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THE USE OF NEMATODES IN MARINE ECOTOXICOLOGY

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ABSTRACT

Free-living nematodes, whether marine, freshwater, or terrestrial, have a life cycle that is normally very entrained to their environments. Their feeding mechanisms are such that they take in both the liquid-phase and the particulates in their environment, and are readily influenced by these factors. The pattern of growth of either natural or laboratory populations of nematodes is a very good indicator of the presence of toxic materials in the environment.

There are two distinct strategies for the use of nematodes as indicators of marine ecotoxicology : the first approach involves the monitoring of nematode populations in situ. Two distinct populations responses of marine nematodes to field exposure to contaminants are observed. Most surveys report that the total numbers of both individual nematodes and the number of nematode species increase in contaminated marine ecosystems, relative to other benthic species. This is due to the fact that the overall survival, growth and reproduction of nematodes is more resistant to most toxic agents than in other invertebrates. The LC50s for nematodes are much higher than LC50s for most other invertebrates. On the other hand, the populations structure of nematodes in contaminated ecosystems is quite different from the structure in uncontaminated ecosystems. The individual

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growth rates and lifespans are decreased in contaminated ecosystems, resulting in populations with a much higher proportion of juvenile stages.

The second approach to marine ecotoxicity involves the utilization of nematodes as laboratory indicators of net toxicity and as a biological monitoring system in the process of identifying the toxic components in a contaminated ecosystem. Nematode bioassays can be performed on water or sediment fractions from the real ecosystems to determine net toxicity, on known dilutions of specific compounds to determine the pattern of toxic effect to the chemical, or on fractions of samples from marine environments to determine the class of chemical contributing to the toxic effect. Laboratory-based nematode bioassays can determine lethal, semilethal, developmental and genotoxic effects.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Nematodes, Review.

INTRODUCTION

Nematodes have not been extensively used as either laboratory or field indicators of toxic effects. This is surprising, since it has been argued that nematodes are the most abundant metazoans in marine (littoral, estuarine, coastal, and oceanic) sediments (Nicholas, 1975), usually comprising more than 90 % of the metazoan fauna (McIntyre, 1969). Both in terms of the numbers of individuals and the number of species present, nematodes represent one of the major groups present in marine environments. Nematodes have also become widely used experimental organisms, used in laboratory studies as in simple model biological systems. Since the publication in 1974 of Brenner's paper on the genetics of Caenorhabditis elegans (Brenner, 1974), free-living nematodes have become widely used experimental organisms (Zuckerman, 1980) in developmental biology, neurobiology, and genetics, greatly adding to the understanding of the basic biology of this group of organisms. There is a tremendous potential for utilization of nematodes as indicators in marine toxicology. We will examine some of this potential in this review.

Toxicological studies using nematodes fall into two broad classes. First because they are important components of marine ecosystems, they can be used as indicator species for determining damage to the specific ecosystem ; nematodes can be used to obtain ecologically relevant information. Secondly, because there are well-established laboratory stocks of nematodes, nematodes can be used as laboratory "yardstick" test-organisms, of use in determining the relative toxic potential of specific contaminants, or in the evaluation and priorization of samples from a contaminated ecosystem.

We will first review the "life-strategy" of nematodes, presenting both sensitive and insensitive parameters of interest in marine ecotoxicology, then review the use of nematodes in both laboratory and field ecotoxicological studies.

NEMATODE LIFE-STRATEGIES

ANATOMY OF SIGNIFICANCE IN ECOTOXICOLOGY

The anatomical organization of nematodes consists of two concentric cylinders. The innermost cylinder is the digestive system, while the outermost cylinder, consist of an internal layer of muscle tissue and an external layer of hypodermal tissue. The hypodermis secretes a collagenous cuticle, providing a significant barrier to transport of dissolved substances between environment and hypodermis. The digestive tube consists of three distinct regions. The anterior portion of the digestive tube, the esophagus, has a cuticle-lined lumen. At the posterior end of the esophagus is a valved muscular pharynx, normally functioning as a pump, bringing material in through the mouth, drawing material through the esophagus, and passing the material through the pharynx to the intestine, the posterior portion of the digestive tube. Absorption of material only occurs in the intestine. Under adverse conditions, the pharynx ceases pumping, preventing the passage of materials to the intestine, thereby blocking the entry of materials and exposure of tissue to materials from the environment (Popham and Webster, 1979a).

DEVELOPMENT AND DEVELOPMENTAL STRATEGIES

Nematodes typically have significant barriers between themselves and their environment during all stages of development. The developing embryo is normally enclosed within a highly impermeable eggshell, and the juvenile that emerges from the eggshell has its own fully-developed cuticle. Postembryonic development involves growth through four juveniles stages through to the adult stage. The juvenile stages are usually designed as L1, L2, and L3. In some species the L1 emerges from the eggshell, while in other species the L2 emerges from the eggshell. The transition from one stage to the next is associated with molting, consisting of two distinct phases : cuticle formation in which a new cuticle is deposited beneath the old cuticle, and ecdysis, in which the old cuticle is cast off. There is, therefore, no point in the life cycle during which the tissues of the animal are in direct contact with the environment. Therefore, there is no highly sensitive phase of the life cycle.

The later stages of postembryonic development usually have thicker cuticles, and there is a general increase in resistance to toxicants in older stages of a populations. Haight et al. (1982) demonstrated that the LC50 for copper, cadmium, zinc, chromium, and nickel was significantly greater for adults than for second stage juveniles of the free-living Panagrellus silusiae. No such difference was observed with mercury or lead, however.

Animals that have completed cuticle formation, but have not yet begun ecdysis represent the most resistant stage in the life cycle, and many parasitic nematodes arrest their development just prior to the ecdysis of a specific molt, to enter a resistent infective stage. Some free-living nematodes utilize a similar strategy and arrest their postembryonic development just prior to ecdysis when environmental conditions are suboptimal. Such an arrest functions to entrain postembryonic development to environmental conditions, arresting development under adverse conditions, and continuing development under improved conditions.

Some nematode species can enter a highly resistant "dauer" stage (Cassada and Russell, 1975 ; Popham and Webster, 1979b). In this stage, the animal reduces its activity, lowers its water content, closes its esophagus

and ceases feeding, and forms an extremely impermeable cuticle (Popham and Webster, 1979b). The "dauer" larvae will resume development to the adult stage under more favorable conditions.

The above brief discussion of the general anatomy of nematodes points out the structural features of nematodes that confer on this group an extremely high resistance to environmental contaminants. As a general rule, the nematodes are the hardiest group in a specific ecosystem. This is one of the reasons that these organisms have not been widely used in ecotoxicological studies. Under field conditions, nematode populations, as a whole, are usually the last groups to decline, in terms of the number of individuals in the populations, as a consequence of contamination. Under laboratory conditions, nematodes would appear to be very insensitive bioassay organisms. However, as we will show, nematodes provide extremely efficient laboratory and field indications of toxic effects. We will now review the laboratory and field utilization of nematodes in ecotoxicological studies.

THE USE OF NEMATODES IN TOXICOLOGICAL STUDIES

NEMATODES AS LABORATORY INDICATORS OF ECOTOXICITY

The most extensively studied free-living nematodes are Caenorhabditis elegans and Panagrellus redivivus. Both species are good laboratory organisms, with a short (approximately 4 days) life cycle, and for which standardized genetically homogeneous stocks have been established. However, there are significant differences between these two species. Caenorhabditis is a self-fertilizing hermaphrodite, that has been extremely well characterized genetically. Under adverse conditions C. elegans enters a "dauer" stage, and can survive under conditions of severe environmental stress (Cassada and Russell, 1975). This species has not been widely used for toxicological studies. Panagrellus, is dioecious and does not have a "dauer" stage, but does entrain its postembryonic development to environmental conditions. Development of P. redivivus through each molt, therefore, is an indicator of the overall quality of the environment. Both species are normally grown on agar, feeding on bacteria or yeast, but both can be grown in liquid medium. Both xenic and axenic cultures can be maintained.

There are three potential uses of free-living nematodes for laboratory toxicological studies ; 1) as biological models to examine the biological responses to specific compounds, 2) as indicators of specific biological effects ranging from acute lethality to mutagenesis, and 3) as indicators of net toxic effects of mixtures of environmental samples.

Popham and Webster (1979a) used Caenorhabditis to examine the effects of cadmium, and showed that the primary response was a consequence of a cessation of feeding ; the cadmium was not entering the animals but was promoting starvation. The cessation of feeding in response to heavy metals was also shown by Mudry et al. (1982) using P. silusiae. This cessation of feeding is a specialized response, representing an aspect of the nematode survival strategy in a deleterious environment.

Bioassays using acute lethality of nematodes as an endpoint are not sensitive indicators of the potential risk of environmental contaminants (Samoiloff, 1980). Haight et al. (1982) point out that P. redivivus is 400, 600, and 900 times more resistant than the freshwater cladoceran Daphnia magna to cadmium, mercury, and nickel, respectively, in terms of the relative acute lethal levels. However, because nematodes are so resistant, any environmental contaminant that produces lethality in nematodes should probably be considered a very high-risk material.

Because there are numerous genetically characterized stocks of both C. elegans and P. redivivus, with a large number of specific genetic markers, these species can be used to detect the mutagenic potential of specific compounds or of environmental samples. Lew et al. (1983) have developed a Caenorhabditis mutagenesis bioassay using the frequency of reversion of a mutation producing small animals as an indicator. Small animals will pass through a filter system, while revertants resulting from mutagenic events will be of normal size and not pass through the filter. The revertants will be expressed at a frequency equal to the reversion frequency. This C. elegans mutagenesis bioassays has yielded results similar to those produced by the Ames test with Salmonella typhimurium (Ames et al., 1973), and the nematode appears to have the metabolic components, lacking in the bacteria, that convert promutagens to mutagens. A mutagenesis bioassay using P. redivivus has also been reported by Samoiloff et al. (1980). This assay detects the frequency of recessive X-linked lethal mutations to determine the induced mutation rate.

These mutagenesis bioassays using nematodes are rapid, relatively inexpensive, and are of use in detecting mutagenesis in multicellular animals, bridging the gap between the bacterial Ames test and expensive, long-term mammalian studies. However, while the technologies for detecting mutagenesis are among the more sophisticated bioassay methods, mutagenesis is much more a human health problem than it is an ecotoxicological problem, in that the consequences of mutagenesis are of little significance to the survival of a population as a whole.

Samoiloff and co-workers have developed and utilized a sensitive general toxicity assay using Panagrellus redivivus. This assay exploits the fact that P. redivivus entrains its development to environmental conditions. Under adverse conditions, there will be an increased number of animals that arrest their development at a specific molt. The bioassay uses a synchronously growing population of nematodes, started as a population of L2 animals. The population is grown in the presence of tested material under standard conditions until approximately 50 % of a parallel control population reaches the adult stage, usually after a period of approximately 96 h. At the end of the growth period, the total number of animals and the number of animals at each postembryonic developmental stage is recorded for both the exposed and control populations. Since the numbers of individuals in each population is known at the start of the growth period, the percentage survival of test and control populations can be determined. Three parameters of growth are also determined for each population. These parameters are : 1) P1, the frequency at which second stage juveniles of the initial population molted to the third juvenile stage, 2) L2, the frequency at which those animals that reach the third juvenile stage molt to the fourth stage, and 3) P3, the frequency at which those animals that reach the fourth juvenile stage complete the molt to the adult stage.

These three parameters have proven to be sensitive indicators of the overall toxic effect of the tested sample. Reduction of P1 and P2 of test populations, relative to the values in the control populations, reflects toxic effects that inhibit the overall metabolism of the animals, while reduction of P3 in test populations, relative to the control value of P3, with no reduction of P1 and P2, indicates an inhibition of the utilization of genetic information, by either mutagenesis, inhibition of transcription, or inhibition of translation. This distinction can be made due to the fact that it is primarily at the final molt that extensive gene activity occurs

(Samoiloff, 1980). The selective inhibition of P3 produces an effect termed phenotoxicity reflecting the fact that this toxic effect interferes with the normal flow of genetic information.

The P. redivivus bioassay can therefore discriminate three classes of effects : lethality, inhibition of normal physiological function, and phenotoxicity. Each of these effects can be quantitatively measured, and the combined effects can be expressed as a single value, termed "fitness".

The P. redivivus bioassay was initially used to determine the toxicity of numerous organic compounds and heavy metals (Samoiloff et al., 1980). The test is rapid, simple, and cost-effective, producing results comparable to those obtained by more expensive, more time-consuming bioassays. The P. redivivus bioassay has its greatest applicability, however, for examining the toxic effects of complex mixtures obtained from environmental samples.

By providing quantitative data, covering a wide range of toxic effects, the P. redivivus bioassay has been used to determine the relative toxic effects of sediments in a highly contaminated river system (Samoiloff et al., 1983a). The sediments were extracted and eight fractions prepared by differential solubility. Each fraction was tested using the P. redivivus bioassay, and the effects of each fraction ranked on the basis of observed toxic effect. Those fractions producing significant lethality in the P. redivivus bioassay are higher priority contaminants than those producing inhibition (second priority), or phenotoxicity (third priority). The major components of each fraction were than tested and priorized using the P. redivivus bioassay. This has permitted the priorization of contaminants, on the basis of the importance of the biological effects, in this ecosystem. As well, the toxicity of the fractions obtained over a series of sites was determined, to establish the spatial distribution of contaminants and to aid in establishing the point sources of the contaminants.

The P. redivivus bioassay has also been used to localize toxicity in fish tissues resulting from both heavy metal and organic contaminants (Samoiloff et al., 1983b). The long-term effects of mercury contamination and subsequent clean-up efforts was observed by examining the toxicity of extracts of nervous tissue predatory fish of varying age from the contaminated river system, and the effects of a spill of a nonpolar organic

contaminant could be observed in fatty tissue of bottom-feeding fish downstream of the spill, compared to the same tissues from fish upstream of the spill.

Bogaert et al. (1984) developed bioassays parallel to the P. redivivus bioassay, using the marine nematode species Monhystera microphthalma and Diplolaimelloides bruciei. These species also entrain their postembryonic development to their environmental conditions, and have proven to be relatively sensitive bioassay organisms, yielding results comparable to the P. redivivus bioassay. However, as these species have a slightly longer growth period, (7 days) compared to P. redivivus (4 days), some speed of bioassay is lost. These studies demonstrate, however, that marine nematodes can be used for laboratory bioassays.

These laboratory bioassays using nematodes show promise for ecotoxicological studies, since they do focus on complex mixtures from the environment, and measure a range of biologically significant toxic effects. However, care must be excercised in extrapolating these results to actual field conditions, where conditions such as bioavailability, microbial conversion, and oxidizing conditions may be quite different from the controlled laboratory growth conditions. The laboratory assays using nematodes must be considered to be "yardsticks" upon which ecological studies can be based.

THE USE OF NEMATODES IN FIELD BIOMONITORING

Nematodes offer several major advantages, and several disadvantages for use in field studies. One feature, that is both an advantage and a disadvantage is the high species diversity of nematodes in a particular ecosystem. The number of species present in any one habitat is usually an order of magnitude greater than for any other taxon (Platt and Warwick, 1980), and there are about 4 000 species in 450 genera described(Heip et al., 1982). This makes identification very difficult for a nonspecialist, and there is only a limited number of nematode taxonomists specializing in free-living nematodes. The diversity of nematodes is compounded by the very complex and confusing systematics of nematodes. However rarely will surveys for biomonitoring require the identification of nematodes in the field to the species level.

The diversity of nematodes within an ecosystem is due, in part, to the fact these animals occupy a range of different trophic levels. Free-living nematodes occupy many different roles in aquatic ecosystems as consumers of bacteria, as grazers of primary producers, and as predators (Herman et al., 1984). Wieser (1953) divided the marine nematodes into four feeding groups according to the structure of the buccal cavity. These groups are : 1) selective deposit-feeders, 2) nonselective deposit-feeders, 3) epigrowth-feeders, and 4) predators and omnivores. Because of this great diversity within an ecosystem, nematodes offer promise as indicator species.

Within this diverse group are species with generation times ranging from days to one year. Obviously, the consequences of contamination on the species composition will be different between those species with a very short life-cycle and those with very long life cycles, especially in view of the fact that the sensitivity varies through the life cycle.

Many physical properties of the ecosystem will affect the diversity of nematode populations in the field. There is a very strong influence of sediment characteristics on population density, and the diversity of species, genera and families of nematodes over several trophic levels (Heip et al., 1984 ; Herman et al., 1984). Futhermore, there are seasonal fluctuations in the species composition of estuarine nematode communities. Tietjen (1969) found variation in two New England estuaries, with epigrowth-feeders reaching maximum densities in spring and summer, and deposit-feeders and omnivores reaching their maximum in fall and winter. Warwick and Price (1979) on the Isles of Scilly, UK, and Skoolmun and Gerlach (1971) in Bremerhaven, West Germany, found a fairly constant species composition over the year. The biomass however was lowest in late autumn to early winter, peaked in May and declined again towards minimum. Earlier Warwick (1977) found in a littoral phytal nematode association detrivivores, omnivores, deposit-feeders more abundant in spring, and epigrowth-feeders predominant later in the year. There is also a significant influence of bottom temperature on species composition (Tietjen, 1976). This variability, coupled to the patchy distribution in nature (Vitiello, 1968 ; Gerlach, 1977) might interfere with sampling.

Tietjen (1977) reports that two basal faunal units have been found repeatedly for nematodes in shallow subtidal systems. In the "mud unit" there is a high species dominance, low species diversity, and low species endemism, while in the "sand unit" there is low species dominance, high

species diversity, and high species endemism. Tietjen gives habitat preferences on the family level that can be extended to nearly all coastal regions examined.

Several generalizations about the responses of natural nematode communities to pollution can be made (Vanderhorst and Wolfe, 1980). The intertidal nematode fauna is more easily affected than subtidal nematode fauna. Following contamination nematodes are often the only surviving metazoans, and often there is an increase in populations of tolerant nematode species. In contaminated ecosystems, the nematode communities contain fewer species, genera, and families. There may be an invasion of fine-sediment species into the polluted larger-grain sediment.

The nematode abundance and diversity in the intertidal zone was still decreased 7 months after the Amoco Cadiz spill (Boucher, 1980). However no decrease was a 2 to 4-fold increase in population density of the tolerant species. Renaud-Mornant and Gourbault (1980) found a similar increase in nematode population density, with little damage to the meiofauna in general. Elmgren et al. (1980) found that after the Tsesis spill all meiofauna was reduced except the nematodes.

Van Damme and Heip (1977) reported that in an area with a mixed type of pollution in the Southern Bight of the North sea, nematodes were often the only meiobenthos present in the samples. An analysis of the meiofauna of the Belgian waters presented in Herman et al. (1984), demonstrates that the distribution of the various nematode feeding types and the species diversity are highly correlated to the sediment composition. Based on cluster analyses using all families of nematodes found in the samples the Southern Bight was divided into six zones which show a strong correlation to the sediment composition. In these waters the nematodes comprise 95.7 % of the total fauna, with the harpacticoids being the second most abundant group (2.5 %). The Belgian coastal area is characterized by a large amount of non-selective deposit-feeders. The latter represent over 95 % of the nematode population in the very polluted coastal area northeast of Ostend (silty sand). In the less polluted southwest region of Ostend (silty sand) epigrowth-feeders and omnivores gain some importance. In the open sea (fine sand as well as coarse sand) the four feeding types are more equally distributed, with epigrowth-feeders being the most numerous. The number of nematode species is much lower in the region northeast of Ostend ($x = 5.4 \pm 1.5$) than in the region southwest of Ostend ($x = 14.3 \pm 1.9$). The number of families is also

significantly higher southwest of Ostend. Only three to seven families were found in the strongly polluted area northeast of Ostend compared to 11.9 families (± 3.1) in the southwest area.

Lorenzen (1974) found only short-term effects of industrial wastes (10 % H_2SO_4 and 14 % $FeSO_4$) on the nematode community. Tietjen (1977, 1980) found that nematode species that normally are characteristic of silty bottoms (fine sediments) establish themselves in medium sands with heavy contamination of heavy metals and/or organic matter. Several genera disappear from the medium sands to the advantage of the species usually inhabiting the fine sediments.

Howell (1982, 1983) studied the incidence of copper, lead, zinc, cadmium, and mercury in specimens of two Enoplus species collected from three sites on the northeast coast of England (Budle Bay, the Blyth estuary, and the Tees estuary). He concluded that the metal concentrations measured in the nematodes fell close to values reported for other organisms collected in the same area, and were higher in the Blyth estuary and the Tees estuary, two polluted sites, than in the unpolluted Budle Bay.

Recognizing the need to include meiofauna in biomonitoring programs, but realizing the high degree of taxonomic expertise required for the identification of pollution-sensitive genera or species at this group, Raffaelli and Mason (1981) examined the response of nematodes and copepods in the meiobenthos to sewage pollution and concluded that the ratio of nematodes to copepodes in the sediment could be a useful biomonitoring tool. There was a negative correlation between the ration of nematodes to copepods and the median particle size of the sand. Ratios from clean sampling stations were, however, always less than 100, while those from polluted sampling stations were always more than 100. Seasonal variation in the ratios were small. Between-core differences however could be marked. The observed high ratio on polluted beaches can be explained by an increase of deposit-feeding nematodes growing on the large amount of added organics and a decrease of the more sensitive copepods. The use of the ratio for biomonitoring might not be usable for such events as oil pollution, although data from the literature show a high ratio after oil pollution and a subsequent decrease to pre-pollution values (Boucher, 1980). However, copepods may be absent from unpolluted very fine sediments and beaches which are highly organically enriched.

If meiofauna are to be used in biomonitoring programs then they should respond to pollution before pollution is obvious through visual detection or through detection of an effect on the macrofauna. Warwick (1981) proposed a refined nematode/copepod ratio for biomonitoring using meiofauna, whereby a predicted nematode/copepod ratio is compared to an observed ratio. To compensate for the various effects of the granulometry of the sediment on the distribution of body size of copepods and nematodes, a weighed mean size of an average copepod and an average nematode for each site has to be determined. From the latter the respiration rate is calculated of nematodes and copepods on each site. Assuming that the population respiration is proportional to the adult respiration, that the energy flow through both groups is equal, and that both groups are equally efficient in utilizing the various kinds of food present, a predicted nematode/copepod ratio for each site is calculated. Warwick takes only the epigrowth-feeding nematodes into account for the calculation of his ratio since copepods compete for food in the sediments mainly with 2A nematodes.

Warwick's preliminary results indicate that his ratio of 2A (nonselective deposit-feeding) nematodes/copepods is more sensitive than Raffaeli's ratio. Warwick's ratio suggests that an indication of pollution might be given by ratios of approximately 40 for fine sediments, and approximately 10 for sands, while Raffaeli's ratio identifies a site as polluted only if the ratio is larger than 100. The application of Warwick's methodology, however, also involves more work and requires a fair amount of taxonomic expertise by the surveyer.

Coull et al. (1981) contest the validity of the use of Raffaeli's ratio as a biomonitoring tool since errors can be introduced due to the patchy distribution of the meiofauna in the sediments (Vitiello, 1968), daily, tidal, and seasonal variations in meiofauna distribution or density, selective seasonal predation of copepods, and a nonuniform reaction of copepods to environmental stress. Coull et al. (1981) state that the assumption that all species of such a varied group as the copepods have a single type of response is misleading (as it would be for nematodes), especially when considering the diverse nature of their life-history characteristics and physiological adaptations. With the present knowledge of meiofauna these authors doubt that a method that reduces the very complex set of interactions to a single ratio is meaningful.

The major drawbacks of the use of the nematode/copepods ratio for biomonitoring are the following : 1) the distribution of nematodes and copepods in the sediment is very patchy, 2) sampling procedures will affect the observed ratios, 3) meiofaunal density ratios vary with season, and 4) seasonal predation on copepods will influence the observed ratios. Raffaelli (1981) emphasizes his earlier conclusion (Raffaelli and Mason, 1981) that it is unlikely that the nematode/copepod ratio in its original form, could be used by any but the most naive statutory monitoring body. He believes, however, that the potential of this indicator should be further explored. Raffaeli (1981) calculated Warwick's (1981) improved ratio of 2A nematodes/copepods for the sites studied in Raffaeli and Mason (1981), and concluded that the observed ratio ranks the sites, except for some details, in a similar order. While Warwick's work adds a theoretical base to the ratio it is questionable whether the limited precision gained justifies the increased cost and effort required to determine the more refined ratio.

Amjad and Gray (1983) tested the validity of the nematode/copepod ratio along a known gradient of organic pollution in the Oslofjord (Norway). The results indicate that the pollution gradient in the Oslofjord can be expressed as a trend in increased numbers of nematodes or, conversely, in decreased numbers of copepods, and that the ratio merely amplifies this trend. No correlation was found between the ratio and any sediment parameter. The latter is in accordance with the findings of Coull et al. (1981). The nematode/copepod ratio in the Oslofjord shows the same trends as macrofaunal data. The determination of the nematode/copepod ratio is more complicated and laborious than using macrofauna as indicators. Amjad and Gray (1983) consider the nematode/copepod ratio a possible useful additional tool to those already available. They are much in support of the use of taxon diversity as a biomonitoring tool as proposed by Van Damme and Heip (1977), mainly because of the simplicity of the method.

Field studies have examined either the species distribution, or the population sizes, and have not focused on the stage distribution of the nematodes within a population. Exposure to contaminants has, however, distinct effects on the stage distribution of natural populations. On one hand, in those species that entrain to their environment, there will be a cessation of the postembryonic development, resulting in a higher proportion of juveniles relative to adults. On the other hand, because the adult stage is more resistant than the juveniles, the more sensitive juvenile stages will die off more rapidly under lethal conditions than the adults, resulting

in an increased number of adults relative to juveniles, but an overall decreased population size. A decrease in the number of adults relative to juveniles in an indicator of sublethal effects, while a decrease in the number of juveniles relative to adults is an indicator of lethal effects.

CONCLUSIONS

This review has examined the uses of nematodes as indicators in ecotoxicological research. The major operational consideration must be the fact that nematodes are extremely resistant organisms. They are not, however, unresponsive to their environment, and the utility of this group in ecotoxicology will very much depend on our ability to detect these responses. The P. redivivus bioassay, as a yardstick indicator of toxicity in complex samples, provides an example of the exploitation of a nematode response to toxic materials that has widespread potential application in ecotoxicology for localizing, identifying, and priorizing contaminants and contaminated sites.

In biomonitoring studies, nematodes, as resistant populations, can be compared to more sensitive groups of organisms, as e.g. in the nematode/copepod ratio. While this feature has not yet been explored, the changes in stage distribution in natural populations, might be much better contamination indicators than the reduction in either the number of individuals in a population of the number of species in a community.

We hope this short overview will stimulate further interest in the use of nematodes as both field and laboratory indicator-species in ecotoxicology.

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