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Life History and Feeding Habits of the Marine Nematode, *Chromadora macrolaimoides* Steiner* **

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Summary. *Chromadora macrolaimoides* Steiner, a free-living nematode present in the aufwuchs assemblages of several marine macrophytes located in North Sea Harbor, Southampton, New York, was isolated from *Enteromorpha intestinalis* and established in laboratory culture, where its life history and feeding habits were studied. Under the experimental conditions (25 C and 26‰ S) the worm has an average generation time (22 days) and average life span (45 days) similar to other chromadorids which have been studied in the laboratory.

Tracer-feeding experiment with ^{32}P-labelled bacteria, diatoms and chlorophytes indicate selectivity by the worm in both the ingestion and apparent digestion of potential food organisms, with the diatoms and chlorophytes being the preferred foods. Out of a total of 20 species of algae and 14 species of bacteria, two species of diatoms (*Nitzschia acicularis* and *Cylindrotheca closterium*) were found which are capable of sustaining indefinite growth. Bacteria-free culture has not been established, however, due to the extreme sensitivity of the worm to antibiotics.

A comparison of the feeding habits of *C. macrolaimoides* with *Rhabditis marina*, another marine nematode fed the same potential food organisms is made, and the influence of selective feeding on the spatial and temporal distribution of marine nematodes is discussed.

Introduction

In order to asses the importance of free-living nematodes in the marine benthic environment, qualitative and quantitative data on the feeding habits, nutrition and life histories of these omnipresent organisms are necessary. Despite a recent surge of interest in the ecology of these and other meiobenthic organisms, detailed information on the nutrition and life histories of marine nematodes is scant.

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Von Thun (1968) has summarized the early studies of Allgen, Bütschli, Cobb and others on the feeding habits of marine nematodes, studies which were based on the observations of gut contents. Perkins (1958), Hopper and Meyers (1967), and Tietjen (1969) also have given information on nematode feeding habits based on gut content studies but, as noted by McIntyre (1969) and Tietjen (1969), conclusions on feeding habits and food preferences based on gut content studies alone may be misleading because food materials of known origin are rarely found in the gut.

In order to accurately ascertain what nematodes are eating, the organisms must be maintained in laboratory culture on known food organisms. Marine nematodes have been maintained in laboratory culture on bacteria and yeasts (Chitwood and Murphy, 1964; Hopper and Meyers, 1966a, b; Tietjen, 1967; Tietjen *et al.*, 1970; Tietjen and Lee, 1972; Gerlach and Schrage, 1971), and algae (Chitwood and Timm, 1954; Webb, 1956; Tietjen, 1967; von Thun, 1968). Tietjen *et al.* (1970) have maintained *Rhabditis marina* in monoxenic culture with a single bacterium (*Pseudomonas* sp.) for over four years at the time of this writing, and currently have the organism in axenic culture. To our knowledge, *R. marina* is the only marine nematode for which quantitative data on feeding habits are available.

More information is extant on the life histories of marine nematodes. Gerlach (1971) has summarized all of the data available up to 1970 and, more recently, Gerlach and Schrage (1971, 1972), Tietjen *et al.* (1970), and Tietjen and Lee (1972) have shown that the life histories of marine nematodes may vary significantly in response to variations in temperature and salinity.

As part of our continuing study of the meiofauna occurring as aufwuchs on marine macrophytes, we are examining the nutrition and life histories of marine nematodes. This paper will report on the nutrition and life history of one nematode, *Chromadora macrolaimoides* Steiner, which was isolated from collections of the aufwuchs present on *Enteromorphia intestinalis* in North Sea Harbor, Southampton, New York.

Methods

Isolation. Small samples (0.2 g DW) of *E. intestinalis* and its epiphytes were collected and brought to the laboratory. Aliquots of these samples were streaked out on agar plates composed of solid differential growth media (see Lee *et al.*, 1970; Tietjen *et al.*, 1970; for media composition) and incubated in front of a light bank at 20–25 C. The samples were examined 2–3 times weekly, and those aliquots showing good growth were separated and the worms subcultured in tissue culture flasks, petri dishes or thin agar slants incubated on their sides.

The nematodes to be inoculated into fresh culture media were washed in 9-hole Pyrex spot plates containing sterile sea water, and transferred to fresh media with potential food organisms (algae and bacteria) carried over from the original cultures. It was found that *C. macrolaimoides* grew best in a medium consisting of an artificial sea water base plus soil extract (see Lee *et al.*, 1970; Tietjen *et al.*, 1970). Once established in continuous culture with one or more species of algae and bacteria serving as food source, it became possible to study the animal's nutrition and life history.

Nutrition. The basic tracer-feeding technique of Lee *et al.* (1966), as modified by Tietjen *et al.* (1970), was followed. Briefly, the technique is as follows: Experimental animals were harvested from stock cultures, washed by serial transfer in sterile sea water and transferred to 20×125 borosilicate experimental test tubes containing 10 ml of sterile Millipore-filtered (HA, 0.45 μ) sea water. All experimental animals inoculated into each tube were of approximately the same size, and each tube contained 20–25 nematodes. The experimental cultures were incubated under conditions identical to those of the stock cultures for 24 hours, to starve the animals before feeding.

The organisms to be tested as potential food sources were grown in appropriate media with ^{32}P added as label. After incubation, the potential food organisms were harvested by centrifugation, aseptically washed and diluted to concentrations of 1×10^6 or 1×10^7 cells /ml. The food organisms were labelled with $\sim$0.1–5.0 dpm/organism and placed in culture with the experimental nematodes. After 24 hours when bacteria were used, or 72 hours when algae were used, the nematodes were harvested, washed, and transferred to scintillation vials, where they were counted suspended in a PPO-POP-POP-Cab-O-Sil counting mixture. Dead nematodes were used as controls. After measuring the uptake of labelled foods, the number and weight (μg) of food organisms ingested per nematode per day were calculated.

Life History. Again, the basic technique outlined by Tietjen *et al.* (1970) was followed. Single individuals of *C. macrolaimoides* were inoculated into each well of 9-hole Pyrex spot plates. Each well was filled with 0.1 ml of solid Erdschreiber medium overlaid with a thin film of sterile sea water. A small inoculum of the animal's preferred food source (as obtained from the results of the tracer-feeding experiments) was added to each well. Daily observations on the growth, molting, reproduction, egg development, etc. were made according to methods outlined by Tietjen *et al.*

Results

Life History

Observations on the life history of *Chromadora macrolaimoides* were made at 25 C and $26^0/_{00}$ S. Eggs are deposited by the females either singly or in pairs and, at the time of deposition, have an average measurement of 26×38 μm. The total number of eggs deposited per female ranges from 9 to 18, and averages 9–11. Egg deposition begins 24–48 hours after copulation, and continues over a 4–5 day period. The eggs generally hatch about four days after they are deposited; at the time of hatching the worms have an average length of 115–123 μm. The first molt occurs two days after hatching (Fig. 1). Three additional molts follow, the last

one in the females occurring about nine days after hatching at a length of 510 μm, the last one in the males occurring 11 days after hatching at a length of 425–435 μm. The females become secually mature about 13 days after hatching at an average length of 700–720 μm, and will continue to grow to a maximum length of 780–820 μm. Maximum length is generally attained by the 16th day after hatching. Males become sexually mature about 16 days after hatching, and attain their maximum length

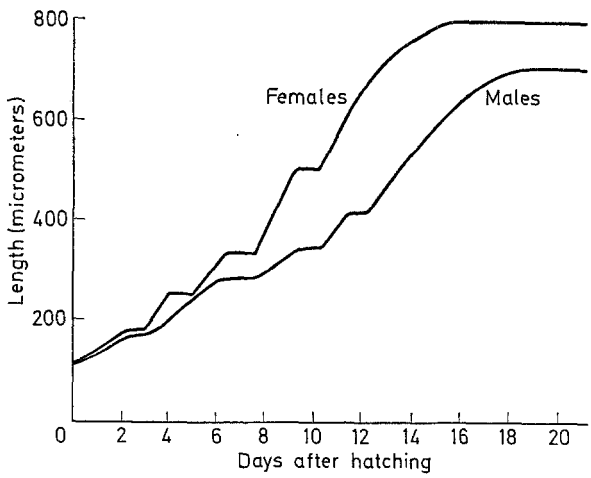

Fig. 1. Growth of *Chromadora macrolaimoides* under culture conditions (25 C and 26‰ salinity)

(700–750 μm) about 18 days after hatching. Thus the average total time of development (the time from egg deposition to onset of maximum length) averages 20 and 22 days in the females and males, respectively.

Copulation may occur at sexual maturity or anytime thereafter. During the course of these observations there was no evidence that males and females may copulate more than once, as has been observed for other marine nematodes (Tietjen *et al.*, 1970; Tietjen and Lee, 1972). Under culture conditions, approximately 90% of the eggs hatched successfully, and the female:male sex ratio was 1:1. The life span of *C. macrolaimoides* at 25 C and 26‰ S averages 45 days, and has a range of 35–54 days. Generation time (measured as the time elapsed between first egg depositions) is 18–25 days, and averages 22 days.

Table 1. Consumption of food by *Chromadora macrolaimoides*

Food organism	No. organisms nematode^{-1} day^{-1}	µg nematode^{-1} day^{-1}
Amphora sp. 1	2.4×10^3	1.4×10^0
Amphora sp. 2	1.3×10^3	2.1×10^{-1}
Amphora sp. 3	1.3×10^3	2.1×10^{-1}
Amphora sp. 4	7.9×10^2	1.3×10^{-1}
Cylindrotheca fusiformis	2.1×10^3	9.7×10^{-1}
Cylindrotheca closterium	7.0×10^3	5.6×10^0
Nitzschia acicularis	2.3×10^4	1.4×10^1
Nitzschia closterium	4.2×10^4	2.7×10^1
Chlorococcum sp.	8.0×10^2	7.5×10^{-1}
Dunaliella salina	6.9×10^2	6.5×10^{-1}
Dunaliella parva	1.0×10^3	9.4×10^{-1}
Dunaliella quartolecta	1.7×10^2	1.6×10^{-1}
Nannochloris sp. 1	1.2×10^4	2.2×10^0
Nannochloris sp. 2	1.7×10^4	2.6×10^0
SH-1	1.0×10^2	9.4×10^{-2}
94	1.7×10^1	1.6×10^{-2}
95	6.9×10^2	6.5×10^{-1}
98	3.0×10^1	2.8×10^{-2}
105	1.1×10^2	1.0×10^{-1}
Chlamydomonas subehrengerii	1.2×10^1	1.1×10^{-2}
Micrococcus sp.	background	—
Flavobacterium marinum	background	—
Pseudomonas sp.	background	—
Salmonella pullorum	background	—
Mycobacterium phlei	background	—
Escherichia coli	background	—
Alcaligenes faecalis	9.0×10^0	3.3×10^{-6}
Proteus sp.	1.6×10^1	6.4×10^{-6}
Serratia marcescens	3.0×10^0	6.6×10^{-7}
Sarcina lutea	2.2×10^1	1.3×10^{-5}
Klebsiella sp.	5.4×10^1	2.3×10^{-5}
Bacillus megatherium	4.0×10^0	8.8×10^{-6}
Streptococcus faecialis	1.2×10^1	2.4×10^{-6}
Pseudomonas fluorescens	1.5×10^1	4.9×10^{-6}

Feeding Habits and Nutrition

The uptake by *C. macrolaimoides* of 20 species of algae and 14 species of bacteria is shown in Table 1. Eleven of the 20 algal species tested (all of which occur in the same environment as *C. macrolaimoides*) were ingested at a daily rate equal to or greater then the body weight of the nematode.

Five of these were diatoms and six were chlorophytes. The two species of *Nitzschia* used (*closterium* and *acicularis*) were ingested at the highest daily rate, both on the basis of cell number and weight of cells ingested per nematode per day. The rates of ingestion, for *N. closterium* and *N. acicularis*, respectively, were: 4.2 and 2.3×10^4 cells nematode^{-1} day^{-1}, and 2.7 and 1.4×10^1 μg nematode^{-1} day^{-1}. Another way of indicating this is to say that *C. macrolaimoides* ingested, per diem, about $50\times$ and $30\times$ its own body weight of *N. closterium* and *N. acicularis*, respectively. *Cylindrotheca closterium* was next in order of ingestion on a weight basis (5.6×10^0 μg nematode^{-1} day^{-1}, or about $10\times$ the body weight of the nematode).

Of the chlorophytes, the two species of *Nannochloris* were ingested at a high daily rate with regard to cell numbers (1.7 and 2.4×10^4 cells nematode^{-1} day^{-1}), but the small size of the individual cells reduces their importance on a weight basis (these were ingested at a daily rate of about $5\times$ the body weight of *C. macrolaimoides*). Other potential food organisms ingested at daily rates equal to or greater than the body weight of the nematode included *Cylindrotheca fusiformis*, an *Amphora* species, *Dunaliella parva* and *D. salina*, a species of *Chlorococcum* and a small, unidentified chlorophyte (No. 95).

Three species of bacteria, which were isolated from the aufwuchs assemblage along with *C. macrolaimoides* (*Micrococcus* sp., *Pseudomonas* sp. and *Flavobacterium marinum*), were not taken up by *C. macrolaimoides* in quantities above background level. Other (non-marine) species of bacteria tested were taken up either at, or just above, background level (Table 1). These experiments suggest that bacteria may not constitute a significant portion of the diet of *C. macrolaimoides*, at least on a cell number or cell weight basis.

Establishment of Gnotobiotic Culture

From the above tracer-feeding experiments, several algae suggested themselves as good potential food sources (*Nitzschia closterium*, *N. acicularis*, *Cylindrotheca closterium*, and the two *Nannochloris* species). By means of aseptic washing, cultures of *C. macrolaimoides* were eventually established on the five last-named species of algae. Growth was best on *N. acicularis* and *C. closterium*, each alga by itself being able to support continuous growth for over 20 generations. When *N. closterium* or either of the *Nannochloris* species were used as sole algal food, continuous growth was supported for between 10 and 20 generations.

Despite the low uptake of bacteria by *C. macrolaimoides*, bacteria-free cultures of the nematode have not been obtained to date. Various concentrations and combinations of Colymycin, Chloramphenicol, Polymyxin B, Novobiocin, Erythromycin and Aureomycin have been used, without success, to eliminate the bacteria from the cultures. This is due to the extremely high sensitivity of the nematode to the antibiotics. *Chromadora macrolaimoides* will tolerate only extremely low concentrations of antibiotics, concentrations which are too low to eliminate bacteria from the cultures. The nematode is, in fact, killed at concentrations of antibiotic below the minimum required for the antibiotic to be an effective anti-bacterial agent. Since death of the worm occurs within 24 hours in bacterized algal culture at sub-bacteriocidal concentrations of antibiotic, it appears that death is due to the antibiotic itself and not starvation.

Discussion

Chromadora macrolaimoides attains its highest population density in North Sea Harbor in early summer, when it comprises about 10–15% of the total number of nematodes present in the area. Together with *Chromadora axi*, *Chromadorina germanica*, *Euchromadora gaulica* and other members of the family Chromadoridae it dominates the nematode assemblages present in the aufwuchs of the local macrophytes. Various members of the family have been shown to undergo seasonal variation in abundance in shallow temperate waters, with maximum abundances tending to occur in the summer months (Tietjen, 1969; Skoolmun and Gerlach, 1971). Field data from these two studies have suggested a life cycle of approximately 30 days for most species of the Chromadoridae encountered. Hopper and Meyers (1966a) and von Thun (1968) have both observed life cycles of approximately one month for several chromadorid species maintained in laboratory culture. Data obtained from the present study of *C. macrolaimoides* are in agreement with the previous studies made of this and other members of the Chromadoridae; that is, *C. macrolaimoides* has an average generation time (22 days) and an average life span (45 days) approximately equal to those that have been observed for other members of the Chromadoridae inhabiting shallow temperate waters. Furthermore, the generation time of *C. macrolaimoides* reported here corresponds quite closely to the approximated time of one month observed by Hopper and Meyers (1966a) for individuals of the same species maintained in laboratory culture in Florida.

The summertime peak abundance of *Chromadora* and other members of the Chromadoridae in shallow New England waters was attributed

by Tietjen (1969) to observed increases in the numbers of benthic microalgae and increased benthic primary productivity. The chromadorids are provided with small teeth in the buccal cavity which, according to Wieser (1953), are used to scrape algae and other food particles off sand grains and other substrata. If the chromadorids do feed in this manner, it is to be expected that they will attain their highest population densities during the summer months, when their preferred food sources are also expected to be most abundant.

The ingestion of algae by marine nematodes is well-documented. Of the 49 species listed by von Thun (1968) in his review, 35 are described as having algae or other vegetable material in their guts. In addition, Webb (1956), Perkins (1958), Hopper and Meyers (1967), Tietjen (1969) and others have observed algae in the guts of nematodes, and Tietjen (1967) observed the ingestion of small flagellates by *Monhystera filicaudata* in laboratory culture.

There is no doubt that many marine nematodes ingest algae. Two questions that are suggested, however, are: 1. Do marine nematodes exhibit any selectivity in the ingestion of algae and 2. do they utilize all of the algae which they ingest?

The only quantitative work that has been done on selective feeding in marine nematodes is that of Tietjen *et al.* (1970), who observed that *Rhabditis marina* exhibited marked selectivity in both the ingestion and apparent digestion of algae and bacteria. Although several algae and bacteria were taken up at daily rates of from one third to ten times the body weight of *R. marina*, continuous culture on a single food source was possible with only one food organism, a bacterium (*Pseudomonas* sp.), apparently indicating that the nutritional requirements of *R. marina* might be satisfied by but a few of the large number of microorganisms available to it in its natural habitat.

Observations on the feeding habits and nutrition of *C. macrolaimoides* indicate that it, too, is selective in its ingestion and digestion of algae and bacteria. Although all 20 species of algae tested were taken up to some degree, only 11 were ingested in significant quantity. Of these 11, only five were capable of sustaining growth for as many as ten generations, and only two, *Nitzschia acicularis* and *Cylindrotheca closterium*, were capable of sustaining growth indefinitely. *N. acicularis* and *C. closterium* have also been shown to be good food sources for several species of foraminifera (Muller and Lee, 1969), perhaps indicating that these two diatoms may have a higher nutritional value than other algae which are available for consumption.

The manner in which algae are ingested by *C. macrolaimoides* was not directly observed, but it is probably similar to the puncture-suction manner of feeding observed by von Thun (1968) for *Hypodontolaimus balticus*, another chromadorid. The cell walls of the larger diatoms upon which *C. macrolaimoides* fed were generally not found in their guts, but the chloroplasts were. In a case where the nematode is feeding upon a larger diatom, it probably punctures the cell wall and sucks out the cell contents, much in the manner described by von Thun. Small diatoms (those 10–20 μm in length) were frequently found ingested whole, and the ingestion of chlorophytes, especially the smaller ones, also appeared to be whole. The manner by which marine nematodes actually ingest their food remains essentially unknown at this time, however, and must be studied if we are to understand the actual methods by which these organisms discriminate among certain food particles.

A comparison of the feeding habits of *C. macrolaimoides* and *Rhabditis marina* reveals certain similarities and differences. Both nematodes are highly selective in their ingestion of algae and bacteria, and both nematodes apparently utilize but a few of the large number of food organisms available to them. All of the bacteria, and 12 of the algae included in the present study were also used in the study of *R. marina* (Tietjen *et al.*, 1970). A comparison of their uptake of mutual food sources yields the following: *C. macrolaimoides* ingested bacteria at a daily rate 2–7 orders of magnitude lower than *R. marina*, and did not ingest the preferred food source of *R. marina*, the bacterium *Pseudomonas* sp., at all. Thus it appears that bacteria may not form a significant portion of the diet of *Chromadora macrolaimoides*, at least on a weight basis, However, the bacteria may be supplying needed vitamins or other factors necessary for the growth of the worm. Unfortunately, the extreme sensitivity of the worm to antibiotics makes experiments aimed at answering this question impossible at this time.

Chromadora macrolaimoides and *Rhabditis marina* also exhibited differences in their uptake of algae. For example, *Cylindrotheca closterium*, one of the two algae found capable of sustaining the unlimited growth of *C. macrolaimoides*, was not ingested by *R. marina* at all. *Nitzschia acicularis*, the other source of food preferred by *C. macrolaimoides*, was taken up at a daily rate two orders of magnitude higher by *C. macrolaimoides* than *R. marina*. *Nannochloris* sp. 1 was ingested at the same rate by both nematodes, but while it served as a good food source for *C. macrolaimoides*, it passed through the gut of *R. marina* undigested. There were, in addition, other differences in the uptake of the other algae, but these were not as significant as those noted above.

Marine nematodes have been observed to vary in their abundance, both seasonally (Wieser and Kanwisher, 1961; Hopper and Meyers, 1967; Tietjen, 1969; Skoolmun and Gerlach, 1971), and spatially (Wieser, 1959a, b, 1960; Tietjen, 1969; Warwick, 1971; Warwick and Buchanan, 1970). Recent studies by Gerlach and Schrage (1971, 1972), Tietjen *et al.* (1970) and Tietjen and Lee (1972) have shown that the life histories of shallow-water marine nematodes may be greatly influenced by variations in temperature and salinity, variations to which the organisms are regularly exposed. If all marine nematodes are as selective in their feeding habits as *R. marina* and *C. macrolaimoides* are, then spatial and temporal variations in the abundances of benthic microorganisms, which are well-documented phenomena, could also account for the spatial and temporal variations in abundance that nematodes and other meiofaunal organisms often exhibit in shallow water.

Furthermore, selective feeding by different nematodes on different food sources would tend to reduce, or perhaps even eliminate, competition for a few chosen food sources. This would enable to co-hatitation of a small area by a relative large number of species which, on the basis of buccal morphology alone, would appear to be feeding on the same foods.

We do not know the manner by which marine nematodes may discriminate among a large number of different food particles. We do not know the role that size of the food plays in its ingestion, although it would appear that individual size of the food particle may be important. We do not know how, when confronted with two species of algae or bacteria which are identical in size and perhaps also closely related taxonomically, *Rhabditis marina* and *Chromadora macrolaimoides* discriminate between them.

In any event, data from the few experimental studies that have been made of shallow-water marine nematodes suggest that these organisms may be quite selective in their ingestion and digestion of potential food particles. Coupled with existing experimental data on responses to temperature and salinity variations, it appears that shallow-water nematodes (and probably other meiofauna) are affected by the same gross temporal changes in environment that other temperate benthic organisms are. While this may seem rather obvious, data from the necessary experiments are only now beginning to be obtained.

References

Chitwood, B. G., Murphy, D. G.: Observations on two marine monhysterids-their classification, culture and behavior. Trans. Amer. micr. Soc. **83**, 311–329 (1964).

Chitwood, B. G., Timm, R. W.: Free-living nematodes of the Gulf of Mexico. Gulf of Mexico, its origin, waters and marine life. Fishery Bull. Fish. Wildl. Serv. U.S. **55**, 313–323 (1954).

Gerlach, S. A.: On the importance of marine meiofauna for benthic communities. Oecologia (Berl.) **6**, 176–190 (1971).

Gerlach, S. A., Schrage, M.: Life cycles in marine meiobenthos. Experiments at various temperatures with *Monhystera disjuncta* and *Theristus pertenuis* (Nematoda). Mar. Biol. **9**, 274–280 (1971).

Gerlach, S. A., Schrage, M.: Life cycles at low temperatures in some free-living marine Nematodes. Veröff. Inst. Meeresforsch. Bremerh. **14**, 5–11 (1972).

Hopper, B. E., Meyers, S. P.: Aspects of the life cycle of marine nematodes. Helgoländer wiss. Meeresunters. **13**, 444–449 (1966a).

Hopper, B. E., Meyers, S. P.: Observations on the bionomics of the marine nematode, *Metoncholaimus* sp. Nature (Lond.) **209**, 899–900 (1966b).

Hopper, B. E., Meyers, S. P.: Population studies on benthic nematodes within a sub-tropical sea-grass community. Mar. Biol. **1**, 85–96 (1967).

Lee, J. J., McEnery, M., Pierce, S., Freudenthal, H. H., Muller, W.: Tracer experiments in feeding littoral foraminifera. J. Protozool. **13**, 659–670 (1966).

Lee, J. J., Tietjen, J. H., McEnery, M., Stone, R. J., Rullman, J., Muller, W. A.: Cultivation and physiological ecology of members of salt marsh epiphytic communities. Helgoländer wiss. Meeresunters. **20**, 136–156 (1970).

McIntyre, A. D.: Ecology of marine meiobenthos. Biol. Rev. **44**, 245–290 (1969).

Muller, W. A., Lee, J. J.: Apparent indispensibility of bacteria in foraminiferan nutrition. J. Protozool. **16**, 471–478 (1969).

Perkins, E. J.: The food relationships of the microbenthos, with particular reference to that found at Whitstable, Kent. Ann. Mag. Nat. Hist. Ser. 13 (**1**), 64–77 (1958).

Skoolmun, P., Gerlach, S. A.: Jahreszeitliche Flukuationen der Nematodenfauna im Gezeitenbereich des Weser-Ästuars (Deutsche Bucht). Veröff. Inst. Meeresforsch. Bremerh. **13**, 119–138 (1971).

Thun, W. von: Autokologische Untersuchungen an freilebenden Nematoden des Brackwassers. Thesis, Kiel University (1968).

Tietjen, J. H.: Observations on the ecology of the marine nematode *Monhystera filicaudata* Allgen, 1929. Trans. Amer. micr. Soc. **86**, 304–306 (1967).

Tietjen, J. H.: The ecology of shallow-water meiofauna in two New England estuaries. Oecologia (Berl.) **2**, 251–291 (1969).

Tietjen, J. H., Lee, J. J.: Life cycles of marine nematodes. Influence of temperature and salinity on the development of *Monhystera denticulata* Timm. Oecologia (Berl.) **10**, 167–176 (1972).

Tietjen, J. H., Lee, J. J., Rullman, J., Greengart, A., Trompeter, J.: Gnotobiotic culture and physiological ecology of the marine nematode, *Rhabditis marina* Bastian. Limnol. Oceanogr. **15**, 535–543 (1970).

Warwick, R. M.: Nematode associations in the Exe estuary. J. Mar. Biol. Ass. U.K. **51**, 439–454 (1971).

Warwick, R. M., Buchanan, J. B.: The meiofauna off the coast of Northumberland. I. The structure of the nematode population. J. Mar. Biol. Ass. U.K. **50**, 129–146 (1970).

Webb, M. G.: An ecological study of brackish water ciliates. J. An. Ecol. **25**, 149–175 (1956).

Wieser, W.: Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Ark. Zool. **4**, 439–484 (1953).

Wieser, W.: The effect of grain size on the distribution of small invertebrates inhabiting the beaches of Puget Sound. Limnol. Oceanogr. **4**, 181–194 (1959a).

Wieser, W.: Free-living nematodes and other small invertebrates of Puget Sound beaches, 179 p. Seattle: Univ. of Washington Press 1959b.

Wieser, W.: Benthic studies in Buzzards Bay. II. The meiofauna. Limnol. Oceanogr. **5**, 121–137 (1960).

Wieser, W., Kanwisher, J.: Ecological and physiological studies on marine nematodes from a small salt marsh near Woods Hole, Massachusetts. Limnol. Oceanogr. **6**, 262–270 (1961).

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