previously thought (Zullini, 2018).

Studies of nematode ecology and distribution in the biosphere should be approached by taking into account the fundamental difference of scale between our macroscopic world and the microscopic world of nematodes. The human body is thousands of times larger and billions of times heavier than the typical body of nematodes, and human lifespan is also hundreds to thousands of times longer. For a tobrilid nematode, a season in a lake bottom corresponds to an entire life, one that is spent foraging and reproducing within an immense region. From this perspective, how a single nematode species can achieve a global distribution continues to puzzle researchers. However, the scale difference also implies the ability of nematodes to disperse very effectively over long distances. It is also likely that the filter made up of local environmental conditions and species interactions is a stronger constraint to the broader distribution of a species than is the spatiotemporal filter. Once juveniles have hatched, they seek out their food, which drives their active dispersal to favorable patches. Eggs, juveniles, and adults can also be passively dispersed by, for example, water currents, wind, rain, and other animals such as birds (Ptatscheck and Traunspurger, 2020).

1.2.3 Role of free-living nematodes in food webs

Free-living nematodes are an important component of belowground food webs in agro-ecosystems, shrublands, forest (Ferris, 2010; Heidemann *et al.*, 2014; Pausch *et al.*, 2016), and both warm and polar deserts (Liang *et al.*, 2002; Shaw *et al.*, 2018). They are also important contributors to

food webs in lake bottoms, streambeds, and microbial biofilms (Majdi and Traunspurger, 2015; Weitere *et al.*, 2018) as well as one of the few links in the food chains in the myriad of karstic environments (Du Preez *et al.*, 2017), pores, and interstices that make up the Earth's crust. In fact, nematodes have even been found in the microbial biofilms that form in the fracture water of a deep mine at a depth of ca. 1 km belowground (Borgonie *et al.*, 2011). The ubiquity, small size, diversity, and abundance of nematodes support their role as intermediaries in food webs, given their ability to exploit essentially all microscopic resources (bacteria, heterotrophic eukaryotes, fungal spores and mycelia, nano- and micro-algae, ultra-fine particulate organic matter and even dissolved organic matter) and in turn serve as food for other invertebrates and many very small vertebrates (Majdi and Traunspurger, 2015). However, the position of nematodes in food webs is complex (e.g. Majdi and Traunspurger, 2017; Wu *et al.*, 2019) because many nematode taxa bear a stylet that allows them to feed and even parasitize plants and animals much larger than themselves. Thus, nematodes are also able to partially exploit the production of higher plants (Pausch *et al.*, 2016) and to serve as 'top predators'. They have also been shown to switch diet opportunistically (Wardle and Yeates, 1993; Moens and Vincx, 1997; Wu *et al.*, 2019). Conversely, relatively large animals such as carp and water fowl can feed massively on nematodes, by filtering them out of the mud (Ptatscheck *et al.*, 2020), thereby reducing both the length of the food chains and energy losses between trophic levels. Interestingly, some common microbial groups, including amoebae, have been shown to pack-hunt and feed voraciously on nematodes (Yeates and Foissner, 1995; Geisen *et al.*, 2015), further challenging the notion that body size determines food web position.

The many pathways through which nematodes in the field acquire their food and, conversely, serve as food for other organisms remain to be fully disentangled, but this is an exciting avenue of research. At the scale of ecosystems, the most important role of nematodes may well be their active participation in carbon and nutrient cycles (Yeates *et al.*, 2009), based on the ability of nematodes to mobilize and package organic matter by burrowing and foraging through microbial mats (Weitere *et al.*, 2018), upgrading the quality of the obtained organic matter by synthesizing essential fatty acids, and then transferring these improved nutrients to higher trophic levels (Menzel *et al.*, 2018). Furthermore, in degraded ecosystems, after a collapse in ecosystem functions (e.g. after organic amendments, Biederman *et al.*, 2008; desertification, Guan *et al.*, 2018; or droughts, Majdi *et al.*, 2020a), nematodes may be among the first organisms in the ecological succession that eventually restore a complex trophic dynamic.

1.3 Why This Book?

1.3.1 The relevance of ecology in nematology

As emphasized in the chapters of this book, free-living nematodes are very small, ubiquitous organisms, have a high species diversity and population

turnover rate, readily disperse, play multiple trophic roles, and can be cultured in laboratory microcosms. Given these remarkable attributes, nematodes are a nearly ideal model organism for studies in a broad range of research fields. In agricultural science, nematology has moved from a focus on the development of synthetic nematicides to biological control, such as the use of entomopathogenic species of nematodes to impede insect pests or designing solutions to control plant-parasitic nematodes (Webster, 2012). The emergence of molecular technologies has supported investigations based on nematode models (especially *Caenorhabditis elegans*) in medical research and in a variety of other areas of research, including genetics, physiology, neurobiology, and developmental biology (Rankin *et al.*, 1990; Leung *et al.*, 2008; Ferris *et al.*, 2012; Webster, 2012). The 21st century has seen an immense increase in the public's interest in ecology, as the consequences for the planet of decades of urbanization, pollution, and the overuse of natural resources become clear.

We performed a literature search of scientific papers that included the words 'nematode' or 'nematodes' or 'nematoda' in their titles between 1960 and 2019 (analysis last performed on Google scholar 13 October 2020) to get an overview of nematological research during the past 60 years. In general, the number of scientific papers published each year has tended to grow over time (Fig. 1.3), with an average annual rate of 8–9% (Bornmann and Mutz, 2015).

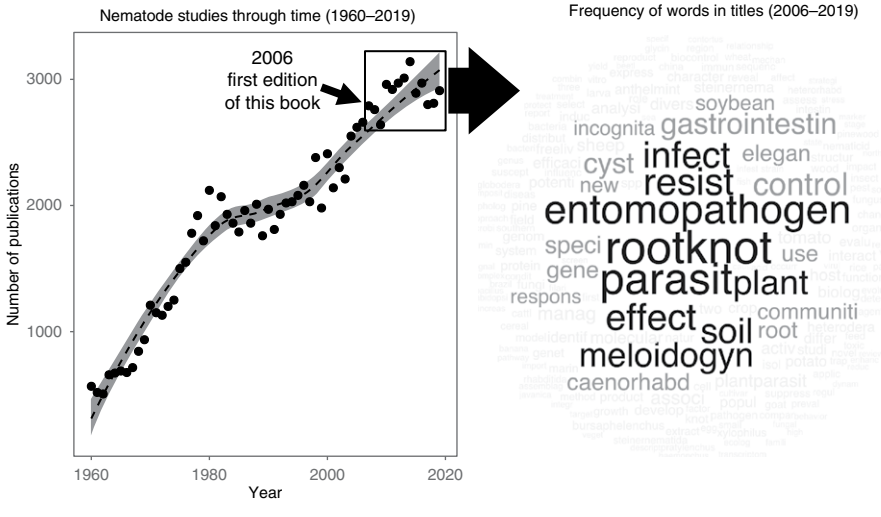

Fig. 1.3. (Left panel) Yearly number of publications with titles containing the words 'nematode' or 'nematodes' or 'nematoda'. The dashed line shows a smoothed conditional means model with the 95% confidence interval (gray). (Right panel) Word cloud representing the relative occurrences of the 200 most frequently used words in publication titles between 2006 and 2019 (N = 40,230 titles screened using 'tm' package in R). Words with darker and larger fonts occur more often. The extracted word lists were cleaned by stemming (e.g. 'parasit' instead of 'parasite') before their analysis, by changing all text to lower case, and removing numbers, common English stop words, punctuation, and extra white spaces. (Author's own figure.)

We then used a text-mining approach and focused on the word content of the titles that included 'nematode' or 'nematodes' or 'nematoda' over the period 2006–2019.

Figure 1.3 clearly shows that the recent nematological literature remains heavily focused on parasitic nematodes, especially plant-parasitic species such as *Meloidogyne* spp. (root-knot nematodes). The stem word 'parasit' was the second most frequently found across publication titles, whereas 'freeliv' ranked 46th. The concentration of research on parasitic nematodes reflects the continued need to mitigate economic losses in agriculture and to improve animal and human well-being, as indicated by the substantial number of publications on gastrointestinal parasites of cattle and humans. In terms of the type of habitats studied, most attention has been devoted to soils ('soil' ranked 7th) whereas free-living aquatic nematodes are a relatively marginal field of research, with the stem words 'marin' and 'freshwat' ranking 185th and 315th, respectively. In the field of ecology, the related stem words were of low ('ecolog', 156th) or very low ('ecosystem', 375th; 'web', 394th; 'trophic', 415th; 'dispers', 436th) ranking, although generic keywords related to biodiversity and population dynamics fared better ('communit', 19th; 'divers', 31st; 'popul', 38th; 'distribut', 66th). Thus, our text-mining analysis sadly demonstrates that nematologists have yet to recognize the potential of nematodes in ecological research. These results are in line with the findings of McSorley (2011), who analyzed topical trends in the *Journal of Nematology* and determined a steady decline in the relative importance of ecological topics in nematology papers published in that journal (22.5% in the 1970s vs. 15.8% in the 2000s), whereas topics associated with biological control increased sharply during the same period (from 1.7% to 12.4%).

1.3.2 An overview of the book's content

This book is a follow-up of the original, 2006 edition, which was dedicated to the taxonomy and ecology of freshwater nematodes (Eyualem-Abebe *et al.*, 2006), but it takes into account the important books and reviews from the past 15 years that concentrated on free-living nematodes. Among the former, three important volumes on the taxonomy of freshwater nematodes were published by Andrássy (2005, 2007, 2009); Ahmad and Jairajpuri (2010) and both Geraert (2008, 2010, 2011, 2013) and Ghaderi *et al.* (2016) focused, respectively, on Mononchida and the taxonomy of Tylenchida; and Schmidt-Rhaesa (2014) edited a *Handbook of Zoology* covering all taxonomic groups. Other authors have addressed the ecology of freshwater nematodes or, more generally, the ecology of the meiobenthos in books, special issues, and reviews. These include books on nematode behavior (Gaugler and Bilgrami, 2004), nematodes as environmental indicators (Wilson and Kakouli-Duarte, 2009), the second edition of *Meiobenthology* (Giere, 2009), *Perspectives in Meiobenthology* (Giere, 2019), book chapters on the ecology of freshwater nematodes (Traunspurger, 2009, 2014) and

on meiofauna in stream ecology (Traunspurger and Majdi, 2017), a special issue of *Hydrobiologia* devoted to the patterns and processes of meiofauna in freshwater ecosystems (Majdi *et al.*, 2020b), reviews of nematodes in aquatic environments (Tahseen, 2012), free-living nematodes in the freshwater food web (Majdi and Traunspurger, 2015) and in aquatic biofilms (Weitere *et al.*, 2018), the biodiversity of aquatic nematodes (Luc *et al.*, 2010; Decraemer and Backeljau, 2015), experimental studies with nematodes in ecotoxicology (Haegerbaeumer *et al.*, 2015), nematodes in caves (Du Preez *et al.*, 2017), and the impacts of plastic particles on benthic invertebrates, including nematodes (Haegerbaeumer *et al.*, 2019).

With this wealth of recent publications, one could ask: why another nematode book? The answer lies in our search of the nematological literature (Fig. 1.3) and a re-occurring take-home message in those publications: despite the recognized importance of nematodes across ecosystems and their undeniable ecological and evolutionary success, many of the world's free-living nematodes have yet to be fully characterized or even discovered. Moreover, important knowledge gaps remain, including studies of the autecology of free-living nematode species, the taxonomic and functional structure of nematode populations and assemblages, the spatiotemporal factors driving nematode dispersal and distribution patterns, the nematode diet, the relevance of nematodes as prey for other organisms, nematode participation in the mineralization of organic matter, and the consequences of habitat degradation on nematode assemblages.

This book focuses on the ecology of free-living nematodes. Our aim is to foster ecological research from the 'perspective of nematodes', as a means to better understand how ecosystems function and thereby devise measures to mitigate current threats to freshwater environments. Because reproducible experimental procedures are the basis for sound science, many chapters offer detailed protocols and case studies. The topics covered by the chapters that follow this Introduction are summarized below.

Chapter 2 'Sampling and Processing of Freshwater Nematodes with Emphasis on Molecular Methods' is an introduction to current methods of nematode sampling and processing, with the latter including molecular methods as well. **Chapter 3** 'Species Composition and Distribution of Free-living Nematodes in Lakes and Streams' provides an overview of the distributional patterns of nematodes in lakes, rivers, and streams worldwide and of the factors that affect the structuring of nematode communities in the field. **Chapter 4** 'Nematodes from Extreme and Unusual Freshwater Habitats' introduces the reader to the intriguing nematode communities that abound in remote or cryptic habitats as well as those found in extreme environments where multicellular life is pushed to its limits. **Chapter 5** 'Dispersal of Free-living Nematodes' considers the possible reasons underlying the ubiquity of nematodes and their high abundances in nearly all of their habitats. **Chapter 6** 'Feeding Ecology of Free-living Nematodes' details how nematodes acquire their food and interact directly, or not, with microbial processes. **Chapter 7** 'Role of Nematodes in the Food Web:

Nematodes as Predator and Prey' places nematodes in a broader food web context, including their participation in matter and energy fluxes within ecosystems. **Chapter 8** 'Production of Freshwater Nematodes' is a guide to measuring biomass production by nematode communities and to interpreting its ecological significance. **Chapter 9** 'Freshwater Nematodes in Metacommunity Studies' examines the relevance of nematodes as models in theoretical ecology studies of metacommunity. **Chapter 10** 'Single- and Multi-species Toxicity Testing with Nematodes' demonstrates the use of nematode physiological endpoints in analyses of the toxicity of potentially harmful chemicals and heavy metals. **Chapter 11** 'Freshwater Nematodes as Bioindicators in Field Studies – the NemaSPEAR[%]-index' presents an index based on the structure of nematode communities that can be used as a bio-indication tool by conservation ecologists and environmental managers. **Chapter 12** 'Case Studies with Nematodes from the Individual to Ecosystem Level' details four laboratory and field experiments in which nematodes were used to test hypotheses at different ecological scales (individual, population, community, and ecosystem).

Through these chapters, we seek to provide a solid introduction to the ecology of aquatic nematodes for scientists, graduate, and undergraduate students, as well as anyone curious about this evolving field.

1.4 Species Diversity and an Overview of Nematode Classification

Few other groups of animals are likely to harbor so many as yet undiscovered species as Nematoda. Currently, the number of valid species of free-living Nematoda is between 12,000 and 14,000 (Andrássy, 2005; Hodda *et al.*, 2009; Zhang, 2013), such that Nematoda are the fourth most diverse phylum after Arthropoda, Mollusca, and Vertebrata. However, the predicted number of species is at least 500,000 (Andrássy, 1976; May, 1988; Hammond, 1992; Hugot *et al.*, 2001, Blaxter, 2011).

Compared with other groups of animals, the classification of Nematoda is in many respects challenging, as the majority of nematodes are very small and hence difficult to study and identify. Furthermore, nematode classification has itself undergone several revisions, especially since the explosive development of molecular identification, which has opened up new perspectives in systematics (Andrássy, 2005). A gross system of Nematoda classification was compiled in 2005 by Andrássy (2005). In the following we present a short overview of this classification system, but for details of this system (and also other classification systems) the reader should consult Andrássy (2005, 2007, 2009). Among the three main groups of free-living nematodes, of the estimated 1530 known valid genera 33% belong to Torquentia, 29% to Secernentia, and 38% to Penetrantia (Andrássy, 2005). The list below includes several ecological considerations for groups that are major players in limno-terrestrial ecosystems. The number of validated free-living species (freshwater and terrestric), if

available, is also reported (in square brackets) and is based on Andrássy (2005, 2007, 2009). Genera and species found in brackish waters are only partly considered in this overview. Some representatives of free-living nematodes are shown in Fig. 1.4.

1.4.1 Torquentia Andrássy, 1974

The species comprising Torquentia are free-living *sensu stricto*, meaning that obligate/facultative plant- or animal parasites are not included. The large majority of the species (~92%) are strictly marine, with the remainder mostly found in continental aquatic environments but a minor proportion adapted to (non-saprobic) terrestrial habitats. In terms of the number of genera and species, Torquentia are nearly as rich as Secernentia and Penetrantia. So far, 673 genera have been established within this class, but how many are 'well-diagnosed' and how many are synonyms (or homonyms) remain to be painstakingly determined. Currently, the decision whether a given genus is valid or synonymous with another is often subjective, such that taxonomic assertions may long remain unchecked. A conservative estimate is that at least three-quarters of the genera (~500–520) are taxonomically valid. Of these, 460–470 are strictly marine and ~50 include but are not limited to continental species. At the species level, there are 3750–3800 'well-diagnosed' species recognized as belonging to Torquentia. Those nematodes are mostly microbivorous or detritivorous, feeding on bacteria, fine particles, protozoans, and algae. Although the feeding preferences of Torquentia have yet to be fully characterized, thus far no predators of other metazoans occur in this group (Andrássy, 2005).

Monhysterida De Coninck & Schuurmans Stekhoven, 1933

Most Monhysterida are aquatic nematodes, predominantly marine, although some species of this group numerically dominate nematode communities in continental waters. Examples of the latter include species from the genera *Eumonhystera* and *Monhystera*, which are systematically found in high numbers in a variety of limnetic habitats.

Monhysterina De Coninck & Schuurmans Stekhoven, 1933
Monhysteroidea de Man, 1876
Xyalidae Chitwood, 1951
Cylindrotheristus [6], *Daptonema [5]*, *Mesotheristus [3]*, *Mongolotheristus [1]*, *Penzancia [2]*, *Sacrimarinema [3]*
Monhysteridae de Man, 1876
Anguimonhystera [2], *Monhystera [20]*, *Eumonhystera [36]*, *Geomonhystera [16]*, *Monhystrella [32]*, *Tridentulus [7]*, *Sinanema [2]*, *Diplolaimelloides and Sitadevinema*
Sphaerolaimoidea Filipjev, 1918
Sphaerolaimidae Filipjev, 1918
Hofmaenneria [6]

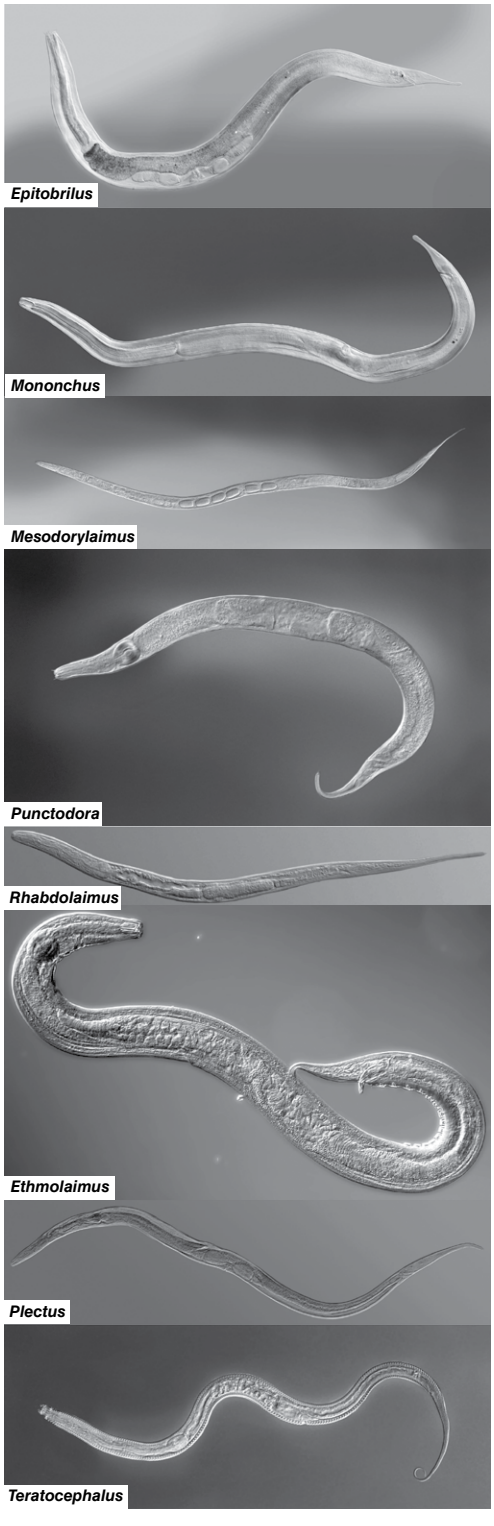

Fig. 1.4. Some representative genera of free-living nematodes. (Photo: Animal Ecology, Bielefeld University.)

Linhomoeina Andrássy, 1974 (marine)

Desmoscolecida Filipjev, 1929

Desmoscolecoidea Shipley, 1896
Desmoscolecidae Shipley, 1896
Desmoscolex [6], *Pareudesmoscolex [3]*
Greeffiellidae Filipjev, 1929
Cyartonematidae Tchesunov, 1989
Meyliidae De Coninck, 1965

Araeolaimida De Coninck & Schuurmans Stekhoven, 1933

Araeolaimina De Coninck & Schuurmans Stekhoven, 1933
Araeolaimoidea De Coninck & Schuurmans Stekhoven, 1933
Cylidrolaimidae Micoletzky, 1922
Cylindrolaimus [19]
Bastianiidae De Coninck, 1935
Bastiania [11]
Odontolaimidae Gerlach & Riemann, 1974
Odontolaimus [4]
Leptolaimina Lorenzen, 1979
Leptolaimoidea Örley, 1880
Leptolaimidae Örley, 1880
Adelonema [1], *Adenolaimus [1]*, *Deontolaimus [1]*, *Hemiplectus [1]*, *Leptolaimus [2]*, *Leptoplectonema [1]*, *Pakira [1]*, *Paraplectonema [7]*, *Prodomorganus [1]*
Aphanolaimidae Chitwood, 1936
Aphanolaimus [25], *Paraphanolaimus [12]*, *Aphononchus [8]*, *Anonchus [10]*
Haliplectoidea Chitwood, 1951
Rhabdolaimidae Chitwood, 1951
Rhabdolaimus [7], *Udonchus [3]*, *Rogerus [1]*
Aulolaimidae Jairajpuri & Hooper, 1968
Aulolaimus [12], *Pseudoaulolaimus [1]*
Plectoidea Örley, 1880
Chronogastridae Gagarin, 1975
Chronogaster [46], *Keralanema [1]*
Plectidae Örley, 1880
Anaplectus [12], *Arctiplectus [1]*, *Ceratoplectus [8]*, *Chiloplectus [6]*, *Perioplectus [1]*, *Plectus [78]*, *Ereptonema [4]*, *Tylocephalus [10]*, *Wilsonema [4]*, *Wilsotylus [1]*
Metateratocephaloidea Eroshenko, 1973
Metateratocephalidae Eroshenko, 1973
Euteratotecephalus [3], *Metateratocephalus [4]*

Chromadorida Chitwood, 1933

Chromadorida are also mostly marine; however, they further include limnic and, to a lesser extent, terrestrial forms. While mainly they are microbivorous, presumably feeding on fine particles and bacteria, there are reports from marine and freshwater ecosystems of species capable of feeding on diatoms (e.g. Jensen, 1982; Majdi *et al.*, 2012), by using their small teeth to crack the frustules and either swallow the organism in parts or suck out its inner cell contents. Chromadoridae may attain extremely high abundances such that they numerically dominate periphytic algal biofilms of lakes and rivers.

Desmodorina De Coninck, 1965
Desmodoroidea Filipjev, 1922 (marine)
Microlaimoidea Micoletzky, 1922
Microlaimidae Micoletzky, 1922
Microlaimus [60], *Prodesmodora [9]*
Chromadorina Chitwood & Chitwood, 1937
Cyatholaimoidea Filipjev, 1918
Cyatholaimidae Filipjev, 1918
Achromadora [17], *Paracyatholaimus [3]*
Ethmolaimidae Filipjev & Schuurmans Stekhoven, 1941
Ethmolaimus [14]
Chromadoroidea Filipjev, 1917
Hypodontolaimidae De Coninck, 1965
Chromadorita [5], *Dichromadora [2]*, *Neochromadora [1]*
Chromadoridae Filipjev, 1917
Chromadorina [7], *Prochromadora [1]*, *Punctodora [6]*

Draconematina De Coninck, 1965 (marine)

1.4.2 Secernentia Linstow, 1905

This is the second largest taxonomic group and it nearly exclusively consists of nematodes adapted to continental conditions. Thus, Secernentia is the only one of the three classes with essentially no marine members. Specifically, thus far <10 species are known to secondarily occur in marine or brackish habitats. The overwhelming majority of Secernentia are terrestrial, but some members may occur in inland waters. There are at least 440 genera, representing 3300–3400 valid species, according to the classification of Andrássy (2005). Stylet-bearing nematodes are capable of asymmetric predation (i.e. they can feed on prey larger than themselves), with fungal hyphae and the root system of lower or higher plants contributing to their diet. Plant-parasitic nematodes belonging to the Secernentia feed on important crops and are therefore considered to be agricultural pests. Other species are parasites of invertebrates or vertebrates, including humans. Species without a stylet are thought to be microbivorous or mycetophagous, but rarely carnivorous. They occur in various terrestrial habitats but also exhibit varying affinities for saprobic habitats (Andrássy, 2007).

Rhabditida Chitwood, 1933

Rhabditid nematodes occur predominantly in terrestrial environments (e.g. soil, humus, compost, decaying organic matters, dung), where they typically outnumber other nematode groups. Strictly aquatic (limnic and hyaline) species are rare, but the detection of rhabditids in many samples suggests that they are important albeit ephemeral inhabitants of inland waters. The majority of Rhabditida are bacterial feeders but some species are carnivorous.

Teratocephalina Andrássy, 1974
Teratocephalidae Andrássy, 1958
Steratocephalus [1], *Teratocephalus [15]*

Cephalobina Andrássy, 1974
Cephaloboidea Filipjev, 1934
Cephalobidae Filipjev, 1934
Bunobus, *Cephalobus [23]*, *Eucephalobus [12]*, *Heterocephalobellus [2]*, *Heterocephalobus [9]*, *Pseudacrobeles*, *Acrobeles [21]*, *Acrobeloides [29]*, *Acrobelophis [7]*, *Acroukrainicus*, *Cervidellus [16]*, *Chiloplacoides*, *Chiloplacus [29]*, *Nothacrobeles [15]*, *Paracrobeles*, *Pentjatinema*, *Placodira*, *Scottnema*, *Seleborca [13]*, *Stegelletina*, *Stegelleta [5]*, *Triligulla*, *Zeldia [14]*, *Acrolobus [1]*, *Cribonema*, *Metacrolobus*, *Panagroteratus*, *Teratolobus*, *Panagroteratus*, *Teratolobus*, *Metacrobeles*
Elaphonematidae Heyns, 1962
Acromoldavicus [2], *Kirjanovia*, *Elaphonema*
Osstellidae Heyns, 1962
Panagrolaimoidea Thorne, 1937
Panagrolaimidae Thorne, 1937
Procephalobus [4], *Propanagrolaimus [2]*, *Panagrolaimus [44]*, *Panagrobeles [4]*, *Panagrobelium*, *Brevistoma*, *Panagrellus [13]*, *Anguilluloides*, *Tricephalobus [3]*, *Halicephalobus [9]*, *Turbatrix [2]*, *Baujardia*
Alirhabditidae Suryawanshi, 1971
Brevibuccidae Paramonov, 1956
Brevibucca, *Cuticonema*, *Plectonchus*
Chambersielloidea Thorne, 1937
Chambersiellidae Thorne, 1937
Diastolaimus, *Macrolaimellus*, *Macrolaimus*, *Catoralaimellus*, *Chambersiella*, *Cornilaimus*, *Geraldius*, *Bicirronema*, *Tricirronema*, *Trualaimus*
Alloionematoidea Chitwood & McIntosh, 1934
Alloionematidae Chitwood & McIntosh, 1934
Alloionema, *Rhabditophanes [3]*

Myolaimina Inglis, 1983
Myolaimidae Andrássy, 1958
Myolaimus [9]

Rhabditina Chitwood, 1933
Rhabditoidea Örley, 1880
Stomachorhabditidae Andrássy, 1970
Rhabditonema [1], *Stomachorhabditis [4]*
Rhabditidae Örley, 1880
Rhabditis [28], *Curviditis [2]*, *Rhabditella [8]*, *Cuticularia [7]*, *Ablechroiulus [10]*, *Cephaloboides [3]*, *Diploscapteroides [5]*, *Discoditis [2]*, *Metarhabditis [1]*, *Oscheius [8]*, *Poikilolaimus [3]*, *Rhitis [9]*, *Rhabditoides [1]*, *Amphidirhabditis [1]*
Protorhabditidae Dougherty, 1955
Protorhabditis [13], *Prodontorhabditis*
Peloderidae Andrássy, 1976
Coarctadera [9], *Pelodera [10]*, *Rhomborhabditis [5]*, *Caenorhabditis [15]*, *Dolichorhabditis [10]*, *Pellioditis [16]*, *Phasmarhabditis [6]*, *Xylorhabditis [2]*, *Heterorhabditis*
Mesorhabditidae Andrássy, 1976
Crustorhabditis, *Cruznema [5]*, *Distolabrellus*, *Lesjan*, *Marispelodera*, *Mesorhabditis [21]*, *Operculorhabditis*, *Rhabpanus*, *Teratorhabditis [5]*, *Bursilla [6]*, *Parasitorhabditis*
Diploscapteridae Micoletzky, 1922
Diploscapter [13], *Carinoscapter*
Bunonematoidea Micoletzky, 1922
Bunonematidae Micoletzky, 1922
Bunonema [15], *Rhodolaimus [15]*, *Serronema*, *Rhodonema*, *Aspidonema [5]*, *Sachsium*, *Craspedonema*
Pterygorhabditidae Goodey, 1963
Pterygorhitis [2], *Pterygorhabditis [2]*

Diplogastrina Micoletzky, 1922
Cylindrocorporoidea Goodey, 1939
Cylindrocorporidae Goodey, 1939
Cylindrocorpus, *Goodeyus*, *Myctolaimus*, *Protocylindrocorpus*
Longicuccidae Poinar, Jackson, Bell & Wahid, 2003 (parasitic in vertebrates)
Odontopharyngoidea Micoletzky, 1922
Odontopharyngidae Micoletzky, 1992
Diplogastroidea Micoletzky, 1922
Pseudodiplogasteroididae Körner, 1954
Pseudodiplogasteroides
Diplogasteroididae Filipjev & Schuurmams Stekhoven, 1941
Demaniella, *Diplogasteroides [4]*, *Demaniella [4]*, *Goffartia [5]*, *Fuchsnema [9]*, *Rhabditidoides*, *Rhabditolaimus*
Diplogastridae Micoletzky, 1922
Acrostichus [13], *Anchidiplogaster*, *Butlerius [10]*, *Cephalobium*, *Costanemella*, *Diplogaster [2]*, *Diplogasteriana [2]*, *Diplogasteritus [22]*, *Eudiplogasterium [1]*, *Monobutlerius [5]*, *Parasitodiplogaster*, *Paroigolaimella [9]*, *Peterngus*
Neodiplogastridae Paramonov, 1952

Diplenteron [1], *Fictor [14]*, *Glauxinema [9]*, *Koerneria [29]*, *Micoletzkya*, *Mononchoides [29]*, *Neodiplogaster*, *Oigolaimella [4]*, *Pareudiplogaster*, *Pristionchus [17]*
Heteropleuronematidae Andrássy, 1970
Heteropleuronema
Tylopharynidae Filipjev, 1934
Tylopharynx [2]

Aphelenchida Siddiqi, 1980

Representatives of the Aphelenchida are adapted to a wide range of ecological and biological conditions. Most of these nematodes are free-living in soils, mosses, decaying organic matter, the undersurface of bark, and plant roots, and are mycetophagous or predacious. Some species are phytoparasitic and cause crop losses; however, phytoparasitism is much less well developed in Aphelenchida than in its large sister group, the Tylenchida. The remaining species are associates or obligate ecto- or endoparasites of insects (Andrássy, 2007).

Aphelenchina Geraert, 1966
Aphelenchoidea Fuchs, 1937
Aphelenchidae Fuchs, 1937
Aphelenchus [13]
Paraphelenchidae Goodey, 1951
Paraphelenchus [25]
Aphelenchoidoidea Skarbilovich, 1947
Aphelenchoididae Skarbilovich, 1947
Aphelenchoides [150], *Berntsenus*, *Laimaphelenchus [14]*, *Robustodorus [1]*, *Punchaulus [1]*, *Ruehmaphelenchus [2]*, *Schistonchus [9]*, *Sheraphelenchus [2]*, *Tylaphelenchus [7]*, *Bursaphelenchus [80]*, *Rhadinaphelenchus [1]*, *Anomyctus [1]*
Seinuridae Husain & Khan, 1967
Aprutides [2], *Papuaphelenchus [1]*, *Seinura [47]*
Ektaphelenchidae Paramonov, 1964 (semi-parasites of insects)
Parasitaphelenchidae Rühm, 1956 (in bark beetles)
Acugutturidae Hunt, 1980 (ectoparasites of insects)
Entaphelenchidae Nickle, 1970 (parasites of beetles)

Tylenchida Thorne, 1949

Tylenchids are present worldwide, with most species free-living in the soil, usually in association with plants, leaf litter, humus, mosses, or other terrestrial habitats. Unlike aphelenchids, they largely avoid decaying material. Aquatic or semi-aquatic species are rare. A majority of tylenchids live on or inside plants, feeding on their fluid cell contents. Other species are commensal or parasitize insects and other arthropods (Andrássy, 2007).

Tylenchina Chitwood & Chitwood, 1950
Tylenchoidea Örley, 1880
Tylenchidae Örley, 1880

Aglenchus [8], *Allotylenchus [1]*, *Coslenchus [37]*, *Cucullitylenchus [1]*, *Discotylenchus [6]*, *Filenchus [75]*, *Fraglenchus [2]*, *Irantylenchus [1]*, *Polenchus [3]*, *Tylenchus [30]*, *Basiria [42]*, *Boleodorus [25]*, *Neopsilenchus [10]*, *Duosulcius [2]*, *Malenchus [32]*, *Miculenchus [2]*, *Mukazia [1]*, *Ridgellus [1]*, *Silenchus [1]*, *Zanenchus [8]*, *Arborityncbus [1]*, *Campbellenchus [2]*, *Cephalenchus [19]*, *Gracilancea [1]*, *Pleurotylenchus [2]*, *Tylodorus [2]*, *Cervoannulatus [1]*, *Neothada [5]*, *Thada [1]*

Ecphyadophoridae Skarbilovich, 1959

Ecphyadophora [8], *Mitranema [2]*, *Ultratenella [1]*, *Chilenchus [1]*, *Ecphyadophoroides [2]*, *Epicharinema [1]*, *Lelenchus [3]*, *Tenunemellus [6]*, *Tremonema [1]*

Atylenchidae Skarbilovich, 1959

Atylenchus [1], *Eutylenchus [5]*

Anguinidae Nicoll, 1935

Anguina [12], *Diptenchus [1]*, *Ditylenchus [58]*, *Indoditylenchus [1]*, *Nothanguina [1]*, *Nothotylenchus [45]*, *Orrina [1]*, *Pseudhalenchus [4]*, *Pterotylenchus [1]*, *Safianema [6]*, *Subanguina [28]*, *Halenchus [3]*, *Neoditylenchus [24]*, *Sychnotylenchus [6]*

Hoplolaimoidea Filipjev, 1934

Psilenchidae Paramonov, 1967

Antarctenchus [1], *Atetylenchus [6]*, *Psilenchus [17]*

Dolichodoridae Chitwood & Chitwood, 1950

Brachydorus [3], *Dolichodorus [16]*, *Neodolichodorus [10]*

Belonolaimidae Whitehead, 1960

Belonolaimus [5], *Carphodorus [1]*, *Ibipora [4]*, *Morulaimus [8]*

Telotylenchidae Siddiqi, 1960

Bitylenchus [30], *Histotylenchus [6]*, *Neodolichorhynchus [17]*, *Paratrophurus [16]*, *Quinisulcius [15]*, *Sauertylenchus [4]*, *Telotylenchus [17]*, *Trichotylenchus [4]*, *Trophurus [14]*, *Tylenchorhynchus [106]*, *Uliginotylenchus [7]*, *Meiodorus [3]*, *Amplimerlinius [20]*, *Geocenamus [14]*, *Merlinius [30]*, *Nagelus [25]*, *Scutylenchus [21]*, *Macrotrophurus [1]*

Pratylenchidae Thorne, 1949

Hirschmaniella [33], *Pratylenchus [85]*, *Zygotylenchus [2]*, *Achlysiella [6]*, *Apratylenchoides [2]*, *Hoplotylus [7]*, *Pratylenchoides [29]*, *Radopholus [20]*, *Zygradus [2]*, *Naccobus [2]*

Hoplolaimidae Filipjev, 1934

Antarctylus [1], *Aphasmatylenchus [4]*, *Orientylus [4]*, *Helicotylenchus [194]*, *Orientylus [4]*, *Pararotylenchus [15]*, *Rotylenchoides [11]*, *Rotylenchus [75]*, *Varotylus [11]*, *Aorolaimus [7]*, *Basirolaimus [18]*, *Hoplolaimus [14]*, *Peltamigratus [22]*, *Scutellonema [45]*

Rotylenchulidae Husain & Khan, 1967

Acontylus [1], *Rotylenchulus [11]*, *Senegalonema [1]*

Heteroderidae Filipjev & Schuurmans Stekhoven, 1941

Bilobodera [2], *Meloidodera [10]*, *Verutus [2]*, *Atalodera [9]*, *Bellodera [1]*, *Camelodera [1]*, *Cryphodera [6]*, *Ekphymatodera [1]*, *Hylonema [1]*, *Rhizonemella [1]*, *Sarisodera [1]*, *Afenestrata [6]*, *Betulodera [1]*, *Cactodera [12]*, *Dolichodera [1]*, *Globodera [14]*, *Heterodera [71]*, *Punctodera [3]*

Meloidogynidae Skarbilovich, 1959
Bursadera [1], *Meloinema [4]*, *Meloidogyne [88]*, *Spartonema [2]*

Criconematina Siddiqi, 1980
Tylenchuloidea Skarbilovich, 1947
Paratylenchidae Thorne, 1949
Tylenchocriconema [1], *Cacopaurus [1]*, *Gracilacus [45]*, *Paratylenchus [74]*, *Tanzanius [1]*
Sphaeronematidae Raski & Sher, 1952
Goodeyella [1], *Meloidoderita [3]*, *Sphaeronema [7]*, *Tumiota [1]*
Tylenchulidae Skarbilovich, 1947
Trophotylenchulus [14], *Tylenchulus [4]*
Criconematoidea Taylor, 1936
Criconematidae Taylor, 1936
Criconemoides [38], *Discocriconemella [31]*, *Mesocriconema [94]*, *Nothocriconemoides [2]*, *Xenocriconemella [2]*, *Amphisbaenema [1]*, *Bakernema [2]*, *Blandicephalanema [3]*, *Criconema [84]*, *Croserinema [1]*, *Crossonema [30]*, *Lobocriconema [18]*, *Neolobocriconema [21]*, *Ogma [58]*, *Orphreyus [3]*, *Pateracephalanema [8]*, *Hemicriconemoides [49]*
Hemicycliophoridae Skarbilovich, 1959
Aulosphora [11], *Colbranium [1]*, *Hemicycliophora [126]*, *Caloosia [15]*

Hexatylina Siddiqi, 1980
Sphaerularioidea Lubbock, 1861
Neotylenchidae Thorne, 1941
Deladenus [22], *Hadrodenus [2]*, *Hexatylus [10]*, *Anguillonema [2]*, *Gymnotylenchus [2]*, *Fergusobia [7]*, *Rubzovinema [1]*
Sphaerulariidae Lubbock, 1961
Prothallonema [20], *Sphaerularia [1]*, *Tripius [2]*, *Bealius [2]*, *Ipiluella [1]*, *Misticius [1]*, *Neomisticius [1]*, *Paurodontella [8]*, *Paurodontoides [2]*, *Paurodontus [9]*
Allantonematidae Pereira, 1931
Allantonema [8], *Anandranema [1]*, *Bradynema [8]*, *Elaeolenchus [1]*, *Formicitylenchus [1]*, *Howardula [20]*, *Metaparasitylenchus [12]*, *Neoparasitylenchus [27]*, *Parasitylenchoides [8]*, *Pratinema [1]*, *Proparasitylenchus [6]*, *Protylenchus [2]*, *Scatonema [1]*, *Sulphuretylenchus [13]*, *Thripinema [5]*, *Aphelenchulus [1]*, *Bovienema [4]*, *Contortylenchus [29]*
Iotonchioidea Goodey, 1953
Iotonchiidae Goodey, 1953
Fungiotonchium [4], *Iotonchium [4]*, *Paraiotonchium [6]*, *Skarbilovinema [2]*
Parasitylenchidae Siddiqi, 1986
Coprotylenchus [1], *Parasitylenchus [9]*, *Kurochkinitylenchus [1]*, *Heterotylenchus [3]*, *Paregletylenchus [1]*, *Wachekitylenchus [4]*,

Incurvinema [1], *Psyllotylenchus [20]*, *Spilotylenchus [8]*, *Heteromorphotylenchus [2]*

1.4.3 Penetrantia Andrássy, 1974

Penetrantia contains several important groups of free-living nematodes, but parasites also occur. Roughly 35% of the species are marine, with the rest successfully adapted to continental ecosystems. True saprobiontes are not members of the Penetrantia. Thus far, ~740 genera have been diagnosed. A rough estimate is that among the nominal genera more than 75% (580–590 genera) can be considered as taxonomically validated. At the species level, 4600 valid free-living species have been proposed (Andrássy, 2007). Free-living species likely play a versatile and important role in benthic food webs. They include forms that feed on bacteria, but also species that attain relatively large sizes at maturity such that their diet includes larger prey, such as protozoans, algae, larger organic particles, other nematodes, and other small metazoans. Stylet-bearing species are assumed to be omnivorous, feeding on prey ranging from algal mats to plant roots and other animals. Some species transmit phyto-viruses and thus cause crop damage. There are also species that live as endoparasites in vertebrates. Species with large buccal cavities armed with teeth include predators of small invertebrates and even other nematodes.

Enoplida Filipjev, 1929

Enoplida are a species-rich, mostly marine group of nematodes that mostly include microbivores, bacteria and algae feeders, but also detritivores, and to a lesser degree, carnivores.

Enoplina Chitwood, 1933 (mostly marine)
- Leptosomatoidea Filipjev, 1916
  - **Anoplostomatidae** Gerlach & Riemann, 1974
  - **Leptosomatidae** Filipjev, 1916
  - **Thoracostomatidae** De Coninck, 1965
- Enoploidea Dujardin, 1845
  - **Anticomidae** Filipjev, 1918
  - **Enoplidae** Dujardin, 1845
  - **Phanodermatidae** Filipjev, 1927
  - **Thoracostomopsidae** Filipjev, 1927

Oncholaimina De Coninck, 1965
- Enchelidoidae Filipjev, 1918
  - **Belbollidae** Andrássy, 1974
  - **Enchelidiidae** Filipjev, 1918
  - *Calyptronema [1]*
  - **Eurystominidae** Filipjev, 1934
- Oncholaimoidea Filipjev, 1916

Oncholaimidae Filipjev, 1916
Viscosia [2], *Adoncholaimus [3]*, *Oncholaimus [7]*
Pelagonematidae De Coninck, 1965
Thalassogenus [5]

Ironina Siddiqi, 1983
Ironoidea de Man, 1876
Thalassironidae Andrássy, 1976
Ironidae de Man, 1876
Ironus [21]
Oxystominoidea Filipjev, 1918
Halalaimidae De Coninck, 1965
Leptosomatidae Filipjev, 1916
Oxystominidae Filipjev, 1918
Andrassyidae Chesunov & Gagarin, 1999
Andrassya [2], *Malakhovia [1]*

Tripyloidina De Coninck, 1965 (mostly marine)
Tripyloididae Filipjev, 1928
Tripyloides [1]

Tripylina Andrássy, 1974
Prismatolaimoidea Micoletzky, 1922
Prismatolaimidae Micoletzky, 1922
Prismatolaimus [34]
Onchulidae Andrássy, 1963
Caprionchulus [1], *Limonchulus [3]*, *Onchulus [8]*, *Stenonchulus [1]*, *Kinonchulus [1]*
Tripyloidea de Man, 1876
Tobrilidae De Coninck, 1965

Tobrilids have very successfully adapted to limnetic environments and may be found abundantly in lake bottoms all over the world. Their armed buccal cavity suggests that they can occupy several trophic niches. Adults are probably omnivorous or predators of other nematodes and meiofauna.

Tobrilus [23], *Eutobrilus [27]*, *Epitobrilus [19]*, *Paratrilobus [8]*, *Kurikania [2]*, *Semitobrilus [4]*, *Neotobrilus [19]*, *Macrotobrilus [1]*, *Quasibrilus [2]*, *Asperotobrilus [3]*, *Tobriloides [2]*
Triodontolaimidae De Coninck, 1965 (marine)
Rhabdodemaniidae Filipjev, 1934 (marine)
Pandolaimidae Belogurov, 1980 (marine)
Tripylidae de Man, 1876
Tripyla [24], *Tripylina [6]*, *Tripylella [3]*, *Trischistoma [4]*, *Tobrilia [2]*

Campydorina Jairajpuri, 1983
Campydoroidea Thorne, 1935
Campydoridae Thorne, 1935
Campydora [1]

Trefusiida Lorenzen, 1981 (marine)

Alaimida Siddiqi, 1983

Alaimina Clark, 1961
Alaimoidea Micoletzky, 1922
Alaimidae Micoletzky, 1922
Alaimus [51], *Cosalaimus [4]*
Amphidelidae Andrássy, 2002
Amphidelus [22], *Caviputa [10]*, *Etamphidelus [9]*, *Laxamphidelus [6]*, *Megamphidelus [1]*, *Metamphidelus [3]*, *Paramphidelus [23]*, *Postamphidelus [1]*, *Scleralaimus [1]*, *Scleramphidelus [1]*, *Cristamphidelus [8]*

Diphterophorida Loof, 1991

Diphtherophorina Coomans & Loof, 1970
Diphtherophoroidea Micoletzky, 1922
Diphtherophoridae Micoletzky, 1922
Diphtherophora [33], *Longibulbophora [2]*, *Tylolaimophorus [14]*
Trichodoridae Thorne, 1935
Allotrichodorus [6], *Ecuadorus [2]*, *Monotrichodorus [8]*, *Paratrichodorus [34]*, *Trichodorus [55]*

Mononchida Jairajpuri, 1969

Representatives of Mononchida are free-living and exclusively continental. They are found in a wide range of limno-terrestrial biotopes across the world, but not in saprobic environments. Many species are predators, seemingly with low specificity as exemplified by their ability to feed on protozoans, rotifers, oligochaetes, other small invertebrates, and particularly on other nematodes (Ahmad and Jairajpuri, 2010). Cannibalism also occurs, as some species may also feed upon plant-parasitic nematodes. These carnivorous species are thus potent biocontrol agents that play an important role in maintaining biological balance in agroecosystems (Andrássy, 2009).

Bathyodontina Coomans & Loof, 1970
Cryptonchoidea Chitwood, 1937
Cryptonchidae Chitwood, 1937
Bathyodontus [3], *Cryptonchus [4]*
Mononchuloidea De Coninck, 1965
Mononchulidae De Coninck, 1965
Mononchulus [1], *Oionchus [4]*

Mononchina Kirjanova & Krall, 1969
Mononchoidea Filipjev, 1934
Mononchidae Filipjev, 1934
Actus [5], *Clarkus [12]*, *Coomansus [28]*, *Granonchulus [5]*, *Judonchulus [3]*, *Mononchus [19]*, *Nigronchus [1]*, *Paramononchus [3]*, *Prionchulus [33]*, *Sporonchulus [4]*, *Tectonchus [4]*, *Cobbonchulus [1]. Cobbonchus [33]*, *Comiconchus [2]*, *Tricaenonchus [1]*

Mylonchulidae Jairajpuri, 1969
Brachonchulus [1], *Crestonchulus [1]*, *Margaronchulus [2]*, *Megaonchulus [1]*, *Mylonchulus [79]*, *Oligonchulus [1]*, *Polyonchulus [2]*
Anatonchoidea Jairajpuri, 1969
Anatonchidae Jairajpuri, 1969
Nullonchus [4], *Caputonchus [1]*, *Hadronchoides [2]*,*Hadronchulus [3]*, *Hadronchus [3]*, *Iotonchulus [4]*, *Iotonchus [89]*, *Jensenonchus [8]*, *Mulveyellus [5]*, *Parahadronchus [12]*, *Prionchulellus [1]*, *Prionchuloides [1]*, *Crassibucca [4]*, *Doronchus [2]*, *Miconchus [33]*, *Paracrassibucca [1]*, *Promiconchus [3]*, *Anatonchus [15]*, *Micatonchus [3]*, *Tigronchoides [9]*

Dorylaimida Pearse, 1942

Members of this group are highly abundant in terrestrial and aquatic habitats extending from the tropics to Antarctica. They include continental and free-living nematodes, but not marine or animal parasitic forms (Andrássy, 2009). Dorylaimida are remarkably diverse in terms of the number of species but also in their roles in ecosystems. This is reflected in their wide range of sizes and their possession of a stylet, which allows these nematodes to feed on a variety of food items, which includes other nematodes (Andrássy, 2009).

Nygolaimina Ahmad & Jairajpuri, 1979
Nygolaimoidea Thorne, 1935
Nygolaimidae Thorne, 1935
Afronygus [1], *Aquatides [13]*, *Clavicauda [2]*, *Clavicaudoides [11]*, *Feroxides [1]*, *Laevides [13]*, *Nygolaimus [34]*, *Paranygolaimus [2]*, *Solididens [8]*, *Paravulvus [17]*, *Nygolaimellus [6]*
Aetholaimidae Jairajpuri, 1965
Aetholaimus [5]
Nygellidae Andrássy, 1958
Nygellus [6]

Dorylaimina Pearse, 1936
Dorylaimoidea de Man, 1876
Thorniidae De Coninck, 1965
Nygolaimoides [5], *Thornia [11]*, *Thorniosa [1]*, *Loofilaimus [1]*, *Sphaeroamphis [1]*, *Thorneella [1]*
Dorylaimidae de Man, 1876
Amphidorylaimus [3], *Kunjudorylaimus [2]*, *Prodorylaimus [20]*, *Prodorylaimium [6]*, *Protodorylaimus [2]*, *Dorylaimus [29]*, *Halodorylaimus [2]*, *Idiodorylaimus [7]*, *Ischiodorylaimus [11]*, *Laimydorus [43]*, *Baladorylaimus [1]*, *Calcaridorylaimus [5]*, *Calodorylaimus [11]*, *Chrysodorus [5]*, *Crocodorylaimus [10]*, *Fuscheila [2]*, *Kittydorylaimus [1]*, *Mesodorylaimus [145]*, *Miodorylaimus [2]*, *Namaquanema [1]*, *Afrodorylaimus [6]*, *Apodorylaimus [2]*, *Drepanodorylaimus [13]*, *Paradorylaimus [7]*
Thornenematidae Siddiqi, 1969

Coomansinema [5], *Indodorylaimus [4]*, *Lagenonema [6]*, *Opisthodorylaimus [11]*, *Prothornenema [1]*, *Sicaguttur [3]*, *Thornenema [25]*, *Anadorella [1]*, *Paratimminema [2]*, *Sclerolabia [5]*, *Willinema [7]*

Actinolaimidae Thorne, 1939

Trachactinolaimus [3], *Trachypleurosum [6]*, *Actinolaimus [6]*, *Afractinolaimus [4]*, *Egitus [22]*, *Mactinolaimus [10]*, *Metactinolaimus [2]*, *Neoactinolaimus [17]*, *Paractinolaimoides [2]*, *Paractinolaimus [27]*, *Scleroactinolaimus [1]*, *Stopractinca [4]*, *Westindicus [6]*, *Actinca [5]*, *Afractinca [4]*, *Brasilaimus [7]*, *Parastomachoglossa [3]*, *Practinocephalus [3]*

Qudsianematidae Jairajpuri, 1965

Discolaimium [30], *Discolaimoides [16]*, *Discolaimus [41]*, *Filidiscolaimus [1]*, *Latocephalus [9]*, *Mylodiscoides [1]*, *Mylodiscus [1]*, *Salimella [1]*, *Carcharolaimus [20]*, *Caribenema [5]*, *Caryboca [4]*, *Allodorylaimus [28]*, *Amblydorylaimus [1]*, *Baqriella [1]*, *Boreolaimus [7]*, *Crassogula [1]*, *Crassolabium [34]*, *Dorydorella [3]*, *Epidorylaimus [14]*, *Eudorylaimus [95]*, *Kallidorylaimus [1]*, *Kolodorylaimus [1]*, *Labronema [41]*, *Labronemella [11]*, *Microdorylaimus [17]*, *Scalpellus [1]*, *Skibbenema [1]*, *Talanema [7]*, *Torumanawa [2]*, *Arctidorylaimus [3]*, *Ecumenicus [4]*

Aporcelaimidae Heyns, 1963

Akrotonus [1], *Aporcelaimellus [57]*, *Aporcelaimus [21]*, *Aporcella [2]*, *Epacrolaimus [2]*, *Makatinus [10]*, *Metaporcelaimus [14]*, *Silvallis [1]*, *Tubixaba [5]*, *Nygolaimium [3]*, *Scapidens [2]*, *Sectonema [24]*, *Aporcedorus [2]*

Paraxonchiidae Dhanachand & Jairajpuri, 1981

Gopalus [1], *Tendinema [2]*, *Parapalus [1]*, *Paraxonchium [13]*

Crateronematidae Siddiqi, 1969

Chrysonema [6], *Crateronema [2]*, *Oonaguntus [2]*, *Lordellonema [4]*, *Moshajia [5]*, *Poronemella [4]*, *Sicorinema [3]*, *Sicorinemella [3]*

Nordiidae Jairajpuri & Siddiqi, 1964

Inbionema [1], *Malekus [2]*, *Oriverutoides [1]*, *Oriverutus [27]*, *Actinolaimoides [10]*, *Acunemella [1]*, *Longidorella [39]*, *Thornedia [3]*, *Californidorus [4]*, *Enchodelus [23]*, *Enchodorus [2]*, *Heterodorus [25]*, *Kochinema [8]*, *Lanzavecchia [2]*, *Lenonchium [6]*, *Pungentella [15]*, *Pungentus [21]*, *Rhyssocolpus [10]*

Longidoroidea Thorne, 1935

Longidoridae Thorne, 1935

Australodorus [1], *Longidoroides [18]*, *Longidorus [150]*, *Paralongidorus [28]*, *Paraxiphidorus [3]*, *Siddiqia [34]*, *Xiphidorus [9]*, *Xiphinema [248]*

Belondiroidea Thorne, 1939

Belondiridae Thorne, 1939

Amphibelondira [1], *Axonchoides [1]*, *Belaxellus [1]*, *Belondira [3]*, *Belondirella [2]*, *Bullaenema [1]*, *Helicobelondira [1]*, *Immanigula [1]*, *Porternema [1]*, *Probelondira [1]*, *Anchobelondira*

[1], *Axonchium [33]*, *Dactyluraxonchium [2]*, *Heynsaxonchium [1]*, *Metaxonchium [19]*, *Nimigula [1]*, *Phallaxonchium [5]*, *Syncheilaxonchium [9]*, *Uniqaxonchium [2]*

Swangeriidae Jairajpuri, 1964

Durinemella [1], *Oxybelondira [4]*, *Oxydirus [13]*, *Paraoxybelondira [1]*, *Paraoxydirus [6]*, *Qudsiella [1]*, *Swangeria [2]*, *Falcihasta [4]*, *Hulqus [3]*, *Mitoaxonchium [1]*, *Paraqudsiella [1]*, *Lindseyus [4]*, *Roqueus [2]*

Dorylaimellidae Jairajpuri, 1964

Axodorylaimellus [6], *Dorylaimellus [63]*, *Ibadanus [1]*, *Mesodorylaimellus [4]*

Tylencholaimoidea Filipjev, 1934

Leptonchidae Thorne, 1935

Apoleptonchus [1], *Bertzuckermania [1]*, *Caveonchus [3]*, *Clavigula [1]*, *Funaria [12]*, *Incanema [1]*, *Leptonchus [11]*, *Loncharionema [2]*, *Meylis [3]*, *Paraleptonchus [1]*, *Proleptonchoides [3]*, *Proleptonchus [6]*, *Sclerolaimus [1]*, *Aculonchus [4]*, *Basirotyleptus [26]*, *Glochidorella [6]*, *Sclerostylus [2]*, *Trichonchium [3]*, *Zetalaimus [3]*, *Gymnotyleptus [3]*, *Scalpenchus [1]*, *Tyleptus [8]*, *Utahnema [3]*, *Kantbhala [5]*, *Xiphinemella [15]*

Tylencholaimidae Filipjev, 1934

Capilonchus [2], *Chitwoodielloides [3]*, *Chitwoodiellus [5]*, *Chitwoodius [8]*, *Cricodorylaimus [2]*, *Meylonema [2]*, *Pseudotylencholaimus [1]*, *Rostrulium [1]*, *Tylenchodoroides [1]*, *Tylenchodorus [1]*, *Tylencholaimus [52]*, *Mumtazium [1]*, *Promumtazium [7]*, *Tantunema [5]*, *Discomyctus [10]*, *Lawtonema [1]*, *Oxydiroides [3]*, *Wasimellus [1]*, *Curvidorylaimus [2]*, *Metadorylaimus [1]*, *Neometadorylaimus [1]*, *Vanderlindia [2]*, *Pachydorylaimus [7]*, *Heynsnema [3]*

Mydonomidae Thorne, 1964

Calolaimus [7], *Timmus [1]*, *Dorylaimoides [69]*, *Morasia [5]*, *Mydonomus [4]*

Tylencholaimellidae Jairajpuri, 1964

Athernema [1], *Agmodorus [4]*, *Doryllium [14]*, *Goferus [1]*, *Oostenbrinkella [3]*, *Phellonema [1]*, *Dorella [4]*, *Margollus [2]*, *Tylencholaimellus [36]*

Aulolaimoididae Jairajpuri, 1964

Adenolaimus [6], *Aulolaimoides [6]*, *Cladocephalus [1]*, *Oostenbrinkia [2]*

Encholaimidae Golden & Murphy, 1967

Encholaimus [2], *Helmabia [6]*, *Nemabia [1]*, *Acephalodorylaimus [1]*, *Cephalodorylaimus [1]*, *Echinodorus [1]*

Mermithida Hyman, 1951

This group includes a few species (isolaimiids) free-living in soils but the vast majority are obligate animal parasites during their larval stages.

They parasitize a wide variety of soil and freshwater invertebrates, such as crustaceans, insects, spiders, slugs, and snails. The final molt, mating, and oviposition of the nematode occur in the soil or in the aquatic habitat of the host.

Isolaimiina Inglis, 1983
Isolaimiidae Timm 1969
Isolaimium [12]

Mermithina Andrássy, 1974 (no free-living genera)

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2 Sampling and Processing of Freshwater Nematodes with Emphasis on Molecular Methods

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Highlights

- Nematode sampling and processing for different habitats will require different sampling methods.
- Nematode identification can be achieved with morphological or molecular approaches.
- The analysis of specific gene fragments can be used to delimitate nematode species.
- Molecular species identification can give further information about phylogenetic background and cryptic species.

2.1 Introduction

Nematodes can be sampled from various freshwater habitats (e.g. lakes, ponds, streams, rivers), both from soft substrates, such as sediments, and from hard substrates, including stones, wood, and plant material. For most studies, the samples have to be preserved in an appropriate fixative and the nematodes extracted, such as by density centrifugation, filtration, or decanting, for further processing. The extracted nematodes can be counted and identified to the species or genus level. Accurate nematode sampling and counting provide important information on the ecological status of the source habitat in terms of species diversity, abundance, biomass, the distribution of feeding types, and secondary production. The techniques commonly used for sampling, preservation, extraction, and mounting are described in several books or book chapters (e.g. Higgins and Thiel, 1988; Traunspurger, 2002; Hodda and Eyualem-Abebe, 2006; Giere, 2009; Traunspurger and Majdi, 2017). Nematode identification can be achieved based on morphological features or molecular techniques.

This chapter consists of a short overview of the general methods used to process nematodes from samples and the morphological and molecular methods used in the identification of freshwater nematodes. Molecular techniques, including single-specimen barcoding, next-generation sequencing (NGS), and environmental DNA (eDNA) approaches, are based on the amplification of short gene fragments that can then be used to identify a specimen by comparing its DNA sequence with reference sequences from a taxonomically known and sequenced specimen. The preferred method will depend on the aim of the study. The questions that can be addressed with molecular analyses, such as the identification of cryptic species and phylogenetic analyses with tree-building approaches, are also considered herein.

2.2 Sampling

Freshwater nematodes are most frequently sampled in sediments of lakes, streams, and rivers using a plastic corer (diameter 1–6 cm). Studies investigating the local structuring mechanisms of species distribution require core sizes <3 cm (Somerfield and Gage, 2000). The corer maintains

the different sediment layers such that the obtained samples can be used to investigate the vertical distribution (e.g. 0–2 cm, 2–5 cm, 5–10 cm) of nematodes (Traunspurger and Drews, 1996). In general, the upper 5 cm of sediment of lakes are preferentially sampled, as the absolute number of nematode individuals will decrease with increasing sediment depth and, thus in deeper sediments only low nematode densities will occur (Traunspurger, 1997). For streams and rivers, however, at least the upper 10 cm of sediment are recommended because several studies have shown that nematodes in these habitats penetrate deep into the sediment (e.g. Griffiths *et al.*, 1990; Eisenmann *et al.*, 1998; Traunspurger, 2000; Beier and Traunspurger, 2003; Traunspurger *et al.*, 2015).

The structure of the sediment can vary enormously, ranging from a very muddy to a coarse sandy structure, and will also affect the abundance and diversity of nematodes in the sample. Hard substrates, such as stones or wood pieces below the water surface, are frequently covered with periphyton (biofilm, Aufwuchs), which mainly consists of bacteria, algae, protozoa, and meiofauna (Weitere *et al.*, 2018). Scraping off the periphyton with a brush sampler, which samples a standardized, defined area of 3.14 cm^2 (= area of a circle with a diameter of 2 cm), allows the quantitative sampling of hard substrates (Peters *et al.*, 2005). The brush sampler uses small bristles to scrape the substrate. Afterwards the sample can be placed in a container and closed for transport to the laboratory, where the sample can be transferred to a bottle filled with fixative. Several 3.14-cm^2 areas may be sampled and pooled to increase the sampled area, achieve sufficient nematode sample sizes, and reduce the variability of sampling owing to small-scale patchiness. The major advantages of this approach are accuracy, as specific areas may be sampled (such as only areas of cobbles facing flow direction), and the option to sample a wide variety of non-removable hard substrates, such as large submerged trunks and root systems, rocks, and other large concrete structures. For deeper locations, sampling is typically carried out by scuba divers.

Leaf litter, macrophytes, and other plant material provide a habitat for a large number of organisms, including nematodes (Brüchner-Hüttemann and Traunspurger, 2020). Leaf litter or parts of macrophytes can be sampled by thoroughly rinsing the material in a white tray. The water in the tray is then poured over a mesh with appropriate mesh size (1 mm) and the retained material transferred into a sampling bottle containing the preservation agent. The collected material is then typically dried, weighed or flattened, and photographed with a scale (or scanned) to estimate abundances as a function of dry weight or area of plant material sampled. Mosses are also inhabited by a large number of metazoans, as this habitat furthermore provides a relatively isolated and protected biocoenosis. Nematodes are among the most abundant metazoans found in moss samples (Lazarova *et al.*, 2000; Schenk *et al.*, 2016). Sampling can be easily conducted, by stamping out a predefined area of the moss with a corer. The sample is then rinsed and poured over the appropriate mesh to extract the organisms (Schenk *et al.*, 2016). Submerged moss samples can

be slid underwater into a plastic bag and the area defined with respect to the dry weight of the moss.

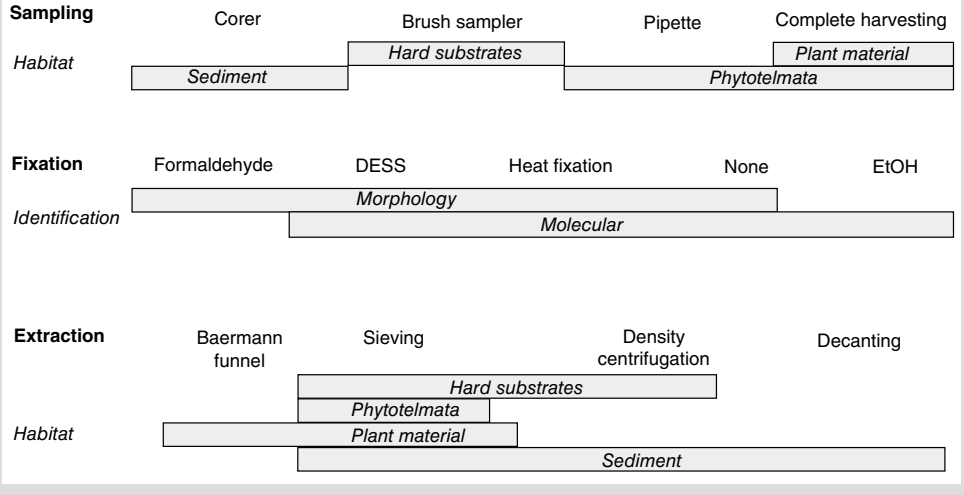
Small waterbodies, such as phytotelmata (e.g. water-filled tree holes or bromeliads), can be sampled as a whole, as the amount of water is relatively small (Ptatscheck and Traunspurger, 2014; Robaina *et al.*, 2015). Sampling is either conducted non-invasively, using a pipette to draw up the water column, or by the removal of the entire plant, if possible (Zotz and Traunspurger, 2016) (Box 2.1).

2.3 Preservation

If living nematodes are required, of course preservation has to be avoided. This can be especially convenient for molecular analysis but also for stable isotopic analysis and other biomarker studies (e.g. fatty acids, gut pigment analyses), as the risk of DNA damage by the fixative is thus avoided. Living nematodes can, for example, be directly transferred into DNA lysis buffer. However, many types of analyses require preservation of the samples. This is especially useful if the period of time between sampling and sample processing will be long, such as when samples are collected in the field over the course of several days or the number of samples exceeds the daily manpower.

Sample preservation directly after sampling will preserve the current organismal composition quickly and accurately. Several fixatives can be used to preserve organisms for later morphologically or molecularly based identification (Riemann, 1988). Diluted formaldehyde (4%) is the most commonly used agent for nematode preservation (Higgins and

Box 2.1. Overview of sampling, fixation, and extraction for freshwater nematodes.



Thiel, 1988) (Box 2.1), as it provides the best conditions for morphological identification, although it might preclude relaxed (e.g. curled) nematodes. However, molecular analyses are not possible from formalin-fixed samples as formaldehyde induces denaturation and crosslinks between DNA and proteins, thus inhibiting the amplification of marker genes (Bucklin and Allen, 2004; Zimmermann *et al.*, 2008). For the preservation of molecular samples, pure ethanol (80–100%) is commonly used (Fonseca and Fehlauer-Ale, 2012) (Box 2.1). Ethanol-preserved nematodes, however, often shrink or are distorted, which hampers a morphological identification. Although, the shrinking can be reversed to a certain degree by storing nematodes for 24 h in distilled water, ethanol is not appropriate for the morphological identification of nematodes. Thus, if a sample needs to be analyzed molecularly and morphologically, it should be split into equal parts, with one part preserved in formalin (morphological analysis) and the other in ethanol (molecular analysis).

An alternative preservation agent is DESS (dimethyl sulfoxide (DMSO), edetic acid disodium salt (EDTA), and saturated NaCl), which allows the morphological identification of nematodes but without hampering molecular amplification (Yoder *et al.*, 2006). DESS inactivates naturally occurring nuclease activities (Creer *et al.*, 2010) and is a good short-term preservative for morphological identification. However, especially freshwater specimens may become distorted, with collapsed bodies, after DESS preservation (Yoder *et al.*, 2006). A major disadvantage is that the high specific density of DESS interferes with agents used for density centrifugation, such as LUDOX® (see Section 2.4.3), thus ruling out the use of this extraction technique (Creer *et al.*, 2010). Nevertheless, for studies that do not require nematode extraction, such as those of nematodes from periphytic or epiphytic habitats, DESS may be a valuable alternative. Further studies of DESS-fixed freshwater nematodes are required to determine the utility of this preservation method, especially for morphological identification.

2.4 Extraction

After sampling, nematode specimens need to be removed from the (preserved) sample for further processing, including molecular or morphological analyses. However, extraction is not needed for eDNA applications as extraction kits are available that overcome the problems posed by humic acids and other inhibitors present in the sample that inhibit polymerase chain reaction (PCR) amplification performed for single-specimen barcoding and metabarcoding (Peham *et al.*, 2017).

In general, the methods used to extract nematodes from the sample vary enormously. Descriptions can be found in several books and book chapters (e.g. Higgins and Thiel, 1988; Giere, 2009; Hodda and Eyualem-Abebe, 2006; Traunspurger and Majdi, 2017; Box 2.1).

2.4.1 Sieving

Sieves between 10 and 70 µm are commonly used to isolate nematodes, but nematode losses even using very fine sieves are inevitable (Seinhorst, 1956; Ptatscheck *et al.*, 2020). The selection of a sieve with a suitable mesh size is crucial as it will influence the results in terms of diversity and species composition (Hodda *et al.*, 2009). The upper cut-off mesh size that retains larger macrofauna but allows micro- and meiofauna to pass through is generally accepted to be 1–2 mm. The mesh size used to retain meiofauna while allowing the passage of all microfauna, organic debris, and other suspended particles or colloids that impede nematode detection is even more important. Based on the size class definition of meiofauna, such meshes have a cut-off of 42 µm or 45 µm (Fenchel, 1978; Higgins and Thiel, 1988; Giere, 2009), but a considerable fraction of the meiofauna, especially nematodes, will still be lost, as juvenile stages and even the adults of small nematode species might not be retained by the sieve. This may lead to an underestimation of certain taxa and thus a biased study outcome (Hodda *et al.*, 2009; Ptatscheck *et al.*, 2020).

Within the past 40 years, roughly two-thirds of all studies have used a mesh size <44 µm (Fig. 2.1), with mesh sizes <30 µm used in less than 10%. However, in a recent study by Ptatscheck *et al.* (2020) a mesh size of 41 µm failed to retain 23% of the nematodes (Table 2.1), whereas with smaller mesh sizes more nematode species were captured, accompanied by a shift in the age structure toward juvenile nematodes. Thus, the choice of mesh size in the sieving process is crucial and a mesh size of at least 20 µm is recommended to retain smaller and juvenile nematodes that may otherwise be lost.

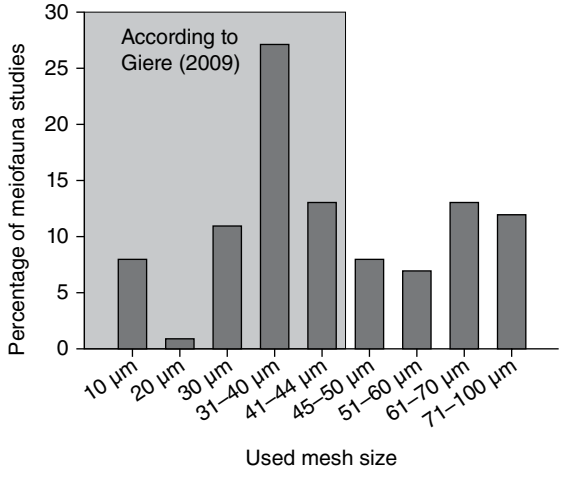


Fig. 2.1. Percentage distribution of the mesh sizes used in studies (n = 100) of meiofaunal community composition performed between 1980 and 2020. The gray box indicates the mesh sizes recommended in Giere (2009). (From Ptatscheck *et al*. (2020) with the permission of Springer.)

Table 2.1. Relative abundance (%), species number, and percentage of juveniles of nematodes retained on cascading sieves with five different mesh sizes. The mean (n = 10) is shown, with the standard deviation. (Table modified from Ptatscheck *et al*. (2020).)

	Mesh size				
	41 µm	30 µm	20 µm	10 µm	1 µm
Abundance (%)	77.0 ± 6.1	85.4 ± 4.3	90.4 ± 3.1	97.1 ± 3.0	100.0
Species number	26.4 ± 3.4	32.2 ± 3.5	33.6 ± 3.0	35.2 ± 2.4	35.5 ± 2.5
Percentage of juveniles	56.5 ± 4.4	69.0 ± 3.0	74.3 ± 4.1	79.3 ± 3.3	81.3 ± 2.6

2.4.2 Decanting

Decanting is a simple and quick method that is frequently used in nematodes extraction (Hodda and Eyualem-Abebe, 2006) and requires only a cylinder or flask and a mesh. Since nematodes are generally less dense than mineral sediment substrates, for sediment samples suspended in water their large heavy mineral particles, such as sand grains, will typically sink faster than the resident nematodes (Stokes' law). Decanting involves pouring off the water and still-suspended nematodes after the heavier soil particles have sunk but before the nematodes sink. If the supernatant containing nematodes and small particles is poured through a fine mesh (recommended: 10–20 µm), the nematodes will be retained and the small particles will pass through. Repeating the decantation steps several times will increase the likelihood of a quantitative extraction of the nematodes from the sediment (Hodda and Eyualem-Abebe, 2006). However, the grain size of the sediment sample may influence the extraction success (Giere, 2009), with sandy sediments most appropriate because they settle faster (van Bezooijen, 2006).

2.4.3 Flotation

Density flotation and centrifugation provide a standardized method that quantitatively extracts nematodes within a short period of time (Box 2.1). Moreover, it can be applied to preserved samples as well as living nematodes, as shown by Ptatscheck *et al.* (2015). The principle of this commonly used method is to create a density gradient by mixing the sample with a colloidal suspension whose specific density is similar to that of nematodes (approx. 1.12–1.15 g/ml). Subsequent centrifugation forces the nematodes into the supernatant, while the sediment/soil particles settle to the bottom. Little mineral material falls within the density range of nematodes, although organic matter often does. The best flotation medium is LUDOX® (or Levasil®), a colloidal silica solution of low viscosity and osmolarity (Pfannkuche and Thiel, 1988; Griffiths *et al.*, 1990). Roughly 70% of nematodes can be extracted within a single extraction, and 95%

after three rounds of extraction (Hodda and Bloemers, 1995). The number of repetitions needed to obtain a quantitative evaluation must be assessed in test runs (Giere, 2009). The size of each subsample depends on the size of the centrifugation tube or bottle, but for sufficient accuracy a LUDOX®/sediment sample ratio of at least 4:1 (or, better, 10:1) and thorough suspension by careful mixing are required (Giere, 2009).

2.5 Counting

In general, nematodes can be counted under a stereomicroscope after removal of the preservation agent (DESS; ethanol or formaldehyde). To facilitate counting, the nematodes should be placed in a standardized Petri dish (5- to 10-cm diameter) with marked grids (e.g. nematode counting dish; https://www.wur.nl/en/show/Nematode-counting-dishes-2.htm) and observed at a magnification of at least 30- to 40-fold. To facilitate the recognition of smaller specimens, staining with rose Bengal is recommended to better distinguish nematodes from sediment particles (Hulings and Gray, 1971). Rose Bengal stains the nuclei and cytoplasm and will therefore only stain organisms, not sediment or hard substrate particles. However, it cannot be used with samples that will be subsequently processed with molecular methods, as the dye enormously hampers amplification success (Fonseca and Fehlauer-Ale, 2012).

Natural nematode densities vary enormously. Typical densities of natural nematode communities are around 100 individuals per 10 cm^2 for sediment and periphyton samples but they may reach >1000 individuals per 10 cm^2 in other habitats (see Chapter 3). In the case of very high numbers of nematodes (>500 individuals/sample), an aliquot of the sample can be analyzed and the results then extrapolated to the total volume of the sample. However, to limit potential extrapolation errors owing to the uneven distribution of nematodes in the sample, ≥100 individuals should be present in the analyzed aliquot. If the sample contains very few specimens (<100 individuals), the whole sample should be analyzed.

Depending on the topic of interest, not only the nematodes but also other meiofaunal taxa in the sample can be recorded. Several protocols (e.g. Table 2.2, Traunspurger and Majdi, 2017) are available for determining the abundance of nematodes, copepods, ostracods, tardigrades, chironomids, and other meiobenthic organisms. Measurements of the length of the different meiofaunal groups can later be used to calculate their biomass (see Chapter 8).

2.6 Morphological Identification

Nematodes to be identified morphologically are mounted on permanent slides for microscopic inspection. Nematodes that were stored in preservative need to be prepared with glycerol for permanent slides. An

Table 2.2. Exemplary protocol for counting meiofaunal communities. For each group, different size classes are given that can be used to calculate biomass (protocol Animal Ecology, Bielefeld University, Traunspurger and Majdi, 2017). (Author's own table.)

Sample ID			
Nematoda	<0.25 mm	Oligochaeta	<1 mm
	0.25–0.5 mm		1–1.5 mm
	0.5–0.75 mm		1.5–2 mm
	0.75–1 mm		2–2.5 mm
	1–1.25 mm		>2.5 mm
	1.25–1.5 mm	Chironomida	<0.25 mm
	1.5–1.75 mm		0.25–0.5 mm
	1.75–2 mm		0.5–1 mm
	>2 mm		1–1.5 mm
Rotatoria type 1	<0.125 mm		>1.5 mm
	0.125–0.25 mm	Cladocera	>0.2 mm
	0.25–0.375		0.2–0.37 mm
	0.375–0.5 mm		0.37–0.5 mm
	>0.5 mm		>0.5 mm
Rotatoria type 2	<0.125 mm	Copepoda	<0.5 mm
	0.125–0.25 mm		0.5–1 mm
	0.25–0.375		<1 mm
	0.375–0.5 mm	Nauplii	<0.25 mm
	>0.5 mm		0.25–0.375 mm
Rotatoria type 3	<0.125 mm		0.375–0.5 mm
	0.125–0.25 mm		>0.5 mm
	0.25–0.375	Gastrotricha	<0.5 mm
	0.375–0.5 mm		0.5–1.0 mm
	>0.5 mm		1.0–1.5 mm
Tardigrada	<0.2 mm		>1.5 mm
	0.2–0.5 mm	Notes	
	>0.5 mm		

often-applied method is that of Seinhorst (1959), which uses sequential replacement of fixative and water with glycerol, using various mixtures of water, ethanol, and glycerol to prevent nematode shrinkage. The nematode specimens are first transferred into a solution of distilled water (79 parts), 96% ethanol (20 parts), and glycerol (1 part). Watch glasses can be recommended as container for the solutions. After evaporation of the ethanol, the nematodes are either transferred into a second solution consisting of 96% ethanol (95 parts) and glycerol (5 parts) or the second solution is added to the first. Evaporation of the ethanol leaves the nematodes finally preserved in glycerol. Nematodes should stay in the first solution for at least 3–4 days. If adding the second solution to the nematodes, a careful drop-by-drop addition from the border of the watch glass is recommended, as turbulences caused by mixing the two solutions can occur. The ethanol of the second solution evaporates within 1–2 days, but nematodes can be stored after processing for a longer time period.

A higher temperature (approx. 30°C) will accelerate evaporation if the preparation is urgent, but it may increase shrinkage (Seinhorst, 1959). The prepared nematodes can be mounted on microscopic slides, optimally with 10 nematodes per slide, and covered with round cover slips held in place with a fine wax. In general, nematodes are transferred with a pipette that has a small hair (e.g. a lash) glued to its tip.

Whereas nematodes can be counted using a stereomicroscope, their identification requires a microscope capable of higher resolution (400× to 1200× magnification) and equipped with an optimal light source. Differential interference contrast microscopy (also referred to as Nomarski interference contrast or Nomarski microscopy) is an optical microscopy technique that enhances the contrast in unstained, transparent samples.

For the characterization of specific nematode body parts, including the oral cavity, amphids, esophagus, gonads, and tail, an oil immersion lens is needed. Free-living aquatic nematodes are usually small (~0.5–5 mm) and because they are mostly translucent much of their internal anatomy is observable by light microscopy. Specimens are identified by characteristic body features, as described in detail in the identification literature (e.g. Loof, 1999, 2001; Andrássy, 2005, 2007, 2009; Ahmad and Jairajpuri, 2010). In addition to a taxonomic assignment, feeding types (see Chapter 6) or life stages (gravid female, female, male, stage of juveniles) are often documented to study nematode communities in their whole functionality (Moens *et al.*, 2006). Precise characterizations are an important prerequisite for species identification and should include: general appearance ('habitus'), external structures such as the body cuticle and ornamentations, head shape and width, body length and width, length of the cephalic and somatic setae, amphid position, length and width of the oral cavity, stylet length, esophagus length, positions of the esophageal glands (or nuclei), the excretory pore, and the vulva, length of the anterior/posterior female gonad, length of the egg, prerectum and rectum length, anal body width, tail length, spicula length, and vulva–anus distance. De Man's formula or indices, which are measurements and ratios generally used in nematode species descriptions (Andrássy, 2005), should be determined as well. The indices are summarized in Table 2.3; in addition to L, a, b, c, and V, the measurement of c´ is highly recommended.

The basis of any study of biodiversity is knowledge of species morphology, as it allows the identification of other specimens of the described species (Luc *et al.*, 2010). Although each specimen is unique, specimens can nonetheless be sorted and grouped into taxonomic units, ordered (classified) on the basis of phenotypic, usually morphological characteristics, similarity, and contiguity, a methodology referred to as α-taxonomy (Decraemer and Backeljau, 2015). For estimations of nematode species diversity, ca. 100 nematodes per replicate/sample are usually identified, although for some purposes 200 nematodes are recommended. A study by Schenk *et al.* (2020) showed that 100 nematodes per replicate are likely the best trade-off between time and accuracy, as the probability of finding a new species within the next individual was <5% and lower than in replicates of 50 or 75 nematodes

Table 2.3. Measurements and ratios frequently used in nematode identification. (Author's own table.)

Abbreviation	Measurement
L	Total body length, expressed in mm or µm
a	Relative thickness of the body, determined by dividing body length by the widest body width
b	Relative length of the esophagus, determined by dividing body length by the distance between the anterior and posterior margins of the esophagus
c	Relative length of the tail, determined by dividing body length by tail length
V	Position of the vulva, measuring the distance from the anterior body end to the vulva as a percentage of the entire body length
c´	Relative length of the tail, determined by dividing tail length by anal width

(Fig. 2.2). Of course, the more individuals identified, the more accurate the determination of nematode diversity will be. Thus far, some 28,000 nematode species have been morphologically described (Luc *et al.*, 2010).

2.7 Molecular Identification of Nematodes

Molecular samples can generally be treated the same as morphological samples for the extraction of nematodes from sediment, plant material, or hard substrates (Box 2.2). However, as residuals from LUDOX® may inhibit successful DNA amplification, a thorough cleaning step with water or ethanol is needed after density centrifugation.

2.7.1 Single-specimen DNA extraction

Single specimens isolated from ethanol- or DESS-fixed samples are transferred to distilled water in order to remove the ethanol and reverse shrinkage. This step is only necessary if light microscopy will be used to identify the specimens first to the lowest possible taxonomic resolution prior to sequencing.

Specimens are individually transferred into small Eppendorf tubes containing nematode lysis buffer (50 mM KCl, 10 mM Tris (pH 8.5), 2.5 mM $MgCl_2$, 0.5% Triton X-100, 0.5% Tween20) (Derycke *et al.*, 2005; Ristau *et al.*, 2013; Schenk *et al.*, 2017) and then frozen for at least 24 h at −20°C to −80°C. After the samples are thawed proteinase K (20 mg/ml) is added. The amount depends on the size of the nematodes but commonly 1.5 µl proteinase K per nematode is sufficient. After 70 min of incubation at 65°C followed by 10 min at 95°C, the released genomic nematode DNA can be stored at 4°C for several days or frozen until further use (note that several rounds of freezing and thawing can affect DNA quality). This DNA extraction protocol is easy to use, inexpensive, and does not require special chemicals. Other similarly inexpensive protocols, such as NaOH extraction (Floyd *et al.*, 2002)

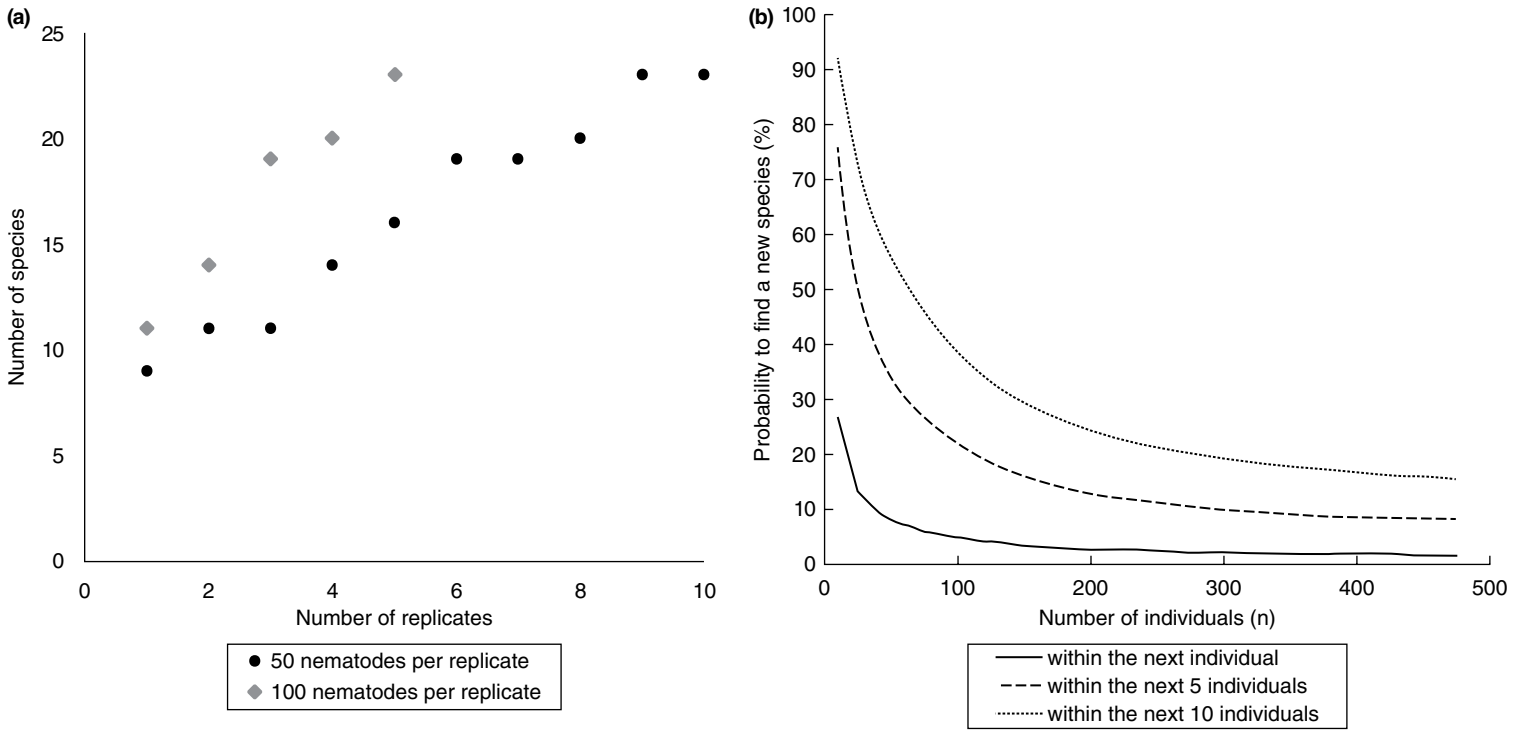


Fig. 2.2. (**a**) Cumulative number of new species found within the next replicate for 50 nematodes per replicate (black circle) and 100 nematodes per replicate (gray diamond). (**b**) The probability of finding a new species within the next individual (black line), the next 5 individuals (dashed line) and the next 10 individuals (dotted line) for 485 nematodes and a varying nematode number per replicate (e.g. 50, 75, 100, and 200) as indicated by the marks on the curve. (Data from Schenk *et al*. (2020), *n* = 485. Author´s own figure.)

or a combination of Chelex and proteinase K (Fontaneto *et al.*, 2015), are also suitable for nematodes. As an alternative, several commercial DNA extraction kits can be used to extract nematode DNA. However, these DNA extraction kits are often more expensive and require a minimum amount of tissue (e.g. 10 mg), which is critical regarding the small amount of nematode DNA.

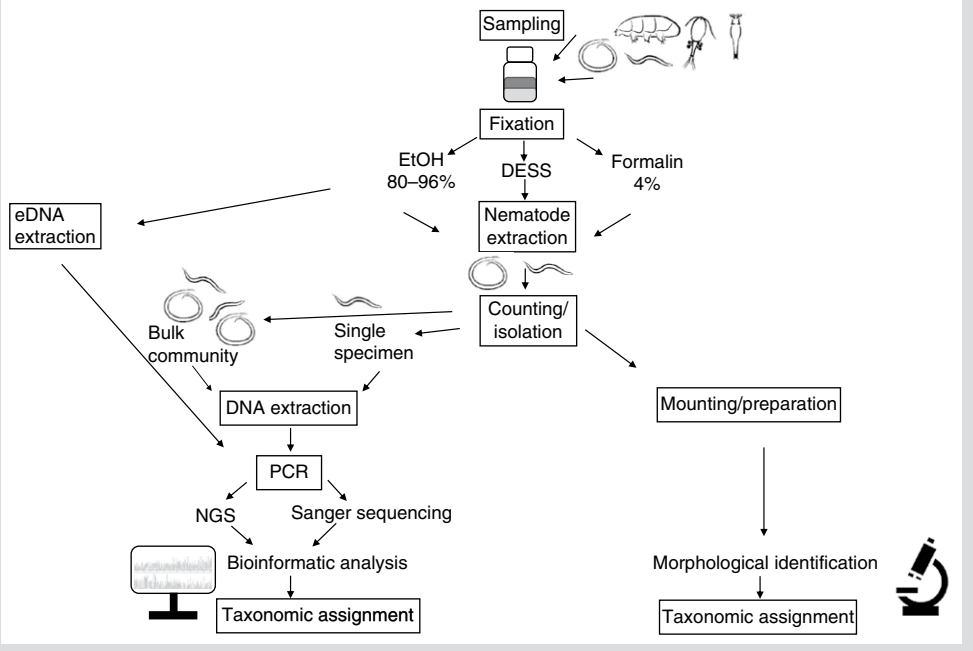
2.7.2 Community DNA extraction

Besides nematode DNA extraction from single specimens, DNA can be extracted from a whole community as a so-called bulk sample. This extraction method is mostly conducted using commercial kits, as the extracted genomic DNA is usually of good quality and lacking in inhibitors, allowing its use in NGS analyses. The DNA is bound to a small silica membrane and then washed with ethanol to obtain pure genomic DNA. In a bulk DNA extraction, the nematodes of multiple species are transferred into the lysis buffer as a whole community (e.g. 100 or 200 nematodes, the same sample size recommended for morphological identification). Several commercial DNA extraction kits are available that can be used for small amounts of DNA (DNeasy Spin Tissue, Qiagen; NucleoSpin Tissue XS, Macherey and Nagel). The total time needed for the extraction is between 30 and 60 min, depending on the number of samples (and excluding a lysis time of 4–8 h).

2.7.3 eDNA extraction

Besides DNA extracted from isolated individuals, DNA can also be extracted directly from (preserved) samples (Box 2.2). This eDNA is used to analyze all of the organisms present in the sample (often ranging from bacteria to larger invertebrates), without the risk of losing them in the isolation process. Several commercial kits can be used to bind the DNA present in the sediment or soil (e.g. DNeasy PowerMax soil kit, Qiagen). The capacity of these kits is limited, as currently only between 1 and 10 g of sediment can be reasonably extracted, and larger amounts of sediment mean higher costs (€10–25 per 10 g sediment). Alternative approaches, such as salt extraction, can be used if many samples need to be sequenced (Weigand and Macher, 2018). eDNA studies have mostly focused on complete meiofaunal communities, whereas those focusing solely on nematodes are rare. An investigation of marine sediments showed that among the meiofaunal phyla, OTU (operational taxonomic unit) numbers were highest for nematodes (Guardiola *et al.*, 2016), and in an ecotoxicological study that investigated the meiofaunal response to copper in mesocosms nematodes were determined to be one of the more sensitive taxa (Gardham *et al.*, 2014). However, a study using the cytochrome c oxidase I (COI) gene could not detect nematode OTUs in the eDNA samples, probably owing to primer failure for the nematode COI gene (see Section 2.8) or a lack of COI reference sequences for nematodes (Weigand and Macher, 2018).

Box 2.2. Overview about workflow for morphological or molecular identification.



2.8 Amplification of Specific Gene Fragments: PCR

The extracted genomic DNA is used to amplify short gene regions in a PCR. Specific primers, i.e. short sequences of between 15 and 25 base pairs (bp) that anneal to conserved regions in the genome, are used for amplification. Several gene regions have proven to work well for nematodes, among them ribosomal rDNA genes, which are present in multiple copies in the nematode genome (Sonnenberg *et al.*, 2007). The 18S rDNA gene consists of the V1–V9 regions (Fig. 2.3), all of which include conserved regions that can be exploited for primer binding, as well as variable regions for species identifications. However, the slow evolutionary rate of the 18S rDNA gene allows a broader range of primer binding but may result in a lower taxonomic resolution, especially for closely related species (Hebert *et al.*, 2004; Sahraean *et al.*, 2017). The V1–V2 region (primers: F04/R22; Blaxter *et al.*, 1998) and the V4 region (primers: 3NDf/C_1132f; Geisen *et al.*, 2018) work well for nematodes but they are not suitable for distinguishing closely related species. For example, several *Plectus* species were shown to have identical 18S rDNA sequences, such that taxonomic resolution using this region was possible only at the genus level or higher (Schenk *et al.*, 2019). Nevertheless, the 18S rDNA gene is useful in the reconstruction of deeper phylogenetic relationships across Nematoda, as shown by

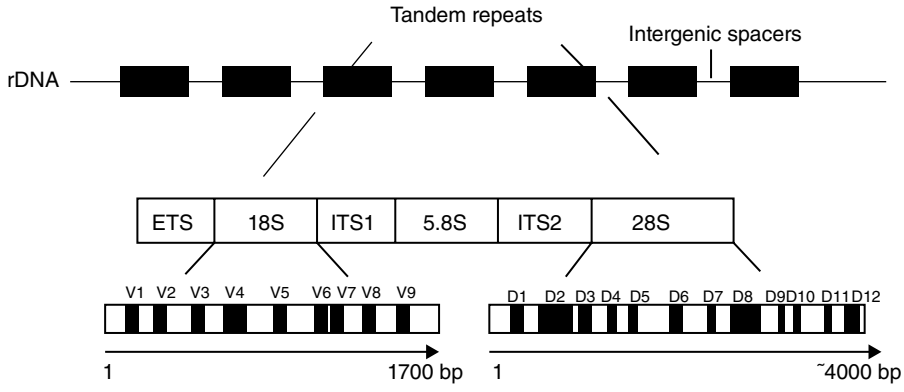


Fig. 2.3. Schematic overview of the ribosomal gene clusters tandemly arranged and separated by intergenic spacers. 18S rDNA consists of 9 variable regions, and 28S rDNA of 12 variable regions. 18S and 28S rDNA are separated by internal transcribed spacer (ITS) 1, 5.8S, and ITS2. (Author's own figure.)

Holterman *et al.* (2008) and van Megen *et al.* (2009) for over 1200 species of terrestrial nematodes.

Another potential marker region is the 28S rDNA gene (Fig. 2.3). Its D1–D12 gene regions are less conserved than the 18S rDNA gene and have thus been used in nematode investigations. While very divergent, the D1–D2 region is nonetheless a valuable tool in a wide range of meiofaunal taxa (Sonnenberg *et al.*, 2007). The D2–D3 region has mostly been used in studies of marine nematode species (Ley *et al.*, 2005; Derycke *et al.*, 2008; Fonseca *et al.*, 2008), and the D3–D5 region in freshwater nematodes (primers: 1274/706; Schenk *et al.*, 2017; Markmann and Tautz, 2005). The D3–D5 region may be a good compromise between divergent and conserved regions and applicable for several other eukaryotes. However, resolution at the species level may be limited for some taxa (Subbotin *et al.*, 2008), as demonstrated by Schenk *et al.* (2019) for two *Plectus* species.

The internal transcribed spacer (ITS) is located between the 18S rDNA and the 28S rDNA (Fig. 2.3). The ITS1 region is separated by the 5.8S rDNA from the ITS2 region (Powers *et al.*, 1997). Use of the whole ITS region has been investigated for marine nematodes (primers: VRAIN2f/VRAIN2R; Derycke *et al.*, 2005). The ITS region varies enormously in size, owing to many insertions and deletions. For nematodes, sizes range between 700 and 1300 bp but the large variations complicate alignments of this genetic marker. Additionally, given its large size, this fragment is better suited for single-specimen barcoding than for metabarcoding. Another limitation is that phylum-wide primer pairs are lacking for the ITS gene (Prosser *et al.*, 2013; Janssen *et al.*, 2017).

Besides ribosomal DNA, mitochondrial DNA can be used for genetic analyses. The COI gene is ubiquitous, as it is present in every cell, and is characterized by a high inter- and low intraspecific genetic variation. COI primer pairs (primers: JB3/JB3; I3-M11 region; Derycke *et al.*, 2005)

frequently used in studies of marine nematodes have shown potential for the genera *Tobrilus* (Ristau *et al.*, 2013) and *Plectus* (Schenk *et al.*, 2016). However, as mutations in the primer binding sites for the COI gene are known for several nematode species (Blaxter *et al.*, 2005; Ley *et al.*, 2005), COI primers may be better suited to investigating specific groups of nematodes rather than obtaining a broader overview of all species present in the sample.

PCR is conducted by mixing ultrapure water, the forward and reverse primers, nucleotides (dNTPs), PCR buffer, Taq-polymerase, and the genomic DNA from the sample in specific proportions. Optional additions may include $MgCl_2$, to stabilize primer annealing and boost Taq-polymerase function. However, many PCR buffers already contain $MgCl_2$, typically at concentrations of 1.5 mM.

Amplification conditions consist of a denaturation phase, in which the template DNA is denatured, achieved by incubation at high temperatures (>94°C). The primers attach to the DNA during the annealing phase, the temperature of which depends upon the annealing temperature of the specific primers used (commonly between 52 and 60°C). Generally, the annealing temperature should be a few degrees below the melting temperature of the primer pair. The polymerase then prolongs the DNA strand in the extension phase, which is conducted at temperatures of between 68 and 72°C. These steps comprise one PCR cycle, with a typical PCR protocol of 30–35 cycles used to amplify the DNA adequately (Schenk *et al.*, 2016).

In the recently developed NGS-amplification (metabarcoding), two PCR steps may be included. In the Illumina platform, the first PCR (amplicon PCR) is similar to the common PCR used in single-specimen barcoding, with the exception that specific sequences for the sequencing platform are attached to the primer sequence. The amplicons need to be cleaned after the first PCR step so that only fragments of the desired amplicon length are retained, as too long or too short fragments will hamper further processes. In the second PCR (index PCR), a specific index is attached to each sample that allows sample identification after sequencing, as all samples are pooled prior to Illumina sequencing on one flow cell. However, there are many alternative protocols for metabarcoding, also depending on the choice of sequencing platform.

2.9 Sequencing

Single-specimen sequencing is frequently based on Sanger sequencing, which was developed in 1970. In short, this method uses specifically modified nucleotides (didesoxynucleotides) that, owing to their missing hydroxyl group, result in a programmed termination of the polymerase enzyme. In Sanger sequencing, the whole sequence can be determined by electrophoresis and a single sequence for each individual is created.

NGS amplifies several samples in parallel, thus greatly accelerating throughput. Current NGS sequencers are capable of creating up to 1.5 Tb of data in 3 days. Depending on the sequencer, several thousands of sequences ('reads') can be generated for each sample. Both the data output and the sequencing length differ between systems, as they rely on different technologies. Despite the large amount of data, sequencers in use today are limited regarding the amplicon length, with up to 600 bp (Illumina: MiSeq: 15 Gb with 2 × 300 bp, compared with HiSeq 2500: 9 Gb with 2 × 250 bp) currently possible with paired-end sequencing (a forward and a reverse read that are merged into a final read). However, as technologies are constantly improving, even larger data outputs and longer amplicon lengths will no doubt soon be possible.

2.10 Processing

Sanger sequences can be analyzed with several programs, including ChromasPro, SeqMan, and Bioedit, by merging the forward and reverse contigs into the final sequence and then checking them for ambiguous and erroneous base calls in an electropherogram (Fig. 2.4). Sequencing errors (e.g. an 'N' in the sequence) in one contig can be corrected if the other contig is unambiguous. The consensus sequence can then be used to generate genetic alignments with several other sequences for purposes of comparison or to calculate genetic distances between and within species, or to construct phylogenetic trees.

NGS data must be preprocessed in bioinformatics pipelines in order to analyze the large amount of data. Bioinformatics pipelines were originally created for bacterial 16S rRNA analysis, but they also process eukaryotic genetic data (Bourlat *et al.*, 2016). Regardless of the choice of pipeline, in most the basic steps are similar. The primer and adaptor sequences need to be removed from the raw reads delivered by the sequencer and the forward and reverse read (if paired-end were used) are then merged. Further steps remove erroneous reads from the dataset, reads that are too long or too short, or chimeric sequences, which are sequencing errors often consisting of two parent reads. The reads can be clustered at a predefined threshold (chosen based on genetic distances) into OTUs. Alternative pipelines use denoising strategies and do not cluster the reads into OTUs, as variations may be lost. The final output is referred to as amplicon sequence variants (ASVs; Callahan *et al.*, 2016). The latter approach works well for marine nematodes (Macheriotou *et al.*, 2019) while for freshwater nematodes the traditional NGS approach is preferred (Schenk *et al.*, 2020). It should be noted that traditional OTU approaches are highly prone to false negatives and false positives. False negatives are taxa that were not recovered but were present in the initial sample, and false positives are taxa that are recorded but were not present in the sample (Birk and Hering, 2006). Either one may alter the study outcome and these issues are discussed in detail in the literature.

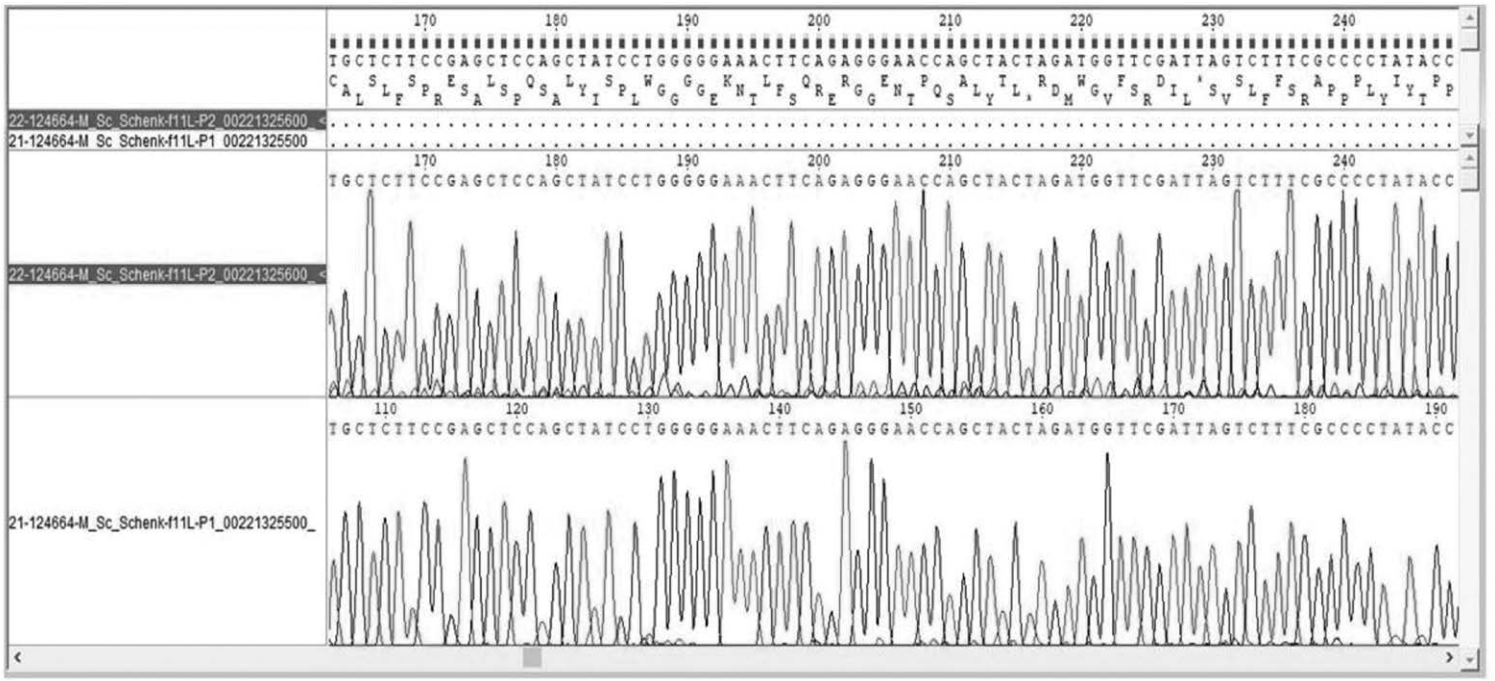


Fig. 2.4. Electropherogram for a merged forward and reverse contig. The overlap between the contigs should be high in order to generate an unambiguous consensus sequence. (Author's own figure.)

2.11 Annotation

The taxonomic assignment of an unknown sequence is achieved by comparison of the query sequence against a reference database, such as BLAST (Basic Local Alignment Search Tool), which aligns the query sequence against the sequences in the database. The results are reported as the query cover, identity score, and expect value (e-value). While the query cover and identity score should be as high as possible, an e-value closer to zero is more reliable. Among the largest databases are that of the National Center for Biotechnology Information (NCBI; Benson *et al.*, 2013) and the Barcode of Life Database (BOLD; Ratnasingham and Hebert, 2007). Recent expansions of reference databases include the Barcode of Life (BOL) initiative, the aim of which is to representatively record large proportions of the world's biodiversity (Stoeckle and Hebert, 2008). However, although freshwater nematodes are extremely abundant, their sequences are still rare in public databases and query sequences can be identified only if a close match is found. Currently, most sequences are available for the 18S gene fragment (27,462 sequences in July 2020).

In general, the BLAST algorithm is most reliable for complete reference sets, but sequences can be further annotated using other programs, such as MEGAN, which is based on the lowest common ancestor algorithm (Huson *et al.*, 2007). This method is computationally fast, while it also assigns the data to different taxonomic levels. Another option is the RDP classifier, which was originally developed for 16S rRNA analyses but can also be used for other gene regions, if properly trained (Schenk *et al.*, 2020). It applies a naïve Bayesian approach to classify sequences and its high assignment accuracy has been demonstrated (Wang *et al.*, 2007; Lan *et al.*, 2012).

2.12 Applications of Molecular Methods

2.12.1 Description of new species

Newly discovered species of nematodes are traditionally described morphologically, by measuring and describing characteristic body features, such as body length, body width, the presence of head bristles, the features of the oral cavity, and those of the tail bristles (see the Section 2.6). Specimens are frequently photographed or illustrated in order to facilitate identification by other researchers.

With the advent of molecular techniques, morphologically based descriptions of new species have been supported by molecular sequences, as this approach is highly reproducible. The utility of molecular analyses was demonstrated in the description of the newly discovered *Tripylella subintermedia* sp. nov. using 18S rDNA sequences (Zhao *et al.*, 2014). The parallel deposition of reference sequences with new species descriptions

will facilitate determinations of the phylogenetic background of the species and thereby yield insights into evolutionary patterns (Pereira *et al.*, 2010).

2.12.2 Cryptic species and genetic distances

Genetic data can be used to infer genetic distances within and between species, an approach frequently used to identify cryptic species, i.e. species that cannot be distinguished based on morphology and/or initially identified based solely on the COI gene together with a (ribosomal) marker (Derycke *et al.*, 2005; Geiger *et al.*, 2016). Intraspecific genetic distances (genetic distances within members of the same species) are usually very low, while interspecific distances (genetic distances between two species) are higher. Therefore, the presence of a very high genetic variability in one or more gene fragments of a 'species' suggests cryptic diversity. Nematodes, among other metazoan taxa, have a high degree of cryptic diversity. This has been demonstrated in marine nematodes but also in a study of freshwater *Tobrilus*, in which three genetically distinct lineages within this genus in northern Germany and Sweden were identified (Ristau *et al.*, 2013). The three lineages differed by up to 16% in the COI gene for the 100 individuals inspected. Although cryptic species cannot usually be distinguished morphologically, in some cases a detailed morphological inspection may reveal small variations in body features that in the absence of molecular analysis might have been attributed to natural variations (Armenteros *et al.*, 2014).

A study of plectids across Germany revealed that closely related species differ by up to 15.42% based on their COI sequences, while 28S rDNA genetic sequences vary by only 0.58% (Schenk *et al.*, 2016). The authors distinguished 24 COI haplotypes among the 53 specimens analyzed. This high genetic variability strongly suggested restricted gene flow, which could be detected even in samples obtained across short geographic distances, including two samples collected only 7 km apart (Fig. 2.5).

Although fixed genetic thresholds at which species can be distinguished have often been discussed, this is unlikely as the evolutionary rate between taxa may not be the same (Holovachov, 2016). Overall, investigations of nematode diversity to the best possible resolution require an integrated approach, in which genetic data are combined with morphological data, ecological information, and habitat coordinates (Fonseca *et al.*, 2008; Fontaneto *et al.*, 2015).

2.12.3 Phylogeny

Among the several methods used to investigate phylogenetic relationships and calculate phylogenetic trees, the most popular are computationally fast methods, such as the neighbor joining (NJ) algorithm (Saitou and

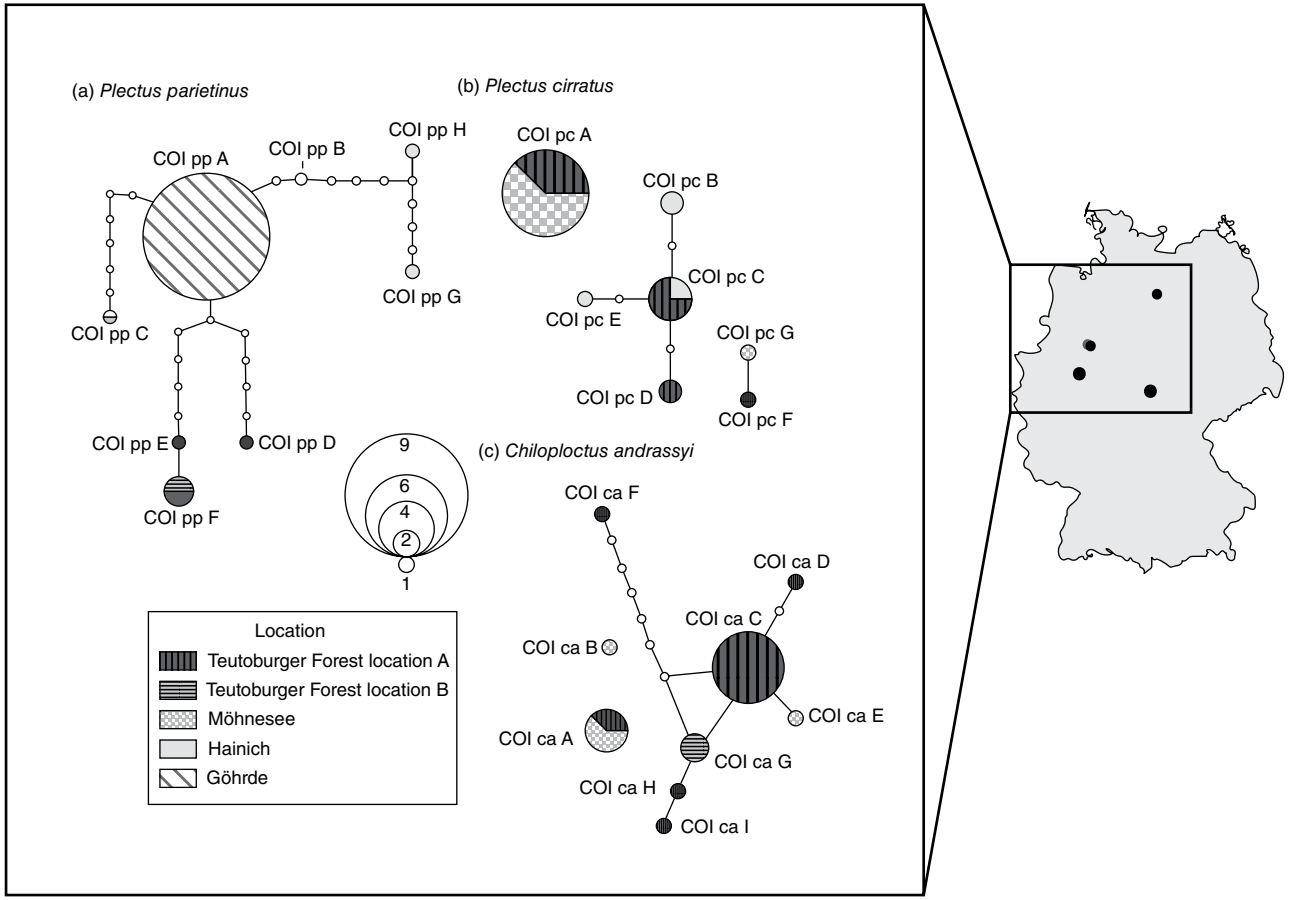


Fig. 2.5. Haplotype networks for the species *Plectus parientinus* (**a**), *Plectus cirratus* (**b**), and *Chiloplectus andrassyi* (**c**), based on the cytochrome c oxidase I (COI) gene fragment. The size of the circles is proportional to the haplotype frequency. The five different sampling locations are coded in different gray shades and patterns, with the pie slices showing the frequency of a particular haplotype at a given location. Small open dots indicate hypothetical haplotypes, and the connecting lines a single mutational step. (From Schenk *et al*. (2016) with the permission of Elsevier.)

Nei, 1987). NJ trees are based on genetic distances and a clustering algorithm (Lemey *et al.*, 2014). Other methods, such as maximum parsimony (MP) and maximum likelihood (ML), are slower but, especially the latter, have the advantage that several trees are generated in parallel and the most likely one is chosen as the final tree (Nei and Kumar, 2000; Tamura *et al.*, 2011). Furthermore, different evolutionary models can be considered, such that MP and ML are often-chosen methods for phylogenetic analyses (van Megen *et al.*, 2009). Another commonly used method to reconstruct phylogenetic relationships is Bayesian inference (BI). Instead of the bootstrap support values of ML and MP analyses, BI reports posterior probabilities while also allowing different models of evolution. In addition, BI enables Markov chain Monte Carlo (MCMC) integration and is computationally more efficient than ML and MP. BI has been used to reconstruct phylogenetic relationships evaluated using the ribosomal 18S rDNA gene fragment (Holterman *et al.*, 2008). Different tree-building methods are discussed in detail in several books (e.g. Page and Holmes, 1998; Lemey *et al.*, 2014).

2.13 Conclusions and Perspectives

Environmental samples containing nematodes can be processed using a wide variety of extraction and identification methods. However, the most appropriate one will depend on the aim of the study. While taxonomic assignments of nematode communities can be achieved based on morphological features, this method is time consuming and requires a high amount of taxonomic expertise. By contrast, molecular approaches are fast, accurate, and nowadays standardized. The introduction of NGS, especially bulk samples and eDNA, allowed a large number of individuals that can now be analyzed in parallel. However, the disadvantages of molecular assignments include flawed reference databases and biases in the amplification process, especially for NGS approaches. Thus, a combined (integrative) approach is, at least for now, the most accurate way to distinguish nematode species.

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3 Species Composition and Distribution of Free-living Nematodes in Lakes and Streams

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Highlights

- Mean nematode abundance ranges from 10^3 to 10^6 individuals per m^2 but may be 10-fold higher.
- Species richness range from 10 to 100 but >200 species per habitat can be found over the year.
- Total biomass is usually between 1 and 100 mg wet weight per m^2 (up to 10 g).
- Deposit feeders, feeding mainly on bacteria, typically dominate aquatic habitats.
- Species structure reacts to different abiotic and biotic drivers.
- Species distributions form patterns evident at microscopic to global scales.

3.1 Introduction

Free-living aquatic nematodes, a group of organisms previously widely neglected by ecologists, play important roles in ecosystems, by interacting with microbial communities and serving as prey for macro-invertebrates and fish juveniles. As such, nematodes connect the microscopic and macroscopic worlds (Majdi and Traunspurger, 2015). Free-living nematodes are present in virtually all limnetic sediments, including those subject to the hot, acidic, and anoxic conditions that exclude many other benthic invertebrates. However, despite the almost ubiquitous distribution and diverse ecological roles of nematodes, for most habitats their taxonomic composition, global, regional, and local distributions, and functional roles are largely unknown.

One of the relatively better-studied aspects of aquatic habitats is their nematode species composition, evidenced by the large number of published papers that include taxonomic descriptions of species from lakes, ponds, rivers, streams, and other habitats, such as hot springs, volcanic lakes, and phytotelmata, from temperate and tropical regions (see Chapter 4). In fact, more papers have been published on nematode taxonomy than on nematode ecology from the respective habitat. Nonetheless, most surveys of nematodes in aquatic habitats, especially those in the tropics, remain incomplete, mainly because the majority of studies have been based on single moments. Another issue plaguing nematode studies is the incomparability of their data on abundance, biomass, or diversity, for reasons that include differences in the number of samples collected and in the techniques employed for sample collection and extraction. As a result,

many questions regarding the ecological role of nematode communities in those ecosystems are still unanswered.

We begin this chapter with an overview of the species richness, density, biomass, and functional structure of the nematode communities that colonize soft and hard substrates, using examples from a selection of lentic (stagnant waters from ponds to large lakes) and lotic (flowing waters from streams to large rivers) ecosystems. We then examine potential drivers of the variability in species composition and nematode distribution at different geographic scales (from centimetric to worldwide). The chapter concludes with a consideration of areas requiring further research.

3.2 Nematode Communities in Lentic Ecosystems

3.2.1 Background: lentic ecosystems

In lentic ecosystems, the water residence time is long and autochthonous production (i.e. organic matter produced within the lentic system itself) is a predominant resource for benthic organisms (Lindeman, 1942). This was demonstrated for nematodes dwelling in a lake bottom, whose abundance increased after the deposition of a decayed plankton bloom in spring (Goedkoop and Johnson, 1996). Within lentic environments, two communities can be distinguished: (i) those colonizing hard substrates (periphyton) and soft substrates (sediment) in the shallow areas (littoral) of lakes and ponds, where the temperature fluctuates, important physical disturbance can occur due to wave action or the activity of limno-terrestrial animals, and where light (photosynthetic active radiation is >1%) reaches the bottom such that benthic primary production is a significant input for food webs; and (ii) communities dwelling in soft substrates in deeper depositional zones (profundal), where light is mostly absent, the water temperature is stable and mostly cold, physical disturbance is limited to the bioturbation activity of benthic organisms, anoxia is common in eutrophic lakes, and heterotrophic processes dominate, given the constant recycling of sedimenting detritic particles by a diversity of benthic microbial decomposers.

3.2.2 Species richness, abundance, biomass

In a recent review, Traunspurger *et al.* (2020) listed the species richness, abundance, and biomass of freshwater nematodes in lakes, both on hard and in soft substrates (Table 3.1). The authors showed that species diversity in the studied lakes varied widely, ranging from a few to about 200 species per lake. However, repeated sampling over time provides a clearer picture of the maximum nematode diversity in a lentic system. For example, 152 species were recorded in Lake Obersee, a shallow eutrophic lowland lake in northern Germany. This estimate was based on sediment samples obtained over 3 years from a homogeneous muddy area of ca. 2 m^2 (Michiels and

Table 3.1. Overview of the species richness, abundance, and biomass of nematode communities inhabiting the hard and soft substrates of a selection of lakes (modified from Traunspurger *et al*., 2020).

Lake – Periphyton	Country	Depth	Lake area (ha)	Mesh size (µm)	Species richness	Abundance (ind./10 cm^2)	Biomass (µg wet weight/ 10 cm^2)	References
Eutrophic								
Hosjön	SWE	L	240	30	14	194		Peters and Traunspurger (2005)
Längsjön	SWE	L	550	30	8	23		Peters and Traunspurger (2005)
Limmaren	SWE	L	540	20	24	1699		Kazemi-Dinan *et al*. (2014)
Limmaren	SWE	L	540	30	23	1145		Peters and Traunspurger (2005)
Limmaren	SWE	L	540	10	42	1740	800	Schroeder *et al*. (2012a, 2013)
Söder Giningen	SWE	L	310	30	14	1570		Peters and Traunspurger (2005)
Tämnaren	SWE	L	3600	30	34	345		Peters and Traunspurger (2005)
Vendelsjön	SWE	L	440	30	20	181		Peters and Traunspurger (2005)
Mesotrophic								
Erken	SWE	L	2400	20	23	386		Kazemi-Dinan *et al*. (2014)
Erken	SWE	L	2400	30	21	1279		Peters and Traunspurger (2005)
Erken	SWE	L	2400	10	32	2710	920	Schroeder *et al*. (2012a, 2013)
Exarbysjön	SWE	L	20	30	15	1615		Peters and Traunspurger (2005)
Gimodamm	SWE	L	310	30	19	507		Peters and Traunspurger (2005)
Österby Stordamm	SWE	L	310	30	33	418		Peters and Traunspurger (2005)
Sakadas	HRV	L	10	24	17	461		Vidakovic *et al*. (2011)
Tomtasjön	SWE	L	50	30	16	1078		Peters and Traunspurger (2005)
Trehörningen	SWE	L	70	30	12	608		Peters and Traunspurger (2005)
Viren 2	SWE	L	140	30	11	34		Peters and Traunspurger (2005)
Viren 1	SWE	L	140	30	15	569		Peters and Traunspurger (2005)
Oligotrophic								
Längsjön	SWE	L	250	30	22	517		Peters and Traunspurger (2005)
Königssee	DEU	L	5200	10	29	44	7	Traunspurger (1992)
Largen	SWE	L	150	20	17	244		Kazemi-Dinan *et al*. (2014)
Largen	SWE	L	150	30	18	964		Peters and Traunspurger (2005)
Largen	SWE	L	150	10	29	1540	400	Schroeder *et al.* (2012a, 2013)
Storsjön	SWE	L	80	30	14	1223		Peters and Traunspurger (2005)

Continued

Table 3.1. Continued.

Lake – Periphyton	Country	Depth	Lake area (ha)	Mesh size (µm)	Species richness	Abundance (ind./10 cm²)	Biomass (µg wet weight/ 10 cm²)	References
Lake sediment								
Eutrophic								
Balaton	HUN	L	59,200	25	46	31	4–20	Bíró (1973)
Czarna Kuta	POL	P	25.2	45	3	16	50	Prejs (1977a,b)
Donghu	CHN	L	3200	45	26	24		Wu *et al*. (2004)
Funtensee	DEU	L	3.5	40	32	53.1	59	Traunspurger (1991)
Havgardssjön	SWE	L	50	35	57	352	730	Ristau and Traunspurger (2011)
Hopfensee	DEU	L	194	35	63	14.2		Traunspurger (2001)
Krageholmssjön	SWE	L	210	35	34	181	372	Ristau and Traunspurger (2011)
Krankesjön	SWE	L	330	35	52	109	308	Ristau and Traunspurger (2011)
Lake Höllerer See	AUT	L		35	26	27.3		Traunspurger (unpubl.)
Löptinersee	DEU	L	10	40	15	165	1919	Traunspurger (unpubl.)
Luterskie	POL	P	691	45	7	36	100	Prejs (1977a,b)
Mikolajskie	POL	L	460	45	39	195		Prejs (1970)
Mikolajskie	POL	L/P	460	45	27	45		Prejs (1970)
Mikolajskie	POL	P	460	45	13	46		Prejs (1970)
Mikolajskie	POL	L	460	45	52	304		Prejs (1977a,b)
Mikolajskie	POL	P	460	45	13	20	110	Prejs (1977a,b)
Narie	POL	P	1240	45	2	1.5	7	Prejs (1977a,b)
Neusiedlersee	AUT	L	32,000	50	26	348	36–144	Schiemer *et al*. (1969), Schiemer (1978)
Obersee	DEU	L	15	35	152	978	400	Michiels and Traunspurger (2004)
Piecek	POL	P	23.3	45	3	2	9	Prejs (1977a,b)
Postsee	DEU	L	276	40	22	1323	5504	Traunspurger (unpubl.)
Sasek	POL	P	869	45	4	11	45	Prejs (1977a,b)
Smolak	POL	P	5.3	45	2	1	5	Prejs (1977a,b)

Mesotrophic								
Älgsjön	SWE	L	40	35	54	80	130	Ristau and Traunspurger (2011)
Dadaj	POL	P	977	45	10	137	500	Prejs (1977a,b)
Faulersee	DEU	L	1472	30	35	133.4	655	Traunspurger (unpubl.)
Fiolen	SWE	L	160	35	98	248	496	Ristau and Traunspurger (2011)
Fjärasjö	SWE	L	30	35	66	117	86	Ristau and Traunspurger (2011)
Haussee	DEU	L	44	40	29	179.8	553	Traunspurger (unpubl.)
Nehmitzsee	DEU	L	171	40	39	114.5	394	Traunspurger (unpubl.)
Schöhsee	DEU	L	78	40	31	123.7	386	Traunspurger (unpubl.)
Skärsjön	SWE	L	280	35	89	212	588	Ristau and Traunspurger (2011)
Spitzingsee	DEU	L	33.6	35	67	31.6		Traunspurger (2001)
Starnberger See	DEU				75	537		Traunspurger (unpubl.)
Stechlinsee	DEU	L	412	35	36	313.9	998	Traunspurger (unpubl.)
Sulzbergersee	DEU	L	35.8	35	55	30.1		Traunspurger (2001)
Tiefersee	DEU	L	50	40	54	209.4	1073	Traunspurger (unpubl.)
Zarnowieckic	POL	L	1431	45	16	90		Prejs (1977a,b)
Zarnowieckic	POL	L/P	1431	45	14	10		Prejs (1977a,b)
Zarnowieckic	POL	P	1432	45	12	6	40	Prejs (1977a,b)
Oligotrophic								
Brunnsee	DEU	P	5.5	35	41	385	340	Bergtold and Traunspurger (2004)
Char	CAN	L	52.6	45	21	425		Prejs (1977a,b)
Char	CAN	L/P	52.6	45	9	25		Prejs (1977a,b)
Char	CAN	P	52.6	45	16	675	750	Prejs (1977a,b)
Constance	DEU	L(0–8 m)	53,600	40	106	141	289	Witthöft-Mühlmann *et al*. (2006)
Constance	DEU	P(13–250 m)	53,600	35	172[a]	627	1190	Traunspurger *et al.* (2021)
Czarn Gasienicowy	POL	P	17.8	45	17	96	300	Prejs (1977a,b)
Ferchensee	DEU	L	15	35	24	1011	245	Michiels and Traunspurger (2005a)
Fereinsalm	DEU	L	1	35	47	864	401	Michiels and Traunspurger (2005a)
Finsevann	NOR	P	325	45	7	74		Prejs (1977a,b)
Froschhausersee	DEU	L	38	35	39	265	97	Michiels and Traunspurger (2005a)

Continued

Table 3.1. Continued.

Lake – Periphyton	Country	Depth	Lake area (ha)	Mesh size (µm)	Species richness	Abundance (ind./10 cm^2)	Biomass (µg wet weight/ 10 cm^2)	References
Grünsee	CHE	L	1	40	17	17.4	12	Traunspurger (1991)
Hökesjön	SWE	L	50	35	67	96	154	Ristau and Traunspurger (2011)
Königssee	DEU	L	5200	40	90	296	70	Traunspurger (1996a)
Königssee	DEU	L/P	5200	40	71	59	30	Traunspurger (1996b)
Königssee	DEU	P	5200	40	60	38	20	Traunspurger (1996b)
Lautersee	DEU	L	14	35	34	974	361	Michiels and Traunspurger (2005a)
Lustsee	DEU	L	5.9	35	47	42.8	6	Traunspurger (2001)
Mirror	USA	L			20	680	210	Strayer (1985)
Morskie Oko	POL	P	34.9	45	14	64	280	Prejs (1977a,b)
Rehbach	AUT	L	1	35	68	666	463	Michiels and Traunspurger (2005a)
Schmalsee	DEU	L	8	35	28	801	250	Michiels and Traunspurger (2005a)
Schwarzensee	DEU	L	10	40	32	57.5	65	Traunspurger (1991)
Soiemsee	DEU	L	3	35	31	2190	863	Michiels and Traunspurger (2005a)
Traunsalpsee	AUT	L	10	35	48	1003	1486	Michiels and Traunspurger (2005a)
Vilsalpsee	AUT	L	60	35	33	1658	451	Michiels and Traunspurger (2005a)
Vilslache	AUT	L	8	35	75	6703	2829	Michiels and Traunspurger (2005a)
Wielki	POL	P	34.3	45	12	34	200	Prejs (1977a,b)
Wildensee	DEU	L	3	35	38	148	74	Michiels and Traunspurger (2005a)
Zadni	POL	P	6.5	45	10	45	120	Prejs (1977a,b)
Zielony Gasienicowy	POL	P	3.8	45	16	702	2080	Prejs (1977a,b)

[a]Lake Constance: total species richness 198 (Witthöft-Mühlmann *et al*., 2006; Traunspurger *et al*., 2021). L, littoral; L/P, littoriprofundal; P, profundal.

Traunspurger, 2004). A considerable proportion of nematodes in this small lake are primarily terrestrial, suggesting large-scale or repeated exchanges with terrestrial ecosystems. A thorough sampling of Lake Königssee, a small (surface area of 5.2 km^2) but deep (mean depth 98 m; maximum depth 190 m) lake in southern Germany revealed 116 species (Traunspurger, 1991), including 90 species in the littoral zone (1–10 m) (Traunspurger, 1996a), 71 in the littoriprofundal, and 60 in the profundal zone (Traunspurger, 1996b). In Lake Constance, the largest (surface area of 539 km^2) and deepest lake of Germany (maximum depth 250 m), 198 species were recorded: 106 in the littoral (0–8 m), 129 in the sublittoral (13–30 m), 113 in the profundal (31–99 m), and 92 in the deep profundal (100–250 m) (Witthöft-Mühlmann *et al.*, 2006; Traunspurger *et al.*, 2021). By contrast, in lakes sampled only once, species numbers have been generally lower, as was the case in eight lakes in Sweden that differ in their trophic state, where the species number varied between 34 in eutrophic Lake Krageholmssjön and 98 in mesotrophic Lake Fiolen (Ristau and Traunspurger, 2011). Very similar results were obtained by Michiels and Traunspurger (2005a), who found between 24 and 75 species in 11 (oligotrophic) alpine lakes of Germany and Austria. Collectively, those studies demonstrate the high diversity of lentic nematode communities, even in small water bodies. They also show that nematode communities are highly dynamic and may stratify along environmental gradients (e.g. depth, seasonality, trophic state).

Overall, those studies showed that species richness is almost twice as high in soft (sediment) than on hard substrates (periphyton), but abundance is almost twice as high in the latter (Fig. 3.1). Schroeder *et al.* (2012a) examined biofilms (periphyton) growing on large stones in the littoral zone of Lake Erken (Sweden) and found an abundance of freshwater nematodes that was higher than had been reported in the literature: up to 10,140 individuals (ind.)/10 cm^2, i.e. 10.14×10^6 nematodes per m^2 in a single sample.

Given that the periphyton is a relatively thin habitat (a few millimeters), this represents a very high agglutination of individuals. In shallow littoral zones of 1–2 m depth, the area that may be coated with periphyton is 1.55×10^6 m^2 (Source: Erken Bathymetric Map, freely available at

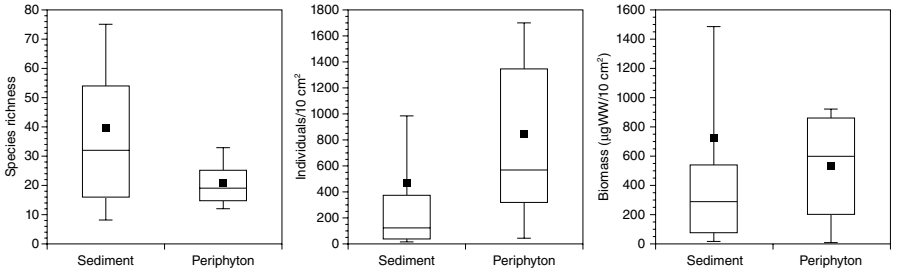

Fig. 3.1. The species richness, abundance, and biomass of nematodes inhabiting the sediment and periphyton of several lakes (modified after Traunspurger *et al.*, 2020 with permission of Springer, data from Table 3.1). The black line indicates the median, the black square the mean value, the boxes show the interquartile range, the whiskers the ±3σ range.

https://www.ieg.uu.se/digitalAssets/629/c_629966-l_1-k_bathymetric-map-erken.pdf); thus, the littoral of Lake Erken could theoretically house up to 15,717 billion nematodes. Moreover, in the study by Schroeder *et al.* (2012a) one species, *Punctodora ratzeburgensis*, accounted for almost the entire community. This finding suggests that hard substrates may constrain nematode diversity, perhaps through competitive exclusion, in which, for example, a species with a lifestyle particularly adapted to hard substrates outcompetes other species. However, it must also be recognized that the number of species is generally reported per area, not per volume of habitat. This would obviously be a limitation for hard substrates, where the habitable layer provided by the biofilm is much thinner than the potential habitat offered by sediments (unless limited by a steep oxygen gradient that confines aerobic life to a very thin layer of mud). The high density of nematodes (based on habitable volume) housed on periphyton can be explained by the fact that the microbial biofilms growing on hard substrates in littoral zones are hotspots of primary production and thus offer a wealth of resources with high nutritional quality, including the diatoms exploited by *Punctodora ratzeburgensis* and other algivorous nematodes (Kazemi-Dinan *et al.*, 2014).

Biomass varies from a few milligrams to several grams of nematode fresh weight per m^2 of substrate (Table 3.1, ×1000 to obtain values per m^2). Although nematode abundance is higher on hard than on soft substrates, the amount of biomass on the former is unremarkable, possibly owing to a lack of data and to the fact that soft sediment communities consist of a relatively higher number of large, omnivorous and predatory species.

3.2.3 Responses of species and feeding type composition to habitat type and trophic state

The trophic status of lentic systems is defined by the bioavailability and ratio of nitrogen and phosphorus, as key nutrients for algal growth. It is a fundamental driver of community structure, as it determines the amount of biomass that may be produced and further utilized by lake consumers. We therefore examined the structure of nematode communities with respect to (i) habitat type (hard vs. soft substrate) and (ii) lake trophic status (oligo-, meso-, eutrophic). A multilevel pattern analysis of the nematode assemblages dwelling on hard and soft substrates in a selection of lakes (for details, see Traunspurger *et al.*, 2020) revealed five genera significantly associated with hard substrates: *Crocodorylaimus*, *Chromadorina*, *Rhabdolaimus*, *Punctodora*, and *Epidorylaimus*, and four genera significantly associated with sediment: *Monhystera*, *Ethmolaimus*, *Ironus*, and *Theristus* (Fig. 3.2). An analysis of the influence of trophic status showed that the genera associated with a specific trophic state were less distinct, although an association of *Prodesmodora* and *Rhabdolaimus* with oligotrophic lakes, the genera *Chromadorina* and *Punctodora* with mesotrophic lakes, and the genera *Dorylaimus* and *Tobrilus* with eutrophic lakes was nonetheless determined (Fig. 3.3).

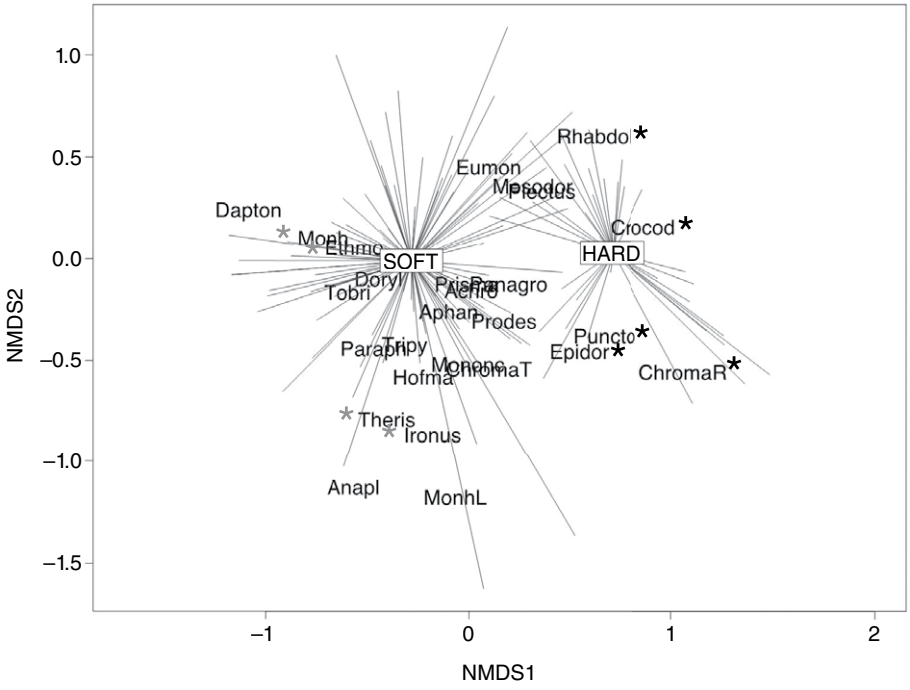

Fig. 3.2. Non-metric dimensional scaling (nMDS) of the Bray–Curtis similarities of nematode genera structure in a selection of lakes (from Table 3.1). Stress value = 0.19. Nematode genera are ordered on the biplot; the radiating lines show the genera, and the centroids the lake trophic status group to which those genera belong. Genera significantly associated with hard substrates are indicated by a black asterisk, and those significantly associated with soft substrates by a gray asterisk. The plot is based on the results of a multilevel pattern analysis (9999 permutations, de Cáceres and Legendre, 2009). Abbreviations: Achro, *Achromadora*; Anapl, *Anaplectus*; Aphan, *Aphanolaimus*; ChromaR, *Chromadorina*; ChromaT, *Chromadorita*; Crocod, *Crocodorylaimus*; Dapton, *Daptonema*; Doryl, *Dorylaimus*; Ethmo, *Ethmolaimus*; Epidor, *Epidorylaimus*; Eumon, *Eumonhystera*; Hofma, *Hofmaenneria*; Ironus, *Ironus*; Mesodor, *Mesodorylaimus*; Monh, *Monhystera*; MonhL, *Monhystrella*; Mononc, *Mononchus*; Panagro, *Panagrolaimus*; Parapl, *Paraplectonema*; Plectus, *Plectus*; Prisma, *Prismatolaimus*; Prodes, *Prodesmodora*; Puncto, *Punctodora*; Rhabdol, *Rhabdolaimus*; Theris, *Theristus*; Tobri, *Tobrilus*; and Tripy, *Tripyla*. (Modified after Traunspurger *et al.* (2020), with permission of Springer.)

Interestingly, lake trophic status had an effect on species richness, with a larger number of species found in sediments of oligotrophic lakes than in eutrophic lakes (Fig. 3.4). Surprisingly, in lakes where more biomass is produced, fewer species were found. Experiments in microcosms yielded conflicting results, in that nutrient depletion led to a reduction in the number of nematode species, especially bacterivores (Michiels and Traunspurger, 2005b). However, in whole-lake systems, where nematodes are not the only animals, competition for rare food resources also impacts larger invertebrates and vertebrates. Under these conditions, small nematodes, with their

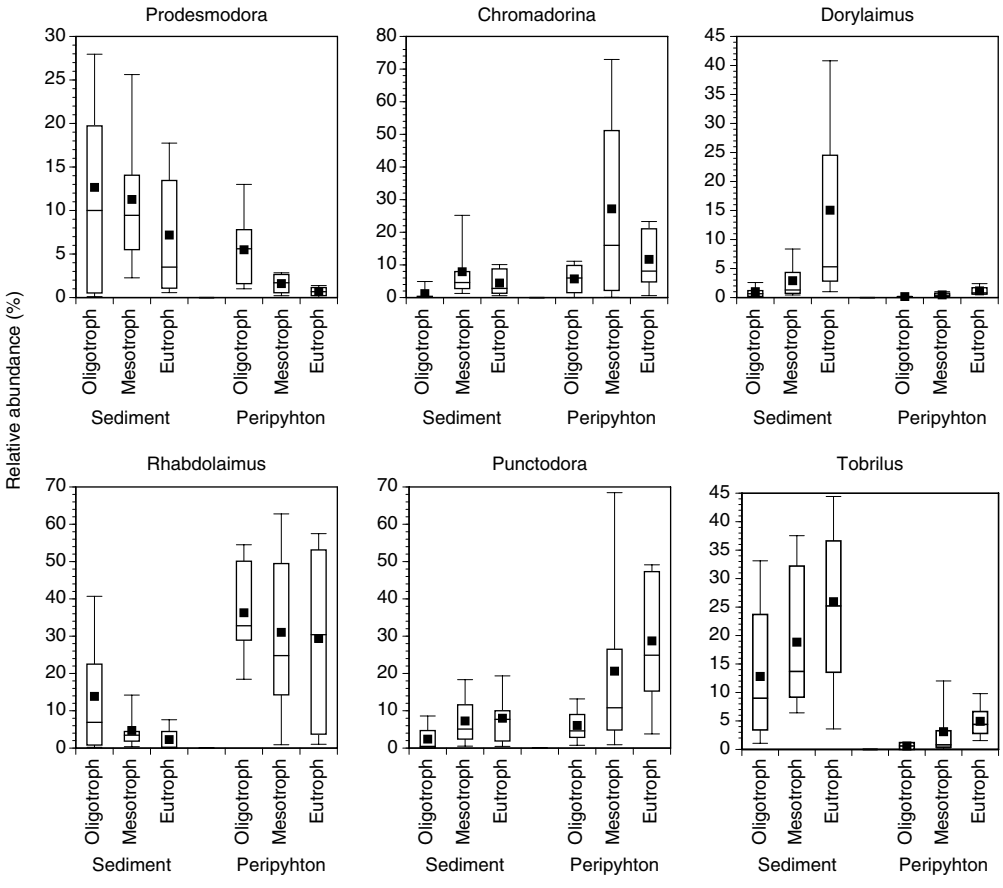

Fig. 3.3. The relative abundance of a selection of nematode genera, distinguished by habitat type (sediment, periphyton), in lakes differing in their trophic status (oligo-, meso-, and eutrophic). The black line indicates the median, the black square the mean value, the boxes show the interquartile range, the whiskers the ±3σ range. (Author's own figure.)

much lower energetic requirements, may have an advantage over larger animals and would thus be better competitors than would larger nematode species and other lake-bottom invertebrates. Accordingly, in oligotrophic lakes nematodes would occupy a wider range of ecological niches, including those left unoccupied by animals with higher energetic needs, and would thus be more diversified.

An analysis of the distribution of freshwater nematodes differing in their feeding types (Traunspurger, 1997a) can provide insights into the role of nematodes in the benthic food web (Fig. 3.5, see also Chapters 6 and 7). Usually, deposit feeders (mainly bacteria-feeding nematodes) dominate in both the sediment and periphyton of lakes, especially in those with oligotrophic rather than eutrophic waters. The proportions of epistrate feeders (mainly feeding on algae) and chewers (predators and omnivores) are similar, ranging between 15 and 38% in the sediment, although chewers

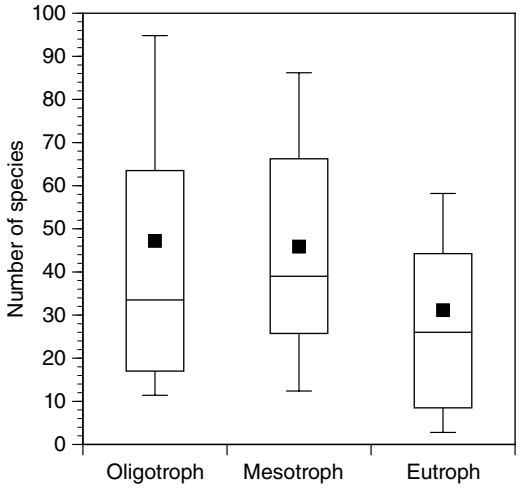

Fig. 3.4. Number of nematode species according to the trophic status of several lakes. The black line indicates the median, the black square the mean value, the boxes show the interquartile range, the whiskers the ±3σ range. (Author's own figure.)

become more numerous with increasing trophic status. This supports the hypothesis that a low availability of basal resources limits the success of larger species with higher positions in food chains while increasing the success of bacterivore species. The proportion of chewers on periphyton is much lower than that in the sediment. Suction feeders (feeding on plants, roots, fungi and also omnivore) account for 5–18% (Fig. 3.5).

3.3 Nematode Communities in Lotic Ecosystems

3.3.1 Background: lotic ecosystems

Habitats with flowing water are referred to as lotic. In lotic ecosystems, the water residence time is short, with water, nutrients, and organic matter constantly introduced from direct rainfall, runoff, subsurface flows, and groundwater. However, depending on climatic and anthropogenic pressures, some rivers may run dry, leaving pools of stagnant water or totally dry riverbed in riffle sections. The dendritic nature of catchments implies a substantial exchange surface between streams and terrestrial ecosystems. Consequently, lotic ecosystems are closely coupled to the dynamics of riparian plant production, both aboveground (leaves and trunks falling in streams) and belowground (rhizosphere-associated processes). In addition, allochthonous resources (i.e. those not produced in the stream but originating from terrestrial ecosystems as particulate or dissolved organic matter, POM and DOM, respectively) play a prominent role in the nutrition of stream organisms. The downstream transport of these organisms by flowing water influences many aspects of their life strategies and may determine their range of dispersal.

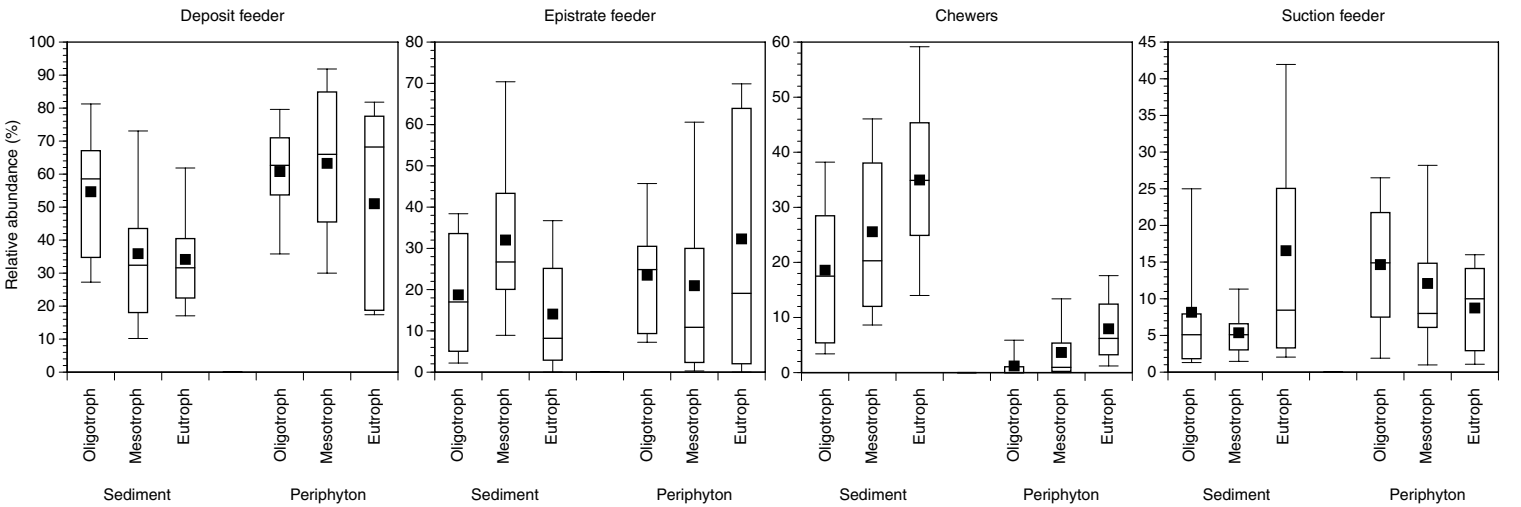

Fig. 3.5. Relative abundance (%) of feeding types of nematodes in the sediment and periphyton of lakes differing in their trophic status. The black line indicates the median, the black square the mean value, the boxes show the interquartile range, the whiskers the ±3σ range. (Author's own figure.)

Lotic ecosystems also face considerable anthropogenic pressures. For example, they receive discharges of chemical elements, such as heavy metals, in addition to POM or DOM from wastewater, nutrients used in agriculture such as nitrates and phosphates, as well as a plethora of xenobiotics, especially pesticides, nano-materials, and plastics. Following their entry into rivers, these substances may be transported downstream after a prolonged residence in the water column or stored in sediments and biofilms for longer periods of time. They may also be processed by stream organisms and either entirely or partially transformed. Another consequence of the flow of water is that streambeds may be scoured by floods. However, while the chemical, physical, and biological characteristics of lotic systems can change dramatically, their oxygen level, salinity, and temperature are generally not limiting at any time. Consequently, river landscapes comprise a constantly changing, highly complex mosaic of possible habitats for nematodes, as described below. Note that because changes in nematode fauna due to the shifting of stream channels are considered the province of terrestrial nematology, they are not discussed in this book.

3.3.2 Species richness and abundance patterns

Nematological studies in lotic systems have used different sampling procedures but the ecological data have not always allowed habitat comparisons. Table 3.2 lists the studies employing similar sampling (e.g. mesh size ≤50 µm) and quantitative extraction procedures, and that reported lotic nematode species diversity as well as, whenever possible, abundance and biomass. In assembling this dataset, our aim was to provide an overview of the diversity, abundance, and biomass patterns of lotic nematodes that could be used in comparisons with the results presented above for lentic nematodes. The difference in the sampling efforts was striking across lotic sites, with sampling conducted only a handful of times in some cases and extensively in others. In a study from the Garonne River, up to 202 samples were collected over two 18-month periods (Majdi *et al.*, 2011, 2012a). Fewer studies have focused on nematodes from epi-benthic hard substrates (biofilms growing on stones, fallen trunks, leaf litter, twigs, macrophytes) than on sediment-dwelling nematodes (Table 3.2). Biomass has also been only rarely reported. Hyporheic and phreatic nematodes, particularly their quantitative composition, are not well known and were omitted from our selection mainly due to the technical and methodological problems in obtaining quantitative samples. While omitting studies may limit the elaboration of a representative overview, our aim in this chapter was to compile quantitative and comparable datasets facilitating comparisons and analyses of ecological patterns.

In soft substrates there is a clear correlation between the number of species reported and the sampling effort, in terms of both number of samples collected and the number of nematodes identified to the species level (Fig. 3.6).

Table 3.2. Overview of nematode species richness, abundance, and biomass from samplings of the hard and soft substrates of several rivers and streams. (Author's own table.)

Hard substrates	Country	Main habitat sampled	Samples collected	Individuals identified	Substrate depth (cm)	Mesh size (µm)	Mean species richness	Mean abundance (ind./10 cm^2)	Mean biomass (µg ww/ 10 cm^2)	References
River site										
Garonne	FRA	Cobbles	202	2875	0	40	28	300.0		Majdi *et al*. (2011, 2012c)
Stream site										
Bergnassonne	FRA	Twigs, leaf litter	4	102	0	50	8	98.7		Majdi *et al*. (2015)
Bernazobre	FRA	Twigs, leaf litter	4	90	0	50	6	27.0		Majdi *et al*. (2015)
Fraissègne	FRA	Twigs, leaf litter	4	48	0	50	7	41.5		Majdi *et al*. (2015)
Furlbach	DEU	Large woody debris	52	858	0	10	46	64.0	4.00	Brüchner-Hüttemann and Traunspurger (2020)
Furlbach	DEU	Leaf litter	52	1294	0	10	66	4.1	0.40	Brüchner-Hüttemann and Traunspurger (2020)
Furlbach	DEU	Macrophyte	52	348	0	10	43	0.7	0.08	Brüchner-Hüttemann and Traunspurger (2020)
Lampy	FRA	Twigs, leaf litter	4	96	0	50	9	155.0		Majdi *et al*. (2015)
Linon	FRA	Twigs, leaf litter	4	83	0	50	5	65.0		Majdi *et al*. (2015)
Mouscaillou	FRA	Twigs, leaf litter	4	125	0	50	7	92.0		Majdi *et al*. (2015)
Nienhagen	DEU	Glass slides	12	600	0	20	23	105.0		Majdi *et al*. (unpubl.)
Orbiel	FRA	Twigs, leaf litter	4	110	0	50	8	106.0		Majdi *et al*. (2015)

Pesquié	FRA	Twigs, leaf litter	4	90	0	50	7	77.0		Majdi *et al*. (2015)
Peyreblanque	FRA	Twigs, leaf litter	4	92	0	50	8	76.0		Majdi *et al*. (2015)
Sant	FRA	Twigs, leaf litter	4	108	0	50	9	13.6		Majdi *et al*. (2015)
Soft substrates										
River site										
Ahr	DEU		3	450	2.5	10	61	479.7	844.85	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Anoia1	ESP		5	85	15	0	15	9.5		López-Doval *et al*. (2010)
Anoia2	ESP		5	50	15	0	2	19.9		López-Doval *et al*. (2010)
Anoia3	ESP		5	114	15	0	7	4.8		López-Doval *et al*. (2010)
Danube	AUT		33	7518	135	0	51			Eder (1983)
Danube	DEU		24	3600	2.5	10	102	2139.4	3103.61	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Elbe	DEU		72	10,800	2.5	10	205	193.5	205.42	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Elbe	CZE		40	4000	10	10	45			Wolfram *et al*. (2010)
Elbe	CZE		25	2500	10	10	45	17.0		Wolfram *et al*. (2010)
Lahn	DEU		3	450	2.5	10	55	290.1	273.77	Heininger *et al.* (2007), Höss *et al*. (2011, 2017)
Llobregat1	ESP		5	17	15	0	7	0.1		López-Doval *et al*. (2010)

Continued

Table 3.2. Continued.

Hard substrates	Country	Main habitat sampled	Samples collected	Individuals identified	Substrate depth (cm)	Mesh size (µm)	Mean species richness	Mean abundance (ind./10 cm^2)	Mean biomass (µg ww/ 10 cm^2)	References
Llobregat2	ESP		5	55	15	0	4	0.3		López-Doval *et al*. (2010)
Llobregat3	ESP		5	53	15	0	5	1.3		López-Doval *et al*. (2010)
Llobregat4	ESP		5	159	15	0	13	27.6		López-Doval *et al.* (2010)
Mosel	DEU		5	750	2.5	10	78	108.4	89.16	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Mueritz-Elde-Wasserstrasse	DEU		13	1950	2.5	10	70	148.3	329.69	Heininger *et al.* (2007), Höss *et al*. (2011, 2017)
Münster	DEU	Mixed substrates	196	25,079		20	90			Niemann (1992)
Nahe	DEU		3	450	2.5	10	47	443.4	815.73	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Oder	DEU		33	4950	2.5	10	141	438.7	351.68	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Rhein	DEU		34	5100	2.5	10	180	638.4	936.12	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Rhein	NLD		16	1600	11.6	0	50	187.0		Bongers and van de Haar (1990)

Saale	DEU		11	1650	2.5	10	94	520.2	458.68	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
Saar	DEU		3	450	2.5	10	43	228.9	199.24	Heininger *et al*. (2007), Höss *et al*. (2011, 2017)
WienLI	AUT	Mixed substrates	16	1036	5	40	45	10.4		Eisendle (2009)
WienRR	AUT	Mixed substrates	16	1036	5	40	43	68.1		Eisendle (2009)
Stream site										
Albereda	ESP	Coarse sand	3	149	5	20	30	513.7		Majdi *et al.* (2020a)
Anyet	ESP	Coarse sand	3	125	5	20	30	1157.8		Majdi *et al.* (2020a)
Baltanes	ESP	Coarse sand	3	64	5	20	17	333.0		Majdi *et al.* (2020a)
Breitenbach	DEU	sand-gravel	105	30,660	10	20	241			Christl (2008)
Cantonigros	ESP	Coarse sand	3	151	5	20	24	3147.9		Majdi *et al.* (2020a)
Castanyet	ESP	Coarse sand	3	20	5	20	11	66.7		Majdi *et al.* (2020a)
Ems	DEU	Sand	70	2509	10	30	92	11.8		Traunspurger *et al*. (2015)
Fuirosos	ESP	Coarse sand	3	143	5	20	23	1005.1		Majdi *et al*. (2020a)
Furlbach	DEU	Sand	70	3416	10	30	69	57.3		Traunspurger *et al*. (2015)
Furlbach	DEU	Sand	52	1935	2	10	79	40.0	20.80	Brüchner-Hüttemann and Traunspurger (2020)
Gellerhagener	DEU	Fine sand	3	88	5	10	17	18.0	3.43	Ptatscheck *et al*. (2020)
Glacier_Möll	AUT		55	5955	5	40	77	86.0	500.00	Eisendle (2008)
Grenzbach	DEU	Fine sand	3	137	5	10	29	79.7	6.56	Ptatscheck *et al.* (2020)
Johannisbach	DEU	Fine sand	3	116	5	10	25	27.0	3.36	Ptatscheck *et al*. (2020)

Continued

Table 3.2. Continued.

Hard substrates	Country	Main habitat sampled	Samples collected	Individuals identified	Substrate depth (cm)	Mesh size (µm)	Mean species richness	Mean abundance (ind./10 cm^2)	Mean biomass (µg ww/ 10 cm^2)	References
Körsch	DEU	Fine sand	48	12,042	5	35	113	473.0	111.30	Beier and Traunspurger (2003a)
Krähenbach	DEU	Coarse sand	48	1207	5	35	71	47.0	17.00	Beier and Traunspurger (2003b)
Llemena	ESP	Coarse sand	3	151	5	20	21	571.6		Majdi *et al*. (2020a)
Montnegre	ESP	Coarse sand	3	72	5	20	25	180.7		Majdi *et al*. (2020a)
Orlina	ESP	Coarse sand	3	41	5	20	19	118.2		Majdi *et al*. (2020a)
Pontons	ESP	Coarse sand	3	151	5	20	32	1612.5		Majdi *et al*. (2020a)
Sant_Celoni	ESP	Coarse sand	3	150	5	20	26	1600,0		Majdi *et al*. (2020a)
Sant_Daniel	ESP	Coarse sand	3	140	5	20	34	955.6		Majdi *et al*. (2020a)
Schlosshofbach	DEU	Fine sand	8	312	5	10	35	75.1	19.00	Ptatscheck *et al*. (2020)
Soval	ESP	Coarse sand	3	149	5	20	16	4875.9		Majdi *et al*. (2020a)
Sudbrackbach	DEU	Fine sand	3	146	5	10	35	81.9	39.14	Ptatscheck *et al*. (2020)
Vilamaniscle	ESP	Coarse sand	3	116	5	20	22	740.7		Majdi *et al*. (2020a)
Vilardell	ESP	Coarse sand	3	152	5	20	29	3623.4		Majdi *et al*. (2020a)
WienNN	AUT	Mixed substrates	16	1036	5	40	35	26.9		Eisendle (2009)
WienSI	AUT	Mixed substrates	16	1036	5	40	34	0.9		Eisendle (2009)

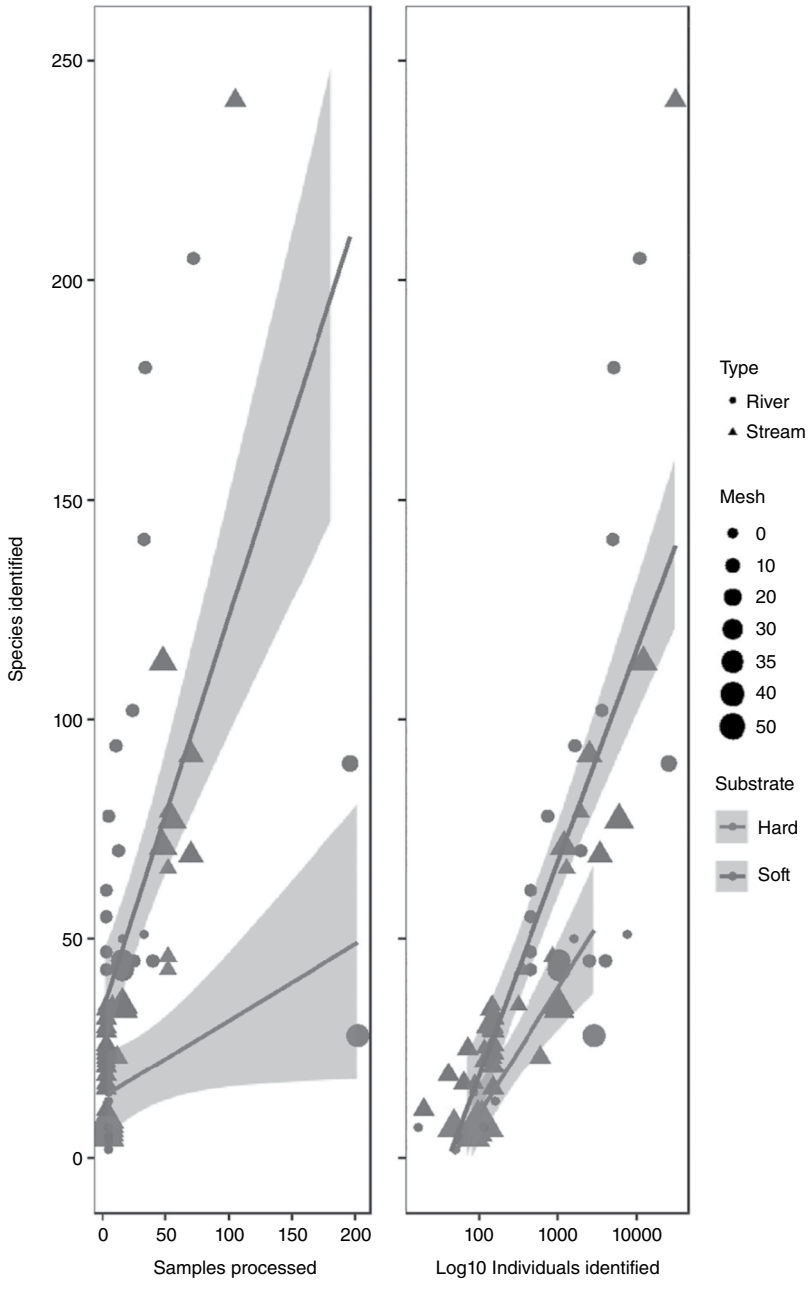

Fig. 3.6. Nematode diversity in a selection of streams and rivers (see Table 3.2) as a function of the number of samples processed (left panel) and the number of individuals identified (right panel). Hard and soft substrates were distinguished as was the mesh size used and the type of lotic system sampled. Regression lines and 95% confidence interval are shown. Among nematodes from soft substrates, the number of species correlated significantly with the number of samples processed (adj. $R^2 = 0.4$, $P < 0.001$) as did the number of species and the number of individuals identified (adj. $R^2 = 0.54$, $P < 0.001$). (Author's own figure.)

This exemplifies the methodological constraints on our understanding of nematode diversity in lotic ecosystems, as only a handful of studies have been based on a meaningful number of samples collected over time or from an adequate number of streams reaches (>100 samples) or have identified a sufficient number of individuals (>10,000) to provide a comprehensive account of nematode diversity in a stream or river. However, the maximum number of species that can be expected in a lotic site may be fairly high, especially in soft substrates (a trend also observed in lentic ecosystems). In an extensive survey of the small Breitenbach stream (105 samples, 30,660 individuals identified), nematode diversity was up to 241 species identified (Christl, 2008). Other extensive surveys of the sediments of the Elbe, Rhein, and Oder rivers yielded up to 245, 231, and 141 species, respectively (Heininger *et al.*, 2007; Gansfort and Traunspurger, 2019). In contrast, over an 18-month period, Majdi *et al.* (2011) found only 28 nematode species within the biofilms coating large cobbles in the Garonne River, supporting the pattern that nematode diversity was lower in hard substrates in comparison with soft substrates. Nevertheless, in their 1-year study, Brüchner-Hüttemann and Traunspurger (2020) reported relatively high species numbers from the organic hard substrates of a small forested stream, reporting 66, 46, and 43 species in the biofilm coating leaf litter, large trunks, and macrophytes, respectively. These examples illustrate the numerous limitations and opportunities for nematode species assemblages colonizing inorganic and organic hard substrates. The latter may provide more food niche opportunities (the substrate itself) and thus seems to house a greater diversity of nematodes than cobbles or large rocks, but further inferences are not possible without more extensive studies or laboratory experiments.

The potential of lotic ecosystems to harbor large numbers of nematode species may be due to the remarkable spatiotemporal heterogeneity of streambeds and their close lateral connections with adjacent riparian ecosystems. Few of the species found in lotic systems are restricted to this habitat, as most are also commonly found in others. In small streams in Germany, only about one-third of the species are restricted to streams, with most species frequently identified in surrounding terrestrial habitats as well (Niemann *et al.*, 1996; Christl, 2008). Many species in the lower reaches of rivers have also been detected in adjacent estuarine habitats (Riemann, 1966). Vertical exchanges with hyporheic and rhizosphere nematofauna are also possible, although this has not been well studied so far.

There seems to be only a slight gradient between the diversity found in deeper sediments and that near the surface. In the Krähenbach, a small, fine-grained stream, 59 species were detected in the upper 2 cm of sediment and 49 in the deeper sediment (2–5 cm) (Beier and Traunspurger, 2003a). In the coarse-grained Körsch stream, 104 species were found in the upper 2 cm of the sediment and 88 in a deeper layer (2–5 cm) (Beier and Traunspurger, 2003b). However, in two sandy springs, Furlbach and Ems, nematode species richness was significantly higher in the deeper (5–10 cm) layer than in the superficial (0–5 cm) layer (Traunspurger *et al.*, 2015). Furthermore,

while diversity in the deeper layer tended to be stable throughout the year, the authors found a greater fluctuation of nematode diversity in the superficial layer, which might fit with fluctuating resource availability (e.g. chlorophyll *a* 'bloom' in spring–summer). In hard substrates, the thickness and 'nutritional quality' of the biofilm matrix in which free-living nematodes dwell seem to be important factors driving nematode diversity. However, in the Garonne River, nematode diversity was minimal during winter low-flow periods, characterized by thick, nutritious diatom biofilms growing on cobbles; instead, the nematode community was almost a monoculture of *Chromadorina bioculata*, able to attain high densities (Majdi *et al.*, 2011). A more diverse community became established in summer, when the biofilm was thinner due to the grazing pressure of aquatic insect larvae and to self-detachment because of bacterial overgrowth. These examples suggest that nematode communities can track ever-changing lotic landscapes, and that studies of the responses of nematode species can lead to a better understanding of the structure and function of lotic ecosystems.

Since a higher abundance implies a higher availability of resource or a lower level of competition, ecologists use this parameter to infer fundamental features of lotic ecosystems. Nematodes may be ideal models for this purpose because they are basal consumers, have fully benthic, short life cycles, and can be found in large numbers in the vast majority of lotic habitats. As such, they are most likely to closely track the changes in lotic ecosystem productivity. Moreover, in contrast to nematode diversity, nematode abundance is simple to measure. Nonetheless, the interpretation of nematode abundance patterns has been hindered by the enormous variation across studies and mean reported values spanning three to four orders of magnitude (Table 3.2). This may be due to different methodological procedures, such as the use of different mesh sizes, sample collection methods, or extraction techniques. In addition, abundances may be reported per area, per volume, or per weight of substrate, further complicating comparisons. For example, the highest abundance of lotic nematodes reported thus far was in a study of free-floating cyanobacterial mats in the Llobregat River, northeast Spain (7520 ind./10 cm^2), by Gaudes *et al.* (2006). The lowest nematode abundance in a lotic system (0.1 ind./10 cm^2), was reported a few years later, in a study by López-Doval *et al.* (2010), who surveyed the sediment of the same river. Although nematode abundance in lakes tends to be higher on hard than in soft substrates, in streams and rivers the pattern is blurred by the considerable variation of abundances both within and across stream sites.

Nematode abundance patterns may emerge during the course of seasons, when the abiotic and biotic environment of lotic nematodes undergoes dramatic changes. The influence of different environmental drivers is discussed in detail below, but seasonality implies a set of changes ranging from resource shifts to hydrological extremes. It may result in indistinguishable changes in nematode abundance (Bott and Kaplan, 1989; Strayer and O'Donnell, 1992; Christl, 2008); or consistent trends, such as the many studies in sandy streambeds that reported peak nematode

abundances in spring and summer (Palmer, 1990; Eisenmann *et al.*, 1998; Beier and Traunspurger, 2003a,b; Traunspurger *et al.*, 2015; Brüchner-Hüttemann and Traunspurger, 2020). In an alpine glacial stream, however, nematode abundance peaked in October (Eisendle, 2008), as was the case in Mediterranean intermittent streams subjected to different degrees of drought severity, where nematode abundance reached 4875 ind./10 cm^2 and was highest in streams with the highest frequency of drying events during summer (Majdi *et al.*, 2020a). As noted above, in epilithic biofilms of the Garonne River, nematode abundances peaked during autumn–winter low-flow periods (Majdi *et al.* 2011, 2012a), mostly as a result of the positive correlations with biofilm thickness and diatom content. Thus, consistent patterns in total nematode abundance may be due to relationships with the abundances of prey and competitors, although even under the least stable or favorable conditions, the abundance of nematodes is consistently higher than that of other invertebrates that may feed on or compete with them. In sediments that become anoxic or are exposed to highly variable flows, the total abundance of nematodes may be higher than that of oligochaetes and chironomids, whereas this is not the case in sediments subject to less severe or variable conditions (Palmer, 1990; Ward and Voelz, 1990; Wolz and Shiozawa, 1995; Hakenkamp and Morin, 2000; Giere, 2009). In contrast with other, less tolerant invertebrates, nematodes are extremely abundant in harsh habitats, such as free-floating cyanobacterial mats that release odor repellents and toxins (Gaudes *et al.*, 2006), streambeds subject to droughts (Majdi *et al.*, 2020a), and biofilms heated by effluents from a nuclear power plant (Majdi *et al.*, 2020b). Indeed, nematodes may be useful sentinel organisms, with unusually high abundances suggesting conditions that are unfavorable for most other animals.

3.3.3 Biomass, comparison with lakes

As in lakes, the biomass of nematodes in lotic ecosystems can vary from milligrams to grams of nematode fresh weight per m^2 of substrate (Table 3.2). While biomass seems to be lower on hard substrates than in lake sediments (Fig. 3.1), this might be due to the fact that, to date, the only report of nematode biomass on hard substrates in lotic ecosystems is that of Brüchner-Hüttemann and Traunspurger (2020), which examined organic hard substrates (leaf litter, wood, and macrophyte) with low abundances, whereas in studies on lakes biomass was measured on inorganic (stony) hard substrates crowded with nematodes. The reasons for these differences in the biomass of nematodes dwelling on hard substrates remain to be determined (see also Chapter 8).

A compilation of the data on mean abundance vs. biomass derived from measurements in lotic and lentic systems (Tables 3.1 and 3.2) revealed significant power relationships between these two parameters and allowed a determination of the average wet weight of an individual lotic or lentic nematode, 0.71 and 1.42 µg, respectively (see exponents representing the

slope of the power law relationship, Fig. 3.7). Lentic nematodes are thus twice as heavy as lotic nematodes, although the majority of the data were from nematodes in soft substrates (Fig. 3.7). However, it is unlikely that this substantial difference between nematodes from the two ecosystems was due to a bias in mesh size or to an inappropriate calculation of nematode biomass, since only studies using mesh sizes <50 μm were included in the determinations and all of those studies used Andrássy's (1956) formula to derive nematode wet weight from body length and width. The fact that the relationship between abundance and biomass follows a power law implies scale invariance properties; in other words, very dense nematode assemblages comprise the same proportion of small and large individuals as sparse assemblages, a finding with interesting functional implications.

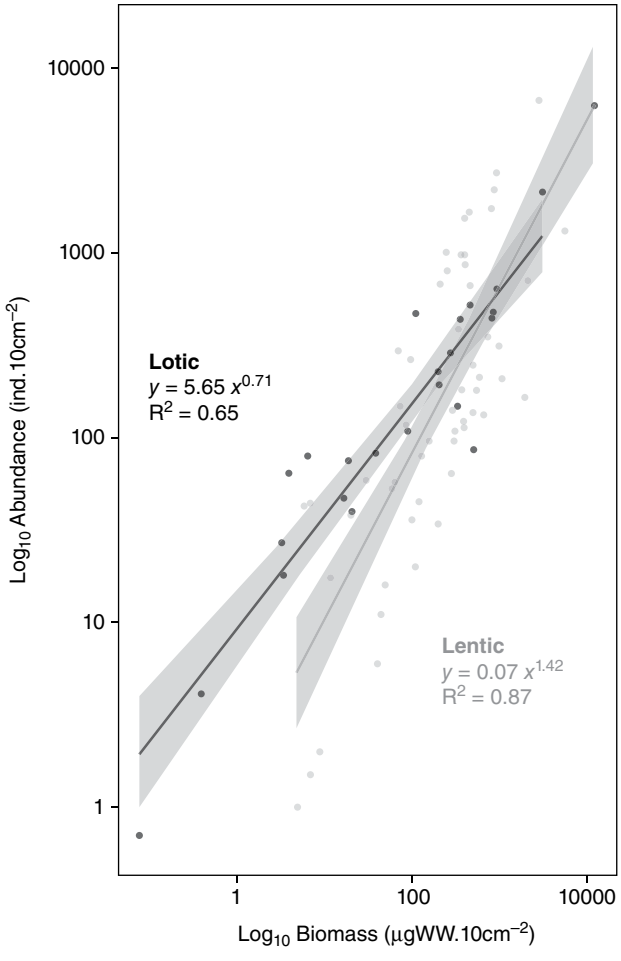

Fig. 3.7. Log-log abundance-biomass plot of nematode assemblages in a selection of lentic and lotic sites (see Tables 3.1 and 3.2). Hard and soft substrates were not distinguished. The power law fit ($P < 0.001$) and 95% confidence interval are shown. (Author's own figure.)

3.3.4 Feeding types

An examination of the relative distributions of feeding types within a nematode community may yield more generalizable insights because with this approach the patchiness of individual species distributions is evened out. We compared the feeding type distribution reported from a selection of 12 lotic sites for hard substrates and 48 lotic sites for soft substrates. In many streams, particularly those with fine sediments, nematodes able to ingest smaller microbes and organic particles (deposit feeders) were dominant (e.g. Zullini, 1976; Bongers and van de Haar, 1990; Beier and Traunspurger, 2003a,b; Traunspurger *et al.*, 2015). This finding provides further support for the dominance of deposit feeders in nematode communities, both in sediment and on hard substrates (Fig. 3.8).

In the latter, most of the available studies examined the feeding types present on organic hard substrates, such as decomposing litter, where deposit feeders dominated strongly (86% on average). By contrast, in a study

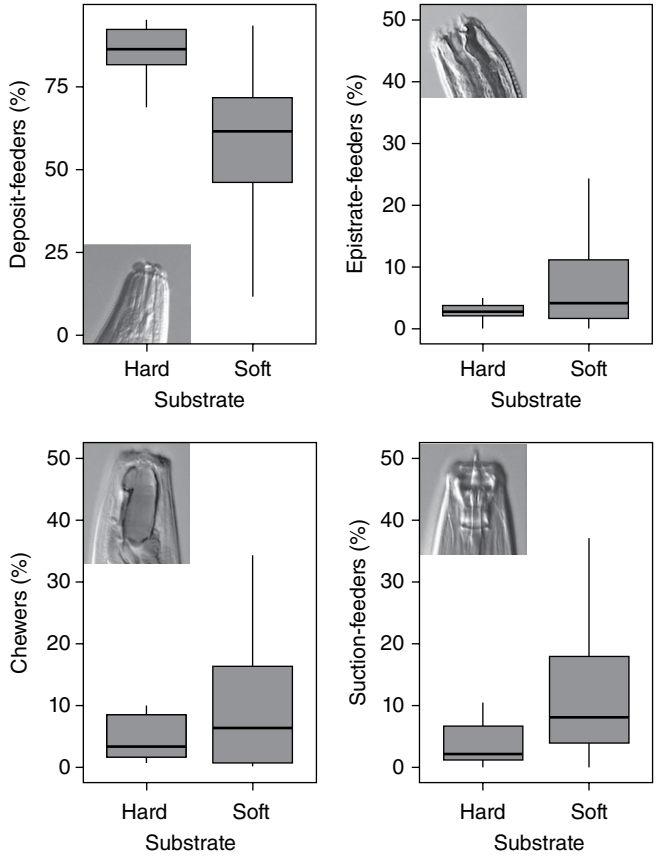

Fig. 3.8. Relative distribution of the main feeding types (following Traunspurger, 1997a) of lotic nematodes dwelling on hard or soft substrates. (Author's own figure.)

of nematodes on the cobbles in the Garonne River (Majdi *et al.*, 2011), a hard inorganic substrate, deposit feeders accounted for an average of only 11%. Those cobbles were coated with phototrophic biofilms that favored epistrate feeders (comprising, on average, 86% of the community and up to 100% during winter low-flow periods). In soft substrates, deposit feeders still typically account for the majority (58.5%) but other feeding types are generally more evenly represented (on average 7.7, 18.1, and 15.2% for epistrate feeders, chewers, and suction feeders, respectively). Suction feeders, comprising omnivorous as well as specialized taxa feeding on plant roots or fungal hyphae, are well represented in intermittent streams (on average 30%, but they can dominate, representing up to 76% in Fuirosos) (Majdi *et al.*, 2020a).

3.3.5 Species distribution

Most species have very patchy distributions. In an analysis of 600 samples from seven different streams, about half the species occurred in less than 20 samples (Niemann *et al.*, 1996) whereas a few species occurred in many samples from similar habitats. On an even wider scale, in a study of the nematodes from the Murray–Darling River Basin, which covers one-seventh of the Australian continent (>1 million km^2), more than half of the species were found in <5% of the samples (Hodda, 2006). Thus, the diversity of lotic nematodes on a scale larger than an individual sample (often termed α-diversity) is typically very high. Consequently, only a few nematode species from lotic systems occur sufficiently frequently to allow broad conclusions about their distribution. Genera ubiquitous in lotic systems include *Tobrilus* and *Eutobrilus* (Tobrilidae), *Monhystera* and *Eumonhystera* (Monhysteridae). Species of these genera have been found throughout the Murray–Darling Basin but also in the Danube River in Austria, the lower Rhine in the Netherlands, and in much smaller forested catchments in Spain, France, and Germany (Eder and Kirchengast, 1982; Bongers and van de Haar, 1990; Beier and Traunspurger, 2003a,b; Hodda, 2006; Majdi *et al.*, 2015, 2020a; Traunspurger *et al.*, 2015; Brüchner-Hüttemann and Traunspurger, 2020).

3.4 Drivers of Variability in Species Composition

A thorough discussion on the possible sources of variation in genus and species numbers and in the composition of the nematofauna in different lakes and rivers is, at present, premature for two reasons. First, information on the factors that play a role in the distribution of free-living nematodes in inland water bodies is still scarce. Instead, the factors influencing the distribution of comparatively well-studied marine nematodes (e.g. grain size, oxygen, temperature, food availability) are assumed to also affect nematodes in inland water bodies. Second, in a large proportion of the

studies of the nematofauna of inland water bodies only a few samples collected from a relatively small proportion of lakes and rivers have been used for taxonomic purposes. A scientific standard based on detailed descriptions of the sampling sites and measurements of a minimum contingent of abiotic parameters is required for taxonomic studies of nematodes that can be used for comparative purposes. Given the generally high temporal and spatial variability of meiobenthos and nematodes in freshwater habitats, whether lakes or rivers (Pennak, 1988; Traunspurger, 1996a,b; Michiels and Traunspurger, 2004; Majdi *et al.*, 2012a, 2017; Schroeder *et al.*, 2012b, Traunspurger *et al.*, 2020, 2021), conclusions based on comparisons drawn from data extracted from snapshot samples obtained at different seasons are likely to be misleading.

3.4.1 Abiotic factors

3.4.1.1 Habitat texture

For interstitial organisms, the size of the interstitial pores where these animals dwell, forage, and reproduce is clearly among the decisive physical properties of the sediment. The structuring effect of sediment granulometry on nematode communities is well documented in both marine and limnetic habitats (e.g. Wasilewska, 1973; Zullini, 1974; Schiemer, 1978; Tudorancea and Zullini, 1989; Eyualem-Abebe *et al.*, 2001; Traunspurger *et al.*, 2021). A muddy environment is homogeneous and offers fewer microhabitats for nematodes, although Tobrilids and other taxonomic groups seem to thrive in muddy depositional zones (Zullini, 1974; Beier and Traunspurger, 2003a,b). By contrast, Tylenchids are among the groups with greater affinity for coarser sediments (Beier and Traunspurger, 2003a,b). Eyualem-Abebe *et al.* (2001) found that the nematode communities at silty sites contained fewer species than did sandy sites. Wasilewska (1973) and Tudorancea and Zullini (1989) also determined a relatively high number of species at sites with medium-grained sand (0.25–0.50 mm). The overall size of nematode populations may also be affected by the physical limitations due to the size of interstices, but consistent patterns have yet to be identified in lotic systems, where sediment grain size may be of less importance than vital constraints such as oxygen penetration. For example, some studies have found higher abundances in finer sandy sediments (Callahan *et al.*, 1979; Bott and Kaplan, 1989), whereas in others higher abundances were detected in coarser sands (Anderson, 1992) and fine gravels (Beier and Traunspurger, 2001, 2003b). For the nematode communities on hard substrates, the nutritional quality of the biofilm may be a more important driver than the area available for colonization. In the previously cited study of the Garonne River, the thickness of the biofilm and the availability of diatoms were important determinants of nematode abundance and of the taxonomic/functional structure of the nematode community (Majdi *et al.*, 2011). Similar conclusions were reached in studies of

the biofilms growing on stony lake shores (Peters and Traunspurger, 2005; Schroeder *et al.*, 2013). In addition, the presence of sand grains within a biofilm matrix was shown to allow the accommodation of more nematodes (Majdi *et al.*, 2014).

3.4.1.2 Flow rate

In streams, hydrodynamics is a key driver of community assembly, as an excessive flow velocity during floods or a lack thereof during drought will affect the dispersal and reproduction of organisms as well as their ability to acquire food. For benthic organisms in soft substrates, the subsurface sediment offers a refuge against bed-scouring floods but also dry phases (Palmer *et al.*, 1992; Boulton and Stanley, 1995; Clinton *et al.*, 1996; Dole-Olivier, 2011). As noted above, in Mediterranean regions streambeds that frequently dry out are surprisingly inhabited by a very diverse and abundant nematofauna once flow returns – evidence of the resilience of nematode communities (Majdi *et al.*, 2020a). For nematodes dwelling within the biofilms on hard substrates, the hydrological regime may be decisive in that relatively moderate bottom-flow velocities (>30 cm/s) may detach biofilms from cobbles, resulting in the displacement of massive numbers of nematodes (Gaudes *et al.*, 2006; Majdi *et al.*, 2012a). In the lake littoral, nematode assemblages in biofilms subjected to the sheer stress generated by wave action will differ from those in biofilms growing in deeper locations (Kazemi-Dinan *et al.*, 2014; Kreuzinger-Janik *et al.*, 2015). However, the potential negative effect of sheer stress (detachment) may be outweighed by the positive effect of greater light availability for primary producers, which in turn benefits nematode grazers (Kreuzinger-Janik *et al.*, 2015).

3.4.1.3 Temperature

Nematodes are poikilotherms, and the productivity of their microbial prey also depends on temperature. Hence, temperature is an obvious constraint for many aspects of the biology and ecology of nematodes. Several studies have demonstrated the temperature dependence of the physiological and biological processes essential for nematodes (Tietjen and Lee, 1977; Anderson and Coleman, 1982; Moens and Vincx, 2000; Majdi *et al.*, 2019). Some species were shown to be eurythermic (able to cope with a broad range of temperatures) and others stenothermic (with a narrow temperature optimum). There are also species with affinities for low temperatures, such as *Plectus velox*, which has a maximum population fitness at temperatures between 10 and 15°C (Majdi *et al.*, 2019). Although *Monhystera stagnalis* and *Rhabdolaimus terrestris* thrive in hot spring habitats (ca. 40°C), very few nematode species can be considered as truly thermophilic, since most species found in hot springs are in fact cosmopolitan (Ocaña, 1991a). It is likely that temperature specialization can be acquired by populations exposed to unfavorable conditions, but otherwise many freshwater nematode species, including Monhysterids, are flexible with respect to temperature and can be found in habitats ranging from

glacier streams to hot springs. A more important aspect may be the temperature fluctuation regime (Vafeiadou *et al.*, 2018; Majdi *et al.*, 2020b). With respect to global climate change, while it is difficult to predict which nematode species will thrive or decline, the outstanding resistance and resilience of nematode populations to environmental extremes suggest that they are likely to persist and that their essential functions in freshwater ecosystems should be preserved.

3.4.1.4 Water chemistry, nutrients, and organic matter

Water chemistry, especially the concentrations of carbonate, calcium, and magnesium ions (often referred to in combination as 'water hardness') and of chloride ions, has been shown to impact nematode assemblages (Venkateswarlu and Das, 1980; Eder and Kirchengast, 1982; Beier and Traunspurger, 2003a,b; Bert *et al.*, 2007). This was the case for nematodes in the Krähenbach, a small, fine-grained, submontane carbonate stream, where abundance correlated negatively and significantly with the chloride concentration (Beier and Traunspurger, 2003a). In freshwater systems, changes in ionic concentrations are usually evaluated by assessing the electrical conductivity. A number of studies have reported significant effects of conductivity on nematode assemblage structure, attributable in most cases to an increased proportion of dissolved nutrients (Tudorancea and Zullini, 1989; Ocaña, 1991b; Barbuto and Zullini, 2005; Schabetsberger *et al.*, 2009; Traunspurger *et al.*, 2015; Gansfort and Traunspurger, 2019). For example, in the study of Barbuto and Zullini (2005) epistrate feeders correlated positively and deposit feeders negatively with the conductivity of the Taro River (Italy). The authors hypothesized that higher conductivities were associated with higher nutrient contents and greater algal coverage, thus benefiting epistrate feeders as they can effectively exploit algae. Gansfort and Traunspurger (2019) compared the effects of various environmental drivers on nematode assemblages through an extensive survey of the Rhine and Elbe Rivers (Germany) and showed that the elemental composition of the sediment, especially its nitrogen content, was the most important driver of nematode community composition. Earlier studies have shown a relationship between the level of organic enrichment of the sediments and the species composition of freshwater nematodes (Zullini, 1976; Eder and Kirchengast, 1982; Bongers and van de Haar, 1990; Niemann *et al.* 1996, Bergtold and Traunspurger, 2005a; Michiels and Traunspurger, 2005b; Witthöft-Mühlmann *et al.*, 2005a,b; Witthöft-Mühlmann *et al.*, 2007; Ristau and Traunspurger, 2011; Ristau *et al.*, 2013a), although while some species seem to be affected, others are not (Zullini, 1974, 1976; Beier and Traunspurger, 2003a,b; Michiels and Traunspurger, 2005b; Traunspurger *et al.*, 2015). Similarly, other authors attempted to codify the affected taxa with respect to the degree of organic enrichment, whether at the species, genus, family, or other level (Bongers, 1990; Bongers and Ferris, 1999; Heininger *et al.*, 2007; Höss *et al.*, 2011, 2017). By surveying the nematode communities dwelling in

ten headwater streams under various levels of riparian forest management in the Montagne Noire massif (France), Majdi *et al.* (2015) showed that deposit feeders were positively associated with the concentration of dissolved organic carbon, which in turn correlated with the extent of riparian canopy cover. Although increasing proportions of nematodes belonging to the orders Rhabditida and Diplogasterida with increasing organic enrichment have been frequently reported (e.g. Zullini, 1976; Ocaña and Picazo, 1991; Beier and Traunspurger, 2003a,b), many of the attempts to attribute the responses of nematodes at lower taxonomic levels to differences in organic enrichment have had limited geographic validity (e.g. Niemann *et al.*, 1996; Beier and Traunspurger, 2001).

3.4.1.5 Oxygen and vertical distribution of nematodes in the sediment

That nematodes tend to stay in the surface sediment layer and avoid the low availability of oxygen in deeper layers was first reported in studies of marine habitats (e.g. Tietjen, 1969; Bryant and Laybourn, 1974; Platt, 1977; Blome, 1982) but the relevance of that finding for lentic habitats as well is now clear. Many studies on freshwater habitats have assessed the vertical distribution of nematodes in terms of species numbers (e.g. Traunspurger, 1996a,b; Traunspurger and Drews, 1996; Eyualem-Abebe *et al.*, 2001; Traunspurger *et al.*, 2015). Their general conclusion has been that species numbers are highest at or close to the sediment surface while decreasing in deeper sediment layers. A study of 106 nematode species in the profundal of Lake Königssee showed that only a few species (e.g. *Ethmolaimus pratensis*, *Ironus tenuicaudatus*, *Tobrilus gracilis*, and *Monhystera paludicola*) inhabited the deeper sediment layers (5–10 cm and 10–20 cm) (Traunspurger, 1996c, 1997b, 1998; Traunspurger and Drews, 1996). Low oxygen availability presumably severely constrains the ability of nematodes to live in deeper sediment layers, especially muddy lake bottoms with minimal mixing or bioturbation activity by larger animals. Nevertheless, several species of nematodes well tolerate hypoxia or anoxia (Kitazume *et al.*, 2018), with adaptations ranging from acute oxygen sensing/migration to suspended animation for several days in response to anoxia. However, most species exhibit a clear preference for well-aerated substrates, as demonstrated for nematodes in the Körsch, a small, coarse-grained, submontane carbonate stream, where the abundance of most nematode species, especially suction feeders (mainly hyphal- and plant feeding), correlated positively with the oxygen level (Beier and Traunspurger, 2003b).

3.4.1.6 Water depth in lakes

Species numbers as a function of water depth did not differ in the littoral zone of Lake Königssee (Traunspurger, 1996a) and varied only slightly in its littoriprofundal and profundal zones (Traunspurger, 1996b). This was also shown for Lake Constance, from the sublittoral to the deep profundal (13–250 m water depth) (Traunspurger *et al.*, 2021). Nevertheless, in

the latter study a simple relationship between species numbers and water depth could not be established. Eyualem-Abebe *et al.* (2001) reported similarly inconclusive results with respect to depth-specific communities at some sites in Lake Tana, Ethiopia. However, there is also abundant evidence for an association of nematode communities with specific parts or depths of lakes (Schiemer, 1978; Traunspurger, 1995, 1996a,b,c, 1997b, 1998; Eyualem-Abebe *et al.*, 2001). For example, in Lake Constance *Eumonhystera* dominated at all depths, whereas the prevalence of *Tobrilus*, *Ethmolaimus*, and *Monhystera* increased with increasing depth, while *Paraplectonema* was mostly found in littoral zones (Fig. 3.9).

In an attempt to explain nematode distribution patterns in the Neusiedlersee, Schiemer (1978, pp. 171–172) identified mixing as one of the fundamental factors affecting nematode distribution. Five zones were defined through a 'horizontal distribution pattern, [which] correlated with the wind influence on benthic conditions', thus highlighting the role of local conditions in determining mixing and sediment suspension. Although these factors (wind, mixing) may be closely related to depth, they are not controlled by water depth alone but by the combined impact of several factors that interact with the mixing process, including wind regime, water depth, and local physical conditions. Of these, the wind regime may be seasonal in its impact and should therefore be considered as such at the specific site (Witthöft-Mühlmann *et al.*, 2005a,b). Moreover, water depth *per se* is a meaningless parameter unless it is evaluated within the context of local conditions. Eyualem-Abebe *et al.* (2001) demonstrated that the nematode communities in two closely located sites in a shallow

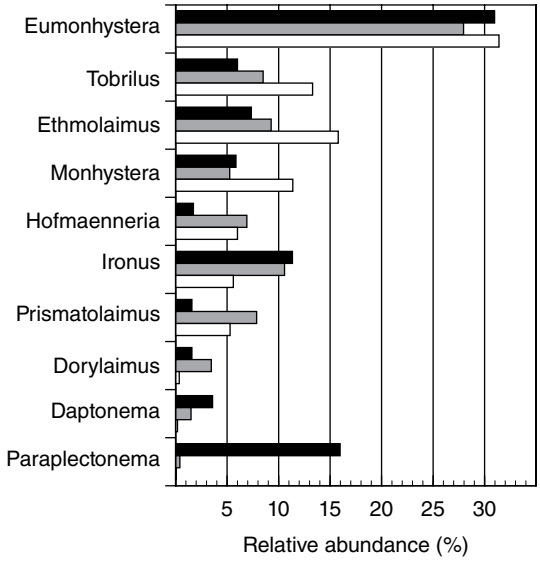

Fig. 3.9. Relative abundances of the dominant nematode genera in the sublittoral (13–30 m), profundal (31–99 m), and deep profundal (100–250 m) of Lake Constance. Black: sublittoral; gray: profundal; white: deep profundal. (Author's own figure.)

part of a lake could differ if one community was protected from wind-induced mixing but the other was not, while nematode communities in distantly situated shallow sites may be similar if both are exposed to regular, strong, wind-induced mixing. The authors showed that species numbers were higher at sites less prone to disturbance than at exposed sites.

3.4.2 Biotic factors

3.4.2.1 Primary producers: microphytobenthos and macrophytes

Prejs (1977a,b) was the first to link the variation in the number of nematode species in a group of lakes with the level of primary productivity. Those studies demonstrated that oligotrophic lakes contain more species than eutrophic ones, a finding later confirmed in an analysis of several other studies (Fig. 3.4; Traunspurger *et al.*, 2020). Other reports provided ample evidence of a strong positive relationship between nematode abundance and the amount of chlorophyll *a* in biofilms growing on hard substrates, both in lotic and in lentic ecosystems (Peters and Traunspurger, 2005; Vidakovic and Bogut, 2006; Majdi *et al.*, 2011, 2012a; Schroeder *et al.*, 2012a, 2013). This correlation mostly concerned the abundances of Chromadorid nematodes (*Chromadorina bioculata*, *Chromadorina viridis*, *Chromadorita leuckarti*, *Punctodora ratzeburgensis*), which seem to thrive especially well in phototrophic biofilms. Additional evidence was provided in studies of marine and freshwater environments that identified Chromadorids as potent algal feeders (e.g. Tietjen and Lee, 1973; Jensen, 1982; Moens and Vincx, 1997; Majdi *et al.*, 2012b,c; Kazemi-Dinan *et al.*, 2014). Nematodes living as epiphytes on submerged macrophytes and bryophytes feed directly on these plants or use epiphytic particles and microalgae. Wu and Liang (1999) compared the nematofauna of two shallow lakes: Lake Houhu, with a phytoplankton-dependent system, and Lake Biandantang, with a macrophyte-dependent system. The total species number was significantly higher in the macrophyte-dependent than in the phytoplankton-dependent system (51 vs. 36 species), consistent with the important effects of habitat heterogeneity and the presence of substantial macrophyte cover for limno-nematofauna. The conclusions drawn from observations of nematodes feeding on aquatic vascular plants in other freshwater aquatic systems can most likely be extended to nematodes in lotic habitats.

3.4.2.2 Heterotrophic microbes

Microcosm experiments have demonstrated the interactions of nematodes with heterotrophic microbes. In incubations of sediment bacteria with microbivorous nematodes, microbial activity was much higher than in the controls, in which nematodes were absent, or with fungivorous nematodes (Traunspurger *et al.*, 1997). Field studies showed that leaf litter containing the largest amounts of bacterial and fungal biomass was colonized by

more nematodes (Palmer *et al.*, 2000; Swan and Palmer, 2000; Gaudes *et al.*, 2009; Majdi *et al.*, 2014). A clear relationship between the production of nematodes and that of heterotrophic ciliates was determined in lakes throughout the year (Bergtold and Traunspurger, 2005b). However, note that only a small proportion of the available field studies reported both microbial and nematode abundances, such that most inferences have been made by considering organic matter as a proxy for the biomass of microbial decomposers (see Majdi and Traunspurger, 2015 and references therein).

3.4.2.3 Interspecific competition

Whether freshwater nematode species compete, with a subsequent shift in the nematode community during the year, is unclear. Competition was demonstrated in a study of the nematodes in Lake Königssee, including between *Monhystera* and *Eumonhystera* (Traunspurger, 2002). *Monhystera* dominated during winter and *Eumonhystera* reached high abundances during the summer. Further analysis of the spatial distribution of the dominant species of *Eumonhystera* in Lake Königssee suggests competition and/or the coexistence of species of the same genus. The mean abundance of the dominant *Eumonhystera* species varied in the ten investigated water depths of Lake Königssee, with *E. filiformis*, *E. similis*, and *E. simplex* dominating at 1 and 5 m and *E. longicaudatula* at 10 m (Traunspurger, 1996a). The examples of *Eumonhystera* and *Monhystera* suggested that biotic factors such as population dynamics and life cycle play decisive roles in the distribution of nematode species. In epilithic biofilms of the Garonne River, *Chromadorina bioculata* and *Chromadorina viridis* clearly dominated the nematode community for most of the year, but their shared dominance seemed to be affected by water temperature, with *C. viridis* being relatively more abundant at temperatures <15°C, *C. bioculata* dominating at 15–20°C, and the replacement of both species by others at higher temperatures (Fig. 3.10). Further studies in the laboratory have confirmed that interspecific competition shapes nematode population dynamics, even among closely related species of bacterial-feeding nematodes (Schroeder *et al.*, 2010; Gansfort *et al.*, 2018b).

3.4.2.4 Predation

The effects of predators on nematode abundance and species composition were shown for a predatory nematode (*Anatonchus dolichurus*) by Prejs (1993) and for fishes by Spieth *et al.* (2011) and Weber and Traunspurger (2015) (see also Chapter 7). Michiels *et al.* (2004) showed that the ratio of predatory to non-predatory nematode abundance correlated positively with nematode species numbers in limnetic habitats (Fig. 3.11). In this study it was shown that the number of nematode species in 13 investigated alpine lakes increased from 24 to 75 when the proportion of predatory to non-predatory nematodes increased from 0.02 to 0.26. Although the relationship between nematode predator–prey ratios and species

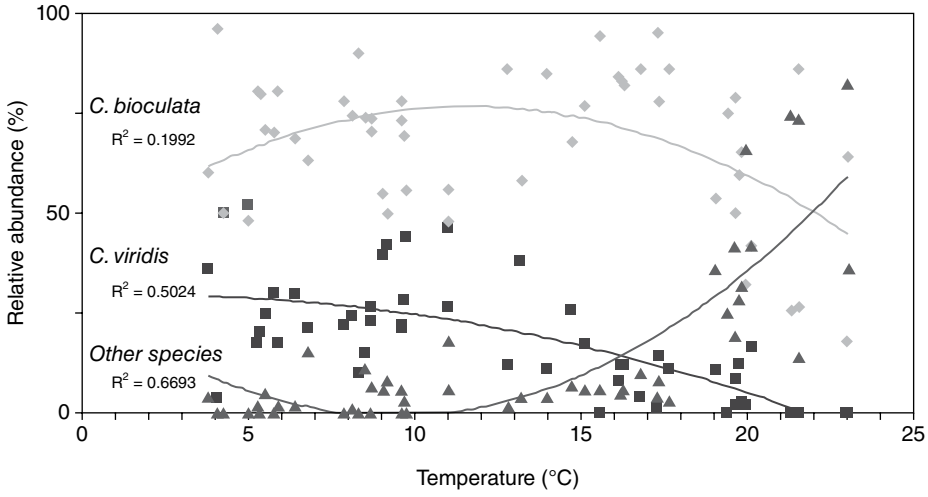

Fig. 3.10 Mean relative abundance of *Chromadorina bioculata* (gray diamonds), *Chromadorina viridis* (squares), and other nematode species (triangles) in biofilms of the Garonne River over an 18-month period (*N* = 51 sampling occasions, *N* = 202 samples). After Majdi *et al*. (2011). (Author's own figure.)

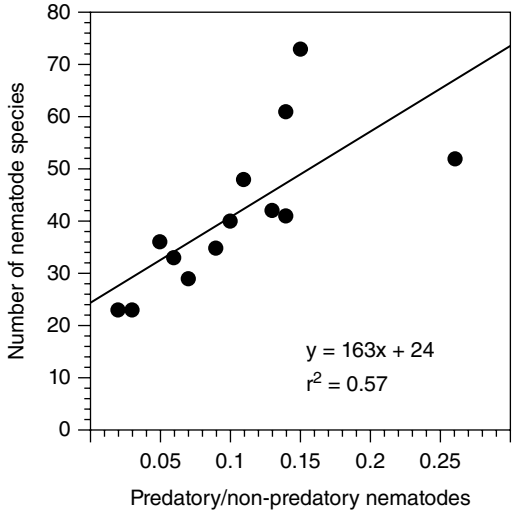

Fig. 3.11. Number of nematode species vs. the predatory/non-predatory ratio of nematodes in a selection of alpine lakes, after Michiels *et al*. (2004). (From Traunspurger *et al*., 2006.)

richness is not completely independent, species richness was not limited by the number of identified individuals per lake, and there was no correlation between nematode abundance and species richness in that dataset. The decisive determinant of an increase in the number of coexisting species was predator abundance, not the number of predatory species. This finding suggests that, among other factors, predation within nematode communities strongly prevents competitive exclusion.

3.5 From Microscopic to Worldwide Patterns in Species Composition

3.5.1 Micro-distribution

Very few studies have investigated the micro-distribution patterns of free-living nematodes in their natural habitats, although this is a prerequisite to better understand nematode community ecology and to refine sampling procedures such that small-scale variability is taken into account. Most knowledge in this field comes from studies in marine systems. The relevant studies include those of Findlay (1981), who reported that mudflat nematodes could aggregate in small patches of ca. 5 cm^2; Gallucci *et al.* (2009), who also measured aggregations, but in small patches (<4 cm^2) in the deep sea; and those of Blanchard (1990) and Pinckney and Sandulli (1990), who reported larger aggregations at patch sizes of 113–201 cm^2. These aggregations seem to respond to patchy distributions of resources on the seafloor, but they may also result from interspecific interactions and the segregation of niches across nematode species, although experimental evidence is lacking (but see Gansfort *et al.*, 2018b). Recently, Gansfort *et al.* (2018a) analyzed the distribution of nematode species over a 27 × 27 cm (729 cm^2) area of a pond floor. Following a grid-like design, they collected 125 samples from 3 layers (see Fig. 3.12) for a total of 375 samples that were used to measure the size of agglutination

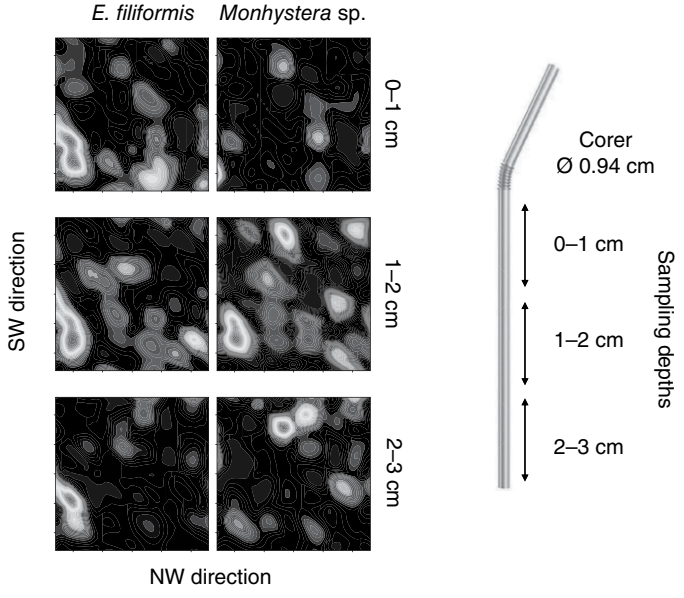

Fig. 3.12. Heat map of a 27 × 27 cm area of a pond bottom sampled in a grid-like design using a straw (corer). The heat map shows the density hotspots of the two dominant nematode species, *Eumonhystera filiformis* and *Monhystera* sp., at three different sediment depths. (Modified after Gansfort *et al.* (2018a), with permission of Wiley.)

patches (if any) and the drivers of patch formation. The authors hypothesized that patch formation would respond positively to organic matter patches and species segregation. The results showed that nematodes formed patches containing 28, 154, and up to 201 individuals/cm^2 in the deeper, middle, and upper layers, respectively (Fig. 3.12). Patch locations did not correlate with the distribution of organic matter but for the dominant species *Eumonhystera filiformis* and *Monhystera* sp. their patch locations and sizes differed (Fig. 3.12), suggesting distinct preferences.

3.5.2 Local- and regional-scale patterns

Several studies have compared nematode species composition in different lakes. In the lakes from two massifs in the Alps, the Tannheimer and Karwendel Alps, 38% of the 156 identified species were found in both (Michiels and Traunspurger, 2005a), whereas in nearby lakes within the two alpine areas the species compositions were of low similarity. In the Tannheimer Alps, which include the lakes Vilslache, Traunsalpsee, Vilsalpsee, and Rehbach, 14% of the 130 identified species were present in >50% of all cores. In the Karwendel Alps, 7% of 85 species were found in >50% of all cores, sampled in the lakes Soiernsee, Fereinsalm, Wildensee, Schmalsee, Ferchensee, and Lautersee. The similarity between cores within lakes was by far higher and ranged between 21 and 41%. Most species occurred in several alpine lakes, but only three (*Eumonhystera filiformis*, *E. longicaudatula*, and *Tripyla glomerans*) in all lakes (Michiels and Traunspurger, 2005a). Thus, the resemblance of species compositions was higher at local (within lakes) and regional (between massifs) scales than at intermediate scales (within a massif).

Identifying the source of the high nematode diversity characteristic of streams (Table 3.2) is central to our understanding of nematode ecology. Many environmental factors, such as grain size, temperature, oxygen, flow regime, and biotic interactions, especially predation and competition for resources, are known to be important drivers of nematode communities, as discussed above and in other chapters of this book. However, the impact of the spatial structuration of nematode metacommunities at regional scales has only very recently been acknowledged (Gansfort and Traunspurger, 2019). In rivers and streams, the spatial patterning of nematode populations may be connected by the network of flowing water; hence, it will be affected by the hydrological background of those water bodies and the potential for their disconnection during periods of drought. For example, in the permanent streams studied by Majdi *et al.* (2020a) typical river nematode communities were dominated by bacterivores, whereas intermittent streams (having one or more dry phases during the year) contained a higher proportion of plant- and hyphal feeders (especially *Filenchus vulgaris*), omnivores (mostly *Tobrilus* spp. and *Trischistoma gracile*), and predators (mostly *Mononchus truncatus*). The occurrence of dry phases in intermittent streams may allow the immigration of soil species from

the nearby forest floor and the coexistence of a greater number of species, by reducing competitive exclusion from typical aquatic species. Frequent considerable disturbances (e.g. total desiccation of the habitat), as in this example, may increase taxonomic and functional diversity. Yet, after partitioning, based on the variance explained by the different categories of environmental predictors, spatial structuring was the most important driver of nematode community structure in those intermittent streams as well, followed by resource availability. The intermittency regime played a larger role than streambed topology, but it explained roughly half of the variance compared with the spatial predictors alone (Fig. 3.13).

Ptatscheck *et al.* (2020) recently analyzed the distribution of nematodes at a local scale using a homogeneous design. They found evidence of the spatial structuration of nematode communities at a local scale (<1 km), through the significant explanation of species distribution by several principal coordinates of neighborhood matrices.

3.5.3 Global-scale patterns

Nematodes in aquatic habitats have also been studied at the continental scale. Freshwater nematodes from Africa were reviewed by Jacobs (1984), and Eyualem-Abebe *et al.* (2001), those from Europe by Andrássy (1978), from North America by Cobb (1914) as well as Esser and Buckingham (1987), from the former Soviet Union by Tsalolikhin (1988), and from China by Wu and Liang (2000). Gerlach and Riemann (1973) produced an invaluable work listing all species reported from all limnetic habitats up to that point. Andrássy (1981, 1984, 1992, 1999) and Loof (1999, 2001) also provided a census of free-living genera and subgenera. These reviews remain extremely useful at the genus level and also to some extent on the species level. In most other reviews, however, the surveys were confined to within the borders of a single nation or the focus was a single nematode group (e.g. Coomans, 1989; Eyualem-Abebe, 2000; Traunspurger, 2003; Andrássy, 2005, 2007, 2009). Nonetheless, in addition to the above-mentioned incomparability, the published data were acquired over a wide span of time and space such that a readily available standard reference for researchers in this field is still lacking.

A few studies have, however, considered the worldwide geographic distribution of freshwater nematodes (Eyualem-Abebe *et al.*, 2008; Zullini, 2014, 2018), but discussions of the global distribution or endemicity of nematode species are largely premature, mainly because nematological surveys from the southern hemisphere are either lacking or incomplete (Jacobs, 1984; Decraemer and Coomans, 1994). Moreover, biogeographic studies of continental free-living nematodes usually focus on soil species. This was the case in the study of van den Hoogen *et al.* (2019), whose modeling approach used published data representing several biomes. Their results shed light on the global abundance and feeding type distribution patterns of free-living nematodes living in soils. The bias toward soil

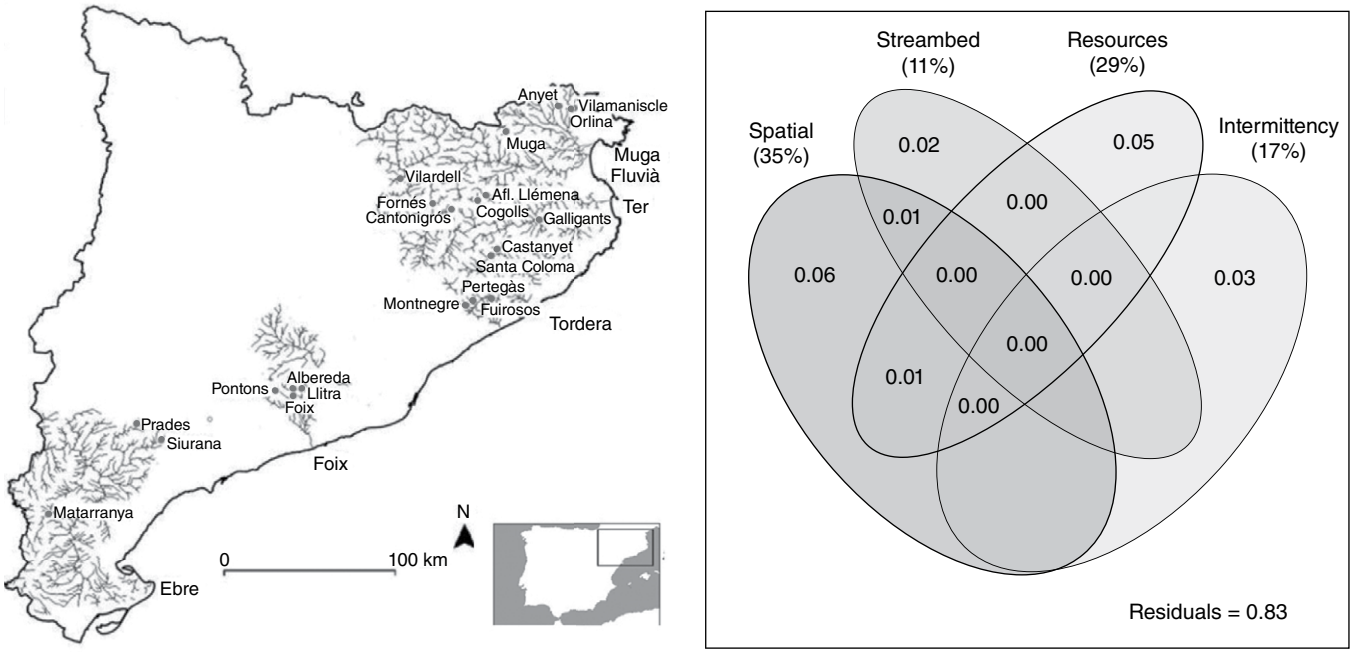

Fig. 3.13. Distribution of study sites across five river basins in the northeastern Iberian Peninsula. The right panel shows a Venn diagram of the variation partitioning analysis among four categories of environmental predictors of nematode community structure. The four explanatory matrices were spatial distances, streambed granulometry, dissolved resources characteristics, and water intermittency temporality. The outer rectangle represents the total variation in the response data, and each circle the portion of the variation accounted for by an explanatory matrix or a combination of explanatory matrices. The values are adjusted-R^2 values from the redundancy analysis model and indicate the amount of variation explained by a given single or shared partitioning. The percentages enclosed in the parentheses are the relative contribution of each independent category to the overall variance explained. (Modified after Majdi *et al.* (2020a) with permission of Wiley.)

nematology may be a consequence of better funding opportunities given the potential practical applications of plant-pathogenic and insect-parasitic nematodes in agro-ecosystems (see Chapter 1), but it may also reflect the dearth of competent taxonomists and continuing controversy on the identity of certain species in freshwater habitats. Andrássy (1978) listed 160 genera and 605 species, including Tylenchids, from European limnetic habitats. In 2008, Eyualem-Abebe *et al.* calculated 1808 freshwater nematode species (1391 free-living species, without Mermithida) in the world, corresponding to ca. 7% of all known nematode species.

Zullini (2014) reviewed 174 papers and species lists from 1905 to 2011, covering 14 geographic areas and 7 lakes. After several taxonomic corrections, and excluding Tylenchomorpha and Mermithida, a total of 1020 nominal species reported from freshwater were reported. Zullini (2014) listed the following results for 14 regional nematode biotas: North America (Canada + USA), 187 species; Costa Rica and Nicaragua, 47; Amazon region, 99; Spain, 123; Scandinavia, 66; Germany and Austria, 301; Poland, 69; Italy, 215; European Russia and Ukraine, 202; Himalaya, 34; Mongolia, 46; China, 88; East Africa, 142; South Africa, 178. The author considered geographic regions as large composite units, and lakes as more compact and comparable units. Accordingly, seven lakes with the best studied nematode fauna were included in the survey (Lake Leman, Lake Königssee, Lake Balaton, Lake Ohrid, Lake Issykul, Lake Baikal, Lake Biwa). The 312 lacustrine species were obviously all strictly freshwater. The main results were as follows. First, a clear east–west gradient emerged, except for Lake Baikal, owing to its exceptional antiquity and rich endemic species content. Second, a Leman–Königssee cluster was identified; these two alpine lakes are exactly 500 km apart. A third finding was the peculiar position of Lake Baikal, whose nematode community strongly differed from the communities in all the other studied lakes. However, based on genera instead of species (reducing the 312 species to their 139 genera), no reasonable biogeographic pattern emerged, as ca.45% of the freshwater genera were present in all seven lakes (Zullini, 2014). Although the widespread distribution of a species within similar habitats but in different geographic regions is certainly plausible, reports of a single species in a wide range of habitats and geographic regions seem unlikely. For example, within Africa, *Dorylaimus stagnalis* was reportedly detected in the bottom sediment of freshwater and brackish water, in terrestrial habitats as well as in the sediment of thermal springs, in addition to also being planktonic (Jacobs, 1984). Until a refined taxonomy is available, Jacobs' (1984) results should be considered with caution.

3.5.4 Do nematodes have a ubiquitous distribution?

Most free-living genera are widespread if not ubiquitous in their distribution (Andrássy, 1978; Jacobs, 1984; Andrássy, 2005, 2007, 2009; Schabetsberger *et al.*, 2009). Jacobs (1984) cautiously suggested that not

only most free-living genera but also a large number of species were cosmopolitan (see Chapter 5), while at the same time acknowledging that the distribution of many species is based on 'doubtful identification' and thus likely to be revised. Generally, a number of these cosmopolitans may in fact have acquired specialized reproductive strategies, including parthenogenesis, drought- (and ingestion-) resistant stages, rapid maturation upon hatching, and short generation times, which contribute to maximal species dispersal and therefore to their cosmopolitanism (Jacobs, 1984). Zullini (2014) considered the biogeography of freshwater nematodes and concluded that their communities differed at the continental level. While the uniqueness of the Lake Baikal community was also noted in that study, data from other lakes, especially those in the tropics, are still needed.

In their study of a remote aquatic habitat within the Galápagos archipelago, Eyualem-Abebe and Coomans (1995) found that 10 of the 18 identified species were cosmopolitan, 6 were widely distributed in the southern hemisphere, and 2 were new records. The presence of freshwater nematodes on the Galápagos was explained as the product of passive and very occasional transport by birds. Support for this explanation came from a study of three previously unexplored lakes in the caldera of the Cerro Azul volcano, Galápagos Island (Muschiol and Traunspurger, 2008).

Caves are another interesting example of disconnected habitats in which the omnipresence of some nematode species can be examined (see also Chapter 4). In a review of nematode species identified from cave systems around the world, Du Preez *et al.* (2017) found that only 7 out of 295 species recorded in caves could be considered as true troglobiotic species. This contrasts with the overall high endemicity of larger invertebrates or vertebrate organisms inhabiting caves. In fact, most cave ecosystems should be quite permeable to nematode species entering accidentally from adjacent soils and aquifers. Most of these species will thus be ephemeral inhabitants, but others may persist and occupy ecological niches in caves. However, community turnover may be stochastic and too fast to allow the emergence of speciation mechanisms (Du Preez *et al.*, 2017).

Finlay (2002) proposed that the abundance of individuals belonging to microbial species (e.g. protozoa) is so large that geographic barriers rarely restrict dispersal. This perspective requires an alternative interpretation of the scale and dynamics of biodiversity at the microbial level, wherein global species numbers are relatively low and local species richness is always sufficient to drive ecosystem functions. One obvious explanation is that microbial species are simply so abundant that their continuous large-scale dispersal sustains their global distribution. According to Finlay (2002), there should be a size range at which ubiquitous dispersal becomes less likely such that species are more likely to be geographically restricted. The organismal size range at which this change occurs is generally believed to be between 1 and 10 mm. Thus, free-living eukaryotes with a body size of <2 mm are probably sufficiently abundant to enable their worldwide distribution. The abundance of different size ranges (according to body length) of freshwater nematodes as determined in the

littoral of 11 selected lakes in Germany is shown in Figure 3.14. A strong decline (r^2 = 0.6–0.9) in freshwater nematode abundance with increasing size characterizes most of those lakes (exception: Lake Lautersee, where the dominant (57%) nematode species is *Prodesmodora circulata*, which has a body length of 785 µm), where at least 80% of all the nematodes have a body length of <2 mm. Moreover, only 7 out of 90 species in the littoral of Lake Königssee have a body length >2 mm.

3.6 Conclusions and Perspectives

Despite the intense interest in biodiversity, species inventories are still missing due to the scarcity of taxonomists with the skills needed to

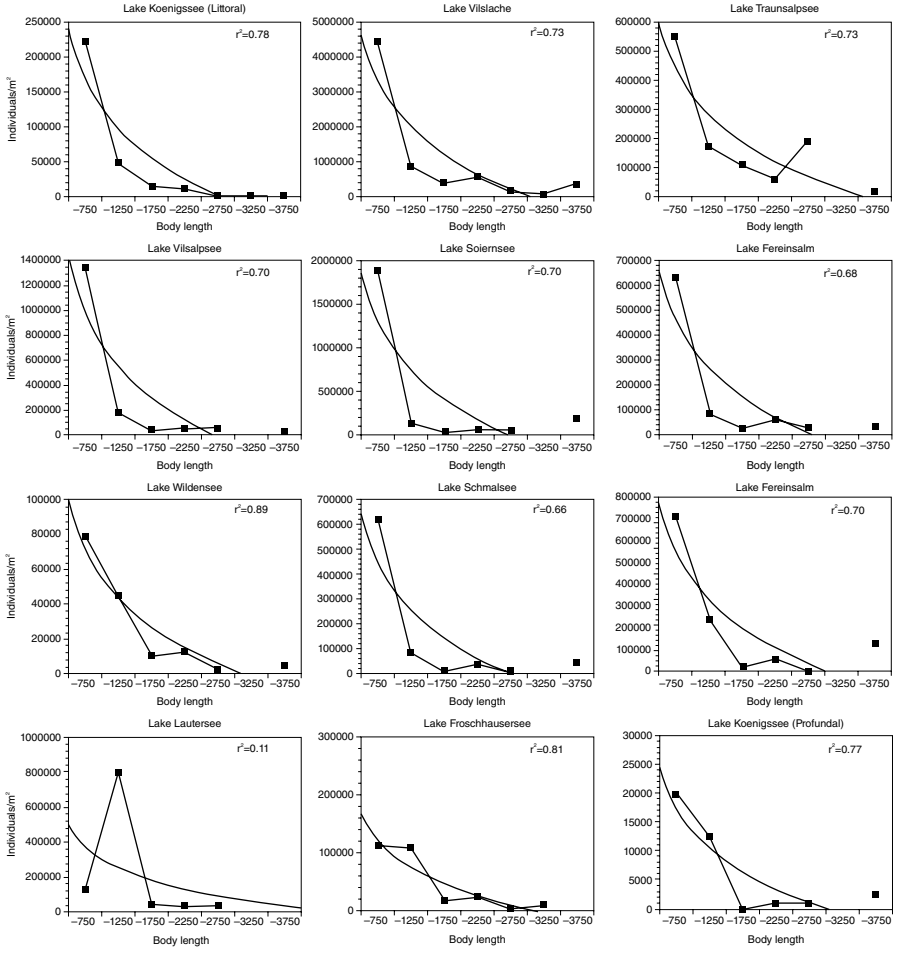

Fig. 3.14. Abundance (individuals/m²) of nematodes with different body lengths (µm) in the littoral of 11 selected lakes and in the profundal of Lake Königssee, Germany. (From Traunspurger *et al*., 2006.)

characterize species communities and to recognize new species. This is the case in nematology, where only a small fraction of the estimated 10^5 to 10^8 existing species has been described (Luc *et al.*, 2010). The impact of correct identification on the resolution power of the resulting ecological studies cannot be overemphasized. In a book chapter on the ecology of freshwater meiofauna, Pennak (1988, pp. 44–45) wrote, '(…) nematodes are perhaps the most highly adaptable organisms from ecological and physiological stand point'. Indeed, the same nematode species may be found from the tropics to the sub-arctic, from warm springs to cold alpine lakes, and on many types of substrates. However, although some species are undoubtedly ubiquitous in their geographic distribution (Jacobs, 1984), in most cases the biological flexibility conferred by a wide range of environmental variation may overwhelm the genetic flexibility of a single species.

A new methodology based on molecular identification has been successfully applied to freshwater nematodes (see Chapter 2) and may expand current knowledge on their biogeography (e.g. Markmann and Tautz, 2005). Other capabilities include the recognition of cryptic species (Ristau *et al.*, 2013b), assessments of whether nematode diversity is sufficient to allow a response to polluted sediments (Schenk *et al.*, 2020b), and determinations of species identities (e.g. van den Elsen *et al.*, 2009; Schenk *et al.*, 2020a). If ubiquity is a general phenomenon in freshwater nematodes, it must be confirmed by methods more sophisticated than those that rely on morphology. Recent discussions of taxonomic challenges and the advantage of molecular identification techniques emphasized the shortcomings and pitfalls of α-taxonomy, questioning its utility and future (Tautz *et al.*, 2002; Decraemer and Backeljau, 2015). Nonetheless, an understanding of aquatic ecosystem functioning must be predicated on knowledge of nematode diversity (Decraemer and Backeljau, 2015). In addition, any described DNA sequence should always be linked to a named species whose morphology has been correctly ascertained by a trained taxonomist (Luc *et al.*, 2010). Extensive sampling of tropical and equatorial regions is also needed, as is the use of inter-disciplinary approaches to define freshwater nematode species richness at a global scale.

Freshwater nematodes, because of their high abundances and species richness, are an ideal group of organisms to test ecological hypotheses and to describe the diversity of an ecosystem. In many lakes, the abundance of freshwater nematodes decreases with the increasing size of these organisms. If the ubiquity of small species is a general phenomenon in limno-nematodes, this must be further demonstrated by molecular identifications. Species compositions are more similar at small (within lakes) and large (between areas) scales than at intermediate scales (within regions). The number and proportions of species of lotic, hyporheic, and phreatic nematodes must still be determined, preferably in larger, longer-term studies able to address the strong seasonal dynamics of nematodes and the variety of possible habitats. Stream nematodes are tightly linked to terrestrial ecosystems and thus play important roles in the functions and connectivity of multiple ecosystems. At a time when most lotic ecosystems,

and their fauna and flora, are at extreme risk of collapse, nematodes can serve as sentinels and in models developed by ecologists to better assess patterns and processes related to climate change. Several avenues of research are open to those eager to better understand the biology and ecology of free-living nematodes in inland water bodies. It is our wish that this review of the literature and the examples provided herein will foster the emergence of comprehensive and useful investigations.

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4 Nematodes from Extreme and Unusual Freshwater Habitats

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Highlights

- Nematodes inhabit all extreme habitats investigated.
- Nematodes can reach high abundances (e.g. in intermittent streams) and high numbers of species.
- Nematodes can be found in springs and deep boreholes at temperatures at or above 40°C.
- A high diversity of over 100 nematode species has been found in the epiphytic tank water of bromeliads.
- Stemflow connects the vegetative canopy with the soil as habitat for nematodes.
- Many nematodes in caves have also been reported in other aquatic and terrestrial habitats; endemic species are rare.
- Some typically marine forms are also found in freshwater environments, and some typically freshwater forms also in saline waters.

4.1 Introduction

This chapter discusses the ecology and biogeography of nematodes from freshwater environments that are extreme in temperature, chemical composition, variability, or isolation. Described and compared are the compositions of nematode faunas from hot or mineral springs, pools and bogs in polar regions, intermittent lakes or pools or streams, freshwater pools in bromeliads or tree hollows, stemflow, fresh groundwaters, and caves. Comparisons of the nematode faunas from these extreme habitats with those from more typical freshwater environments are also provided. Also discussed are nematodes with evolutionary affinities to freshwaters that are found in estuarine sediments along with nematodes from freshwaters with evolutionary affinities to otherwise marine taxa. The emphasis is on broad ecological patterns rather than on detailed species interactions with the various freshwater environments. Thus, the chapter focuses on genera or higher taxa rather than species.

4.2 Springs

Among the 'extreme' freshwater habitats for nematodes, springs are perhaps the best-studied. Springs can be considered ecotones between the hypogaean and the epigaean aquatic environment and, like caves, are of special interest because of their typically near-constant abiotic parameters (Zullini *et al.*, 2011). European springs have been the focus of many studies (Granada in Spain: Ocaña, 1991a,b,c, 1993; Ocaña and Morales, 1992; Germany and Austria: Pax and Soos, 1943; Paetzold, 1958; Andrássy, 1978; Schiemer, 1978; Seiml-Buchinger and Traunspurger, 2006; Traunspurger *et al.*, 2015; the Italian Isle of Ischia: Meyl, 1953a,b, 1954a; Italian alpine springs in Trentino: Zullini *et al.*, 2011; and Yugoslavia: Schneider, 1940). Nematodes in springs have also been studied in Central Africa (De Coninck, 1935), the USA (Hoeppli, 1926), Kyrgyzstan (Gagarin and Lemzina, 1992), and China and Taiwan (Hoeppli and Chu, 1932) have been investigated as well.

Springs are unique freshwater habitats because their physical and chemical environments are relatively stable. This is in marked contrast to most

lakes, rivers, and marine and terrestrial environments, whose physical and chemical parameters are more internally variable. However, the chemical composition, water velocity, and temperature of different springs may vary widely (Botosaneanu, 1998). Other differences include the history of a spring at a particular location, the spring's remoteness from other springs, and the nature of the surrounding habitat. Because of their ecological characteristics, springs have long been the focus of ecological, biogeographic, evolutionary, and genetic studies (Hynes, 1970; Odum, 1971). Furthermore, as they include some of the few habitats unimpacted by human activities, springs have also been used as controls or reference points for comparisons involving aquatic habitats with varying degrees of pollution (Ocaña and Picazo, 1991).

Springs have been used as model ecosystems in very influential studies of productivity, energy flow, and trophic relationships (Odum, 1957; Teal, 1957; Minckley, 1963; Minshall, 1967; Tilly, 1968; Iversen, 1988; Traunspurger *et al.*, 2015). However, in those studies nematodes were considered, if at all, as part of gross trophic groups. Moreover, in the broad but relatively shallow approach of the studies that did include nematodes, the techniques used in their sampling and enumeration were inefficient. As a result, conclusions about the ecological importance and trophic roles of nematodes were associated with large uncertainties.

In springs with increasingly extreme environmental conditions, nematodes account for an increasingly large proportion of metazoan populations, with respect to both number and biomass. This is not necessarily because nematodes become more abundant; rather, it reflects the fact that nematodes are among the multicellular organisms most tolerant of extreme conditions, which allows them to survive when other organisms cannot. Nevertheless, nematodes do not occur where conditions become too extreme: at temperatures above 43°C and in waters with high ionic concentrations, particularly chloride ion concentrations >7600 meq/l and a total ionic conductivity >17,000 µS/cm (Ocaña, 1991a,b).

The nematode genera found in springs in Granada, Spain, and Germany are listed in Table 4.1, which clearly shows the highly variable abundance, diversity, and distribution of individual species in these locations. In Spain, most species were found in <10% of the 38 springs that were sampled (Ocaña, 1991a; Ocaña and Morales, 1992). Many species belonging to the Orders Dorylaimida, Rhabditida, Tylenchida, and Aphelenchida were recorded in those springs only once and are considered accidental inhabitants (Ocaña *et al.*, 1986). In the springs of Granada, ca. 65 species were identified (Ocaña, 1991a,b, 1992; Ocaña and Morales, 1992).

By contrast, an extensive study of the nematodes in 94 alpine springs in Trentino, Italy, identified 90 species (Zullini *et al.*, 2011). In Italy, the 10 species with the largest number of individuals were *Eumonhystera filiformis-vulgaris* (11.0%), *Dorylaimus stagnalis* (9.5%), *Epitobrilus allophysis* (8.2%), *Ethmolaimus pratensis* (6.7), *Monhystera paludicola* (5.2%), *Tripyla filicaudata* (4.6%), *Plectus parietinus* (4.1%), *Eumonhystera barbata* (3.9%), *Paractinolaimus macrolaimus* (3.3%), and *Tylencholaimus minimus* (3.0%) (Table 4.1). The major abiotic factors influencing nematode community composition are water temperature and lithology

Table 4.1. Nematode genera from different springs in Italy (Trentino), Spain (Granada), and Germany (Nationalpark Berchtesgaden). Italy: Zullini *et al*. (2011); Spain: Ocaña (1991a,b, 1992; Ocaña and Morales, 1992); Germany: Seiml-Buchinger and Traunspurger (2006). (Author's own table.)

	Italy	Spain	Germany		Italy	Spain	Germany
Achromadora	X	X	X	*Mesorhabditinae*		X	
Aglenchus			X	*Mesocriconema*			X
Alaimus	X	X	X	*Mesodorylaimus*	X	X	X
Anaplectus	X			*Metateratocephalus*	X		
Anatonchus	X			*Monhystera*	X	X	X
Aphanolaimus	X	X		*Monhystrella*	X	X	
Aphelenchoides		X	X	*Mononchus*	X	X	X
Aporcelaimellus	X	X		*Mylonchulus*	X		
Bastiania	X			*Odontolaimus*	X	X	
Brevitobrilus		X	X	*Oxydirus*	X	X	
Bunonema			X	*Panagrellus*	X		
Butlerius		X		*Panagrolaimus*	X		
Cephalenchus			X	*Paractinolaimus*	X	X	
Cephalobus	X	X		*Paracyatholaimus*		X	
Ceratoplectus	X			*Paramphidelus*	X		X
Chromadorita		X		*Paraplectonema*		X	
Chronogaster		X		*Paravulvus*	X		
Clarkus	X			*Paratylenchus*			X
Criconema			X	*Paraxonchium*	X		
Criconemoides			X	*Plectus*	X	X	X
Crocodorylaimus	X			*Prionchulus*	X		
Cuticularia		X		*Pratylenchus*			X
Cylindrolaimus	X	X		*Prismatolaimus*	X	X	X
Daptonema		X		*Prodesmodora*	X	X	X
Diplogasteritus		X		*Prodorylaimus*	X		X
Diploscapter		X		*Proleptonchus* sp.		X	
Dorylaimus	X		X	*Protorhabditis*	X	X	
Doryllium			X	*Rhabdiitidae*		X	
Enchodelus	X		X	*Rhabdolaimus*		X	X
Epidorylaimus	X	X	X	*Rhyssocolpus*	X		
Epitobrilus	X		X	*Rotylenchus*			X
Ethmolaimus	X	X	X	*Semitobrilus*	X		X
Eucephalobus	X			*Teratocephalus*	X		X
Eudorylaimus	X	X	X	*Theristus*	X		
Eumonhystera	X	X	X	*Thonus*		X	
Fictor	X			*Tobrilus*	X	X	X
Filenchus			X	*Tripyla*	X	X	
Geomonhystera	X			*Trischistoma*	X	X	
Helicotylenchus			X	*Tylencholaimellus*			X
Hemicycliophora			X	*Tylencholaimus*	X		X
Heterocephalobus		X		*Tylenchidae*		X	
Hofmaenneria	X			*Tylenchus*			X
Ironus	X	X	X	*Udonchus*		X	
Laimydorus	X			*xiphinema*		X	
Macroposthonia			X				

(carbonate vs. crystalline). On average each spring hosted 9 (range: 1–26) nematode species (Zullini *et al.*, 2011), all of which were common in freshwater habitats, with a wide geographical range on a continental scale.

In Nationalpark Berchtesgaden (Germany), 54 nematode species were identified in 5 springs, with an additional 30 taxa identified only to the genus level. Abundance varied between 250 and 500 individuals/10 cm^2. Most species were known to be widely distributed and not restricted to springs or groundwater, with 9% of the species typical of terrestrial habitats, 13% found only in aquatic habitats, and 78% in both habitats (Seiml-Buchinger and Traunspurger, 2006).

4.2.1 Hot or thermal springs

Hot or thermal springs are among the most often studied types of springs. Their faunas have been surveyed in Ruwenzori (Democratic Republic of Congo), the USA, China, Taiwan, Japan, Italy, Germany, Spain, and Indonesia (Hoeppli, 1926; Hoeppli and Chu, 1932; De Coninck, 1935; Schneider, 1937; Pax and Soos, 1943; Meyl, 1953a,b; 1954a; Ocaña, 1991b; Eyualem-Abebe *et al.*, 2001; Suzuki *et al.*, 2017). Species descriptions from Spain and Austria have also been reported (Schiemer, 1978; Ocaña, 1991c).

The occurrence of particular nematode species in hot springs is for the most part highly variable (Ocaña, 1991a,b) and seems to be largely governed by chance. However, at least two species are found in hot springs all over the world, albeit not in all hot springs: *Rhabdolaimus terrestris* (Ocaña, 1991a in Spain; Schiemer, 1978 in Austria; and Schneider, 1937 in Sumatra, Java, and Bali) and *Udonchus tenuicaudatus* (De Coninck, 1935 in Ruwenzori, Democratic Republic of Congo; Meyl, 1953a,b; 1954a in Italy; Ocaña, 1991b in Spain; and Paetzold, 1958 in Germany).

Total nematode abundance and species richness in hot springs are also highly variable, perhaps associated with the particular species of both nematodes and other organisms present in a particular spring (Fig. 4.1). In general, total nematode abundance tends to decrease in springs with a water temperature ≥25°C. Nonetheless, in at least one very hot spring (>40°C) very high abundances consisting almost entirely of *Rhabdolaimus terrestris* have been found. Species richness follows a similar trend: within the Orders Monhysterida, Araeolaimida, Chromadorida, and Enoplida, eight species were detected in springs with a water temperature >40°C, 11 species in those with a water temperature of 30–40°C, and an even larger number in those with a water temperature of 20–30°C (Ocaña, 1991b).

4.2.2 Low-oxygen springs

The characteristics of the nematode faunas are less variable in springs whose waters differ in their oxygen potential than in their temperature. This is perhaps related to the lower productivity and diversity of other organisms in springs with low oxygen availability, which results in fewer opportunities for nematodes to colonize successfully. Fewer species are

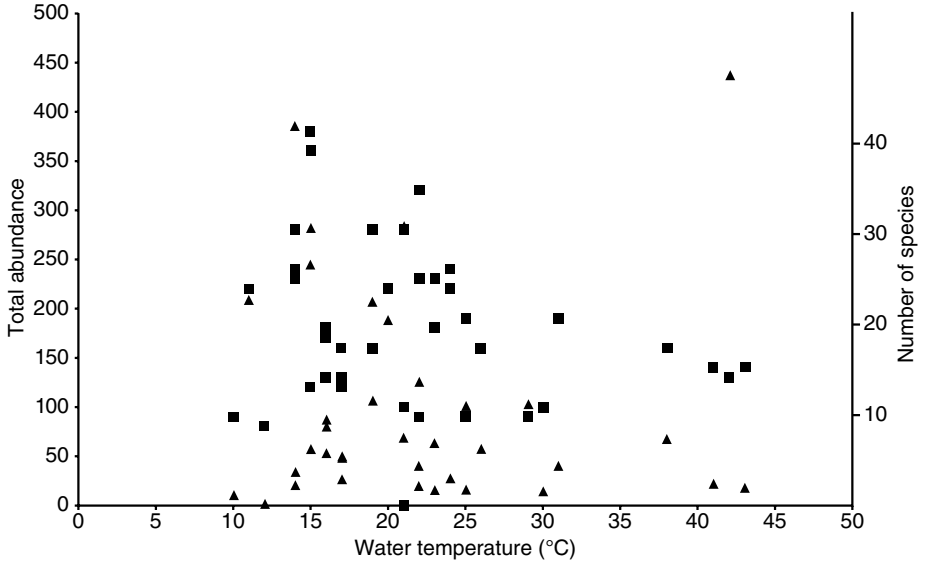

Fig. 4.1. Total abundance and species richness of nematodes in hot springs as a function of water temperature (data from Ocaña, 1991b). Triangles represent abundance, and squares species richness. (From Hodda *et al.*, 2006.)

found in low-oxygen springs than in other springs, and the total abundance of nematodes in the former is low. In springs where the oxygen availability is always low, nematode species of the Order Rhabditida are often the most abundant (Ocaña, 1993). Rhabditids have a highly opportunistic ecological strategy: they are easily dispersed, rapidly increase their population size under favourable conditions because of their high fecundity and short generation times, and they are very resistant to adverse conditions (Zullini, 1976; Bongers, 1988; Zullini and Pagani, 1989; Muschiol and Traunspurger, 2007).

Where the amount of oxygen is often low but more variable, nematode species differ. One species each of the monhysterid genera *Eumonhystera* and *Monhystrella*, several species of the genus *Plectus*, and a single species of the genus *Prismatolaimus* were observed (Ocaña, 1993). The physiological adaptations of Monhysterida allow them to thrive in low-oxygen environments; the genus *Plectus* shares many of the ecological characteristics of Rhabditida and the genus *Prismatolaimus* occurs in very diverse habitats, indicative of its tolerance of a wide range of conditions.

4.2.3 Mineral springs

Mineral springs contain significantly more ions than is usual in freshwater. The concentrations of particular ions may vary considerably among these springs, which in turn affects many other aspects of their chemistry and biology. Among the most common ions present are chloride, sulfate, carbonate or bicarbonate, calcium, magnesium, sodium, potassium, and iron.

As in other types of springs, in mineral springs the total abundance and number of species present are highly variable and correlate very poorly with a single environmental parameter. The general trend is toward a higher abundance and species richness in springs with a lower ionic content (Ocaña, 1991a; Ocaña and Morales, 1992) (Fig. 4.2)

Overall, mineral springs host about the same numbers of species as hot springs (Ocaña, 1991a; Ocaña and Morales, 1992). Most of the species are extremely sporadic, but a few occur frequently and under a wide range of conditions. The latter group includes those tolerant of fluctuating oxygen conditions, such as species of the genera *Eumonhystera*, *Monhystrella*, *Chronogaster*, *Plectus*, *Prismatolaimus*, *Ironus*, *Aphanolaimus*, and *Tobrilus* s.l. Most of the more sporadically occurring species are not limited to a specific ionic composition or range, but a few species have been found only in springs with a high concentration of particular ions (Ocaña, 1991a; Ocaña and Morales, 1992). These include springs in which chloride was the predominant anion (*Monhystera*, *Monhystrella*, *Paracyatholaimus*, and *Chromadorita*) and some in which sulfate was predominant (*Ironus* and *Paraplectonema*). In springs in which carbonate or bicarbonate was the predominant ion, nematodes were detected only in those with a relatively low ionic concentration (*Eumonhystera*, *Chronogaster*, *Plectus*, *Tobrilus* s.l., and *Achromadora*) (Ocaña and Morales, 1992). Species in the latter group are common in many rivers and streams (e.g. Ocaña and Picazo, 1991; Traunspurger, 2000; Beier and Traunspurger, 2003a,b; Traunspurger *et al.*, 2015; see also Chapter 3).

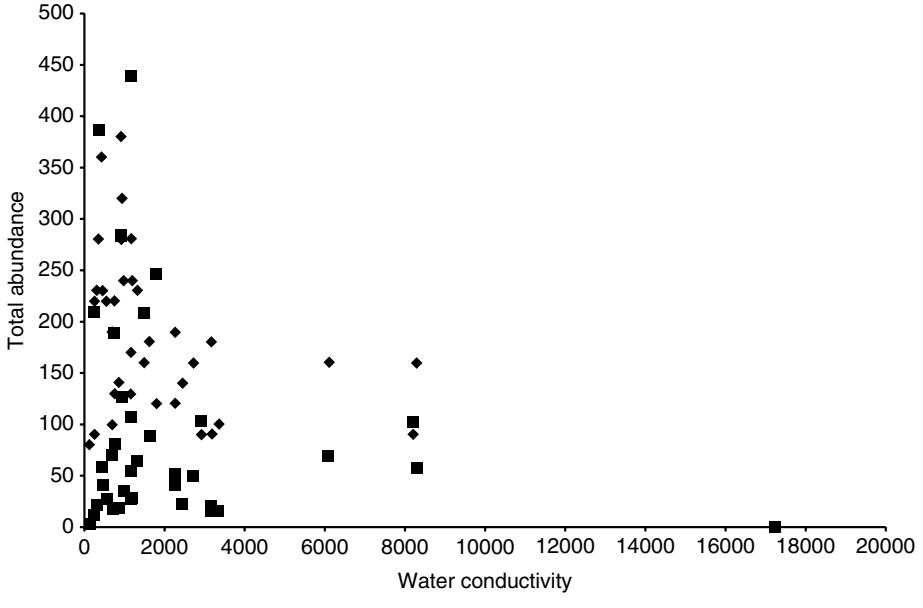

Fig. 4.2. Total abundance and species richness of nematodes in springs differing in their water conductivity (data from Ocaña, 1991a). Diamonds represent abundances, and squares species richness. (From Hodda *et al*., 2006.)

4.2.4 Freshwater springs

Of the nematodes inhabiting springs, only a few are restricted to those with low ionic concentrations, moderate to high oxygen levels, and near-ambient temperatures. Instead, one of these parameters may be limiting and sometimes two, but rarely all three. For example, *Udonchus tenuicaudatus* is restricted to springs with low ionic concentrations, but is widespread in hot springs. *Monhystrella lepidura* is restricted to springs with moderate oxygen levels, but present in hot springs and those with high ionic concentrations. These observations strongly suggest that many, if not most, of the species found in springs are eurytopic to some extent.

Among the various types of springs, freshwater springs generally support the largest numbers of species and the highest abundances (Ocaña, 1993). Many of those species also occur in other types of springs as well as in other habitats (Gerlach and Riemann, 1974; Ocaña and Picazo, 1991; Seiml-Buchinger and Traunspurger, 2006).

4.3 High-latitude Freshwaters

Polar regions in the northern and southern hemispheres commonly contain freshwater habitats. Freshwaters in polar regions are, by definition, frozen for much of the year, but thaw during the brief summer, forming small pools and bogs that host freshwater nematodes.

4.3.1 Subpolar islands

Although extensive studies have been conducted on the sub-Arctic and sub-Antarctic islands of Spitzbergen (78°N), and Macquarie Island (54°S) (Bunt, 1954; Loof, 1971; Nunn, 1993; Marchant and Lillywhite, 1994; Hodda and Keith, unpublished), freshwater aquatic habitats were poorly represented in all of them. Nevertheless, the results of those studies provide preliminary insights into freshwater nematodes in subpolar islands.

Fifteen species were recorded in a small pool on Spitzbergen, including two species also found in European spring waters and other habitats (*Monhystera stagnalis* and *Eumonhystera vulgaris*) (Loof, 1971; Table 4.2). Sampling in a similar habitat, including fine sediment, many years later produced a similar ecological suite of species (Nunn, 1993) (Table 4.2).

Many of the same genera were present in the later study, although it did not include species-level characterizations. In general, the fauna was dominated by microbial feeders, followed by omnivores. Three samplings of a small stream on Macquarie Island in 1952 consistently revealed a high nematode abundance but the species were not identified (Bunt, 1954). Single samples from streams on Macquarie Island investigated by Hodda and Keith in 1998 produced 11 species. The trophic composition was similar to that of the samples from Spitzbergen (Table 4.2), and many of the genera were also the same. The similarity in faunal composition at the generic level is remarkable given the geographic isolation of both

Table 4.2. Relative abundances (%) of nematode genera found in subpolar freshwaters. (From Hodda *et al.* (2006).)

Order	Genus	Loof (1971)	Nunn (1993) (fine sediments)	Nunn (1993) (coarse sediments)	Hodda and Keith (unpub.)
Enoplida	*Enoploides*				5
Monhysterida	*Eumonhystera*	20		5	30
	Monhystera	4			
Araeolaimida	*Plectus*	8		22	10
Triplonchida	*Prismatolaimus*	1		2	2
	Tripyla	4			
Dorylaimida	*Dorylaimus*		2		
	Eudorylaimus s.l.	25	29	19	23
	Prodorylaimus	1			
Mononchida	*Mononchus*		7	4	5
Rhabditida	*Rhabditis* s.l.		20	12	5
	Heterocephalobus	12	4	26	
	Eucephalobus	4	5	3	15
	Chiloplacus	1		5	
Tylenchida	*Tylenchus* s.l.		7		3
	Neotylenchidae	4			
	Ditylenchus	1			
Aphelenchida	*Aphelenchoides*	18	27		2

islands and the likelihood that chance events strongly influence species composition, as noted above for spring waters (see Section 4.2).

The freshwater aquatic nematodes of both Macquarie Island and Spitzbergen are separated from the nearest freshwater habitat by large distances of sea water. The results from Spitzbergen can be explained by the presence of land bridges during recent glaciation and lower sea levels. Alternatively, nematodes may have been deposited with snow, as determined for nematodes in the high mountains of southern Europe and northern Africa (Duval *et al.*, 1999). Unlike Spitzbergen, Macquarie Island has never been connected to a continental land mass and is very remote even from the freshwaters of other islands, much more so than Spitzbergen. Human transport may account for at least some of the species detections, since one species found on Macquarie Island, *Enoploides stewarti*, is known from southern Australia (Nicholas, 1993; Marchant and Lillywhite, 1994; Nicholas and Marples, 1995; Hodda and Keith, unpublished), from which many early visitors to Macquarie Island departed.

4.3.2 Polar freshwaters

Nematodes from polar freshwaters have been recorded from continental Antarctica and from islands south of the Antarctic convergence considered as 'polar', including King George Island (62°S), Adelaide Island (67°S), Alexander Island (70°S), and Ross Island (77°S), but only a very few species, representatives of very simplified freshwater nematode communities, are

known from these waters (Table 4.3). While the respective genera are a subset of those found in the less extreme, less isolated subpolar islands, many of the species seem to be endemic to the continent, such as the terrestrial nematodes from Antarctica (Maslen, 1979).

The trophic structures of the nematode communities in polar freshwaters are remarkably similar to those of subpolar freshwaters. This may seem unusual as many of the species are also found in 'terrestrial' polar habitats (Treonis *et al.*, 1999; Sinclair and Sjursen, 2001), albeit with different relative abundances. There are three possible explanations for this finding: (i) nematode species living in the polar environment are extreme physiological and ecological generalists, (ii) the limited number of species in this environment allows species to occupy niches from which they would normally be excluded, or (iii) the temporal and spatial variability of polar habitats is such that only highly vagile species able to colonize a variety of habitats can survive.

Nematodes have also been found in meltwaters and other habitats associated with Arctic ice, but as this is sea ice derived from salt water, the nematodes present are all typically marine (Portnova *et al.*, 2019).

Table 4.3. Freshwater aquatic nematode genus and number of species from polar regions. (From Hodda *et al*. (2006).)

Location	Habitat	Genus/species	Reference
King George Island	Water puddle	*Eudorylaimus* 5 spp.	Tsalolikhin (1989)
		Eumonhystera 1 sp.	Tsalolikhin (1989)
	Lake	*Rhabditis* s.l. 1 sp.	Tsalolikhin (1989)
Antarctica	Nunataks	*Panagrolaimus* 1 sp.	Swart and Harris (1996)
		Rotylenchus 1 sp.	Van Den Berg and Harris (1996)
		Plectus s.l. 1 sp.	Heyns (1995)
		Chiloplacoides 1 sp.	Heyns (1994)
		Eudorylaimus 1 sp.	Heyns (1993)
Adelaide Island	Pool	*Monhystera* 1 sp.	Dartnall (1980)
McMurdo	Stream	*Panagrolaimus* 1 sp.	Treonis *et al.* (1999)
		Eudorylaimus 1 sp.	Treonis *et al.* (1999)
		Plectus 1 sp.	Treonis *et al.* (1999)
	Small pool with algae	*Scottnema* 1 sp.[a]	Andrássy (1998)
		Plectus s.l. 1 sp.	Andrássy (1998)
	Mire	*Eudorylaimus* 1 sp.	Andrássy (1998)
McMurdo Dry Valleys	Lake	*Monhystera* s.l.	Timm (1971), Wharton and Brown (1989)
Bunger Hills	Deep lakes	*Plectus* s.l. 1 sp.	Andrássy (1998), Kirjanova (1958)
Alexander Island	Pond	*Mesodorylaimus* 1 sp.[b]	Maslen (1982)
Ross Island	Intermittent stream	*Panagrolaimus davidi*	Sinclair and Sjursen (2001)

[a]In a very extensive survey of the same area, Treonis *et al.* (1999) found this species in drier areas only, not in streams.
[b]Found in 45 of 50 samples (Maslen, 1982).

4.3.3 Permafrost

Nematodes occupy some of the most extreme habitats on Earth, but the survival of living and viable – although completely quiescent – nematodes for thousands of years in frozen permafrost is still astonishing. These nematodes (*Panagrolaimus* sp. aff. *detritophagus* (Rhabditida) and *Plectus* sp. aff. *parvus* (Plectida)) were isolated from samples of 30,000- and 42,000-year-old Pleistocene permafrost deposits of the Kolyma River lowland in the Siberian far east (Shatilovich *et al.*, 2018). Their burial deep in the permafrost made it unlikely that their presence was the result of contamination with modern organisms: nematodes generally do not burrow deep into the Siberian permafrost, as seasonal thawing only reaches a depth of about 1 m (Solly, 2018). Nonetheless, while the sampling procedures were designed to ensure complete sterility, the samples were located adjacent to burrows of hibernating squirrels such that contamination was a possibility.

Other records of nematodes from permafrost have been from closer to the surface, but still of impressive age. Thus, viable nematodes have been found close to the upper boundary of the permafrost with a material radiocarbon date between 2100 and 4500 years (Gubin *et al.*, 2016). The authors of that study hypothesized that the ecological conditions of some zones near the boundary between the permafrost and overlying soils promoted the preservation of a diverse microfauna, including protists and nematodes. They also proposed that a considerable part of the community (50% of nematodes) maintained its viability over the short term by entering a dormant state, whereas the viability of some of the nematodes and other microfauna in the upper layer of the permafrost was ensured over the long term through cryptobiosis.

4.4 Estuarine Sediments

For the purposes of this chapter, estuarine is defined as characterized by a salinity of >0.1%.

More than 30 species, either mostly from freshwaters or else with close taxonomic affinities to freshwater forms, have been recorded in estuaries (Table 4.4). Although this is a considerable number, it is small compared with the much larger number of typically estuarine or marine species present in estuarine habitats (Hodda *et al.*, 2009). Moreover, some freshwater species have been detected in estuaries only once, suggesting that they were washed accidentally downstream. As nematodes have been found suspended in irrigation waters and surface flows of rainwater (Cadet and Albergel, 1999; Cadet *et al.*, 2002; Hugo and Malan, 2010), their being washed from terrestrial soils or freshwater streams into estuaries may be reasonably common. Other species, however, have been observed frequently enough to be classed as occupants, or at least as occasional inhabitants and not just as accidental occurrences.

Table 4.4. Freshwater nematodes from estuaries (listed alphabetically by genus). (Author's own table.)

Place	Habitat	*Genus or species*	Trophic group	Reference
Europe	Sediment	*Aphelenchoides* sp.	F	Bongers and van der Haar (1990)
Thailand	Sublittoral	*Aphelenchoides* sp.	F	Timm and Franklin (1969)
Bangladesh	Coastal pool	*Aphelenchoides gynotylurus*	F	Timm and Franklin (1969)
Florida	Sea grass	*Aphelenchoides marinus*	F	Timm and Franklin (1969)
Vietnam	Mangrove	*Aquatides thornei*	O	Gagarin and Nguyen (2008)
Vietnam	Mangrove	*Brevitobrilus stefanskii*	M	Gagarin and Nguyen (2012)
Mexico	Salt marsh	*Cactodera salina*	P	Baldwin *et al*. (1997)
Australia	Mangroves	*Criconemella avicenniae*	P	Nicholas and Stewart (1984a)
Vietnam	Delta channel	*Dolichodorus heterocephalus*	P	Gagarin (2018)
Vietnam	Mangrove	*Dolichodorus orientalis*	P	Gagarin and Nguyen (2015)
Australia	Sublittoral	Dorylaimidae sp.	O	Nicholas *et al*. (1992)
Vietnam	Mangrove	*Dorylaimus stagnalis*	A	Gagarin and Nguyen (2012)
Australia	Mangroves	*Enchodelus* sp.	O	Nicholas and Stewart (1984a)
Vietnam	Mangrove	*Eucephalobus oxyuroides*	M	Gagarin (2018)
Vietnam	Mangrove	*Eudorylaimus productus*	O	Gagarin and Nguyen (2012)
Greenland	Sublittoral	*Eudorylaimus maritimus*	O	Ditlevsen (1913)
Australia	Sublittoral	*Eutobrilus heptapapillatus*	M	Nicholas *et al*. (1992)
Bangladesh	Sublittoral	*Filenchus marinus*	P	Timm (1956)
Europe	Sea weed	*Halenchus* sp.	A	De Man (1892), Davide (1980), Garrad (1978)
Europe	Sea weed	*Halenchus fusicola*	P	Siddiqi (2000)
Europe	Sea weed	*Halodorylaimus*	O	De Man (1892)
Vietnam	Delta channel	*Helicotylenchus crenacaudata*	P	Gagarin and Nguyen (2007)
Vietnam	Delta channel	*Helicotylenchus falcatus*	P	Gagarin and Nguyen (2007)
South Africa	Salt marsh	*Helicotylenchus* sp.	P	Furstenberg and de Wet (1983)
New Zealand	Salt marsh	*Heterodera litoralis*	P	Wouts and Sturhan (1996)
Netherlands	Salt marsh	*Heterodera spinicaudata*	P	Wouts *et al.* (1995)
Texas, USA	Sediment	*Hirschmaniella mexicana*	P	Sher (1968)
Florida, USA	Marine plant	*Hirschmaniella marina*	P	Sher (1968)

Continued

Table 4.4. Continued.

Place	Habitat	*Genus or species*	Trophic group	Reference
Australia	Mangroves	*Labronema* sp.	O	Hodda and Nicholas (1985)
Vietnam	Mangrove	*Laimydorus agilis*	O	Gagarin (2018)
Vietnam	Mangrove	*Laimydorus oxurus*	O	Gagarin and Nguyen (2005a,b, 2008)
Vietnam	Mangrove	*Lanzavecchia mangrovi*	O	Gagarin (2014)
Vietnam	Mangrove	*Longidorus* sp.	O	Gagarin and Nguyen (2008)
Brunei	Mangrove	*Meloidogyne mersa*	P	Siddiqi and Booth (1991)
Eastern USA	Salt marsh	*Meloidogyne spartinae*	P	Lamondia and Elmer (2007)
France	Salt marsh	*Meloidoderita salina*	P	Ashrafi *et al.* (2012)
Vietnam	Mangrove	*Mesodorylaimus hofmaenneri*	O	Gagarin (2018)
Vietnam	Mangrove	*Mesodorylaimus lutosus*	O	Gagarin and Nguyen (2005a,b), Gagarin (2018)
Iran	Mangrove	*Neodolichodorus persiangulfus*	P	Gharahkhani *et al*. (2019)
Vietnam	Delta channel	*Panagrolaimus rigidus*	M	Gagarin and Nguyen (2007)
Netherlands	Salt marsh	*Pratylenchoides maritimus*	P	Bor and S'Jacob (1966)
Vietnam	Mangrove	*Prodorylaimus andrassyi*	A	Gagarin (2013)
Canada	Sea weed	*Rhabditis littorea*	M	Sudhaus and Nimrich (1989)
Europe	Sublittoral	*Rhabditis marina* spp. complex	M	Derycke *et al*. (2008)
Vietnam	Mangrove	*Rhabdolaimus terrestris*	M	Gagarin (2018)
China	Beach	*Tobrilus* sp.	M	Wu *et al.* (2002)
Australia	Sublittoral	Tylenchida sp.	P	Nicholas *et al*. (1992)
Australia	Mangroves	*Tylenchus* sp.	P	Hodda and Nicholas (1985)
South Africa	Salt marsh	*Tylenchus* sp.	P	Furstenberg and de Wet (1983)

A = algal feeding; F = fungal feeding; M = microbial feeding; O = omnivorous; P = plant feeding.

Among the estuarine nematodes with freshwater affiliations, some are most abundant at the upper edge of the intertidal zone where the marine influence is often weak (Nicholas *et al*., 1992); others occur primarily in fully saline conditions, such as among mangroves or in estuarine sediments (Table 4.4), and still others, particularly those associated with plants, in salt marshes with at least periodic hypersaline conditions

(Furstenberg and de Wet, 1983; Wouts *et al.*, 1995; Wouts and Sturhan, 1996; Baldwin *et al.*, 1997).

Many (ca. 40%) of the normally freshwater or terrestrial nematode species in estuaries are associated with the flowering plants that have evolved adaptations to tidal flats bordering estuaries, such as mangroves and salt marsh herbs or grasses. These nematode species include plant-root feeders associated with vascular plants (e.g. species of *Dolichodorus*, *Helicotylenchus*, *Hirschmanniella*, *Heterodera*, *Meloidogyne*, *Neodolichodorus*, *Pratylenchoides*, and *Tylenchus*) (Table 4.4). The related genus *Halenchus* is the only tylenchid completely absent from freshwaters, as it feeds exclusively on sea weeds (Siddiqi, 2000). The effects of estuarine species on their hosts are mostly unknown. While other species from most of these genera cause severe damage, limited testing suggests that estuarine nematodes only occasionally adversely affect their host plants (Dormann and Van Der Wal, 2001).

Other large groups of nematodes with freshwater affinities found in estuaries are omnivores (ca. 30%, especially Dorylaimida) or microbivores/omnivores (ca. 20%). The latter include Tobrilidae, a family very typical of freshwater sediments. At least one estuarine species of Tobrilidae, *Eutobrilus heptapapillatus*, is also widespread in the rivers and lakes upstream of the respective estuary (Nicholas *et al.*, 1992; Hodda and Nobbs, 2008) and has also been found in salt lakes of the same river system (Hodda and Nobbs, 2008). This distribution suggests an ability of some freshwater nematode species to tolerate a wide range of conditions and thus to invade estuarine sediment.

Dorylaimida too are very diverse in most terrestrial and freshwater aquatic habitats, indicative of their ability to tolerate a wide range of conditions (Jairajpuri and Ahmad, 1992). Likewise, the normally freshwater and terrestrial genus *Aphelenchoides* is very diverse ecologically, including plant-parasitic, entomophilic, and mycetophagous species and thus is likely to have sufficient plasticity to colonize estuarine environments as well.

4.5 Seasonal and Temporary Freshwaters

The faunas of seasonal and temporary freshwaters have been studied in Africa, Europe, North America, and Australia. Their composition is distinct from that of the fauna of most other freshwaters, consisting of a mixture of terrestrial, aquatic, and specialized groups (Table 4.5). The apparent predominance of different groups in different regions may be related to the differing patterns of inundation.

Until recently, investigations of the influence of inundation pattern were limited and emphasized different aspects of the nematode fauna, such that the data were not directly comparable. However, in a recent study, Majdi *et al.* (2020) directly compared the nematode faunas from 15 intermittent streams (IS) with those of nearby permanent streams (PS)

Table 4.5. Nematode genera from seasonal or intermittent freshwater aquatic habitats. (Author's own table.)

Genus	Sahara 1	Sahara 2	Australia LI	Australia MI	Spain IS	Spain PS
Acrobeloides					x	
Acrobelophis						x
Actinolaimus			x			
Achromadora	x		x		x	x
Afrodorylaimus			x	x		
Anaplectus					x	
Alaimus		x			x	x
Aphanolaimus					x	x
Aphelenchoides					x	x
Aphelenchus					x	x
Aporcelaimellus			x	x	x	x
Bastiania					x	
Bursilla					x	x
Cephalobus					x	x
Chromadorita					x	x
Coslenchus					x	x
Crassolabium					x	
Cylindrolaimus					x	x
Diplogaster					x	
Diplolaimella	x					
Diplolaimelloides	x					
Diploscapter					x	x
Ditylenchus					x	x
Dorylaimus	x		x		x	x
Ethmolaimus					x	x
Eucephalobus					x	
Epidorylaimus					x	x
Epitobrilus					x	x
Eudorylaimus					x	x
Eumonhystera			x		x	x
Euteratocephalobus					x	
Fictor					x	x
Filenchus					x	x
Goffartia					x	
Helicotylenchus					x	
Hemicycliophora					x	
Heterocephalobus					x	
Ironus		x			x	
Laimydorus		x	x			
Longidorus					x	
Malenchus					x	x
Mesodorylaimus			x	x	x	x
Monhystera		x			x	
Monhystrella	x				x	x
Mononchoides					x	

Continued

Table 4.5. Continued.

Genus	Sahara 1	Sahara 2	Australia LI	Australia MI	Spain IS	Spain PS
Mononchus	x		x		x	x
Mylonchulus					x	
Neoactinolaimus			x			
Odontolaimus					x	
Oncholaimus	x					
Panagrolaimus					x	
Paractinolaimus			x		x	x
Paracyatholaimus	x					
Paramphidelus					x	
Paraphelenchus					x	
Plectus	x		x	x	x	x
Prismatolaimus					x	x
Prodesmodora					x	
Prodorylaimium					x	x
Protorhabditis					x	
Psilenchus					x	
Rhabditis s.l.				x	x	
Rhabdolaimus					x	x
Semitobrilus					x	x
Teratocephalobus					x	
Theristus					x	
Thornia					x	x
Tobrilus	x				x	x
Tripyla					x	x
Trischistoma					x	x
Tylencholaimellus					x	
Tylencholaimus					x	
Tylenchorhynchus				x	x	
Tylenchus					x	
Tylocephalobus					x	
Udonchus					x	x
Xiphinema					x	

Sahara 1: Grootaert (1976); Sahara 2: Goossens (1976); south-east Australia, less intermittent (LI) and more intermittent (MI): Hodda (1999); Spain, intermittent (IS) and permanent streams (PS): Majdi *et al*. (2020). X = genus found in study listed.

in Spain (Table 4.5). They found nearly twice as many species in IS than in PS (108 vs. 58), although 83 of the species in IS were rare (<1% of the fauna), and just three species accounted for >25% of the total nematode abundance (*Eumonhystera vulgaris* 11.6%, *Monhystrella paramacrura* 10.0%, and *Filenchus vulgaris* 4.5%). In PS, there were fewer rare species, but the most abundant three species accounted for >35% of total nematode abundance (*Monhystrella paramacrura* 15.1%, *Eumonhystera vulgaris* 11.6%, and *Rhabdolaimus aquaticus* 10.7%). Few species were found in PS only (five species including the algivore *Chromadorita leuckarti*,

or 9% of the total), and most of the species in PS also occurred in IS (91%). By contrast, half of the fauna of IS (55 species, 51% of the total) did not occur in PS. Thus, the fauna of both types of streams seemed to consist of a core of 'freshwater' taxa, supplemented in IS by low numbers of taxa from outside (two of the three most abundant species in both habitats were the same, typical freshwater inhabitants). Ecologically, differences in trophic composition related to the consistency of the stream flow were observed, with relatively more bacterivores (76.8% of the total number of individuals), fewer fungivores and omnivores, and low percentages of algivores and predators in PS. Abundance also differed with inundation and was higher in IS (1421 ± 315 individuals per 10 cm^2) than in PS (Majdi *et al.*, 2020).

In the Sahara, nematodes have been sampled in many small freshwater bodies (Goossens, 1976; Grootaert, 1976), many of which were highly intermittent or seasonal, with periods of little or no water alternating with periods of inundation. Nematode abundance data were limited in both of the cited studies, but despite their small size the sampled water bodies contained a surprisingly large number of species (up to 23). Unlike the faunas of the Spanish streams, most of the species were classified as typically freshwater and cosmopolitan (Goossens, 1976; Grootaert, 1976). A few species were distinctively Ethiopian in their distribution (Goossens, 1976; Coomans and Jacobs, 1983).

Data on the species composition of nematodes in seasonal/temporary freshwaters in North America are rare, but abundance data are available, not only from different localities but also covering both dry and wet periods (Boulton and Stanley, 1995; Leeper and Taylor, 1998). In studies conducted at two different locations, in Arizona and North Carolina, total nematode abundance showed a small initial increase upon wetting of the site, but then increased further as the temporary surface water dried (Boulton and Stanley, 1995; Leeper and Taylor, 1998). The fauna in these intermittent waters may have been largely ecological opportunists rather than specialized aquatic species, but detailed species lists were not compiled.

In Australia, nematode abundance and species composition have been studied in several intermittent lakes and pools of various sizes but data on the timing of the occurrences were not included (Hodda and Nobbs, 2008; Hodda *et al.*, 2021). In the most frequently filled lakes, the generic composition of the fauna was similar to that reported in the Saharan studies (Table 4.5), whereas in the least frequently filled lakes, many of the species were the same as those in the surrounding arid areas (Table 4.5). Nematode abundance was high, but the nematodes that were present may have been active only during wet periods. In all of the intermittent lakes studied, most species seemed to be common and widespread.

By contrast, in isolated, but relatively constant freshwaters in the Australian desert, the number of species is much smaller, but those that are present represent typical freshwater genera and are often endemic (Nicholas and Hodda, 2000). Endemic species have also been reported from oases in the Sahara (Coomans and Heyns, 1983).

4.6 Bromeliads and Tree Hollows

Tank bromeliads are a major structural component in neotropical forests (e.g. Benzing, 2000) and the biota inhabiting these natural microcosms provide an attractive model system in ecological studies (e.g. Srivastava *et al.*, 2004; Dezerald *et al.*, 2014). Bromeliads are epiphytic monocotyledonous plants with large upright leaves and are especially common in Central and South American rainforests (Fig. 4.3). The water retained in the leaf axils (up to 8 l) supports a distinctive fauna and flora. Most studies have focused on insects and other arthropods, but protists, crustaceans, other invertebrate groups, and algae have been investigated as well. Information on nematodes in bromeliad tanks is, by contrast, very sparse. Zullini (1977) and Zullini *et al.* (2002) identified nematode species in bromeliads from Costa Rica and southern Mexico, and Jacobs (1984) presented a list of nematode species associated with plants in Africa. The nematode genera found in the waters retained by bromeliads in Africa include genera typical of other freshwaters (*Tobrilus* s.l. and *Trischistoma*), species often found in freshwaters but not confined to them (*Mesodorylaimus* and other Dorylaimida, *Mononchus* and other Mononchida, *Plectus*, *Chronogaster*, and *Ironus*), terrestrial genera associated with plants or fungi (*Aphelenchoides*, *Aphelenchus*, and *Helicotylenchus*), and genera probably opportunistic in their ecological characteristics (*Caenorhabditis*, *Prismatolaimus*, *Plectus*, and *Cephalobus*). Most of the nematodes detected by Jacobs (1984) were from microbivorous or omnivorous genera.

Fig. 4.3. *Werauhia sanguinolenta* on Barro Colorado Island, Republic of Panama. (Photo: Gerhard Zotz; University Oldenburg.)

The meiofauna of 54 epiphytic tank bromeliads were investigated during the wet and dry season in the lowlands of Panama, with nematodes examined in detail (Zotz and Traunspurger 2016; Table 4.6). In terms of individuals, rotifers were by far the most abundant group in both the dry and the wet season (up to 960 individuals per milliliter), followed by nematodes, annelids, and harpacticoid copepods. Individual plants hosted up to 25 nematode species and 89 morphospecies of nematodes were identified in total.

In terms of ubiquity, only rotifers, nematodes, and mites were found in all samples irrespective of the season (Zotz and Traunspurger, 2016). These nematodes represented a diversity of feeding guilds, with suction feeders (about 38%) and deposit feeders (27–40%) as the most abundant. Both species richness and abundance correlated strongly with the size of the phytotelmata and the season. More species were found in the wet season (67) than in the dry season (43). Of the most abundant species, only two were abundant in both seasons: *Prismatolaimus* cf. *intermedius* (14.8% of the total nematode population in the wet season and 8.6% in the dry season), and *Aphelenchoides* sp. (9.3% and 9.8%, respectively; Table 4.6).

4.7 Stemflow

Stemflow, that is, the portion of rainwater falling on the leaves or branches of trees which then drains down the stem, connects the vegetative canopy with the soil. This hydrological process has an important impact on the biogeochemical cycles of forest ecosystems.

Ptatscheck *et al.* (2018a) showed that stemflow contains nematodes and other metazoans. Over 8 weeks, the authors collected the stemflow from three species of trees (*Fagus sylvatica*, *Carpinus betulus*, and *Quercus robur*) beginning in spring, when the trees were leafless. A mean of ca. 160 individuals/l stemflow was found for the three species, but there was considerable variation between them. Nematodes, rotifers (bdelloidea), tardigrades, mites, and collembolans were the most common, with rotifers and nematodes present in nearly all samples from *F. sylvatica* and *C. betulus* (94.1–100%), but much less frequently in samples from *Q. robur* (27% and 63% of samples for nematodes and rotifers, respectively), in which mites and collembolans were dominant.

All 15 species of nematodes identified were colonizers of the soil and trees of forest ecosystems (Fig. 4.4). The two predominant nematodes (*Chiloplectus andrassyi* and *Laimaphelenchus penardi*) were also very abundant in epiphytic moss from the same sampling site (Schenk *et al.*, 2016), in water-filled tree holes from other locations (Ptatscheck *et al.*, 2015), and in aeroplankton collected at the same site (Ptatscheck *et al.*, 2018b). These findings provide important insights into how nematodes enter tree habitats (see also Chapter 5). The most frequent nematode feeding types were bacterial feeders and hyphal feeders. The root-hair feeders and large

Table 4.6. Compilation of the nematode genera found in the tanks of four bromeliads species growing in the lowlands of Panama during wet and dry seasons. (Modified from Zotz and Traunspurger (2016) with permission of *BMC Ecology*.)

	FT	Wet season (%)	Dry season (%)
Achromadora	EF	0.4	0.2
Alaimus	DF	0.2	
Aphelenchoides	S-P/R	10.5	9.8
Aphelenchus	S-P/R	0.1	
Aporcelaimellus	S-Omn	0.2	
Butlerius	DF	0.1	
Cephalobus	DF	1.8	
Ceratoplectus	DF	0.1	
Chiloplectus	DF	0.1	
Chromadorina	EF	0.1	
Clarkus	Ch		
Diplogaster	Ch	0.1	
Diplogasteridae	Ch		9.1
Diploscapter	DF	0.1	5.8
Ditylenchus	S-P/R	3.7	11.7
Dolichorhabditis	DF	0.1	
Ereptonema	DF	0.1	
Ethmolaimus	EF		0.2
Eudorylaimus	S-Omn	1.7	1
Eumonhystera	DF	0.3	1.1
Geomonhystera	DF	2.5	5.2
Heterocephalobus	DF	3	7.3
Laimaphelenchus	S-P/R	0.1	
Malenchus	S-P/R	0.1	
Mesodorylaimus	S-Omn	0.3	2.3
Mesorhabditis	DF		0.7
Monhystrella	DF		0.2
Mononchoides	Ch		0.2
Mylonchulus	Ch	5.4	1.2
Neoactinolaimus	S-Omn		0.3
Panagrolaimus	DF	0.6	3.3
Paraphelenchus	S-P/R	0.8	
Plectus	DF	6.6	0.9
Prismatolaimus	EF	15.6	8.6
Prodesmodora	EF	3.4	1.9
Rhabdolaimus	DF	0.2	
Rhabditis	DF	0.3	
Protorhabditis	DF	3.6	
Rhabditidae	DF	0.9	11.5
Teratocephalus	DF	3.2	0.7
Theristus	DF		0.3
Thornia	S-Omn	0.7	0.5
Tripyla	Ch		1
Tripylella	Ch	9.4	
Tripylina	Ch	0.8	

Continued

Table 4.6. Continued.

	FT	Wet season (%)	Dry season (%)
Trischistoma	Ch		
Tylencholaimellus	S-P/R	14.2	
Tylencholaimus	S-P/R		3
Tylocephalus	DF	2.7	2.8
Westindicus	S-Omn	5.1	
Wilsonema	DF	0.1	
Tylenchus	S-P/R	0.2	8.9
Seven unidentified species	DF	0.5	0.3

Feeding types (FT): DF = deposit feeders; EF = epistrate feeders; S-P/R = suction feeder – plant/roots; S-Omn = suction feeder – omnivore; Ch = chewers.

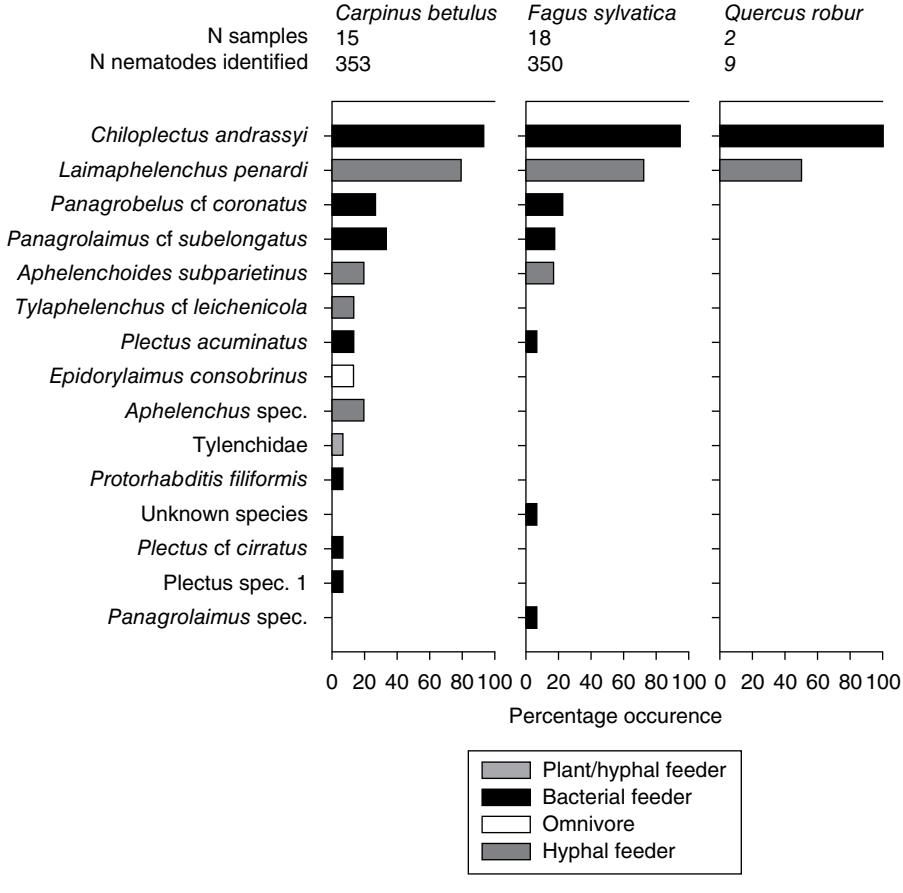

Fig. 4.4. Percentage occurrence of the nematode taxa identified in the stemflows of *C. betulus, F. sylvatica*, and *Q. robur.* The nematodes are listed from the overall most common to the rarest species and according to feeding type. (From Ptatscheck *et al*. (2018a) with permission of *BMC Ecology*.)

predacious and omnivorous species that are frequently detected in soil and moss were not present in the stemflow.

Overall, these results suggest that the individuals collected from the stemflow had been flushed from other parts of the trees and consisted of species able to survive in soil systems as well. In addition, many small juvenile nematodes were found in the stemflow, perhaps because their small size and weight allowed them to be more easily washed free of the stem.

4.8 Groundwaters

Groundwater nematodes have usually been studied in association with caves or flowing waters, but they have also been found in other subterranean waters, such as mines, boreholes, and aquifers.

4.8.1 Caves

Caves and cave-dwelling biota have fascinated scientists for centuries. While the ecological limits of a cave ecosystem have been defined (Moseley, 2009), the habitats and biota associated with caves remain poorly studied. Cave-dwelling (cavernicolous) organisms include those inhabiting the water and sediment of subterranean pools, streams, and lakes, as well as soil and bat guano deposits. The freshwaters of caves share some of the habitat characteristics of springs, such as a very stable temperature, but with their highly variable flow rates they also resemble seasonal or intermittent freshwaters. Some caves, like some springs, are chemo-autotrophic, with the ultimate energy source being chemical rather than electromagnetic radiation from the sun.

As with many other unusual freshwater habitats, the fauna in caves consists of species restricted to this or very similar habitats but also of accidental occupants and species with wide environmental tolerances. In the case of nematodes, it is difficult to differentiate between accidental (temporary) and true (permanent and obligatory) cave-dwelling species since many nematodes have attributes similar to those of cave-adapted species (Poinar and Sarbu, 1994). However, most nematodes in caves are probably accidental inhabitants (Andrássy, 1965; Cayrol, 1973; Poinar and Sarbu, 1994; Hodda *et al.*, 2006).

Du Preez *et al.* (2017) conducted an in-depth literature review of all reports related to cave-dwelling nematodes, to provide a sound basis for future studies. A survey of 78 different cave systems in 18 countries reported in 41 scientific works over the past 138 years revealed 295 unique nematode taxa, a substantial increase over the numbers reported in two studies (Poinar and Sarbu 1994; Hodda *et al.*, 2006) conducted prior to that of Du Preez *et al.* (2017). Accounting for most of the difference was the >136 different taxa added during the past 10 years as a result of studies in 24 different Spanish and southern African caves.

Of the 295 nematode taxa known from caves, only seven species appear to be true cave-dwellers (Stammer, 1935; Schneider, 1940; Andrássy, 1965; Poinar and Sarbu, 1994; Du Preez *et al.* 2017). Some of these species are truly remarkable because the evidence suggests that they are relict marine organisms from the Tertiary period, with no aquatic or terrestrial relatives (Andrássy, 1965). This implies their persistence in a cave environment for millions of years. Three of these species, *Desmoscolex aquaedulcis* Stammer 1935, *Thalassoalaimus aquaedulcis* Schneider 1940, and *Halalaimus stammeri* Schneider 1940, were discovered in Slovenia some time ago (Stammer, 1935; Schneider, 1940). Two others are from typical freshwater taxa and are characterized by their frequent occurrence as well as their large numbers: *Stenonchulus troglodytes* Schneider 1940 (found in Laibach Jama in Slovenia by Schneider (1940) and again later in Eggerloch in Austria by Eder (1975)) and *Mylonchulus cavensis* (found in Baradla-Barlang Cave in Hungary by Andrássy (1959)). The sixth species, *Hemicycliophora aquatica* (Micoletzky 1913) Loos 1948, belongs to a typically terrestrial plant-feeding genus and was found in both Gallerie d'Ans-lez-liège and Grotte de Chaudfontaine (Belgium) (Leruth, 1939). The seventh species, *Chronogaster troglodytes* Poinar and Sarbu 1994, has been isolated from the floating mats of stratified fungal mycelia and sulfide-oxidizing bacteria in Movile Cave (Romania) many times over multiple years and studies. The species is uniquely adapted to the unusual conditions associated with its cave habitat (Poinar and Sarbu, 1994; Sarbu *et al.*, 1996; Riess *et al.*, 1999; Muschiol and Traunspurger, 2007; Muschiol *et al.*, 2015).

The remainder of the 295 species identified thus far in caves comprise a mixture of all trophic types, but with more bacteriovores, omnivores, predators, and plant parasites than fungivores, eukaryote feeders, and entomophilics (Du Preez *et al.*, 2017). They include species known from all major habitats (terrestrial, freshwater, and in a few cases marine habitats), those from genera associated with these habitats, and species with wide environmental tolerances.

4.8.2 Other subterranean freshwater environments

Nematodes are also found in other subterranean environments, both natural and man-made, associated with terrestrial and aquatic (freshwater/marine) habitats (Culver and Pipan, 2009; Borgonie *et al.*, 2011; Bonaglia *et al.*, 2014). The latter include aquifers, which present a vast subterranean landscape that can host a highly diverse biota, the importance of which has only recently been recognized (Eisendle-Flöckner and Hilberg, 2015). Only small species and small developmental stages are observed in the hyporheic fractions of porous aquifers (Feral *et al.*, 2005; Kawanishi *et al.*, 2013), but, in general, nematodes, along with arthropods and annelids, are common (e.g. Meleg *et al.*, 2012; Gutjahr *et al.*, 2013). Nonetheless, nematodes have been largely neglected in groundwater research (Andrássy, 1978; Boulton *et al.*, 2004; Hahn and Matzke, 2005), with most studies of aquifers reporting only total group abundances and lacking detailed observations at finer taxonomic levels (Eisendle-Flöckner and Hilberg, 2015).

Boreholes and wells provide access to groundwater, but the biotic and abiotic components associated with these man-made structures can be substantially different than those of the aquifer they draw from (Sorensen *et al.*, 2013). In the few reports of nematodes from boreholes, only microbivorous *Plectus aquatilis* Andrássy 1985 and a monhysterid species were noted (Andrássy, 1985; Borgonie *et al.*, 2011). If a well or borehole is not cased or the surface opening is not sealed, runoff water carrying substrates (soil, sediment, detritus) can contaminate a groundwater system with a diversity of nematode taxa; however, even under these circumstances water from boreholes and wells may be free of plant-parasitic nematodes (Hugo and Malan, 2010).

More nematode species have been described from underground mines (Hnatewytsch, 1929; Altherr, 1938; Borgonie *et al.*, 2011). The 33 species reported in the literature include many that were previously unknown and their discovery followed very extensive sampling (Hnatewytsch, 1929; Altherr, 1938). The dominant species were bacterial feeding: *Plectus cirratus* Bastian 1865, *Colporhabditis* (*Teratorhabditis*) *coronigera* (Altherr 1938) Andrássy 1976, and *Cuticularia oxycerca* (de Man 1895) Andrássy 1983 (Hnatewytsch, 1929; Altherr, 1938). The latter were particularly abundant in biofilms consisting of filamentous bacteria growing in sulfur-rich thermo-mineral waters similar to those inhabited by *Chronogaster troglodytes* in Movile Cave (see Section 4.8.1). In deep galleries without a trace of water infiltration, very few nematode species have been found (Altherr, 1938), but even in very deep, very old borehole water taken from mine shafts in South Africa there has been evidence of nematodes (unidentified monhysterids detected using DNA; Borgonie *et al.*, 2011).

With their detection at 3600 m below the Earth's surface, nematodes from deep mine shafts are the deepest-living multicellular organism ever recorded. However, this is not the only nematode species discovered from waters deep in the Earth. A new species, *Halicephalobus mephisto* Borgonie, García-Moyano, Litthauer, Bert, Bester, van Heerden, Möller, Erasmus and Onstott 2011, was discovered at a depth of 1300 m below the surface, and *Plectus aquatilis* Andrássy 1985 was found nearly 1000 m below the surface (Borgonie *et al.*, 2011). Temperatures at these depths can be very high (48°C for the 3600 m depth and 37°C for the 1300 m depth, respectively), implying the ability of these species to tolerate very high temperatures. A mixture of aerobic and anaerobic bacteria from palaeometeoric water probably served as a food source for these nematodes (Borgonie *et al.*, 2011).

Sampling of stalactites, consisting primarily of $CaCO_3$, growing on the ceilings of mine tunnels at a depth of 1.4 km beneath the surface contained around 10 living nematodes in each of two samples (Borgonie *et al.*, 2015). They were identified morphologically as *Monhystrella parvella* (Filipjev 1931) Jacobs 1987, previously found only in brackish and marine waters in Namibia, Ethiopia, and Bulgaria (Filipjev, 1931; Jacobs, 1987). The nematodes were 96–100% genetically similar to the monhysterids collected in other South African mines, but only 91% similar to the

closest surface nematode for which sequence information was available (the marine nematode *Diplolaimelloides* sp.). Sequences of *M. parvella* from the surface were not available for comparison. Tests of survival in culture media differing in their ionic concentrations showed that the nematodes recovered from the mine stalactites could survive only in salty water ionically similar to that in the mine, thus showing that the nematode was truly adapted to the subterranean waters it inhabited.

The nematode-containing stalactites consisted of lamellae carrying bacterially precipitated mineral structures and arranged in a complex pattern. The nematodes were detected between the layers along with their presumed bacterial food (Borgonie *et al.*, 2015). In other words, the nematodes and bacteria clearly lived inside the stalactites and not only in the central straw. Furthermore, both survived long after the water that originally formed the stalactite had stopped flowing.

4.9 Marine Nematodes in Freshwater Environments

Just as a few species from 'freshwater' taxa have been found in estuarine or marine habitats (see Section 4.4), a few nematodes from 'marine' taxa have been found in freshwaters (Table 4.7). These include genera with several species often found in freshwaters but also those with just one freshwater species. There are also many single records of marine nematodes in freshwaters, but these undoubtedly represent accidental occurrences.

The genus *Oncholaimus* (Oncholaimida) is abundant and diverse in marine and estuarine sediments, but has also evolved several species in freshwater habitats (Table 4.7). Few other freshwater forms belong to this order or to the respective family. The number of freshwater species of *Oncholaimus* suggests a genuine trend for species in this genus to move into freshwater habitats, perhaps related to the morphological and ecological similarities between *Oncholaimus* and the common freshwater nematodes of the Order Mononchida. Both are large omnivorous predators with a barrel-shaped buccal cavity armed with teeth. The ecological niche or niches occupied by these taxa may allow survival in either environment. Another genus in the overwhelmingly marine Oncholaimida to have moved into freshwaters is the enigmatic *Thalassogenus* Andrássy 1972. This genus is definitely related to marine Oncholaimida, but all five known species occur exclusively in freshwaters (Andrássy, 1972; Loof and Zullini, 2000).

Species of the genera *Cylindrotheristus*, *Mesotheristus*, and *Penzancia* (all of which were originally regarded as subgenera of the very large genus *Theristus* in the Family Xyalidae) are very abundant and diverse in marine and estuarine sediments, but there are also several freshwater species (Table 4.7). While in some cases their records probably represent accidental occurrences, in others the number of records and species involved suggests either an ecological type or evolutionary lineage that was particularly able to transfer between marine and freshwater biotopes. The species

Table 4.7. Numbers of nematode species found in freshwaters but from genera common in marine habitats. (Author's own table.)

Genus	Only in freshwater	In both marine and freshwaters	Total no. of species known[a]	Freshwater habitat[b]	References[b,c]	Survived transition to freshwater over 1 year[d]
Adoncholaimus	1	2	23	Cave, Yugoslavia	Andrássy (1973)	
				Lake, Vietnam	Gagarin and Nguyen (2005a,b)	
				Lake, Australia	Hose *et al.* (2008)	
				River delta, Vietnam	Nguyen *et al.* (2012)	
Anoplostoma	1	2	19	Various	Gerlach and Riemann (1974)	
Axonolaimus[e]	1		41	C, Yugoslavia	Andrássy (1973)	
Caribplectus[e]		1	2	Lake, Colombia	Riemann (1975)	
Chromadorina	2	1	32	Cave, Cuba	Andrássy (1973)	
				River, Hungary	Andrássy (1962)	
Chromadorita		2	35	Cave, Cuba	Andrássy (1973)	
				Spring, Spain	Ocaña (1990)	
Cyatholaimus[e]		1	26	Reclaimed river, China	Wu *et al.* (2002)	Yes
Cyatolaimium[e]	1	0	3	Lake, Colombia	Riemann (1975)	
Daptonema	2	12	139	Spring, Spain	Ocaña (1990)	
				Various	Tsalolikhin (2017)	
Desmolaimus[e]	1		14	Cave, Cuba	Andrássy (1973)	Yes
Desmoscolex s.l.	1	5	99	Cave, Slovenia	Stammer (1935)	
				Soil, Guatemala	Thames (1966)	
Dichromadora[e]	1		34	Various streams, rivers, and lakes	Hodda (1999)	
Diplolaimella[e]	2		12	Lake, Colombia	Riemann (1975)	
				Rivers, Australia	Hodda (1999)	
				Pools, Sahara	Grootaert (1976)	
Diplolaimelloides[e]		2	10	Reclaimed river, China	Wu *et al.* (2002)	Yes
				Pools, Sahara	Grootaert (1976)	
Enoploides	1		8	Streams, sub-Antarctic	Hodda and Keith (unpub)	

Ethmolaimus	1	2	16	Springs, Europe	Zullini *et al.* (2011), Ocaña (1991a), Seiml-Buchinger and Traunspurger (2006)	
				Streams, Europe	Zotz and Traunspurger (2016)	
				Bromeliads, Panama	Majdi *et al.* (2020)	
Halalaimus	3		76	Cave, Yugoslavia	Andrássy (1973)	
				Oasis, Sahara	Coomans and Jacobs (1983)	
				Lake, Siberia	Alekseev and Linnik (1994)	
Haliplectus[e]	1		25	Caves, Cuba	Andrássy (1973)	
Mesacanthion	2	1	48	Lake, Colombia	Riemann (1975)	
				Lake, Australia	Nicholas (1993)	
Microlaimus	2	1	83	Cave, Yugoslavia	Andrássy (1973)	
				River, Italy	Meyl (1954b)	
				Lake, Hungary	Biro (1972)	
				Hot spring, USA	Hoeppli (1926)	
Neochromadora		2	32	Reclaimed river, China	Wu *et al.* (2002)	Yes*
				Lake, Hungary	Biro (1968)	
Oncholaimus	6	2	121	Waterfall, Java	Schneider (1937)	Yes[f]
				Moss, sub-Antarctic Campbell Island	Allgen (1929)	
				Insect, Holland	Fuchs (1937)	
				Lake, Colombia	Riemann (1975)	
				Crater lakes, New Guinea	Nicholas and Stewart (1984b)	
				Oasis, Sahara	Coomans and Heyns (1983)	
				Stream, South Africa	Heyns and Coomans (1977), Coomans and Heyns (1986)	
				Stream, Surinam	Loof (1973)	
Oncholaimellus[e]		1	10	Reclaimed river, China	Wu *et al.* (2002)	Yes
				River, Sahara	Grootaert (1976)	
				Springs, Spain	Ocaña (1991a)	
Paracyatholaimus	3		30	Lake, Colombia	Riemann (1975)	
				Pool, Solomon Is.	Coomans *et al.* (1985)	

Continued

Table 4.7. Continued.

Genus	Only in freshwater	In both marine and freshwaters	Total no. of species known[a]	Freshwater habitat[b]	References[b,c]	Survived transition to freshwater over 1 year[d]
Parodontophora[e]	1		11	River, China	Wu *et al.* (2000)	Yes
Polygastrophora		2[g]	15	Reclaimed river, China	Wu *et al.* (2002)	Yes
				Lake, Nicaragua	Meyl (1957)	
Prodesmodora	3	1	10	Springs, Europe	Zullini *et al.* (2011), Ocaña (1991a), Seiml-Buchinger and Traunspurger (2006)	
				Streams, Europe	Colomba and Vinciguerra (1980), Traunspurger *et al.* (2015), Majdi *et al.* (2020)	
				Lakes, Europe	Ristau and Traunspurger (2011), Witthoeft-Muehlmann *et al.* (2007)	
				Bromeliads, Panama	Zotz and Traunspurger (2016)	
Setoplectus[e]		1	4	Lake, Colombia	Riemann (1975)	
Terschellingia		1[g]	29	Cave, Cuba	Andrássy (1973)	
Theristus s.l.[h]	6	4[j]	110	Lake, Colombia	Riemann (1975)	Yes
				River, Europe	Schiemer (1984)	
				Lake, Mongolia	Tsalolikhin (1985)	
				Stream, Namibia	Heyns and Coomans (1989)	
				Springs, Europe	Zullini *et al.* (2011)	
				Bromeliads, Panama	Zotz and Traunspurger (2016)	
Thalassoalaimus	1		24	Cave, Yugoslavia	Andrássy (1973)	
Thalassogenus	5		5	Cave, New Guinea	Andrássy (1972)	
				Stream, Costa Rica	Loof and Zullini (2000)	
				Lake, Equador	Vinciguerra and Orselli (2004)	
				Taro pond, Solomon Island.	Orton-Williams and Jairajpuri (1984)	
				Stream, India	Ahmad *et al.* (1992)	

Tripyloides	2	3	12	Lake, Colombia	Riemann (1975)	Yes
Viscosia	1	1[fg]	78	Stream, Austria	Micoletzky (1925)	
				Lake, Nicaragua	Gerlach (1957)	Yes
				Pool, Solomon Island	Coomans *et al.* (1985)	

[a]Hodda (2021a).
[b]Only freshwater habitats listed, brackish and marine not listed.
[c]Only references for freshwater habitats listed, brackish and marine not listed.
[d]Wu *et al*. (2002).
[e]Not listed as occurring in freshwater by Eisendle-Flöckner *et al*. (2017).
[f]Estuarine, sometimes found in its freshwater portions.
[g]Possible accidental occurrence.
[h]Includes former subgenera now regarded as valid genera (*Cylindrotheristus*, *Mesotheristus* and *Penzancia*), but excluding *Daptonema*, which is listed separately because of a large number of freshwater species.
[j]Two species in estuarine or freshwater, three in completely marine or freshwater.
*survived transition to freshwater soybean field over 7 years.

of Xyalidae are overwhelmingly marine, but the Order Monhysterida contains many freshwater and marine taxa. Xyalidae seem particularly adept at crossing the marine/freshwater boundary. This can be attributed to the physiological, ecological, and evolutionary characteristics of the lineage, which include an ability to feed on bacteria, algae, or organic detritus (selective deposit feeders).

A third 'marine' genus that seems to have genuine freshwater species is *Paracyatholaimus* (Chromadorida: Cyatholaimidae) (Table 4.7). As there are a few closely related genera that are entirely freshwater or terrestrial (e.g. *Achromadora*), this genus may represent a third evolutionary crossing of the marine/freshwater boundary.

Many of the other marine taxa found in freshwaters are estuarine and may have been washed into freshwaters accidentally. Since such accidents have occurred frequently enough to have been observed, it is perhaps significant that few evolutionary lineages other than the three noted above have apparently radiated in freshwater environments. The discussion in the proceeding paragraphs on freshwater nematodes in marine environments illustrates a continual interchange of species between the two environments. The involved species are of evolutionary and ecological interest, as is the fact that the flow of species from marine to freshwater environments and vice versa seems to be of similar magnitude. Marine nematodes are much more diverse at higher taxonomic levels than freshwater or terrestrial nematodes and represent the biotope in which nematodes originally evolved. On this basis a greater flow of taxa from marine to freshwater may be expected over evolutionary time.

Over short, ecological timescales, distinct changes in nematode faunas have been observed in habitats transformed from marine to freshwater, as occurs, for example, when land is reclaimed from the sea (Wu *et al.*, 2002). In such cases, most of the taxa transitioned from 'marine' to 'freshwater' or 'terrestrial' within a few years, as the land lost all marine influence (Table 4.7). During the transition, within about 1 year after the dykes were built, some of the 'marine' fauna were still present but only one marine species, from the genus *Neochromadora*, seemed to have survived the transition to a fully freshwater environment. Like *Paracyatholaimus*, this genus is in the Order Chromadorida, but the two genera are not closely related (different families) (Lorenzen, 1994; Hodda, 2021a).

Ecologically, in the above-cited study the reclamation of estuarine land involved a shift from a fauna dominated by bacterial feeders and predators to one dominated by plant feeders and omnivores (Wu *et al.*, 2002). There was little change in the proportion of nematodes thought to be ecological opportunists versus those with a preference for stable conditions.

4.10 Inland Hypersaline Waters

Hypersaline waters (salinity >40 g/l) not in contact with the open sea may contain nematode species with a wide range of habitat affinities,

including freshwater. While few nematodes are adapted to these extreme habitats, the scattered detections lend weight to the 'nematologist's creed', first enunciated by Cobb (1914): that nematodes occur in every conceivable habitat where free water occurs. In the following, habitats characterized by a rapid increase in salinity or a much more gradual increase are considered.

Of the nematodes from inland saline waters, some are freshwater nematodes that have evolved adaptations allowing survival in these conditions, but others are marine species. The long-term changes in the nematode fauna in Sivash Bay (Sea of Azov), Russia, the largest hypersaline lagoon worldwide, were investigated by Sergeeva *et al.* (2019). A political decision to stop supplying water from the River Dnieper to the canal was made in 2014, and the discharge of freshwater into the lagoon accordingly ended, resulting in an increase in its salinity from less than 40 g/l (1979 and 2013) and up to 60–75 g/l (2015). Benthic samples were taken in 1979, 2013, and 2015: 50 nematode species were identified in 1979, 32 in 2013, and 21 in 2015. Most species were typically marine but two freshwater species, *Chromadorita leuckarti* (de Man, 1876) and *Eumonhystera filiformis* (Bastian, 1865), were recorded in 1979 and 2013 but disappeared by 2015. The typical saline nematode species *Ethmolaimus multipapillatus* Paramonov, 1926 showed the same pattern.

In the nearby naturally saline Lake Elton there were fewer species (14) but many had marine affinities (Gusakov, 2019). However, there were also a number of species from typically freshwater genera (five), some the same as found in Sivash Bay. Among the 14 species, five were local endemics. Significantly, the endemics included a species of *Oncholaimus* (Gagarin and Gusakov, 2012), one of the otherwise overwhelmingly marine genera with several species apparently found in freshwaters (see Section 4.9).

Inland saline lakes in Australia are mostly filled very intermittently, but when filled there is a huge burst of biological activity. Nematode genera found in these lakes include *Monhystera*, *Prodesmodora*, *Mesodorylaimus*, and a plectid (Hodda, unpublished). The occurrence of these species is very sporadic. Moreover, they are quiescent most of the time, making species identifications very difficult.

In African soda lakes, only a single species has been reported, a member of the genus *Mesodorylaimus* (Tudorancea and Zullini, 1989), but no nematodes were found in a hypersaline lake in Africa. Nematodes were detected in a study of salt streams, springs, and water holes in Namibia but the species and genera were not identified (Procter, 1982).

In North America, the fauna of an artificial inland hypersaline lake, the Salton Sea, includes marine nematodes (Warwick *et al.*, 2002). While this lake is barely 100 years old, its salinity has increased rapidly. The nematodes are thought to have been introduced from nearby marine habitats on the Pacific coast or Sea of Cortez through human influence.

In Asia, a natural freshwater lake in a closed drainage basin that is very slowly becoming saline provides a contrast to the rapid salinity increase in the Salton Sea. Lake Issyk-kul', located in Kyrgyzstan, is thought

to be ca. 6000 years old and currently has a salinity of ca. 6 ppt (below that of sea water, but well above the salinity of most freshwaters). Its nematode fauna is part freshwater and part marine (Tsalolikhin, 1979), with the local freshwater species, including those of *Tobrilus* s.l., having seemingly adapted to the slow increase in salinity. Genera with closer evolutionary links to marine environments (*Leptolaimus*, *Monhystera*, and *Pseudoncholaimus*) may become more abundant as the lake becomes more saline. Because the lake is now isolated from the sea, these genera may be relicts of the marine conditions that existed far back in the geological past (Tsalolikhin, 1979).

4.11 Conclusions and Perspectives

There are several nematode groups that stand out because of their occurrence in many of the extreme habitats discussed in this chapter: the closely related *Monhystera*, *Eumonhystera*, and *Monhystrella* (Monhysterida), the genera or subgenera listed as *Plectus* s.l. (Plectida), *Rhabditis* s.l. (Rhabditida), and the closely related *Mesodorylaimus* and *Eudorylaimus* (Dorylaimida). All are ecological generalists: monhysterids, *Plectus* s.l., and *Rhabditis* s.l. are microbivorous but lack specialized structures in their stoma, dorylaimids are highly omnivorous (Yeates *et al.*, 1993; Hodda, 2021b). Rhabditids have highly developed forms of quiescence, food storage, and dispersal (Bird and Bird, 1991) whereas monhysterids and *Plectus* s.l. seem to have considerable physiological plasticity. Dorylaimids are ubiquitous in freshwaters and terrestrial soils, which facilitates their colonization of freshwater habitats not readily colonized by other taxa. They must also readily enter a state of quiescence, given their abundance in completely dry desert lakes (i.e. lakes that remain dry but are filled with water once in a few years).

Other genera that are prominent in multiple extreme freshwater habitats, but less so than those listed above, include *Chronogaster* (Plectida; related to *Plectus*), *Tobrilus* (Triplonchida), and *Mononchus* s.l. (Mononchida). *Chronogaster* is characterized by a high degree of physiological plasticity (Heyns and Coomans, 1980; Poinar and Sarbu, 1994), and *Tobrilus* s.l., which is ubiquitous in freshwaters, by morphological plasticity (a large number of similar taxa have been described but they may also represent a few variable taxa; e.g. Tsalolikhin, 1981a,b, 2001).

Nematodes from extreme freshwater habitats are able to cope with the hostile conditions of those environments, both the highly variable or extremes of salinity, oxygen or moisture and the isolation in space and time. The nematode taxa that have managed to adapt to these conditions can be classified into three groups. First are those with attributes that allow adaptation to the particular extreme conditions. In this group are clusters of closely related genera: *Monhystera*, *Eumonhystera*, *Monhystrella* (Monhysterida), *Plectus* s.l., *Chronogaster* (Plectida), *Rhabditis* s.l. (Rhabditida), *Mesodorylaimus*, *Eudorylaimus* (Dorylaimida), *Tobrilus* s.l.

(Triplonchida), and *Mononchus* s.l. (Mononchida). Second are taxa that probably arrived accidentally in the extreme habitat but were then able to take advantage of a largely vacant niche. These taxa encompass a wide range of taxonomic groups, including some normally considered 'marine' or 'terrestrial'. Third is a large group of nematodes found accidentally or opportunistically in the various habitats but which have not adapted and are thus only temporary residents.

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5 Dispersal of Free-living Nematodes

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Highlights

- Through their different adaptions and dispersal modes, nematodes are able to reach even the most remote and isolated habitats.
- Previous investigations indicated that active movement (crawling or swimming) by nematodes is mainly restricted to low-flow environments, which allows spreading over short distances; water drift, rafting, zoochory, and anthropogenic factors mediate long-range dispersal.
- Although only a few nematodes may be transferred from a community, their continuous dispersal, ability to survive unfavorable environmental conditions, and fast reproduction make nematodes good pioneer organisms.
- With their broad range of dispersal modes and their diverse emigration and immigration strategies, nematodes provide a model organism for investigations of dispersal.
- Owing to the lack of studies on the dispersal of freshwater nematodes, determinations of dispersal rates, the influence of dispersal on community structure, and the drivers of dispersal remain largely unknown.

5.1 Introduction

Dispersal describes the active or passive movement of populations, individuals, or specific dispersal units (propagules) that leads to gene flow. Three successive phases are involved in dispersal: emigration from a source habitat, transfer to another habitat, and, finally, immigration (Ronce, 2007). Dispersal is a central component in the life history of any organism (Bonte and Dahirel, 2017) as well as a decisive factor in population dynamics and biodiversity (Leibold *et al.*, 2004; Howeth and Leibold, 2010; Matthiessen *et al.*, 2010). As accurately phrased by Bonte and Dahirel (2017, p. 477): 'Without dispersal […], any deme is doomed to go extinct.' Dispersal enables organisms to emigrate from unsuitable habitats and to escape detrimental conditions, such as a shortage of food resources, increased competition, predation, disturbance, or adverse environmental parameters. Dispersing organisms can exploit new habitats or recolonize locations after a disturbance, such that local diversity can be increased by the immigration of new species or reduced because more competitive invaders become predominant. The frequency and intensity of dispersal are decisive factors that determine the effect of dispersal on the stability, composition, and distribution of organisms. Both factors are defined by habitat connectivity and the ability of species to cross the distances between habitats (see Chapter 3).

As nematodes cannot cover long distances by crawling, they are unable to actively reach other water bodies; they are also unable to swim against strong flow velocities. Furthermore, free-living nematodes in aquatic environments are confined to the substrate and pelagic larval stages are lacking. Nonetheless, nearly all substrates in aquatic systems (e.g. sediments, periphyton macrophytes, or moss) worldwide, from high-latitude lakes to groundwaters and deep-sea environments as well as ephemeral and isolated island habitats (e.g. ponds, puddles, or phytotelmata), are colonized by nematodes. Giere (2009) posed the question: 'How can we explain a wide distribution of animals with almost no dispersive capacity?'

Numerous nematode taxa are ubiquitous. For example, 10 out of 18 nematode species collected on the Galapagos Islands were cosmopolitans and 6 were at least widespread in the southern hemisphere (Eyualem-Abebe and Coomans, 1995). Among the former was *Rhabdolaimus terrestris*, encountered in a permanent lake and in an anthropogenic freshwater reservoir on San Cristobal (Galapagos), but also known from tank bromeliads in Panama, several lakes in Europe, Ethiopia, and the Himalayas, and from Vietnamese streams. Nematodes also quickly appear in new or disturbed habitats, whether they are part of larger ecosystems containing numerous source habitats, such as lakes or streams, or inhabitants of isolated and remote sites (ponds, phytotelmata, rain barrels) that preclude in-water exchange with other nematode populations. This suggests that nematodes can disperse both within aquatic environments (either the substrate or the water column) and by overland vectors that enable transport over longer distances. However, despite their more or less global distribution, nematode species often have distinct spatial distributions, with endemic species occurring in isolated habitats (islands) or ancient lakes (e.g. Lake Baikal) (Zullini, 2018). These physical detections are supported by studies of the genetic diversity of nematodes. For example, Ristau *et al*. (2013) investigated the genetic structure of *Tobrilus gracilis* in nine postglacially formed European lakes. Although the authors predicted a widespread distribution (>250 km), they found high intraspecific differences even between populations within the same lake.

In this chapter, we discuss the three phases of dispersal as follows. The first section considers the drivers and mechanisms of the *emigration* of nematodes from their original habitat. The second section outlines active and passive modes of *transfer* and provides estimates of transfer distances. In the last section, *immigration* is addressed, including an overview of the density and species composition in developing nematode communities during colonization (Fig. 5.1).

5.2 Emigration

The first phase of dispersal is the emigration of an organism from its original habitat. In case of active dispersal, the individual can choose to leave or stay within a local site. The main factors that trigger the active emigration behavior of nematodes are (i) population density, (ii) the presence and quality of food resources, (iii) oxygen content, (iv) the threat from predators, and (v) the hydrodynamics above the substrate.

Laboratory experiments have demonstrated that, with increasing densities, more nematodes from marine environments leave the source population. In the simultaneous emigration of nematodes from different species, weak competitors emigrate earlier and at higher densities than strong competitors (Meester *et al*., 2012, 2015). The presence of food resources in the source habitat reduces emigration, while the presence of more suitable food resources in adjacent habitats leads to a higher exodus within the sediment and even out of the substrate. For example, in the

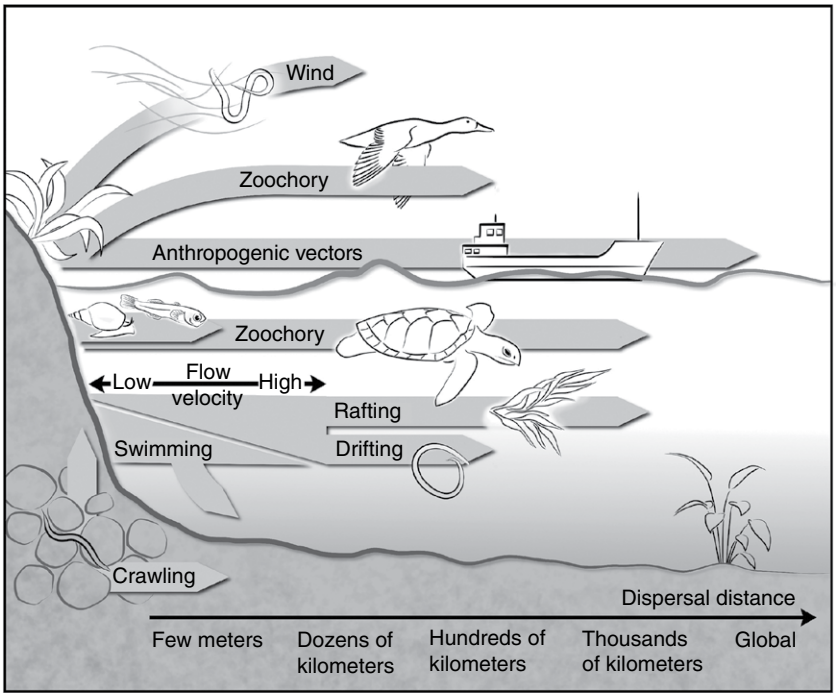

Fig. 5.1. Overview of the distribution modes and possible ranges of nematodes, based on studies conducted in marine and freshwater environments. (From Ptatscheck and Traunspurger (2020), modified.)

laboratory experiments conducted by Jensen (1981) using the marine nematode *Chromadorita tenuis* placed in aquaria, nearly all of the worms left the sediment and swam upward in the direction of food sources placed 5 cm above. Conversely, the presence of suitable food induced the directed immigration of swimming nematodes into the sediment under no-flow conditions whereas in the absence of such attractors only a very small proportion of nematodes in sediments (<6%) emigrated into the open water. These observations suggest that high densities of nematodes in open water are mainly due to passive processes (see the next section). However, this may not apply to all meiobenthic organisms, since under the same conditions in which the nematodes did not emigrate, 96%, 65%, and 35% of the copepods, ostracods, and flatworms from the same marine sediment actively emigrated within 24 h. Another crucial factor in the vertical distribution of nematodes is the oxygen content, as determined in both marine and freshwater environments. Nematodes can survive in deeper layers with a lower oxygen content, but the upper layers of the sediment, characterized by higher oxygen levels, are preferentially colonized. Nonetheless, in well-aerated habitats, such as streams with a coarse sediment size, relocation to deeper layers is possible (Schmid-Araya, 1997; Eisenmann *et al.*, 1998; Traunspurger *et al.*, 2015). The presence of other benthic organisms may affect the oxygen content within the sediment by bioturbation, thus indirectly leading to the active relocation of

nematodes. However, whether the oxygen concentration in sediment directly triggers nematode migration or the change in nematode communities is due to ecological filtering requires further investigation. A better investigated factor is the presence of predators, which can trigger the escape behavior of nematodes. For example, the presence of copepods and chironomids was shown to induce increasing densities of mainly juvenile nematodes in upper sediment layers (Traunspurger *et al.*, 2006), and Ólafsson (2003) observed the downward movement of nematodes when crustaceans, annelids, and mollusks were present. In the case of larger predators, for example bottom biting fish, a change in nematode distribution will be primarily due to a physical reworking of the sediment (Weber and Traunspurger, 2016).

In passive dispersal, an individual is limited to behavioral adaptions that enhance or reduce the probability of being dispersed. Examples of the former include crawling to the upper layer of the sediment to be more available for dispersal vectors, such as water drift or other animals, or adhering to potential rafts. Dispersal avoidance by nematodes and other meiobenthic organisms in streams may include vertical movement to reduce the risk of erosion by a strong flow velocity (Palmer and Gust, 1985; Palmer, 1986, 1988a; Palmer and Molloy, 1986; Fegley, 1987; Traunspurger *et al.*, 2015). The passive dispersal of nematodes has been demonstrated in studies from coastal regions, where daily, tide-dependent vertical migrations were observed. However, the upwards migration of some nematodes toward the strongest flow (Steyaert *et al.*, 2001; Gallucci *et al.*, 2005; Brustolin *et al.*, 2013) suggests that vertical migration offers more than just protection from erosion.

5.3 Transfer: Active Dispersal

5.3.1 Locomotion

Free-living nematodes are able to move within substrates (horizontal and vertical) but they can also swim in the open water. Their locomotion is characterized by typical undulatory movements achieved by alternating contractions of their longitudinal muscles. Then, depending on the physical resistance of the surrounding environment and the particle size of the substrate, the nematode body is pushed forward.

While larger nematodes move faster within sediments than smaller ones, in fine sediments without a consistent interstitial system very thin individuals with long tails have a higher mobility and maneuverability than larger or thicker worms (Traunspurger and Drews, 1996; Schratzberger *et al.*, 2007). Due to its multilayer cuticula, the nematode body is poorly deformable, which can restrict movement under certain circumstances. The ratio of particle diameters to body length provides a guideline for a more precise assessment of nematode mobility in sediments (Wallace, 1968; Soetaert *et al.*, 2002). Therefore, the maximum speed is reached when the particles diameter is three times smaller than the nematode's body length. In sediments with smaller particle sizes, the nematode must slow down and

burrow, while larger pore spaces reduce the repulsion resistance and enable swimming locomotion. Within the open water a nematode has to overcome its own bodyweight and, because propulsion is low, strongly increase the wavelength, amplitude, and thus the frequency of undulatory winding. Thomas and Lana (2011) observed the movement patterns of marine nematodes captured from the water column. They postulated that nematodes with a slim body (body length/body width >40) and long tail (tail length/tail width >10) are better active swimmers than lethargic species with more compact body shapes. Similar movement patterns of nematodes with different body shapes have been demonstrated for vertical crawling within sediments. Slim, highly flexible nematodes were also observed to enhance their sinking rate by reducing their projected body area via coiling (Eskin and Palmer, 1985; Tita *et al.*, 1999). Nematode movement patterns also correspond to specific feeding types, as bacteria-feeding nematodes (deposit feeders), with typically slim body shapes, move faster than algae-feeding (epigrowth feeders) and predators with high body length/body width ratios.

How fast can a nematode move in its natural environment? This question is not easily answered, because, especially in sediment, movement is difficult to monitor. Thus, most studies on the movement speed of nematodes have been conducted under laboratory conditions using artificial media such as agar or gelatin (Table 5.1). In those studies, crawling rates of ca. 3 cm/min were determined whereas swimming speeds may be up to 220% faster. The fastest swimming speed measured thus far is that of the marine nematode *Chromadorita tenuis* (50 mm/min), but continuous swimming for 1–2 h was demonstrated even for the terrestrial nematode *Caenorhabditis elegans*. However, compared with copepods, with swimming speeds far exceeding 400 mm/min and burrowing speeds of up to 16 mm/min, as well as oligochaetes, whose burrowing speeds may reach 13 mm/min (Enright, 1977; Yen, 1988; Palmer *et al.*, 1992), nematodes are slow-moving representatives of the meiofauna.

5.3.2 The role of active movement for nematode distribution

Nematode dispersal is considered to be mostly passive, but active dispersal, whether by swimming in open water or horizontal and vertical crawling, is not negligible. During active transfer by swimming, the flow velocity is a main determinant of the efficiency of active dispersal, especially in lentic habitats because even low-flow conditions can prevent active progress (Hagerman and Rieger, 1981; Palmer and Gust, 1985). The transfer of nematodes in lotic environments occurs primarily via water drift or rafting (see the following sections). The scientific challenge is to separate clearly whether dispersal is active or passive, as in the field it is difficult to distinguish whether a nematode is actively swimming or is being transferred in the water column by passive processes. So far, observations of the active entry and transfer of nematodes into open water have mainly been obtained in microcosm experiments without any

Table 5.1. Summary of studies that estimated the velocity (crawling or swimming) of different nematode species in different media. (From Ptatscheck and Traunspurger (2020), open access.)

Taxon	Body length (µm)	Medium	Distance (mm) moved per minute	Reference
Caenorhabditis elegans (Maupas, 1900)	Different sizes	In/on agar	1–15	Ramot *et al*. (2008)
	500–1500	In/on agar + food	1–13.5	
Chromadorita tenuis (G. Schneider, 1906)	ca. 1000	Water/swimming	50	Jensen (1981)
Haemonchus contortus (Rudolphi, 1803)	575	On 2% agar	3.1	Gray and Lissmann (1964)
		Water/swimming	9.9	
Heterodera schachtii (A.Schmidt, (1871)	larvae (<500)	On alginate jelly +		Wallace (1958)
		<1 µm water film	<0.05	
		2–5 µm	1.7	
		5–10 µm	0.7	
		10–20 µm	0.3	
		50 µm	0.2	
Panagrellus silusiae (de Man, 1913)	1190–1340	On 1% agar	30.1	Gray and Lissmann (1964)
		In 1% agar	27.6	
		On 1% gelatin	17.5	
		In 1% gelatin	23.2	
		Water/swimming	38.4	
Phasmarhabditis hermaphrodita (A. Schneider, 1859)	ca. 1000	On 1.2% agar	9.7	Hapca *et al.* (2007)
		On 1.2% agar + sand	6.9	
Rhabditis		In a suspension of starch grains	13.2–31.2	Gray and Lissmann (1964)
Turbatrix aceti (Müller, 1783)	1500–1570	On 0.5% agar	15.4	Gray and Lissmann (1964)
		In 0.5% agar	17.2	
		Water/swimming	43.1	

flow, thereby excluding passive processes (Ullberg and Ólafsson, 2003a; Schratzberger *et al.*, 2004; Gallucci *et al.*, 2008).

The active distribution of nematodes by crawling has been monitored in the field and in the laboratory. Field studies typically consist of placing azoic sediment in natural environments while ensuring that immigration corridors are restricted to the sediment (Williams and Hynes, 1976; Williams, 1977; Schmid-Araya, 2000; Ullberg and Ólafsson, 2003a; Schratzberger *et al.*, 2004). In the laboratory, test arenas can be designed that enable the manipulation of specific parameters (Meester *et al.*, 2012, 2015). Only a few studies have focused exclusively on the immigration of nematodes via the sediment. In those examining the recolonization of azoic sediments by nematodes, the immigration of sediment-crawling organisms was typically prevented by impassable barriers, such as trays, or superimposed by other dispersal modes, such as water drift. A direct comparison of several dispersal modes of nematodes in streams showed that ca. 60% of the individuals immigrate via horizontal or vertical crawling (Williams and Hynes, 1976; Williams, 1977).

Nonetheless, little is known about the active distribution of nematodes in their natural environment by crawling or swimming, including the distances covered and the contribution of active movement to nematode dispersal. Despite the morphological ability of nematodes to swim and actively enter the water column, it is generally accepted that their occurrence in open water is due to transport by water drift (see next section) and that nematodes are incapable of influencing the direction of their journey, even under low-flow conditions, but can only delay their sedimentation. For example, Palmer (1984) showed that anesthetized nematodes have the same sinking/swimming rate as non-anesthetized individuals. Compared with other dispersal modes (Fig. 5.1), the distance of active movement is restricted to a few meters at most. In the study of Ullberg and Ólafsson (2003b), only 50% of emigrating nematodes arrived at a location 36 cm away from the source habitat.

5.4 Transfer: Passive Dispersal

5.4.1 Morphological adaptions

During their passive dispersal, nematodes are often subjected to adverse conditions, such as desiccation, digestive juices, food shortage, or strong flow. Furthermore, the simultaneous immigration of a large number of passively dispersed individuals to a habitat is the exception, rather than the rule. The presence of dispersal units that enable long-term resistance and fast, asexual reproduction are essential requirements for the passive long-range dispersal of microscopic aquatic organisms (Fontaneto, 2019).

Eggs or anhydrobiotic (almost complete dehydration) stages are the common dispersal units of nematodes. These so-called propagules can withstand adverse environmental conditions (e.g. dryness, cold), pass

unharmed through the digestive tract of other organisms (see Section 5.4.4), enable survival over several decades (van Gundy, 1965; Watanabe, 2006) and are particularly important for the overland transfer of nematodes. The hatching of nematodes from their eggs is triggered by factors that include temperature or moisture and may thus be delayed until environmental conditions are favorable (van Gundy, 1965). The oviposition of egg clusters with sticky membranes offers additional protection from dehydration or erosion and enables dispersal by rafting or zoochory (Micoletzky, 1922). However, while eggs are common dispersal units of aquatic nematodes, anhydrobiosis has so far only been demonstrated for terrestrial or semi-aquatic species from ephemeral habitats (e.g. puddles, ponds, or phytotelmata), not for species from permanently aquatic environments (Crowe and Madin, 1974; McSorley, 2003; Tahseen, 2012). The presence of caudal glands or sticky eggs enables nematodes to withstand strong flow velocity (Micoletzky, 1922). With these adhesion organs, nematodes can anchor on substrates (Zullini and Croll, 1972), avoid erosion, disperse by rafting, or colonize lotic habitats (waters with high flow velocity). Once nematodes reach a suitable habitat, their asexual reproduction by parthenogenesis, fast sexual maturity, short generation times, and high numbers of descendants allow rapid colonization (Eyualem-Abebe *et al.*, 2008).

5.4.2 Water drift

Despite their benthic lifestyle and the lack of pelagic life stages, nematodes have been captured regularly from the open water. For example, up to 11,000 nematodes per cubic meter were collected from the littoral of different lakes (Eddy, 1927; Abdel-Aziz and Aboul-Ezz, 2004) and up to 46,000 nematodes per cubic meter were found drifting in the Grand River (Michigan, USA) (Mott and Harrison, 1983). However, compared with the number of nematodes typically present in a cubic meter of sediment, this density is not particularly high. Indeed, numerous studies from different aquatic habitats indicate that only a very small fraction (<3%) of nematodes in the substrate enter the open water (Ptatscheck and Traunspurger, 2020), even in lotic habitats (Palmer, 1992). In freshwater environments, copepods and, especially in streams, rotifers and chironomid larvae are encountered above the substrate in significantly higher percentages than nematodes. Nevertheless, considering their predominance in benthic environments, a small percentage of drifting nematodes may be sufficient for successful dispersal. Moreover, in a single day the transport of nematodes may be substantial. Mott and Harrison (1983) showed that even under low-flow conditions (20 m^3/s), 1 billion nematodes per day may be transported. Artois *et al.* (2011) documented the transport of 300 million nematodes of 38 species down the Adda River (Italy) in one day. The species composition of the drifting nematodes may not be congruent with that in the substrate below, which indicates dispersal also from distant habitats (Commito and Tita, 2002; Fonsêca-Genevois *et al.*, 2006).

Most studies describing nematodes in open waters have been conducted in streams or the shore areas of lakes, both characterized by high flow velocities. Although nematodes in the open water of lakes are often discussed only as bycatch, obtained by resuspension of the sediment during sampling, they have been detected at a height >15 m above the lake bottom (Khalifa *et al.*, 2015). However, as observed by Sibert (1981) in marine environments, the majority of nematodes in open water are confined to <5 cm above the ground (67%), while at a height of 6.5 m very few are detected, for example, only 2% in the study of Boeckner *et al.* (2009).

The requirement for successful dispersal via water drift is a water connection between the emigration source and the immigration target. In river systems, the connectivity of sites along the watercourse is a more relevant factor than environmental constraints in determining nematode community structure, which indicates the importance of dispersal within a water body (Gansfort and Traunspurger, 2019). Accordingly, even flooding events, by temporarily connecting rivers, lakes, ponds, and other freshwater bodies, can play a significant role in nematode dispersal, as also shown for zooplankton (Dias *et al.*, 2016) and macrobenthos (Petsch *et al.*, 2017).

The most important factor influencing the degree of drift across connected sites is the flow velocity. For a wide range of waters with high flow velocity, such as lake littorals, streams, and tidal coastlines, the abundance of nematodes in the water column is greater at high than at low flow velocities. In lotic habitats, the emigration and transfer of nematodes can be attributed to passive particles, although this is not the case for all meiobenthic organisms. Copepods, but also flatworms, can be found in the open water in higher numbers during low-flow events, evidence of their higher potential for active dispersal. Both near-bed flow and shear stress impact erosion (emigration) from the sediment and passive immigration by deposition. Additional factors that affect local hydrodynamics include substrate topography and above-ground structures (Eckman, 1983).

Flow velocities of 9–12 cm/s and even <3 cm/s are enough to wash nematodes from the sediment. This is especially the case for nematodes in the upper sediment layer, as they lack the morphological adaptions needed to avoid erosion by vertical movement and are thus highly prone to resuspension. Therefore, the active vertical distribution of nematodes within the substrate determines their passive dispersal by water drift. The critical erosion threshold is lowest in fine sediments (e.g. sand) such that small benthos will be eroded with the surrounding sediment, while coarser sediment may offer greater protection (Alldredge and King, 1977). Plant cover, stones, dead wood, and depressions all lower the shear stress and retain drifting organisms. Atilla *et al.* (2005) demonstrated that, with the increasing complexity of above-ground structures (in that study, bristle density), the retention and diversity of the captured nematodes increase significantly, due to flow reduction. Because larger organisms, including annelids, crabs, and fish, modify the roughness of the substrate surface by dwelling or feeding activity, they also affect the critical erosion velocity

and thus the emigration and immigration of nematodes. For example, recolonization by meiobenthos was shown to be faster in fish feeding pits than in the surrounding sediments (Sherman *et al.*, 1983; Billheimer and Coull, 1988; Cross and Curran, 2004). The feeding activity of fish can also directly impact nematode dispersal by water drift, as shown by Palmer (1988b). In that study, the number of nematodes in the open water of a stream significantly increased when bottom biting fish reworked the sediment.

Once in the open water, the traceability of nematodes and their source habitats as well as a precise determination of the dispersal distances are difficult. A possible approach to follow drift over at least short distances is the targeted exposure of stained nematodes, as done by Thomas and Lana (2011) in their study of marine environments. Their results showed that eroded nematodes can be transferred for >2 m via water drift. However, as this type of monitoring approach is ineffective over longer distances, dispersal distances are typically estimated based on the closest location containing a natural occurrence of the collected species. Such studies, all conducted in marine environments, have shown that nematodes may drift for distances of up to 10 km.

Just as only a small percentage of nematodes from the substrate typically reach the open water, only a small percentage finally immigrates back to the benthos. Palmer (1984) investigated the colonization effort by drifting meiofauna and found that nematodes sediment in still waters much more slowly (0.08 cm/s) than do other meiofaunal representatives. In that study, only 38% of the nematodes in the open water entered the sediment, with a flow velocity of 8 cm/s. However, dispersal is continuous and rates of up to 135 individuals/cm/day have since been determined in intertidal areas (Commito and Tita, 2002). Thus, 24 h of nematode transfer by open water dispersal can result in the settling of a larger number of individuals than previously located in the home substrate (Palmer, 1992).

5.4.3 Rafting

Rafting organisms are transported via their attachment to floating biotic (e.g. parts of plants) or abiotic (e.g. plastic debris) items. Rafting as a dispersal mode has so far mainly been described for organisms in the open sea or washed up in coastal areas (Thiel and Gutow, 2004, 2005), for example mainland organisms that cross the ocean on floats and colonize remote islands. However, the basic features of rafting can be applied to freshwater ecosystems. Biotic rafts (e.g. algae, bacterial mats, or detritus) are often former parts of benthic environments washed off in lotic environments by water flow or wind, and are thus occupied by a complete benthic community. This explains the frequent detection of nematodes on detached substrates. Even transient contact with the benthos, for example, such as ice floes washed up ashore (Macfarlane *et al.*, 2013), enables the

rafting of nematodes. In addition, non-resident organisms may arrive on the raft by swimming or, more likely for nematodes, water drift.

Rafting is often considered to be a dispersal mode that occurs primarily in lotic waters and enables the long-range replacement of organisms between coastlines, mediated by ocean currents. Living nematodes on floating algae have been documented >100 km away from the shoreline (Ingolfsson, 1995; Abe *et al.*, 2013). However, rafting also occurs along rivers. This was shown by Gaudes *et al.* (2006), who found up to 752 nematodes/cm² rafting downstream on a detached cyanobacterial biofilm. In low-flow and shallow shore environments, substrate fragments containing nematodes may float up during the daytime via photosynthetic processes, subsequently relocated by wind and then sink to the bottom at night, thereby enabling at least a short-term dispersal (Phillips, 1958). Floating bacterial mats have been identified as rafts for nematodes, even in cave waters (Riess *et al.*, 1999; Muschiol *et al.*, 2015). In addition to water flow conditions, the durability of the floating item limits the covered distance. For example, floating sea foam is significantly more unstable than driftwood, which persists over many years.

In suitable environmental conditions that favor rafting and a long-lasting raft, three main features determine the rafting success of organisms: (i) the ability to access and cling to the raft, (ii) successful survival on the raft, and (iii) the successful reproduction and establishment of persistent populations during transport (Thiel and Gutow, 2005). The abundances of nematodes on floating items may be significantly lower than in the substrate below and even the species composition may differ. In the study of Faust and Gulledge (1996), only 1% of the nematodes of the upper sediment layer were contained in floating detritus. Therefore, it is likely that not all nematodes associate with the raft or that some individuals and even entire species become lost during transfer. Species-specific morphological adaptions (e.g. caudal glands and adhesive eggs) can increase dispersal success by rafting (Micoletzky, 1922).

Since entire benthic communities can be transported via rafting, the necessary food resources but also possible predators will be on board. Bacteria, protozoans, diatoms, and other algae are common structural components of rafting substrates or become quickly available through the growth of a biofilm on the raft surface. Accordingly, studies on rafting nematode communities mostly identified bacterial- and algal-feeding species (Micoletzky, 1922; Riess *et al.*, 1999; Gaudes *et al.*, 2006; Muschiol *et al.*, 2015). With this nutritional base, nematodes can survive long distances more effectively than if dispersed by water drift. However, during their voyage, rafting nematodes will be exposed to competition and predation as well as to rapidly changing environmental parameters (e.g. flow velocity, temperature, salinity, UV radiation). Nonetheless, given their ability of fast parthenogenetic reproduction, nematodes are able to compensate for losses and establish durable populations. Gaudes *et al.* (2006) observed a high proportion of juvenile nematodes and gravid females in nematodes on floating bacterial mats and significantly higher nematode

densities than found on attached substrates. Reproduction during transfer results in more individuals reaching a new habitat simultaneously, such that priority effects are overcome, and successful colonization is possible (see Section 5.5).

5.4.4 Zoochory

Nematodes and other small organisms are frequently transferred on (epizoochory) or in (endozoochory) larger and more mobile animals, which can greatly increase their dispersal radius and enable overland transport between waters. The transporting organisms may come into contact with nematode-containing water or substrates during swimming, bathing, or drinking, or they may ingest nematodes, by direct predation or as bycatch. In the case of zoochory via terrestrial animals such as mammals or waterfowl, nematodes from littoral zones, wetlands, mudflats, and other shallow waters are the most likely hitchhikers.

5.4.4.1 Endozoochory

The transfer of nematodes via the digestive tract of larger animals is determined by the time required until the next defecation. Therefore, the basic requirement for successful endozoochory is the avoidance of digestion and the ability to otherwise survive intestinal passage. Among the organisms that frequently ingest nematodes are insect larvae, crustaceans, and juvenile fish (Ptatscheck *et al.*, 2020). However, even if the worms survive uptake, most will be digested within a very short time (20 min to hours). An exception may be *Panagrellus redivivus*, which has been found alive in the guts of larval coregonids 2 h after ingestion. The nematodes transited and exited the intestine by their own active movements before digestion (Schlechtriem *et al.*, 2005). Unscathed gut passages have been reported for numerous Rhabditida species ingested by gastropods (Sudhaus, 2018). The latter exert strong top-down effects on the nematode communities of periphyton, with the nematodes surviving in the gastropod gut for as long as 5 days until excretion.

The transport of nematodes via birds traveling hundreds of kilometers a day offers a considerably wider dissemination. Riparian regions are at times simultaneously visited by thousands of birds, as resting places or feeding habitats. Gaston (1992) observed that a single green-winged teal can consume the amount of nematodes (up to 2300 individuals) found in 1 m^2 of top-layer sediment within 20 min. Investigation of waterfowl feces revealed that, depending on the bird species, between 3 and 60% of the samples contained living adult nematodes. When these samples were incubated in water, nematodes appeared after a few days in some (Frisch *et al.*, 2007; Green *et al.*, 2008), indicating that not only living nematodes but also their eggs, which hatch after excretion, are transported by waterfowl. In total, ca. 13 living nematode individuals and an unknown

number of eggs were contained in the feces of a single gray goose (Green *et al.*, 2008).

Secondary dispersal is another mode of transport, such as when a fish eaten by a bird had consumed benthic nematodes. However, this dispersal pathway has yet to be documented for nematodes but it has been shown to enable the long-distance dispersal of other aquatic invertebrates (van Leeuwen *et al.*, 2017).

5.4.4.2 Epizoochory

Frisch *et al.* (2007) suggested that the transport of meiobenthic organisms on the feathers and feet of birds is more decisive than endozoochory. This has been shown for benthic taxa such as cladocerans, copepods, and bryozoans, but it has not been documented for nematodes. Epizoochory of meiobenthic organisms has also been demonstrated in mammals such as wild boar and nutrias (Vanschoenwinkel *et al.*, 2008b; Waterkeyn *et al.*, 2010a). However, in the latter studies, as the collected nematodes made up only ca. 1% of the organisms transported they can be considered as single finds. To best of our knowledge, the only evidence of extensive epizoochory in free-living aquatic nematodes comes from marine environments. Indeed, the shells of marine turtle represent hotspots of nematode diversity and may be colonized by >23,000 nematodes, representing up to 41 genera/species, as well as by numerous other meiobenthic organisms (Corrêa *et al.*, 2014; dos Santos *et al.*, 2018). Since a complete community is transported and long-term survival and reproduction are possible, turtle shells act as a dispersal vector very similar to a raft. Moreover, because turtles, with their standing stock of meiobenthic organisms, can cover distances of thousands of kilometers, they serve as one of the most important epizoochoric dispersal vectors in marine environments.

5.4.5 Human-mediated transfer

Wyatt and Carlton (2002) pointed out that not only natural processes but also anthropogenic vectors must be considered in discussions of the global distributions of microscopic organisms such as nematodes. In fact, since contact between larger organisms, including humans, and the sediment often leads to the displacement of meiobenthos, nematodes are likely to be common 'travel companions'. Aquatic nematodes, especially their eggs, are highly viable in dried sediment (Gerlach, 1977) and both Waterkeyn *et al.* (2010b) and Valls *et al.* (2016) showed that nematodes can be transferred in the mud residue clinging to clothing (in those studies, footwear). Thus, human-mediated transfer can enable the dispersal of organisms across different wetland habitats, especially those in highly frequented areas but also to sites separated by long distances.

Through their transit of lakes, river systems, and oceans, and the emptying of their ballast tanks, ships contribute to the global dispersal of

numerous, sometimes invasive, species. Nematodes are the predominant and most diverse meiobenthic taxon collected from the sediments of ballast tanks (Duggan *et al.*, 2005, 2006; Radziejewska *et al.*, 2006). Duggan *et al.* (2005) identified 48 different freshwater nematode taxa in the tanks of ships traveling the Great Lakes, which border parts of the USA and Canada. Radziejewska *et al.* (2006) reported the transport of >100,000 nematodes within the sediment contained in the ballast tank of a bulk carrier. Moreover, depending on the location of sediment uptake, nematode abundance in transported sediments may be even higher.

Finally, even tap water enables the dispersal of nematodes, as these are present in drinking water distribution systems (Funch *et al.*, 1995; Schreiber *et al.*, 1997; Christensen, 2011; Inkinen *et al.*, 2019). In fact, guideline values for finished water from the Netherlands (in 1993) and North America include the allowance of 0.3 and 2.5 nematodes per liter (reviewed by Christensen, 2011). Other studies found up to 156 individuals, belonging to 41 species, per liter of drinking water (reviewed by Artois *et al.*, 2011).

5.4.6 Wind dispersal

Wind is an omnipresent vector for the overland dispersal of nematodes and other small metazoans (e.g. nematodes, microcrustaceans, flatworms, acari, rotifers) as well as unicellular organisms (e.g. bacteria, algae). Wind erosion primarily affects terrestrial organisms, while inhabitants of benthic habitats are exposed to wind mostly in eulittoral zones, desiccated substrates, and thus especially in temporal waters and semi-aquatic habitats (e.g. ponds or phytotelmata). In contrast to other benthic organisms or zooplankton, there is little consistent information on the wind drift of nematodes. This deficit can largely be explained by the lack of a suitable method for collecting nematodes directly from the air. The methods used thus far include specific above-ground dust collectors (Orr and Newton, 1971), soil samples as well as the contents of open-top chambers and Bundt pan soil traps (Nkem *et al.*, 2006), windsocks (100-µm mesh) (Vanschoenwinkel *et al.*, 2008a), sticky traps (Vanschoenwinkel *et al.*, 2009), and formaldehyde-filled funnels and 5-µm mesh-size filters (Ptatscheck *et al.*, 2018), but this diversity makes the results difficult to compare. Furthermore, most of those studies were conducted in harsh, dry regions (Antarctica and Africa during the dry season) and their focus was the dispersal of terrestrial nematodes, especially plant parasites. However, an extrapolation of their results suggests that drought and the presence of propagules, a life form not known for aquatic nematodes, are the basic requirements for successful wind dispersal, as demonstrated for other meiobenthic organisms such as microcrustaceans (Incagnone *et al.*, 2015). Vanschoenwinkel *et al.* (2008a) collected single individuals (unidentified) next to other wind-drifted meiobenthic organisms and thus suggested that wind can act as a vector also for the transport of nematodes

between waters. Considerably higher percentages of aquatic nematodes and clear indications of their wind dispersal were reported by Ptatscheck *et al*. (2018) from temperate latitudes (central Europe). In that study, aeroplankton were collected in a natural environment (vegetation and ponds) and on the roof of a 10-story building (35 m above the ground); in both sources, nematodes were the dominant taxon (up to 46%) (Fig. 5.2). At least eight of the 26 identified nematode species were known from aquatic environments; only one was an obligate freshwater species. Contrary to previous assumptions, only living individuals were found, perhaps due

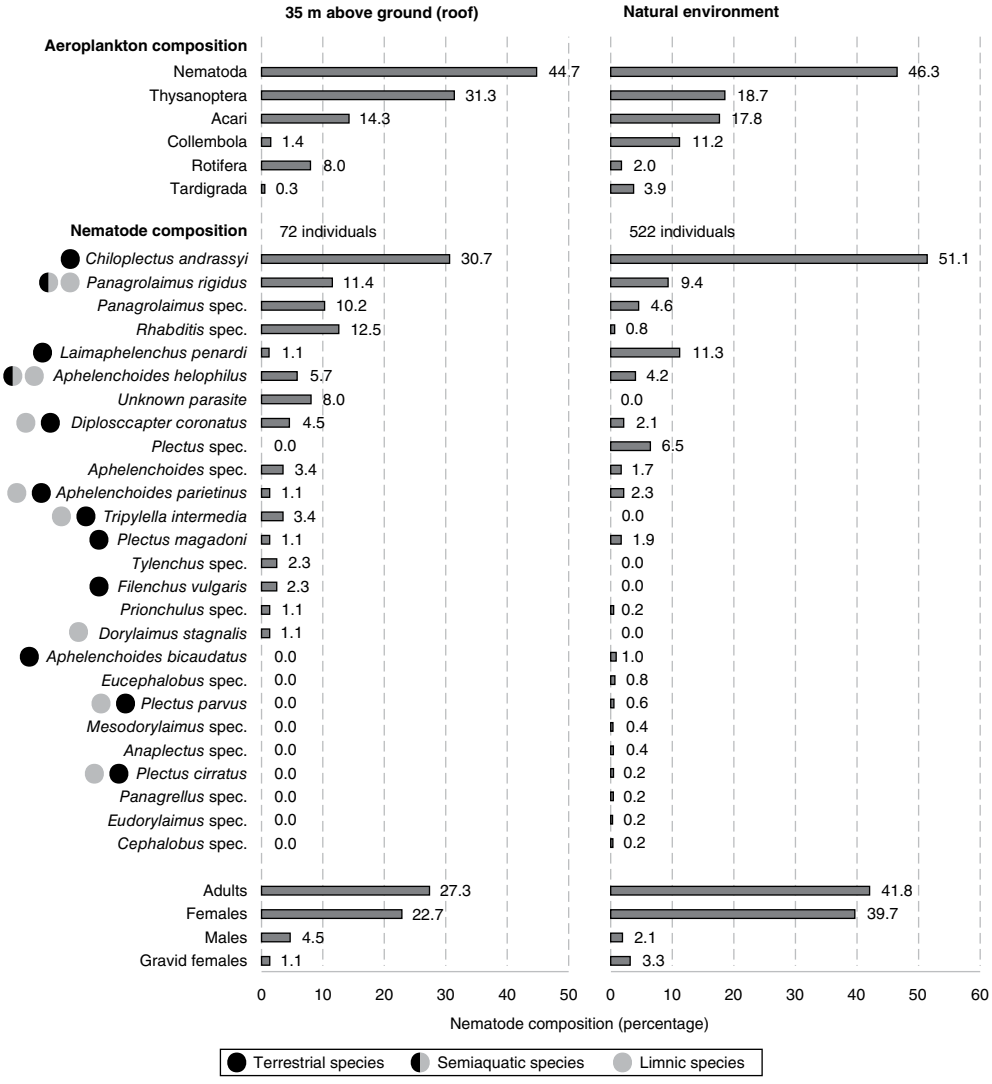

Fig. 5.2. Composition of the aeroplankton, especially the nematode component, collected at two different sites by Ptatscheck *et al*. (2018).

to the more humid climate, whereas propagules would be expected only in harsher environments. With increasing humidity and wind speed, the number of collected wind-drifted nematodes increases, resulting in a seasonal pattern of dispersal (Ptatscheck *et al.*, 2018). The worms' form and weight determine both the wind energy necessary for their erosion and the transfer range. Theoretical calculations have shown that resting eggs are less effectively transported by wind, while the small, light individuals of later stages may be eroded at wind speeds as low as 0.01 m/s and deposited up to 40 km from their original location (Carroll and Viglierchio, 1981). Accordingly, the majority of the nematodes collected by Ptatscheck *et al.* (2018) were juveniles, whose body length is typically <0.75 mm (Fig. 5.2). Besides meteorological factors, the spatial distribution of habitats determines the dispersal success of wind-drifted nematodes. At distances farther from the source habitat the number of individuals and species declines, as shown for the nematodes collected in natural environments and from the roof of the 10-story building (Fig. 5.2). Under favorable meteorological conditions and nearby source habitats, up to 3020 wind-drifted nematodes may be deposited in a square meter within 4 weeks. However, in studies of the colonization of azoic sediments in streams by aerially transported organisms, there was no evidence of the establishment of nematode communities, in contrast to other benthic invertebrates such as ostracods or copepods (Williams and Hynes, 1976; Williams, 1977; Benzie, 1984).

5.5 Immigration

Independent of the dispersal mode and transport vector, for nematodes arriving at a potential habitat the third phase of dispersal, immigration, starts. Potential colonizers of a habitat must survive and reproduce within the environmental conditions of a site and interact with its residents, whether competitors or predators. In the following section, the development of nematode communities in the initial days and weeks of colonization is described and related to the potential role of biotic factors during the colonization process.

5.5.1 The development of nematode communities

5.5.1.1 In terms of density

Studies that have investigated the colonization of empty habitats have mostly employed the same basic experimental set-up, in which a new, more or less artificial substrate is introduced into the natural environment and the abundances of organismal groups and species colonizing this new habitat are documented over a defined time span. For freshwater nematodes, these habitats are typically hard substrates (e.g. aluminum plates in

Peters *et al.*, 2007), natural sediment without the inhabiting community (e.g. eliminated by boiling in Duft *et al.*, 2002), or artificial waterbodies (e.g. artificial tree-holes in Ptatscheck *et al.*, 2015). An overview of these studies is provided in Table 5.2.

Colonization of the introduced habitats may start very quickly. In the study of Schmid-Araya (2000), within 2 h the first meiofaunal individuals had begun to colonize standpipe traps placed in the sediment of a stream bottom. After nearly 5 days, nematode densities were the same in the pipes as in the surrounding natural sediment. Smith and Brown (2006) also found that 5 days were necessary for nematodes in an azoic sediment placed in an artificial stream channel to develop populations with the same densities as those in the natural stream reference site. In other studies, the recolonization of azoic sediment took longer or consisted of only a few individuals (6.2 ind./100 cm^2 in Williams and Hynes, 1976; 11% of nematode background populations in Duft *et al.*, 2002) even 3–4 weeks after colonization. Figure 5.3 depicts the development of nematode densities in dried sediment during the first 4 months after the placement of samples in eight artificial ponds (Traunspurger, unpublished data). Already after 1 month, 177–730 individuals were found in 100 cm^2 of sediment, with a high variability of densities among ponds and the mean density increasing during this time span such that after 4 months of colonization nematode abundance had reached 4500 ind./100 cm^2.

However, in all these cases a basic habitat structure was available, whether the offered azoic sediment (Williams and Hynes, 1976; Schmid-Araya, 2000; Smith and Brown, 2006) or the interstitial sediment that had accumulated in the traps (Schmid-Araya, 2000). By contrast, when a hard substrate or previously empty water bodies are colonized by meiofauna, periphyton or other substrates that serve as three-dimensional habitats and food resources must emerge, with colonization by other organisms accordingly delayed. This was the case in the study of Ptatscheck and Traunspurger (2014), who observed that nematode communities developed significantly faster in artificial habitats in which leaf litter was available from the beginning of colonization than in those initially devoid of substrate. Specifically, after 1 month of colonization a mean of 160 nematode individuals/100 cm^2 was detected in treatments with a high leaf litter content vs. 4 ind./100 cm^2 in those without the addition of leaf litter. A further example is provided by the study of Peters *et al.* (2007), who monitored the colonization of aluminum plates. After up to 6 days no nematodes were found on these artificial hard substrates but a steep increase in nematode abundances then occurred, with densities of 250 and 1000 ind./100 cm^2 determined after 15 and 57 days, respectively, which corresponded to a maximum of 16.5% of the nematode densities on the reference substrates.

Ptatscheck *et al.* (2015) described the development of communities in artificial tree-holes (empty plastic cups), where after 3 months nematode abundances ranged from 12 to as many as 8704 ind./100 cm^2. This wide variability likely reflects both the unsteady, rather random input of

Table 5.2. Summary of studies documenting the colonization of habitats by nematodes. (Author's own table.)

Location/ habitat	Colonization area (grain size)	Sampling period	Nematode abundances per 100 cm^2	Relative abundance (of meio- and macrofauna)	Comparison with background density	Dominant species	Number of species	Pathway	Reference
Littoral zone (lake)	Aluminum plates	Sampling after day 2, 4, 6, 8, 15, 22, 29, 57							Peters *et al*. (2007)
	Bottom		Continuous increase from single individuals during the first few days to 250 on day 15 and 1000 on day 57	<5% (until 20 days) afterwards ca. 10% (until day 57)	about 16.5% after 57 days	*Eumonhystera vulgaris* and *filiformis*, *Chromadorina bioculata* and *viridis, Daptonema dubium*	Continuous increase, from 1–4 species during the first few days up to 10 species per plate from day 15 (total of 22 species)	W, S	
	30 cm above bottom		As above	As above	As above	See above	Continuous increase from 1–4 species during the first few days up to 10 species per plate from day 15 (total of 17 species)	W	
Littoral zone (lake)	Sediment disturbed by carps	Sampling after 45 days (once)	1200	NA	No difference	*Tobrilus gracilis*, *Eumonhystera filiformis*, *Monhystera paludicola/ stagnalis*	14	AP	Weber and Traunspurger (2015), Weber and Traunspurger (2016)

Continued

Table 5.2. Continued.

Location/ habitat	Colonization area (grain size)	Sampling period	Nematode abundances per 100 cm^2	Relative abundance (of meio- and macrofauna)	Comparison with background density	Dominant species	Number of species	Pathway	Reference
Stream	Standpipe traps (30 µm–9 mm)	Sampling after 2, 4, 6, 12, 24, 48, 72, 96, 192 h	NA	11.5–14.5% in 5 days	95% after 5 days	NA	NA	S	Schmid-Araya (2000)
Stream pool	Azoic sediment (240 µm)	Sampling after 21 days (once)						AP	Duft *et al*. (2002)
			134	7.5%	ca. 11%	Genera *Eumonhystera* and *Plectus*	24 (background 31)	No access for macrobenthos	
			115	4.3%	ca. 11%	*Rhabdolaimus*, *Dorylaimus*/ *Mesodorylaimus*	24 (background 31)	Access for macrobenthos	
Artificial stream	Washed gravel (2.5–3.5 cm) and sand	Sampling until no difference in the density compared to the background communities	62	NA	No differences after 5 days	NA	NA	W	Smith and Brown (2006)
Stream	Azoic sediment	Sampling after 28 days (once)							Williams and Hynes (1976)
			59.3	37.2%	NA	NA	NA	W	
			64.9	30.9%	NA	NA	NA	S	
			22.1	16.1%	NA	NA	NA	SV	
			0	0%	NA	NA	NA	A	
			6.2	3.4%	NA	NA	NA	AP	
Artificial tree-holes	Plastic cups hung on a tree	Sampling every 3 months for 1 year	1924 ± 6780 after first 3 months	5.7-80.1% after 3 months	NA	*Dolichorhabditis dolichura, Laimaphelenchus* sp.	35 (2.7±1.5 per cup)	A, SF	Ptatscheck *et al*. (2015), Ptatscheck and Traunspurger (2015)

Artificial tree-holes	Plastic cups hung on a tree	After 1, 4, 8, 24 weeks	Mean of 11.4 after 1 week; 4.3 after 4 weeks; 68.6 after 8 weeks; 45.7 after 24 weeks	Mean of 13.5% after 1 week; 9.1% after 4 weeks; 3.3% after 8 weeks; 2.0 after 24 weeks	NA	*Plectus cirratus*/*acuminatus*, *Aphelenchoides parietinus*	29 (1–7 per cup)	A, SF	Ptatscheck and Traunspurger (2014)

A = aerial transport; AP = all pathways possible; S = primarily through sediment; SV = vertical upward migration through sediment; SF = stem flow, W = primarily through the water column.

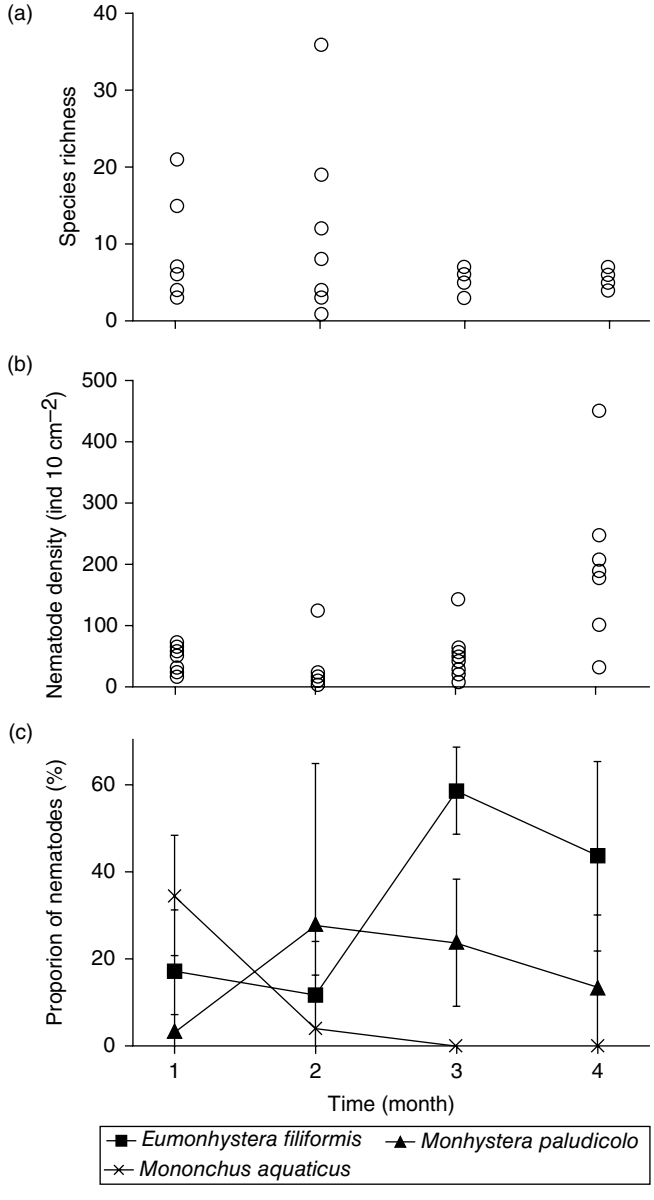

Fig. 5.3. Development of nematode communities in the sediment from eight artificial ponds during four months of colonization, after the sediment had been stored dry for 2 years: (a) the number of nematode species, (b) overall nematode densities, and (c) the proportions of the three most dominant nematode species among all nematode individuals. (Traunspurger, unpublished data.)

individuals and the high exposure of small water bodies such as those in tree-holes to outside impacts (e.g. temperature and precipitation fluctuations) that are less important in larger, more stable aquatic environments such as streams and lakes. However, the bottom-up structuring of

the newly formed communities is clearly a feature of the colonization of hard substrates, both in lakes and in artificial tree-holes (Peters *et al.*, 2007; Ptatscheck and Traunspurger, 2014; Ptatscheck *et al.*, 2015).

The studies discussed above show that compared with other meiofaunal groups nematodes are rather slow colonizers. For example, in the above-cited study by Schmid-Araya (2000), nematodes needed 5 days to reach 95% of their natural densities while rotifers and ostracods reached this same level after only 2 days; harpacticoids were slower immigrants than nematodes. Similarly, in the studies of Duft *et al.* (2002), Peters *et al.* (2007), and Majdi *et al.* (2012) nematodes comprised only 4–10% of the meiofaunal community in newly formed habitats whereas in the ambient habitat they contributed 20–40% and were therefore one of the most dominant groups. The relative abundances of rotifers and crustaceans during the succession of newly colonized habitats are usually higher. In marine environments, the ability of copepods to colonize disturbed habitats rapidly is known from several studies of intertidal zones, where nematodes are slower but nonetheless successful immigrants (e.g. Chandler and Fleeger, 1983; Ólafsson and Moore, 1990). The differences have often been attributed to the better swimming ability of rotifers and copepods, which allows them to reach new habitats earlier than nematodes. Therefore, in discrete habitats in which the source of colonization is not water but, for example, aerial transport, crustaceans are less dominant. Nematodes and rotifers are probably more frequently dispersed by wind (Gansfort *et al.*, 2020) and therefore more dominant colonizers if transport is not mediated by water. This was shown by Ptatscheck and Traunspurger (2014), who found that the colonization of tree-hole communities was dominated by rotifers and nematodes but excluded copepods.

5.5.1.2 In terms of species composition

The characteristics directly related to colonization success, such as fast reproduction, parthenogenesis, and a high tolerance of harsh environmental conditions, are found in many taxa of freshwater nematodes. Those species were classified as typical colonizers by Bongers and Bongers (1998). They include members of the family Monhysteridae, such as *Eumonhystera* (Duft *et al.*, 2002; Peters *et al.*, 2007; Weber and Traunspurger, 2016; Fig. 5.3) and *Monhystera* (Weber and Traunspurger, 2016; Fig. 5.3), which are dominant first colonizers in streams and lakes. However, most abundant immigrants in artificial tree-holes belong to the families Plectidae (*Plectus cirratus/acuminatus*, Ptatscheck and Traunspurger, 2014) and Aphelenchoididae (*Aphelenchoides parietinus, Laimaphelenchus* sp., Ptatscheck and Traunspurger, 2014, 2015). The dominance of bacterial (Monhysteridae, Plectidae) and hyphal (Aphelenchoididae) feeders in the first phases of succession reflects the important role of bottom-up structuring in the establishment of nematode communities. However, if basal resources are available in abundance, then a larger number of species belonging to different trophic groups will be present during the same period

of colonization (Ptatscheck and Traunspurger, 2014). This was demonstrated by Ptatscheck and Traunspurger (2014), who detected predatory nematode species (*Prionchulus muscorum*, *Coomansus parvus*) solely in treatments in which leaf litter had been added to the colonized artificial tree-holes. By contrast, the predator *Mononchus aquaticus* was present at high relative abundances in artificial ponds (Fig. 5.3) during the first months of their colonization but its densities rapidly declined to almost zero, which indicated the inability of this species to establish a stable population within the newly formed habitat; however, the densities of the bacterial feeders *Eumonhystera filiformis* and *Monhystera paludicola* increased continuously over time.

Two scenarios can be proposed to explain the development of the number of nematode species during colonization: (i) as the number of immigrants increases, so does the species richness due to the progressive development of the habitat, including the availability of more and different resources, which allows the colonization by a wider range of species; (ii) conversely, while a large number of species may initially arrive at a new habitat not all of them persist over the long term, due to competition for resources and space, such that species richness decreases during the colonization period. The main difference between the two scenarios is that the first depends on the development of a habitat structure and the availability of the necessary resources whereas in the second a basic stock of substrate and resources as well as a rich source of species inputs are assumed. Accordingly, Peters *et al.* (2007) described an increase in species richness from one to four nematode species during the early days of colonization, with a constant species number of 10 reached after 2 weeks of colonization. By contrast, in the study of the colonization of artificial ponds, species richness decreased during the first month of colonization from over 20 species to 5–10 species, a number that remained basically unchanged during the rest of the year (Fig. 5.3 and unpublished data).

5.5.2 The role of biotic interactions

5.5.2.1 Predation, competition, and indirect effects

When individuals immigrate to already established communities, both the abiotic characteristics of the habitat and biotic interactions become important determinants of their establishment success. Biotic factors include the presence of a predator or competitor. For example, Weber and Traunspurger (2016) could show that the presence of a fish, acting as a predator, reduces nematode densities and mean species richness in addition to altering the community composition. For soil nematodes the ability of a predator to reduce the densities of its prey was shown in an experimental set-up in which the successful invasion by nematodes of a local community was reduced by the presence of predatory mites (Kotiaho and Sulkava, 2007).

The establishment success of a species can likewise be negatively affected by the presence of a competitor. In general, most species of a local community compete with each other for space and, depending on the competing species, also for resources (Tilman, 1982). Successful colonization in the latter case depends on the competitive ability of the competing species, although it can be compensated by the initial density of each of the competitors, which in terms of colonization reflects propagule pressure and the effectiveness of the other phases of dispersal. Such interactions may have a negative effect on population survival, as shown for the bacterial feeders *Acrobeloides nanus* and *Dolichorhabditis dolichura* (Ilieva-Makulec, 2001). In that study, rapid food depletion by one species reduced the population growth of the other. However, interactions between two bacteria-feeding nematode species can also positively impact the population growth of each one. This was demonstrated in the study of Gansfort *et al.* (2018), in which *Panagrolaimus thienemanni* and *Poikilolaimus regenfussi* achieved higher growth rates in combined culture than in monocultures (more details of this study are given in Chapter 12).

In addition to these direct interactions, indirect effects can play a role in the establishment of communities. Duft *et al.* (2002) documented the colonization of azoic sediment in a stream when either meio- or macrofauna could enter the sediment or when macrofauna were excluded. Although nematode densities and species numbers were similar in the two treatments, community assembly differed. An indirect effect of the presence of gastropods was proposed, as they reduced algal biomass by grazing such that the nematode species composition changed to a larger proportion of species capable of reproducing under low food conditions.

Gray *et al.* (2015) systematically investigated the role of multiple biotic and abiotic factors on the establishment success of protozoans. In their experimental set-up, predator presence, the competitive dominance and initial density of the different species, and resource availability were manipulated to gain insights into factors contributing to the successful establishment of a species. The authors found that, for an intermediate level of resource availability, the initial density and competitive dominance of a species were the most important predictors of establishment success. However, the presence of a predator did not inhibit establishment success. Similar studies in freshwater nematodes are largely lacking even though nematodes are ideal organisms for investigating analogous dynamics (see Chapter 12).

5.5.2.2 Priority effects

Not only the number of propagules but also the timing of their arrival at a habitat can affect species establishment success. This temporal effect of the immigration of one species on that of another is referred to as priority effects (Fukami, 2015). Priority effects may be facilitative or inhibitory, such that the first species to arrive increases or decreases the probability of establishment success for a later arriving species. Most research on

priority effects has thus far focused on the inhibitory type (Fig. 5.4). In these cases, later-arriving species are negatively affected, as the availability of resources and/or space is reduced by the previously arrived species, or the latter is a predator of the arriving individuals.

Thus, differences in the timing of a species' arrival leads to different alternative stable states of communities (Beisner *et al.*, 2003; Schröder *et al.*, 2005). In an experimental set-up that included a freshwater community made up of different species, ranging from algae to micro- and meiofauna to macrofauna (but not nematodes), Drake (1991) showed that the sequence of species invasions resulted in different final community states, even though the initial species pool and environment were the same. While for freshwater nematodes comparable studies are not available, priority effects can be expected to impact their community dynamics as well. For example, an analysis of the genetic diversity of marine nematodes illustrated the importance of priority effects in the population structuring even of closely located patches (Derycke *et al.*, 2013).

5.6 Conclusions and Perspectives

Dispersal can be separated into three phases: emigration, transfer, and immigration, each of which is influenced by numerous factors that determine the outcome of a dispersal event. Consequently, direct investigations

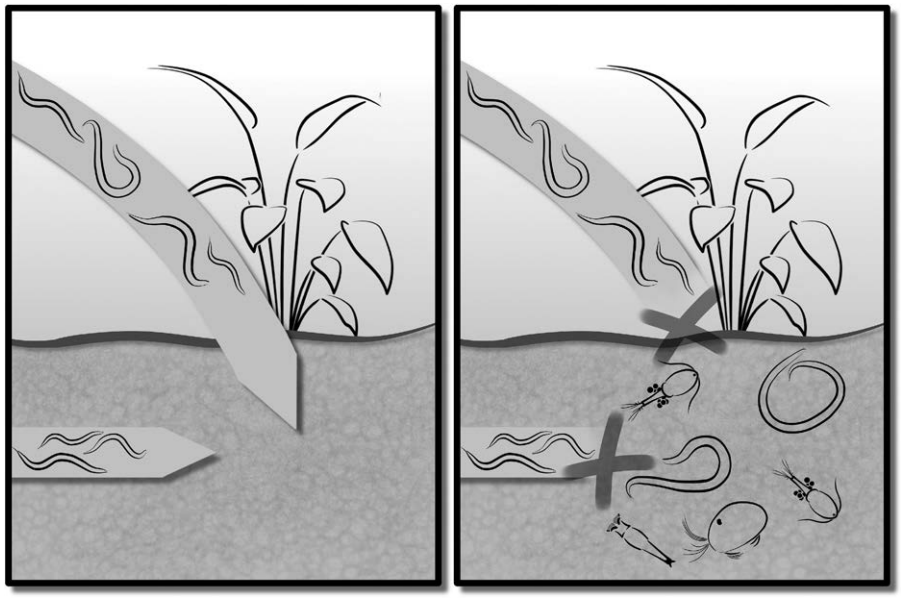

Fig. 5.4. Illustration of inhibitory priority effects: the immigration of a species to a habitat is more effective in the absence (left) than in the presence (right) of an already established community. (Author's own figure.)

of dispersal are often challenging. While emigration and immigration can be meaningfully studied in lab experiments or under controlled conditions in the field (for which freshwater nematodes are ideal, see Chapter 12), most information on transfer (e.g. dispersal pathways, rates, and distances) can be gained only from field observations. However, exploring transfer processes in the field for animals as small as nematodes is 'a logistical nightmare' (Kneitel and Chase, 2004), which in part accounts for the paucity of studies of the dispersal of freshwater nematodes. Yet, detailed insights into the dispersal characteristics of freshwater nematodes are essential to meaningfully interpret and eventually explain nematode distribution pattern (see Chapter 3). While dispersal is recognized as a central factor shaping nematode communities, metacommunities, and biodiversity, an understanding of *how* is a goal for future studies. Freshwater nematodes can serve as model organisms in studies aimed at answering this question, as they exhibit a broad range of dispersal modes, emigration, and immigration strategies. Moreover, with their well-established cosmopolitan distribution, it is clear that they are extremely successful dispersers.

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6 Feeding Ecology of Free-living Nematodes

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Highlights

- Nematodes are intermediaries in benthic food webs.
- The trophic structure of nematode communities may reflect ecosystem features.
- Various behavioral responses hinder omnivory and diet flexibility.
- Nematodes may stimulate microbial prey populations through ecological engineering effects.
- The development of molecular and isotopic biomarkers will facilitate studies of nematode trophic interactions.

6.1 Introduction

Nematodes are an integral part of benthic food webs and thereby important mediators of matter and energy fluxes (Majdi and Traunspurger, 2015), but compared with other biota little is known about the role of free-living

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nematodes in benthic ecosystems. Nematodes feed on bacteria, microalgae, plants, fungi, detritus, protozoans, and other metazoans, and may even benefit from dissolved organic matter. While the quantitative importance of any of these resources in the nutrition of nematodes has yet to be determined (Majdi and Traunspurger, 2015), numerous studies have reported correlations between the chlorophyll *a* content in biofilms and the abundance of nematodes (e.g. Peters and Traunspurger, 2005; Gaudes *et al.*, 2006; Vidaković and Palijan, 2010; Majdi *et al.*, 2011; Schroeder *et al.*, 2012; Kazemi-Dinan *et al.*, 2014), suggesting that nematodes use autotrophic biomass.

Nematodes that derive their energy from feeding on living autotrophic biomass are referred to as 'grazers' and belong to 'green food webs', although the term 'grazers' is also frequently applied to nematodes feeding on (heterotrophic) bacteria. Food webs based on the decomposition of dead organic matter are referred to as 'brown food webs'. However, as green and brown food webs are rarely well separated (Ruess and Ferris, 2004), disentangling the two sources of energy can be especially challenging in the case of nematodes that dwell both in phototrophic mats and in subsurface environments dominated by heterotrophic processes. For heterotrophic organisms such as nematodes, the ingestion of energy and material is closely coupled. In Box 6.1, some of the main processes relevant to a nematode's carbon and energy budgets are defined.

Given their size and ecological habits, nematodes establish complex trophic connections with microbial food webs, and their feeding behaviors may indirectly affect microbially driven ecosystem processes. Beneficial effects of nematodes on photosynthesis (Mathieu *et al.*, 2007) and bacterial activity (Traunspurger *et al.*, 1997) have been reported. In addition, the presence and activity of nematodes may increase ecosystem

Box 6.1. Trophic processes relevant to a nematode's carbon and energy budgets. (Author's own box.)

Ingestion (I)[a]: total uptake of energy or mass.
Absorption (A)[b]: that part of I that crosses the gut wall or epidermis.
Defecation: that part of I that is not absorbed, but leaves the gut as feces.
Growth: that part of A that is incorporated into the body tissue of the organism.
Reproduction: that part of A that is released as gametes or reproductive bodies.
Excretion: that part of A that is released from the body in the form of urine or other exudates (with the exception of reproductive bodies).
Respiration: that part of A that is released in association with the oxidation of organic compounds and thus causes a net loss of CO_2.

[a]The term 'consumption' is also used, and could be considered synonymous with 'ingestion'.
[b]The term 'assimilation' is often used as a synonym of absorption but it is likewise often poorly defined. According to Penry (1998), assimilation relates to anabolic processes, that is, the incorporation of absorbed products into animal tissue (see 'Growth'). Absorbed products that are not assimilated are used for catabolic processes (with the end products voided through respiration, excretion, and defecation).

productivity by enhancing the porosity of substrates and biofilm matrices to nutrients and oxygen. This process is referred to as 'bioturbation' but 'micro-bioturbation' may be a more accurate description as nematodes cannot push aside large sediment particles but they do dig holes in interstitial biofilms. This grazing activity of nematodes may also contribute to the dispersal of bacterial clumps via external or internal transport or the 'skim-off' of senescent bacterial layers, thereby maintaining bacterial activity and exponential growth (Ingham *et al.*, 1985; Neher, 2010). These indirect effects of nematode presence and feeding activity could explain why populations of bacteria exposed to nematode grazing are more productive and more diversified than populations of bacteria unaffected by nematodes (Traunspurger *et al.*, 1997; Jiang *et al.*, 2017).

The high species diversity of free-living nematodes is often attributed to a high level of niche specialization, with food as an important driving factor. The classification of marine, terrestrial, and freshwater nematodes into feeding types or feeding guilds (see, respectively, Wieser, 1953; Yeates *et al.*, 1993; Traunspurger, 1997) has been widely used to address the lack of species-level information on feeding behavior (Fig. 6.1). Nonetheless, the many unresolved questions on food sources and feeding rates limit the accuracy with which nematodes can be included in benthic food web models (Moens *et al.*, 2004).

The remarkable presence of nematodes worldwide (Van Den Hoogen *et al.*, 2019, see Chapter 3) requires a synthesis of the many facets of nematode feeding ecology in order to obtain a complete picture of the functioning of benthic ecosystems. Technological improvements in microscopy- and molecular-based techniques are enabling new and exciting avenues of research in the field of nematode trophic ecology (see Chapter 2). The aim of this chapter is to foster this research by providing an overview of the

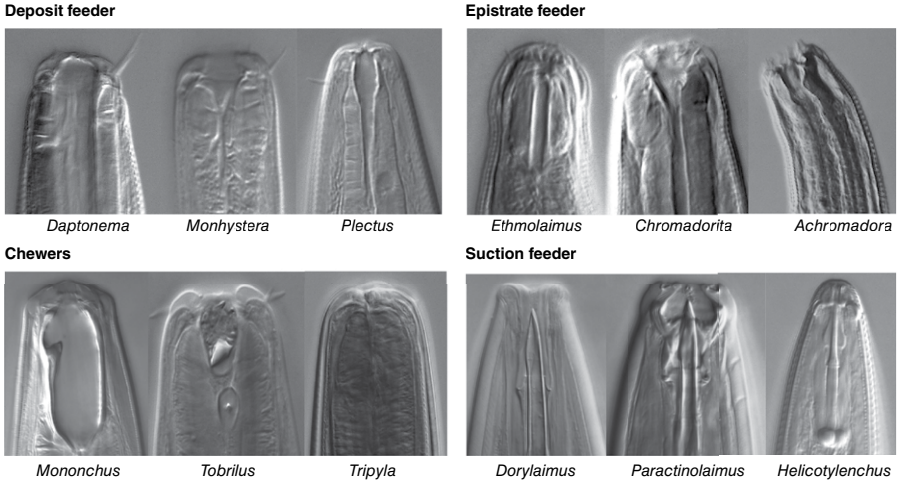

Fig. 6.1. Feeding types of freshwater nematodes. (Photo: Walter Traunspurger, Animal Ecology, Bielefeld, University.)

feeding habits and food sources of aquatic nematodes. The environmental constraints on feeding, food recognition, and feeding selectivity are also addressed, together with the complex, indirect trophic interactions between nematodes and their microbial prey. To raise awareness of the inherent methodological and/or interpretational problems in studies of nematode feeding ecology, the chapter ends with a brief look at the methods that have been adapted to quantify feeding rates in nematodes.

6.2 The Feeding Type Classifications of Free-living Nematodes

Species' biological traits can be used to describe biodiversity along functional features rather than in terms of taxa (e.g. Ristau *et al.*, 2015). These trait-based data can then be used to infer the ecological functions carried out by a community of species. In some cases, the recognition of biological traits requires empirical knowledge of the behavior and life history of a species in the field. In nematology, such knowledge is often lacking because the behavior and life history of nematode species are extremely difficult to assess in field studies. Some of those traits, however, can be studied using laboratory populations (e.g. Muschiol and Traunspurger, 2007; Muschiol *et al.*, 2009; Schroeder *et al.*, 2010; Fueser *et al.*, 2018), although transfer of the insights gained to the field is rarely straightforward due to the ever-changing, complex nature of biological interactions.

By contrast, the morphological traits of specimens collected in the field and mounted on slides can be measured relatively easily. In nematology, this has allowed buccal morphology to be linked to (presumed) feeding habits, thus compensating in part for the lack of species-level information on feeding behavior and other life-history traits. For nematodes in marine and brackish waters, the feeding type classification proposed by Wieser (1953), which is nearly exclusively based on stoma morphology, is still the most frequently used system. Wieser (1953) distinguished four trophic types. A primary subdivision splits nematode without (group 1) and with (group 2) a buccal armature (e.g. teeth, onchia, jaws); a secondary subdivision discriminates within each primary group mainly on the basis of small (group A) or large (group B) mouth cavities (Box 6.2).

In soils, nematode succession and competition between different nematode species for resources are well documented, which has allowed the use of some nematode taxa as functional indicators of soil status. For example, peaks in the abundances of Rhabditidae typically follow resource pulses (Yeates, 2003). Yeates *et al.* (1993) proposed one of the most comprehensive grouping of soil nematodes according to their feeding habits, with documented or presumed food sources listed by genus. Many soil nematode species bear stylet-like structures and are thus less restricted by the size of their mouth cavities for food intake. The classification of Yeates uses numbers (1–8) and differs from that of Wieser by focusing on observed or inferred food habits rather than stoma morphology. A summary of the

Box 6.2. Feeding-type classification of nematodes in marine and brackish waters, modified after Wieser (1953).

(1A) *Selective deposit feeders* have minute to small unarmed buccal cavities, allowing only particles in the bacterial size range to be ingested.
(1B) *Non-selective deposit feeders* also have unarmed buccal cavities, but they are more spacious than in the previous group. These nematodes ingest bacteria as well as larger-sized protozoan and occasionally even metazoan organisms.
(2A) *Epistrate feeders* possess a tooth, teeth, onchia, denticles, or similar structures in a relatively narrow buccal cavity and use them to scrape off particles from a substrate and/or to puncture algal cells before feeding on their contents.
(2B) *Predators/omnivores* typically have a more pronounced buccal armature and/or pharyngeal musculature than type 2A nematodes. Many feed as predators on protozoans or other metazoans, including other nematodes (Moens and Vincx, 1997; Moens *et al*., 1999b; Hamels *et al*., 2001a).

feeding types making up the classification by Yeates *et al*. (1993) and of the modifications proposed by Moens *et al*. (2004) is provided in Box 6.3.

Nematologists interested in freshwater species draw on either or both of the above schemes, or establish their own approach to the classification of trophic types. The scheme for classifying freshwater nematodes developed by Traunspurger (1997) is presented below and includes the main freshwater nematode families belonging to each feeding group (Fig. 6.1). This classification recognizes four major feeding types based on mouth morphology, and a total of six subcategories based on expected or reported feeding habits. For example, the feeding type *suction feeders* contains the subcategories *plant and fungal feeders* and *omnivores*.

(i) *Deposit feeders* or *bacterivores* have small unarmed buccal cavities (mouth opening diameter typically 1–5 µm). The absence of a stylet apparatus or sclerotized mouth cavity precludes these species from feeding on prey larger than their buccal cavity. Hence, only particles in the bacterial size range are likely to be ingested. In most freshwater ecosystems, deposit feeders are remarkably diverse and are generally the numerically dominant component of nematode communities (Fig. 6.2).

Nevertheless, since deposit feeders are generally small, accurate assessments of their relative abundance vs. that of other feeding types will be affected by the mesh size used to sieve the samples (see Chapter 2). The fact that deposit feeders possess no teeth also implies that their microbial prey are swallowed whole and thus either subsequently assimilated or dispersed via defecation. Also, feeding selectivity (if any) may be driven by mouth size, pumping rate, gut passage time, and digestive abilities. While there is little evidence that deposit feeders selectively feed on bacteria, bacterial density seems to affect the reproductive success of some species reared in the laboratory, which suggests their occupation in the wild of trophic niches based on fluctuations of bacterial density during ecological successions (Muschiol and Traunspurger, 2007; Muschiol *et al.*, 2015).

Box 6.3. Soil and aquatic nematode feeding-type classification, after Yeates *et al.* (1993) and the modifications proposed by Moens *et al.* (2004). (Author's own box.)

(1) *Plant feeders* comprise stylet-bearing nematodes that mostly feed on vascular plant tissues and fluids. This group is further subdivided according to feeding specialization (Yeates *et al*., 1993; Bongers and Bongers, 1998).
(2) *Hyphal feeders* comprise nematodes with a stylet that is used to pierce fungal hyphae. These species include obligate hyphal feeders but also the alternate life cycles of some parasites of invertebrates. This group also includes nematodes that feed on yeasts or fungal spores if the cells are pierced, not ingested.
(3) *Bacterial feeders* feed on prokaryotes and usually ingest bacterial cells whole. However, especially in species with a broad stoma, bacteria may not be the only food (see Moens *et al*., 2004, for examples).
(4) *Substrate ingesters* feed an organic substrate together with their associated microflora and -fauna, but whether the substrate, the microflora, or both are digested is unclear. Examples include the consumption of agar covered with a bacterial lawn by *Diplenteron colobocercus* (Yeates, 1998) and the ingestion of sediment particles by *Metoncholaimus scissus* (Meyers and Hopper, 1966). The term 'non-selective deposit feeders', often used in marine nematology (Wieser, 1953), suggests a more or less random ingestion of suitably sized and shaped particles, including the substrate, and is also applicable to freshwater and terrestrial nematodes.
(5) *Carnivores* are relatively large nematodes that use their teeth, stylet, and mandibles to feed on a variety of invertebrate metazoans.
(6) *Unicellular eukaryote feeders* feed on microalgae, fungal spores, yeasts, flagellates, ciliates, and/or other protozoa. This group comprises nematodes with 'unarmed' but fairly spacious mouth openings as well as epistrate feeders, both of which have (relatively small) teeth and/or teeth-like structures. The former typically ingest cells whole, while the latter pierce (e.g. Romeyn and Bouwman, 1983; Nehring, 1992; Moens and Vincx, 1997) or crack (Jensen, 1982; Moens and Vincx, 1997) the cells and then suck up their contents. The size and shape of the food particles greatly affect feeding success.
(7) *Free-living stages of animal parasites* are of little importance in aquatic systems and their feeding behaviors are those described above. Alternatively, these organisms are simply inactive and hence mostly irrelevant for studies on the trophic ecology of aquatic nematodes.
(8) *Omnivores*. Some dorylaimid and most oncholaimid nematodes are often cited as representatives of this feeding type. However, since omnivory refers to organisms feeding at more than one trophic level, the term equally applies to a variety of nematodes covered by feeding groups 3–6.

Typical *deposit-feeding* nematode families:

Mainly aquatic: Monhysteridae, Xyalidae, Cylindrolaimidae, Leptolaimidae, Aphanolaimidae, Rhabdolaimidae, Chronogastridae, Plectidae, Metateratocephalidae, Teratocephalidae, Alaimidae, Amphidelidae.

Mainly terrestrial: Cephalobidae, Panagrolaimidae, Rhabditidae, Protorhabditidae, Peloderidae, Mesorhabditidae, Diploscapteridae, Bunonematidae, Diplogastridae, Neodiplogastridae.

(ii) *Epistrate feeders* or *algivores* possess a tooth, teeth, onchia, denticles, or similar structures in the buccal cavity, which they use to scrape off particles from a substrate and/or to puncture cells before feeding on their contents. Observations with marine nematodes have demonstrated that food organisms

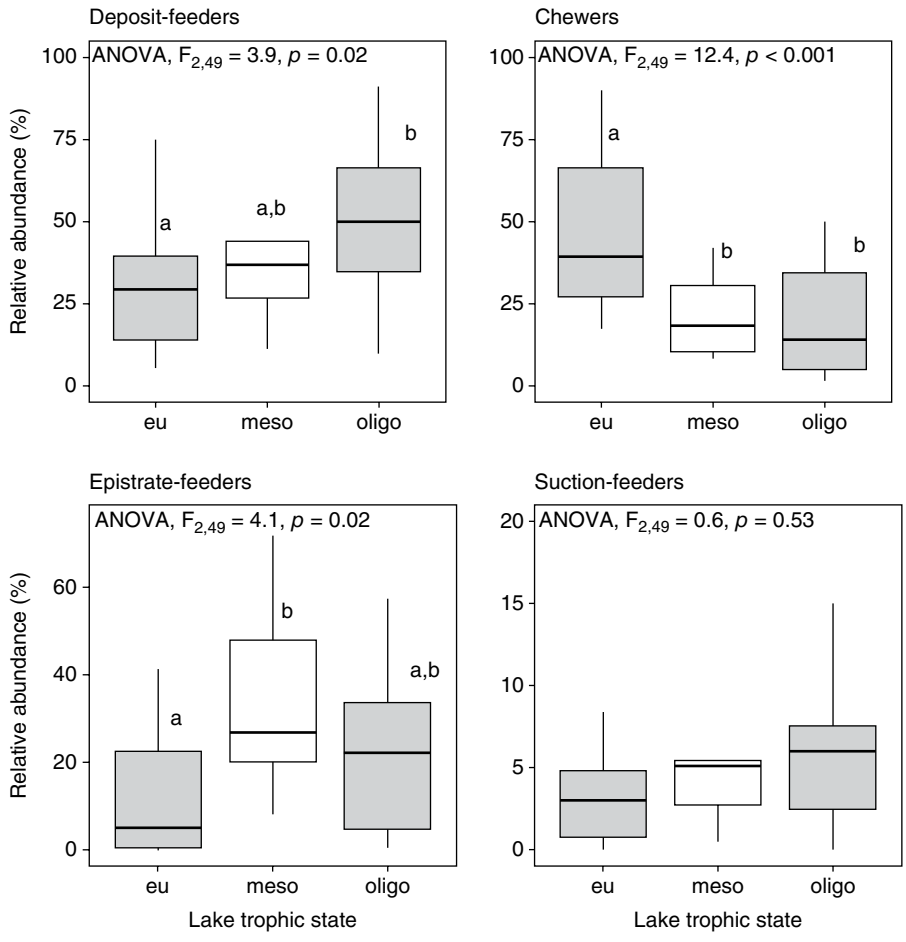

Fig. 6.2. Relative distribution of nematode feeding types in the sediment of 52 lakes differing in their trophic state, after the classification of Traunspurger (1997). Values are the averages determined from 11 published studies (see Table 6.1) and concern nematofauna in sediment samples collected using corers. Sampling was performed using mesh sizes between 35 and 45 µm. Nematofauna was extracted from the sediment using quantitative flotation methods (e.g. Ludox flotation). In most cases, the samples were stained, preserved in 4% formaldehyde, counted, and mounted on slides for species identification. Details of the literature search can be found in Traunspurger *et al*. (2020). (Author's own figure.)

can be pierced by a partly everted tooth, as in *Pareudiplogaster*, *Dichromadora*, or cracked after partial intake into the mouth, as in *Hypodontolaimus*, *Chromadorita* (Jensen, 1982; Romeyn and Bouwman, 1983; Romeyn *et al.*, 1983; Moens and Vincx, 1997). In freshwater ecosystems, epistrate feeders such as *Chromadorina*, *Chromadorita*, and *Punctodora* are extremely abundant in the algal biofilms growing on hard substrates in lakes and rivers (e.g. Gaudes *et al.*, 2006; Majdi *et al.*, 2011; Schroeder *et al.*, 2012). Common food sources for epistrate feeders include diatoms, other unicellular microalgae, and filamentous green and blue-green algae (Croll and Zullini, 1972;

Table 6.1. Average relative contribution (%) of feeding types of freshwater nematodes in the sediment of a selection of 52 lakes differing in their trophic state. (Author's own table.)

Lake	Country	Trophy	Mesh	DF	EF	C	SF	Reference
Obersee	GER	Eu	35	75	5	17	3	Michiels and Traunspurger (2004, 2005)
Mikolajskie	POL	Eu	45	6	7	84	3	Prejs (1977a,b)
CzarnaKuta	POL	Eu	45	80	0	20	0	Prejs (1977b)
Luterskie	POL	Eu	45	50	26	20	4	Prejs (1977b)
Narie	POL	Eu	45	10	0	90	0	Prejs (1977b)
Piecek	POL	Eu	45	21	0	78	1	Prejs (1977b)
Sasek	POL	Eu	45	37	1	62	0	Prejs (1977b)
Smolak	POL	Eu	45	10	0	90	0	Prejs (1977b)
Havgardssjön	SWE	Eu	35	32.7	29	27.1	11.3	Ristau and Traunspurger (2011)
Krageholmssjön	SWE	Eu	35	42.4	15.1	34.1	8.4	Ristau and Traunspurger (2011)
Krankesjön	SWE	Eu	35	19.5	41.5	31.8	7.2	Ristau and Traunspurger (2011)
Funtensee	GER	Eu	40	27.3	2.9	27.1	42.7	Traunspurger (1991)
Hopfensee	GER	Eu	35	31.6	3.2	62.5	2.7	Traunspurger (2001)
Löptinersee	GER	Eu	40	5.5	18.8	48.8	27	Traunspurger (unpubl.)
Postsee	GER	Eu	40	15.1	41.4	39.9	3.6	Traunspurger (unpubl.)
Donghu	CHN	Eu	45	38.5	0	38.5	23.1	Wu *et al*. (2004)
Älgsjön	SWE	Meso	35	44	20.2	30.6	5.2	Ristau and Traunspurger (2011)
Spitzingsee	GER	Meso	35	69.8	9.4	9	11.8	Traunspurger (2001)
Sulzbergersee	GER	Meso	35	78	8.2	10.2	2.7	Traunspurger (2001)
Faulersee	GER	Meso	35	15.1	71.7	8.1	5.1	Traunspurger (unpubl)
Haussee	GER	Meso	40	36.8	20.7	42.1	0.5	Traunspurger (unpubl)
Nehmitzsee	GER	Meso	40	29.8	47.8	18.5	4	Traunspurger (unpubl)
Schöhsee	GER	Meso	40	26.9	28.1	39.6	5.4	Traunspurger (unpubl)
Stechlinsee	GER	Meso	35	11	69.5	17.5	2.1	Traunspurger (unpubl)
Tiefersee	GER	Meso	40	42.1	26.7	20.3	11	Traunspurger (unpubl)
Brunnsee	GER	Oligo	35	72.3	2.1	25.2	0.4	Bergtold and Traunspurger (2004)
Ferchensee	GER	Oligo	35	72.4	23.7	2.1	1.8	Michiels and Traunspurger (2005)
Fereinsalm	GER	Oligo	35	80.9	2.2	9.2	7.7	Michiels and Traunspurger (2005)

Froschhauersee	GER	Oligo	35	49.2	33.3	15.5	1.9	Michiels and Traunspurger (2005)
Lautersee	GER	Oligo	35	35.1	57.3	4.8	2.8	Michiels and Traunspurger (2005)
Rehbach	AUT	Oligo	35	34.4	3.5	30.9	31.2	Michiels and Traunspurger (2005)
Schmalsee	GER	Oligo	35	53.6	34.6	3.6	8.2	Michiels and Traunspurger (2005)
Soiemsee	GER	Oligo	35	91.1	0.4	3.4	5.1	Michiels and Traunspurger (2005)
Traunsalpsee	AUT	Oligo	35	64.1	8.4	14	13.6	Michiels and Traunspurger (2005)
Vilalpsee	AUT	Oligo	35	66.4	25.1	2.2	6.3	Michiels and Traunspurger (2005)
Vilslache	AUT	Oligo	35	58.5	11.7	6.3	23.5	Michiels and Traunspurger (2005)
Wildensee	GER	Oligo	35	82.7	0.6	10	6.7	Michiels and Traunspurger (2005)
Char	CAN	Oligo	45	45	45	5	5	Prejs (1977a,b)
CzarnGasienicowy	POL	Oligo	45	10	32	44	14	Prejs (1977b)
MorskieOko	POL	Oligo	45	50	6	38	6	Prejs (1977b)
Wielki	POL	Oligo	45	15	20	50	15	Prejs (1977b)
Zadni	POL	Oligo	45	40	22	38	0	Prejs (1977b)
Zielony Gasienicowy	POL	Oligo	45	18	27	44	11	Prejs (1977b)
Fiolen	SWE	Oligo	35	21.2	33.7	38.2	6.9	Ristau and Traunspurger (2011)
Fjärasjö	SWE	Oligo	35	44	34	17.5	4.6	Ristau and Traunspurger (2011)
Hökesjön	SWE	Oligo	35	27.2	38.6	28.1	6.1	Ristau and Traunspurger (2011)
Skärsjön	SWE	Oligo	35	25.7	27	39.9	7.4	Ristau and Traunspurger (2011)
Grünsee	SWZ	Oligo	40	48.5	12	4.5	35	Traunspurger (1991)
Schwarzensee	AUT	Oligo	40	63.7	2.2	9.1	25	Traunspurger (1991)
Königssee	GER	Oligo	40	66.1	17.3	14.5	2.1	Traunspurger (1996 a,b)
Lustsee	GER	Oligo	35	58.6	38.4	1.3	1.7	Traunspurger (2001)
Constance	GER	Oligo	40	81.3	3.1	11.5	4.1	Witthöft *et al*. (2006, 2007)

Eu, eutrophic (N = 16); Meso, mesotrophic (N = 9); Oligo, oligotrophic (N = 27); DF, % deposit feeders; EF, % epistrate feeders; C, % chewers; SF, % suction feeders.

Deutsch, 1978; Gaudes *et al.*, 2006; Majdi *et al.*, 2012a,b; Estifanos *et al.*, 2013), but bacteria, ciliates, and other groups of organisms may be an equally important part of their diet (Moens and Vincx, 1997; Esser, 2006).

Typical *epistrate-feeding* nematode families:

Cyatholaimidae, Ethmolaimidae, Hypodontolaimidae, Chromadoridae, Microlaimidae, Prismatolaimidae.

(iii) *Chewers* (predators and omnivores) typically have a more pronounced buccal armature and/or pharyngeal musculature and a larger buccal cavity than epistrate feeders. This ability to handle a variety of prey items suggests a broad diet. Nevertheless, predation on other meiobenthic metazoans (e.g. other nematodes, tardigrades, rotifers, small oligochaetes) may be obligate in some species (e.g. Mononchidae, Ironidae, Oncholaimidae), and facultative in others (e.g. Tobrilidae, Tripylidae). These groups are classified as *chewers-predators* and *chewers-omnivores*, respectively. For example, some predatory nematodes may switch between protozoan and metazoan prey depending on their availability (Hamels *et al.*, 2001a). The scavenging of dead metazoans, for example, by marine oncholaimid nematodes, is also included within this feeding category (Jensen, 1987; Moens and Vincx, 1997). Quist *et al.* (2017) observed that predacious and omnivorous nematodes tend to show a higher degree of microscale patchiness than bacterivorous and fungivorous nematodes, indicative of a more complex and selective diet in the field. Generally, however, the feeding behavior of chewers is poorly understood and the role of this feeding type in benthic food webs remains to be determined.

Typical nematode families of *chewers*:

Mainly predator: Ironidae, Mononchidae, Mylonchulidae, Anatonchidae.

Mainly omnivore: Tobrilidae, Tripylidae.

(iv) *Suction feeders* comprise stylet-bearing nematodes with a tylenchoid stomatostylet or a dorylaimoid odontostylet. Both mouth structures are used in asymmetrical predation and are presumably important prerequisites for adaptation to ecto-parasitism. Suction feeders are extremely abundant in the rhizosphere of plants, where parasitic as well as free-living species feed on vascular plant tissues, roots, fungi, and fluids. In addition, some species may pierce algae, lichens, or mosses (e.g. Dorylaimidae). In soil ecosystems, these species are grouped as 'plant feeders' obtaining their food from living plant cells, and further subdivided according to feeding specialization and practical aspects of their parasitic lifestyles (Yeates *et al.*, 1993; Bongers and Bongers, 1998; Bilgrami and Gaugler, 2004). In freshwater ecosystems, species with particularly delicate stylets may feed on plant epidermal cells and root hairs (e.g. Tylenchidae). Suction feeders also include facultative or obligate hyphal feeders feeding on fungal hyphae and with a relatively narrow prey range (Dighton *et al.*, 2000). Fungal feeders may be grouped together with other root feeders as *suction feeders-plant and fungal feeders*. Finally, some large dorylaimid species are able to pierce and penetrate the cuticles of other animals and

are considered typical omnivores (feeding on a variety of prey ranging from algae and ciliates to larger invertebrates); these nematodes can be subcategorized as *suction feeders-omnivores*.

Typical *suction-feeding* nematode families:

Mainly plant and fungal feeders: Aphelenchidae, Paraphelenchidae, Aphelenchoididae, Tylenchidae, Dolichodoridae, Pratylenchidae, Hoplolaimidae, Paratylenchidae, Criconematidae, Hemicycliophoridae, Neotylenchidae, Trichodoridae, Longidoridae.

Mainly omnivore: Dorylaimidae, Actinolaimidae, Qudsianematidae, Aporcelaimidae, Nordiidae.

A detailed overview of the trends in the relative abundances of the different nematode feeding types in freshwater habitats is beyond the scope of this chapter (see Chapter 3). It is clear, however, that important differences exist between different freshwater environments as well as within habitats, for example, with season, bathymetry, and sediment depth (e.g. Traunspurger, 1997). As an example of those trends, the box plots in Fig. 6.2 show the average relative distribution of nematode feeding types in the sediment of 52 lakes, mostly from central and northern Europe and spanning a gradient from oligo- to eutrophic. A detailed analysis that includes hard substrates can be found in Traunspurger *et al.* (2020). In summary, deposit feeders have a stronger presence in oligotrophic than in eutrophic lakes whereas the opposite is true for chewers (Fig. 6.2). Interestingly, epistrate feeders, which likely derive most of their energy from green food webs, seem to thrive best in mesotrophic lakes. These observations, although quite variable and requiring additional experimental validation (but see Chapter 3, and Traunspurger *et al.*, 2020), raise questions regarding the effect of lake trophic state on benthic biota and the simple segregation of feeding types along a trophic gradient. This opens interesting perspectives on the use of the feeding-type structure of nematode communities as a bioindicator of lake eutrophication.

Nevertheless, assignment to feeding categories is in part subjective. In the field, nematode communities are often characterized by a high number of species with similar feeding types but coexisting in small patches (Gansfort *et al.*, 2018a). Furthermore, although feeding habits are clearly linked to stoma morphology, the extrapolation of this relationship to specific feeding strategies may be misleading. Very similar stomal structures can be used for very different feeding behaviors, as exemplified by the range of feeding strategies exhibited by suction feeders. Conversely, different morphologies may serve very similar feeding behaviors (Moens *et al.*, 2004). For example, stylets may be used by suction feeders to feed on other animals, but chewers can do so using their large, sclerotized mouth cavities. Nonetheless, feeding type-based classifications may help to infer the most probable interactions occurring within a community. For instance, Sieriebriennikov *et al.* (2014) used nematode feeding traits to evaluate soil ecosystems. However, we recommend caution and, if possible, the inclusion of additional measures of diet, such as stable isotopes (Estifanos *et al.*, 2013) or gut content analyses (Kazemi-Dinan *et al.*, 2014), in drawing conclusions based on feeding types.

6.3 Particulate versus Dissolved Food

Nematode feeding-type classifications imply that nematodes feed on particulate organic matter (POM). However, concentrations of dissolved organic matter (DOM) by far surpass those of POM. The DOM concentration is generally higher in freshwater than in marine environments (Wetzel, 2001) and especially concentrated in sediment pore water (Thomas, 1997). DOM includes non-humic substances such as carbohydrates, proteins, peptides, free amino acids, fats, waxes, pigments, and other low-molecular-weight compounds that are generally labile, easily degradable, and characterized by rapid flux rates. DOM also includes higher-molecular-weight humic substances that are complex mixtures of molecules bearing a diversity of phenolic and carboxylic groups. DOM is an important source of energy for bacteria, but also for other consumers (Hipp *et al.*, 1986; Thomas *et al.*, 1990; Wilcox *et al.*, 2005; Augspurger *et al.*, 2008).

Montagna (1984) used radiolabeled glucose to show that free-living nematodes from intertidal sediments can incorporate DOM. In laboratory studies, glucose was also absorbed by the marine nematodes *Pontonema vulgare* and *Adoncholaimus thalassophygas* (Chia and Warwick, 1969; Lopez *et al.*, 1979). The latter species is also capable of utilizing acetate, a product of microbial fermentation (Riemann *et al.*, 1990). Acetate and glucose are used for amino acid synthesis by some secernentean nematodes (*Ditylenchus triformis*, *Meloidogyne incognita*, *Caenorhabditis briggsae*, *Aphelenchoides rutgersi*) (summarized in Nicholas, 1984). Acetate, stearate, and oleate are also used by *C. briggsae* and *Panagrellus redivivus* in the *de novo* synthesis of polyunsaturated fatty acids (PUFAs) (Rothstein, 1970), which represents a unique biosynthetic capability in multicellular animals. Nematode uptake of dissolved compounds such as acetate for the synthesis of essential PUFAs may have interesting implications for trophic transfers (Menzel *et al.*, 2018). Specifically, this source of PUFAs may represent an alternative for larger invertebrates and fishes to essential algal-derived PUFAs. This was demonstrated in experiments showing that mortality was lower in carp larvae fed a *P. redivivus* diet than a diet of zooplankton (Schlechtriem *et al.*, 2004) and that growth and fertility were reduced in collembolans fed PUFA-defective vs. non-PUFA-defective *C. elegans* (Menzel *et al.*, 2018).

DOM composition exerts differential effects on nematode growth and/or reproduction. This was shown in laboratory experiments in which the effects of different components of refractory DOM (humic substances) on the reproduction of *C. elegans* were examined. The negative effects of fulvic acids isolated from a humic lake were attributed to a reduction in bacterial activity; whereas positive effects were determined for other fulvic acids and ultrafiltrates isolated from other sources such as soil leachates and groundwater (Höss *et al.*, 2001). Humic substances were also shown to influence behavior (chemotaxis) and genome expression in *C. elegans* (Menzel *et al.*, 2005).

However, a major methodological difficulty in assessing the potential contribution of DOM to nematode nutrition is separating direct effects from

bacterially mediated uptake processes. DOM may be ingested directly by nematodes, but it may also be taken up via adsorption to (bacterial) cells, exudates (Höss *et al.*, 2001; Steinberg *et al.*, 2002), exopolymeric secretions from bacteria and/or microalgae (Decho and Lopez, 1993; Lundqvist *et al.*, 2012), and even via the mucus secreted by nematodes themselves (Riemann and Schrage, 1978). The direct and indirect consumption of DOM can be disentangled in time-series labeling experiments in which, for example, the accumulation of ^{13}C assimilated by nematodes and their prey from an enriched ^{13}C-DOM source is measured. Immediate and ephemeral enrichment in nematodes indicates the direct assimilation of DOM, and delayed and longer-lasting enrichment the uptake of the label by grazing on DOM-fixing microbes. This was the approach followed by Pape *et al.* (2013), who showed greater enrichment in deep-sea nematodes than in bacteria fed ^{13}C-glucose, indicative of the direct assimilation of glucose by nematodes. It thus seems likely that DOM contributes directly to the nutrition of nematodes, but its uptake routes (cuticular vs. gut uptake) and their significance at broader scales remain to be established in further experiments.

6.4 Abiotic and Biotic Constraints on Nematode Feeding

Nematode feeding activity is influenced by a variety of environmental factors. These include substrate characteristics, such as sediment texture and pore size, both of which may affect the activity (and efficiency) of consumers and resources. For example, Elliott *et al.* (1980) observed soil texture-dependent trophic-level switching by *Mesodiplogaster*, from bacterivory to predation on amoebae. Temperature has obvious effects on nematode metabolism and reproduction rates and therefore on the energetic requirements of nematodes (e.g. Laybourn, 1979; Venette and Ferris, 1997; Moens and Vincx, 2000; Ayub *et al.*, 2013; Majdi *et al.*, 2019). However, temperature may also determine the daily behavior and spatial position of nematodes in soils, sediments, and biofilms (see Section 6.5).

Another crucial constraint on nematode feeding rates is the capacity of nematodes to react to changes in resource availability and quality. Analyses of gut pigment contents in biofilm-dwelling nematodes showed a linear correlation of the concentrations of chlorophyll *a* and its degradation products with the concentration of chlorophyll *a* in the biofilm throughout the year, suggesting that nematode grazing increased with the increasing availability of phototrophs in the biofilm (Majdi *et al.*, 2012b).

Interestingly, ingestion (consumption) and absorption rates may show different food density-dependent patterns, as may absorption and respiration rates (Schiemer, 1985). This was demonstrated in studies of the ingestion (Moens *et al.*, 1996) and absorption (Moens and Vincx, 2000) rates of *Litoditis marina* as a function of bacterial (food) density, which in both cases showed that absorption efficiencies are food density-dependent. Absorption rates in nematodes may also be influenced by the efficiency of

the gut microbiome in converting inedible food items into digestible food, with both food quantity and quality impacting energy budget parameters. For example, Derycke *et al.* (2016) found that nematode gut microbiomes differed between species and even between individuals of the same species, and that the composition of the gut microbiome changed according to the type of food ingested. These characteristics have far-reaching consequences for nematode feeding strategies, the structure of bacterial communities, and, ultimately, microbially driven ecosystem processes.

Foraging involves a variety of trade-offs, such as between energy expenditure on feeding (ingestion and digestion) vs. on searching for optimal feeding conditions (Weber and Traunspurger, 2013). Independent of other environmental variables to maximize energy gains a nematode in a patchy environment must choose between two options: moving to a better feeding location or foraging within a given patch. The choice will depend on the nematode's response to a given type of food but also on the presence, suitability, and detectability of alternative food sources.

A better understanding of the constraints on feeding rates requires a consideration of the interactions (competition or facilitation) between different nematode species sharing resources within the same habitat patch, as is frequently the case in the field (Jensen, 1987; Ettema, 1998; Michiels *et al.*, 2004; De Mesel *et al.*, 2006; Gansfort *et al.*, 2018a,b). In the case of bacterivorous nematodes, the coexistence of *Panagrolaimus* cf. *thienemanni* (Panagrolaimidae) and *Poikilolaimus* cf. *regenfussi* (Rhabditidae) within the microbial mats in the chemotrophic environment of Movile Cave (Romania) is a remarkable example. In a laboratory study of these two species, Schroeder *et al.* (2010) showed that *Panagrolaimus* grows best at high densities (>10^9 cells/ml) of its *E. coli* food, and *Poikilolaimus* at lower densities (5×10^8 cells/ml) of this food source. Weber and Traunspurger (2013) conducted a food-choice experiment to examine the food preferences of these nematodes, which showed that the two species were rarely found sharing the same *E. coli* density spot. These results provide further support for niche differentiation based on food density levels. In the field, *Panagrolaimus* cf. *thienemanni* and *Poikilolaimus* cf. *regenfussi* occupy different ecological succession stages of floating microbial mats that differ in their bacterial densities, thus corroborating the laboratory observations (Muschiol *et al.*, 2015). More recently, Gansfort *et al.* (2018b) manipulated food density levels and starting density of these nematode species in monospecific and mixed cultures to investigate the magnitude and trajectory of potential competitive effects. A positive interaction was observed between the two species, in which the presence of one consistently promoted the population growth of the other. Moreover, the results confirmed those of Schroeder *et al.* (2010), in that *Panagrolaimus* was competitively superior under high, and *Poikilolaimus* under low bacterial densities. Gansfort *et al.* (2018b) also found a strong shift in the expected growth rates of both species when grown on culture plates. As the bacterial food density did not change during the course of the experiment, the observed positive influence of coexistence on

the growth rate of each species implied a facilitative association of the two species. It is plausible that kairomones, used by nematode species for mating (Choe *et al.*, 2012) and food tracing (Hong and Sommer, 2006), boosted the growth of *Panagrolaimus* and *Poikilolaimus*, regardless of food competition constraints.

6.5 Living in an Information-rich Context: Food Recognition

Nematodes are capable of extracting many different types of information from and about their immediate environment. Their responses to different stimuli, especially taxis toward food and food-related factors (Huettel, 1986; Perry, 1996; Baldwin and Perry, 2004) and their vertical position in response to temperature (Dusenbery, 1974, 1989) have been the focus of many studies. In a study conducted in tidal flats subject to a submersion/emersion cycle, the observed positional changes were interpreted as a response to drying disturbance or to the migration of competitors and their resources (Steyaert *et al.*, 2001). Interestingly, nematodes can 'learn', exemplified by their ability to respond to temperatures associated with optimal food conditions (Hedgecock and Russell, 1975; Mohri *et al.*, 2005). So far, chemical olfaction in nematodes has been investigated mostly in terrestrial species, such as studies on the taxis of parasitic nematodes toward their mammalian, insect, and plant hosts (Dillman *et al.*, 2012; Gang and Hallem, 2016). *Caenorhabditis elegans* can distinguish between olfactory (volatiles or odor compounds) and gustatory (water-soluble or dissolved compounds) cues, which are linked to separate receptor sets (Bargmann and Mori, 1997). The complex behavioral responses of nematodes to external cues are triggered by relatively simple neuronal circuits that are partly conserved across different nematode species (Rengarajan and Hallem, 2016).

The chemically based sense of orientation in free-living aquatic and limno-terrestrial nematodes has not been well investigated. Recognition of food hotspots may provoke the aggregation of individuals, as observed *in vivo* in food-choice experiments (e.g. Jensen, 1982; Moens and Vincx, 1997; Höckelmann *et al.*, 2004; Bilgrami *et al.*, 2005; Salinas *et al.*, 2007; Hohberg and Traunspurger, 2009; Weber and Traunspurger, 2013). Those studies provided evidence for an attraction toward a given food. In more focused experiments, the limno-terrestrial nematode *Bursilla monhystera* and other freshwater nematodes isolated from a lake littoral exhibited clear chemotaxis to the volatile compounds produced by cyanobacterial mats. The responses of *B. monhystera* and *Plectus* sp. were not elicited by a single compound but rather by mixtures of different volatiles (Höckelmann *et al.*, 2004). Other lines of evidence come from observations of nematode movements in response to indirect proxies for food availability. For example, *Adoncholaimus thalassophygas* moves up gradients of (dissolved) CO_2, which may guide nematodes to patches of intense microbial activity and mineralization (Riemann and Schrage,

1988). Whether chemical recognition in nematodes is sufficiently well developed such that it can explain the often highly species-specific preferences determined in multiple food-choice experiments (e.g. Weber and Traunspurger, 2013) remains to be seen. Moens *et al.* (1999a) found that four coexisting species of Monhysteridae responded differently to the presence of bacteria in their immediate environment, including not only to the bacterial strains offered but also to the density, age, growth conditions, and activity of the bacteria.

Prey recognition by predacious nematodes has also been investigated. Some mononchid predators apparently locate living prey from a distance (Jairajpuri and Bilgrami, 1988), whereas *Aporcelaimellus nivalis* largely depends on chance encounters with its prey (Jairajpuri *et al.*, 1991). The latter species, however, is able to perceive injured or dead prey from a distance, as do several other predacious nematodes (Jairajpuri and Bilgrami, 1988; Bilgrami *et al.*, 2001). Moreover, olfaction can be used to avoid the deleterious effects of cannibalism on fitness. This was demonstrated in *Pristionchus pacificus*, which depending on environmental conditions, can switch to become a potent nematode predator. This capability includes the secretion of small, hyper-variable peptide compounds that adhere to this nematode's surface and confer self-recognition and prevent predation by members of the same species (Lightfoot *et al.*, 2019). Additional studies are needed to explore the suite of behaviors exhibited by predatory nematodes when sensing prey from a distance, as well as the potential predator avoidance behaviors adopted by nematode prey when exposed to 'alarm' cues. In fact, these topics can be readily addressed by applying the simple and inexpensive experimental design proposed by Stilwell *et al.* (2017) for classroom science.

6.6 Feeding Selectivity and Intraguild Diversity

The information-rich environment inhabited by nematodes, with its variety of potential food sources, implies an ability of these organisms to select the food that best meets their energetic needs but with the lowest acquisition cost. Trophic groupings tend to consider all members of one group as potential consumers of the entire resource class. The high species diversity of nematodes, by contrast, is often attributed to a high level of niche specialization, with food as a major driving factor. Thus, feeding selectivity and flexibility may allow intraguild resource partitioning in nematodes.

The wide-ranging morphological differences in buccal cavities, lips, appendages, etc., among bacterivorous nematodes suggest a variety of strategies for obtaining bacterial food and a specialization for parts of, rather than the entire bacterial resource. Rhabditidae and Panagrolaimidae feed continuously when sufficient food is available and obtain bacteria mostly from biofilms or suspensions of free-living cells in interstitial water. Cephalobidae and Wilsonematidae (Plectidae), by contrast, feed

more intermittently even when food availability is low. The complex cephalic structures and active sweeping motions of these species during feeding suggest that they are specialized for collecting bacteria attached to or associated with a substrate (Moens *et al.*, 2004).

Differences in digestive efficiency may also affect resource partitioning between closely related species, consistent with the biochemical and ultrastructural differences between the intestinal cells of, for example, different chromadorid and rhabditid nematode species (Deutsch, 1978; Borgonie *et al.*, 1995). In a laboratory study, Estifanos *et al.* (2013) offered an isotopically labeled diet consisting of either *E. coli*, *Matsuebacter* sp., or both to *C. elegans*, *Acrobeloides tricornis*, *Panagrolaimus*, and *Poikilolaimus* sp. Differences in the isotopic signatures were linked to differences in the capacity of these nematodes to assimilate the offered monobacterial diet. Feeding selectivity was examined in mixed bacterial diet experiments, which showed that the largest contribution to the carbon supply of *C. elegans* was by *E. coli*, and to that of *A. tricornis* by *Matsuebacter* sp. In a food-choice experiment with *C. elegans*, Abada *et al.* (2009) observed clear feeding selectivity for certain soil bacteria over the standard *E. coli* food and a longer lifespan of worms feeding on their preferred food.

Other experiments have examined the algal feeding selectivity of biofilm-dwelling nematodes from rivers and lakes, through the detection and quantification of algal biomarker pigments in their guts (Majdi *et al.*, 2012b; Kazemi-Dinan *et al.*, 2014). The detection of diatom biomarkers in nematode guts suggested preferential grazing on diatoms over other available microphytes (cyanobacteria, chlorophytes). However, in some cases (shallow littoral zones of Lake Erken and Lake Largen), chlorophytes were ingested preferentially.

The diet of predatory nematodes in freshwater ecosystems has not been well examined, but field experiments and laboratory observations of the feeding behaviors of soil and marine predatory nematodes have provided compelling evidence of marked inter-specific and even intra-specific selectivity (Bilgrami *et al.*, 1984, 2005; Bilgrami, 1993; Prejs, 1993; Khan *et al.*, 1995; Moens *et al.*, 1999b, 2000; Bilgrami and Gaugler, 2005; Leduc *et al.*, 2015). Predatory nematodes tend to select their prey based on their availability in the habitat but also on their chemical, behavioral, and morphological traits (e.g. the ability to secrete deterrent substances or make use of escape strategies, protection by a thick cuticle, defensive structures).

Although several experiments have yielded clues regarding the feeding selectivity and flexibility of free-living nematodes, insights into the underlying mechanisms have yet to be gained. However, an understanding of the consequences of the feeding selectivity of free-living nematodes at meaningful scales is pivotal to recognizing the functional implications of diversity and of species redundancy within trophic levels. Species are functionally redundant if they share the same function or trophic level and do so with similar efficiency (Lawton, 1994). Mikola and Setälä (1998) demonstrated that bacteria-feeding nematodes perform species- rather than guild-specific functions in soil food webs, which implies that functional

redundancy is low. This is further supported by the observation that selective grazing by monhysterid nematodes results in shifts in bacterial community composition that may differ even among congeneric nematodes (De Mesel *et al.*, 2004, 2006; Derycke *et al.*, 2016). Functional redundancy does, however, occur and may be facilitated by patchy population dynamics (Gansfort *et al.*, 2018a,b). Together, nematode species diversity and trophic niche differentiation mechanisms thus provide an intriguing system for fundamental ecological studies on the relationship between biodiversity and ecosystem functioning (Moens *et al.*, 2004).

6.7 Complex Interactions between Nematodes, Their (Microbial) Resources, and Microbially Driven Ecosystem Processes

Many studies have documented correlations between nematode abundances and both nutrient recycling and organic matter turnover. Rather than being passively dependent on the availability of resources, free-living nematodes affect both the availability of labile organic matter and production by the microorganisms on which they feed. For example, nematode densities increase rapidly after the sedimentation of a phytoplankton bloom on a lake bottom (Goedkoop and Johnson, 1996), but decrease if the sedimentation of organic matter is hindered, as shown in field and laboratory experiments (Bergtold and Traunspurger, 2005). Similarly, nematode densities positively react to the deposition of river-borne detritus, and the magnitude of this positive response may even exceed that of benthic microbial communities (Witthöft-Mühlmann *et al.*, 2005). Bioturbation by nematodes and other meiofauna may help to increase the fluxes of oxygen and nutrients to microbial decomposers and thus stimulate mineralization (Cullen, 1973; Alkemade *et al.*, 1992; Aller and Aller, 1992; Nascimento *et al.*, 2012; Bonaglia *et al.*, 2014). Several experiments in phototrophic biofilms have shown that the presence of nematodes tends to stimulate photosynthesis and primary production (Pinckney *et al.*, 2003; Mathieu *et al.*, 2007; D'Hondt *et al.*, 2018), presumably through the maintenance of biofilm porosity to nutrients and light or through the limitation of microbial overgrowth.

Other interactions between nematodes and microbiota, especially in aquatic ecosystems, have been documented, with the most important including: (i) the secretion of mucus, which by trapping sediment particles contributes to biofilm and sediment structure and stability, perhaps also to DOM uptake and transformation (see Section 6.3), and in some cases to the increased growth of microbiota locally (Riemann and Schrage, 1978; Nehring *et al.*, 1990; Hubas *et al.*, 2010; D'Hondt *et al.*, 2018); (ii) effects on bacterial community composition and diversity through (selective) grazing, the founder effects of mucus production, or both (De Mesel *et al.*, 2004; Moens *et al.*, 2005); and (iii) intricate nematode–ectosymbiotic chemotrophic bacteria interactions, which have been particularly well documented in some groups of marine nematodes (Polz *et al.*, 1994;

Nussbaumer *et al.*, 2004; Ott *et al.*, 2004; Bellec *et al.*, 2019). Riemann and Helmke (2002) demonstrated enzymatic activity in the mucus trails of the nematode *Adoncholaimus thalassophygas* and proposed that both nematodes and microbiota attracted to the mucus contribute to a common exoenzyme pool from which they benefit through the dissolved nutrients released by its enzymatic activity ('enzyme-sharing'). The general validity of these observations and their relevance for benthic systems remain to be established.

6.8 Measuring Feeding Rates: An Overview of Methodologies

The methods used to assess the feeding rates of nematodes can be roughly divided into four categories: (i) direct observations, (ii) gut content analyses, (iii) measurements of (decreases in) food density, and (iv) tracer techniques, including those based on the use of a fluorescent tracer and on radioactive or stable isotopic signatures. The latter elemental markers have the advantage that they are incorporated into nematode tissues, which facilitates measurements of absorption rates. Some of the applications of these approaches but also the problems inherent to them are briefly considered in the following. For a detailed discussion of the methodologies used in trophic ecology, the reader is referred to Majdi *et al.* (2018, 2020) and to the topical papers cited below.

Direct observations of living nematodes constitute the most straightforward approach to examining feeding behaviors, but given the minute size of free-living nematodes such studies are mostly restricted to the laboratory and conducted in artificial media. They are most feasible when the food particles are not too small compared with the consumer. Direct observations can be used to study attraction toward a food source, inter- or intra-specific interactions, and other behavioral traits. Miniaturized microcosms that can be mounted under a microscope (e.g. micro-flow chambers/micro-fluidic slides; see Esser, 2006; Stilwell *et al.*, 2017) or larger Petri dishes inoculated with semi-natural communities (Moens and Vincx, 1997) and observed under an inverted microscope or stereomicroscope enable the behavior of nematodes to be monitored in an environment relatively close to field conditions. However, the information that can be obtained from direct observations is qualitative rather than quantitative. An exception is the well-established counting of pharyngeal pumping rates to quantify food consumption by rhabditid nematodes (Mapes, 1965; Moens *et al.*, 1996). Rhabditids are bacterivores that possess a valve apparatus in the pharyngeal metacorpus, the movements of which are fairly easy to observe. Other nematodes, however, may lack these features such that accurate counts of pharyngeal pulsation rates are only possible when the organism moves slowly and in a plane. However, Duncan *et al.* (1974) were able to count the pumping movements of the freshwater *Plectus palustris* (Plectidae), from which the authors estimated a mean grazing rate of 5000 bacterial cells/min and a gut-filling time between 3 and 10 min.

The volume of medium ingested per pulsation can be estimated from the volume dilation of the pharynx (De Soyza, 1973; Woombs and Laybourn-Parry, 1984); if the food particles are evenly distributed through the medium, food consumption can be calculated as well.

Gut content analyses also generate qualitative information, but caution is warranted in their interpretation, since this approach provides only a snapshot of the diet, the information relates to substrate ingestion rather than utilization, and the method is chiefly limited to recognizable food items transiting in the gut. Biomarker-based analyses of gut contents, such as using pigments or DNA, can overcome the latter drawback and have been used to study microalgal grazing, with gut pigments identified by high-performance liquid chromatography (HPLC) or confocal laser-scanning microscopy (CLSM) (Majdi *et al.*, 2012b; Mialet *et al.*, 2013; Kazemi-Dinan *et al.*, 2014). The sample size needed for reproducible measurements mainly depends on the targeted pigment(s) and the technique: while several hundreds of nematodes will have to be cleaned and extracted for HPLC-based analysis, the autofluorescence of chlorophyll and carotenoid pigments can be detected from a single specimen mounted on a slide and studied under CLSM. The two techniques have their advantages and disadvantages but their combined use can provide robust conclusions. Specifically, HPLC is quite labor-intensive in terms of nematode sorting and cleaning, but it allows a more detailed quantification of the different mixture of pigments (including biomarker carotenoids) present in the guts of a population of nematodes. CLSM needs less sorting and yields qualitative information on the spatial distribution of pigmented items in the gut of a single individual, but only broader categories of pigments will be detected (e.g. the technique is probably not accurate enough to discriminate among biomarker carotenoids). Consumption rates can be estimated if the gut passage time is known, but this information is scarce (Duncan *et al.*, 1974; Avery and Thomas, 1997; Ghafouri and McGhee, 2007). In addition, several fixation procedures commonly used to kill and preserve nematodes tend to induce partial voidance of the gut contents and are thus incompatible with analyses of gut pigment contents (Moens *et al.*, 1999a). As an alternative, field samples can be fixed in liquid N_2 followed by long-term storage at –80°C, which causes the gut contents to congeal while the pigments remain unaltered.

Gut content analyses can also be improved by the use of molecular tools to help identify ingested items. For example, nematode DNA can be amplified from other organisms as evidence of predation on nematodes. Heidemann *et al.* (2014) found nematode DNA in a variety of soil mites preying and scavenging upon nematodes in soils. Polymerase chain reaction (PCR) amplification in reactions that included nematode primers revealed micro-turbellarians as potent predators of nematodes in marine ecosystems (Maghsoud *et al.*, 2014). Alternatively, microbial 16s rRNA genes can be sequenced from nematode specimens to investigate microbiomes associated with nematodes, such as their gut microflora and its response to different offered diets (Derycke *et al.*, 2016).

Measurement of (decreases in) food density in laboratory microcosm experiments is a fairly straightforward method to evaluate food consumption by consumer organisms, provided adequate controls without consumers are included in the experimental design. This approach has, for instance, been used to quantify bacterivory in *Caenorhabditis briggsae* (Nicholas *et al.*, 1973), diatom and ciliate grazing in *Chromadorina* sp. (Esser, 2006), predation rates of *Enoploides longispiculosus* on other nematodes and ciliates (Moens *et al.*, 2000; Hamels *et al.*, 2001a), and *Prionchulus muscorum* predation on other nematodes (Kreuzinger-Janik *et al.*, 2019). If prey organisms do not multiply over the time course of the incubation, the ingestion rate can be readily calculated from the rate of disappearance of prey organisms per unit time. If prey items do multiply, as would be the case for bacteria and protozoans during experiments of more than 1 h, the prey growth rate in the absence of predators also has to be determined. To allow extrapolation to field conditions, the prey and predator densities (as well as environmental conditions) of the incubations should closely match those found in the field. An extension on this approach, potentially yielding feeding rates relevant to field conditions, is a comparison of changes in food density in micro- or mesocosms in which consumer densities are manipulated vs. experimental units with natural consumer densities. This design has been applied to evaluate the ecosystem-level effects of the presence of nematodes (Traunspurger *et al.*, 1997; Mathieu *et al.*, 2007).

Tracer techniques include the addition of tracer as free label (inorganic or organic) or as prelabeled food particles. In the latter case, experiments conducted under controlled conditions are particularly useful for studying food selectivity, but less so for determining absolute grazing rates pertinent to field situations. Most experiments on aquatic meiofauna reported in the literature have been performed to determine (community) grazing rates on bacteria or microalgae after the direct addition of radioactive label. However, the use of radioactive tracers requires specific safety procedures and generates radioactive waste. Moreover, since free label may enter grazers through non-feeding pathways, extensive controls are a prerequisite for relevant grazing experiments using radioactive tracers (Montagna, 1983; Montagna and Bauer, 1988). Stable isotopes are a powerful alternative to radioactive tracers, in addition to being much easier to handle safely. Stable isotope ratios in consumers tend to be very similar to those of their food sources, the offset between the two (fractionation) typically being very small (on average 1‰) for carbon but more substantial for nitrogen (3–4‰). The isotopic signature of tissues is the result of the progressive absorption of a diet, such that fluxes of biomass can be quantified over meaningful periods of time. However, the isotopic signature also depends on the pathway from the resource to the consumer's tissues (e.g. selective digestion). Another drawback of the use of stable isotopes is the lack of sensitivity of standard elemental analyzer-stable isotope mass ratio spectrometers (EA-IRMS), which may thus necessitate the sorting and cleaning of hundreds of nematodes to get a reliable signal,

although the sensitivity of EA-IRMS can be adjusted to reduce the number of individuals needed to obtain a signal (Carman and Fry, 2002; Melody *et al.*, 2016). If bulk stable isotopic signatures are of interest, the power of natural stable isotope ratios to provide information on diet depends in large part on whether the isotope ratios of different potential resources are sufficiently distinct. Moreover, natural isotope ratios usually do not allow an assessment of selectivity among different prey species. To increase the definition of isotopic niches, Wu *et al.* (2019) monitored additional assimilation biomarkers like fatty acids. In addition to natural stable isotope analyses, enrichment experiments using either prelabeled food (e.g. pre-cultured ^{13}C-enriched diatoms) or free tracer (e.g. $NaH^{13}CO_3$ to label microphytobenthos in the field, and then track its fate in consumers) can also be designed. Consumption and/or absorption rates can then be calculated from the mass balance of the added tracer (e.g. Moens *et al.*, 2002; Majdi *et al.*, 2012a).

Fluorescently labeled food (bacteria or diatoms) or food analogues (similarly sized but inert microbeads) have been applied in studies on nematode feeding (Epstein and Shiaris, 1992; Borchardt and Bott, 1995; Murfin *et al.*, 2012; Fueser *et al.*, 2019). Sediment may also be stained in its entirety, revealing both free-living and attached microbiota, which may partly overcome the problem of consumer selectivity for or against prelabeled food particles (Starink *et al.*, 1994; Hamels *et al.*, 2001b). However, this procedure kills sediment microbiota, but some microbivores exhibit selection against dead microbiota. Furthermore, the quantification of ingested particles in nematode guts may be difficult, hampered by the autofluorescence of consumer organisms (a problem strongly aggravated by certain commonly used fixatives such as glutaraldehyde). More generally, formaldehyde, glutaraldehyde and ethanol are commonly used to fix nematodes, but they cause substantial label loss from nematodes through leakage of low-molecular weight metabolites, alteration of the isotopic signature, or the voidance of gut contents upon addition of the fixative (Moens *et al.*, 1999a). As noted above, liquid N_2 fixation followed by long-term storage at –80°C is recommended to avoid gut voidance or label alteration.

6.9 Conclusions and Perspectives

Freshwater nematodes use a broad range of food items, ranging from DOM to other nematodes. However, given the tiny size of nematodes, their trophic interactions with other biota are difficult to elucidate. Most often, resource use is best followed using indirect indices, such as based on mouth morphology, which presumes a diet consisting of a particular category of prey. Methodological advances such as the detection of isotopic or molecular biomarkers may further help to dissect trophic interactions and fluxes and thus place nematodes in an ecosystem perspective. Additional insights would be gained from studies of whether, how, and when nematodes switch their diet or feed selectively depending on food

availability or nutritional quality. Like their human researchers, nematodes are capable of sophisticated dietary choices. Learning more about these choices and, more generally, the influence of nematodes on microbial communities will reveal important features of ecosystems.

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7 Role of Nematodes in the Food Web: Nematodes as Predator and Prey

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Highlights

- Nematodes are both predators and prey for organisms ranging from protozoans to vertebrates, based on gut analyses and direct observations.
- Nematodes have numerous trophic links in benthic food webs, but the implications for benthic communities are still unclear.
- Functional response experiments, microcosm studies, and enclosures/exclosures in the field can be used to investigate the intensity of these trophic interactions and their impact on individual species as well as entire communities.

7.1 Introduction

Nematodes are an abundant and species-rich taxon in benthic environments and their diversity is reflected in their feeding types. Depending on the species-specific morphology of their mouth cavity (see Chapter 6), the diet of nematodes mainly consists of bacteria, algae (microphytobenthos), plants and roots, and fungi, but omnivorous and predatory species that feed on protozoans or other meiofaunal organisms are also known (Majdi and Traunspurger, 2015). The occurrence of predatory nematodes is often related to the trophic state of the water body inhabited by these species, with higher abundances occurring in more nutritious environments (Michiels and Traunspurger, 2005; Schroeder *et al.*, 2012; Ptatscheck and Traunspurger, 2014). Thus, while the relative proportion of predatory species within a nematode community is usually about 10%, it may exceed 51% (e.g. Barbuto and Zullini, 2005). Nonetheless, little is known about the feeding ecology of predatory nematodes in freshwater environments (Majdi and Traunspurger, 2015).

In addition to their role as predators, nematodes contribute substantially to metazoan abundance, biomass, and secondary production in benthic environments and their sheer quantity can provide a standing stock of suitable food for larger meio- and macrofauna, vertebrates (especially juvenile fish), but also other nematodes (Fig. 7.1).

Studies conducted in the middle of the last century convincingly showed that meiofauna are part of the diet of larger organisms, ranging from insect larvae to fish, in freshwater and marine environments. Coull (1990) was the first to point out the importance of nematodes and other meiofaunal organisms as links in marine food webs. In 2000 and 2002, both Schmid-Araya and Schmid and Schmid-Araya *et al.* integrated these organisms into a realistic freshwater food web, as previous iterations had primarily focused on macrofauna and fish while smaller taxa were ignored. Adding meiofauna to the food web strongly increased the number of trophic links and intermediate species (feeding on more than one trophic level), but the connectivity between the linked organisms declined because of their large size disparity. This pattern well demonstrated that, with increasing size, consumers find it difficult, if not impossible, to

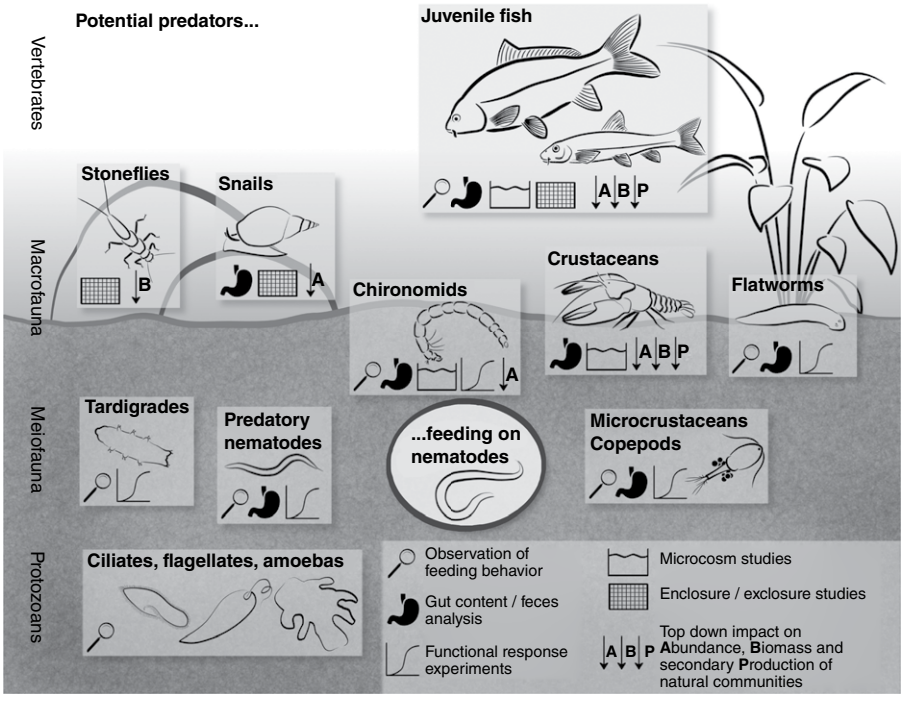


Fig. 7.1. Overview of some of the organisms that consume nematodes. The mode of investigation and the influence of the identified predators on nematode communities in natural sediments are also depicted. (Author's own figure.)

catch and retain small prey organisms. Therefore, meiofauna, including nematodes, are often considered as a dead end in benthic food webs. In addition, as their sampling, processing, identification, and classification are labor-intensive, nematodes have been rarely considered even in more recent studies of food webs.

7.2 Nematodes, a Nutritious Meal?

The importance of nematodes or other meiofaunal organisms, such as copepods and rotifers, as food for juvenile marine and freshwater fish in aquaculture has been well studied. With their high proportion of polyunsaturated fatty acids and amino acids, meiofaunal organisms are a 'good-quality food' that promotes growth (Watanabe *et al.*, 1983; Coull, 1990, 1999; Sargent *et al.*, 1997). Nicholas (1975) listed the lipid proportions of the nematode species *Panagrellus redivivus* (15–24%), *Caenorhabditis elegans* (33–36%), *Aphelenchus avenae* (32–36%), and *Ditylenchus dipsaci* (28–38%) (Fig. 7.2), but the nutritional value of nematodes and other meiofaunal taxa is probably influenced by their own diet (Rottmann *et al.*, 1991; Santiago *et al.*, 2003). Coull (1999) examined the impact of bacterial and algal diets on the fatty acid and amino acid compositions of nematodes and copepods.

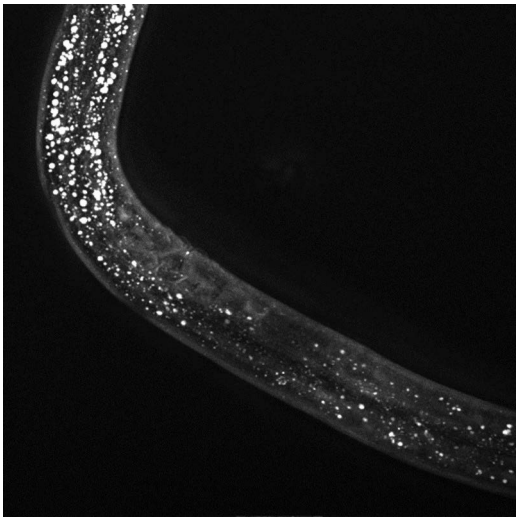


Fig. 7.2. Lipid distribution in *Caenorhabditis elegans* as recorded by CARS (Coherent Anti-Stokes Raman Scattering) microscopy. (Photo: Hendrik Fueser, Animal Ecology, Bielefeld University.)

Such studies are relevant for aquaculture, in which marine fish are often fed copepods or *Branchionus* rotifers whereas in the cultivation of freshwater fish of different species nematodes are the main food (Ptatscheck *et al.*, 2020 and literature therein). Unlike in the field, fish raised in aquaculture have access to nearly unlimited quantities of nematodes, obviating the need to search for food in the sediment. But do nematodes in natural sediments have a similar nutritional value as food? According to several studies conducted in marine environments, the ingestion of up to 750 copepods and 7000 nematodes per day covers the daily nutrient needs of young fish (27–33 mm) (Ceccherelli *et al.*, 1994; Feller and Coull, 1995; Street *et al.*, 1998). Translated to freshwater environments, an area of <400 cm² of the upper sediment layers (0–2 cm) would suffice as a feeding habitat for a single, young fish (Traunspurger and Drews, 1996).

7.3 Investigating the Trophic Interactions of Nematodes

There are several methods that can be used to get an insight into the trophic interactions of nematodes, both as predator and as prey for other organisms (Box 7.1).

7.3.1 Gut content analysis and observations of feeding behavior

Much of our knowledge on the trophic relationships between benthic vertebrates and invertebrates has been obtained by examining the food components in the intestines of those organisms (Table 7.1).

Box 7.1. Investigating the trophic interactions of nematodes.

Observation of feeding behavior

- Most practicable for microscopic organisms
- Detailed descriptions of the feeding process, handling time, and attack rate
- Difficult to investigate under natural conditions (e.g. in sediment)

Gut content / feces analysis

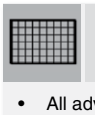
- Fundamental method to determine the ingestion of nematodes
- Provides information on the proportion of nematodes in the whole diet
- Nematodes tend to be underrepresented because they are rapidly digested
- Difficult to perform for small predators (meiofaunal size and smaller) because only parts or body fluid of the prey are ingested

Functional response

- Easy method for the calculation of handling time and attack rate
- Impractical for large predators (e.g. juvenile fish)
- Certain behaviors, such as digestion times or resting periods, are not considered
- Not usable in natural environment (e.g. no sediment, only one prey species)
- Does not allow a representation of trophic interactions within an entire food web

Microcosm studies

- Top-down effects on whole benthic communities within sediment
- Good comparability with natural systems
- Suitable for long-term studies



Enclosure / exclosure studies

- All advantages of microcosm studies
- Additional natural parameters, such as weather, waves, dispersal of prey organisms, and recolonization processes, can be documented

Table 7.1. Nematodes as the prey of other organisms, based on the results of gut and feces analyses from individuals feeding on natural meiofaunal communities. (Modified from Ptatscheck *et al*. (2020), open access.)

Predator	Prey	Reference
Nematodes		
Prionchulus punctatus (Andrássy 1958)	Rotifers (two species)	Schmid-Araya and Schmid (2000)
Anatonchus tridentatus (de Man 1876)	Rotifers (four species), oligochaetes	Schmid-Araya and Schmid (2000)
Anatonchus dolichurus (Ditlevsen 1911)	Nematodes, oligochaetes	Prejs (1993)
Mononchus sp.	Rotifers (2 species)	Schmid-Araya and Schmid (2000)
Crustaceans		
Neocaridina davidi (Bouvier 1904)	Nematodes, oligochaetes, microcrustaceans	Weber and Traunspurger (2016b)
Procambarus clarkia (Girard 1852)	Nematodes, oligochaetes, microcrustaceans	Weber and Traunspurger (2017)
Astacus astacus (Linnaeus 1758)	Nematodes, oligochaetes, microcrustaceans	Weber and Traunspurger (2017)
Flatworms		
Polycelis tenuis (Ijima 1884)	Nematodes, rotifers, oligochaetes, microcrustaceans	Reynoldson and Young (1963), Young (1981)
Dipterans		
Chironomidae		
Chironomus riparius (Meigen 1804)	Nematodes[a]	Ptatscheck *et al*. (2017)
Trissopelopia longimana (Staeger 1839)	Rotifers, tardigrades, microcrustaceans, nematodes (2 species), oligochaetes	Schmid-Araya and Schmid (2000)
Limnophila	Oligochaetes, nematodes (2 species)	Schmid-Araya and Schmid (2000)
Gastropods		
Bellamya unicolor (Olivier, 1804)	Rhabditids[a] (1 species)	Sudhaus (2018)
Biomphalaria alexandrina (Ehrenberg, 1831)	Rhabditids[a] (1 species)	Sudhaus (2018)
Biomphalaria glabrata (Say, 1818)	Rhabditids[a] (1 species)	Sudhaus (2018)
Bulinus truncatus (Audouin, 1827)	Rhabditids[a] (1 species)	Sudhaus (2018)
Cleopatra bulimoides (Olivier, 1804)	Rhabditids[a] (1 species), *Mononchus* sp.	Sudhaus (2018)
Lanistes boltenianus (Röding, 1798)	Rhabditids[a] (1 species)	Sudhaus (2018)
Melanoides tuberculata (Müller, 1774)	Rhabditids[a] (2 species)	Sudhaus (2018)
Planorbella (*Helisoma*) *duryid* (Wetherby, 1879)	Rhabditids[a] (1 species)	Sudhaus (2018)
Fish		
Common carp *Cyprinus carpio* (Linnaeus 1758)	Nematodes, oligochaetes, microcrustaceans	Weber and Traunspurger, (2015), Weber *et al*. (2018)

[a]Only nematodes were investigated.

This method provides insights into (i) whether certain organisms are ingested by a predator, (ii) their proportional contribution to the total diet and thus whether (iii) there are clear food preferences or (iv) ingestion of the organism occurs incidentally, for example, as bycatch (Box. 7.1). However, an analysis of the gut or feces content of a predator requires that the prey organisms have not been digested beyond recognition, such that at least clearly assignable morphological structures are preserved, for example, the shells of hard-bodied microcrustaceans or the mastax of rotifers. In the case of suction-feeding nematodes, which only ingest the soft components of their prey, documentation of the food spectrum via gut examinations is less promising if not impossible. Dietary determinations are simplified if the prey has been swallowed whole, which primarily occurs at high predator:prey size ratios, rather than shredded and the hard components have not been spat out. However, nematodes consumed by chironomid larvae, copepods, and fish are no longer identifiable a few hours after their digestion, as demonstrated in the studies of Hofsten *et al.* (1983), Muschiol *et al.* (2008a), and Ptatscheck *et al.* (2015). In dietary analyses, this may lead to a general underestimation of nematodes and other soft meiofaunal organisms. Moreover, realistic interpretations of the gut or feces content must take into account the environment of the predator. For example, many of the investigations that reported nematodes in the digestive tract of juvenile fish were conducted under aquaculture conditions, in which the fish were placed in experimental vessels without sediment and unnaturally high densities of nematodes were offered directly via the water column. Thus, while the results demonstrated that fish are able to ingest nematodes, whether and to what extent this happens in nature and in benthic environments remains unresolved. However, laboratory approaches do allow exact observations and a documentation of the feeding process, neither of which is possible in studies conducted under natural conditions with small endobenthic organisms (Box. 7.1).

7.3.2 Functional response assessments

This classical approach to evaluating the trophic relationship of a predator–prey pair assumes that the number of prey organisms ingested per unit time (*ingestion rate*, *IR*) is a function of the prey density and increases as the number of available prey increases (see Box 7.2). Beyond a certain threshold density, no more prey can be ingested during the given time, and the *IR* thus remains constant (Fig. 7.3). Functional response assessments also enable an evaluation of the *handling time* (T_h, the time a predator needs to capture, ingest, and digest its prey) and the *attack rate* (a, the searching efficiency), which can provide important information about predation behavior (Fig. 7.3; Table 7.2). Handling times are usually significantly shorter if they are estimated by direct observation rather than derived from the results of functional response assessments, since in the former the digestion time is not included (Hohberg and Traunspurger, 2005; Muschiol *et al.*, 2008a,b).

Box 7.2. Theoretical background of functional response.

Holling (1959) classified three different types of functional response. A type I functional response is characterized by a linear increase of the ingestion rate followed by a sharp transition to a plateau. The requirements for a type I functional response are (i) a negligibly short handling time or the ability of an organism to simultaneously handle food while searching for further food and (ii) a maximally high and successful searching rate (Jeschke *et al*., 2004). Only filtrating organisms fulfill both criteria and they are therefore the only organisms of this type. A type II functional response describes a hyperbolic curve that progresses until the ingestion rate gradually slows down and a plateau is reached (Fig. 7.3). While it was long assumed that type II was the most common functional response, an increasing number of studies indicate that a type III response is in fact the most common. In a type III functional response, when the *attack rate*, which is constant under type II, is a function of the prey density ($a = bN$; b = *searching coefficient*), the curve becomes sigmoidal, with a slow increase in the number of ingested prey organisms at low prey densities. The possible reasons for this variable attack rate include the learning behavior of the predator and a switching of either the feeding mode (e.g. from selective to unselective) or the predator:prey size ratio. For example, small prey may only become attractive to a relatively large predator when they exceed a certain density, because they are less nutritious but also harder to find and catch than larger prey at comparable densities. Furthermore, the habitat structure can offer retreats and, together with environmental factors (e.g. temperature, oxygen, light), will affect the traceability, mobility, and distribution of the prey (see Chapter 5).

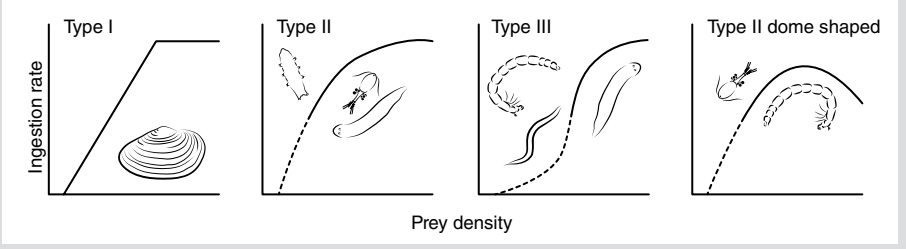


Fig. 7.3. Different types of functional responses and examples of the benthic organisms that exhibit them. With exception of type I, nematodes were used as prey. For type II and type III functional responses, the dotted curve section indicates a predominant impact of the *attack rate* (a), while continuous sections indicate that the *handling time* (T_h) determines curve progression. (Author's own figure.)

A special type of type II functional response results in a dome-shaped curve. In this case, swarming effects result from prey densities that are high enough to hinder predators in their feeding activities (Jeschke *et al.*, 2002, 2004) (Fig. 7.3). Ptatscheck *et al.* (2015) showed that high numbers of *C. elegans* offered to chironomid larvae induce cleaning movements and less feeding by the latter because the nematodes become entangled on the predator's body. Slight swarming effects were also observed in experiments with copepods (Muschiol *et al.*, 2008b), whereby the transition of the curve to saturation is slow and a calculation of functional parameters accordingly challenging.

Table 7.2. Results of functional response experiments with meio- and macrofaunal predators and nematodes as prey. (Author's own table.)

Predator	Prey nematodes	Mean predator: prey size ratio	Time	Ingested nematodes: Maximal (*n*)	Ingested nematodes: % of predator's biomass	Density at saturation (per cm²)	*Attack rate* (*a*, for type II) *Searching coefficient* (*b*, for type III)	T_h (s)	Type	Reference
Nematodes										
Prionchulus muscorum (Dujardin 1845)	*Caenorhabditis elegans*									Kreuzinger-Janik *et al*. (2019)
	Juveniles	5.3	4 h	73	59.3	48	0.173	43	III	
	Adults	4.2	4 h	52	77.0	48	0.814	86	III	
	Juveniles[a]	5.3	4 h	86	70.0	64	0.781	43	III	
	Adults[a]	4.2	4 h	54	80.0	64	0.433	58	III	
Copepods										
Eucyclops subterraneus scythicus (Plesa 1989)										Muschiol *et al*. (2008a)
	Panagrolaimus sp.	1.2	20 min	26	28.7	20	4.39×10^{-4}	53	II	
	Poikilolaimus sp.	3.2	20 min	38	31.1	26	5.44×10^{-4}	37	II	
Diacyclops bicuspidatus (Claus 1857)										Muschiol *et al*. (2008a)
	Panagrolaimus sp.	1.3	2 h	45	43.5	46	1.87×10^{-4}	174	II	
Tardigrades										
Macrobiotus richtersi (Murray 1911)	*Pelodera teres*									Hohberg and Traunspurger (2005)
	Juveniles	2.2	4 h	105	22.0	316	4.7×10^{-3}	132	II	

Continued

Table 7.2. Continued.

Predator	Prey nematodes	Mean predator: prey size ratio	Time	Ingested nematodes: Maximal (*n*)	Ingested nematodes: % of predator's biomass	Density at saturation (per cm²)	*Attack rate* (*a*, for type II) *Searching coefficient* (*b*, for type III)	T_h (s)	Type	Reference
	Adults *Acrobeloides nanus*	1.8	4 h	54	32.0	127	6.4×10^{-3}	385	II	
	Juveniles	2.7	4 h	99	26.0	190	4.4×10^{-3}	205	II	
	Adults	2	4 h	58	34.0	127	5.4×10^{-3}	362	II	
Flatworms										
Planaria torva (Mueller 1774)	*C. elegans*									Kreuzinger-Janik *et al*. (2018)
	Juveniles	32.3	3 h	143	0.2	9	0.409	22	III	
	Adults	11.1	3 h	46	2.0	5	0.887	43	III	
Polycelis tenuis (Ijima 1884)	*C. elegans*									
	Juveniles	26.5	3 h	927	1.6	142	0.745	11	III	
	Adults	9.1	3 h	51	3.4	12	0.356	32	III	
Dugesia gonocephala (Duges 1830)	*C. elegans*									Beier *et al*. (2004)
	Juveniles	76.0[a]	3 h	197	–	111	0.568	65	II	
	Adults	15.8[a]	3 h	94	–	74	0.418	130	II	
Chironomidae										
Chironomus riparius (Meigen 1804)	*C. elegans*									Ptatscheck *et al*. (2015)
	Juveniles	43.5	4 h	763	3.2	387	7.16×10^{-2}	5	III	
	Adults	15.0	4 h	557	92.5	–	–	–	II dome shaped	

[a]Functional response experiments with substrate.

7.3.3 Functional response assessments using nematodes

Several functional response experiments have examined the interactions between different macro- or meiobenthic predators and their meiobenthic prey. In all cases, nematodes of different species (Table 7.2) were offered in defined densities as prey to a single predator until the *IR* reached saturation. Since low food densities are decisive in determining a type II vs. type III functional response, a sufficient spectrum of differing low prey densities must be included in the experiment. Other considerations are the length of the trial, the size of the experimental arena, and the range of prey densities offered, all of which must be adapted to the feeding behavior of the studied predator. In general, shorter test times and vessels that are as small as possible will allow lower prey densities and result in fewer swarming effects, less reproduction, and less amount of work, but these conditions can either limit the predator, due to the short acclimatization period, or restrict its movement. Larger predators (e.g. juvenile fish or crustaceans) will require larger test vessels and the number of offered prey necessary to achieve *IR* saturation will therefore increase (Table 7.2). This may explain why fish have yet to be investigated in functional response experiments. Instead, with the appropriate design, functional response experiments are easily carried out in organisms that can be confined to small vessels such as Petri dishes, in which case insights into the intensity of trophic interactions will be obtained after just a few hours (Table 7.2). A standardized laboratory setup allows the inclusion or exclusion of the biotic (e.g. trophic interactions with other species, competition, dispersal) and abiotic (oxygen, temperature, light) factors that directly influence predation in natural environments, making the results easier to compare and interpret.

The results of such functional response experiments have indicated strong predator–prey interactions between meio- and macrobenthic organisms and nematodes (Table 7.2). Muschiol *et al.* (2008a,b) and Hohberg and Traunspurger (2005) showed that, at small predator:prey size ratios (1.2–3.2), predatory copepods and tardigrades can consume up to 38 nematodes in 20 min and 105 nematodes within 4 h. For larger organisms such as flatworms and chironomid larvae and high predator:prey size ratios (up to 76), 927 individuals were ingested in 3 h (Ptatscheck *et al.*, 2015; Kreuzinger-Janik *et al.*, 2018). In other functional response experiments, predatory nematodes such as *Prionchulus muscorum* were shown to present a serious threat to smaller worms, evidenced by *IR*s of up to 54 adult *C. elegans* in 4 h, corresponding to 80% of the predator's own biomass (Kreuzinger-Janik *et al.*, 2019). The copepod *Diacyclops bicuspidatus* (<1 mm body length) consumed 43.5% of its own biomass in just 20 min (Muschiol *et al.*, 2008a).

The handling time and thus the efficiency of nematode ingestion does not necessarily depend solely on the predator's size but may also be a function of its feeding behavior (see Section 7.3.1). However, as shown by the studies listed in Table 7.2, with increasing prey size the *handling time* increases and the number of ingested nematodes decreases,

regardless of whether the predator is large or small. In general, small prey lead to high encounter rates, high attack rates, a high degree of capture success, short handling times, and consequently to higher *IR*s (Hohberg and Traunspurger, 2005 and studies therein).

Finally, functional response experiments examining the intensity of the trophic relationship between two species under laboratory conditions cannot account for the complex interactions that occur within the food web of an entire benthic community. While Kreuzinger-Janik *et al.* (2019) were able to demonstrate the effect of the substrate on the functional response of a nematode system, such experiments are only a small step in understanding a food web and their findings must be supported by studies conducted in natural environments and which include whole benthic communities.

7.3.4 Impact of the sediment on predation

Substrates (e.g. sediment, leaf litter, periphyton) are an essential refuge for benthic organisms and they determine their horizontal and vertical distribution. The distribution patterns of nematodes reflect a trade-off between residence in the high-risk (Traunspurger *et al.*, 2006) but productive sediment surface and the safer but physiologically harsher conditions (e.g. low oxygen content; Traunspurger and Drews, 1996; Traunspurger *et al.*, 2015; Majdi *et al.*, 2017) of the deeper sediment. Above all, substrates offer protection from predation, by hiding prey or hindering their predator. In the study of Kreuzinger-Janik *et al.* (2019), moss was added to a three-dimensional habitat and the impact on the trophic interaction between the predatory nematode *Prionchulus muscorum* and its prey (*C. elegans*) was then examined. The authors found that compared with the no-substrate treatments, the *IR*s were not affected by the presence of added substrate but the *searching coefficient* was significantly higher when small prey were offered, suggesting an improvement in the locomotion of larger nematodes (both predator and larger prey) in the pore spaces (see Chapter 5).

Laboratory experiments that included functional response determinations have shown that significantly fewer nematodes are ingested by tardigrades, flatworms, and chironomid larvae when sediment is added to the testing arena (Beier *et al.*, 2004; Hohberg and Traunspurger, 2005; Ptatscheck *et al.*, 2015; Kreuzinger-Janik *et al.*, 2018) (Fig. 7.4). In combination with substrate complexity, the feeding behavior of benthic invertebrate predators determines the intensity of nematode predation. For example, for chironomids, which are endobenthic organisms that build their tubes in soft sediments, feeding on nematodes is more efficient in sand than in coarse-grained substrates (Ptatscheck *et al.*, 2015). By contrast, fewer nematodes are consumed by epibenthic flatworms in fine sediment than in either coarse gravel or leaf litter (Beier *et al.*, 2004; Kreuzinger-Janik *et al.*, 2018). The small interstices of fine sediment offer

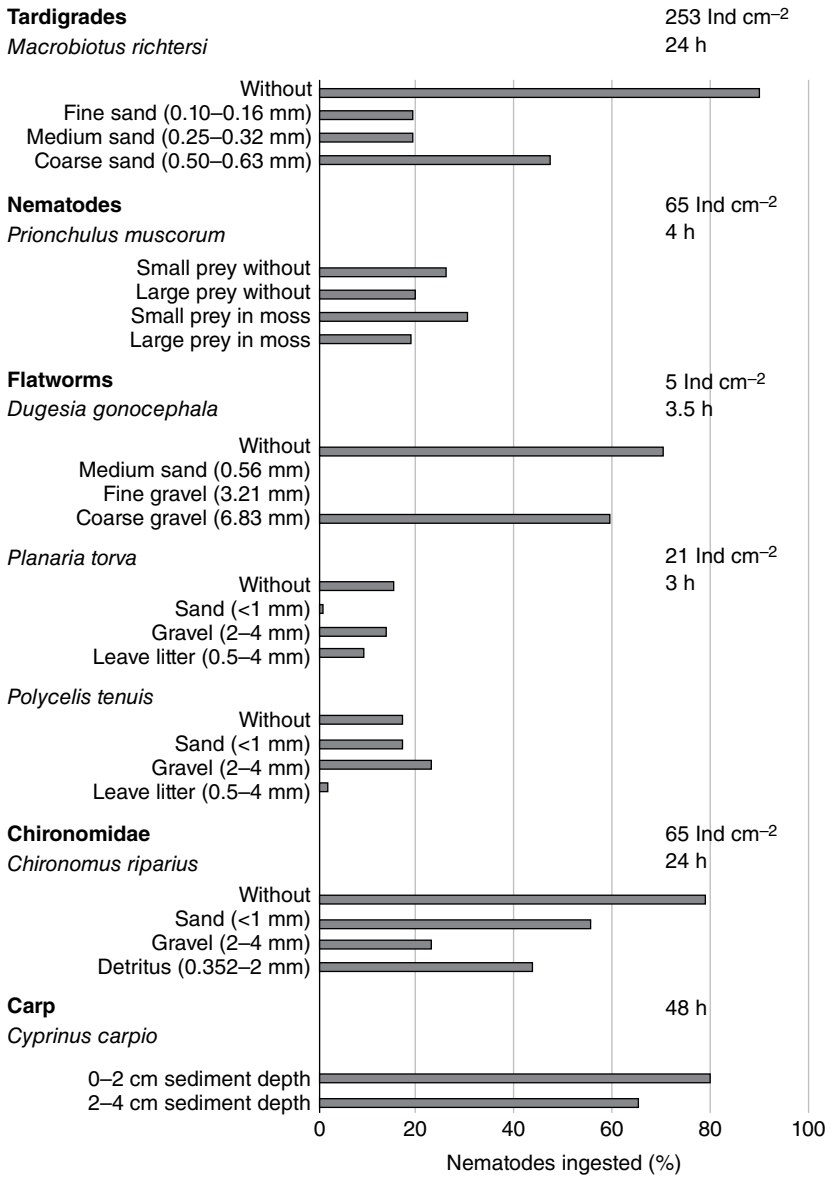


Fig. 7.4. Percentage of nematodes (*C. elegans*) ingested by different predators in laboratory experiments using different types of sediment. Additionally, the number of offered nematodes and the duration of the experiment are shown. (Author's own figure.)

considerable protection for meiofaunal prey and hinder deep penetration by their predators. Consequently, in fine sediments the nematode feeding rates of smaller flatworm species will be higher than those of larger species. Movement to deeper layers also offers protection against predation by juvenile fish (Weber *et al.*, 2018). In the latter study, common carp

(6–8 cm body length) caused a greater reduction of nematodes in the upper sediment layers (0–2 cm) (80%) than in deeper layers (2–4 cm, 65%) (Fig. 7.4).

7.3.5 Experimental settings using microcosms and enclosures/exclosures

While investigations of gut contents and feeding behaviors very clearly link nematodes to the benthic food network, empirical approaches have evidenced the strong feeding pressure exerted on nematodes by fish, crustaceans, and organisms of macrofaunal and meiofaunal (even other nematodes) size (Fig. 7.4). However, those studies only examined the influence of predators on single prey species and were thus unable to determine predator preferences within a whole nematode/meiofauna community. A complete picture of the top-down impact of predation on nematodes requires studies in which the prey is representative of the larger meiofaunal community and monitoring is conducted over longer time periods (see Section 7.5.6).

Model ecosystems (microcosms) offer nearly optimal conditions for such approaches because their low investments of time, space, and cost enable reproducibility. Microcosms are often used in population- and community-level studies and provide a bridge between theory and the natural environment (Fraser and Keddy, 1997). However, they cannot reproduce the influence of environmental factors, such as wave action and current velocity, nor of factors such as emigration and immigration (see Chapter 5) (Blanchet *et al.*, 2008; Englund and Leonardsson, 2008; Ludlam and Magoulick, 2009). Thus, an extrapolation of their results to natural conditions (field studies) is problematic (Aarnio, 2000; Petersen and Englund, 2005; Meissner and Muotka, 2006). Attempts to close the knowledge deficits resulting from the gap between highly replicable small-scale experiments and field studies with respect to the effects of fish predation on meiobenthic communities in natural freshwater ecosystems (Weber and Traunspurger, 2015) have been limited. Nevertheless, single studies on fish and macrofauna using field enclosures and exclosures have shown that the presence of these predators significantly influences the structure and composition of natural nematode communities (Table 7.3).

7.4 Nematodes as Predators

Nematodes that feed on other benthic organisms are generally *chewers* and *suction feeders* that either hunt, swallow, or shred their prey or suck out the contents of their body shells (Fig. 7.5).

Analyses of the gut contents of freshwater nematodes of the genera *Anatonchus*, *Mononchus*, and *Prionchulus* (Table 7.1) revealed a wide spectrum of potential prey organisms, including oligochaetes, chironomids, rotifers, and other nematode species (Prejs, 1993; Schmid-Araya

Table 7.3. Studies of the predation effect of different organisms on nematode communities in natural sediments. (Modified from Ptatscheck *et al*. (2020).)

Predator	Design	Substrate (particle size)	Effects on nematodes	Study duration	Reference
Nematodes					
Anatonchus dolichurus	LE	Diverse	Abundance –32% (only individuals <2 mm)	12 days	Prejs (1993)
Crustaceans					
Neocaridina davidi (Bouvier 1904)	LE, GA	Fine-grained sediment	Abundance –44% Biomass –40% Secondary production –28%	42 days	Weber and Traunspurger (2016b)
Procambarus clarkia (Girard 1852)	LE, GA	Gravel (3–5 mm)	No effect	14 days	Weber and Traunspurger (2017)
Astacus astacus (Linnaeus 1758)	LE, GA	Gravel (3–5 mm)	No effect	14 days	Weber and Traunspurger (2017)
Chironomidae					
Chironomus riparius (Meigen 1804)	LE, GA	Sand (<1 mm)	Abundance –55%	8 days	Ptatscheck *et al*. (2017)
Stoneflies					
Chloroperlidae					
Unspecified	FS	Leaf litter	Biomass –37%	18 days	Majdi *et al*. (2015)
Snails					
Theodoxus fluviatilis (Linnaeus 1758)	FS	Periphyton	Abundance of total meiofauna –79%, with the largest reductions in nematode and oligochaete abundances	6 weeks	Peters and Traunspurger (2012)
Common carp					
Cyprinus carpio (Linnaeus 1758) 6–8 cm (juvenile)	LE	Fine-grained, natural sediment	Abundance –82% Biomass –94% No effect on nematode diversity	48 h	Weber and Traunspurger (2014b)
	FE	Mud and woody and leafy debris	Sec. production –77% No effect on nematode diversity	80 days	Weber and Traunspurger (2015)

Continued

Table 7.3. Continued.

Predator	Design	Substrate (particle size)	Effects on nematodes	Study duration	Reference
	FE	Mud and woody and leafy debris	Abundance and biomass reductions of the most common species Nematode composition but not diversity was affected by fish	80 days	Weber and Traunspurger (2016a)
	FE, GA	Mud and woody and leafy debris	0–2 cm sediment depth Abundance –80% Biomass –80% 2–4 cm sediment depth Abundance –65% Biomass –67%	32 days	Weber *et al.* (2018)
Gudgeon					
Gobio gobio (Linnaeus 1758) 5–7 cm (juvenile)	LE	Mud and woody and leafy debris	Abundance –56% Biomass –80% Effects on meiofaunal size structure but not on diversity	48 h	Weber and Traunspurger (2014b)
Roach					
Rutilus rutilus (Linnaeus 1758) 1–2 cm (juvenile)	LE, GA	Coarse sediment overlain with fine material	No effect	11 days	Dineen and Robertson (2010)
10–13 cm (juvenile)	LE	Fine-grained, natural sediment	No effect	2 months	Spieth *et al.* (2011)
Bream					
Abramis brama (Linnaeus 1758) 7–12 cm (juvenile)	FE	Sand (0.5–2 mm)	Abundance significantly reduced	14 days	Spieth *et al.* (2011)

FS, field study in natural environments; FE, field enclosures; LE, laboratory experiment; GA, gut analysis.

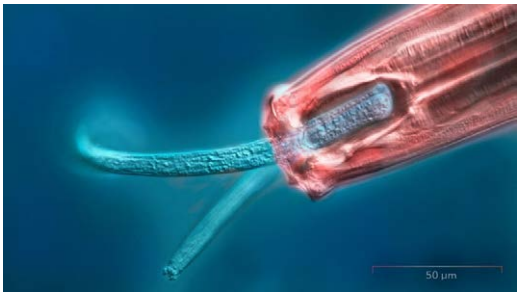


Fig. 7.5. Predatory nematode *Prionchulus muscorum* feeding on *Teratocephalus terrestris.* (Photo W. Traunspurger, Animal Ecology, Bielefeld University.)

and Schmid, 1995, 2000). Studies of the diet of nematodes from marine environments yielded very similar results but also identified protozoans (Moens and Vincx, 1997). In a survey of the intestine of the predatory nematode species *Anatonchus dolichurus*, the remains of oligochaetes were found in 45%, those of nematodes in 10%, and 'undefined material', possibly the body fluid of sucked prey, in 88% (Prejs, 1993). Direct observations of *Anatonchus dolichurus* and *Prionchulus muscorum* showed that small prey (less than half of the predator's body size) were mainly swallowed whole but in some cases they were ruptured and their soft components sucked out (Prejs, 1993; Lightfoot *et al.*, 2016; Kreuzinger-Janik *et al.*, 2019; see Fig. 7.5). Since larger prey cannot be easily devoured, the latter feeding behavior is particularly important (Nelmes, 1974). Moens and Vincx (1997) observed the marine nematode *Enoploides spiculohamatus* and showed that it bites through the integument of oligochaetes to reach the intestine, the contents of which are then sucked out. Additional mechanisms of predation may include paralyzing prey or the release of deadly substances, but these have been poorly investigated so far.

An unusual adaption was identified in members of the genus *Pristionchus*, whose oral cavity exhibits a morphological dimorphism related to the nematode's development. Thus, the eurystomatous mouth of *P. pacificus* is characterized by an additional tooth, a heavily serrated plate of denticles and a wide mouth diameter, which together allow the effective hunting and killing of other nematodes (Wilecki *et al.*, 2015). By contrast, the tenostomatous and narrow-mouthed form of this species is specialized for feeding on bacteria.

7.5 Nematodes as Prey

The inventories of Schmid-Araya and Schmid (2000) and Ptatscheck *et al.* (2020) obtained from field collections or microcosm studies using natural substrates listed 55 benthic invertebrates whose guts contained meiofauna. These consumers included protozoans, meiofauna (e.g. rotifer, nematodes, microcrustaceans), macrofaunal representatives such

as insect larvae (mainly dipterans), oligochaetes, flatworms, crustaceans, and juvenile fish (carp and roach). Although only eight taxa (14.5%) contained nematodes in their guts, they represented a wide taxonomic range (crustaceans, 3; insect larvae, 3; flatworm, 1; and fish, 1; Table 7.1).

7.5.1 Nematodes as prey for protozoans

If their numbers are high enough, some prey can pose a serious threat to their predators, even significantly larger ones. This was shown by Bjørnlund and Rønn (2008) for terrestrial nematodes (*C. elegans*) feeding on flagellates. While at low prey density the flagellates were consumed by adult worms, at higher densities they began to kill their former predator. Geisen *et al.* (2015) observed the hunting strategy of amoebas on nematodes: a single amoeba prevented the escape of a nematode at least 10 times larger by attaching itself both to the worm and to the substrate. Within the following 12 h, other amoebas were attracted to the site and completely fixed, dissolved, and ingested their prey. In contrast to this type of pack hunting, ciliates of the genus *Urostyla* (300 µm body length) are able to ingest living nematodes of 1 mm body length in their entirety, which is not without danger to the predator itself and frequently ends with its rupture and disintegration (Doncaster and Hooper, 1961).

7.5.2 Nematodes as prey for invertebrates (meio- and macrofauna)

Predator–prey relationships between various meiofaunal organisms and nematodes have so far been documented primarily through direct observations or functional response experiments (see Section 7.3.3). Muschiol *et al.* (2008a,b) reported that copepods actively hunt prey nematodes, devouring small individuals (<0.6 mm) within a few seconds, but prey (body length of up to 1.7 mm) larger than the predator itself require as long as several minutes. Tardigrades also feed on nematodes, but are less limited by the size of their mouths. Doncaster and Hooper (1961) as well as Sayre (1969) documented, including visually, that tardigrades cling to nematodes with their claws. The victim's integument is then punctured – sometimes several times and by many tardigrades occupying a single prey – and the offal sucked out. Smaller nematodes are partially drawn into the predator's mouth. The tardigrade *Macrobiotus richtersi* (650 µm body length) is able to consume juveniles of *Acrobeloides nanus* (240 µm) in 18.6 s and adults (330 µm) in 80.8 s (Hohberg and Traunspurger, 2005). Interestingly, with increasing nematode densities, tardigrades exhibit increasingly 'wasteful feeding', ingesting less biomass from trapped nematodes and instead switching to other prey organisms (Hohberg and Traunspurger, 2009).

Insect larvae and crustaceans (Weber and Traunspurger, 2016b, 2017) ingest other meiofaunal organisms, such as oligochaetes and copepods, as well as detritus much more frequently and in larger amounts than

nematodes, most likely due to the morphology, feeding behavior, and associated selectivities of these predators. Among insects, meiofauna are mostly consumed by young stages with smaller body lengths, while larger organisms increasingly switch to macrofauna although meiofauna remains a minor part of their diet (Schmid-Araya and Schmid, 1995; Schmid and Schmid-Araya, 1997). The feeding behavior of the larvae of the non-predatory chironomid *Chironomus riparius* is continuous but very unselective, as all nematodes in their path are sucked up in a few seconds. However, this feeding mode does not allow the consumption of prey larger than the diameter of a larvae's mouth. With the exception of predatory Tanypodinae (Chironomidae), whose members specifically hunt for benthic organisms and shred larger prey before ingesting them, chironomids and possibly also other dipterans exhibit unselective feeding, which leads to a constant uptake of detritus (Walshe, 1951; Pinder, 1986). Kreuzinger-Janik *et al.* (2018) observed the feeding behavior of flatworms, which under natural conditions mainly feed on oligochaetes and arthropods (Reynoldson and Young, 1963; Young, 1981). In laboratory experiments using nematodes as prey, smaller individuals moving over the substrate became stuck in the flatworm's mucus. At certain intervals, depending on the prey density, the predator ingested both the mucus and the entrapped prey. These results suggest that nematodes are frequently consumed unselectively or as bycatch by macroinvertebrates.

As shown in Table 7.1, flatworms, crustaceans, and chironomid larvae consume nematodes. In addition, Sudhaus (2018) reported that free-living nematodes, rotifers, and tardigrades may be incidentally ingested by terrestrial gastropods but may survive gut passage (see Chapter 5). For example, nematodes were found in the guts of eight freshwater gastropods (Table 7.1). Other mollusks are also likely to ingest nematodes. Vaughn *et al.* (2008) described the diverse diet of mussels, which consume, but not exclusively, organisms from the water column, including rotifers. Hicks and Marshall (1985) collected microcrustaceans from the guts of marine bivalves. While, to our knowledge, there are no studies of the gut contents of freshwater mussels that definitively demonstrate the ingestion of meiofauna, the majority (80%) of the diet of mussels consists of deposited material (Raikow and Hamilton, 2001) and thus likely includes attached meiofauna.

7.5.3 Impact of invertebrate predation on natural nematode communities

Little is known about how invertebrate (micro-, meio-, or macrofauna) feeding affects the composition of nematodes in freshwater environments. Most studies have been empirical in their design (Table 7.3). To our best knowledge, the only study of the effect of predatory nematodes on the meiofaunal composition in freshwater sediments was that of Prejs (1993). In that work, within 12 days 20 individuals of the nematode *Anatonchus dolichurus* had reduced the abundance of nematodes (≤2 mm), oligochaetes

(<3 mm), and chironomids (<2 mm) over a 20-cm² area of sediment by 31% (nematodes) and 60% (oligochaetes and chironomids).

Both Peters and Traunspurger (2012) and Majdi *et al*. (2015) found that plecopterans and gastropods in field enclosures reduce the biomass and abundance of nematodes. However, whether the reductions are caused by predation, migration, or indirect engineering effects (e.g. changes in habitat structure by grazing or the predator's movements) could not be determined with certainty given the experimental settings. Firmer results were provided by Weber and Traunspurger (2016b, 2017), who set up small water tanks containing natural sediment to investigate the impact of crustaceans on meiofaunal composition. All examined species of crustaceans reduced the biomass and secondary production of the total meiofauna as well as the abundance of single taxa such as oligochaetes and microcrustaceans. However, while all crustaceans ingested nematodes (Table 7.1), only the smaller sherry shrimp *Neocaridina davidi* (15–20 mm cephalothorax length) significantly reduced the abundance, biomass, and secondary productions of the nematode community already after 14 days; for the larger crayfishes *Procambarus clarkii* and *Astacus astacus* (20–30 mm cephalothorax length), no effects were observed during the same interval (Table 7.3). A strong and immediate trophic impact on nematodes was shown for the detritus-feeding chironomid larvae of *Chironomus riparius*, investigated in microcosms (Ptatscheck *et al*., 2015, 2017). In the direct vicinity of its tube, one larva reduced nematode abundance by 39% within 1 day. An extrapolation of these results indicates that in benthic environments, where the density of chironomid larvae can reach >10,000 per m², the trophic impact of these organisms on nematodes and even other meiobenthic organisms, such as rotifers, copepods, and oligochaetes, is strong. Consistent with their non-selective feeding behavior, chironomid larvae ingest large numbers of the most common nematode species but with a specificity for body size. Thus, in the above experiment, conducted in natural sediments, larvae with a body length of 11.4 mm significantly reduced only medium-sized nematodes (0.125–1 mm), while larger larvae (13.5 mm) were able to consume larger nematodes (body length of 1–2 mm) but those >2 mm were not ingested, probably because they were too large for the mouth of *C. riparius* (Ptatscheck *et al*., 2015, 2017). Overall, those studies showed that detritus-feeding chironomids in natural sediments are able to shape the meiofaunal community, especially nematode composition, and significantly reduce nematode diversity, which contrasts with the feeding preferences reported in the literature. In an earlier study, however, the predatory flatworm *Dugesia gonocephala* had no effect on the nematode species composition in the sediment (Beier *et al*., 2004).

7.5.4 Nematodes as prey for vertebrates

In a study conducted in 1989, Gee examined the meiofaunal intake of numerous marine and brackish-water fish, mainly juveniles of bottom

biting taxa, and reported that a body length of 3–6 cm was the threshold for the switch from a diet of meiofauna to one of macrofauna. Freshwater fish have not been examined as intensively, but the available studies suggest that even juvenile carp (*Cyprinus carpio*) with body lengths up to 14 cm include nematodes and other meiofaunal organisms in their diet (Ptatscheck *et al.*, 2020). The ratio of meiofauna, macrofauna, and detritus in the stomachs of young fish can greatly differ. For example, the gut of young carp may contain nematodes but the proportion of detritus is higher (Weber *et al.*, 2018); in the gut of young brook charr, the proportion of detritus is small and the content of macroinvertebrates is higher than that of meiofauna (McNicol *et al.*, 1985). Meiofaunal-sized organisms (but not nematodes) comprise the major part of the roach's diet (Dineen and Robertson, 2010), despite the availability of larger organisms and therefore more rewarding food sources in the sediment. Ahlgren (1990) showed that the composition of prey organisms fluctuates strongly over the course of the year, as in summer microcrustaceans account for 95% of the ingested food of juvenile fish but in winter the proportion is lower. Accordingly, the author concluded that juvenile fish can specifically separate detritus from invertebrates and that detritus is ingested intentionally when preferred invertebrate prey are scarce. Decisive factors in the selective absorption by fish of nematodes and other meiofauna from the sediment are: (i) the feeding behavior of the fish, based on the morphology of its gill apparatus, and (ii) the fish's body size (Fig. 7.6).

In fish, the feeding process (food searching, intake, selection, and further processing), including the selective consumption of nematodes and other meiofaunal organisms, consists of a highly complex sequence of behavioral patterns that are based on species-specific morphological features. The ability to detect small endobenthic organisms and to separate them from less suitable particles is crucial for the successful and selective ingestion of meiofauna. In detrivore fish this is aided by the high density of taste buds on their forehead and in their mouth cavity, which is much higher than the density on the foreheads of plankton feeders (Gomahr *et al.*, 1992). For example, ca. 95 taste buds per mm^2 are found on the forehead of a minnow (*Phoxinus phoxinus*) vs. 10 per mm^2 on the forehead of a planktivorous bitterling (*Rhodeus sericeus*). The palatal organ of carp is equipped with 820 taste buds per mm^2; by comparison, the density on the human tongue is only 5 per mm^2. While the external taste buds of fish trigger penetration and sampling of the substratum, internal sensors initiate filtering of the ingested particles (summarized by Sibbing and Nagelkerke, 2000). Repeated backwashing by oral compression when the fish's mouth is closed pumps the suspension over the branch sieve. The size range of the retained prey organisms is determined by the morphology of the mouth cavity of the fish and especially by the structure and mesh width of the branchial basket, formed by the gill rakers (Fig. 7.6) (Sibbing, 1988).

Weber and Traunspurger (2014a) and Spieth *et al.* (2011) measured the branchial apparatus of different fish species and determined mesh

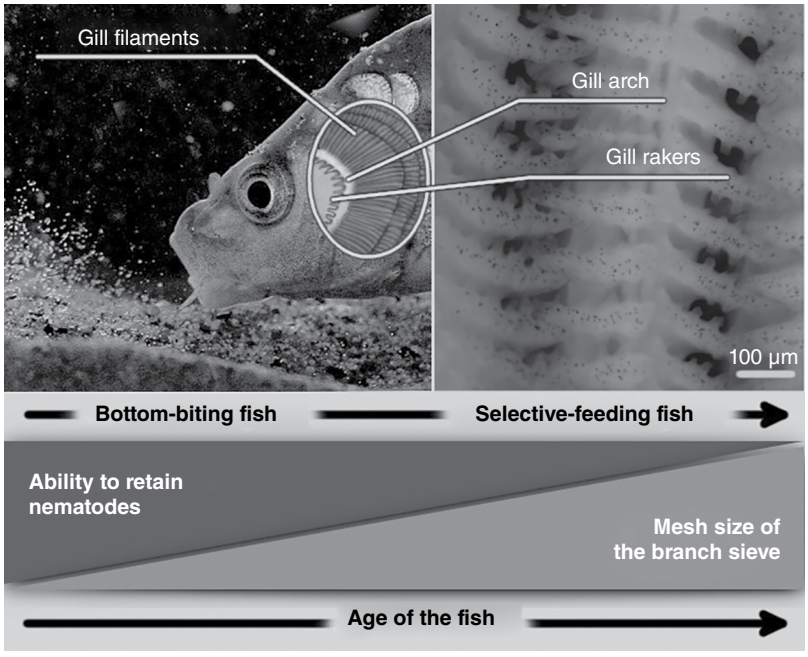


Fig. 7.6. Structure of the gill apparatus in juvenile carp (*Cyprinus carpio*) and the factors influencing the filter properties of this structure. (Author's own figure.)

sizes of <30 µm for dabbling carp (*Cyprinus carpio*) and gudgeon (*Gobio gobio*) and significantly larger (>200 µm) mesh sizes for roach (*Rutilus rutilus*), bream (*Abramis brama*), and ninespine stickleback (*Pungitius pungitius*). For comparison, a mesh size of 44 µm is required to extract meiofauna by sieving (Giere, 2009). Accordingly, bottom biting carp (3–4 cm body length) and gudgeon (2.5–5 cm) are able to consume considerable amounts of nematodes (*C. elegans*), with feeding rates reaching 234,000 and 183,000 nematodes per day, respectively (Spieth *et al.*, 2011; Weber and Traunspurger, 2015) (Fig. 7.3). A single carp with a body length between 11 and 14 cm was shown to reduce the number of *C. elegans* in a 680-cm² sediment area (1 cm thickness) by 67% within 24 h (Spieth *et al.*, 2011). By contrast, roach, bream, and ninespine stickleback do not or only slightly feed on nematodes in sediments and show clear preferences for larger meiofaunal organisms, such as copepods and oligochaetes (Dineen and Robertson, 2010; Spieth *et al.*, 2011; Weber and Traunspurger, 2014a), probably because the branchial apparatus of these fish is too large to retain nematodes (Spieth *et al.*, 2011).

In general, with increasing nematode body length, fish ingest progressively fewer nematodes as the mesh size of the gill apparatus decreases (Fig. 7.6). Weber and Traunspurger (2014a) reported that the size of juvenile carp (3–4 cm) and gudgeon (2.5–5 cm) triples during development while the branchial mesh size doubles, such that the ingestion of nematodes is reduced by 14 and 6%, respectively. An extrapolation from what

is known about marine fish (Coull *et al.*, 1995) suggests that in freshwater only small fish species or juveniles will be able to retain meiofaunal organisms. In the course of their development, young bottom biting fish become less effective in retaining meiofauna and increasingly prefer macrofaunal-sized prey (Fig. 7.6).

For the sake of completeness, it should be mentioned that, besides fish, waterfowl are another vertebrate group that ingests meiofauna. Their ability to rework sediment (Cadée, 1990) and filter even small organisms (Gurd, 2007) enables waterfowl to retain consumed meiofauna. Gaston (1992) examined the guts of different bird species and found that a single green-winged teal can consume the nematodes inhabiting 1 m^2 of top-layer sediment (up to 2300 individuals) within 20 min. However, it is unlikely that nematodes provide a nutritious meal for such large organisms; rather, they are probably consumed incidentally.

7.5.5 Impact of predation by juvenile fish on natural nematode communities

Studies investigating the impact of fish on natural sediments have shown that the clearest top-down influence of freshwater fish on meiofaunal abundance and biomass is that of bottom biting fish (carp and gudgeon) (Table 7.3), with the largest reductions occurring in populations of oligochaetes, microcrustaceans, and nematodes. Juvenile carp (6–8 cm) and gudgeon (5–7 cm) caused a maximum reduction in nematode abundance of 82 and 56% and a biomass reduction of 94 and 80%, respectively (Weber and Traunspurger, 2014b). An effect of bream on nematode abundance in natural sediment, whether by feeding or sediment disturbance, was detectable after 14 days, and a significant top-down impact of carp already after 4 days (Spieth *et al.*, 2011). Carp also reduced the total secondary production of the nematodes down to 23% within 80 days (Weber and Traunspurger, 2015).

However, the largest reductions imposed by carp and gudgeon were observed in large-bodied meiofauna such as copepods and oligochaetes, while within the nematode's individuals >0.5 mm were preferentially consumed, which led to a shift in nematode size composition and a predominance of smaller worms. Similar effects were reported in a study of Nile tilapia (*Oreochromis niloticus*) (Abada *et al.*, 2017). Juvenile roach (1–2 mm) feeding on meiofauna reduced the abundances of oligochaetes and copepods but had no effect on either the nematode or the rotifer community (Dineen and Robertson, 2010; Spieth *et al.*, 2011) (Table 7.3).

Intensive predation by juvenile fish is particularly evident in the upper sediment layers (see Section 7.3.4) but the impacts on the meiofaunal community and specific prey sizes do not necessarily result in a change in nematode diversity, as demonstrated by Weber and Traunspurger (2014b) in a study of predation by juvenile gudgeon and carp (Table 7.3). In another study by Weber and Traunspurger (2015, 2016a), conducted in an enclosure/exclosure in a natural freshwater

pond (Fig. 7.7), the presence of carp depressed the abundance and biomass of free-living freshwater nematode assemblages, especially those of the dominant nematode species, resulting in changes in species density, biomass, and species composition (Fig. 7.8). The density and biomass of the nematode community changed significantly over time. At the beginning (day 0) of the field study, nematode density and biomass were similar between all treatments (Fig. 7.8). However, after 4 days the rapid

Fig. 7.7. Cages (enclosures) placed in a pond, which allow the contained fish to exert top-down effects on the benthic community in a natural environment and in a standardized area (Weber and Traunspurger, 2015, 2016a). (Photo: Sebastian Weber, Animal Ecology, Bielefeld University, Animal Ecology.)

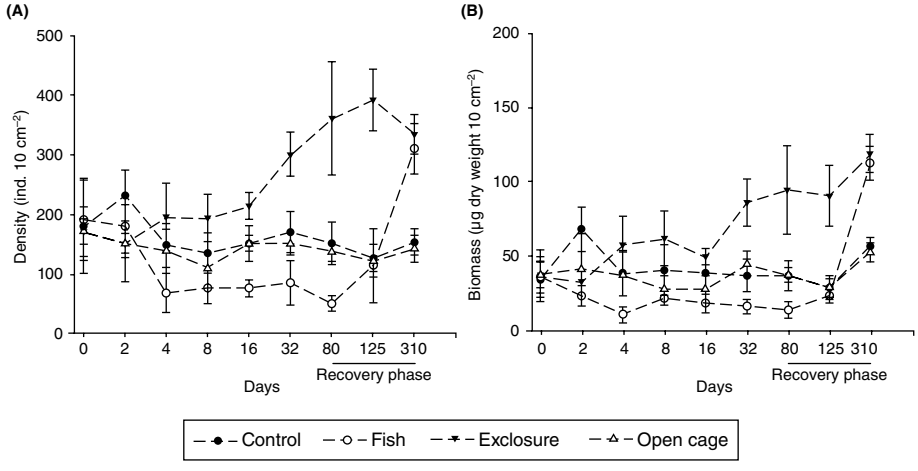


Fig. 7.8. Mean density (ind./10 cm^2) (**A**) and mean biomass (µg dry weight/10 cm^2) (**B**) of nematodes in response to different treatments during a field study (n = 5; ± standard deviation (SD)). The fish were removed from the fish treatment after 80 days to allow recovery of the nematode community (shown in the figure as the recovery phase). Note the scale change of the y-axis between figure parts. (From Weber and Traunspurger (2016a), with the permission of Brill.)

response of the nematode community to the fish treatments led to strong decreases in both density and biomass (Fig. 7.8). After 80 days, the differences between the treatments were significant; nematode density and biomass were lowest in the fish treatment, followed by the open cage and control treatments, and highest in the exclosure treatment (Fig. 7.8). Within the nematode community, the three most abundant nematode species, *Tobrilus gracilis*, *Eumonhystera filiformis*, and *Monhystera paludicola/stagnalis*, were greatly reduced after 80 days of fish predation, with the largest reductions occurring in the fish treatment, followed by the control and open cage treatments, and the smallest reductions in the exclosure treatment. By contrast, fish predation had no effect on either the diversity or the feeding type of the nematode assemblages (Weber and Traunspurger, 2015, 2016a).

7.5.6 Long-term effects of predation on nematode population dynamics

The studies presented above demonstrate the ability of benthivorous fish and macroinvertebrates to change the structure and composition of meiobenthic invertebrate communities in natural ecosystems. Their results, especially those from field investigations, provide evidence of at least a short-term top-down effect of larger organisms on nematodes in freshwater ecosystems. Consequently, nematode abundance, biomass, secondary production, size structure, and species composition will be shaped by predation by larger organisms (Table 7.3).

However, those studies were conducted over days, weeks, or a few months and whether the same results, that is, a clear impact of top-down control on nematode population dynamics, are obtained over the long term remains to be determined. Potential pitfalls were demonstrated in studies conducted in marine environments, where in microcosms or enclosure experiments young fish were shown to consume meiofaunal organisms and reduce their abundance, but field studies failed to evidence top-down control. It is very likely that large-scale events, such as flow disturbances and, especially, the migration of meiofaunal organisms, overwhelm the effects observed under controlled conditions (Coull, 1999; Dineen and Robertson, 2010). Meiofauna can recolonize disturbed substrates within hours (e.g. within one tidal cycle) or a few days, by active movement or passive drift by water or even air (see the Chapter 5). Their short generation times, high reproduction rates, and for some species their asexual reproduction strategies enable a rapid population recovery. The only enclosure/exclosure study in freshwater, by Weber and Traunspurger (2015, 2016a), suggested that young carp significantly shape meiofaunal communities. However, after removal of the carp from the fish enclosures (6 ind./m^2) on day 80 (Fig. 7.8), the meiofaunal and nematode communities recovered, and within 45 days post-removal meiofaunal abundance,

biomass, and secondary reproduction as well as nematode species composition were not significantly different in the open cage, control, and enclosure treatments (Weber and Traunspurger, 2015, 2016a). A determination of top-down control by larger organisms on meiofauna is thus highly dependent on predation intensity and frequency. Predation that occurs continuously and at high intensity will leave a larger impact than either a single predation event or even seasonal predation. In the case of nematodes and other meiofaunal organisms, predation is highly variable as it reflects the many predations strategies available to predators, such as moving to adjacent areas and changing feeding preferences (as occurs with growth), leaving the habitat (adult insects), but also consumption of the predator by an even larger one. Finally, periodic predation enables the benthic community to regenerate. Further investigations must therefore consider the influence of seasonality but also of species turnover and habitat structure.

7.6 Conclusions and Perspectives

Nematodes represent a nutritiously and omnipresent food source in benthic habitats. Indeed, gut analysis and direct observations revealed that they are consumed by a wide range of unicellular organisms, members of the meiobenthos including predacious nematodes, macrobenthos, and even vertebrates like juvenile fish (Fig 7.1). This list is certainly far from complete and it can be assumed that many more taxa feed on nematodes than previously thought. However, the examination of food components in the testiness of potential nematode feeding organisms or the observation of feeding behavior can only be the first step to complete the full mosaic and must be supplemented by further empirical studies. Laboratory approaches (e.g. functional response experiments) can help to investigate the trophic interactions between single species while microcosm experiments or enclosure/exclosure settings enable the examination of entire communities in natural environments and over long periods. Beyond that, analyses of stable isotopes and fatty acids are suitable tools to determine trophic interactions. All these studies have so far only been carried out for very few organisms that have been shown to ingest nematodes. Accordingly, there are still large gaps in our knowledge on the intensity and consequences of trophic interactions. For example, the quantity of ingested nematodes, the resulting (long-term) effects on the population dynamics (abundance, species composition, biomass, or secondary production) of nematode communities in natural environments and the benefits for organisms that selectively or unselectively consume nematodes need further scientific attention. Only the combination of different experimental approaches will ultimately deliver further insights into the role of nematodes in the food web.

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8 Production of Freshwater Nematodes

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Highlights

- The presence of so many varied sampling and modeling approaches used in the literature makes it so much harder to fully understand nematode production patterns in aquatic ecosystems.
- Here we give guidelines for sampling and we compare different modeling approaches through a case study to help standardize estimates of nematode production.
- Estimation of nematode annual production ranged from 0.28 to 1.77 gDW/m^2 of biofilm, depending on seasonal regime, sampling frequency, and allometric model used.
- Models taking into account temperature dependency of metabolism seem more sensitive to seasonal fluctuation, but they also show high variance.
- The model proposed by Schmid-Araya *et al.* (2020) tracks well with seasonal variation and is less skewed, suggesting it has the highest consistency.

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8.1 Introduction

Secondary production is a function of population density, biomass, growth rate, reproduction, and the rate of biomass turnover (production divided by biomass, *P*/*B*). As such, the estimated secondary production of a given taxonomic group reflects the ecological role of the group, its trophic level, and even its habitat. Benke (1993), Dolbeth *et al.* (2012), and Benke and Wallace (2015) suggested that estimates of a population's production provide a measure of its fitness, and if applied to functional groups or entire species assemblages serve as a measure of energy flow through the system. Moreover, the estimate will be comparable with others provided that the methods used in their calculation are comparable. Other applications of production estimates are quantifying the consequences of a toxic pollutant on ecosystem fluxes of energy and matter (Whiles and Wallace, 1995; Benke and Huryn, 2010; Faupel and Traunspurger, 2012).

However, for benthic ecosystems, there is little knowledge about the amount of carbon they produce, because ubiquitous and numerically dominant animals, such as nematodes, are rarely included in freshwater production budgets (reviewed in Schmid-Araya *et al.*, 2020). There are several reasons for this omission: (i) counting, identifying and measuring microscopic animals such as nematodes is a time-consuming task; (ii) for populations of free-living nematodes, there have been few controlled, laboratory determinations of the rate of biomass turnover and its dependency on key external drivers such as temperature; and (iii) as most nematode species are able to reproduce continuously in the field, natural populations lack discernible cohorts, which calls into question the utility of production estimates derived from methods based on cohort-interval frequencies. Moreover, it highlights the importance of developing a coherent framework to estimate nematode production in the field using standard sampling, measurement procedures, and comparable allometric models.

In this chapter we focus on the methods used to determine nematode production. We begin by briefly describing the methods used in sampling and measuring nematodes, followed by a summary of the common allometric models developed to estimate secondary production. We then present a case study in which the results of those models are compared. Finally, we evaluate the drivers of nematode production in different lakes and streams and compare nematode secondary production with that achieved by macrobenthos, other meiobenthic taxa and microbes.

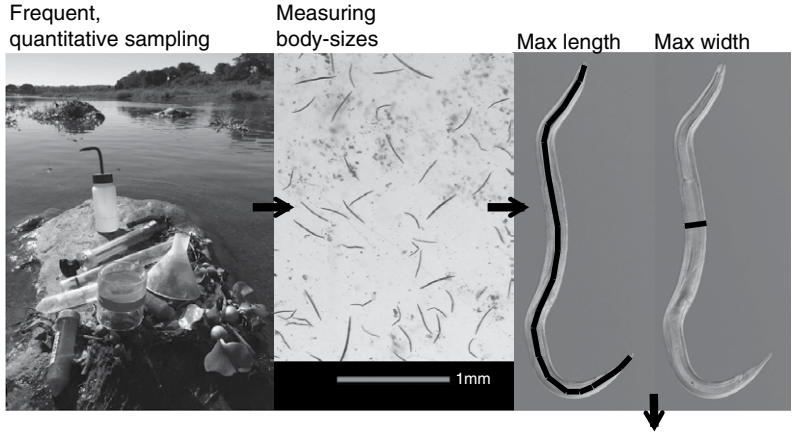
8.2 Measuring Nematode Production

8.2.1 Sampling and measuring nematodes

The critical step in estimating secondary production by microscopic animals such as nematodes is the use of a standardized and quantitative sampling design. Quantitative sampling methods are described elsewhere in this book (Chapter 2). Here we emphasize (i) the importance of using

quantitative sampling (e.g. a corer, or brush-sampler in case of hard substrates) to estimate the secondary production of nematodes; (ii) that sampling should be spatially easily replicable in order to take into account the small-scale heterogeneity of nematode distribution; and (iii) that sampling should be temporally easily replicable to capture the fluctuations in abundance (and thus production) that may occur in a given habitat. A useful rule of thumb to capture potential variations in abundance is to perform at least seasonal samplings (e.g. every 3 months, over 1 year), but more frequent sampling (weekly, monthly, or bi-monthly) will ultimately provide a more accurate picture of the fluctuations in nematode standing stocks, especially in very dynamic environments such as streams and rivers (e.g. Majdi *et al.*, 2011; Traunspurger *et al.*, 2015; Brüchner-Hüttemann *et al.*, 2020).

Nematodes should be extracted from samples quantitatively (e.g. via Ludox flotation), using relatively small mesh sizes (10 or 20 µm) in order to retain adequately both the smallest species and juvenile stages (Ptatscheck *et al.*, 2020), which show the highest rates of biomass turnover. When nematodes are counted, they may be directly categorized in size classes or photographed 'en masse' against a millimetric gridded background. Staining with Rose Bengal facilitates processing and allows individuals to be readily counted from images using the line segmentation program available in image analysis freeware, such as FiJi (Fig. 8.1).



The formula of Andrássy (1956) and a calculation table are used to estimate wet weight (WW) as:

$WW = (L*D^2)/(1.6*10^6)$

WW is wet weight (µg),
L is max length (µm),
D is max width (µm)

	Date	Sample	Specimen	L	D	WW
5940	A214	M19	12048	0,60802	0,03046	3,53E-01
5941	A214	M19	12049	0,59590	0,03119	3,62E-01
5942	A214	M19	12050	0,52490	0,03578	4,20E-01
5943	A214	M19	12051	0,41421	0,02400	1,49E-01
5944	A214	M19	12052	0,77433	0,03388	5,56E-01
5945	A214	M19	12053	0,70450	0,03103	4,24E-01
5946	A214	M19	12054	0,61373	0,03109	3,71E-01
5947	A214	M19	12055	0,65371	0,03195	4,17E-01
5948	A214	M19	12056	0,70442	0,02749	3,33E-01
5949	A214	M19	12057	0,79571	0,03582	6,38E-01
5950	A214	M20	12058	0,59315	0,03330	4,11E-01
5951	A214	M20	12059	0,47998	0,03133	2,94E-01
5952	A214	M20	12060	0,47419	0,02943	2,57E-01
5953	A214	M20	12061	0,76903	0,03264	5,12E-01
5954	A214	M20	12062	0,65123	0,03211	4,20E-01
5955	A214	M20	12063	0,44975	0,02666	2,00E-01
5956	A214	M20	12064	0,43065	0,01861	9,32E-02
5957	A214	M20	12065	0,66180	0,03432	4,87E-01

Fig. 8.1. Example of steps to assemble nematode body size datasets necessary to estimate secondary production. Water temperature, individual, and population biomass may further be used to estimate secondary production using allometric relationships from the literature (see main text). (Author's own figure.)

Of course, nematode individuals can also be selected randomly from samples and then mounted on slides for taxonomic identification and morphometric determinations. While this is surely the most accurate method to obtain data on the taxonomic composition and body size distribution within a sample, it requires taxonomic expertise and the laborious processing of each individual. The number of individuals that need to be measured depends on the number of samples or sub-samples from a given habitat as well as the number of sampling occasions. However, a general guide to obtain an accurate estimate of nematode standing stocks is to measure 50–100 individuals (maximum length and width) per sample in each of five replicates, which means between 250 and 500 individuals measured per habitat, per sampling occasion (Fig. 8.1). Once body length and body width are measured, the formula of Andrássy (1956) can be used to infer the fresh body mass (wet weight, WW) of an individual nematode, which can be further converted to dry weight (DW) based on the common assumption that DW = 0.25 × WW.

When body size measures are not available or possible but the species composition is known, then community biomass composition can be inferred from the abundance, species composition, and average body mass per taxa, as reported in the taxonomic literature. Ferris' *Nematode-plant expert information system* (available at http://nemaplex.ucdavis.edu) is a very useful online platform that provides a wealth of nematological information, including a list of adult body mass values for 2840 nematode species that commonly inhabit soils but which may also be found in freshwater habitats.

8.2.2 Estimating secondary production

The laborious and sometimes tedious procedures necessary to measure invertebrate production have led to the development of shortcut approaches: they may be cohort- or non-cohort-based (Benke and Huryn, 2007, 2010). As noted above, the short generation times and continuous reproduction of nematodes result in natural populations that typically lack discrete cohorts. Thus, for nematodes, non-cohort methods, which rely on independent estimates of development or biomass growth rates, will be more appropriate. These are based on allometric models and rely on the use of simple regression analyses that examine the relationships between production (P), biomass (B), biomass turnover (P/B ratio), and one other variable, such as lifespan, temperature, or body size at maturity (maximum B) (e.g. models detailed below: Banse and Mosher, 1980; Vranken *et al.*, 1986; Plante and Downing, 1989; Schmid-Araya *et al.*, 2020). Nevertheless, allometric models of secondary production are effective only in synthesizing information from large datasets (Morin, 1997); they cannot be reliably used for snapshot, discontinuous, or scarce data.

8.2.2.1 Banse and Mosher's model

A commonly used allometric relationship is the power function of Banse and Mosher (1980), which relates the annual rate of specific production (P/B) to the body mass (M): $P/B = aM^b$, where a is −0.19 and b = −0.37 after Banse and Mosher's (1980) equation no. 3 for temperate zone invertebrates (annual mean temperatures comprised between 5 and 20°C). Furthermore, Banse and Mosher (1980) suggest that meiofaunal organisms tend to have a substantially lower P/B as predicted by the general relationship given for macro-invertebrates, because they postulate that meiofaunal organisms are so small that they should be protected from predation. Hence, they suggest a power function of mass dependence of P/B that should be three to five times below that of macro-invertebrates. Strayer and Likens (1986) further applied the maximum (adult) individual body mass (M, µg) measured for each species in Mirror Lake to the modified equation no. 3 of Banse and Mosher. They also assumed that meiofaunal turnover is slower than the rate predicted by Banse and Mosher's equation no. 3 and therefore followed the suggestion of Banse and Mosher, but divided the calculated P/B ratio by 4. This gives the following general equation for meiofauna (and thus nematodes):

$$\frac{P}{B} = \frac{-0.19 \times M^{-0.37}}{4}$$

8.2.2.2 Vranken et al.'s model

Other allometric models have been specifically developed to measure the production of aquatic nematodes. For example, the model developed by Vranken *et al.* (1986) is based on measurements of the duration of the egg-to-egg development of 12 marine nematode species. A regression equation (R^2 = 0.88) relates the duration of egg-to-egg development to body mass and temperature as follows:

$$log_{10}(T) = 2.202 - (0.0461 \times t) + (0.627 \times log_{10}(W))$$

Where T is the generation time (in days), t is the temperature (°C), and W is the adult body weight measured as DW. Assuming a biomass turnover per generation of 3, the annual P/B ratio can be calculated as:

$$\frac{P}{B} = \frac{365}{T} \times 3$$

Multiplying P/B by B (defined as the average standing stock or biomass of the community for a given surface or volume of habitat) gives an estimate of production during the period considered. Hence, nematode production can be calculated if the temperature, the biomass of the population, and the body mass of the adult species are known.

8.2.2.3 Plante and Downing's model

Another allometric approach that has been commonly used with nematodes is that of Plante and Downing (1989). It uses the regression:

$$log_{10}(P) = 0.06 + (0.79 \times log_{10}(B)) - (0.16 \times log_{10}(M)) + (0.05 \times T)$$

This is based on correlations between secondary production (P), the annual mean population biomass (B, gDW/m^2), maximum individual body mass (M, mgDW), and the mean annual temperature (T, °C).

According to Plante and Downing (1989), this equation can be used to predict the logarithm of production with 79% precision. Majdi *et al.* (2017) used Plante and Downing's allometric regression to estimate daily production on each sampling date (using the daily mean temperature, mean, and maximum biomass, instead of annual means). They argued that using annual average temperature values fails to consider the temperature fluctuation regime and can thus miss potentially large variations in productivity.

8.2.2.4 Schmid-Araya et al.'s model

Recently, Schmid-Araya *et al.* (2020) reviewed the different allometric models above and tested the significance of the correlations between temperature-adjusted biomass and production for different taxonomic groups of meiofaunal invertebrates, including nematodes, based on the Boltzmann distribution: $e–E/kT$ where E is the activation energy (in eV), k is Boltzmann's constant (1.380649×10^{-23} J/K), and T is absolute temperature (in Kelvin) (see details in Brown *et al.*, 2004). Based on the values for 29 species of nematodes, Schmid-Araya *et al.* (2020) reported a significant linear regression between ln-transformed temperature-corrected annual production ($P\ e^{E/kT}$) (mgDW/m^2/year) and the ln-transformed temperature-corrected biomass ($B\ e^{E/kT}$) (mgDW/m^2) according to the following equation:

$$Ln\left(Pe^{\frac{E}{kT}}\right) = \left(0.818 \times ln\left(Be^{\frac{E}{kT}}\right)\right) + 8.311$$

The exponent close to 1 they found based on the relationship between P and B indicates that production increases in proportion to biomass. Thus, in this model, standing stock, rather than temperature, is the most important variable explaining the variation in production (86% for nematodes).

8.3 Secondary Production of Nematodes: A Case Study

In the following case study, we estimate the secondary production of free-living nematodes dwelling on epilithic biofilms in the Garonne River (southwestern

France, Fig. 8.2). The dataset was obtained from two sampling campaigns. The first consisted of 16 sampling occasions conducted, to the extent possible, at regular intervals (ca. every 23 days) over a 1-year period (December 2004–December 2005), and the second of 29 sampling occasions conducted at more frequent intervals (ca. every 12 days) also over a 1-year period (September

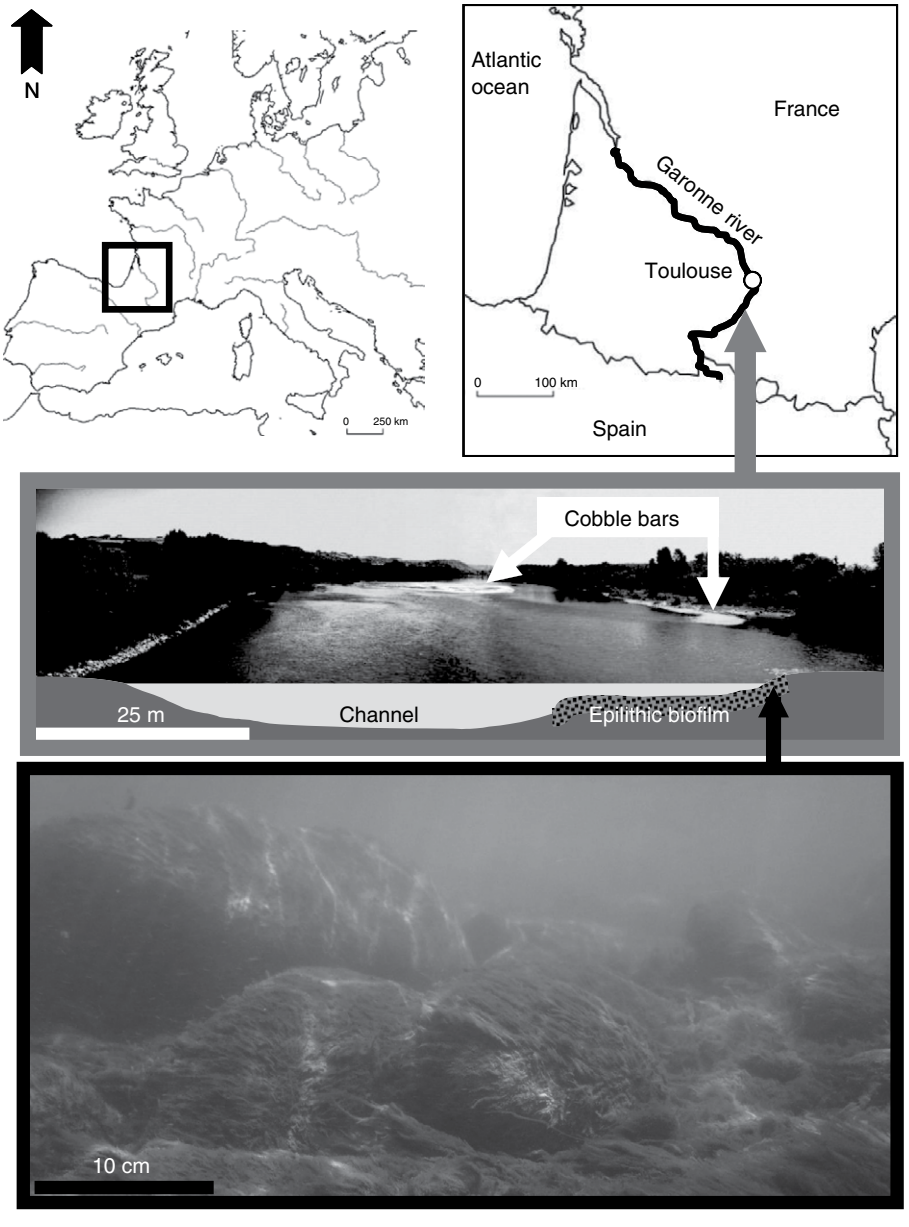


Fig. 8.2. Location of the sampling site, cross-sectional view of the Garonne River at the sampling site and closer subaquatic view on the epilithic biofilm. (Author's own figure.)

2008–September 2009). However, for both, fewer samplings were conducted in winter and spring because of floods or weather conditions that hindered access to the site and proper sampling. At each sampling occasion, the same 150-m-long river reach was sampled (lat. 01°17′53′′E, long. 43°23′45′′N; elevation: 175 masl). At this site, the canopy is wide open and the shallow riverbed is covered by cobbles coated by a productive diatom biofilm that harbors a variety of microscopic animals throughout the year (Fig. 8.2), although floods and heat waves may provoke biofilm detachment and shifts of community structure (see Majdi *et al.*, 2011, 2012 for details). At each sampling occasion, four randomly selected cobbles (mean diameter = 10 cm) were collected by sliding them into a plastic bag underwater (depth = 30–50 cm), which prevented detachment of the biofilm.

The upper surface of the cobbles was entirely scraped off using a toothbrush and the area of biofilm that had been removed was inferred from scaled photographs. The biofilm suspension was sieved on 40-µm meshes and the nematodes on the sieve were counted, followed by measurements of the body length and body width of a total of 4202 and 6370 individuals for the first and second sampling campaign, respectively. The age, sex, and species of the nematodes obtained from the second sampling campaign were determined (Majdi *et al.*, 2011). The latter assessment showed that the nematode community was strongly dominated by *Chromadorina bioculata* and *Chromadorina viridis*, although summer biofilms contained a higher diversity, especially of small bacterivorous species such as *Eumonhystera dispar* and *Monhystrella paramacrura*, which were thus relatively more abundant in summer (Majdi *et al.*, 2011). This seasonal shift was also evident from the mean individual biomass, which in both sampling campaigns tended to be lower during summer (Fig. 8.3). Abundances were substantially higher during the first sampling campaign (mean: 525,276 up to 1.3 million individuals/m^2) in comparison with the second sampling campaign (mean: 164,376 individuals/m^2) (Table 8.1). The first sampling campaign was characterized by a dry autumn without floods allowing the formation of extremely thick biofilms packed with overwintering nematodes. As a result, the biomass was, on average, four times higher during the first than during the second sampling campaign (49.87 vs. 12.29 mgDW/m^2; Table 8.1).

An ‘instant’ daily production value was estimated for each sampling occasion (as some methods rely on the temperature, see above) and then multiplied by 365 to infer ‘instant’ annual production as determined on a given sampling occasion. All of the values were averaged and used for further descriptive statistics. Depending on the method used to determine annual production, the average estimated yearly production was 2.4 to 3.7 times higher during the first than the second campaign (Table 8.1). For both sampling campaigns, the highest mean annual secondary production value was obtained using Plante and Downing’s method (Table 8.1), and the lowest mean estimate was obtained using Schmid-Araya *et al.*’s method (Table 8.1). A comparison of the position of median values in the

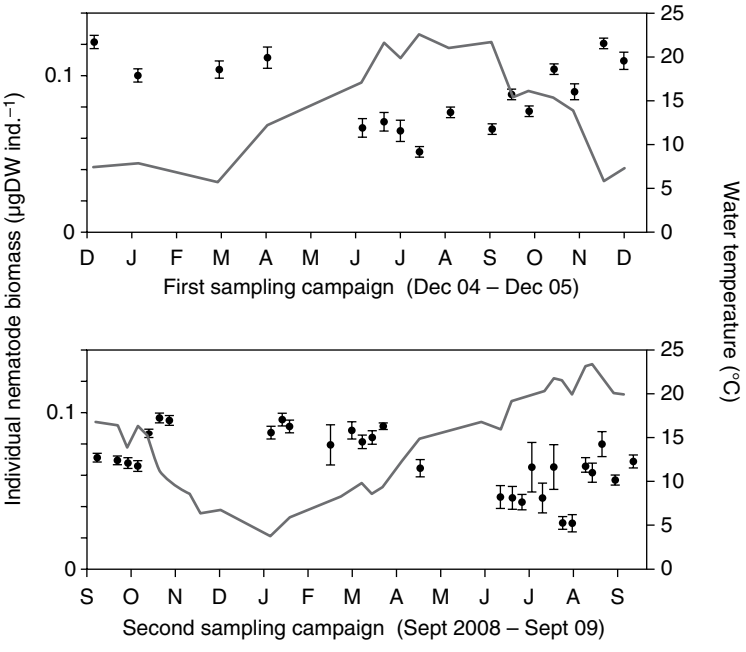


Fig. 8.3. Mean individual nematode biomass (black points, ± standard error) and water temperature (gray line) during the first and second biofilm sampling campaign in the Garonne River. (Author's own figure.)

box plots of Fig. 8.4 suggests that the methods of Banse and Mosher (1980) and that of Schmid-Araya *et al.* (2020) were more conservative (i.e. less skewed dispersions).

By contrast, the methods of Vranken *et al.* (1986) and that of Plante and Downing (1989) reflect the skewed data distribution, with an outlier value >3σ for the first sampling campaign (Fig. 8.4). This was probably due to the fact that those latter methods take into account temperature directly, which in a riverine habitat may fluctuate widely throughout the year (ΔT ca. 20°C) and will therefore result in overestimation and broader variance when the sampling frequency is skewed toward warm periods.

Concerning *P*/*B*, once again Plante and Downing's method gave the highest estimates (see Table 8.1). Nevertheless, in contrast with estimates of *P*, the estimates of *P*/*B* were consistently higher during the second sampling campaign. This may reflect the greater share of small individuals present during the second than the first sampling campaign (average nematode weight of 0.07 vs. 0.09 µgDW/individuals, respectively, Fig. 8.5), especially during summer (see Fig. 8.3). For both sampling campaigns, a seasonal pattern of *P*/*B* can be seen (Fig. 8.6), with the highest values occurring in spring/summer. However, the seasonal pattern was not well discerned using the method of Banse and Mosher (1980), which suggests that this method tends to underestimate the seasonal dynamics of nematode biomass turnover.

Table 8.1. Abundance, biomass, and production of biofilm-dwelling nematodes in the Garonne River as determined during two sampling campaigns. (Author's own table.)

Sampling campaign		Water temperature (°C)	Abundance (ind./m²)	Standing stock biomass (mgDM/m²)	Individual biomass (µgDM/ind.)	Maximal individual biomass (µgDM/ind.)	Production:biomass ratio (*P*/*B*), after				Annual production (gDM/m²/year), after			
							Vranken *et al.* (1986)	Banse and Mosher (1980)	Plante and Downing (1989)	Schmid-Araya *et al.* (2020)	Vranken *et al.* (1986)	Banse and Mosher (1980)	Plante and Downing (1989)	Schmid-Araya *et al.* (2020)
2004–2005	Median	15.45	455,267	42.74	0.0892	0.35	25.93	28.67	39.40	19.88	0.55	1.21	0.91	0.84
	Mean	14.46	525,276	49.87	0.0890	0.41	**32.99**	**28.57**	**79.77**	**24.79**	**1.23**	**1.41**	**1.77**	**0.89**
	Minimum	5.70	4,817	0.32	0.0512	0.19	6.69	18.40	13.06	16.43	0.01	0.01	0.06	0.02
	Maximum	22.70	1,302,705	118.66	0.1219	1.17	92.41	36.39	257.52	48.14	5.16	3.48	6.88	1.95
2008–2009	Median	16.25	152,084	10.88	0.0690	0.27	39.79	31.45	68.61	25.40	0.23	0.33	0.43	0.28
	Mean	15.09	164,376	12.29	0.0676	0.28	**44.49**	**33.20**	**109.54**	**31.69**	**0.40**	**0.38**	**0.74**	**0.28**
	Minimum	3.80	3,476	0.16	0.0287	0.08	8.71	22.83	14.15	20.41	0.00	0.01	0.01	0.01
	Maximum	23.03	502,890	36.0	0.0959	0.65	120.05	49.21	306.02	54.76	1.30	1.08	3.02	0.74

DM, dry mass; ind., individuals.
Mean values are bold.

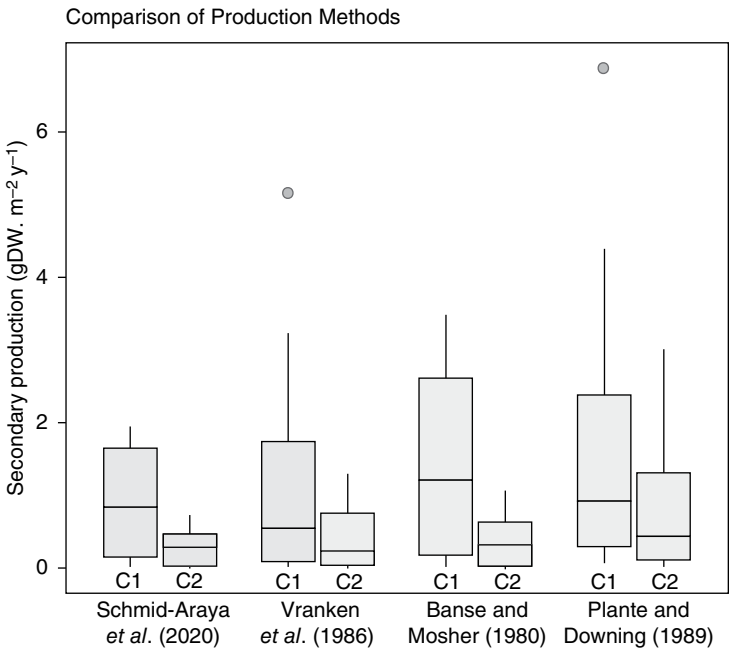


Fig. 8.4. Comparison of annual production of biofilm-dwelling nematodes in the Garonne River estimated using different models during the first sampling campaign (C1) and the second sampling campaign (C2). The black line indicates the median, the boxes show the interquartile range, the whiskers the ±3σ range, and the dots the outliers. (Author's own figure.)

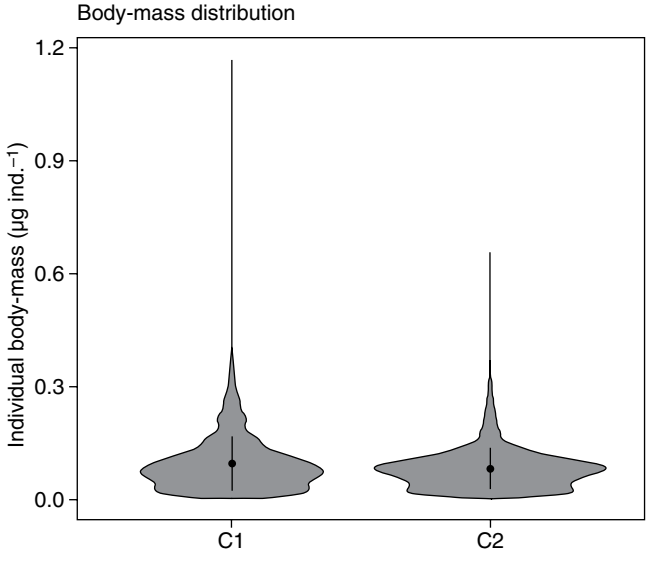


Fig. 8.5. Compact display of the body mass distributions of individual biofilm-dwelling nematodes during the first (C1: December 2004–December 2005; N = 4202 measures) and second (C2: September 2008–September 2009; N = 6370) sampling campaigns. The lines show the density distributions, the dots show the mean values, and the whiskers the standard deviation. (Author's own figure.)

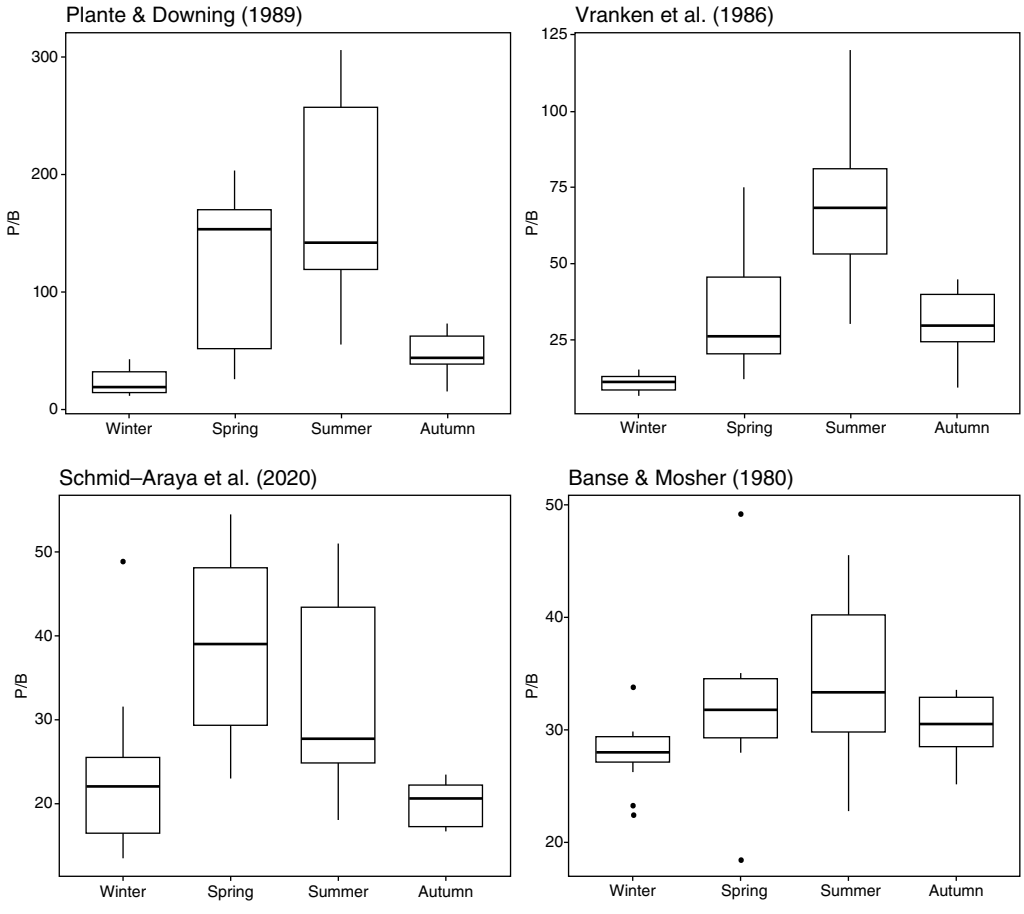


Fig. 8.6. Comparison of the methods used to calculate production and the resulting seasonal production:biomass ratios (*P*/*B*) for biofilm-dwelling nematodes sampled during two year-long field campaigns conducted along the Garonne River ($N = 45$). The black line indicates the median; the boxes show the interquartile range, the whiskers the ±3σ range, and the dots the outliers. (Author's own figure.)

8.4 Nematode Production in Relation to the Environment

8.4.1 Across-habitat comparisons of secondary production

The values of *P* and *P*/*B* measured in the Garonne River samples were in the upper range of those previously reported from other freshwater and marine habitats (Table 8.2).

While annual nematode production estimates may vary considerably depending on the habitat and the method used in their calculation, annual production values reported from marine habitats seem substantially higher, but community *P*/*B* values across freshwater and marine habitats are relatively consistent and often higher than the commonly used

Table 8.2. Comparison of annual production and the production:biomass (*P*/*B*) ratios of freshwater and marine nematodes as reported in the literature. (Author's own table.)

Study site	Habitat	Production (gDW/m²/year)	Community *P*/*B*	Method	Reference
Garonne River C1	Epilithic biofilm	0.89–1.77	25–80	Various	This study
Garonne River C2	Epilithic biofilm	0.28–0.74	33–109.5	Various	This study
Headwater stream Ems	Sandy sediment (0–5 cm)	0.26**	35	Plante and Downing (1989)	Majdi *et al*. (2017)
Headwater stream Ems	Sandy sediment (5–10 cm)	0.12**	10	Plante and Downing (1989)	Majdi *et al*. (2017)
Headwater stream Furlbach	Sandy sediment (0–5 cm)	0.44**	48	Plante and Downing (1989)	Majdi *et al*. (2017)
Headwater stream Furlbach	Sandy sediment (5–10 cm)	0.34**	22	Plante and Downing (1989)	Majdi *et al*. (2017)
Headwater stream Furlbach	Sandy sediment	0.152		Banse and Mosher (1980)	Brüchner-Hüttemann and Traunspurger (2020)
Headwater stream Furlbach	Dead wood	0.06		Banse and Mosher (1980)	Brüchner-Hüttemann and Traunspurger (2020)
Headwater stream Furlbach	Macrophytes	0.007		Banse and Mosher (1980)	Brüchner-Hüttemann and Traunspurger (2020)
Headwater stream Furlbach	Leaf litter	0.02		Banse and Mosher (1980)	Brüchner-Hüttemann and Traunspurger (2020)
Lake Königssee	Littoral (2 m)	0.61[a]	37	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Littoral (5 m)	0.8[a]	28	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Littoral (10 m)	0.1375[a]	10	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Littoriprofundal (15 m)	0.095[a]	11	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Littoriprofundal (20 m)	0.0525[a]	10	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)

Continued

Table 8.2. Continued.

Study site	Habitat	Production (gDW/m²/year)	Community *P*/*B*	Method	Reference
Lake Königssee	Littoriprofundal (30 m)	0.065[a]	6	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Profundal (60 m)	0.0475[a]	5	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Profundal (120 m)	0.0375[a]	9	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Königssee	Profundal (190 m)	0.0275[a]	13	Banse and Mosher (1980)	Bergtold and Traunspurger (2006)
Lake Brunnsee	Profundal (18 m)	0.7825[a]	9	Banse and Mosher (1980)	Bergtold and Traunspurger (2005)
Marine (Belgium)	Littoral	5.55[a]	20	Vranken *et al*. (1986)	Vranken *et al*. (1986)
Marine (Japan)	Aufwuchs	2.275[a]	58	Vranken *et al*. (1986)	Vranken *et al*. (1986)
Marine	Seagrass (*Posidonia oceanica*, Ligurian Sea)	2.1[b]	8.4	Warwick *et al*. (1979)	Danovaro *et al*. (2002)
Marine	Sandbank (North Sea)	1.42[b]	10–35	Production efficiency 40–70%	Heip *et al*. (1990)

[a]recalculated assuming DW:WW = 0.25.
[b]recalculated assuming C:DW = 0.5.

value of 9 proposed by Gerlach (1971), when the species composition is unknown. Moreover, reports in the recent literature indicate that meiobenthic production is much higher than previously estimated and may equal or even exceed that of the macrobenthos (reviewed in Schmid-Araya *et al.*, 2020). Contrary to the assumption of Banse and Mosher (1980), there is no evidence of the existence of a 'size refuge' for nematodes (see Chapter 7) that would trigger a reduced mortality, and thus skip the necessity to have high biomass turnover strategies. Instead, nematodes appear as a quite productive community in aquatic ecosystems, which supports their role as important trophic links. The generally high abundance of nematodes dwelling on biofilms (see also Chapter 3) translates into a substantial production of biomass in comparison with the situation observed in soft substrates (Table 8.2). The net production values reported in our case study are substantially higher than those reported from other types of freshwater habitats, even when considering the same estimation methods. This supports the hypothesis that biofilms are important habitats for nematodes, but one may ask what is the fate of the substantial biomass produced by biofilm nematodes: Is it fueling biofilm food webs? Is it exported when biofilms are detached by floods or grazed by macro-invertebrates and fishes? Answering these questions would require further research.

8.4.2 Temporal variation of production

Biomass turnover (*P*/*B*) is a key feature of communities and ecosystems and closely relates to the abundance and the body size spectrum of the populations involved (Schmid-Araya *et al.*, 2020; Schmid *et al.*, 2020). *P*/*B* may change throughout the year as does community structure and standing stocks, in response to seasonal fluctuations of biotic and abiotic factors (see Chapter 3). In the Garonne River, seasonality has a strong impact on nematode community structure (Majdi *et al.*, 2011): in summer there is a shift toward a community made up of more abundant populations of small opportunistic nematode species, which reflects the change in the availability of resources in the biofilm but also increasing competition from larval insect grazers (Majdi *et al.*, 2011). This shift in body size structure of the community was evident for both sampling campaigns (Fig. 8.3). Small species have a higher *P*/*B* than do large species, hence the highest community *P*/*B* values were measured in summer. As an example, in a study of freshwater nematodes growing in the profundal zone at 10°C in Lake Brunnsee (southern Germany), Bergtold and Traunspurger (2006) estimated that *P*/*B* ranged from 2.3 for the very large *Tripyla glomerans* (4.45 µgDW) to 187 for the small *Eumonhystera simplex* (0.007 µgDW).

As another example of seasonal variation of nematode production, in Lake Königssee (southern Germany), nematode production was high during summer and autumn in the littoral zone (Fig. 8.7), where *Rhabdolaimus terrestris* and *R. aquaticus* dominated. Annual production reached a maximum at 5 m water depth (Table 8.2) whereas monthly

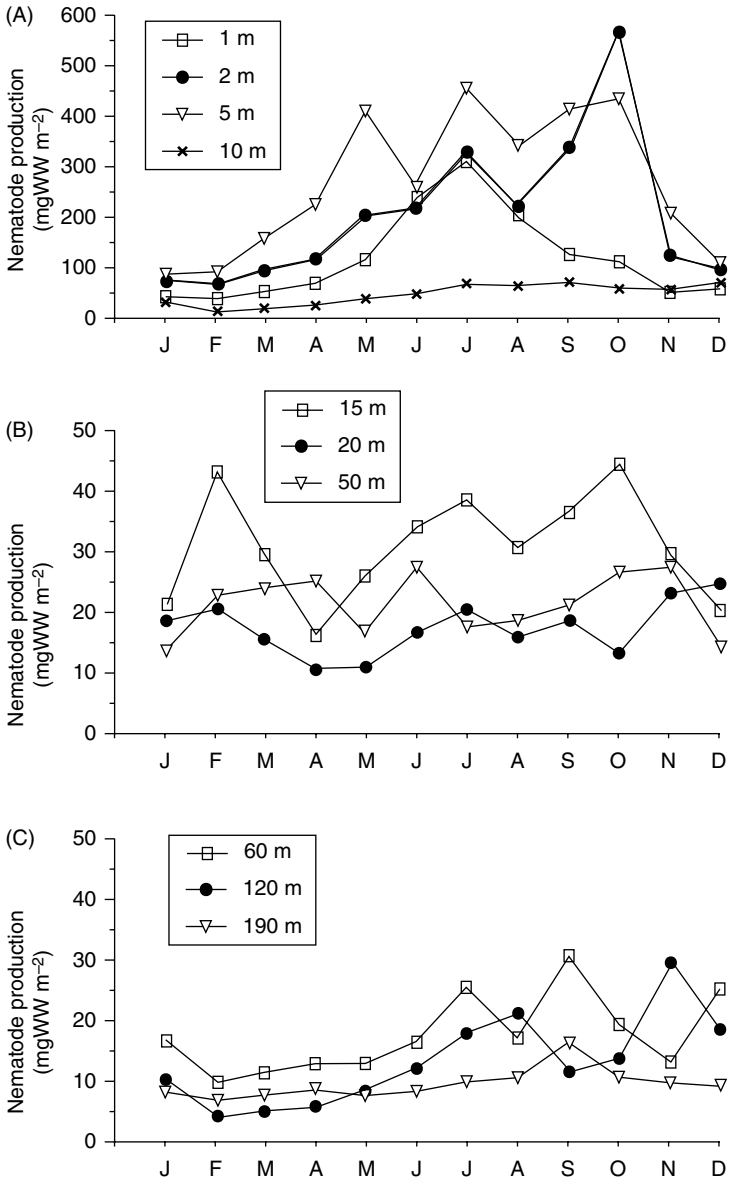


Fig. 8.7. Nematode production in the littoral (**A**), littori-profundal (**B**), and profundal (**C**) zones of Lake Königssee. (From Bergtold and Traunspurger, 2006.)

production was highest at 2 m, in October (Fig. 8.7). Nematode production showed a pronounced seasonal fluctuation, with peaks occurring in February and October at 15 m water depth (Fig. 8.7). At this depth but also at 20 m *Monhystera paludicola* dominated the nematode community. In the profundal zone, nematode production was characterized by moderate fluctuations during the study period, increasing from February toward

autumn (Fig. 8.7). In the profundal zone of another small, alpine lake, Lake Brunnsee, production by most of the species was highest during summer, with another peak reached in autumn, and low during September. In this environment *Tobrilus gracilis* had the highest production, followed by *Eumonhystera filiformis* (Bergtold and Traunspurger, 2005).

8.4.3 Spatial variation of production

While this trend is unlikely to occur in biofilms, or at least has yet to be determined at the microscopic scale, the results of nematological studies investigating soft substrates suggest that nematodes show vertical distribution patterns at centimetric scales in the sediment, clearly preferring the uppermost layers, where food and oxygen are more available. This preference has consequences for production, with higher values measured in the superficial sediment, as demonstrated in the Ems and Furlbach streams (Table 8.2, Majdi *et al.*, 2017), but also in Lake Brunnsee (Fig. 8.8; Bergtold and Traunspurger, 2004, 2005) and in other freshwater (Kirchner, 1975; Traunspurger and Drews, 1996) and marine (Platt, 1977; Soetaert and Heip, 1989) sediments. Figure 8.8 shows nematode production in the different layers. The percentage of total benthic production is highest in the uppermost 0.5 cm (30%) but almost as high (27%) in the deepest layer (2.0–4.0 cm). This contrasts with the large difference in nematode relative abundance: 46 vs. 18%, respectively. It should be noted that in Lake Brunnsee larger nematodes prefer the deeper sediments, with 39% of *T. gracilis* individuals inhabiting the deepest sediment layer (Bergtold, 2001). This species thus contributes substantially to the vertical production pattern observed in the sediment of this lake.

In lakes we may also observe larger-scale spatial patterning as production and *P*/*B* ratios tend to decrease with increasing water depth (Table 8.2).

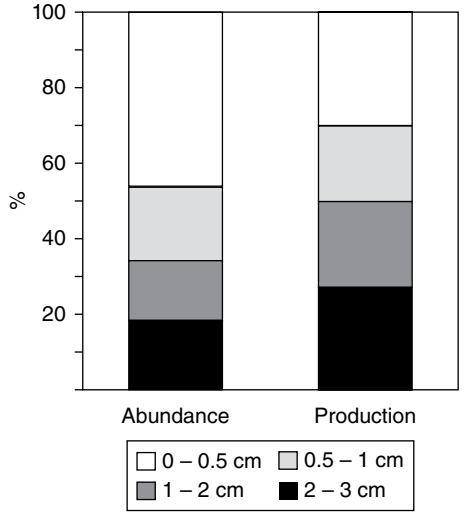


Fig. 8.8. Relative contribution (%) to total nematode abundance and production across different sediment layers in Lake Brunnsee. (From Bergtold and Traunspurger, 2006.)

This may be caused by temperature and resource shifts, but also reflects community structure shifts. For example, the dominance of relatively large species like *T. gracilis* that clearly have affinities with profundal zones. In Lake Königssee, nematode production increased from 1 to 5 m water depth but then decreased substantially in the deeper parts of the lake (Table 8.2). *P*/*B* ratios in the profundal zones of Lake Brunnsee and Lake Königssee were comparable, although production was substantially higher in the former. Trophic status, the temperature regime, and the availability of light to fuel 'green' tropho-dynamics are fundamental drivers of nematode productivity. Thus, in the constantly cold and dark environment of the profundal zone, where nematodes rely on sedimenting organic particles, both species generation time and community *P*/*B* values will be much lower than in the littoral zone (Table 8.2).

8.5 Production by Nematodes vs. Other Benthic Organisms

Larger organisms (macrobenthos) dominate the sediments in terms of biomass. Hakenkamp *et al.* (2002) showed that for some lotic systems, including the Yealm River, UK (Ramsay *et al.*, 1997), 21 streams in the Outaouais region of Canada (Bourassa and Morin, 1995), and Mirror Lake, USA (Strayer, 1985), total biomass generally increases with body mass. Those authors also concluded that the contribution of smaller organisms to overall biomass is higher in lentic than in lotic systems, although variance is large and the number of studies is limited. The large variance of the data can be explained by several factors, including the grain size of the sediment, predation by fish, and food availability. Strayer (1991) suggested that smaller meiofaunal organisms would predominate in food-poor environments, a hypothesis supported by at least two literature reports (Anderson and de Henau, 1980; Särkkä, 1995). However, the share of benthic production among organisms of different size classes has been the topic of only a few studies (e.g. Strayer and Likens, 1986; Schwinghamer *et al.*, 1986; Bergtold and Traunspurger, 2005; Schmid *et al.*, 2020; Brüchner-Hüttemann *et al.*, 2019, 2020). Since the mass-specific metabolic rate of an individual decreases as its body mass increases, a larger biomass of large than of smaller organisms will be needed to obtain an equal rate of total production. In a study conducted at selected Latgalian lakes (Latvia), the ratio of meiobenthic to macrobenthic production varied widely, with meiobenthic surpassing macrobenthic production by a factor as high as 14 (Kurashov, 2002). In contrast, other studies in lakes reported that the metabolic activity of macrobenthos is equal to or up to twice as high as that of meiobenthos (Holopainen and Paasivirta, 1977; Nalepa and Quigley, 1983; Strayer and Likens, 1986). In streams, the contribution of meiobenthos to overall invertebrate production varies between 0.7 and 52% (Hakenkamp *et al.*,

2002 and literature cited therein), but more recent studies collectively report a substantial contribution of meiobenthic organisms to overall invertebrate production in streams (Majdi *et al.*, 2017; Schmid-Araya *et al.*, 2020). A critical issue that arises in comparisons of production by meiobenthos and macrobenthos is the very disparate sampling strategies used in meiofaunal studies. The use of 10-µm rather than 50- to 63-µm meshes to retain meiofauna will strongly affect estimates of the meiofaunal contribution to secondary production, since the smallest meiofaunal taxa are the most productive, but along with juvenile stages of nematodes they will mostly be lost when larger meshes are used (Ptatscheck *et al.*, 2020). Furthermore, inadequate extraction and fixation procedures will lead to the loss of abundant soft-bodied meiofauna, including gastrotrichs and micro-turbellarians (Balsamo *et al.*, 2020), again contributing to a general underestimation of the meiofaunal contribution to secondary production.

In many freshwater and marine sediments, nematodes are the most abundant meiofaunal representatives, with trophic roles ranging from fungivores, bacterivores, and algivores, to predators of other meiofauna (Heip *et al.* 1985; Traunspurger, 2000, 2002; Traunspurger *et al.*, 2020). The production of nematodes has been compared with that of other similarly sized meiofaunal organisms. For example, Majdi *et al.* (2017) estimated that nematodes account for 10–46% of meiofaunal production in the Ems and Furlbach streambeds, followed by chironomid larvae (17–25%) and micro-turbellarians (13–30%). Reviewing production data from German and UK streams, Schmid-Araya *et al.* (2020) estimated significantly higher production by nematodes, oligochaetes, and harpacticoid copepods than by other meiofaunal taxa.

So far only one study has provided an estimate of bacterial, protozoan, and metazoan production within a lake, specifically, in the profundal zone of Lake Brunnsee (Bergtold and Traunspurger, 2005). Strayer and Likens (1986) estimated the total output (g C) of Mirror Lake (New Hampshire). Respiration by macrobenthos and micro-meiobenthos was calculated in Lake Brunnsee and Mirror Lake. In both, benthic bacteria were shown to dominate metabolic activity (Table 8.3). Annual protozoan production in Lake Brunnsee was 22 gC/m^2 and therefore contributed substantially to the overall benthic production. Meiobenthic metabolic activity was relatively higher in Mirror Lake (protozoan not included) than in Lake Brunnsee, where it accounted for only a minor portion of the lake's production (Table 8.3). The overall metazoan abundance in Lake Brunnsee was numerically dominated by nematodes (77%) but their contribution to overall benthic biomass was low (ca. 4%). The nematode contribution to overall secondary production in Lake Brunnsee was ca. 2% but it accounted for ca. 62% of meiobenthic production. To better understand fluxes of energy and matter in aquatic ecosystems, we need more studies estimating the secondary production achieved by the different micro, meio-, and macrobenthic organisms.

Table 8.3. Secondary production (gC/m²/year) of different groups of organisms in Lake Brunnsee and the percentage contribution to production (%P) (Lake Brunnsee) and comparison with contributions to respiration (%R) in (Mirror Lake). Production was calculated after Banse and Mosher (1980), and respiration after Banse (1982). The Mirror Lake data were recalculated after Strayer and Likens (1986). (From Bergtold and Traunspurger, 2006.)

Faunal groups	Annual production (gC/m²/year)	Lake Brunnsee (%P)	Mirror Lake (%R)
Bacteria	27.0	47.1	59.0
Protozoa	22.0	38.4	
Meiobenthos	1.6	2.8	27.3
Macrobenthos	6.7	11.7	13.7
Total	57.3	–	–

8.6 Conclusions and Perspectives

Nematodes are an abundant component of benthic communities and they interact with phototrophic microbes, decomposers, and larger metazoans. The quantitative involvement of nematodes in trophic channels can be inferred from estimates of their biomass turnover and from their contribution to production of biomass in ecosystems. We used abundance and biomass data on nematode dwelling on epilithic biofilms in a temperate river to investigate secondary production and biomass turnover. We found relatively high production values in comparison with values reported previously from soft substrates in other freshwater ecosystems. We found that biomass turnover as expressed by P/B ratio peaked in summer, a season that was characterized by a higher proportion of small bacterivorous species. Furthermore, in this chapter we compared four different allometric models used to calculate secondary production by nematodes and we show that these models performed differently: the model only based on production–biomass correlation (Banse and Mosher, 1980) was not very sensitive to seasonal changes. The models taking into account the temperature dependency of metabolic rates (Vranken *et al.*, 1986; Plante and Downing, 1989) were more sensitive to the seasonal regime; however, they showed high variance and may have overestimated P/B, being biased by the heterogeneous sampling effort through time (sampling was more frequent in summer). In the case of highly dynamic river systems, we recommend using allometric models taking into account the temperature dependence of metabolism. However, in this case, we do also recommend that production estimates should be based on homogeneous sampling strategies whenever possible. Interestingly, the model proposed by Schmid-Araya *et al.* (2020) based on a relation between temperature-corrected biomass and production, gave the lowest estimations of P and P/B but it tracked well seasonal regime and was the least skewed, suggesting it had the highest consistency despite the heterogeneous sampling. Further studies should examine the accuracy of these models against empirical measures of respiration or metabolic rates obtained in the laboratory to further establish

their relevance. A persisting challenge in nematology is the disparate methodology used in this field of research and the limited number of surveys that have reported production values, resulting in difficult comparisons. This chapter tries to fill this gap, highlighting simple procedures aimed at standardizing the estimation of secondary production by nematodes. With standardization of methodologies and refinement of production models, nematologists will be encouraged to compile and report their abundance and biomass data as production or P/B data that have an important ecological meaning.

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9 Freshwater Nematodes in Metacommunity Studies

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Highlights

- The metacommunity concept considers dispersal, niche-based and stochastic processes in a single theoretical framework.
- Freshwater nematodes are well suited to test theories of metacommunity ecology in field and lab studies.
- Freshwater nematodes are largely underrepresented in metacommunity field and lab studies.
- The spatial structure of nematode metacommunities has so far mostly been attributed to a dispersal surplus.

9.1 Introduction

A fundamental question in ecology is: why is a species present at a given site during a particular time? The answer may largely depend on the scale of the investigation. For example, one may ask 'Why does a nematode species occur in this lake?' or 'Why does it occur on that continent?' The approaches used to address these questions will be substantially different, most likely related in the first case to the environmental conditions of the lake and in the second to the biogeographic history of the continent. This problem of scale hampers the formulation of general theories on the distribution of species (Leibold and Chase, 2018). Instead, a theory is needed that accommodates the transition from small to large scales without the need to define where one community ends and another begins (Leibold and Chase, 2018). This is the idea underlying the metacommunity concept.

In this chapter we discuss the application of the metacommunity concept to data on freshwater nematodes. First, the theoretical concepts, terminology, and methods used in metacommunity analyses are introduced. Second, metacommunity studies of freshwater nematodes are summarized and the results are compared with those obtained from studies of other organismal groups. Finally, research gaps in metacommunity ecology in general and freshwater nematodes in particular are highlighted.

9.2 Metacommunity Theory

9.2.1 The general concept

The traditional theoretical framework used to create models of species occurrences is community ecology. An ecological community is defined as an assemblage of two or more different species occupying a specific geographic area at a particular time (Ricklefs and Miller, 2000). This implies that investigations of species occurrences are always limited to one local spatial scale and mostly to local processes. The latter describes the responses of species to abiotic (i.e. resource use) and biotic (i.e. species interactions) environmental conditions on local scales and thus the ability of a species to persist in a community (e.g. Gause, 1934; Hutchinson, 1959;

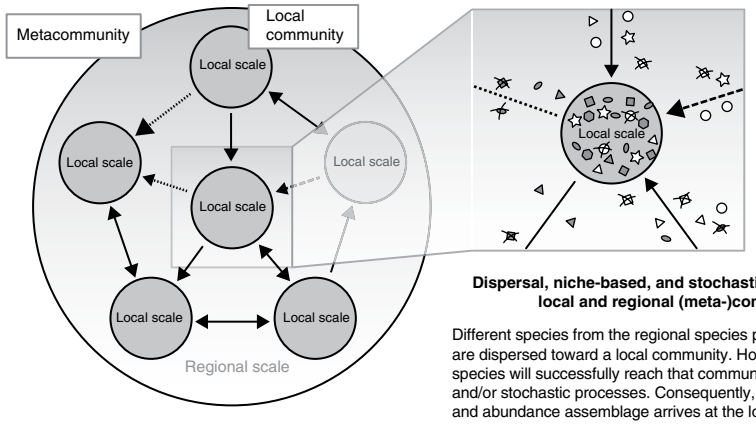
Levin, 1970). This local view, however, ignores the fact that no community is completely isolated from its surroundings, as each community may be connected with others that have arrived from distinct spatial locations by dispersal. Therefore, it is impossible to distinguish whether a community is truly local, supported by resource utilization and species interactions, or whether coexistence is induced by regional processes, especially through the input of organisms by dispersal (see Box 9.1).

This emphasizes the need to expand the community concept in order to include regional processes, and thus by the factor of dispersal. This will allow general patterns of organism distribution to be discerned and rules or theories generally applicable in natural systems to be formulated (Lawton, 1999; Ricklefs, 2008; Fahrig and Triantis, 2013). One such approach is based on the concept of metacommunity, defined as an assembly present within a larger area and made up of local, possibly biotically and abiotically heterogeneous communities that may be connected by dispersal (Wilson, 1992). Within this framework, three types of processes contribute to the species assembly of a local community: dispersal, niche-based and stochastic processes (Box 9.1).

Dispersal determines which species can reach a local community, their ability to do so, at what density and at what time. Therefore, dispersal is a determinant of species occurrences and colonization success (see Section 9.2.2).

Niche-based processes encompass local processes of resource use and species interactions as well as regional environmental heterogeneity.

Box 9.1



Dispersal, niche-based, and stochastic processes shape local and regional (meta-)communities:

Different species from the regional species pool (depicted in white) are dispersed toward a local community. However, not all of these species will successfully reach that community, due to deterministic and/or stochastic processes. Consequently, only a specific species and abundance assemblage arrives at the local community.

In the local community, species are subject to biotic interactions with the resident community (depicted in gray) and to the abiotic environmental conditions. Some individuals (and potentially species) may not persist, due to deterministic and/or stochastic processes, such that some species may become extinct.

The local community serves as source for the emigration of its individuals, some of which are successfully dispersed.

Together, they act as a filter that from the pool of potential residents separates the subset occurring within a community (environmental filtering).

Stochastic processes are difficult to define because they pervade both niche-based and dispersal-based processes but they may also be distinct. Generally, demographic stochasticity is distinguished from environmental stochasticity (Bonsall and Hastings, 2004; Lande *et al.*, 2006). Demographic stochasticity can occur at the scale of individuals that differ in their *birth* and *death* rates, which can lead to ecological drift; that is, random changes in local species abundances (Hubbell, 2001). Environmental stochasticity refers to temporal environmental events, such as weather phenomena or disturbances, but species responses to these events are deterministic and similarly affect the whole population. Both environmental and demographic stochasticity can emerge at the population scale in the context of stochastic *colonization* and *extinction* dynamics (Leibold and Chase, 2018). Dispersal-based processes are especially prone to stochasticity if the dispersed species is a passive disperser (see Chapter 5). In the case of nematodes, they may drift with the wind such that a high wind speed may transport more individuals (Carroll and Viglierchio, 1981; Ptatscheck *et al.*, 2018). Likewise, heavy rain can induce strong water currents that might increase the number of transported individuals (Palmer, 1992) or the temporal connectivity of sites within a floodplain. The time of immigration is also a stochastic component of dispersal-based processes and can be a crucial factor in establishment success (see Chapter 5).

9.2.2 The influence of dispersal-based processes

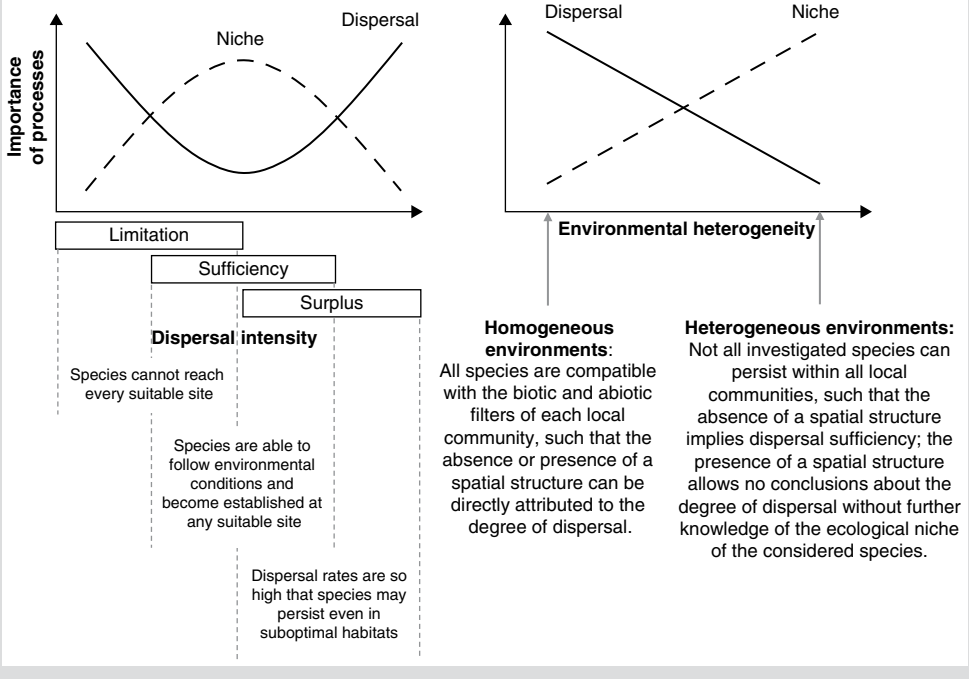
9.2.2.1 On community structure

As noted above, dispersal is the key process allowing the expansion of community ecology on a local scale to metacommunity ecology on a regional scale, because it enables the connection of populations from different localities. Due to this special role of dispersal in metacommunity studies, its characteristics are described in detail in this section (for a more detailed discussion of the dispersal of freshwater nematodes, see Chapter 5). Dispersal can be defined as any movement transporting one population of individuals or propagules to another, with potential spatial consequences for gene flow (Ronce, 2007; Jacobson and Peres-Neto, 2010). Dispersal frequency and strength are defined by *habitat connectivity* and the *dispersal ability* of a species. Habitat structure may be discrete, such that local communities are clear entities and species dispersal is limited by certain boundaries. Examples of such habitats range from lakes and puddles to pitcher plants. However, there are also habitats that lack clear boundaries such that the metacommunity has a more continuous structure, as is the case for stretches within rivers and circumscribed areas of lakes. Since most field studies of metacommunities involve the latter type, researchers are essentially forced to create an artificial boundary

between local and regional (meta)communities (Leibold and Chase, 2018). For continuous metacommunities, spatial extent is an important variable determining dispersal rates, as it may be assumed that dispersal decreases with increasing distance. While active dispersers are able to choose their new habitat, passive dispersers, including nematodes, depend on vectors such as wind, water flow, or other animals for their transport. Therefore, active dispersal is presumed to be more strongly linked to environmental conditions while passive dispersal may have a larger stochastic component (Soininen, 2014). Furthermore, because passive dispersers are often transported with a very high frequency they can in effect 'choose' a suitable habitat (Fenchel and Finlay, 2004) and dispersal-related traits (e.g. body size) should therefore also be considered (De Bie *et al.*, 2012).

Empirical and theoretical studies have identified three ways in which dispersal affects metacommunity structures, depending on the dispersal strength (according to Leibold and Chase, 2018; see Box 9.2): (i) If dispersal rates are low, species cannot reach every possible site in a region where they could persist based on environmental conditions (dispersal *limitations*). (2) In the absence of dispersal barriers, dispersal is *sufficient* and species will track the environmental conditions, becoming established at any suitable site. (3) If dispersal rates are so high that species can persist

Box 9.2



even in suboptimal habitats, due to constant immigration from suitable habitats (i.e. source–sink dynamics), dispersal is considered to be *surplus*.

Dispersal can often be measured directly by tracking or catching individual dispersers, such that the actual dispersal of single individuals in one or all three phases of the dispersal process (emigration, transport, immigration) can be assessed. Alternatively, dispersal can be measured indirectly, by deducing dispersal from data on captured species using measures of habitat connectivity that serve as proxies for dispersal (see Section 9.3). In this case, previous dispersal can be determined based on a much larger number of individuals than is possible by direct assessments (Jacobson and Peres-Neto, 2010). The role of dispersal in metacommunity structure is one aspect of metacommunity analyses. Field observations can fail to distinguish between the three types of dispersal as the spatial structure of the metacommunity may be the product of both very low and very high dispersal intensities or spatial structure of environmental heterogeneity (Box 9.2). Therefore, meaningful interpretations of metacommunity analyses will benefit from knowledge on the dispersal abilities of species and the ecological constraints thereof.

9.2.2.2 *On biodiversity*

Studies of dispersal- and niche-related factors and their role in structuring metacommunities specifically investigate beta-diversity, by comparing the community similarities of sites that differ in their spatial locations and environmental conditions (see Section 9.3). However, for some ecological questions the relationships of those processes to local and regional diversity (alpha- and gamma-diversity) are of greater relevance (Fig. 9.1). It is generally accepted that the number of ecological niches,

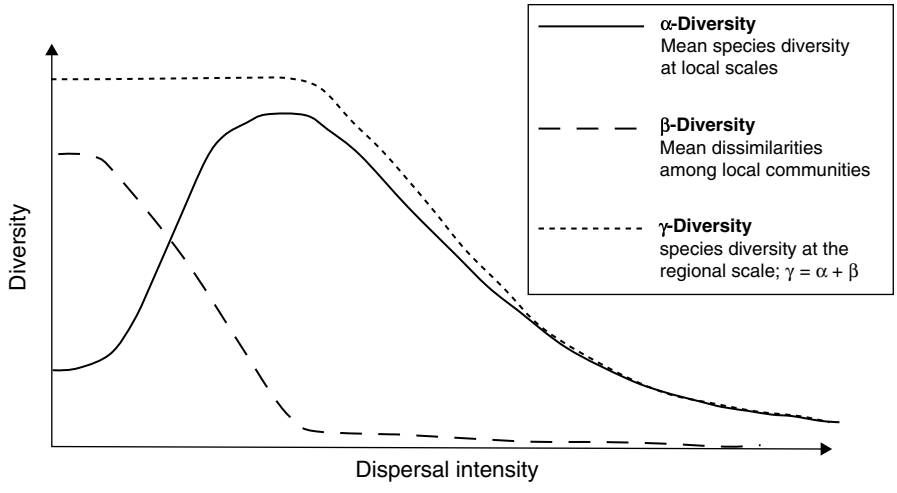


Fig. 9.1. Hypothesized relationship between the dispersal rate and alpha-, beta-, and gamma-diversity. (Modified from Mouquet and Loreau (2003).)

as well as local and regional diversity, increase with increasing local and regional biotic and abiotic heterogeneity. However, less is known about the influence of dispersal-based processes, specifically dispersal intensity. An often-cited theoretical model from Mouquet and Loreau (2003) on the relation of diversity with increasing dispersal is shown in Fig. 9.1. According to this model and in line with empirical data, dispersal generally reduces beta-diversity by homogenizing local communities (Mouquet and Loreau, 2003; Cottenie and De Meester, 2004). For alpha-diversity, theory predicts a positive relationship between dispersal rates and local diversity due to (i) generally lower dispersal limitations, (ii) rescue effects (Brown and Kodric-Brown, 1977; Hanski, 1999), which allow colonists to re-establish previously extinct communities and (iii) source–sink effects (Pulliam, 1988) (also referred to as mass effects (Shmida and Wilson, 1985) and summarized as dispersal surplus). Conversely, dispersal above a specific threshold may cause a decrease in diversity by allowing a regionally dominant competitor to invade local communities, thereby reducing spatial refuges for inferior competitors and reducing local diversity (Amarasekare and Nisbet, 2001; Mouquet and Loreau, 2002; Forbes and Chase, 2002). Regional (gamma) diversity depends on the relationship between alpha- and beta-diversity (Gering and Crist, 2002). As dispersal will decrease beta-diversity, gamma-diversity, as a function of alpha-diversity, may remain unaffected or decrease as well (Mouquet and Loreau, 2002). However, it should be noted that these assumptions on alpha- and gamma-diversity are of a theoretical nature and only partly supported by experimental and observational data (see, for example, the meta-analysis of Cadotte, 2006).

9.3 Methods in Metacommunity Analyses

Given the multitude of research questions in metacommunity ecology, a large array of methods have been used to obtain the answers. In terms of their focus, most studies may be classified into one of the following four study types (Table 9.1).

9.3.1 Environment vs. space

The aim of most metacommunity studies is to determine the relative role of dispersal vs. niche-based processes in the structuring of metacommunities. A statistical approach allowing metacommunity patterns to be related to environmental differences, dispersal, or both is provided by variation partitioning. In this method, direct gradient analyses (canonical correspondence analysis, CCA; redundancy analysis, RDA) (Borcard *et al.*, 1992; Cottenie, 2005) are followed by a partitioning of the variance in species composition (beta-diversity) into components attributable to the pure effect of environmental variables, the pure effect of spatial variables, the

Table 9.1. The study types and associated statistical tests used in observational metacommunity studies. Data comprising the species, space (inclusion of spatial variables), and environment (inclusion of environmental variables) are listed. Information on the statistical method, publications on their operating principles ('Literature'), examples of their applications ('Examples'), and the R packages freely available for these types of calculations are also provided.

	Data				Reference		
Study type	Species	Space	Environ	Method	Literature	Examples	R package
Environment vs. space Assess the role of niche-based and dispersal-based processes on metacommunity structure	Site-species matrix with abundance or presence/absence data	Yes	Yes	Constrained ordination analysis (canonical correspondence analysis (CCA) or redundancy analysis (RDA)) and subsequent variation partitioning	Borcard *et al*., 1992 Peres-Neto *et al*., 2006	Ptatscheck *et al*., 2020 Cottenie, 2005	*vegan* Oksanen *et al*., 2018
				Mantel tests	Mantel and Valand, 1970 Legendre and Fortin, 1989	Michelson *et al*., 2016	*ade4* Dray and Dufour, 2007
				Boosted regression trees	Elith *et al*., 2008	Gansfort and Traunspurger, 2019 Dallas and Drake, 2014	*gbm* Greenwell *et al*., 2020
Metacommunity patterns Identify patterns of metacommunity: mostly turnover (nestedness and replacements)	Site-species matrix with presence/absence data	No	No	Elements of metacommunity structure	Leibold and Mikkelson, 2002 Presley *et al*., 2010	Dümmer *et al*., 2016 Presley and Willig, 2010	*metacom* Dallas, 2014
				Nestedness temperature	Atmar and Patterson, 1993 Rodriguez-Girones and Santamaria, 2006	Omesová *et al*., 2008	*bipartite* Dormann *et al*., 2008

Continued

Table 9.1. Continued.

	Data				Reference		
Study type	Species	Space	Environ	Method	Literature	Examples	R package
Diversity pattern Identify how species diversity is distributed across space and environmental gradients	Diversity indices	Yes	Yes	Usually linear and quadratic regressions	Basic statistics	De Mendoza *et al*., 2017	basic *stats* package R Core Team, 2019
		No	Spatial levels	Diversity partitioning	Crist *et al*., 2003	Matsuda *et al*., 2015	*vegan* Oksanen *et al*., 2018
Testing neutrality Assess the importance of neutral dynamics	Site-species matrix with abundance data	No	No	Zero-sum multinominal distribution	Hubbell, 2001 Alonso and McKane, 2004	McGill, 2003	*sads* Prado *et al*., 2018

shared fraction, and the unexplained variation (residuals). Variation partitioning has gained wide acceptance among ecologists as a suitable method to assess the relative importance of environmentally induced and dispersal-based processes (Soininen, 2014). In metacommunity analyses, it is probably the statistical approach most often used to disentangle the role of spatial and environmental factors. The major advantage of variation partitioning is that it controls for the spatial structure of environmental variables, as the correlation of environment and space is very common in nature and complicates distinguishing the influence of niche-based vs. dispersal processes. The overlap between environment and space may be minimized by the appropriate study design (e.g. Grönroos *et al.*, 2013) but only rarely can it be completely avoided (Zhai *et al.*, 2015).

Nonetheless, the disadvantage of variation partitioning is that the response type of species to the tested environmental gradients is assumed to be linear (RDA) or unimodal (CCA) across the whole community and across all environmental factors. By contrast, Mantel tests, as a non-parametric form of analysis, are not plagued by the need to define the species–environment relationship. Instead, they evaluate the 'relationship between two dissimilarity matrices computed from two sets of multivariate data concerning the same *n* individuals or sampling units' (Legendre *et al.*, 2015, p. 1239). Thus, Mantel tests are used in ecological research to relate any two distance matrices (Legendre, 2000). For metacommunity studies, geographic distance matrices are related to matrices of community similarities (e.g. Michelson *et al.*, 2016). However, there are two basic problems with the use of Mantel tests. First, they do not account for the spatial structure of environmental factors (in contrast to variation partitioning). Second, Legendre *et al.* (2015) found that the use of matrices of geographic distances among sites derived from spatial coordinates may be inappropriate, as most studies have failed to verify the basic assumptions of Mantel tests regarding spatial analyses.

Regression tree analyses have rarely been used in metacommunity analyses. Because single tree models have a relatively poor predictive performance, Elith *et al.* (2008) suggested the use of boosted regression trees (BRTs) – in which many hundreds of trees are combined into one model – in ecological analyses. The advantage of BRTs is that they can fit complex non-linear relationships and automatically handle interactions. Moreover, they are not sensitive to many of the difficulties common in ecological modeling (e.g. different predictor types, outliers, missing data). A disadvantage is that the interpretation of the model outcome is not as straightforward as in common variation partitioning analysis. BRTs offer no *P*-values or percentages of explained variation; rather the results indicate the relative importance of each predictor variable (expressed as a percentage) compared with the other offered predictors. Collinearity between predictor vectors (thus also the spatial structure of environmental heterogeneity: the collinearity between spatial and environmental factors) therefore appears as a shared percentage of the correlated predictors.

9.3.2 Metacommunity pattern detection

Studies of the importance of niche-based and dispersal-based processes in metacommunity structure seek to explain beta-diversity (i.e. community dissimilarities) with respect to environmental and spatial parameters. By contrast, studies aimed at identifying metacommunity structure investigate components of beta-diversity; that is, whether community dissimilarities are due to nestedness or to species turnover (Baselga, 2010). Nestedness occurs when a community at a particular site is composed of a subset of the species pool from the community of another, more species-rich site. In this case, the beta-diversity of those sites is based on species loss. However, if beta-diversity between sites is due to species turnover then this implies the replacement of some species by others (Baselga, 2010).

A relatively widely used approach in studies of metacommunity structure is the analysis of species distribution patterns based on incidence matrices, in which checkerboards, turnover, and boundary-clumping are tested against differently defined null models. Analyses that relied on this method were assessed and integrated by Leibold and Mikkelson (2002) and further improved by Presley *et al.* (2010). These efforts gave rise to the concept of elements of metacommunity structure (EMS), which does not include any gradients (neither environmental nor spatial; Table 9.1) of metacommunity patterns such that no assumptions are made about the processes underlying the observed patterns (Logue *et al.*, 2011). Rather, site scores are calculated from reciprocal averaging (an algorithm also used in correspondence analysis) and are indicative of the similarity of the communities at the respective sites. This approach allows gradients to be related to the ordination axis (site scores in the EMS) and therefore yields preliminary insights into the potentially most relevant structuring forces in the investigated metacommunity (Presley and Willig, 2010).

Other approaches solely focus on the assessment of nestedness (e.g. nestedness temperature; Atmar and Patterson, 1993), which may be pertinent to the specific research question. The respective analyses are usually combined with other tests used to assess the structure of metacommunities.

9.3.3 Diversity patterns

While the focus of the above discussion was beta-diversity, in the following we consider patterns of local and/or regional diversity (e.g. species richness) across spatial and environmental gradients. Distinguishing between niche- and dispersal-based processes as factors driving species diversity requires the use of certain tests. However, in the study of De Mendoza *et al.* (2017), for example, the significant unimodal response of nematode species richness to an altitudinal gradient and the concomitant independence of nematode communities from environmental conditions suggested that dispersal-based processes are an important determinant of nematode

species richness. Investigations of large-scale biodiversity patterns may also reveal the dispersal abilities of species and therefore the role of dispersal-based processes (Fontaneto and Ricci, 2006). Another approach is to use different hierarchical levels (see Section 9.3.6) and then determine whether the contributions to gamma-diversity components (alpha- and beta-diversity) are equal across scales (e.g. Matsuda *et al.*, 2015). In that case, it can be assumed that, if dispersal is effective, alpha-diversity will be the largest contributor to gamma-diversity.

9.3.4 Testing neutrality

As discussed at the beginning of this chapter, metacommunity analyses include investigations of dispersal, niche-based processes as well as stochastic processes. In Hubbel's neutral theory (2001), in which demography and dispersal are considered to be completely stochastic, species are treated as identical in their environmental responses. Accordingly, deterministic dispersal- and niche-based processes are irrelevant. The relevance of neutrality for the observed communities can be tested by evaluating the predictive power of neutral models using real data. Examples of this approach are the zero-sum multinominal distribution as well as general randomization tests (Logue *et al.*, 2011). In addition, neutral dynamics will be supported if environmental factors are irrelevant but spatial factors are relevant for the species distribution in a metacommunity, or if a large proportion of the variance cannot be explained (Gansfort *et al.*, 2020).

9.3.5 Data type

The simple occurrence of a species and its abundance at certain site may be driven by different processes. Consequently, the use of different data types may lead to different results in metacommunity analyses. Generally, species abundance responds more strongly to environmental gradients than does the presence/absence (p/a) of a species, with the latter data thus more sensitive to rare species (Heino *et al.*, 2010; Soininen, 2014). Consequently, it is often the case that variation can be better explained in abundance than in p/a data (e.g. Cushman and McGarigal, 2004; Beisner *et al.*, 2006; Heino *et al.*, 2010). In a study of freshwater nematodes, p/a and abundance data differed in their relationships to environmental and spatial gradients (Gansfort and Traunspurger, 2019). For example, a stronger effect of nitrogen on species composition was determined from abundance than from p/a data, whereas the opposite was true for the factor grain size distribution. Diversity indices that include species abundance composition (e.g. evenness) and not only species number (e.g. species richness) may respond differently to metacommunity processes, with pure species assemblages being more strongly influenced by dispersal

surplus as was shown in a recent experiment using nematode communities in mesocosms (Gansfort *et al.*, 2021).

9.3.6 Spatial variables

Spatial variables can be integrated in metacommunity analyses in several ways. Thus, space can be included implicitly, such that rather than being directly related to spatial structure (meta)communities from different hierarchical levels are compared (e.g. Matsuda *et al.*, 2015; Dümmer *et al.*, 2016). Space can also be implemented explicitly, by using spatial factors as predictors in any type of community or biodiversity model (e.g. in variation partitioning, see above). Here, spatial factors may be coordinates of sites (latitude, longitude, and elevation) or site scores from the metric ordination (Principal Coordinates Analysis, PCoA) of geodesic distance matrices, whether derived from the overland distances of sites or measured along specific dispersal pathways. For example, for freshwater nematodes, this could be the watercourse connection of sites sampled along a river network (Gansfort and Traunspurger, 2019). All of these examples allow broad-scale spatial patterns to be included in the analysis.

A tool to create vectors related to both broad- and fine-scale spatial autocorrelation patterns is Moran's eigenvector maps (Legendre and Legendre, 2012), such as principal coordinates of neighbor matrices (PCNM; Dray *et al.*, 2006). The spatial variables in those analyses are also generated based on the geographic distances among sites and result in vectors indicating more variable spatial patterns (Fig. 9.2). However, 'environment vs. space' analyses (see above) applied in combination with this advanced spatial analysis may overestimate the role of dispersal because spatial variables are analyzed using non-linear pattern detection, whereas for environmental variables only linear relationships are tested (e.g. in RDA and variation partitioning). As a result, non-linear environmental patterns may be falsely captured by the spatial components in variation partitioning (Leibold and Chase, 2018).

9.4 Studying Freshwater Nematodes in a Metacommunity Framework

General interest in metacommunity theory grew rapidly after 2004 (mainly due to the publication of Leibold *et al.* (2004) and a book by Holyoak *et al.* (2005)), but the first metacommunity study focusing on freshwater nematodes was published 10 years later. Currently, in addition to the three metacommunity studies cited in a recent review (Gansfort *et al.*, 2020), three other studies have been published (Table 9.2). Therefore, within this chapter our insights into freshwater nematode metacommunity structures and processes are limited to those derived from just six published studies.

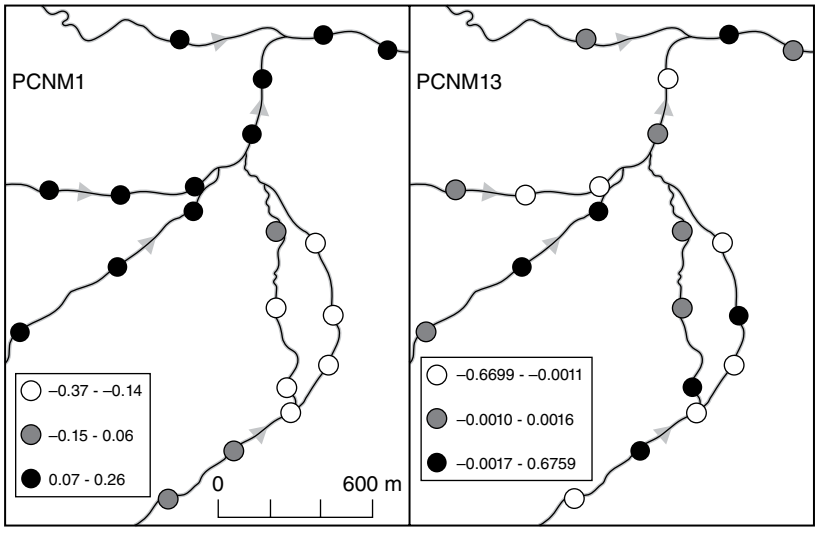


Fig. 9.2. An example of the principal coordinates of neighbor matrices (PCNM) vectors of the river network investigated in Ptatscheck *et al.* (2020). (**Left**) The PCNM vector represents a broad-scale spatial pattern. (**Right**) The PCNM vector represents a small-scale spatial pattern. (Modified from Ptatscheck *et al.* (2020).)

9.4.1 The influence of dispersal processes on metacommunity structure

Two studies investigated the role of spatial factors (as proxies for dispersal-based processes) in nematode metacommunities from stream sediments (Gansfort and Traunspurger, 2019; Ptatscheck *et al.*, 2020). Both identified an important influence of spatial factors on nematode metacommunity structure. Ptatscheck *et al.* (2020) tested fine- and broad-scale spatial vectors (PCNMs) for their relative role in structuring the metacommunities on a small scale of investigation (maximum distance between sites: 2 km). They found that fine-scale spatial patterns alone significantly explained 24% of the community variation. Due to the limited spatial extent and high passive dispersal potential of freshwater nematodes along the watercourse (see Chapter 5), the spatial structure was attributed to dispersal surplus rather than to dispersal limitation. The importance of fine-scale spatial patterns as indicators of a dispersal surplus has also been reported by Heino *et al.* (2015b). Gansfort and Traunspurger (2019) investigated the nematode metacommunity at sites distributed over a large area of the German Elbe River network (maximum distance between sites: 350 km). In that study, only broad-scale spatial factors (distances overland and along the watercourse) were tested for their influence on metacommunity structure. While the authors showed that distance along the watercourse had a notable effect on nematode communities, environmental and temporal parameters were more important. The identified spatial structure was attributed to a dispersal surplus because the sites within a river network

Table 9.2. Summary of metacommunity studies of freshwater nematodes at the species level. (Modified from Gansfort *et al.*, 2020.)

Publication	Habitat	Substrate	Field/ lab	Taxa	Spatial extent (km)	Region	Explanatory variables	Influential variables	No. of sites	Samples (*n*)	Connectivity	Species (*n*)	Data type	Method
De Mendoza *et al*. (2017)	Lakes	All benthic substrates	Field	Nematodes, chironomids, non-chironomid insects, oligochaetes	260	Pyrenees (France/ Spain)	Environ, trophic state, elev, substrate, biotic conditions	Ca, elev (on species richness)	82 lakes	5 per lake ergo 410	Discrete	20	p/a	RDA, regressions on species richness
Dümmer *et al*. (2016)	Lakes	Sediment	Field	Nematodes	0.08–360	South Sweden, northern Germany	Trophic state, lat, long, elev, four spatial scales	Trophic state, lat, long	16 lakes	315	Connected and discrete	174	p/a	EMS, Spearman
Gansfort and Traunspurger (2019)	Streams	Sediment	Field	Nematodes	350	Elbe, Rhine, Germany	Environ, overland and watercourse distances, year, season, ecotox. Index	Year, N, S, substrate, watercourse distances	47 Elbe 42 Rhine	115 (Elbe) 59 (Rhine)	Connected	245 (Elbe) 231 (Rhine) All 314	Both	NMDS, BRT
Gansfort *et al*., 2021	Mesocosms	Sediment	Lab	Nematodes	NA	Sediments taken to the lab from Bielefeld, Germany	Dispersal rate, temperature, degree of regional environmental heterogeneity	Dispersal and heterogeneity p/a	120 mesocosms	120	Artificially connected	35	Both	LM(M) on alpha-, beta-, and gamma-diversity
Ptatscheck *et al*. (2015)	Artificial tree holes	All substrates in the tree holes	Field	Nematodes	20	Eastern Germany	None	NA	300 artificial tree holes	300	Discrete	35	p/a	EMS
Ptatscheck *et al*. (2020)	Streams	Sediment	Field	Nematodes, flying and non-flying insects	2	Bielefeld, Germany	PCNMs, environ, protozoan and bacterial biomass	For nematodes, fine-scale PCNMs	20	100 (5 subsamples per site)	Connected	73	p/a	RDA and var part

Environ, environmental variables; lat, latitude; long, longitude; elev, elevation; N, nitrogen; S, sulfur; BRT, boosted regression trees; EMS, elements of metacommunity structure; p/a, presence/absence; both, abundance and p/a data; NMDS, non-metric multidimensional scaling; PCNMs, principal coordinates on neighbor matrices; RDA, redundancy analysis; var part, variation partitioning; spatial extent, maximum distance of sites.

are presumably highly connected. By contrast, across lentic sites there is usually no watercourse connection and passive overland transport may be subject to limitations, especially over large spatial scales. Dümmer *et al.* (2016) investigated the distribution of sediment-dwelling nematodes in German and Swedish lakes over varying spatial extents (maximum distance of sites in the smallest extent 0.08 km and at the largest 360 km). A primarily spatial structure was determined for metacommunities only on the largest scale. The authors suggested dispersal limitation as the reason, given that the investigated lakes in Germany and Sweden were separated by the Baltic Sea, which could act as a dispersal barrier. De Mendoza *et al.* (2017) conducted their study at the mountain lakes of the Pyrenees over an area of 260 × 40 km^2. They did not explicitly include spatial variables (except of altitude) in their calculations. However, as nematodes showed very little association with the measured environmental parameters but were not random in their occurrence the authors concluded that nematode distribution mainly reflected the topological position of the sampled sites.

9.4.2 The influence of niche-based processes on metacommunity structure

In addition to spatial factors, freshwater nematode metacommunity structure is shaped by environmental parameters, especially resource availability. The degree of habitat productivity has been shown to influence metacommunities in lakes (significance of trophic state index; Dümmer *et al.*, 2016) and rivers (importance of nitrogen and sulfur contents in sediment; Gansfort and Traunspurger, 2019). This finding is in accordance with studies in which nematode metacommunity structure could not be related to environmental parameters in the absence of a productivity gradient, demonstrated in mountain lakes (general low productivity; De Mendoza *et al.*, 2017) and at the very small spatial extent of a river network (very short gradient length due to the small area; Ptatscheck *et al.*, 2020). The bottom-up structuring of nematodes has also been observed in descriptive community studies (e.g. Ristau and Traunspurger, 2011; Ristau *et al.*, 2012), but they ignored the role of dispersal-based processes in their analyses. Because environmental factors are naturally spatially structured to a certain degree, it is essentially impossible to distinguish the roles of dispersal and niche-based processes. In studies that fail to take into account spatial factors, the role of the latter may be overestimated or even misinterpreted. Studies of freshwater nematodes that included both spatial and environmental parameters have shown that, depending on habitat type (connected vs. discrete), spatial extent, and environmental heterogeneity, spatial factors may be more important than environmental parameters in structuring nematode communities. Therefore, even if dispersal processes are not the focus of a study whose aim is to unravel the environmental factors responsible for nematode communities, spatial autocorrelation must be taken into account in the analysis (see Section 9.3).

Niche-based processes refer to the relationships of species with their abiotic and biotic environment. Abiotic environmental gradients determine community composition not only directly, by changing population growth rates, but also indirectly, as changes in population growth will alter the outcome of competitive exclusion (Cadotte and Tucker, 2017). Thus, any reduction in the growth rate of a species due to suboptimal environmental conditions will likely lead to competitive exclusion well before environmentally induced mortality causes extinction (Cadotte and Tucker, 2017). Biotic factors (e.g. the presence of a predator or mutualistic species) can directly influence species occurrence and abundance (for examples, see Cadotte and Tucker, 2017). To determine whether the variation in observational data is due to heterogeneity in biotic or abiotic factors can therefore be problematic. So far, metacommunity studies of freshwater nematodes have not included biotic variables in their analyses of species compositions across sites. However, in a study of zooplankton metacommunities, Gray *et al.* (2012) demonstrated that the predictive ability of biotic factors can be almost as large as or even larger than that of abiotic or spatial variables.

9.4.3 Stochastic and temporal dynamics in nematode metacommunities

Over distances greater than several centimeters or meters, nematodes are dependent on passive dispersal (see Chapter 5) and may thus be prone to a more stochastic distribution than active dispersers (e.g. flying insects). However, none of the freshwater nematode metacommunity studies explicitly examined stochastic dynamics. In the small-scale river network study of Ptatscheck *et al.* (2020), 68% of the variation in nematode community composition remained unexplained, which suggests a role for stochastic dynamics beyond the portion attributable to other factors related to the study design and analytical method (summarized in Gansfort *et al.*, 2020). Fukami (2010) reviewed several lab, field, and theoretical studies to assess the relative importance of stochastic processes within community dynamics and concluded that stochasticity plays a large role in smaller, more isolated, and less heterogeneous communities. The latter conclusion may be applicable to the very small size of the area studied by Ptatscheck *et al.* (2020) (maximum distance between sites: 2 km). The extremely small and potentially isolated communities living in water-filled tree holes were the focus of the study by Ptatscheck *et al.* (2015). The random turnover of nematode species identified using the EMS framework suggested a certain degree of stochasticity within the colonization process.

A potential source of the unexplained variation in metacommunities is temporal dynamics. In the majority of metacommunity studies, sampling was conducted during the same season and/or within a short time period (maximum of 2 years), such that temporal heterogeneity could not be taken into account (Jacobson and Peres-Neto, 2010). However,

Jacobson and Peres-Neto (2010) demonstrated that processes may shift over time, thereby implicating different temporally related variables in the shaping of metacommunity patterns. Furthermore, dispersal rates may have a strong seasonal dynamic that induces temporal shifts in communities. Indeed, in a study of nematode metacommunities in a river network (Gansfort and Traunspurger, 2019) the sampling year was shown to have a very strong impact on the nematode community assemblages. In fact, the large temporal gradient (13 years) had a larger impact on metacommunity structure than did any other spatial or environmental predictor. Such temporal shifts can be related to the transient presence (e.g. through dispersal surplus) and absence (e.g. extinctions induced by extreme events) of species that gives rise to the historical contingency of communities.

9.4.4 The influence of dispersal and niche-based processes on biodiversity

A problem in studying the role of metacommunity processes in nematode diversity is that dispersal rates are very difficult to measure in the field and even more difficult to manipulate. For example, De Mendoza *et al.* (2017) found that the nematode species richness in mountain lakes peaks at middle elevations but whether this was due to the elevation itself or to dispersal intensities inherent to the spatial distribution of the lakes (e.g. isolation effects of high altitudes) could not be determined. One of the rare examples in which the dispersal intensity could be measured and therefore related to diversity in the field was the study of Simonis and Ellis (2014). Those authors investigated a system of freshwater rockpools among which the dispersal rates of invertebrates (no nematodes) attributable to gulls varied. In most other cases, however, the influence of dispersal on biodiversity has been studied in lab experiments (e.g. Matthiessen *et al.*, 2010; Pedruski and Arnott, 2011). In the only metacommunity lab experiment involving freshwater nematodes (Gansfort *et al.*, 2021; Table 9.2), the interactive effects of dispersal and niche-based processes on diversity at different scales were examined. In that study, field sediment containing nematodes was transferred into mesocosms representing local communities while three mesocosms were combined as a metacommunity. Artificial passive dispersal at different intensities was performed by mixing sediments containing different local communities of each metacommunity at varying time intervals. Additionally, the regional environmental heterogeneity of the metacommunities was either maintained (all local communities were subject to the same environmental conditions) or altered (local communities were kept under different temperatures).

In general, the results of the experiment were in accordance with the above-described theoretical assumptions (compare Fig. 9.1 with Fig. 9.3). The influence of dispersal intensity on alpha- and gamma-diversity was

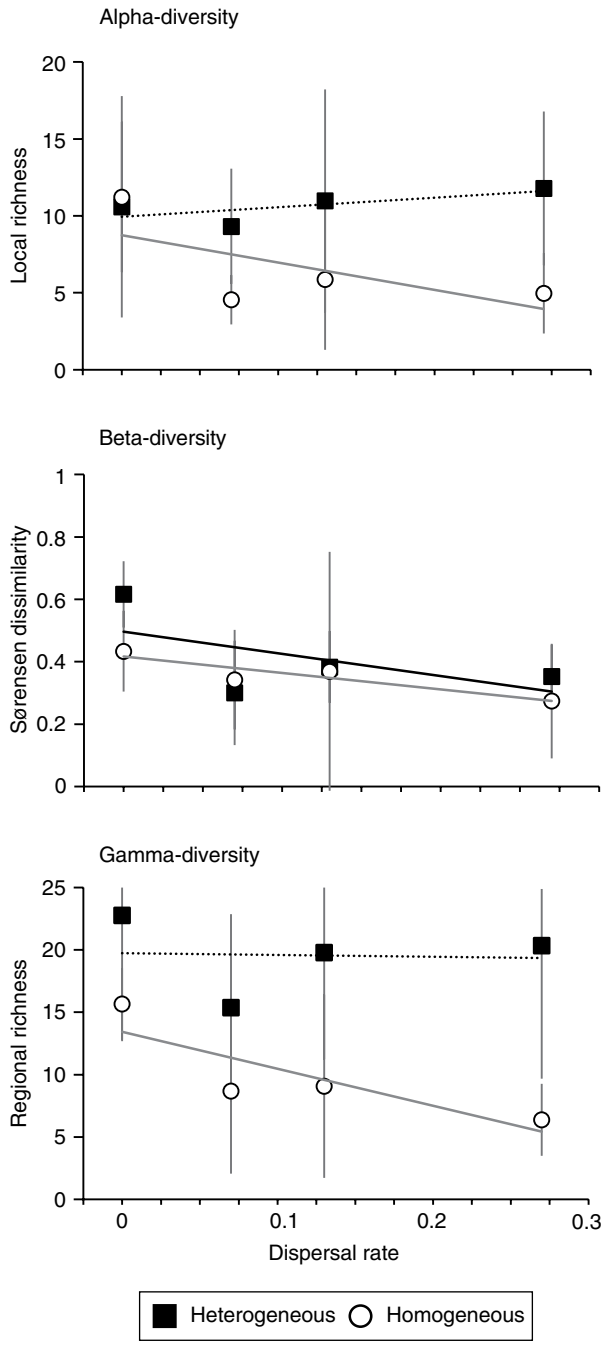


Fig. 9.3. Relationship between mean (± standard deviation) alpha-, beta-, and gamma-diversity in nematode (meta)communities and the dispersal rate (proportion of dispersed sediment in 4 weeks) in an experimental set-up under heterogeneous (black squares) and homogeneous (white circles) environmental conditions. Dispersal was induced by mixing the sediment of three local communities derived from one metacommunity (data from Gansfort *et al.*, 2021).

shown to interact with the degree of environmental heterogeneity such that in homogeneous regional environments the increase of dispersal reduced diversity while in a heterogeneous regional environment diversity was unaffected or even increased with increasing dispersal (Fig. 9.3). An interesting aspect of this experiment was the different responses of the different nematode species to the dispersal treatments. For example, *Daptonema dubium* greatly benefited from a high connectivity of local communities, whereas many other species, including *Eumonhystera vulgaris* or *Monhystera paludicola*, were more effective when local communities were completely isolated from each other.

9.5 Comparisons with Other Freshwater Organism Groups

As discussed in this chapter so far, metacommunity dynamics change in relation to species dispersal abilities, such that species with high dispersal rates will exhibit sufficient dispersal or even a dispersal surplus while those with low dispersal rates or dispersal distances will be dispersal limited. Likewise, freshwater organism groups are differentially affected by the spatial structure of their metacommunities due to differing dispersal abilities. In this section, we position nematodes among these other freshwater organism groups.

The key species traits determining dispersal are body size and dispersal mode (Hájek *et al.*, 2011; De Bie *et al.*, 2012; Padial *et al.*, 2014; Göthe *et al.*, 2017). In freshwater environments, dispersal is less effective in large than in small organisms, which can drift more easily within a water body (Beisner *et al.*, 2006; Shurin *et al.*, 2009; Padial *et al.*, 2014). However, small organisms cannot move actively between suitable patches and are thus reliant on some form of passive dispersal by mobile elements, such as wind, water currents, and other animals (Bohonak and Jenkins, 2003; Fontaneto, 2019). The passive dispersal of microscopic organisms may be highly effective, leading to spatially unlimited distributions either on global (Fenchel and Finlay, 2004; Fontaneto, 2019) or regional (Beisner *et al.*, 2006) scales (see Chapter 5). The threshold between large and small body size determining dispersal success is a matter of debate (Fenchel and Finlay, 2004; Shurin *et al.*, 2009). Body size and dispersal mode are comparable in freshwater nematodes, zooplankton, and other meiofaunal-sized taxa. However, benthic meiofauna are, by definition, associated with the substrate such that their overland and watercourse dispersal are likely to be limited by a poor accessibility to dispersal vectors (flow, animals, wind). In this respect, nematodes are especially interesting because in contrast to benthic and pelagic meiofaunal groups (e.g. rotifers, ostracods), the nematode community is largely restricted to the benthos and enters the water column only during movement or dispersal.

Several studies have investigated the metacommunity structure of organismal groups differing in their size and dispersal modes (e.g. Hájek *et al.*, 2011; De Bie *et al.*, 2012; Padial *et al.*, 2014; Göthe *et al.*, 2017)

but they failed to account for nematodes and other meiobenthic taxa. Nonetheless, studies simultaneously analyzing the metacommunity structures of several groups are highly valuable because the results of different studies performed in different settings (e.g. habitat type, spatial extent, ecological gradient lengths) are not easily comparable (Gansfort *et al.*, 2020). Among the nematode metacommunity studies summarized in this chapter (Table 9.2), two included other freshwater organismal groups (De Mendoza *et al.*, 2017; Ptatscheck *et al.*, 2020). Ptatscheck *et al.* (2020) distinguished between active dispersers (insects) and large (non-flying macroinvertebrates) and small (nematodes) passive dispersers and found that only insects were significantly associated with environmental conditions, while both passively dispersing groups were spatially structured. However, the non-flying macrobenthos had a broad-scale metacommunity structure indicating dispersal limitations while the small-scale spatial structure of nematodes was consistent with a dispersal surplus. In the study of De Mendoza *et al.* (2017), although spatial factors were not explicitly tested, environmental factors poorly explained nematode distribution, in contrast to the distributions of the other tested groups (oligochaetes, chironomids, and other insects), which were significantly associated with environmental conditions. Furthermore, in that study nematodes accounted for the smallest proportion of narrow-ranging species across altitudes, suggesting that they are less affected than other organismal groups by environmental conditions, at least in the nutrient-poor environments of the investigated mountain lakes.

In a recent review of meiofaunal metacommunity studies (Gansfort *et al.*, 2020), the authors positioned meiofauna among other freshwater groups with respect to dispersal ability and the spatial structure attributable to dispersal limitations (Fig. 9.4). This was done in accordance with theoretical assumptions based on dispersal mode and body size and in line with selected metacommunity studies that compared the constraints of metacommunities from several freshwater organismal groups (De Bie *et al.*, 2012; Grönroos *et al.*, 2013; Padial *et al.*, 2014; Rádková *et al.*, 2014). The results of the comparison suggested a strong overlap between the dispersal ability of meiobenthos and that of macroinvertebrates and zooplankton. For macroinvertebrates, dispersal mode may be more important than body size, as active dispersers, although usually larger, seem to track better with environmental conditions than do passive dispersers on regional scales (Grönroos *et al.*, 2013). For passive macroinvertebrate dispersers, a significant spatial structure due to limited dispersal among isolated sites was determined (Rádková *et al.*, 2014). Zooplankton metacommunities seem to be more affected by spatial constraints than are extremely mobile groups with very small body sizes, such as protists, bacteria, and phytoplankton, none of which are usually subjected to dispersal limitations (Beisner *et al.*, 2006; Fontaneto *et al.*, 2006). However, a study of the metacommunities within a floodplain system showed that spatial effects do not play a significant role also in the case of zooplankton (Padial *et al.*, 2014). Instead, spatial structuring is probably stronger for fish and macrophytes (Beisner *et al.*, 2006; Padial *et al.*, 2014) than for meiofauna.

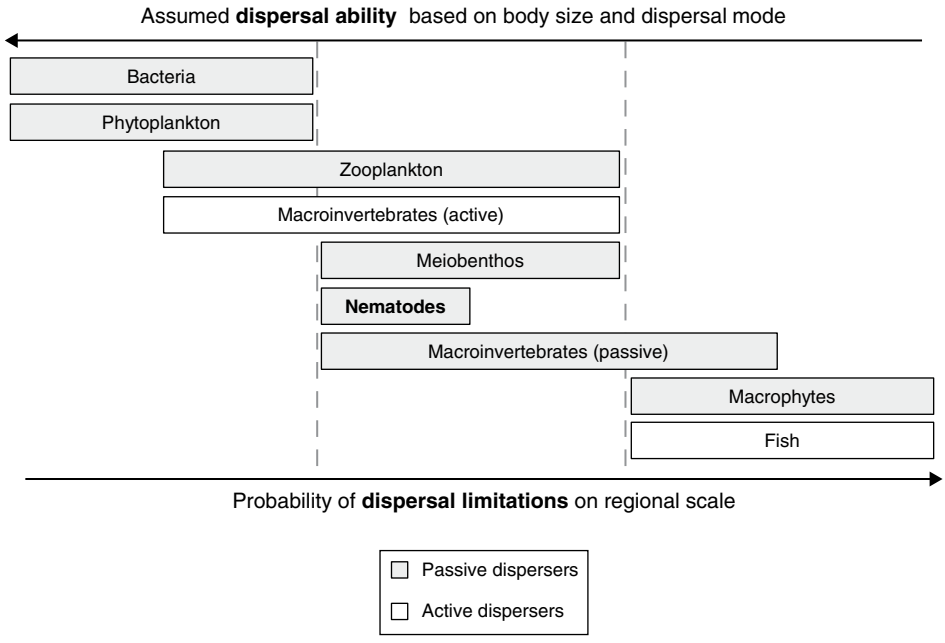


Fig. 9.4. Aquatic organismal groups arranged according to their probability of being dispersal limited on a regional scale, a feature negatively related to the assumed dispersal ability based on body size and dispersal mode. Nematodes are within the group of meiobenthos considered to be good dispersers. (Modified from Gansfort *et al*., 2020.)

Among meiofauna, nematodes are among the better dispersers despite their strong association with the sediment (Fig. 9.4). Specifically, based on the determined dispersal limitations, ostracods, nematodes, and rotifers differ (in ascending order) in their dispersal ability. These differences probably reflect differences in body size and the efficient dispersal of nematodes and rotifers by wind vs. the need of ostracods for animal vectors (Gansfort *et al.*, 2020).

The findings of the few studies of freshwater nematode metacommunities suggest that dispersal surplus (in terms of mass effects) explains their spatial structure (Dümmer *et al.*, 2016; Gansfort and Traunspurger, 2019; Ptatscheck *et al.*, 2020). Cottenie (2005) similarly showed that mass effects in combination with niche-based processes (i.e. species sorting) are the second most relevant metacommunity dynamic for good passive dispersers (here, metacommunities of zooplankton and terrestrial plants). By contrast, Leibold and Chase (2018) noted that mass effects have been demonstrated in very few studies. Thus, nematodes provide a relatively rare opportunity to study the effects of dispersal surplus.

9.6 Conclusions and Perspectives

The metacommunity framework allows simultaneous considerations of multiple processes on multiple scales (Leibold and Chase, 2018) and has

thus become a widely accepted research approach within contemporary ecological research. However, it has been only rarely exploited by freshwater nematologists. This is unfortunate, because nematodes are valuable organisms in studies of metacommunity processes at different scales and in different aquatic habitats. With their high diversity in many habitat types as well as their wide range of ecological dependencies and dispersal modes, nematodes can provide a broad picture of organism–habitat and organism–organism interactions as well as of regional and local dynamics. Accordingly, studies of different freshwater habitats, with different spatial extents and beyond Europe, are needed to assess the role of dispersal as well as niche-based and stochastic processes in nematode metacommunities. Investigations allowing comparisons of different nematode taxa and between nematodes and other freshwater organisms will contribute to obtaining a broader picture of metacommunity processes in freshwater ecosystems.

In addition to their advantages in observational studies, nematodes are valuable organisms to test general aspects of metacommunity theory in laboratory set-ups (see Chapter 12). Freshwater nematodes are diverse, abundant, and relatively tolerant of the conditions typically used in artificial mesocosm communities (e.g. Michiels and Traunspurger, 2005; Ristau *et al.*, 2012; Gansfort *et al.*, 2021). Mesocosm studies would provide insights into the relevance of biotic interactions in the metacommunity context, as disentangling the roles of biotic and abiotic factors in field studies is challenging (Kneitel and Miller, 2003; Cadotte *et al.*, 2006). Experimental approaches are also essential elements in studies of metacommunity dynamics, given that, despite the utility of pattern-based approaches in observational field studies (irrelevant of analysis method), they do not allow unequivocal deductions of a process from an observed pattern, because the same patterns may arise due to different processes (Leibold and Chase, 2018).

In field studies, biotic predictors should be more often included within observational studies of nematode metacommunities (Gray *et al.*, 2012; Göthe *et al.*, 2013) to assess their role in niche-based processes. A serious general objection to metacommunity research is that it is usually based on snapshot data and ignores temporal dynamics (Heino *et al.*, 2015a). As temporal dynamics may be the primary force structuring freshwater nematode communities, they should be strongly considered in metacommunity analyses as well.

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10 Single- and Multi-species Toxicity Testing with Nematodes

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Highlights

- Nematodes offer versatile and relevant tools for an environmental risk assessment of chemicals and environmental samples.
- ISO 10872 using *Caenorhabditis elegans* is one of the few standardized whole-sediment toxicity tests.
- The sensitivity of *C. elegans* to metals and polycyclic aromatic hydrocarbons (PAHs) is well within the range of other, field-relevant freshwater nematode species.
- Nematodes are suitable organisms for toxicity assessments in microcosm systems at the laboratory scale.
- Measurements of structural (abundance, biomass, diversity) and functional (secondary production) parameters provide important information about ecosystem functioning.

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10.1 Introduction

This chapter discusses the utility of nematodes in experimental ecotoxicology, and specifically in the study of freshwaters. Drawing on reports in which nematodes were used as test organisms in single-species tests as well as studies investigating nematode communities in model ecosystems (i.e. microcosms), the suitability of nematode-based experimental approaches in prospective and retrospective risk assessments of chemicals in freshwater sediments was examined. Since the publication of *Freshwater Nematodes: Ecology and Taxonomy* in 2006 (Eyualem-Abebe *et al.*, 2006), research on nematodes as toxicity test organisms has made considerable progress: a book specifically dedicated to the topic *Nematodes as Environmental Bioindicators* (Wilson and Kakouli-Duarte, 2009) and several review articles on the use of nematodes in experimental ecotoxicology (Haegerbaeumer *et al.*, 2015), especially on the model nematode species *Caenorhabditis elegans* (e.g. Meyer and Williams, 2014; Hunt, 2017) have been published. Moreover, the inclusion of *C. elegans* in toxicity tests of water, sediments, and soils was standardized by the International Organization for Standardization (ISO) in its 2010 protocol (ISO 10872:2010; ISO, 2010) and in the recent revision (ISO 10872:2020; ISO, 2020).

10.2 Environmental Risk Assessments of Chemicals

Ecotoxicological methods are used to assess the environmental risk posed by the toxicity of single chemicals or chemical mixtures in water, sediment, and soil (European Commission, 2003; ECHA, 2008; EFSA, 2013; Fig. 10.1). Note that a distinction must be made between prospective and retrospective risk assessments. The former evaluates the risk associated with the authorization and potential restricted release of a chemical into the environment (e.g. plant protection products; EFSA, 2013). A retrospective risk assessment is required for environmental samples that may have been contaminated with chemical substances (e.g. dredged sediments; Manz *et al.*, 2007).

In prospective risk assessments, both *in vitro* and *in vivo* toxicity tests are required to investigate the mechanisms of toxicity and to set accurate toxicity thresholds (European Commision, 2003; EFSA, 2013). With higher-tier testing, toxicity thresholds can be refined to reduce safety risks (Boxall *et al.*, 2002). Toxicity testing can be made more realistic by using (i) chronic toxicity endpoints (e.g. reproduction), (ii) realistic exposure scenarios (e.g. sediments for scarcely soluble substances), or (iii) ecologically more relevant multi-species test systems (model ecosystems), such as micro- or mesocosms.

In retrospective risk assessments, the ecological status or quality of an ecosystem is determined by evaluating the *in situ* fauna or flora (Water Frame Directive (WFD); European Community, 2000). Here, indicator systems can help to identify habitats with a poor ecological status and to link this status

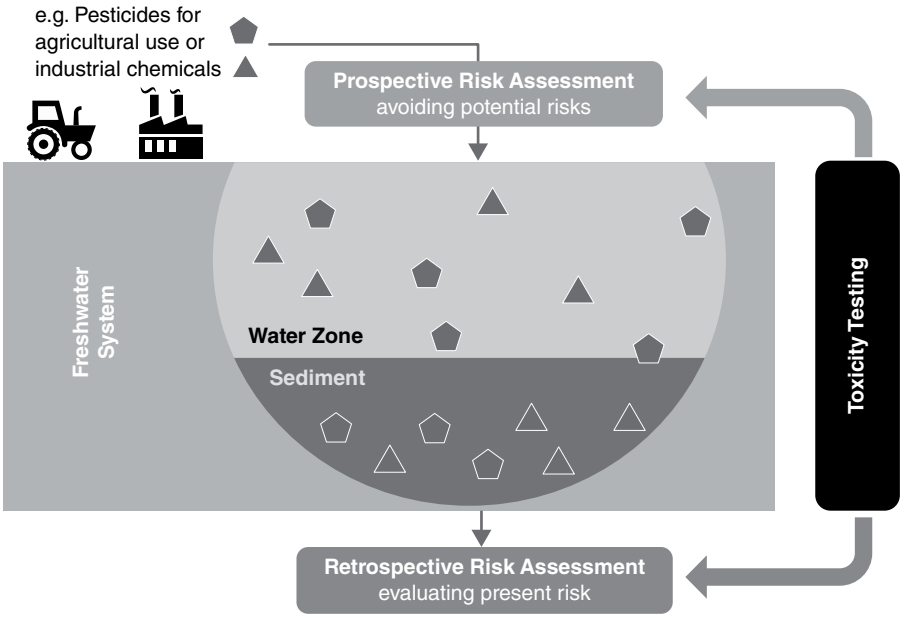

Fig. 10.1. The use of toxicity testing in environmental risk assessments. (Author's own figure.)

to the presence of chemical pollutants or other types of stress, including hydromorphological modifications and climate change (e.g. Von der Ohe *et al.*, 2007; Von der Ohe and Goedkoop, 2013; see also Chapter 11). However, methods that allow the causes of the determined deleterious effects on an ecosystem to be elucidated are a prerequisite of water management policies aimed at improving the ecological status of ecosystems (as mandated by the EU WFD) (De Zwart *et al.*, 2009). In this case, toxicity tests can help to detect the direct toxicity of chemicals in environmental samples (e.g. Tuikka *et al.*, 2011; Feiler *et al.*, 2013) and of contaminated environmental samples, such as dredged sediments (Manz *et al.*, 2007).

10.3 Suitability of Nematodes for Ecotoxicological Studies

The ecological relevance of nematodes for lentic and lotic habitats is described in Chapter 3, but nematodes also offer several advantages as test organisms for toxicity tests performed in the context of ecotoxicological research and environmental risk assessments (see also Chapter 11).

10.3.1 Sediment as natural habitat

Many toxic chemicals that enter aquatic ecosystems (e.g. hydrophobic organic chemicals and metals) bind to settling suspended matter and are therefore transported to the sediment compartment. As a result, the concentrations of pollutants are often considerably higher in sediments than

in the water phase, which leads to higher exposure concentrations for benthic organisms. Accurate determinations of the toxicity of sediment-bound chemical require the use of sediment-dwelling organisms as test organisms (e.g. Chapman and Anderson, 2005; Höss *et al.*, 2010). Nematodes are strictly benthic organisms and are thus well suited for whole-sediment toxicity testing. Moreover, they prefer fine sediments, which are often hotspots of chemical contamination (Salomons and Förstner, 1984). Toxicity tests with *C. elegans* are among the few standardized toxicity tests adapted for invertebrates and validated for toxicity assessments in freshwater sediments (ISO 10872; ISO, 2020). Other tests are based on the use of chironomids (OECD, 2001; ASTM, 2005), amphipods (ASTM, 2005; ISO, 2013), oligochaetes (OECD, 2007), and snails (OECD, 2016).

10.3.2 Accrued knowledge

Caenorhabditis elegans is one of the best-studied multicellular organisms, as it has been used for over four decades as a model organism in a broad range of life science disciplines, such as genetics, developmental biology, biomedical and environment toxicology, and ecology (e.g. Kaletta and Hengartner, 2006; Antoshechkin and Sternberg, 2007; Leung *et al.*, 2008; Höss and Williams, 2009). Recently, *Pristionchus pacificus* has been developed as a satellite model nematode to *C. elegans* for use in detailed genetic and molecular studies (Hong and Sommer, 2006; Sommer, 2015), which has led to pioneering discoveries in neuroscience, development, signal transduction, cell death, aging, and RNA interference (Antoshechkin and Sternberg, 2007). Increasing evidence suggests a high level of genetic and physiological similarity between *C. elegans* and higher eukaryotes (Kaletta and Hengartner, 2006). Therefore, *C. elegans* may also have high predictive power when used as a model organism in assessments of toxicological processes in humans (e.g. Aschner *et al.*, 2010) and serve as a complement to mammalian models in toxicology research and toxicity testing.

10.3.3 Easy culturing and standardization

Cultures of *C. elegans* and *P. pacificus* are easily maintained (e.g. Stiernagel, 1999) and the short life cycles of these species (about 72 h) enable experiments with ecologically meaningful toxicity endpoints, such as reproduction and population growth rates, to be performed within manageable time frames (Traunspurger *et al.*, 1997; Muschiol *et al.*, 2009), even over several generations (Wamucho *et al.*, 2019). Moreover, the use of monoxenic cultures and the hermaphroditism of nematodes facilitate standardization of the test conditions (Traunspurger *et al.*, 1997; Höss *et al.*, 2012).

10.4 Single-species Tests

10.4.1 Nematodes as test organisms

Nematodes have been used as test organisms in laboratory bioassays for nearly 50 years (e.g. Boroditsky and Samoiloff, 1973). Assessments of the potential toxicity of various compounds in aqueous medium (Haight *et al.*, 1982), on agar (Popham and Webster, 1979), and in more complex matrices such as sediments and soils (Donkin and Dusenbery, 1993) have mainly been conducted using free-living, bacterivorous nematodes as test organisms. Thus, ecotoxicological studies have included the marine species *Monhystera disjuncta* (Vranken and Heip, 1986), species of the genus *Panagrellus* (Samoiloff *et al.*, 1980), and *Plectus acuminatus* (Kammenga *et al.*, 1996). An overview of the nematode species used in ecotoxicology research can be found in Haegerbaeumer *et al.* (2015).

Nonetheless, the bacterivorous species *C. elegans* remains the most commonly used nematode. Methods for its application in assessments of waste water (Hitchcock *et al.*, 1997), sediment (Traunspurger *et al.*, 1997; Höss *et al.*, 1999, 2012), soil (Freeman *et al.*, 2000; Höss *et al.*, 2009), and waste (Höss and Römbke, 2019) have been standardized. For these purposes, a variety of toxicity parameters have been defined for *C. elegans*: lethality (Williams and Dusenbery, 1990), growth and reproduction (Traunspurger *et al.* 1997; Höss *et al.*, 1999, 2012), population growth (Vangheel *et al.*, 2014; Mueller *et al.*, 2020a), behavioral endpoints (Chen *et al.*, 2013), and functional endpoints, such as oxygen consumption (Du Preez *et al.*, 2020). Other authors employed *C. elegans* as a bioindicator for studying pollution-induced gene expression (Guven *et al.*, 1994; Menzel *et al.*, 2009b; Kumar *et al.*, 2015), as a test organism for bioconcentration and bioaccumulation studies (Haitzer *et al.*, 1999; Spann *et al.*, 2015), and to screen potential mutagens (Lew *et al.*, 1983). Although *C. elegans* naturally inhabits microbe-rich decaying plant material (Félix and Braendle, 2010) and has rarely been detected in freshwater habitats (Hirschmann, 1952; Zullini, 1988), this species lives in aquatic biofilms and its sensitivity to several chemical substances is comparable with that of true freshwater nematode species (Haegerbaeumer *et al.*, 2018a).

In the following, we present several examples of the use of *C. elegans* as a test organism in single-species bioassays, in order to demonstrate the versatility of this nematode for ecotoxicological investigations.

10.4.2 Standardization (ISO 10872)

In 2010, standardization of the toxicity test using *C. elegans* was finalized with the publication of the ISO standard ISO 10872:2010, in which freshwater sediment, elutriate, and porewater formed the basis of the test (ISO, 2010). In the test, *C. elegans* is exposed to environmental samples for 96 h, sufficient time to allow the development of sub-lethal toxicity endpoints,

such as growth and reproduction, whose performances are compared to those in a negative control (Fig. 10.2). A subsequent revision of the test process involved substantial methodological changes to allow soil testing, such that ISO 10872:2020 is the first ISO standard that combines water, sediment, and soil testing ('Water and Soil Quality', ISO, 2020).

The results of an interlaboratory round-robin (ring) test showed that ISO 10872 is a reliable and reproducible tool for assessing the toxicity of freshwater sediments (Fig. 10.3). The coefficient of variance of reproducibility (CV_R) for the inhibition of growth and reproduction of *C. elegans* exposed in two contaminated sediments was below 30% and thus comparable with or even better than the results reported for other toxicity tests (Höss *et al.*, 2012). For the toxicity of the reference substance (BAC-16; benzalkonium chloride), the ring test yielded EC_{50} values of 15.1 and 7.5 mg/l for the growth and reproduction of *C. elegans*, respectively (Höss *et al.*, 2012). The low CV_R values of these two endpoints, 11.1 and 10.5%, respectively, are indicative of the high precision of the *C. elegans* toxicity test in aqueous medium, as both values were well below the limits considered acceptable by most scientists and permit holders (CV_R <30–40%; e.g. Moore *et al.*, 2000).

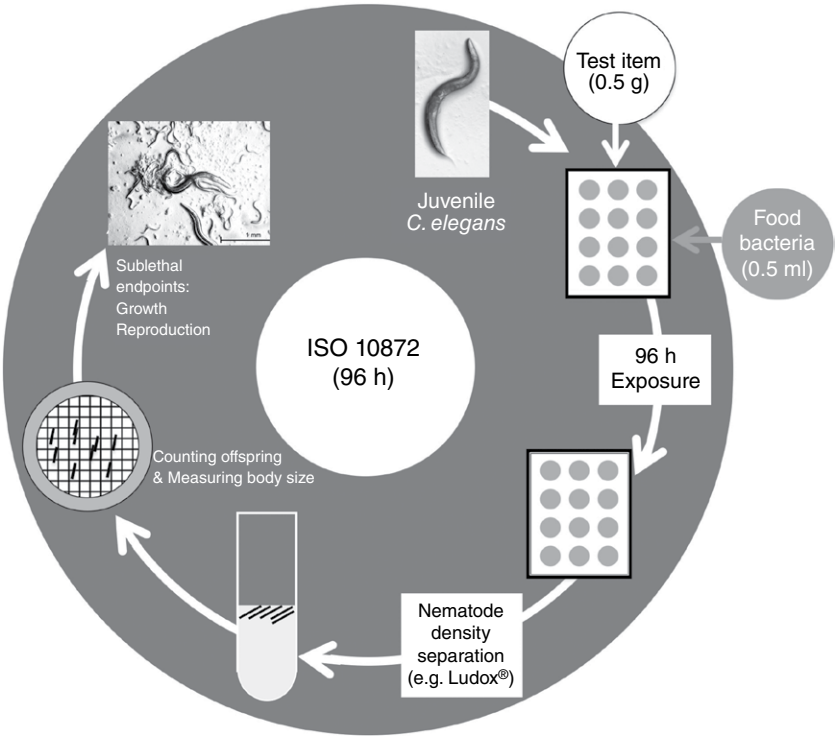

Fig. 10.2. Scheme for toxicity testing of sediments with *C. elegans* according to ISO 10872:2020 (ISO, 2020). (Author's own figure.)

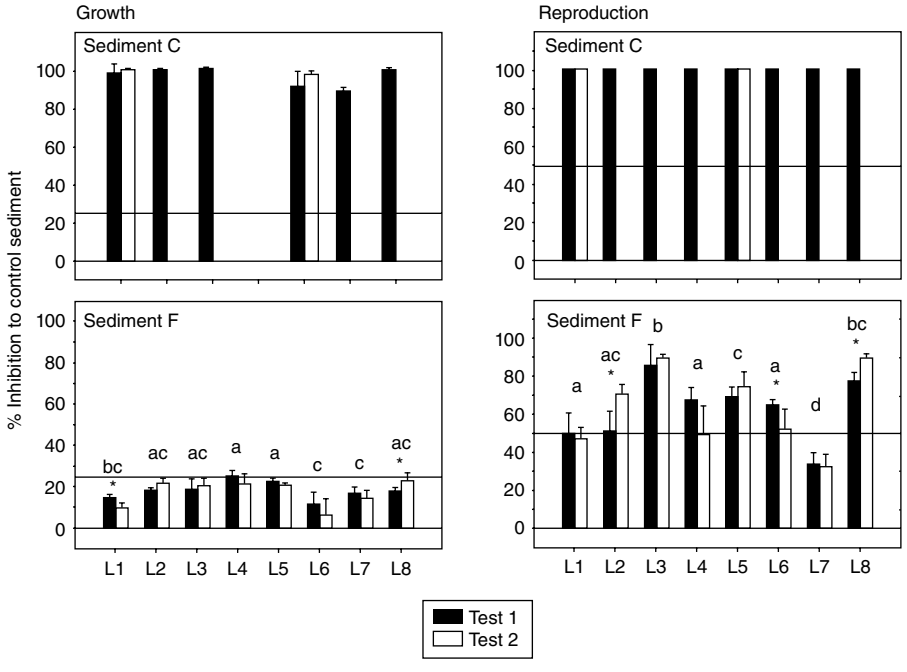

Fig. 10.3. Percentage inhibition of growth (**left**) and reproduction (**right**) of *C. elegans* exposed to different sediment samples (Sediment C, highly toxic; Sediment F, toxic). Horizontal lines indicate toxicity thresholds for sediments and soils based on Höss *et al.* (2010); * = significant difference between tests 1 and 2 ($P < 0.05$; *t*-test); different letters indicate significant difference between the average values obtained by different labs ($P < 0.05$; one-way analysis of variance (ANOVA), post-hoc: Dunnett's test); the same or no letters indicate no significant difference between the average values from the different labs ($P < 0.05$; one-way ANOVA). (Modified from Höss *et al.* (2012) with permission of John Wiley and Sons.)

10.4.3 Testing of chemical substances using *C. elegans*

The ability to maintain *C. elegans* in aqueous or solid substrates provides numerous options for the testing of chemicals in the environment. For example, water and sediment can be spiked with single chemicals or mixtures thereof. Chemical substances and materials of any class can be tested for their effects on *C. elegans* and at levels ranging from the molecular to the whole organism. Such studies have thus far been conducted for endocrine disruptors (reviewed in Höss and Weltje, 2007), pesticides (reviewed in Meyer and Williams, 2014), metals (e.g. Boyd *et al.*, 2003; Swain *et al.*, 2004; Höss *et al.*, 2011; Caito *et al.*, 2012), polycyclic aromatic hydrocarbons (PAHs; e.g. Sese *et al.*, 2009; Spann *et al.*, 2015; Fischer *et al.*, 2016), nano-materials (e.g. Meyer *et al.*, 2010; Höss *et al.*, 2015b; Hanna *et al.*, 2016; reviewed in Wu *et al.*, 2019), microplastics (e.g. Lei *et al.*, 2018; Mueller *et al.*, 2020b), natural toxins (e.g. Wei *et al.*, 2003; Crickmore, 2005; Höss *et al.*, 2013), and the joint toxicity of chemical mixtures (Jager *et al.*,

2014; Vingskes and Spann, 2018; Wittkowski *et al.*, 2019). Toxicity can be assessed using a variety of phenotypic endpoints, such as mortality, growth, reproduction, feeding, and motility (see also Höss and Williams, 2009). In addition, toxico-dynamic approaches, in which life cycle traits, such as population growth rates, are considered, have been used to investigate ecotoxicological effects on a more relevant ecological level (Brinke *et al.*, 2013; Jager *et al.*, 2014). Molecular markers ('omics') have also been exploited in *C. elegans* to evaluate the effects of chemicals (Menzel *et al.*, 2009a; reviewed in Hunt, 2017) and environmental samples (Menzel *et al.*, 2009b). The results of innovative methods, such as high-throughput toxicity testing (Boyd *et al.*, 2010; Helmcke *et al.*, 2010; Du Preez *et al.*, 2020) or passive dosing exposure (Fischer *et al.*, 2016; Roh *et al.*, 2016), indicate that nematode-based ecotoxicology contributes substantially to achieving an overall understanding of the impact of chemicals on ecosystems. Given the suitability and relevance of nematodes in ecotoxicology, the rare use of nematodes in routine risk assessments of chemicals in sediments (e.g. within the framework of the REACH Regulation; Cesnaitis *et al.*, 2014) is difficult to understand.

A recent study of the toxicity of PAHs on *C. elegans* demonstrated the merit of innovative dosing techniques for an accurate definition of toxicity thresholds and to obtain insights into uptake mechanisms. Fischer *et al.* (2016) exposed *C. elegans* to various PAHs via passive dosing and compared the resulting toxicity with that induced by conventional exposure (solvent spiking). In passive dosing, the desired concentrations of freely dissolved (C_{free}) hydrophobic organic chemicals can be established and constantly controlled by the continuous partitioning of the chemical from a reservoir (e.g. silicon O-rings) into the test medium (Mayer *et al.*, 1999). This method compensates for potential chemical losses due to evaporation, degradation, or binding to food or vessel surfaces, since the test compound is continuously supplied from the reservoir. With solvent spiking, there is no such compensation and any chemical loss will lead to a decrease of C_{free} and thus also to underestimations of toxicity. Fischer *et al.* (2016) showed that, by passive dosing, the C_{free} of phenathrene, anthracene, fluoranthene, and pyrene could be kept constant both over time and despite increasing densities of food bacteria, while this was not possible with solvent spiking. Consequently, reproduction-based EC_{50} values for phenanthrene and pyrene were considerably lower when determined under passive dosing (45 and 8.2 µg/l, respectively) than under solvent spiking (115 and 31 µg/l, respectively) conditions. Moreover, with passive dosing not only were the dissolved concentrations of the PAHs kept constant with increasing bacterial densities, but the uptake rate of bacterial-bound PAHs increased (Fig. 10.4B; Fischer *et al.*, 2016). In addition, whereas with increasing food density the toxicity of phenanthrene decreased under solvent spiking conditions (Fig. 10.4A), it increased during passive dosing (Fig. 10.4B). This result demonstrates that not only freely dissolved phenanthrene (as suggested by exposure via solvent spiking; Fig. 10.4A), but also bacterial-bound phenanthrene accounted for the chemical's toxic effect on *C. elegans*.

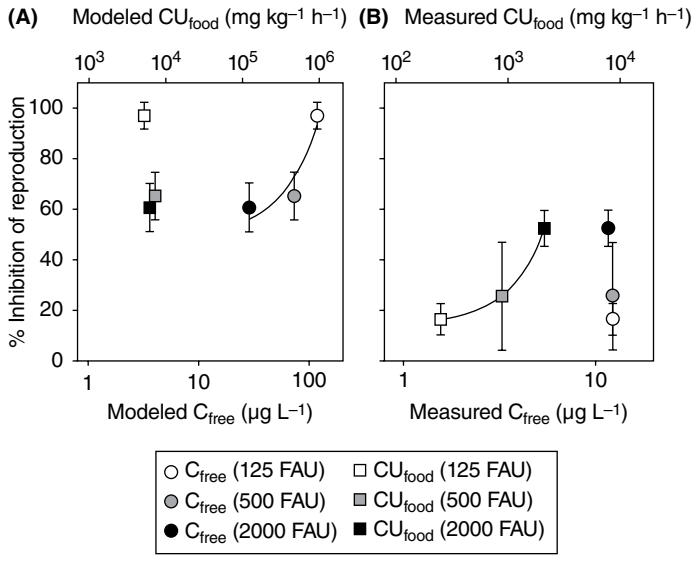

Fig. 10.4. Inhibition of *C. elegans* reproduction (%, mean ± standard deviation, $n = 3$) after a 96-h exposure of the nematode to phenanthrene in the presence of different *E. coli* cell densities (FAU 125, 500, and 2000); phenanthrene was provided by (**A**) solvent spiking or (**B**) passive dosing. Note that with solvent spiking the apparent toxicity was driven by C_{free} whereas with passive dosing it was altered by CU_{food} while C_{free} values remained constant. (From Fischer *et al*. (2016) with permission of the ACS; https://pubs.acs.org/doi/full/10.1021/acs.est.6b02956, further permissions related to the material excerpted should be directed to the ACS.)

10.4.4 Sensitivity of *C. elegans*

To determine whether the chemical sensitivity of *C. elegans* is representative of that of other nematode species, Haegerbaeumer *et al.* (2018a) compared the acute toxicity of various metals, PAHs, and various chemical mixtures on a selection of freshwater nematode species, with *C. elegans* serving as the benchmark species. Nematode species assemblages extracted from pristine freshwater sediments and comprising >100 different species were exposed to the test chemicals in aqueous medium for 48 h, after which the survival rate was determined. Between 21 and 27 species fulfilled the study criteria and could be compared with *C. elegans*. The sensitivity of *C. elegans* to metals and PAHs was well within the range of field-relevant freshwater nematode species (Fig. 10.5a; Haegerbaeumer *et al.*, 2018a). Specifically, while for metals *C. elegans* ranked in the middle of all tested species (Fig. 10.5b), for organic pollutants, such as PAHs, it was one of the more tolerant species (Fig. 10.5c). Thus, there are nematode species in the field that might be more susceptible than *C. elegans* to chemical stress, which could lead to an underestimation of the risks posed by those chemicals, if toxicity testing relies only on a single species.

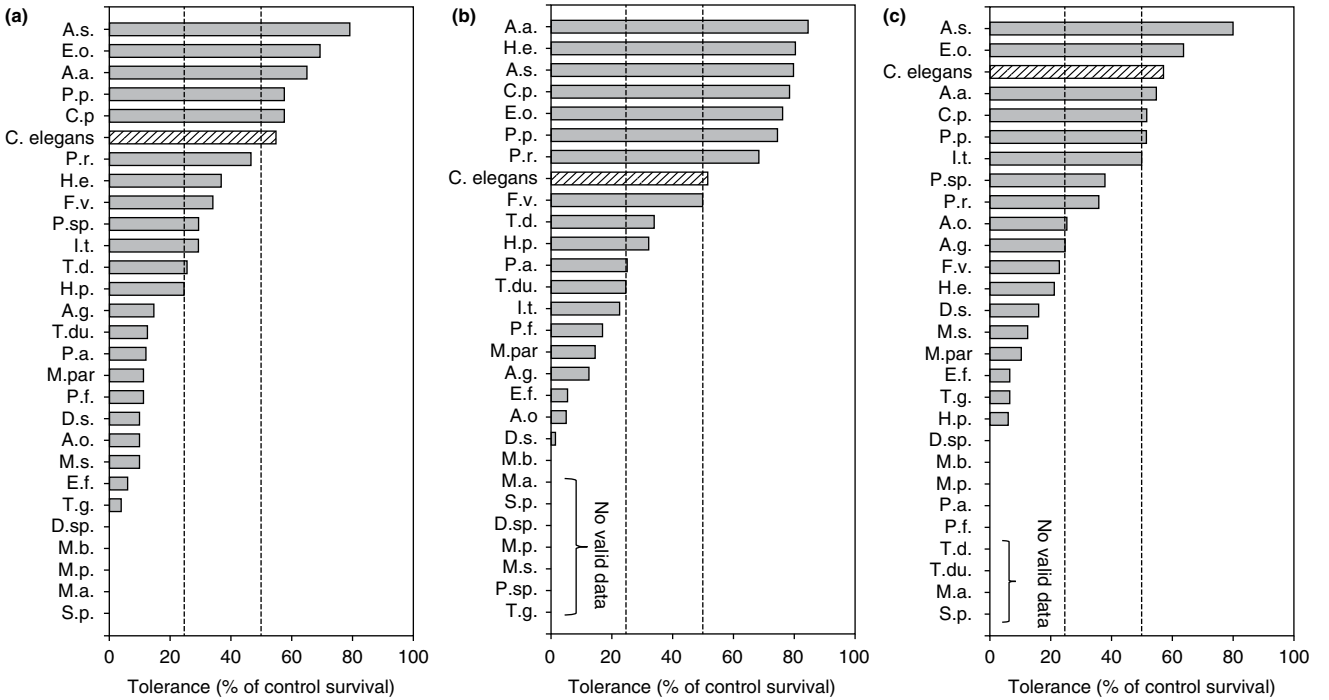

Fig. 10.5. Mean tolerance (% of control survival) of the freshwater nematode species tested in acute multi-species toxicity tests; the results are expressed as the summarized responses to (**a**) all investigated chemicals, (**b**) metals, and (**c**) PAHs; toxicity data of *C. elegans* additionally shown as a reference (white bar with striped pattern); dashed lines mark boundaries between sensitive (<25% of control survival), intermediate (25–50% of control survival), and tolerant (>50% of control survival) freshwater nematode species. A.a., *Aphanolaimus aquaticus*; A.g., *Anaplectus granulosus*; A.o., *Aporcelaimellus obtusicaudatus*; A.s., *Aphelenchoides subparietinus*; C. elegans, *Caenorhabditis elegans*; C.p, *Cephalobus persegnis*; D.s., *Dorylaimus stagnalis*; D.sp., *Diplogasteritus* sp.; E.f., *Eumonhystera filiformis*; E.o., *Eucephalobus oxyuroides*; F.v., *Filenchus vulgaris*; H.e., *Heterocephalobus elongatus*; H.p., *Helicotylenchus pseudorobustus*; I.t., *Ironus tenuicaudatus*; M.a., *Mononchus aquaticus*; M.b., *Mesodorylaimus bastiani*; M.p., *Monhystera paludicola*; M.par *Monhystrella paramacrura*; M.s., *Monhystera stagnalis*; P.a., *Plectus aquatilis*; P.f., *Prodorylaimus filiarum*; P.p., *Pelodera punctata*; P.r., *Panagrolaimus rigidus*; P.sp., *Pratylenchus* sp.; S.p., *Semitobrilus pellucidus*; T.d., *Tylenchus davanei*; T.du., *Tylenchorhynchus dubius*; T.g., *Tobrilus gracilis*. (From Haegerbaeumer *et al*. (2018a) with permission of Springer Nature.)

10.4.5 Testing of environmental samples with *C. elegans*

Sediments are both a sink and a source for contaminants in freshwater ecosystems (Förstner, 2002; Brils, 2004). Accordingly, sediment studies may reveal the extent and history of water pollution while also revealing trends. Toxicity testing using single species can help to identify the toxicity of chemicals in complex environmental samples, such as sediments, where many confounding factors are able to mask toxic effects. Together with chemical analyses and benthic community assessments, whole-sediment tests are a pillar of the sediment quality triad, a holistic weight-of-evidence approach for assessing the risk posed by contaminated sediments (Chapman and Anderson, 2005). Considering the natural variability of sediment toxicity tests due to confounding factors (e.g. grain size, organic matter), whole-sediment toxicity tests can be used to distinguish chemical stress from other causes of ecosystem impairment, such as hydromorphological factors (e.g. Postma *et al.*, 2002; Höss *et al.*, 2010).

Using a battery of sediment contact tests with organisms representing various trophic levels and different uptake routes for contaminants (bacteria, higher plants, nematodes, oligochaetes, fish), Feiler *et al.* (2013) tested the toxicity of sediments differing in their degree of pollution. The test battery showed that for sediments with a low to medium toxic potential (based on sediment quality guidelines), toxicity was underestimated in >50% of the cases. This result underlined the need for toxicity tests, because they are able to take into account all bioavailable, even unknown, chemicals able to affect one of the applied test organisms. Nematodes (*C. elegans*) and fish (*Danio rerio*) were the most sensitive organisms within the test battery (Feiler *et al.*, 2013). Similar results were reported by Tuikka *et al.* (2011), who compared contaminated sites to reference sites in three different European river catchments (Elbe, Llobregat, Scheldt) using a test battery that included different test organisms (bacteria, nematodes, chironomids, oligochaetes, snails, fish). The single toxicity tests provided similar, non-redundant information that filled the gap left by the chemical analysis and revealed the toxicity of the sediments (Tuikka *et al.*, 2011).

Schertzinger *et al.* (2019) applied sediment quality guidelines and two sediment toxicity tests (fish: *D. rerio*; nematodes: *C. elegans*) to investigate the toxicity of sediments at three different sites that received discharge from combined sewer overflows (CSO). Sediments downstream from the CSOs were strongly impacted by discharged particles in terms of pollution, due to the associated contaminants, as well as by oxygen depletion (Schertzinger *et al.*, 2019). While the test with *D. rerio* mainly detected oxygen depletion, the nematode toxicity test was able to detect chemical pollution, as the toxic potential of the metals and polychlorinated biphenyls correlated strongly with the inhibition of *C. elegans*' reproduction (Schertzinger *et al.*, 2019). Furthermore, within the rainwater retention basin the toxicity of the sediment for nematode reproduction was high (>50% inhibition) but it decreased with increasing distance from the CSO (Fig. 10.6).

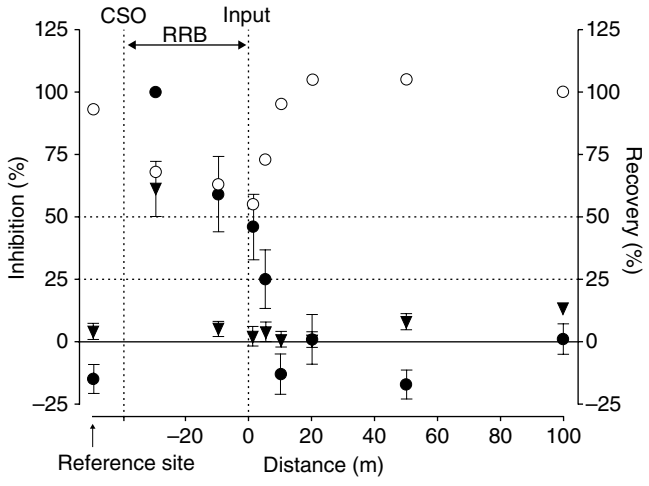

Fig. 10.6. Inhibition of the growth and reproduction (left *y*-axis) of *C. elegans* after 96 h of exposure to sediments from one location (Location 3 as described by Schertzinger *et al*. (2019)) sampled along a transect from the combined sewer overflow (CSO) (mean and standard deviation of 4 replicates, 10 nematodes per replicate) and the recovery (right *y*-axis) of the test organisms. RRB, rain retention basin; vertical dotted lines, location of the CSO and the input from the retention zones into the creek; dashed horizontal lines, toxicity thresholds for growth (25% inhibition) and reproduction (50% inhibition). (From Schertzinger *et al*. (2019) with permission of Elsevier.)

10.5 Model Ecosystems

Model ecosystems established in meso- or microcosms are large enough to study natural communities under controlled conditions but small enough to enable sufficient replication and precise control of relevant experimental conditions. Typically, natural sediments are transferred into the experimental containers (Fig. 10.7) and then manipulated experimentally (e.g. spiking of chemicals) for a certain period of time (usually, several weeks to months). Therefore, experiments in model ecosystems can also be regarded as community-level bioassays. In prospective risk assessments of chemicals, model ecosystems are an effective compromise between standard laboratory tests and outdoor studies. Moreover, they offer a balance between interpretability and practicability on the one hand and ecological relevance on the other (Fig. 10.8).

Nematodes are suitable organisms for microcosm experiments, as the relatively short generation times of most species (several weeks to months) allow community changes to be measured over a time scale that is feasible in model ecosystem studies. Also, problems related to recruitment in microcosm systems are avoided, since nematodes undergo direct benthic development (unlike macrobenthic groups, in which planktonic larval recruitment is common). A typical laboratory microcosm set-up for the study of meiofauna is shown in Fig. 10.7.

Fig. 10.7. Microcosms at Bielefeld University. (Photo: Marvin Brinke, Animal Ecology, Bielefeld University.)

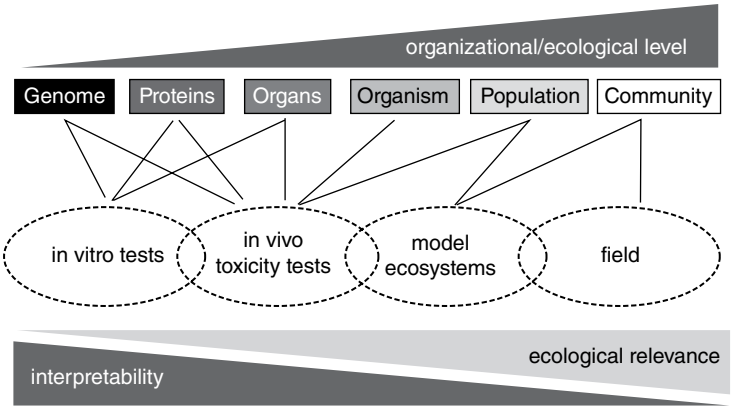

Fig. 10.8. Ecotoxicological methods applicable at different organizational and ecological levels using freshwater nematodes as test or indicator organisms. Model ecosystems offer a balance between interpretability and practicability on the one hand and ecological relevance on the other. (Modified from fig. 1 in Haegerbaeumer *et al*. (2015) with permission of Exeley.)

Recently, Haegerbaeumer *et al.* (2015) reviewed experimental ecotoxicological studies with nematodes in freshwater and marine sediments as well as in soils. Thus far, there have been few investigations using freshwater nematodes (Table 10.1). In those studies, different metals (e.g. cadmium, copper) and organic chemicals (e.g. nonylphenol, fluoranthene, crude oil) were investigated in model ecosystems over a period of

Table 10.1. Selected studies on model ecosystems with freshwater nematodes. (Author's own table.)

Chemical	Duration (days)	Assessment parameters	Reference
Metals			
Cadmium	218	AB, FT, MI, SR, SC	Brinke *et al*. (2011)
Cadmium	215	AB, BM	Faupel *et al*. (2011)
Cadmium	215	SC, SP, SR	Faupel and Traunspurger (2012)
Nickel	180	AB, BM, NS, SC	Haegerbaeumer *et al*. (2017, 2018b)
Copper	180	AB, BM, NS, SC	Haegerbaeumer *et al*. (2017, 2018b)
Iron oxide colloids	189	AB, NS, SC, SR	Höss *et al*. (2015a)
Zinc	180	AB, FT, NS, SC, SR	Haegerbaeumer *et al*. (2016, 2017)
Organics			
Benzo[a]pyrene	180	AB, BM, NS, SC	Haegerbaeumer *et al*. (2017, 2018b)
Crude oil	105	AB, SR, FT	Monteiro *et al*. (2019)
Fludioxonil	84	AB, FT, J/A, SC, SR	Höss *et al*. (2020)
Fluoranthene	180	AB, BM, NS, SC	Haegerbaeumer *et al*. (2017, 2018b)
Fluoranthene	189	AB, NS, SR, SC	Höss *et al*. (2015a)
Ivermectin	56	AB, FT, SC, SR	Brinke *et al*. (2010)
p-Nonylphenol	98	AB, FT, SC, SR	Höss *et al*. (2004)
Tetracycline	28	AB	Quinlan *et al*. (2011)

Abundance, AB; biomass, BM; feeding type composition, FT; Maturity index, MI; NemaSPEAR[%]-index, NS; species composition, SC; the ratio of juveniles to adults, J/A; species richness, SR; and secondary production, SP.

several months. The parameters used to assess effects of chemicals on nematode community were abundance, species richness, species composition (using multivariate methods: e.g. principal response curves, PRC), biomass, feeding type composition, and nematode-specific indices, such as the NemaSPEAR[%]-index and the maturity index. Haegerbaeumer *et al*. (2018b) investigated effects of various metals and PAHs on nematode communities in freshwater microcosms and showed that changes in species composition (revealed by multivariate community analysis), especially if based on individual biomass data, responded most sensitively to the chemical disturbance, if compared with total nematode abundance or biomass and species richness.

Besides structural community measures (species diversity and composition), functional endpoints, such as secondary production, can provide a more detailed understanding of the disturbance of ecosystem functions. However, while earlier risk assessment studies (mostly of marine systems) demonstrated the high susceptibility of nematodes and

the value of taxonomic analyses to the species level, ecologically important functional parameters, such as biomass and secondary production, were largely ignored. Secondary production (see Chapter 8) is a major pathway of energy flow in ecosystems (e.g. Strayer and Likens, 1986; Bergtold and Traunspurger, 2005; Stead *et al.*, 2005). As a functional measure, it provides insights into population, food web, community, and ecosystem dynamics (Benke and Huryn, 2017; Majdi *et al.*, 2017), both in response to a disturbance and during the subsequent recovery (Benke and Huryn, 2010). As one of the rare studies combining structural and functional parameters, Faupel and Traunspurger (2012) studied the impact of Cd on natural freshwater nematode communities and assessed species richness and composition in comparison with the secondary production of nematodes. To gain insights into the long-term effects of toxicants on the functioning of benthic ecosystems, microcosms were spiked with Cd as a model pollutant in two concentrations (50 and 400 mg/kg dry sediment). Over a period of 7 months, the response of the zoobenthic secondary production to the Cd treatment was assessed. While, over the 7 months, the total zoobenthic community showed a mean secondary production of 5.3 g/m^2/year in the controls, Cd treatment caused a dose-dependent decrease of production to 1.5 and 0.63 g/m^2/year at low and high doses of Cd. Interestingly, a comparison of various benthic groups supported a tendency of taxa with a relatively high turnover rate (e.g. rotifers) to be less sensitive to the Cd pollution than taxa with a lower turnover rate (e.g. oligochaetes), whereas nematodes took an intermediate position in terms of sensitivity (Fig. 10.9a). For nematodes, Faupel and Traunspurger (2012) compared the functional endpoint secondary production with structural community measures, such as species richness and species composition. While the species composition showed a similar response to the secondary production of the nematodes, the species richness was considerably less sensitive to Cd (Fig. 10.9b). Thus, a non-taxonomic, functional measure, such as secondary production, might be a good and relevant alternative to elaborate and taxonomy-dependent parameters.

In another microcosm study, Höss *et al.* (2004) investigated the influence of nonylphenol (NP) on freshwater nematode communities. NP, an important class of non-ionic surfactants employed in many detergent formulations for industrial and household use (Nimrod and Benson, 1996), is known to have toxic (Staples *et al.*, 1998) and especially endocrine effects on invertebrates (Baldwin *et al.*, 1997). NP was applied to seven microcosms over a period of 6 weeks using a controlled-release method, such that maximal concentrations in the sediment ranged from 0.3 to 3.37 mg NP/kg dry sediment. Nematode community structure in the microcosms (NP treatments and controls) was observed over a period of 14 weeks (2 weeks before, 6 weeks during, and 6 weeks after application). The communities were characterized in terms of total nematode abundance, species diversity (Shannon-index, evenness), species composition, feeding type, and life history strategies (maturity index). Species composition was analyzed using a multivariate method (principal response curves, PRC), a valuable tool for

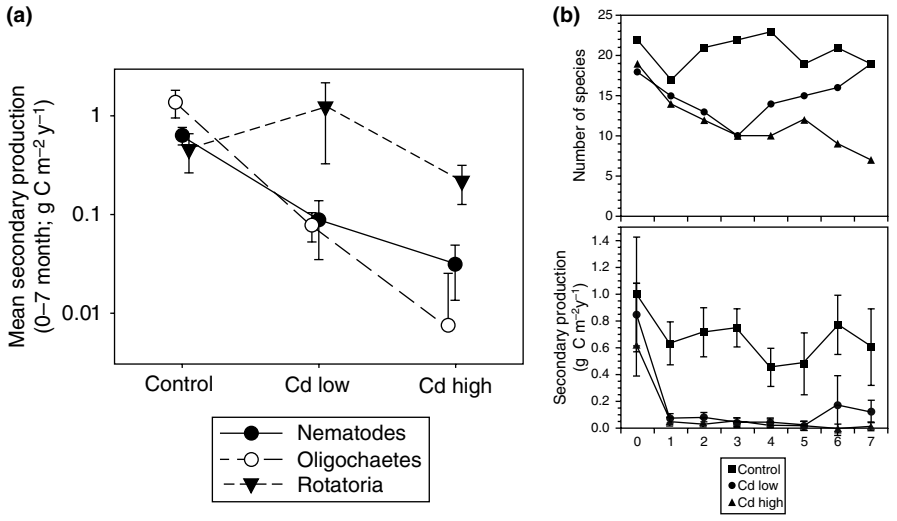

Fig. 10.9. Effects of cadmium (low and high concentrations, 50 and 400 mg/kg dry sediment) in sediments of microcosms on (**a**) the mean secondary production of nematodes, oligochaetes, and rotifers over a period of 7 months (data taken from table 1 in Faupel and Traunspurger, 2012); and (**b**) on species richness (pooled replicates; n = 5) and secondary production of nematode in course of the 7-month experiment. (Modified from Faupel and Traunspurger (2012) with permission of Elsevier.)

visualizing effects on species composition, as time and treatment effects can be assessed simultaneously. The PRC analysis revealed NP-induced changes in species composition over a period of 6 weeks, from the end of the application period until the end of the experiment (Fig. 10.10). In the highest dosed treatment, the abundance of deposit-feeding species (e.g. *Eumonhystera*) increased and that of epistrate feeders (e.g. *Prodesmodora*) and chewers (e.g. *Tobrilus*) decreased compared with the control. In addition, both feeding type composition and the maturity index were affected, albeit only during the last 3 weeks of the study. NP had no effect on nematode abundance and diversity indices throughout the experiment.

Microcosm studies allow the consideration not only of direct toxicity of chemicals on nematodes, but also of indirect food-web effects via other components of the benthos. Höss *et al.* (2015a) investigated the effects of iron oxide colloids and fluoranthene on micro- and meiobenthos in freshwater microcosms. The NemaSPEAR[%], which specifically indicates chemical stress on nematode communities, showed no decrease in the presence of either iron oxide colloids or fluoranthene, suggesting that the treatment did not directly affect the nematode communities. The observed effects of iron oxide colloids on abundance and taxa composition of the meiobenthos, however, could be well correlated with the bacterial activity in the sediments, which was shown to be the most sensitive benthic parameter (Fig. 10.11). This relationship suggested an indirect bottom-up effect of the treatments on the meiofauna via the benthic bacteria (Höss *et al.*, 2015a).

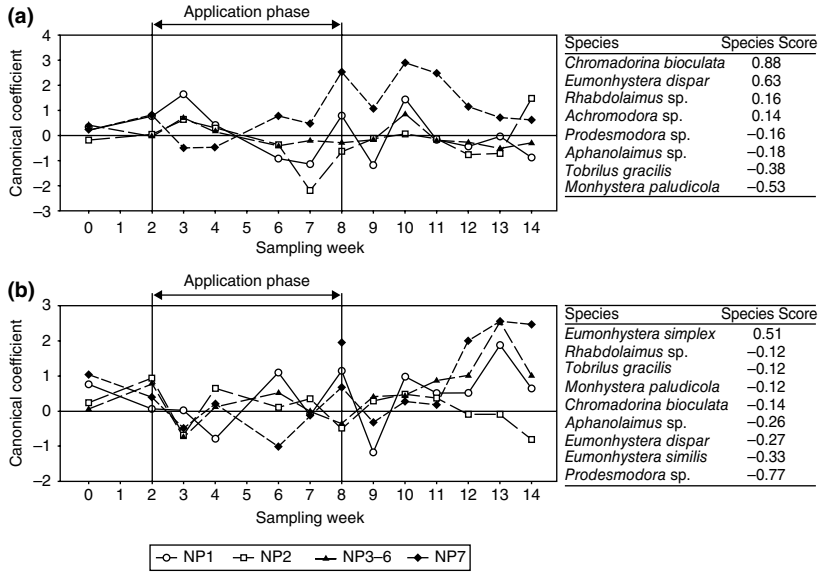

Fig. 10.10. Principal response curves and species scores calculated from the species composition of nematode communities in microcosms dosed with nonylphenol (NP1, NP2, NP3–6, NP7); a, first explanatory variable (22.1%; Eigenvalue: 0.06); b, second explanatory variable (15.4%; Eigenvalue: 0.04). For controls and NP 3–6, *n* = 4; for NP1, NP2, and NP7, n = 1. (From Höss *et al*. (2004) with permission of John Wiley and Sons.)

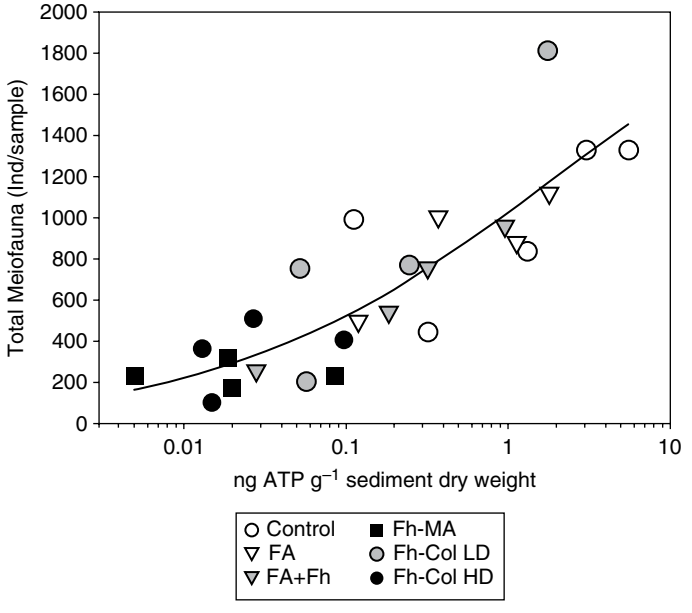

Fig. 10.11. Non-linear correlation of mean total meiofaunal abundance and bacterial activity in microcosms treated with iron oxide (FeOx) and fluoranthene (FA). Fh-Col, ferrihydrite colloids; Fh-MA, ferrihydrite macro-aggregates; ATP, adenosine triphosphate. (From Höss *et al*. (2015a) with permission of John Wiley and Sons.)

These examples show that microcosms are suitable tools to assess direct and indirect toxicity of relevant environmental toxicants on nematode communities. Along with other lines of evidence (sediment quality guidelines, sediment toxicity testing), freshwater microcosms can contribute to a reliable, weight-of-evidence-based risk assessment for sediments, such as mandated by the WFD. Microcosm systems are environmentally realistic experimental units that can be used to assess the impact of chemical stress on natural nematode communities and allow the results to be related to lower-tier toxicity testing (Haegerbaeumer *et al.*, 2016; Höss *et al.*, 2020). As such, they minimize the uncertainty underlying lab to field extrapolations. Among the various measures, functional parameters are not only more sensitive, they are also more meaningful in terms of revealing the consequences of the tested chemical(s) on benthic ecosystems. Furthermore, although taxonomic identifications of nematodes are challenging, species-specific analyses were clearly shown to be more valuable and informative than analyses based on whole nematode communities. Hence, species-level analyses that primarily focus on the functional parameters of nematode assemblages should be included in benthic community analyses performed within environmental risk assessments.

10.6 Conclusions and Perspectives

Freshwater nematodes offer versatile and relevant tools for an environmental risk assessment of chemicals and environmental samples. They can be used as test organisms for single-species toxicity testing, with *C. elegans* being an excellent model organism for identifying reliable toxicity thresholds for chemicals in water and sediment and for unraveling the underlying toxicity mechanisms. With ISO 10782:2020, there exists an international guideline for testing the toxicity of water, sediments, and soils that allows an internationally harmonized and reliable risk assessment for chemicals and environmental samples. It could be shown that *C. elegans* is representative for most dominant freshwater nematode species in terms of sensitivity to metals and organic compounds, although there are species that are more susceptible to environmental pollutants. Here, multi-species test systems can help to cover a broader range of sensitivities among nematodes and, thus, allow a more protective risk evaluation for chemicals in benthic ecosystems. The effects of chemicals on whole nematode communities can be assessed in small-scale microcosms (ca. 2-l systems), which can be set up in the laboratory under well-defined conditions and high statistical replication. These microcosm systems have already been shown to provide ecologically meaningful information on the toxicity of relevant environmental chemicals in complex benthic systems, considering (i) direct toxicity on multiple species, (ii) structural and functional aspects of community ecology, and (iii) indirect effects via the food web. These nematode-based ecotoxicological tools help to fill

the gap between lower-tier toxicity testing and biomonitoring tools in the field and thus provide valuable data for the prospective and retrospective environmental risk assessment.

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11 Freshwater Nematodes as Bioindicators in Field Studies – The NemaSPEAR[%]-index

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Highlights

- Nematodes provide many advantageous features to be used as *in situ* indicators for assessing the ecological quality of freshwater sediments in biomonitoring programs.
- The NemaSPEAR[%]-index has been shown to specifically indicate chemical-induced changes in benthic communities.
- The NemaSPEAR[%]-index allows an accurate ecological quality assessment of fine sediments, where the suitability of routinely used macro-invertebrates is limited.
- Class boundaries defined in the NemaSPEAR[%]-index allow sediments to be categorized according to their ecological status.
- The NemaSPEAR[%]-index can be used in combination with high-throughput DNA-based taxonomic methods.

11.1 Introduction

In this chapter, after a general introduction to quality assessments of freshwater habitats, we review the use of freshwater nematodes as *in situ* bioindicators, including in monitoring the ecological quality of freshwater

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habitats. By drawing on studies of nematode communities in unpolluted and polluted habitats as examples, we highlight both the different methods used to assess the quality of freshwater ecosystems and their applications. A focus of the chapter is the development of a new index that uses freshwater nematodes to assess chemically induced changes in the ecological status of freshwater habitats, the NemaSPEAR[%]-index (Nematode SPEcies At Risk) (Höss *et al.*, 2011).

11.2 Quality Assessments of Freshwater Ecosystems

Anthropogenic pollution is a major stress factor that may affect the important functions of aquatic ecosystems, such as nutrient cycling and biomass production. Pollutants may also accumulate in food webs and thus ultimately affect human health. Recognizing the potential threat posed by the pollution of water resources, scientists and regulators have developed methods to assess the ecotoxicological risks to aquatic ecosystems (ASTM, 2003). An important regulatory basis for the protection of aquatic ecosystems is the European Union Water Framework Directive (EU-WFD), the aim of which is to prevent further deterioration of aquatic ecosystems but also to protect and improve their status (European Community, 2000). However, to date, only ca. 40% of all European surface water bodies have achieved a good ecological and chemical status, with rivers generally having a lower status than lakes and coastal waters (European Environment Agency, 2018).

The broad range of contaminants that are released into rivers and lakes via municipal and industrial emissions requires a variety of ecotoxicological approaches to assess their impacts. Thus, in addition to chemical methods of pollutant analysis, biological methods have been developed that allow estimates of the bioavailability and toxicity of pollutants for biota. Since the late 1960s, these biological methods have been continuously improved and applied to different habitats (pelagial, benthal), organisms (bacteria, plants, animals), trophic levels (decomposers, producers, consumers), organizational levels (from molecules to food webs), and cell structures (specific receptors).

Benthic invertebrate fauna can serve as a biological quality element in determinations of the ecological status of freshwater ecosystems. Many macrofaunal-based indices, such as the saprobic index, are routinely used to detect organic stressors (Hering *et al.*, 2004; Sandin and Hering, 2004). The SPEAR (SPEcies At Risk)-index utilizes the specific traits of macroinvertebrate species to identify the stress on freshwater systems posed by anthropogenic organic pollution (von der Ohe *et al.*, 2007), or specifically by pesticides ($SPEAR_{pesticides}$; Liess and von der Ohe, 2005; Knillmann *et al.*, 2018). To meet the demands of the WFD, the modular design of the macroinvertebrate-based *Perlodes* system has been used to evaluate the impact of different stressors (organic pollution, acidification, and general degradation) on the ecological quality of flowing waters (Meier *et al.*, 2006).

In efforts to improve the ecological status of aquatic ecosystems, the ability to demonstrate cause–effect relationships is crucial. In the case of a poor ecological status, suitable water management actions to achieve an improved ecological status can only be taken if the causes are known. As rivers, streams, and lakes are complex ecosystems, organisms are exposed to multiple stresses, both natural (natural toxins, food depletion, predation, competition) and anthropogenic (e.g. chemical contamination and hydro-morphological modifications). Biological indices that are able to identify specific stressors can help to accurately assess the ecological status, and, thus, to detect deficits and to show trends (Fig. 11.1).

The sediment compartment plays an important role in the achievement of a good chemical and ecological status of freshwater ecosystems (e.g. De Zwart *et al.*, 2009). Sediments provide a habitat for an abundant and diverse benthic fauna as well as important ecosystem functions and services (Apitz, 2012). However, they are also hotspots of chemical contamination, as they serve as both a sink and, when the necessary conditions for remobilization are met (e.g. flood events), a source of sedimentary pollutants (Hollert *et al.*, 2000). Ignoring this ability of sediments to store but also release contaminants can lead to erroneous conclusions in freshwater status assessments (Förstner, 2002; Heininger *et al.*, 2007).

Fine, cohesive sediments often contain high concentrations of chemicals, as their large adsorptive surface area enables the binding of (hydrophobic) compounds (Salomons and Förstner, 1984). Thus, the fauna of fine sediments is at particularly high risk of pollutant exposure. However, to date, biomonitoring programs have neglected typical faunal representatives of fine sediments, including meiofaunal organisms. Indices routinely used in assessments of the ecological quality of aquatic ecosystem

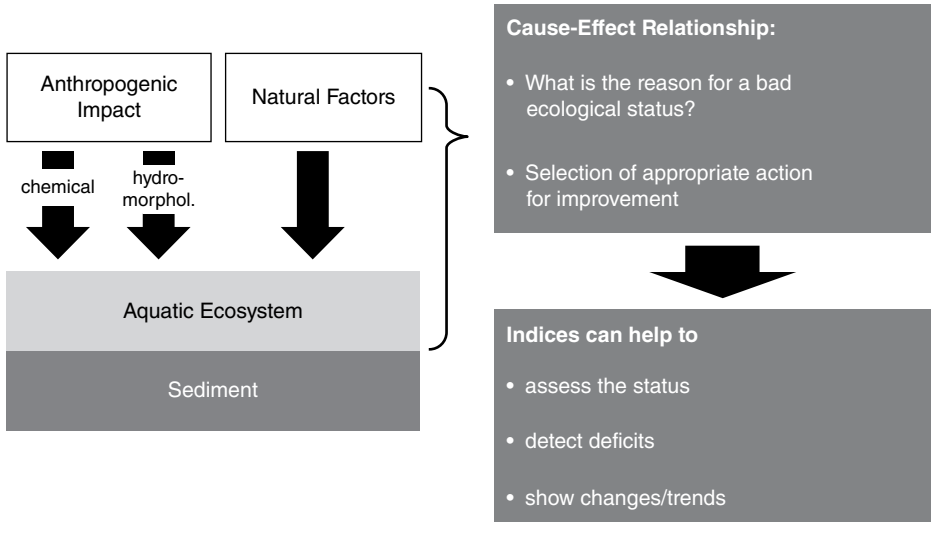

Fig. 11.1. The use of biotic indices to support cause–effect determinations in assessments of the ecological quality of aquatic ecosystems. (Author's own figure.)

are, as noted above, based on macro-invertebrates, but in fine sediments these comprise only a limited set of species (e.g. Wolfram *et al.*, 2010; Lopez-Doval *et al.*, 2010). By contrast, as discussed later in this chapter, meiofaunal organisms, including nematodes, are suitable bioindicators in ecotoxicological assessments that include fine sediments.

11.3 Suitability of Nematodes for Ecotoxicological Studies

The ecological relevance of nematodes in lentic and lotic habitats is described in several chapters in this book. However, nematodes are also of interest in pollution studies, whether conducted in the laboratory or in the field, and their use as bioindicators offers several advantages compared with commonly used macrobenthic organisms (Wilson and Kalkouli-Duarte, 2009) (Box 11.1).

Box 11.1. Features of nematodes beneficial for pollution studies. (Author's own box.)

- **High abundance**: Nematodes are the most abundant metazoans in soils and sediments (Bongers and Ferris, 1999; Traunspurger, 2000, 2002; van den Hoogen *et al*., 2019; Traunspurger *et al*., 2020, 2021), reaching mean densities in freshwater habitats of up to 6.7 million per m^2 (Michiels and Traunspurger, 2005). Due to these high densities, statistically valid determinations based on the acquisition of small, easily processed samples can be achieved, which is rarely the case with macrofauna.
- **Species richness**: Compared with macrofauna, the number of meiofaunal species belonging to a single taxon in a given habitat can be an order of magnitude higher. This diversity covers a broad range of physiological and feeding types and thus allows a balanced assessment of the effects of prevailing conditions on food webs and community processes.
- **Pervasiveness and tolerance**: Nematodes have been found in all environments examined so far, including extremes such as hot volcanic springs, anoxic sediments, sea ice, and polluted sediments. Their ubiquity reflects the ability of nematodes to tolerate a wide range of environmental stresses. However, nematodes also include sensitive, stress-intolerant species. These features of nematodes can be exploited to investigate changes that occur across a broad spectrum of stress conditions in a large number of different ecosystems.
- **Limited mobility:** Nematodes are of limited mobility as they are mostly confined to the sediments. As a result, they are continuously exposed to harmful materials that enter their environment. Although nematodes are able to move relatively rapidly within the sediment (e.g. Croll and Zullini, 1972; also see Chapters 3 and 5), their community structure is generally more directly related to the physico-chemical conditions of the habitat sampled than is the case with more mobile macrofaunal communities.
- **Generation time**: The life cycles of meiofaunal communities range from days to >2 years (most species have a generation time of 1–3 months; Traunspurger, 2002) such that the effects of both short-term and long-term influences can be discerned.
- **Innovative methods**: Given the increasing amount of nematode sequence data (especially for soil nematodes; Holterman *et al*., 2006; Schenk *et al*., 2017, 2020), DNA-barcode-based community analyses hold promise as a high-throughput tool (see Chapter 12).

11.4 Community-level Assessments

Assessments of *in situ* benthic communities or assemblages offer an ecologically more relevant approach than single-species bioassays, as they integrate the biotic and abiotic interactions of the benthos, including the presence of pollutants. However, before pollution-induced changes in nematode community structure can be recognized, the ecology of nematodes in unpolluted aquatic environments must be well understood. In their review of the ecology of nematodes in both unpolluted and polluted habitats, Ferris and Ferris (1979) emphasized the importance of obtaining baseline data on the natural variability within habitats. Since nematode communities are influenced by many different factors, regardless of sediment pollution, a clear demonstration of a cause–effect relationship with a single pollutant will always be challenging. Food availability, particle size, and organic carbon considerably influence nematode community structure (Vanreusel, 1991; Schroeder *et al.*, 2010; Vanaverbeke *et al.*, 2011; Fonseca *et al.*, 2014; Traunspurger *et al.*, 2020, 2021) and may therefore mask pollutant effects. For freshwater nematodes, nutrients were shown to influence community structure (Ristau *et al.*, 2012). In a meta-community analysis, in addition to strong temporal dynamics, environmental factors were found to be more important than spatial factors in structuring riverine nematode communities (Gansfort and Traunspurger, 2019). In an earlier study, the dependence of the community response of nematodes on the environmental conditions that the community normally experiences was demonstrated (Schratzberger and Warwick, 1999).

Therefore, assessments of pollutant effects require the use of indicator species or index systems that, more or less, specifically respond to chemical pollution. However, although total density measurements, diversity indices (e.g. number of species, Shannon index, evenness), and trophic indices (e.g. feeding type composition) are valuable tools with which to describe benthic communities and understand trophic interactions, none of them is unequivocally and unidirectionally influenced by a certain stressor. Species diversity, for instance, can decrease but also increase with habitat disturbance and is therefore not suitable for the detection of ecosystem stressors. Table 11.1 lists several statistical methods and indices applied to relate changes in nematode communities to chemical pollution.

A simple index recommended to detect polluted habitats was a determination of the percentage of Secernentea (Zullini, 1976), a class of nematodes referred to in modern phylogenetics as Rhabditida (De Ley and Blaxter, 2002). However, this index is unlikely to be specific for the detection of chemical pollution, as its strongest correlations are with the organic matter content (measured as dissolved organic carbon) in water. For soil nematodes, nematode-specific indices have been developed to identify chemical disturbances (see also Bongers and Ferris, 2009; Danovaro *et al.*, 2009), including maturity (MI, MI2-5; Bongers, 1990; Bongers and Ferris, 1999), structure (SI), enrichment (EI), and channel (CI)

Table 11.1. Methods applied for analyzing nematode communities. (Author's own table.)

Measures	Information	Reference	Example
Univariate methods			
Species richness: S	Number of species in a defined sampling unit	Magurran (1988)	Den Besten *et al.* (2000)
Shannon index (H′) and Pielou's evenness (J)	Distribution of species abundances: rare species are more heavily weighted; a higher index indicates greater diversity	Pielou (1969); Shannon and Weaver (1949)	Höss *et al.* (2004)
k-dominance curves	Distribution of species abundances: the percentage of cumulative abundance (k-dominance) is plotted against the species rank (k) of two nematode assemblages, A and B, with B more diverse than A if the curve is consistently below or touches that of A	Lambshead *et al.* (1983)	Danovaro *et al.* (1995)
Feeding types	Distribution of feeding types	Jensen (1987); Wieser (1953)	Korthals *et al.* (1996)
Maturity index (MI)	Distribution of the different life history strategies of nematodes: based on the ratio of colonizers to persisters. In the 1–5 scale, a low index indicates disturbance	Bongers (1990)	Korthals *et al.* (1996)
% Secernentea or ratio of Secernentea/ Adenophorea (S/A)	High proportion of nematodes belonging to the subclass Secernentea indicates pollution	Zullini (1976)	Beier and Traunspurger (2001)
Egg index	(log egg size) – (log expected egg size): a negative value indicates organic pollution	Zullini and Pagani (1989)	Zullini and Pagani (1989)
NemaSPEAR[%]-index	Percentage of sensitive species at risk (NemaSPEAR); a low value indicates poor ecological quality	Höss *et al.* (2011, 2017)	Sonne *et al.* (2018)
Multivariate methods			
Cluster analysis	Clustering based on similarities in taxonomic composition	Clarke (1993)	Trett *et al.* (2000)
Multidimensional scaling ordination (MDS)	Biplots with data points based on similarities in taxonomic composition; the distance between data points indicates a similar taxonomic composition	Clarke (1993)	Austen and McEvoy (1997)
Canonical correspondence analysis	Relates taxonomic composition to environmental factors	Ter Braak (1994)	Fiscus and Neher (2002)
Principal response curves	Redundancy analysis based on taxonomic composition, including factor time; useful for experimental studies	Van den Brink and Ter Braak (1999)	Den Besten and Van den Brink (2005)

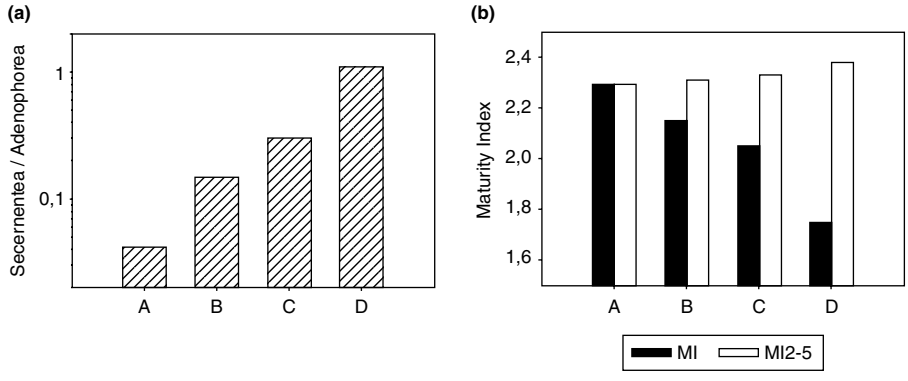

Fig. 11.2. (**a**) Ratios of Secernentea to Adenophorea and (**b**) maturity indices (MI, including *cp-1* nematodes (= enrichment opportunists); MI2-5, excluding *cp-1* nematodes) for nematode communities from the sediments of two unpolluted (A, B) and two polluted (C, D) sites in small streams. (Data taken from Beier and Traunspurger (2001).)

indices (Ferris *et al.*, 2001), all of which use the informative value of certain functional traits of nematode taxa (life history, feeding type) to assess soil health (Table 11.1). Studies in which the MI was used to assess the disturbance of nematodes in freshwater habitats yielded conflicting results that question the suitability of this approach. Den Besten *et al.* (2000) were able to use the MI to demonstrate the success of a remediation action in the river Rhine (MI at remediated and non-remediated sites: 2.5–2.8 and 2.1–2.6, respectively), but it could not be used effectively in the river Meuse. In two small German streams where the nematode communities of reference sites were compared with those of sites impacted by sewage effluents, the MI was sensitive to the effluent's impact (Fig. 11.2) but only with the inclusion of *c-p* 1 nematodes (colonizer-persister scale according to Bongers, 1990), known indicators of organic enrichment independent of chemical contamination (Beier and Traunspurger, 2001). If *c-p* 1 nematodes were omitted from the analysis, reference and polluted sites could not be distinguished using the MI (MI2-5). Heininger *et al.* (2007) observed that in large German rivers nematode groups with high *c-p* values (Tobrilidae, Mononchidae) were more abundant at highly contaminated sites, a contradiction of the theoretical basis of the MI.

11.4.1 NemaSPEAR[%]-index

With the goal of obtaining a robust nematode-based index to assess specifically chemically induced changes in benthic communities, Höss *et al.* (2011) exploited a large data set collected from 103 sites at large German rivers from six river basins (203 samples) and consisting of benthic nematode species data as well as physico-chemical data (grain size, organic matter, metals, and organic pollutants) from the same sites. These data pairs allowed the different nematode species to be classified as 'species at

risk' (NemaSPEAR: species mainly occurring at sites with low-level pollution) and 'species not at risk' (NemaSPE$_{not}$AR: species whose occurrence was not related to chemical pollution but also species mainly occurring at polluted sites). The classification was based on a multivariate correlation of the nematode species composition with the toxic potential of the various samples (Fig. 11.3).

The NemaSPEAR[%]-index was initially separated into metal- and organics-specific indices (NemaSPEAR[%]$_{metal}$; NemaSPEAR[%]$_{organic}$; Höss *et al.*, 2011), but it was revised after it was shown that this distinction was unnecessary, as the contamination patterns seen in environmental samples are rarely clearly dominated by either metals or organic chemicals (Höss *et al.*, 2017). Therefore, a general NemaSPEAR[%]-index was proposed. Moreover, it could be shown that the NemaSPEAR[%]-index was also applicable at the taxonomic level of genera, which increased the overall acceptance of a taxonomy-based index (Höss *et al.*, 2017). All NemaSPEAR-taxa (species and genera) are listed in Table 11.2.

The NemaSPEAR[%]-index is strongly related to the toxic potential of environmental samples. When the index values are plotted against the mean PEC-Q (probable effect concentration quotients) calculated according to the sediment quality guidelines defined by de Deckere *et al.* (2011), a sigmoidal curve can be fitted to the data (Fig. 11.4; Höss *et al.*, 2017). This suggests that NemaSPEAR[%] values decrease dose-dependently with increasing sediment contamination. Data from microcosm experiments in which nematode communities were exposed to single contaminants confirmed the dose-dependent response of the NemaSPEAR[%] with increasing contaminant concentrations, supporting the potential of the index to detect chemical pollution (Haegerbaeumer *et al.*, 2016, 2018; Höss *et al.*, 2017).

To evaluate the ecological status of a freshwater ecosystem with the NemaSPEAR[%], class boundaries must first be defined for the indices to allow a good ecological status to be distinguished from a bad one. Based on an approach developed for the macrofauna-based SPEAR[%]-index (Von der Ohe *et al.*, 2007), Höss *et al.* (2017) used the variation of the NemaSPEAR[%] among reference sites characterized by low-level pollution to define the threshold between an acceptable and unacceptable quality. Therefore, the standard deviation (SD) of the mean NemaSPEAR[%] value over all reference sites was taken as measure of the 'reference variation', with two standard deviations applied as the class size indicating a good ecological status. Höss *et al.* (2017) also defined class boundaries for the NemaSPEAR[%]-index, which could thus be used to distinguish sites with a high (NemaSPEAR ≥54%), good (30% to <54%), moderate (20% to <30%), poor (10% to <20%), or bad (<10%) ecological status (Fig. 11.4).

11.4.2 Case studies using NemaSPEAR

Because the NemaSPEAR[%]-index is a relatively new ecological tool for assessing the risk of chemicals in sediments, few studies in which it was

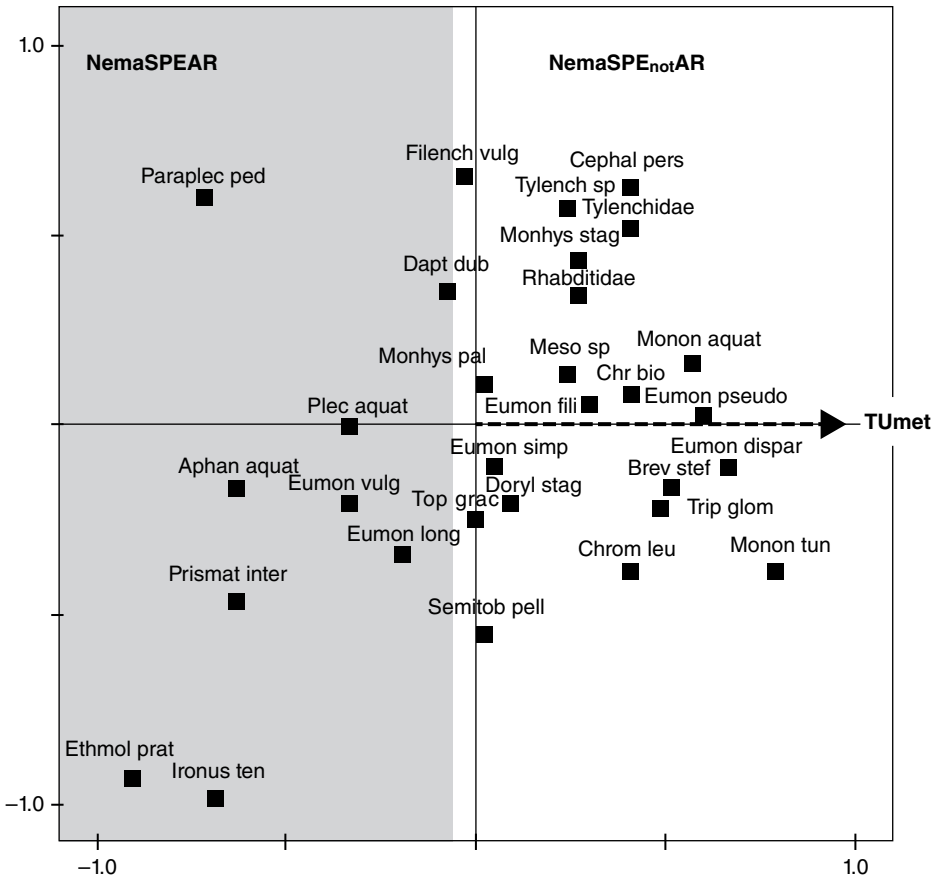

Fig. 11.3. Canonical correspondence analysis (CCA) of nematode species composition (log (x + 1)-transformed relative abundance of species) from 155 samples (training data set): biplot (species and environmental variable) of CCA with only one co-variable: TU_{met} = log $maxTU_{D.magna\_metal}$ (TU of metal x = porewater concentration of metal x/$LC50_{Daphnia\ magna}$ of metal x; maxTU = maximal TU among all metals); for clarity, only the 30 most frequently occurring species are displayed. Aphan aquat, *Aphanolaimus aquaticus*; Brev stef, *Brevitobrilus stefanskii*; Cephal pers, *Cephalobus persegnis*; Chr bio, *Chromadorina bioculata*; Chrom leu, *Chromadorita leuckarti*; Dapt dub, *Daptonema dubium*; Dory stag, *Dorylaimus stagnalis*; Ethmol prat, *Ethmolaimus pratensis*; Eumon dispar, *Eumonhystera dispar*; Eumon fili, *E. filiformis*; Eumon long, *E. longicaudatula*; Eumon pseud, *E. pseudobulbosa*; Eumon simp, *E. simplex*; Eumon vulg, *E. vulgaris*; Filench vulg, *Filenchus vulgaris*; Ironus ten, *Ironus tenuicaudatus*; Meso sp, *Mesodorylaimus* sp.; Monhys pal, *Monhystera paludicola*; Monhys stag, *M. stagnalis*; Monon aquat, *Mononchus aquaticus*; Monon tun, *M. tunbridgensis*; Paraplec ped, *Paraplectonema pedunculatum*; Plec aquat, *Plectus aquatilis*; Prismat inter, *Prismatolaimus intermedius*; Rhabditidae, Rhabditidae gen. sp.; Sem pell, *Semitobrilus pellucidus*; Tob grac, *Tobrilus gracilis*; Tripyla glom, *Tripyla glomerans*; Tylenchidae, Tylenchidae gen. sp.; Tylench sp, *Tylenchus* sp. (From Höss *et al*. (2011), with permission of Elsevier.)

Table 11.2. Nematode species and genera defined as species at risk (NemaSPEAR) by Höss *et al*. (2017). (Author's own table.)

Species			Genus	
Achromadora ruricola	*Eumonhystera barbata*	*Paraplectonema pedunculatum*	*Achromadora*	*Hemicycliophora*
Achromadora terricola	*Eumonhystera longicaudatula*	*Plectus aquatilis*	*Aglenchus*	*Hirschmaniella*
Aglenchus agricola	*Eumonhystera simplex*	*Plectus cirratus*	*Alaimus*	*Hofmaenneria*
Alaimus meyli	*Eumonhystera vulgaris*	*Plectus opisthocirculus*	*Amphidelus*	*Ironus*
Alaimus parvus	*Helicotylenchus pseudorobustus*	*Plectus rhizophilus*	*Aphanolaimus*	*Monhystrella*
Alaimus primitivus	*Hemicycliophora typica*	*Prismatolaimus tenuicaudatus*	*Aphelenchus*	*Paramphidelus*
Amphidelus cf. *elegans*	*Hirschmaniella gracilis*	*Prismatolaimus dolichurus*	*Aporcelaimellus*	*Paraphanolaimus*
Aphanolaimus aquaticus	*Hofmaenneria brachystoma*	*Prismatolaimus intermedius*	*Bastiania*	*Paraplectonema*
Aporcelaimellus obtusicaudatus	*Ironus ignavus*	*Prodesmodora circulata*	*Cephalenchus*	*Pratylenchus*
Coslenchus costatus	*Ironus longicaudatus*	*Punctodora dudichi*	*Coslenchus*	*Prismatolaimus*
Cuticularia oxycerca	*Ironus tenuicaudatus*	*Punctodora ratzeburgensis*	*Crassolabium*	*Prodesmodora*
Cylindrolaimus communis	*Mesodorylaimus conurus*	*Rhabditis gracilicauda*	*Criconema*	*Prodorylaimus*
Epidorylaimus agilis	*Mesodorylaimus subtiliformis*	*Rhabdolaimus terrestris*	*Cylindrolaimus*	*Punctodora*
Epitobrilus medius	*Monhystera lemani*	*Semitobrilus pellucidus*	*Diplogasteritus*	*Rhabditis*
Epitobrilus steineri	*Monhystrella paramacrura*	*Theristus agilis*	*Epidorylaimus*	*Rhabdolaimus*
Ethmolaimus pratensis	*Mononchus truncatus*	*Theristus vesentiniae*	*Epitobrilus*	*Semitobrilus*
Eucephalobus oxyuroides	*Neotobrilus longus*	*Thornia propinqua*	*Ethmolaimus*	*Theristus*
Eudorylaimus acuticauda	*Panagrolaimus* cf. *thienemanni*	*Trischistoma monhystera*	*Eudorylaimus*	*Trischistoma*
Eudorylaimus carteri	*Paramphidelus dolichurus*		(*Eumonhystera*)[a]	
Eumonhystera andrassyi	*Paraphanolaimus anisitsi*		*Fictor*	

[a]*Eumonhystera* is actually classified as NemaSPE$_{not}$AR; however, as there are several *Eumonhystera* species classified as NemaSPEAR the classification on genus level is ambiguous; therefore, this genus should be omitted from NemaSPEAR$_{genus}$ calculation.

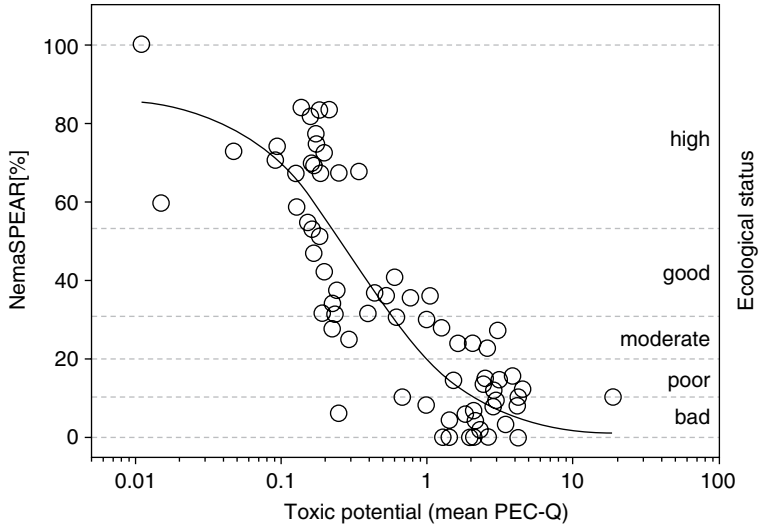

Fig. 11.4. Non-linear correlation of the NemaSPEAR[%]-index at the species level with the toxic potential of sediment samples (mean PEC-Qs based on sediment quality guidelines from de Deckere *et al*., 2011). PEC-Q, quotient of measured sediment concentrations and probable effect concentration (PEC; 'consensus 2'). The data were fitted using a sigmoidal logistic model (Sigma plot; Systat) ($r^2 = 0.74$, $P < 0.001$). (Modified from Höss *et al*. (2017), with permission of Elsevier.)

used have been published so far. Nevertheless, in this section we provide an overview of the results of case studies that used the index to investigate nematode communities. The studies analyzed the nematode communities in the sediments of small and large streams impacted by pesticides, metals, or groundwater plumes.

Wolfram *et al*. (2012) investigated the impact of chemical contamination on the benthic communities of rivers in three different European catchments: Elbe (Czech Republic), Scheldt (Belgium), and Llobregat (Spain). A weight-of-evidence (WoE) approach was applied to evaluate the link between chemical contamination and biological impacts, with various ecotoxicological tools representing different lines of evidence (LoE): (i) chemical sediment concentrations were translated into toxic potentials, using the toxic unit (TU) and the msPAF (multiple-substance potentially affected fraction) approaches. (ii) Direct sediment toxicity was assessed with sediment toxicity tests using test organisms from different trophic levels (bacteria, nematodes, oligochaetes, snails, fish; Tuikka *et al*., 2011). (iii) The structure of benthic communities was analyzed to evaluate the *in situ* response of the benthic biota to chemical pollution. As macro-invertebrates, which are routinely used as bioindicators (Hering *et al*., 2004), showed only low diversity in the investigated fine sediments (mainly chironomids and tubificids), meiofauna (nematodes) were included, as their densities and species diversity at the study sites were considerably higher than those of macro-invertebrates (Lopez-Doval

et al., 2010; Wolfram *et al.*, 2010). The macro-invertebrate and nematode communities could be clearly distinguished between the various catchments but also between sites characterized by lower vs. higher chemical contamination. Moreover, the results of the NemaSPEAR[%]-index were comparable with those of the macro-invertebrate-based SPEAR[%]-index (Wolfram *et al.*, 2012). However, although both indices showed relatively low values for reference and polluted sites, a finding supported by the relatively high toxic potentials of some of the reference sites (Wolfram *et al.*, 2012), at the reference sites the NemaSPEAR[%] values were considerably higher than the SPEAR[%] values, indicative of a better suitability of the nematode-based index in fine sediments (Fig. 11.5). Within the WoE approach, the different chemical and eco(toxico)logical LoEs yielded conflicting indications, with the NemaSPEAR[%]-index contributing valuable information to final classification of the sites.

In a recent study, Brüchner-Hüttemann *et al.* (2021) compared the NemaSPEAR[%]-index with macro-invertebrate-based indices in samples collected from various rivers and showed a clear pollution gradient, with toxic potentials ranging from very low to high. In that study, macro-invertebrates were sampled from multiple habitats rather than only from the bed sediments, where nematodes were sampled. This approach allowed a better comparison of the nematode-based data with the overall

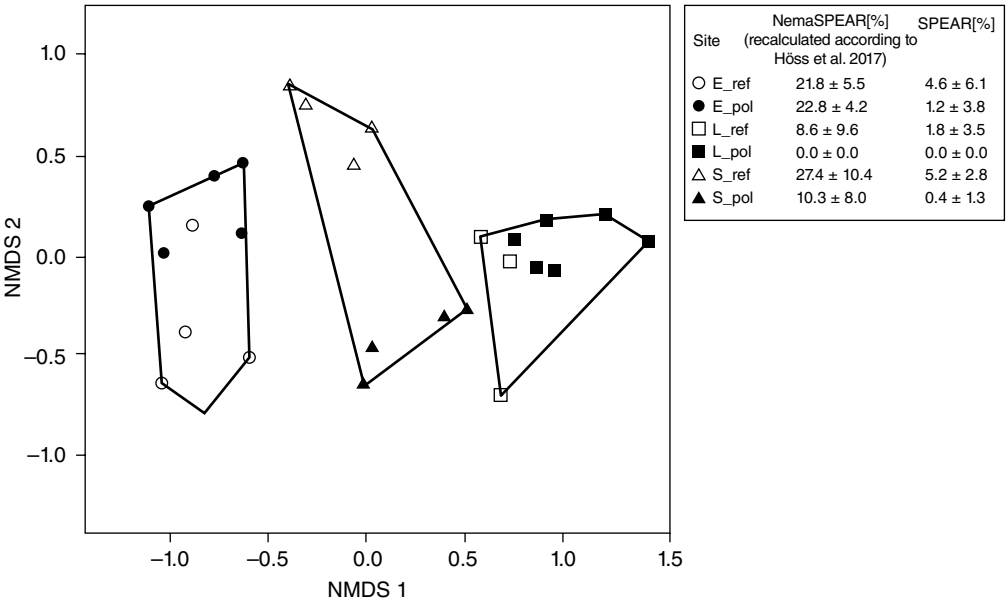

Fig. 11.5. Two-dimensional plot from the non-metric multidimensional scaling (NMDS) ordination of nematode communities. River basins are grouped in convex hulls. Open symbols, unpolluted sites; full symbols, polluted sites; E, Elbe (Czech Republic); L, Llobregat/Anoja (Spain); S, Scheldt (Belgium). (Modified from Wolfram *et al*. (2012) with permission of Elsevier; note that the NemaSPEAR[%]-index values were recalculated according to the classification of Höss *et al*. (2017) and deviate from the values listed in table 5 of Wolfram *et al*. (2012).)

ecological status of the sites (as evaluated by *Perlodes*; Meier *et al.*, 2006). The NemaSPEAR[%] correlated significantly with the toxic potential of the sediments when the mean PEC-Qs based on the sediment quality guidelines according to de Deckere *et al.* (2011) were used (Fig. 11.6a). Moreover, the index appeared to be robust against the seasonal variations in nematode communities, showing no significant differences between the seasons (Brüchner-Hüttemann *et al.*, 2021). However, agreement between the macro-invertebrate-based indices and the NemaSPEAR[%]-index was limited (Fig. 11.6b), perhaps due to the responses of certain indices to specific chemicals, such as pesticides (in which case, for example, the $SPEAR_{pesticide}$ indicated a poor to bad status even at 'clean'

(a)

Site	FB	Ve	Ör	RM	Cu	Hi	Lu
Mean PEC-Q	0.01	0.01	0.02	0.34	0.71	1.20	7.71
Perlodes	g	m	g	b	p	b	b
SPEAR[%]	59.2 (h)	49.6 (h)	64.9 (h)	27.8 (m)	61.0 (h)	63.9 (h)	29.3 (g)
$SPEAR_{pesticide}$	0.98 (h)	0.61 (p)	0.26 (b)	0.33 (b)	0.57 (m)	0.55 (p)	0.39 (p)
NemaSPEAR[%]	51.8 (g)	42.8 (g)	70.9 (h)	31.8 (g)	31.4 (g)	26.7 (m)	16.5 (p)

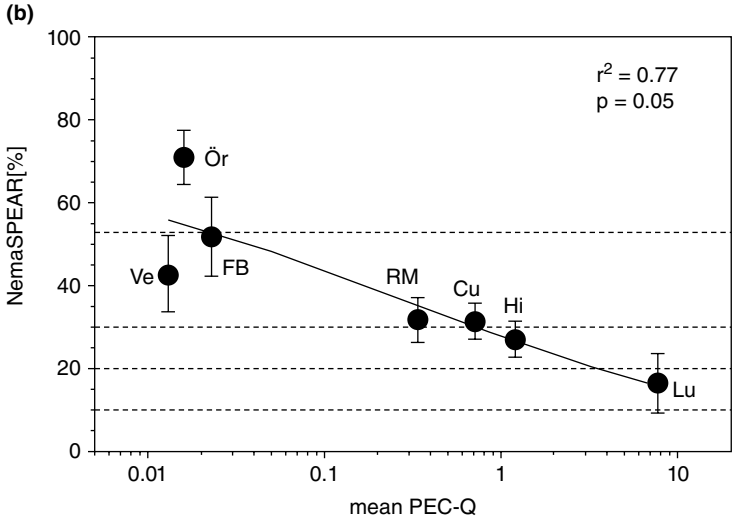

Fig. 11.6. (**a**) Comparison of various measures characterizing the chemical and ecological status of sediments sampled from seven sites showing a pollution gradient: toxic potentials (PEC-Q; probable effect concentration quotient: quotient of a measured sediment concentration and the respective sediment quality guideline according to de Deckere *et al.*, 2011), NemaSPEAR[%], *Perlodes*, SPEAR[%], and $SPEAR_{pesticide}$. Letters refer to ecological status: h = high, g = good, m = moderate, p = poor, and b = bad. (**b**) Non-linear regression of the NemaSPEAR[%]-index (mean ± standard deviation) with mean PEC-Q values. FB, Furlbach; Ve, Veerse; Ör, Örtze; RM, Rischmühlenschleuse; Cu, Cumlosen; Hi, Hitzacker; Lu, Luppe. (Modified from Brüchner-Hüttemann *et al.* (2021), with permission of Elsevier.)

sites), that might not be included in the sediment quality guidelines or detected by endobenthic organisms. Alternatively, nematodes, as endobenthic organisms, might have responded to toxicants confined to the sediments such that epibenthic macro-invertebrates were simply not exposed (e.g. SPEAR indicated a good status even at polluted sites). Therefore, the definition of ecological status should draw on several biotic indices, including those based on strictly endobenthic meiofaunal organisms such as nematodes.

Sonne *et al*. (2018) showed that the inclusion of meiofauna in a monitoring program provides additional information with which to link multiple chemical stressors with ecological impacts. The meio-invertebrate community could be linked to the biogeochemical water quality in the hyporheic zone of a Danish stream, which was not possible for the less-dominant macro-invertebrates. This was probably due to higher exposure concentrations in the deeper layers of the bed sediment than in the surface water. Within that study, the NemaSPEAR[%]$_{genus}$ contributed important information that allowed chemical stress to be distinguished from other factors (e.g. vegetation; physical streambed parameters) that cause deviations in nematode genus composition. Thus, using field evidence, Sonne *et al*. (2018) demonstrated the importance of including meio-invertebrates to obtain a holistic understanding of the potential impacts of chemicals in groundwater.

11.5 Conclusions and Perspectives

The potential of nematodes to serve as bioindicators of the quality of freshwater environments has been demonstrated in several studies. The high abundance and diversity of nematodes together with their short generation times recommend the inclusion of these meiofaunal organisms in routine biomonitoring programs. An advantage of the NemaSPEAR[%]-index, a recently developed biomonitoring tool for assessing the ecological risk of contaminated freshwater sediments (e.g. in the context of the EU-WFD), is that it can be used in combination with DNA-based taxonomic methods (Schenk *et al*., 2020). The negligible seasonal variation of the index is among the features that make it a robust metric able to specifically identify chemical stress in benthic communities. Moreover, the class boundaries defined in the NemaSPEAR[%]-index allow sediments to be categorized according to their ecological status. As nematodes are abundant representatives of the endobenthic habitat of fine sediments, they fill the gap in chemical risk assessments left by the paucity of macro-invertebrates at these sites. The lack of consistent agreement between the NemaSPEAR[%]-index and commonly used macrofauna-based indices demonstrates the added value of this nematode-based measure in providing non-redundant information that can contribute to the protection of benthic fauna.

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12 Case Studies with Nematodes from the Individual to Ecosystem Level

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12.1 Introduction

Organisms smaller than 2 mm in size are ideal candidates for laboratory and field experiments with a theoretical focus. In this chapter, we illustrate this point by drawing on recently published works in which studies of nematodes have informed theories within population and community ecology. Case studies examining the following are presented:

1. Life cycle experiments (individual level).
2. The interactions of two nematode species – competition experiments (population level).
3. Nematode community-based assessments of sediment quality (community level).
4. Nematodes in a detritus-based food web model (food web level).

12.2 Case Study 1: Life Cycle Experiments Can Reveal the Life History Traits of Nematodes and the Responses of Nematode Individuals and Cohorts to Contamination

12.2.1 What is a life cycle experiment and what information can it provide?

Life cycle studies use experimental set-ups that allow the monitoring and accurate measurement of life history traits (LHTs) considering all stages of a test organism's life, from birth until death, and at both the individual and the cohort level. In the case of nematodes, life cycle studies can be performed to assess general biological features, such as lifespan, reproduction, and fecundity (Muschiol and Traunspurger, 2007), but also the impacts of environmental stressors (e.g. chemicals) on LHTs, which can be simultaneously investigated at the desired degree of accuracy (Muschiol *et al.*, 2009). Moreover, in contrast to standard toxicity tests, mostly only providing a snapshot analysis of certain toxicity endpoints (e.g. ISO, 2010), life cycle experiments enable continuous investigations of the effects of a toxicant on both reproductive and survival traits, in individuals and across cohorts (Jager *et al.*, 2005, 2014; Goussen *et al.*, 2015). While this flexibility is helpful to better understand population-level responses to contaminants in the field, it is a prerequisite to quantify the response of individual LHTs to contamination (Kreuzinger-Janik *et al.*, 2017). For this purpose, measures of reproduction, survival, and growth (Table 12.1) are generally the most important and sensitive indicators in experiments examining the impact of prolonged contaminant exposure (Harada *et al.*, 2007). These measures are encompassed in several LHTs, including total reproduction, intrinsic population growth rate, age at sexual maturity, age-specific fecundities/mortalities, mean and maximum lifespan, adult body sizes, and/or juvenile growth rates (e.g. Muschiol *et al.*, 2009; see also Table 12.1).

Table 12.1. The life history traits recorded in a nematode life cycle experiment. (Author's own table.)

Abbreviation (unit)	Life history trait	Equation	Definition
T_{hatch} **(h)**	Hatching time	$T_{hatch} = \frac{N_{eggs}}{N_{juveniles} + N_{eggs}} * T$	Time from egg deposition to nematode hatching
Mean lifespan (days)	Mean lifespan	–	Average age at death of all tested individuals
Max. lifespan (days)	Maximum lifespan	–	Age at death of the longest-lived test individual; used as a species-specific aging parameter
Age_{min} **(h)**	Mean age at first oviposition	–	Start of reproduction
Age_{max} **(h)**	Mean age at maximum daily reproduction	–	Maximum of reproduction
Age_{end} **(h)**	Mean age at last oviposition	–	End of reproduction
Eggs/nematode	Laid eggs per nematode	–	Mean number of eggs laid per nematode
Total eggs	Total laid eggs	–	Total number of eggs laid by all nematodes
Reproductive period (days)	Reproductive period	–	Length of the reproductive period
TFR	Total fertility rate	$TFR = \sum_{x=0}^{d} m_x$	Theoretical number of offspring an individual in a population would produce during its maximum lifespan without any age-specific mortality
R_0	Net reproductive rate	$R_0 = \sum_{x=0}^{d} l_x m_x$	Average number of offspring an individual in a population will produce during its lifetime
r_m **(per day)**	Intrinsic rate of natural increase	$\sum_{x=0}^{d} e^{-r_m x} l_x m_x = 1$	Growth rate of a population with a stable age distribution and growing exponentially in an unlimited environment
PDT **(h)**	Population doubling time	$PDT = \frac{\ln 2}{r_m} * 24\,\text{h}$	Time needed for a growing population to double its number of individuals
T_0 **(h)**	Cohort generation time	$T_0 = \frac{\sum x l_x m_x}{R_0} * 24\,\text{h}$	Mean age at reproduction of a cohort of females; alternative measures of generation time according to Charlesworth (1980)

Continued

Table 12.1. Continued.

Abbreviation (unit)	Life history trait	Equation	Definition
T_1 **(h)**	Mean generation time	$T_1 = \frac{\sum \ln R_0}{r_m} * 24\,\text{h}$	Time needed for a population growing at a constant rate of r_m to increase by a factor of R_0; alternative measures of generation time according to Charlesworth (1980)

x = Time (days); l_x = age-specific survival probability (proportion of the original number of test individuals surviving to the next age class x); m_x = age-specific fecundity (mean number of all fertile eggs laid in a certain time interval or of juveniles of an age class x hatched from those eggs); T = experimental time.

12.2.2 Presentation of case study 1

Many methods can be used to culture nematodes and record their lifespan and fecundity (e.g. Lewis and Fleming, 1995; Muschiol *et al.*, 2009). Despite the numerous population biology and ecotoxicology studies evaluating the LHTs of *Caenorhabditis elegans* (Muschiol *et al.*, 2009), differences in their protocol designs (e.g. cultivation temperature, solid vs. liquid media, food quality and quantity, data acquisition methods) have hampered comparisons of the data obtained in different studies.

The hanging drop method developed by Muschiol and Traunspurger (2007) uses semi-liquid droplets (ca. 10 µl) of Nematode Growth Gelrite® (NGG) as the culture medium and *Escherichia coli* as the food source. Nematodes are placed individually in the droplets, which hang from the underside of the lid of a culture plate (Fig. 12.1) (Kammenga *et al.*, 1996).

The individual nematodes are then followed throughout their lifespan. The advantages of the hanging drop method are: (i) it allows unobstructed observation of the nematodes and the recording of the LHTs of individuals of similar age with reliable accuracy (Muschiol *et al.*, 2009), and (ii) it avoids the limitations common to batch cultures, such as intraspecific interference and the uneven distribution of food in solid medium (Kreuzinger-Janik *et al.*, 2017).

To reduce the inter-individual variability of the recorded LHTs, the droplets are inoculated with individuals from a cohort of age-synchronized first-stage juveniles that hatched within a short time. Synchronous cohorts of juveniles can be obtained from eggs that have been axenized following Stiernagle (2006) by treating nematode culture plates with hypochlorite solution, which kills all individuals not protected by the egg shell. Each of the nematodes is transferred to a new droplet containing a bacterial food supply every 6–24 h (depending on the life stage) to prevent food depletion and to allow the eggs that were laid in the previous droplet to be counted. Regardless of whether those eggs are fertile or sterile, the droplet is incubated for another 24 h after which both the eggs (sterile) and hatched offspring (fertile) are counted, and body sizes are measured. During the experiment, survival and fecundity traits are recorded.

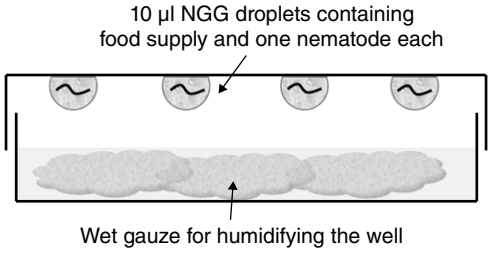


Fig. 12.1. Schematic experimental set-up of the hanging drop method. (Author's own figure.)

The experiment runs until the last adult nematode has died. The recorded survival and reproductive traits (Fig. 12.2) are then used to compile complete and high-resolution life tables and fecundity schedules that provide information on the nematode's age at sexual maturity, mean and maximum lifespan, net reproductive rate (R_0), total fertility rate (TFR), generation time, population doubling time, and intrinsic rate of increase (r_m) (e.g. Muschiol and Traunspurger, 2007; Muschiol *et al.*, 2009; Fueser *et al.*, 2018).

Studies using the hanging drop method have measured standard LHTs for free-living nematode species, such as *Caenorhabditis briggsae*, *C. elegans*, *Plectus acuminatus*, *Panagrolaimus* spp., and *Poikilolaimus* sp. (Muschiol and Traunspurger, 2007; Muschiol *et al.*, 2009; Lancaster *et al.*, 2012; Ayub *et al.*, 2013; Brinke *et al.*, 2013; Fueser *et al.*, 2018), as well as entomopathogenic species (Gilarte *et al.*, 2015; Addis *et al.*, 2016a; Addis *et al.*, 2016b). Their results revealed species-specific differences in lifespan, population growth dynamics, and reproduction.

Additionally, environmental factors, such as temperature (Ayub *et al.*, 2013), food concentration (e.g. Addis *et al.*, 2016a), and toxic stress (e.g. Brinke *et al.*, 2013; Fueser *et al.*, 2018) were shown to alter the life cycle of an individual *a posteriori*. For instance, the chronic exposure of *C. elegans* to copper (Cu) at a slightly toxic concentration (0.5 mg/l; 20% inhibition of reproduction at 96 h exposure (EC_{20}); ISO 10872), significantly modified the LHTs of this nematode species (Fig. 12.3; Table 12.2). The Cu treatment considerably impacted lifespan and fertility of *C. elegans*, as well as the somatic growth of their offspring. Compared with the controls, the hatching time of the exposed nematodes was significantly delayed (by about 18%), the mean lifespan was significantly shorter (10.8 days), the last individual died 10 days earlier (Fig. 12.3), and the first oviposition occurred about 8 h earlier (Table 12.2). Therefore, Cu at low concentrations not only shortened the length of the juvenile period, by decreasing $Age_{min,}$ but also considerably increased the mortality of individuals during the reproductive period. Moreover, hatched juveniles from Cu-exposed nematodes were significantly smaller (3–11%).

12.2.3 Possibilities and limitations

The population growth capacity depends on the fecundity of the population, the generation time, and the survival of adults and juveniles (Wharton, 1986). These parameters form the basis of LHT analyses in life

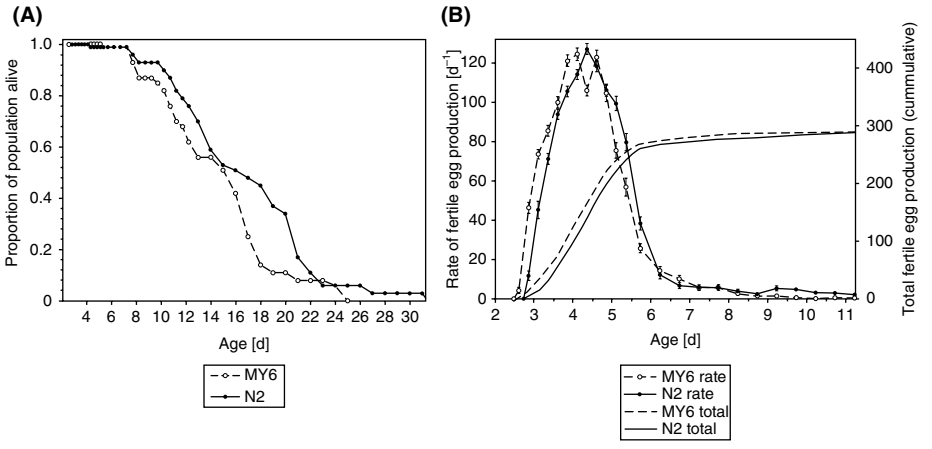


Fig. 12.2. Hermaphrodite survivorship curves of *Caenorhabditis elegans* strains MY6 and N2 (**A**). Lines represent the fraction of the population surviving at the given interval after egg deposition (age $x = 0$). Note that mortality among juveniles and young adults was virtually zero. Population sizes were $n = 72$ (MY6) and $n = 69$ (N2) until age 5.7 days and $n = 36$ (MY6 and N2) thereafter. Fecundities of *C. elegans* strains MY6 and N2 (**B**). Population sizes were $n = 72$ (MY6) and $n = 69$ (N2) until age 5.7 days and $n = 36$ (MY6 and N2) thereafter. (From Muschiol *et al.* (2009) with permission from BMC.)

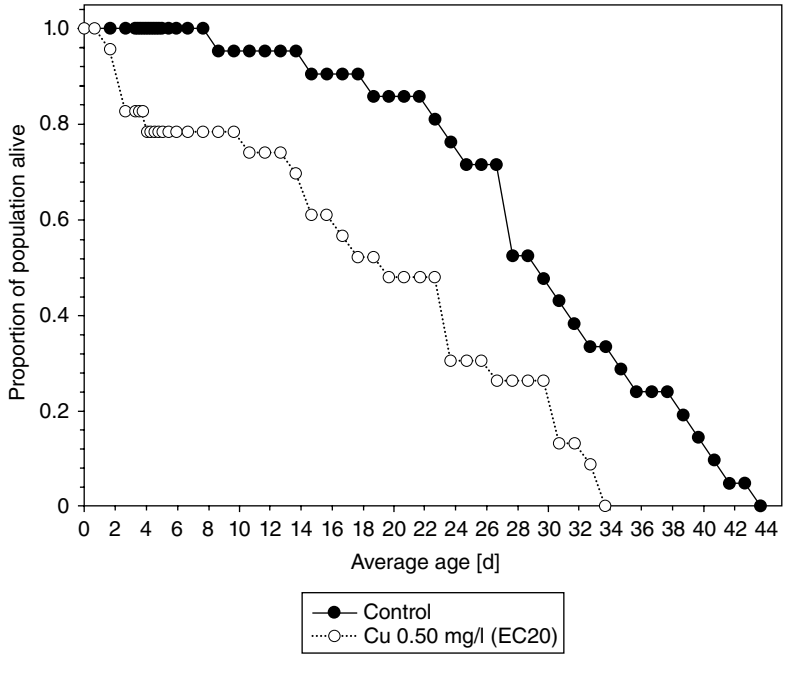


Fig. 12.3. Survivorship curves of *Caenorhabditis elegans* exposed to Cu at EC_{20}. Proportion of nematodes alive at the indicated average age (days). SigmaPlot 12.0 (Systat Software Inc.). (Modified from Fueser *et al*. (2018) with permission from Elsevier.)

Table 12.2. Recorded life history traits of unexposed and Cu-exposed *Caenorhabditis elegans*. (Modified from Fueser *et al*. (2018) with permission of Elsevier.)

	Life cycle experiment		
Parameter	Control	Cu 0.50 mg/l (EC_{20})	Test statistics
Valid individuals	21	23	–
Hatching time (h)	5.9 ± 2.1	7.2 ± 1.9	$t(18) = -1.436$; $P = 0.033$
Mean lifespan (days)	29.7 ± 8.8	18.9 ± 10.7	$t(42) = -3.538$; $P < 0.001$
Max. lifespan (days)	43.7	33.7	–
***Age*$_{min}$ (h)**	89.0 ± 11.7	80.9 ± 2.8	$U = 115.0$; $P = 0.005$
***Age*$_{max}$ (h)**	114.7 ± 12.4	109.2 ± 7.7	$U = 137.0$; $P = 0.118$
***Age*$_{end}$ (h)**	325.7 ± 57.3	316.8 ± 71.0	$U = 167.5$; $P = 0.385$
Eggs/nematode	140.8 ± 40.0	117.8 ± 73.1	$U = 230.0$; $P = 0.796$
Total eggs	2976	2890	–
Reproductive period (days)	9.82 ± 2.45	9.83 ± 2.97	$U = 184.0$; $P = 0.683$
TFR	141.5	158.4	–
R_0	138.7	123.1	–
r_m (per day)	0.858	0.908	–
***PDT* (h)**	19.39	18.32	–
T_0 (h)	183.68	169.45	–
T_1 (h)	137.97	127.22	–

Age$_{min}$ = mean age at first oviposition; *Age*$_{max}$ = mean age at maximum rate of oviposition; *Age*$_{end}$ = mean age at last oviposition; *TFR* = total fertility rate; R_0 = net reproductive rate; r_m = intrinsic rate of natural increase; *PDT* = population doubling time; T_0, T_1 = alternative calculations of the generation time.

cycle experiments and therefore of the calculations employed to determine the intrinsic growth rate of the population (r_m). In nature, where resources may be limiting, the LHTs of a species reflect the compromise made between maximizing population fitness and resource allocation for individual survival (Kreuzinger-Janik *et al.*, 2017). The LHT data of cohorts collected in a life cycle experiment allow population responses to be modeled and the results to be extrapolated to higher ecological levels. Small changes in LHTs (e.g. the intrinsic rate of natural increase r_m) can lead to larger population effects that are observable only after several generations (e.g. Derycke *et al.*, 2007; Lira *et al.*, 2011).

Due to the individual-based design of the hanging drop method, the LHTs of individual nematodes can be observed directly through the transparent lid of the culture dish at any time and with any degree of detail, depending only on the recording and transfer intervals. This method thus overcomes intraspecific interference and the artifacts related to an uneven distribution of food or contaminants. Since the gelation of NGG can be controlled by adding divalent cations, it can be freshly prepared over the course of the experiment (also after adding contaminants) and nematodes can be easily recovered (Brinke *et al.*, 2013).

Another advantage of the hanging drop method is that data on the LHTs of single nematodes can be linked with additional fitness traits, such as lipid storage (Fig. 12.4) (Fueser *et al.*, 2018). The latter can be tracked using

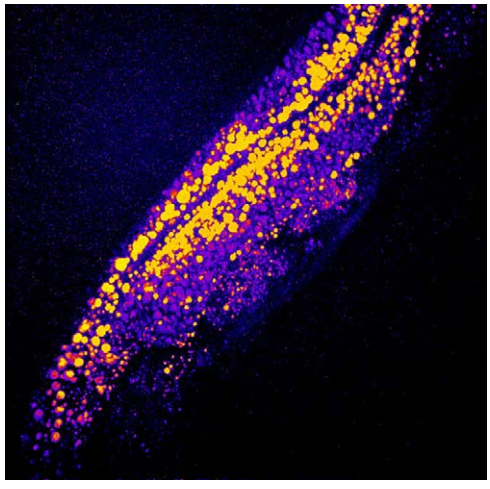


Fig. 12.4. Imaging lipid droplets in *Caenorhabditis elegans* with coherent anti-Stokes Raman scattering (CARS) microscopy. 3D projection of 45 z-stack CARS images (150 × 150 µm) for adult *C. elegans*. (Photo: Hendrik Fueser, Animal Ecology, Bielefeld University.)

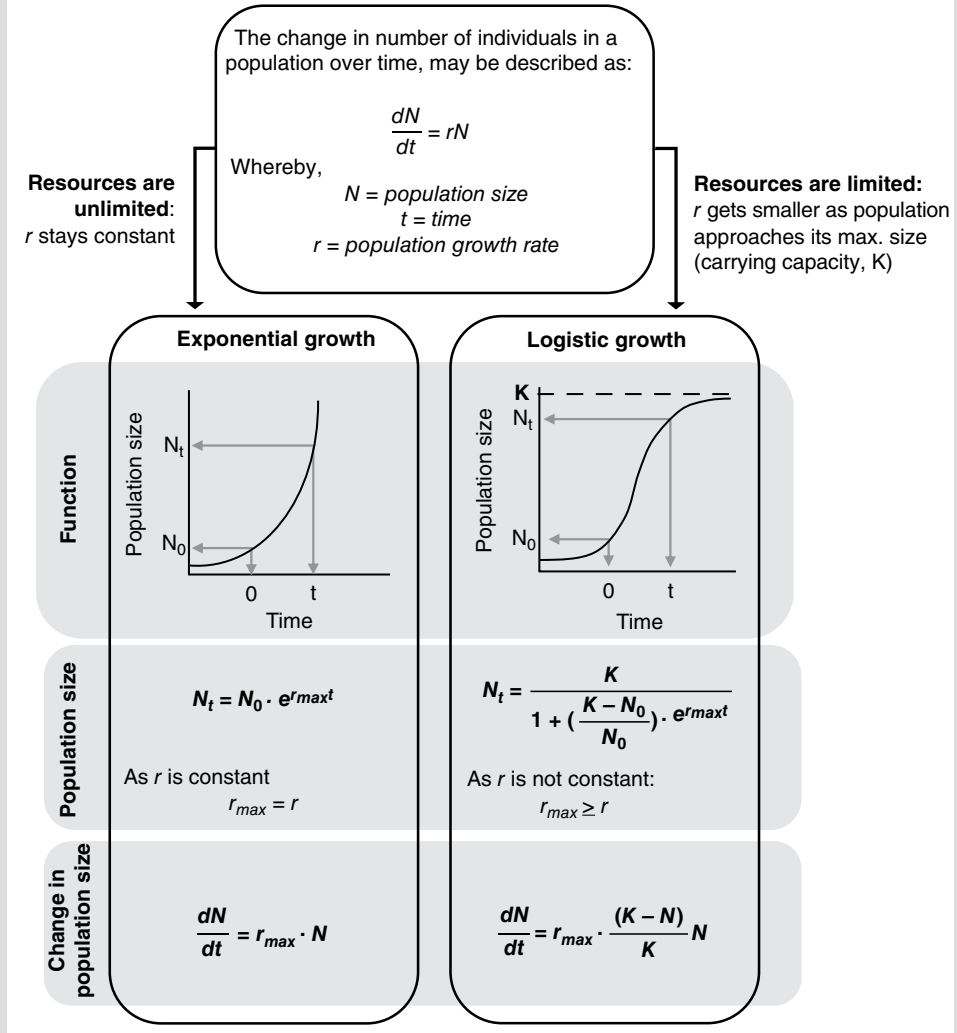
coherent anti-Stokes Raman scattering (CARS) microscopy, which enables chemically selective, label-free, non-invasive imaging of lipids based on their specific signal: the aliphatic 2845 cm^{-1} CH_2 stretching vibration of fatty acid chains (e.g. Hellerer *et al.*, 2007; Yen *et al.*, 2010; Wang *et al.*, 2014; Yi *et al.*, 2014). This method has been used in *C. elegans* to demonstrate the close relationship between lipid storage, oxidative stress, and LHTs (Yen *et al.*, 2010; Fueser *et al.*, 2018). Fueser *et al.* (2018) used CARS microscopy to link population fitness responses with individual ecophysiological responses. The authors conducted a life cycle experiment (Fig. 12.3; Table 12.2) in which freshly hatched juvenile *C. elegans* were exposed to low concentrations of Cu until their death, after which the lipid content of each of the nematodes was quantitatively analyzed using CARS microscopy. Compared with Cu-exposed nematodes, in the control nematodes lipid-filled areas were 88% larger, the individual size of lipid droplets was, on average, 74% greater, and the area fraction occupied by lipids was increased by 93% (Fueser *et al.*, 2018). Copper exposure may have directly interfered with lipid synthesis or with the resource allocation strategies that reflect changes in energy assimilation and demand (Congdon *et al.*, 2001). For an organism, responding to a contamination is energetically costly, such that maintaining reproduction requires additional energy, gained, for example, by burning lipid reserves. The study by Fueser *et al.* (2018) demonstrated that, in senescent *C. elegans*, a significant decrease in population fitness parameters, such as the LHTs recorded in a life cycle experiment, adversely impacts individual fitness, measured as a significant reduction in lipid storage.

12.3 Case Study 2: Population Growth and Competition

12.3.1 What is a population growth experiment and what information can it provide?

The growth of an isolated population is the sum of the reproductive success of each member, such that an increase in the density of a population reflects individual life cycle parameters, including fecundity and life span (see

Box 12.1. Modeling population growth rates.



case study 1). A population growth curve thus represents the responses of many individuals to their environment and changes thereof. This is evidenced, for example, by the population growth rate (Box 12.1), which may then be used as the basis for comparisons (Forbes and Calow, 1999).

A population growth experiment may simply involve counting the number of individuals of a population during a defined time span. From this information a population growth curve can be plotted (Box 12.1) and compared with the curve obtained from a population exposed to different conditions. Given the fast reproduction of many freshwater species (e.g. rhabditids) and their intermediate body size (large enough to be easily counted, but small enough to hatch in the lab) these experiments can be comfortably conducted in the laboratory. In nematode populations, this approach has been used to determine the effects of resource availability (Schroeder *et al.*, 2010), biotic interactions (Gansfort *et al.*, 2018), abiotic conditions (Majdi *et al.*, 2019), and microplastics exposure (Mueller *et al.*, 2020) on population growth rates. For example, when resources are unlimited nematode populations exhibit exponential growth, whereas limited resources result in a logistic growth pattern.

12.3.2 Presentation of case study 2

In this case study, a population growth experiment is presented in which the interspecific interactions of two nematode species, *Panagrolaimus thienemanni* and *Poikilolaimus regenfussi*, under different resource availabilities were of interest (Gansfort *et al.*, 2018). Using this study as an example, we demonstrate how a population growth experiment may be performed and its results evaluated and interpreted.

The experiment made use of two bacterivorous species cultured from samples of the bacterial floating mats found in the groundwater ecosystem of Movile Cave, Romania. The ecosystem had been isolated from the surface for thousands of years and its community of nematode species, including *P. thienemanni* and *P. regenfussi*, had coexisted for hundreds of generations. A previous study (Schroeder *et al.*, 2010) showed that *P. thienemanni* grows better at high bacterial densities (10^{10} cells/ml) and *P. regenfussi* at low bacterial densities (10^{8} cells/ml). Under an intermediate level of resource availability (10^{9} cells/ml) the growth rates of the two species are similar. These responses make this two-species system ideal for investigating the interspecific interactions of coexisting species competing for a mutual resource under conditions in which either one species is a superior/inferior competitor or the growth rates of the two species are balanced.

Ecologists studying the interactions between two species are often interested in the population growth rates and densities of those species (Abrams, 1995). Both parameters can be assessed in population growth experiments. Altering the densities of the species at the start of the experiment will alter both intra- and interspecific interactions, with the influence of one species on the other increasing as its population density

increases relative to that of the other species (Bronstein, 1994). In the experiment using *P. thienemanni* and *P. regenfussi*, monocultures of each species initially containing 48 individuals were compared with mixed cultures at density ratios of 2:1, 1:1, and 1:2. All cultures were grown in NGG (Muschiol and Traunspurger, 2007) containing high (10^{10} cells/ml), medium (10^{9} cells/ml), or low (10^{8} cells/ml) bacterial densities. The full-factorial design of the experiment allowed the influence of differences in resource availability and species ratios (as a proxy for interspecific interactions) as well as the interaction of these two factors to be tested (for a treatment overview, see Fig. 12.5).

The population densities of both species were measured five times (days 4, 7, 11, 15, and 21) by removing one-fourth of the nematode-containing growth medium (NGG) after careful mixing to obtain homogeneous suspensions of the nematodes (the advantages and disadvantages of this method are discussed in the next section). The removed volume was replaced by medium containing the respective bacterial density. The nematodes in the removed medium were counted and the relationship between time and population size was used to determine the growth pattern and the population growth rate, calculated for every replicate. The advantage of using the population growth rate is that it is defined by a single value indicative of population growth irrespective of the time or population size at the start of the experiment (Fig. 12.6).

The results of the experiment showed that the population growth rates of both nematode species significantly depended on the bacterial density in the medium (Fig. 12.6b). The role of species interaction varied across treatments (representing different resource availabilities). For both species, the initial species ratio significantly affected the growth rate, as the increased presence of the one species led to a decrease in its population growth rate (Fig. 12.6b). For *P. thienemanni*, a change in the growth rate as a function of the species ratio was observed under a high bacterial density but not under a low or medium density (Fig. 12.6b). The facilitative effect among the two species provided a rare example of positive interspecific interactions among organisms competing for the same resources (Gansfort *et al.*, 2018). Positive interactions among resource competitors that permit a stable coexistence in the presence of a single resource have been previously described based on simulations (Gross, 2008) but they may also explain the coexistence of these species in the closed system of Movile Cave over thousands of years. However, the experimental design of a population growth experiment cannot provide insights into the mechanism of this interaction, which instead requires other types of investigations.

12.3.3 Possibilities and limitations

In the methodology of population growth experiments using nematodes, several aspects merit greater consideration.

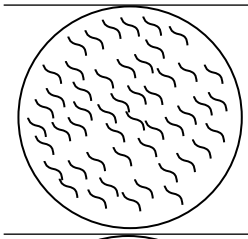
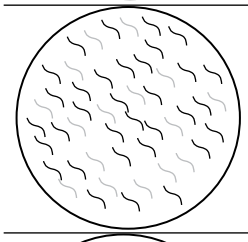
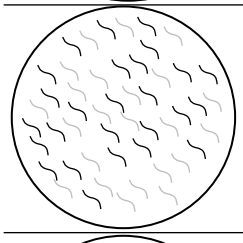
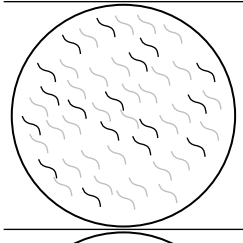
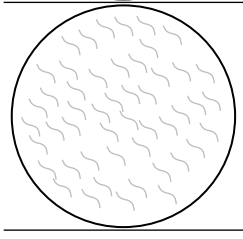
	Treatment		Species startdensities		Species ratio		Bacterial density
			Pan	Poik	Pan	Poik	
Monoculture		1	48	0	1	0	10^8
		2	48	0	1	0	10^9
		3	48	0	1	0	10^{10}
		4	32	16	2	1	10^8
		5	32	16	2	1	10^9
		6	32	16	2	1	10^{10}
Combined culture		7	24	24	1	1	10^8
		8	24	24	1	1	10^9
		9	24	24	1	1	10^{10}
		10	16	32	1	2	10^8
		11	16	32	1	2	10^9
		12	16	32	1	2	10^{10}
Monoculture		13	0	48	0	1	10^8
		14	0	48	0	1	10^9
		15	0	48	0	1	10^{10}

Fig. 12.5. Overview of the experimental treatments used to measure the population growth of two nematode species: *Panagrolaimus thienemanni* (Pan) and *Poikilolaimus regenfussi* (Poik). (Modified from Gansfort *et al*. (2018).)

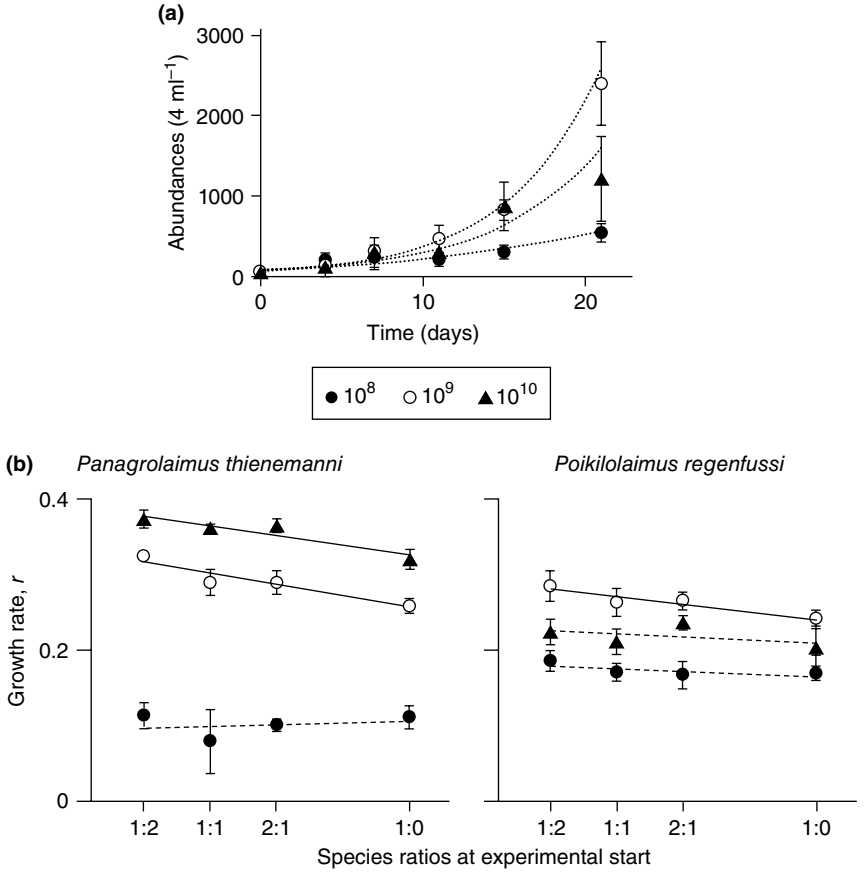


Fig. 12.6. Example of the development of monocultures of *Poikilolaimus regenfussi* under three different bacterial densities (**a**). By fitting the growth function that best approximates the data, r_{max} can be calculated (Box 12.1). The values obtained in the different treatments can be compared (**b**), as done in this experiment for four species ratios and three bacterial densities (black circles: 10^8 cells/ml; white circles 10^9 cells/ml; black triangles 10^{10} cells/ml). The linear regressions are shown, significant slopes determined according to a generalized linear mixed model are indicated as solid lines (for the statistical analysis, see table 1 in Gansfort *et al*., 2018). (Modified from Gansfort *et al*. (2018).)

12.3.3.1 Counting of nematodes

There are two methods to determine the number of nematode individuals in a Petri dish or multiwell plate. First, the individuals can be counted directly, by placing the whole dish under a microscope. However, this option requires that the whole plate can be counted because nematodes do not spread homogeneously on agar plates. Further, the nematodes are usually present in different vertical planes such that they are difficult to count, especially in permeable medium such as NGG. Also, it is difficult if not impossible to control for the food resource of the nematodes (e.g. bacteria, algae) without sampling or replacing the medium. This problem

is resolved by the second counting option, which was applied in the study by Gansfort *et al.* (2018). In this method, liquidation and fixation of the medium in the plates allow the trapped nematodes to be easily counted any time and the density of the food resource to be determined. Replacement of the resource-depleted medium with freshly prepared medium containing a defined level of resource (or other factor of interest) makes it possible to observe and control the experimental conditions. Nonetheless, this method requires that the growing population is disturbed, due to mixing of the medium, such that individuals are relocated. Further this method induces an artificial mortality of the population. The latter must be considered in the subsequent calculation of growth rates (see Online Resource 1 in Gansfort *et al.*, 2018).

12.3.3.2 Resource levels

Most nematode population growth experiments conducted so far have used bacterivorous species and thus bacteria as the food resource. At the start of the experiment, food is typically supplied at the same level for every population, with the bacterial cells either counted or their densities adjusted photometrically. Without new inputs, the number of bacterial cells decreases as the number of nematodes on the plates increases. Replacing the medium during the experiment as described above keeps the bacterial density constant, at least until the nematode population exceeds a certain size (ca. >5000 individuals; Schroeder *et al.*, 2010). In the study of Gansfort *et al.* (2018), there was no measurable decrease in bacterial densities during the experiment. However, food availability must be measured and differences between treatments identified for a more accurate interpretation of the results.

In addition to food, a limiting resource in population growth experiments is space, specifically the space per nematode individual. However, the amount of space in each treatment can be kept comparable over the duration of the experiment by increasing the amount of medium over time (as done in Schroeder *et al.*, 2010). A scarcity of space may result in a decline in growth rates, manifested as a logistic growth curve. Therefore, before the growth rate (r) is calculated, the growth curve that best fits the data must be determined. Further, if initial populations vary in size between treatments, the role of intraspecific interaction must be excluded (as done in Gansfort *et al.*, 2018).

12.3.3.3 Starting population

To ensure the comparability of the treatments, the starting populations must have the same age- and sex structure. While this can be achieved by manually placing the same number of males and females in the experimental plates, it is usually only possible when adult nematodes are used. The alternative is to work with relatively large starting populations and many replicates, which decreases the probability that randomly induced effects of age and sex ratios in the populations influence the experimental results.

The advantages of population growth experiments with nematodes as introduced here are: (i) the acquisition of information about many thousands of individuals summarized in one comparable value, that is, the population growth rate. The growth conditions may be (ii) easily manipulated and (iii) controlled over the duration of the experiment. The limitations are that (i) because the growth rate describes the whole population, more detailed insight into the mechanisms that change the growth rate may not be discernable; (ii) the focus on one or a few populations and their response to a single resource may lead to an oversimplification. Additional insights into single individuals may be needed, obtained as described in case study 1 (life cycle experiment). Including those findings in a wider community concept may help to generalize the outcomes of a population growth experiment, as discussed in case study 4 (benthic food web). Thus, population growth experiments fall in the middle of a gradient of nematode studies that range from the individual to a whole community.

12.4 Case Study 3: Molecular and Microscopic Identification of a Nematode Community

12.4.1 What is metabarcoding and what information can it provide?

A nematode community can be identified to the species or genus level using either microscopic or molecular techniques. Metabarcoding is a relatively new method in the field of molecular diagnostics that allows the parallel high-throughput sequencing of several thousand amplicons (Taberlet *et al.*, 2012; Cristescu, 2014) and can be used to analyze the composition of communities and, thus, the response of communities to environmental impacts. This DNA-based method can be either carried out with a selection of specific taxa (bulk samples) or with unfiltered samples, including the total environmental DNA (eDNA). Specimens for bulk extractions are extracted from the habitat and usually yield larger amounts of DNA than eDNA, which is directly extracted from the sample (Creer *et al.*, 2016; Hering *et al.*, 2018). Metabarcoding has the potential to estimate the diversity of organismal groups, to monitor subtle changes in the community, such as those potentially due to pollution events or climate change, and to identify individuals for bioindication (Shokralla *et al.*, 2012; Cristescu, 2014; Schenk *et al.*, 2020a). When used in combination with the NemaSPEAR[%]-index, as a proxy for sediment quality, a metabarcoding approach enables the rapid and comprehensive evaluation of whole nematode communities for bioindication purposes.

12.4.2 Presentation of case study 3

The NemaSPEAR[%]-index can be calculated for molecular-derived data (Schenk *et al.*, 2020a) and for microscopic-derived data and is appropriate

for both species-level and genus-level assessments (Höss *et al.*, 2011, 2017). Metabarcoding approaches using community DNA for bioindication have been applied to several taxa, but rarely for nematodes. A pilot study used molecular and microscopic identification together with the NemaSPEAR[%]-index to assess seven locations characterized by a pollution gradient (Schenk *et al.*, 2020a). At each location, sediment samples were taken with a corer and preserved in ethanol (80%) for molecular analyses or in formalin (4%) for microscopic analysis. The nematodes were then extracted from the sediment and species were identified by both methods (see Chapter 2). The nematode species composition at the different sites derived by metabarcoding (using two ribosomal markers: 18S rDNA and 28S rDNA) was analyzed using multivariate statistics (non-metric multidimensional scaling; nMDS) based on the relative biomass composition of the nematode species. The results were then compared with those of the morphological taxonomy in terms of their ability to separate the communities of the various sampling sites (Fig. 12.7).

The communities could be clustered according to their location, for the most part independent of the taxonomic approach (Fig. 12.7). Locations classified as unpolluted (VE, ÖR, FB) clustered together, as did the two Elbe locations (HI and CUM), showing a similar degree of pollution. Also, LU and RM built a common cluster. For HI and CUM, it could be shown that the morphological and the molecular approach using the 28S rDNA gene fragment revealed similar structures of the nematode communities, but significantly differing from the 18S approach.

The classification of the ecological quality of sediments using nematode community DNA to calculate the NemaSPEAR[%]-index was accurate and comparable with the morphologically derived values. The agreement between the DNA-based and morphological approaches regarding the NemaSPEAR[%] values was even better if using the genus level as basis of the calculations (R^2 = 0.86, P = 0.0027; Fig. 12.8). For all taxonomic approaches, the ecological status defined by the NemaSPEAR[%]-index properly reflected the toxic potential (using sediment quality guidelines according to De Deckere *et al.*, 2011) of the sediments sampled from the seven locations, ranging from very low (FB, VE, ÖR) to medium (RM, CUM) and high (HI, LU) (Fig. 12.9).

12.4.3 Possibilities and limitations

A major advantage of molecularly based species identification is that it is not dependent upon taxonomic expertise, which is unequally available for several groups, especially those representing small and inconclusive taxa (Tang *et al.*, 2012; Ahmed *et al.*, 2015). In contrast to traditional molecular methods, such as single-specimen barcoding, metabarcoding using next-generation sequencing (NGS) allows the analysis of a whole community, with a higher throughput of individuals. It thus promises to be faster and more accurate than morphological inspection and has the additional advantage of a highly standardized laboratory workflow across labs (Leasi *et al.*, 2018).

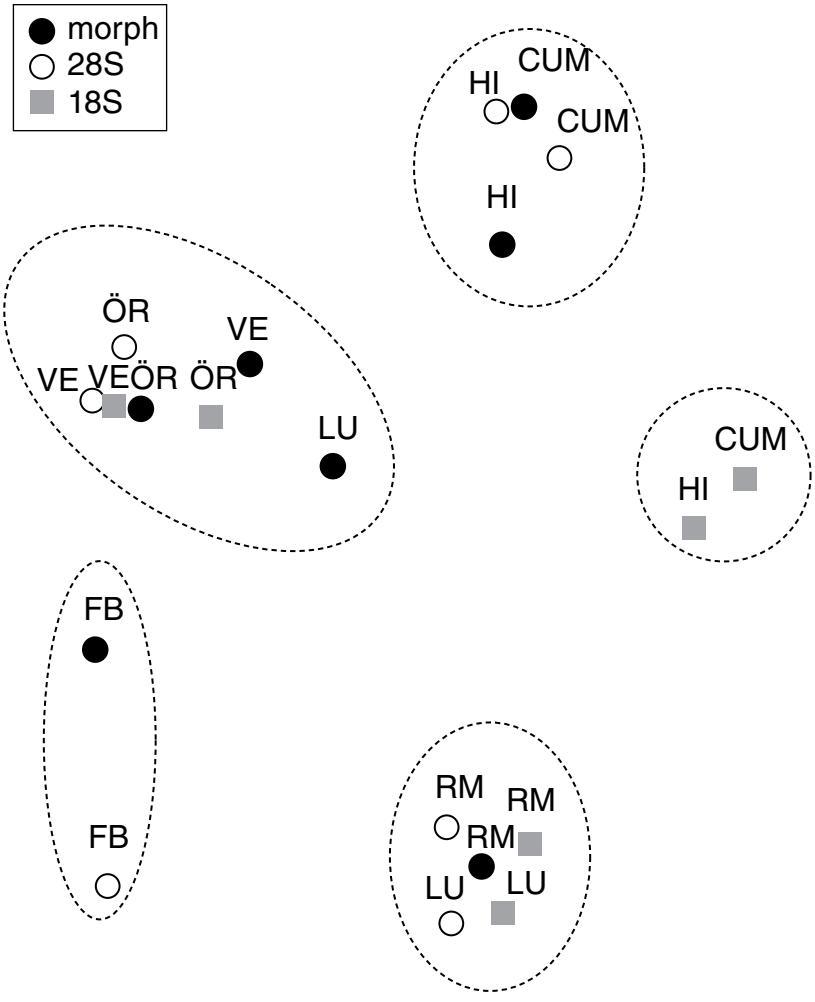


Fig. 12.7. Non-metric multidimensional scaling (nMDS) comparing the nematode genus composition in river sediments sampled from seven sites (CUM = Cumlosen, HI = Hitzacker, RM = Mischmühle, LU = Luppe, ÖR = Örtze, VE = Veerse, FB = Furlbach). The analyses used the Bray–Curtis similarities of the untransformed data based on biomass. A 20%-similarity level was used to define clusters laid over the nMDS plot (identified in a similarity profile analysis (SIMPROF); $P < 0.05$). The analyses compared three different taxonomic methods: morphological taxonomy (morph) and two molecular taxonomies (28S, 18S). Stress of the nMDS plot = 0.20; sites could be ranked in a gradient of pollution (i.e. toxic potential): LU (highest toxic potential) > HI > CUM > RM > ÖR = VE = FB. (From Schenk *et al*. (2020a). With the permission of Elsevier.)

Besides bulk sample sequencing, with the metabarcoding of eDNA all organisms present at the sampling location can be traced, which expands the information that can be obtained within an investigation (Deiner *et al.*, 2016). So far, however, eDNA has not been used to study freshwater nematodes properly at the species or genus level.

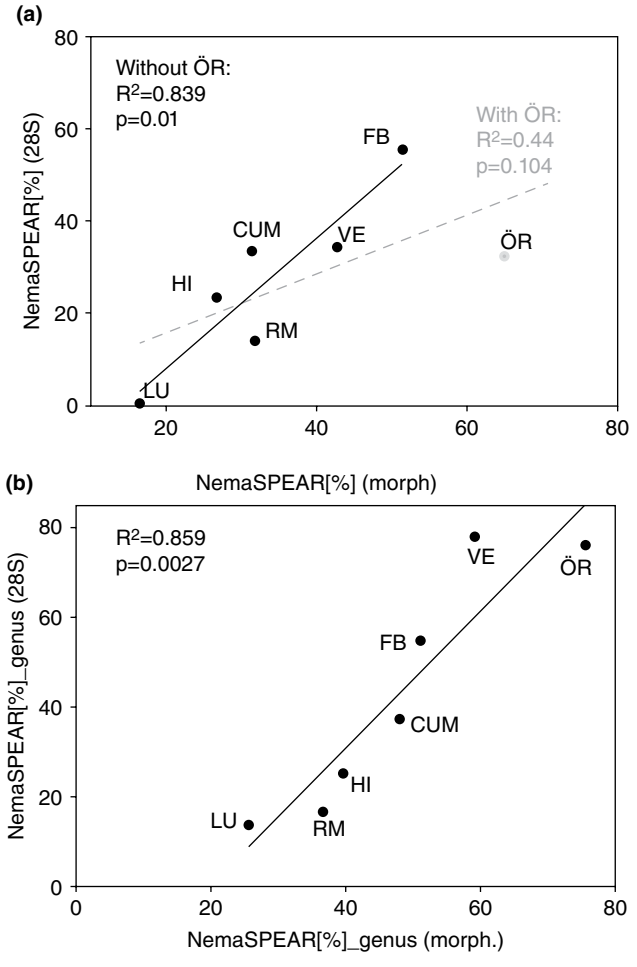


Fig. 12.8. Correlations between NemaSPEAR[%] and NemaSPEAR[%]$_{genus}$ calculated based on morphological (morph) and molecular (28S) taxonomic data. (**a**) NemaSPEAR[%] 28S vs. NemaSPEAR[%] morph; (**b**) NemaSPEAR[%]$_{genus}$ 28S vs. NemaSPEAR[%]$_{genus}$ morph. The dotted line indicates a relatively weak and therefore only partial correlation. (From Schenk *et al*. (2020a). With the permission of Elsevier.)

Despite the accuracy and sensitivity of metabarcoding, its current limitations (and those of other NGS applications) include polymerase chain reaction (PCR) biases that can alter the outcome of the study. Due to primer–template mismatch, differences in (ribosomal) copy numbers and the differential degradation of DNA templates, taxa may be under- or overrepresented (Elbrecht and Leese, 2015; Piñol *et al.*, 2015). For every molecular approach, the choice of the genetic markers will also impact the study outcome, as several markers are limited in terms of their taxonomic resolution or amplification efficiency (Clarke *et al.*, 2017). Furthermore, reference databases are not equally well curated for all taxa, including

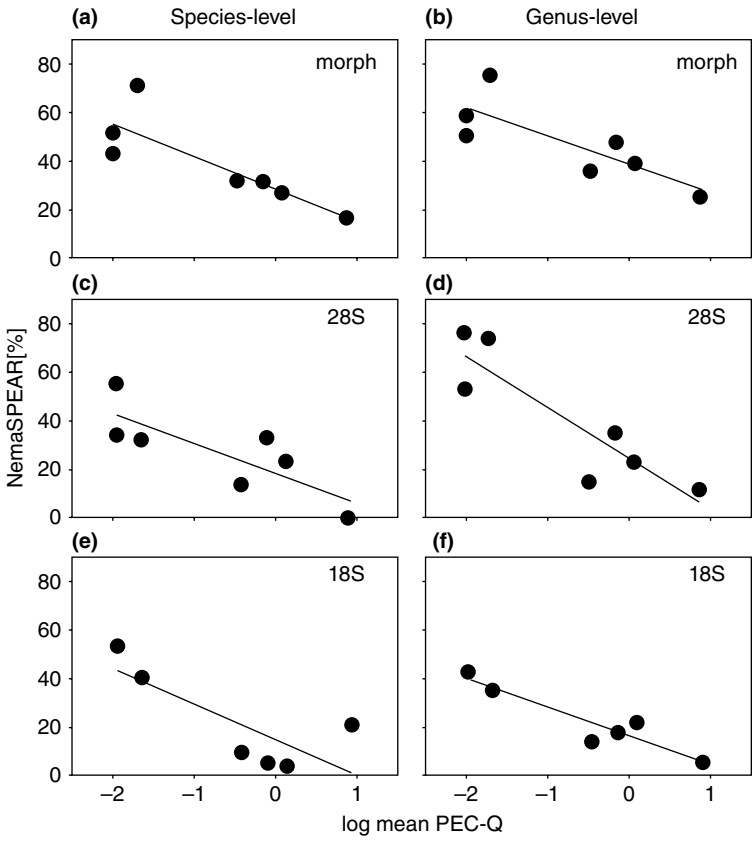


Fig. 12.9. NemaSPEAR[%] (**a**, **c**, **e**) and NemaSPEAR[%]$_{genus}$ (**b**, **d**, **f**) calculated based on morphological (morph; a, b) and molecular (28S-rDNA, c, d; 18S-rDNA, e, f) taxonomic nematode species and genus data of river sediment sampled at seven sites (six sites for 18S-rDNA) plotted against the mean probable effect concentration quotient (PEC-Q) values calculated for the respective sediments. (a) $r^2 = 0.71$, $P = 0.017$; (b) $r^2 = 0.66$, $P = 0.027$; (c) $r^2 = 0.65$, $P = 0.029$; (d) $r^2 = 0.78$, $P = 0.008$; (e) $r^2 = 0.60$, $P = 0.070$; (f) $r^2 = 0.88$, $P = 0.006$. (From Schenk *et al*. (2020b). With the permission of Elsevier.)

freshwater nematodes (Pawlowski *et al.*, 2018), such that many OTUs may not be taxonomically classified, which in turn will therefore influence the list of taxa.

12.5 Case Study 4: The Role of Nematodes in the Benthic Food Web

12.5.1 What role do nematodes play in the benthic food web?

Nematodes are extremely abundant in sediments and in biofilms growing on organic and inorganic substrates (see Chapter 3). With their functionally diverse communities, they are likely to play a central, but complex,

role in benthic food webs, as they are able to exploit a broad range of microbial resources (see Chapter 6) but they also serve as prey for a wide variety of predators (see Chapter 7). Focused studies in the laboratory (e.g. food choice and functional response experiments, see Chapters 6 and 7) and in the field (e.g. food labeling experiments and fish caging, see Chapters 6 and 7) can provide quantitative assessments of the importance of trophic transfer to and from nematodes, and thus a better understanding of the contribution of nematodes to benthic food webs.

However, an appreciation of the causalities of trophic interactions on a meaningful scale requires experimental manipulations that integrate the responses of all biological compartments (including nematodes). In this section we present a case study in which the responses of a comprehensive benthic food web (in terms of standing stocks) within a stream to the presence of a top predator were measured (experiment detailed in Majdi *et al.*, 2014). The aim of the study was to better understand how predator effects (both direct trophic effects and indirect non-trophic effects) can extend to prey and non-prey communities and ultimately affect ecosystem processes like decomposition rate. The experiment was performed directly in a stream to maximize its ecological representativeness, given the complexity of the assemblages considered. However, similar approaches in the laboratory using sediment cores may be envisaged to reduce environmental variability (Majdi *et al.*, 2016) or to better dissect predation mechanisms within simplified food webs (Beier *et al.*, 2004; Kreuzinger-Janik *et al.*, 2018).

12.5.2 Presentation of case study 4

Detritus-based food webs are for the most part cryptic and not fully understood, as empirical findings often deviate considerably from predictions based on classic trophic cascades primarily identified from pelagic examples (Mancinelli and Mulder, 2015). Moreover, although intermediary food web components such as nematodes are generally numerically dominant in detritus-based food webs, they have been largely neglected in the development of conceptual models of detrital dynamics (Gessner *et al.*, 2010). In the present case study, the effects of a top predator (the free-living flatworm *Polycelis felina*) on the litter decomposition rate, litter-associated fauna (including nematodes), and microbial decomposers were assessed experimentally to gain a better mechanistic understanding of a detrital food web.

Stream flatworms are small (body length <3 cm), active predators that prey on a variety of macrofaunal taxa (e.g. Jennings, 1957), although recent functional response experiments conducted in the laboratory have demonstrated that several species may feed voraciously on nematodes as well (see Chapter 7). The functional importance of predatory flatworms is suggested by their high abundance in leaf accumulations deposited along stream margins and by their efficient foraging strategy, which involves group hunting and mucus trapping (e.g. Jennings, 1957).

In our food web model (Fig. 12.10), macrofauna was considered to be a trophically disparate group of prey that feed at all lower trophic levels. Thus, the predator effect on the litter decomposition rate was expected to be contingent upon the effect of the flatworm on the composition of the macrofaunal community. As most fungal biomass is embedded within leaf tissues, fungal mycelium was expected to be consumed solely by leaf-shredding macrofauna, whereas meiofaunal organisms such as

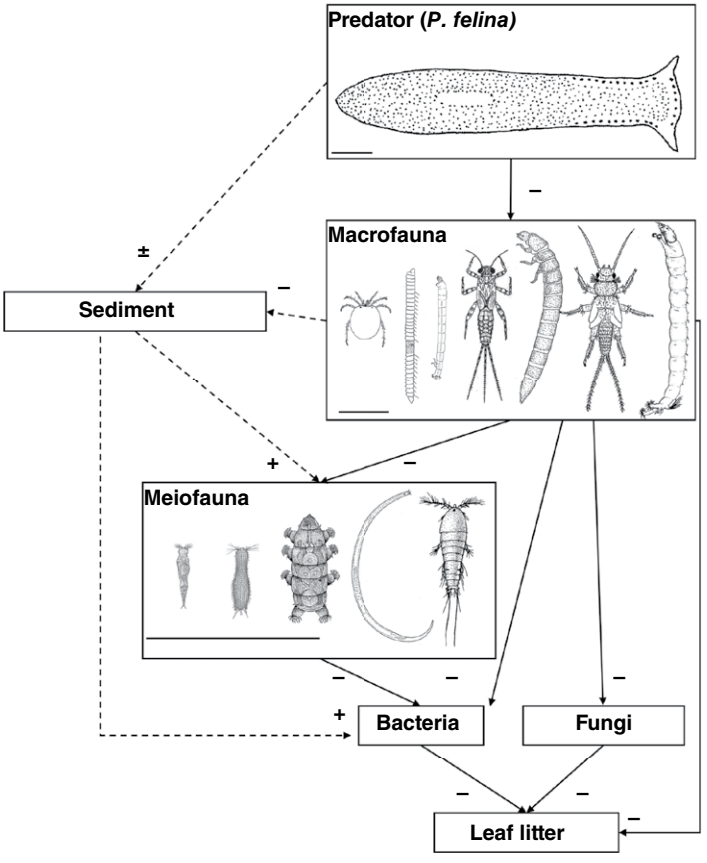


Fig. 12.10. Conceptual model of a detrital food web in experimental enclosures set in a stream. Macrofauna includes water mites, oligochaetes, non-predatory chironomids, biofilm-grazing mayflies and beetles, leaf-shredding stoneflies, and predatory dipterans. Meiofauna includes rotifers, gastrotrichs, tardigrades, nematodes, and harpacticoid copepods. Scale bar is 1 mm. Arrows depict direct and indirect interactions through which the predatory flatworm *Polycelis felina* was hypothesized to influence detrital communities, their habitat (sediment), and the stock of the basal resource (leaf litter). Signs denote the direction of the expected changes in biomass/amount (depletion –, accretion +, undefined ±). Solid lines represent a trophic cascade propagating through multiple pathways, and dashed lines the non-trophic pathways mediated by sediment deposition on leaf surfaces. For instance, the largest animals (flatworm and macrofauna) were expected to influence sedimentation through bioturbation (–) or bioretention/consolidation (+). (After Majdi *et al.* (2014) with permission of Wiley.)

nematodes were expected to primarily exploit the bacterial biofilms colonizing leaf surfaces (Fig. 12.10). Because the leaf litter surface is a homogeneous landscape for nematodes and bacteria, both groups may also heavily rely on the complex interstitial habitat formed by the fine sediment entrapped in the accumulated litter (e.g. Gaudes *et al.*, 2009). The largest invertebrates (flatworm and their macrofaunal prey) may affect the amount of deposited sediments through bioturbation or bioconsolidation (Statzner, 2012; Majdi *et al.*, 2015), thereby providing the basis for a non-trophic pathway through which flatworm predators, by affecting habitat texture, affect the trophic dynamics of non-prey meiofaunal and bacterial communities (Fig. 12.10).

Polycelis felina is the most abundant predatory flatworm species in headwater streams in the French Montagne Noire massif. It was detected in most benthic habitats where leaf packs hosted up to 20 individuals per gram of leaf litter dry mass (data from 11 streams surveyed). In the modeled food web, the density of *P. felina* was manipulated (zero, three, or nine individuals) in small (10 × 10 cm; 50-µm nylon mesh netting) semi-rigid litter bags set in the study stream (Fig. 12.11).

The bags were filled with 2.5 g of air-dried oak leaves, secured on iron sticks, and wedged on the stream bottom for 22 days prior to the addition of the flatworms, to ensure suitable litter colonization by small invertebrates and microorganisms. Eight randomly selected bags per treatment

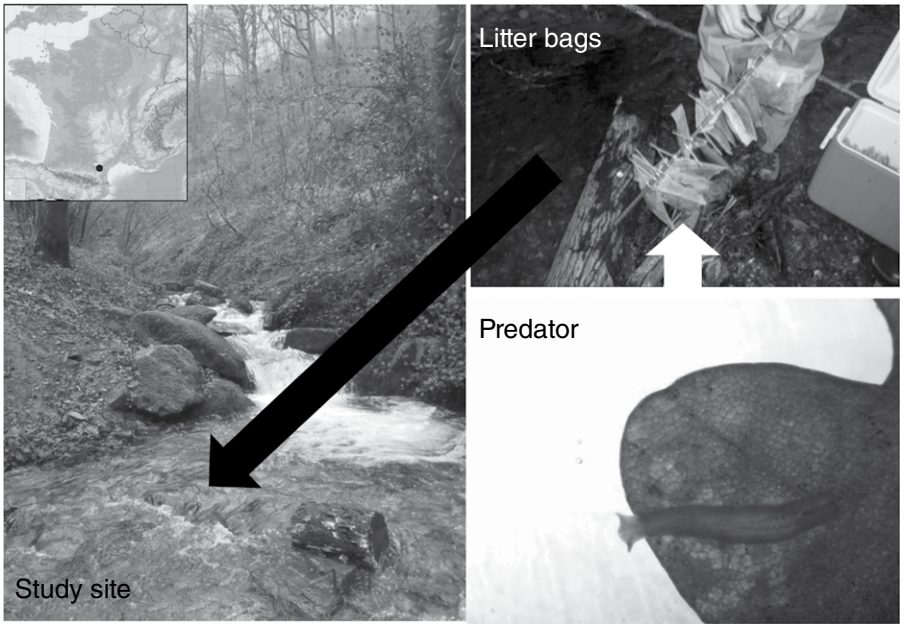


Fig. 12.11. Scheme of the experimental design: Flatworm predators (*Polycelis felina*) were collected from the field and enclosed in fine-mesh bags containing stream-conditioned oak litter. The enclosures were incubated in a stream in southern France and predator effects on detrital communities were monitored. (Author's own figure.)

were sampled 14 and 24 days following the addition of *P. felina*. A third sampling date was planned after 48 days, but a large flood occurred – one of the many potential hazards of field experiments – that damaged most of the remaining bags, such that the assessment was based on the first two sampling dates only. The sampled litter bags were stored individually in plastic zip-lock bags, transported to the laboratory in a cool-box, and processed within 2 h. The amount of sediment trapped on the leaf surfaces, the leaf decomposition rate, bacterial and fungal biomass, as well as the biomass and functional structure of litter-associated invertebrates were measured following standard procedures (further details in Majdi *et al.*, 2014).

The presence of *P. felina* significantly accelerated oak litter decomposition and enhanced bacterial biomass, meiofaunal biomass, and the amount of fine sediment deposited on leaves. Bacteria and meiofauna showed the largest positive response to *P. felina*, with a two-fold difference at the end of the experiment between the highest predator density treatment and the no-predator control (Fig. 12.12). Furthermore, *P. felina* had a significant effect on nematode community structure, especially at the highest predator density, through a net positive effect on bacterivorous nematode species (Fig. 12.13). By contrast, there was no predator effect on either fungal or macrofaunal biomass.

To determine causalities within the hypothetical food web, biomass correlations were analyzed using partial-least squares path modeling (PLS-PM) to quantify direct and indirect predator effects on litter decomposition and litter-associated communities. PLS-PM is a robust form of structural equation modeling (SEM) that allows the estimation of latent variables (Esposito-Vinzi *et al.*, 2010). The latter are multivariate constructs used to condense information encapsulated within several measured variables whose relationships to each other are not fully understood *a priori* or are of lesser interest than the information they represent.

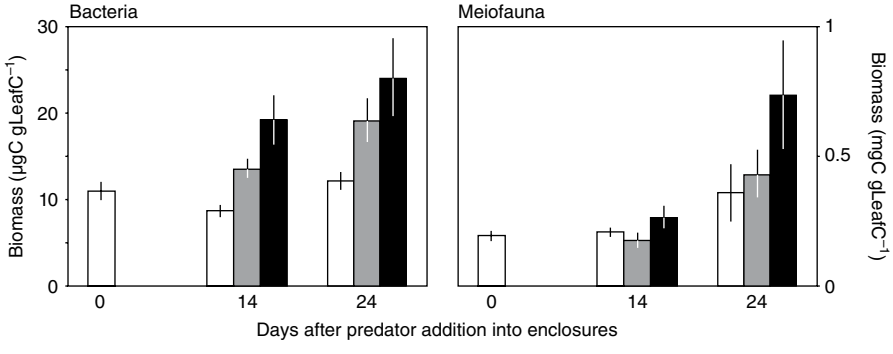


Fig. 12.12. Effects of the presence and density of the predatory flatworm *Polycelis felina* on bacterial and meiofaunal biomass. The values are the means by sampling date (N = 8, ± 1 standard error). Open, shaded, and solid bars show leaf pack enclosures without flatworms, with three flatworms, and with nine flatworms, respectively. Abbreviations: carbon (C). (Modified after Majdi *et al.* (2014) with permission of Wiley.)

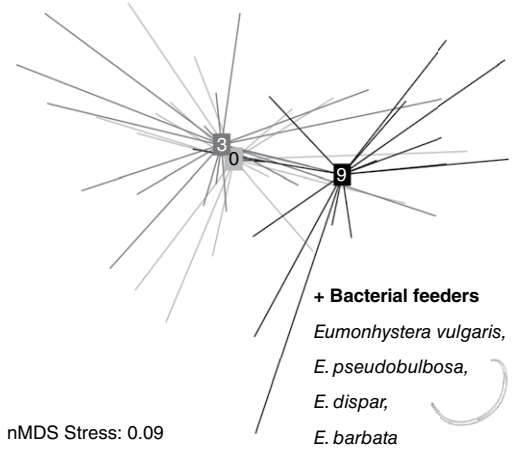


Fig. 12.13. Effects of predator density (zero, three, and nine individuals) on the composition of nematode assemblages analyzed by nMDS ordination based on the Bray–Curtis similarity. The 'spider webs' link each sample to the centroid of the respective predator treatment. Bacterial feeders were especially favored under the highest predator density. (Modified after Majdi *et al.* (2014) with permission of Wiley.)

Contrary to the common SEM approach based on covariance structure analysis, PLS-PM is based on iterative processes and non-parametric hypothesis testing (Esposito-Vinzi *et al.*, 2010). It therefore does not rely on strong assumptions, such as a normal distribution and data independency, nor does it require large datasets to perform optimally (Chin, 2010). PLS-PM was therefore particularly well suited to our study exploring a relatively high density of paths based on a modest dataset (N = 48 samples). Causal diagrams were constructed from a pool of 16 continuous variables, including predator density as the exogenous variable, the biomass of leaf-colonizing biota (with macrofauna and meiofauna as latent variables), the amount of inorganic sediment deposited on the leaf surface, and the remaining leaf litter mass (Table 12.3).

A first (*a priori*) model was constructed following our initial expectations to assess trophic and non-trophic predatory effects propagating exclusively downward through the food web (Fig. 12.14A). The model was validated through PLS-PM after minor adjustments, but the marked positive response of bacterial biomass was not well explained. Especially, a 'fear syndrome' seemed to have propagated downward along the flatworm–macrofauna–bacteria trophic chain, given that in the presence of *P. felina* the top-down control of bacteria by Chironomidae was dampened, suggesting a reduction in Chironomidae grazing activity in response to a perceived predation risk. Thus, an 'alternative' model was developed (see bold paths in Fig. 12.14B): (i) to incorporate the indirect positive effect of flatworms on bacteria through the reduction of chironomid grazing (see the path from flatworm to bacteria in gray in Fig. 12.14B); (ii) to assess the possible control of sedimentation on macrofauna (by reversing the direction

Table 12.3. Model compartments and variables considered in the path models. Habitat and main trophic traits were inferred from Rundle *et al*. (2002) and Tachet *et al*. (2010). (After Majdi *et al*. (2014) with permission of Wiley.)

Model compartment	Variables and abbreviation	Unit	Numerically dominant taxa/morphotypes	Habitat and trophic traits	Mean body mass (µgC)
Flatworm	Flatworm number		*Polycelis felina*	Benthic, fluid feeders of invertebrates	593.0
Macrofauna	Biomass of leaf-shredding Plecoptera (Plecosh)	gC gLeafC^{-1}	Nemouridae, *Leuctra* spp.	Benthic, shredders of leaf tissues	15.6
	Biomass of biofilm-grazing Ephemeroptera and Coleoptera (ECgraz)	gC gLeafC^{-1}	*Ephemerella* spp., *Elmis* spp.	Benthic, grazers of biofilm algae and FPOM	38.0
	Biomass of predatory Diptera (Diptpred)	gC gLeafC^{-1}	Tanypodinae	Benthic/interstitial, predators of small invertebrates	44.7
	Biomass of non-predatory Chironomidae (Npchiro)	gC gLeafC^{-1}	Orthocladinae	Benthic/interstitial, generalist biofilm and FPOM feeders	36.8
	Oligochaeta biomass (Oligo)	gC gLeafC^{-1}	Naididae	Interstitial, deposit feeders (bacteria and FPOM)	5.1
	Hydrachnidia biomass (Mites)	gC gLeafC^{-1}		Benthic/interstitial, predators/ parasites of insect larvae	21.3
Meiofauna	Nematoda biomass (Nema)	gC gLeafC^{-1}	*Eumonhystera* spp.	Interstitial, bacterial feeders	0.029
	Rotifera biomass (Roti)	gC gLeafC^{-1}	Monogononta	Benthic, filter feeders	0.017
	Tardigrada biomass (Tardi)	gC gLeafC^{-1}		Interstitial, microphagous/predators	0.054
	Harpacticoida biomass (Harp)	gC gLeafC^{-1}		Benthic, microphagous	0.053[a]/0.177[b]
	Gastrotricha biomass (Gastro)	gC gLeafC^{-1}		Interstitial, microphagous	0.033
Fungi	Fungal biomass	gC gLeafC^{-1}	*Clavariopsis aquatica*, *Anguillospora filiformis*, *Tricladium chaetocladium*	Endophytic hyphae, exploit exclusively leaf C	Not determined
Bacteria	Bacterial biomass	gC gLeafC^{-1}		Interstitial and epiphytic biofilm, exploit FPOM, DOM, and leaf C	1.34×10^{-10}
Sediment	Inorganic sediment	gDM gleafC^{-1}			
Leaf mass	Percentage leaf mass remaining	%			

FPOM, fine particulate organic matter; DOM, dissolved organic matter; C, carbon; DM, dry mass.
[a]Mean body mass of nauplii larvae.
[b]Mean body mass of copepodite stages and adults.

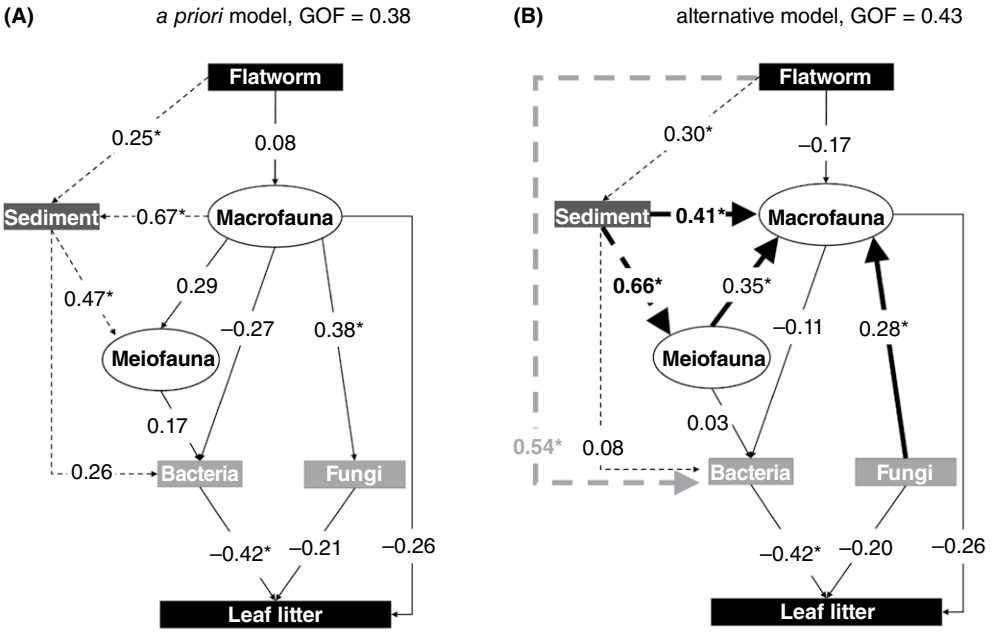


Fig. 12.14. Path diagrams used to assess the direct and indirect effects of predatory flatworm on a detrital food web. The *a priori* model (**A**) presented in Fig. 12.10 was fitted and a second model (**B**) developed to test alternative hypotheses, which improved the match between theory and observations. Solid arrows indicate trophic interactions, and dashed arrows non-trophic interactions. The path linking flatworms to bacteria shown in bold gray in the alternative model represents a fear syndrome, i.e. a reduction in bacterial grazing activity by chironomids in response to a perceived predation risk. The bottom-up stimulation of meiofauna and macrofauna through the bioconsolidation of sediment by mucus-secreting flatworms is represented in bold dashed. Macrofauna and meiofauna were specified as latent variables (circles), defined as a linear combination of trophic groups or taxa. Loadings are not shown in the figure since all taxa or trophic groups correlated positively with their respective latent variable. Values are the path coefficients estimated by partial-least squares path modeling. Asterisks indicate values significantly different from zero based on 95% percentile confidence intervals calculated on 200 bootstrap samples. GOF: goodness-of-fit. (Modified after Majdi *et al.* (2014) with permission of Wiley.)

of the corresponding path); and (iii) to test for the occurrence of bottom-up rather than top-down regulation (by reversing the direction of the paths between macrofauna and meiofauna and between macrofauna and fungi).

The alternative model had a slightly better fit (goodness-of-fit: 0.43) than the *a priori* model (0.38), mostly due to an increase in the explained variation for macrofauna (increase in R^2 from <0.01 to 0.54) and for bacteria (increase in R^2 from 0.03 to 0.27). The alternative model hinted at the prevalence of non-trophic effects in the propagation of predator effects across the detritus-based food web. Interestingly, *P. felina* increased the sedimentation on leaf surfaces, leading to a significant and positive indirect effect on meiofauna. This positive effect propagated upward with the

additional bottom-up regulation of macrofauna by meiofauna, evidenced by the significant positive path coefficient (+0.35) that emerged in the alternative model (Fig. 12.14B). The sum of indirect flatworm effects on macrofauna (sum of the products of the path coefficients after the addition of a direct path linking the predator to bacteria to represent this indirect effect along all indirect pathways: +0.19) balanced out the direct negative impact of the predator on prey biomass (–0.17). The balance between top-down and bottom-up forces provided a reasonable explanation for the non-significant net predator effect on macrofauna indicated by the univariate analysis.

12.5.3 Possibilities and limitations

The major advantage of field-based, comprehensive assessments coupled to path modeling is the broader view of trophic and non-trophic pathways obtained by: (i) the incorporation of intermediate consumer levels (microbial decomposers and meiofauna) below the predator–prey system; (ii) assessing indirect effects of predators on non-prey communities (nematodes and fungi); and (iii) considering the downward and upward propagation of trophic and non-trophic pathways. For example, an important finding of our model was that *P. felina* accelerated litter decomposition mostly through non-trophic interactions; hence this species can be viewed as a 'gardening predator'; that is, top-down regulation of the prey population (Chironomidae) was compensated by predator-induced improvements of the prey's habitat (sediment) and food (nematodes and bacteria). A gardening strategy may stabilize predator–prey dynamics and allow a larger prey pool to be shared among conspecifics, by stimulating the incorporation of detrital material into the food web.

However, enclosure experiments performed directly in-stream suffer the limitations inherent in field-based designs, especially their sensitivity to environmental hazards (such as floods or malevolent acts in non-restricted areas). Other limitations of field experiments are: (i) they do not allow a disentangling of the individual effects of predator traits (as in our study several predators were mixed in each enclosure, and trait-based evaluations of the pool of predatory individuals were not performed *a posteriori*); (ii) they overlook the effects of other potentially important environmental drivers of trophic dynamics (such as temperature and dissolved resource dynamics); and (iii) they cannot distinguish causalities at lower trophic levels (e.g. between bacteria and nematodes) – despite the congruent responses of bacteria and bacterial-feeding nematodes to *P. felina* density, total nematode biomass did not correlate with bacterial biomass. It was therefore not possible to separate the bottom-up effect on nematodes triggered by an increased bacterial biomass from the 'habitat effect' on nematodes triggered by the increased habitat size resulting from the adhesive secretions of the flatworm. Combining comprehensive field manipulations with more focused laboratory investigations would surely help to overcome the limitations of such field-based experimental approaches.

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Ecology of Freshwater Nematodes

Edited by Walter Traunspurger

Nematodes are incontestably the most numerous and the most diverse metazoans in freshwater habitats, and these properties bestow exceptional significance to their role in the environment. An array of functional roles has been attributed to them: they are grazers on bacteria and primary producers, regulators of decomposition of plant material, predators, prey for other animals, and closely associated symbionts of bacteria and other organisms.

Freshwater nematodes are central in the context of environmental monitoring, pollution assessments, global warming and food webs, and this is increasingly being recognized. Moreover, the short generation time (a few days to months) of many species makes nematodes ideal for laboratory studies. This book:

- Provides a follow-up to *Freshwater Nematodes: Ecology and Taxonomy* (2006).
- Offers guidelines for studying the ecology of free-living nematodes, including detailed protocols and case studies.
- Promotes free-living nematodes as model organisms for studies in a broad range of research fields.

Despite the recognized importance of nematodes across ecosystems, many species of free-living nematodes have yet to be discovered, and essential knowledge gaps remain. *Ecology of Freshwater Nematodes* provides an overview of research efforts in this field, and is an important resource for researchers in the field of nematology and ecology

Cover image credit: Walter Traunspurger