ATTRACTION OF THE MARINE NEMATODE, *METONCHOLAIMUS* SP., TO FUNGAL SUBSTRATES[1]

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ABSTRACT

An extremely abundant omnivorous marine nematode, *Metoncholaimus* sp., readily colonizes mycelial-cellulose mats of marine fungi in the field. The meiofauna on the fungal substrate comprises almost entirely the single species of nematode, with the majority of the large population being gravid females. Sediment samples in the immediate vicinity of the test showed proportionate sex ratio of the species. A strong attractive response by the nematode to the fungal-cellulose substrate is indicated. Striking dissimilarities in nematode colonization is noted among closely situated test sites, as well as at single sites at the same and different collection periods.

INTRODUCTION

While nematodes are probably the most abundant meio-metazoans of sublittoral sediments (Wieser, 1959, 1960; McIntyre, 1964), comparatively little is known of their bionomics and the influence of physical and biological factors on their activity and distribution. Even less information is available on the effect of the environment on reproductive processes and life cycles of particular species. In the main, tabulation of occurrences of marine nematodes has been of a survey nature rather than seasonal studies of nematode populations of certain well-defined areas.

Earlier investigations in Biscayne Bay, Florida (Meyers *et al.*, 1963, 1964) demonstrated the ability of various marine fungi to support development of a marine-isolated species of *Aphelenchoides*, a stylet-bearing nematode. Subsequently, studies were begun on the fungal infestation of subtropical seagrass communities (Meyers *et al.*, 1965) and the possible effect of this microbial activity on the nematode population present. Initial observations in 1964 in Biscayne Bay indicated the considerable abundance of a large and extremely active marine nematode, *Metoncholaimus* sp.,[2] which at times comprised the dominant meiofaunal representative of the biomass in the upper sedimentary layer. Experimental data on the ecology of this species, isolated in association with fungal substrates, are reported

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[2]A new species to be described elsewhere.

here. Behavioral characteristics of the animal under laboratory conditions have been described elsewhere (Hopper & Meyers, 1966).

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Methods

Two defined localities in Biscayne Bay were studied extensively. The major collection site (designated A) was within a turtle grass *(Thalassia testudinum* König) flat, situated between the Institute of Marine Science laboratory and the western shore of Key Biscayne. Since access to the site was possible only by boat, the community was relatively undisturbed by human activity. The flat was covered by several feet of water at low tide. The sediment consisted of a fine, loose, sandy silt with large quantities of small shells. Six permanent positions, each approximately 20 yards apart, were established across the area, and were sampled regularly from October 1964 through March 1965. In addition to *Thalassia,* vegetation at Area A included the algae, *Spyridia* and *Acanthophora,* which at times completely covered the turtle grass leaves. Water temperature was recorded during each collection.

Another collection area (designated B) was at the IMS beach just below the intertidal zone. The bottom here varied from a coarse sand, through a soft sediment overlying a hard bottom with *Thalassia, Syringodium,* and *Diplanthera,* to a hard, fine sand. Area B was sampled for a 5½-month period from February to June 1965. Marker stakes were located to indicate sites for successive collections over a period of several months.

A method employing a mycelial mat was developed to study nematode populations in the field. The two marine fungi used were *Dendryphiella arenaria* Nicot (IMS No. 505), a deuteromycetous species, and the Ascomycete, *Halosphaeria mediosetigera* Cribb & Cribb (IMS No. 115) (accession numbers in parentheses).

Both fungi form compact mycelial mats on cellulose and on glass filter paper discs in liquid culture in the laboratory. *D. arenaria* is among the dominant mycota in leaves of *Thalassia* (Meyers *et al.,* 1965). *H. mediosetigera* is abundant in estuarine waters where it rapidly attacks submerged wood and other cellulosic materials (Johnson & Sparrow, 1961; Meyers & Reynolds, 1963; Jones, 1963). Meyers *et al.* (1964) have demonstrated the ability of both fungal species to support growth and reproduction of the marine-isolated, stylet-bearing nematode, *Aphelenchoides* sp.

Fungi were grown in flasks of 0.1 per cent Bacto-Yeast Extract in Gulf Stream sea water with 4.2 cm cellulose (Millipore Filter Corp. No. AP1004700) or glass paper (Whatman GF/C) discs added to the

medium. Cultures were incubated on a reciprocating shaker at room temperature (25°C) for 8 days. This was sufficient time to permit compact uniform development of the fungus over the glass or filter paper disc. The fungal mats were harvested and washed according to previously established procedures (Meyers *et al.*, 1964). Controls consisted of uninoculated substrates.

Special plastic carriers on wooden stakes were used to expose the test material in the field (Fig. 1). The washed fungal mats, or controls, were placed within the chamber of the carrier and the openings covered by a 52 × 52 mesh Saran screen (No. 61009-00, Chicopee Mfg. Co., Cornelia, Ga.). This arrangement permitted ready internal passage of water and entry of the meiofauna present. The carriers were attached to wooden stakes at several positions, singly or in adjacent pairs. The stakes were inserted vertically in the sediment so that the fungal mat was either at the sediment surface or in the subsurface water. Care was taken to insure accurate location of the test substrates. The lower carrier was at the sediment surface while the upper one was approximately 3½ inches higher. Samples were examined microscopically in the laboratory within ½ to 1 hour of collection.

Test substrates were checked to determine approximate numbers, species, and sex of nematodes present. The freshly collected substrates were then transferred to flasks of sterile sea water and incubated at room temperature on a reciprocating shaker. Through this incubation process it was possible to ascertain the presence and growth of other nematode species either deposited as eggs in the field or overlooked during the initial examination. Furthermore, during incubation, *Metoncholaimus* sp. deposited eggs that subsequently developed juveniles.

Results and Discussion

It is noteworthy that the dominant nematode fauna colonizing the mycelial mats consisted, in the main, of a single species of *Metoncholaimus*. Furthermore, this population was composed almost entirely of gravid females of the species. Large numbers of *Metoncholaimus* sp. were observed on the test substrates in Area A from October 1964 through February 1965. Often concentrations as great as 3000 animals were tabulated. They covered both surfaces of the fungal mat as well as the interior portion of the cellulose disc. Maximal numbers of nematodes were present in the interior of the test chamber. Colonization occurred shortly following submergence of the fungal mat, often within a 24- to 48-hour period.

Subsequent incubation of the mycelial mats in the laboratory revealed the occasional development of other species of nematodes, indicating that eggs of these species had been deposited in the chambers in the field. Specimens of *Monhystera* were especially abundant after incubation. Other

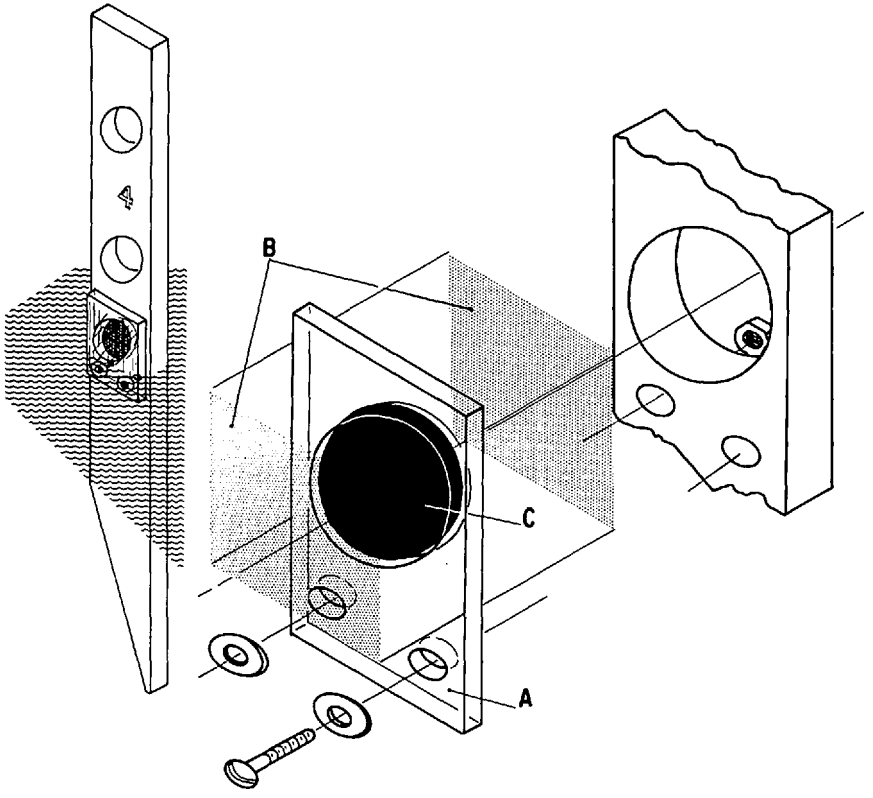

FIGURE 1. Diagram of wooden stake and plastic carrier for nematode isolation: a, plastic carrier; b, Saran screening to enclose chamber; c, test substrate, fungal mat or control.

nematodes that developed included species of *Prochromadorella, Araeolaimus, Acanthonchus, Diplolaimella, Chromadora, Symplocostoma* and *Viscosia.* Occasionally as many as four to five different genera were found in the same culture vessel.

As noted, the populations of *Metoncholaimus* sp. on the mycelial mats were gravid females, with only occasional males and juveniles. Often 100 per cent of the animals present were gravid. One mat submerged for 24 hours showed a population of 98 gravid females and one juvenile, while another submerged for 72 hours showed 599 gravid specimens, 11 males, and 11 juveniles. Fungal mats with several thousand gravid *Metoncholaimus* sp. alone were not uncommon. Regular conventional collections of the sediment in the immediate area of the test stakes showed a 50:50

TABLE 1

COLONIZATION OF *Metoncholaimus* SP. ON *Halosphaeria* MATS AT THREE POSITIONS IN AREA A*

Date Submerged	Total Time of Submergence (In Days)	Number of Nematodes Position No.		
		1	3	5
November 25, 1964	8	1500	0	500
December 11	3	10	500	1000
December 11	5	<25	100	0
December 16	7	–	2000	25
January 6, 1965	7	1000	100	200
January 15	5	500	3000	3000
February 1	2	300	<10	0
February 9	3	500	2000	0

*Selected as representative of the six positions established in this area.

ratio of males and females of the species. In addition, various species of nematodes common in the upper sedimentary layer were rarely found on the fungal mats. These data, and those discussed below, suggested that the fungal-cellulose substrate served as an attractant for almost uniform migration of gravid *Metoncholaimus*.

Cellulose discs with *H. mediosetigera* were more attractive than those of *D. arenaria*. In several tests, where as many as 3000 animals were present on *H. mediosetigera* mats, comparable *D. arenaria* substrates showed approximately 200-500 nematodes. Maximal animal colonization occurred in *H. mediosetigera* mats on cellulose located at the sediment-water interface. Tests submerged within the sediment as well as those positioned several inches above the bottom had far fewer animals. Greater colonization by *Metoncholaimus* sp. occurred when the fungal mat was "sandwiched" between two cellulose discs. A variation of this method was attempted wherein a cellulose disc was placed between two fungal mats in the chamber. In this manner, a greater mycelial surface was exposed compared to that of the single mycelial mat located between two cellulose discs. Nevertheless, the majority of the *Metoncholaimus* sp. were found on the innermost surfaces of the mat rather than on the exposed portions. This migration to the interior of the chamber may indicate a response to the protective environment within the chamber or may suggest a greater concentration of possible attractant material in this confined area.

In other tests, the fungi were grown on glass discs for comparison with the fungus on the cellulose discs. One test showed less than 100 nematodes present on the glass disc with *D. arenaria,* compared with 500 on the cellulose disc, while only 100 nematodes were present on the glass disc with *H. mediosetigera* compared with 3000 animals on the fungal-cellulose

TABLE 2

COLONIZATION OF *Metoncholaimus* SP. ON *Halosphaeria* MATS AT SITE B, APRIL - JUNE, 1965

Period of Submergence	Length of Submergence (days)	Number of Nematodes Various Submergence Positions									
		1	2	3	4	5	6	7	8	9	10
April 1	3	–	–	150	–	<10	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	40	<10
	2	0	0	50	100	–	–	–	–	–	–
April 15	4	–	–	0,20	10,50	–	–	–	–	–	–
	4	–	–	0-75*	–	–	–	–	–	–	–
April 29	3	–	–	–	20	–	–	–	–	–	–
May 1	2	–	–	–	15	–	–	–	–	–	–
	4	–	–	–	50	–	–	–	–	–	–
	5	–	–	–	25	–	–	–	–	–	–
	6	–	–	–	20	–	–	–	–	–	–
	11	–	–	–	<5	–	–	–	–	–	–
May 15	4	0	150	0	15	1	150	50	<5	25	–
	3	–	0,50	–	–	–	<5,75	–	–	–	–
May 30	4	5	0	<10	15	<10	300	<10	0	50	–
June 1	4	10	40	15	0	10	30	15	0	40	–
June 11	4	1	0	0	100	100	200	300	50	100	–

*Represents a series of 1-6 stakes with 0, 0, 10, 20, 25, and 75 animals present respectively.

mat. Similar results were obtained elsewhere indicating that the fungus alone, i.e., on the glass paper disc, was far less effective as a substrate for colonization by *Metoncholaimus* sp. than was the fungal-cellulose combination.

Control discs of cellulose and glass paper usually gave negative results or showed significantly less numbers of animals than were present on the fungal-cellulose mats. In those few instances where as many as 1000 *Metoncholaimus* sp. were found on the control discs, considerable sedimentary material was present on the substrate surfaces. It is possible that this material allowed enhanced bacterial activity and stimulated migration of *Metoncholaimus* in a manner comparable to that observed for the fungal mats.

Great irregularity occurred in numbers of *Metoncholaimus* sp. at the various established positions within Area A over the period of collection. Similar variability in colonization was observed between three closely situated positions within the area (Table 1). Striking shifts in numbers of nematodes at individual positions occurred within periods of less than one week, as indicated by the tremendous increase in numbers of specimens at positions 3 and 5 during the period January 6-15. At position 5, the absence of *Metoncholaimus* sp. after 5 days submergence of *H. mediosetig-*

era mats is noteworthy compared with the large number of animals found after 3 days exposure. It is apparent that the nematode fauna is an extremely dynamic population affected by a complexity of environmental factors. While the topography and vegetation of Area A appeared rather uniform, it is possible that micro-differences in the physical and biological characteristics of the sediment affected the concentration and movement of *Metoncholaimus* on the fungal mats. Elsewhere, Weiser (1959) showed that the nematode fauna varies in several respects with the degree of exposure, content of organic debris, and particle size of the substrate.

At Area B, along the Institute of Marine Science beach, similar striking differences in colonization of fungal mats occurred at positions less than 12 feet apart as well as at adjacent stakes (Table 2). As noted, this area varied from a coarse sand through a soft sediment overlying a hard bottom, with *Thalassia, Syringodium,* and *Diplanthera* growth, to a hard, fine sand. In general, most of the animals were found in the softer, looser areas with vegetation present. The 96-hour test on April 15, representing a series of six stakes within a 2-feet square site, demonstrates the dissimilarity in number of animals tabulated on the mats. The concentration of animals during the early spring period was considerably less than that noted in the previous fall and winter months. Activity and numbers of gravid females at Area A decreased in March 1965, and subsequently we have not been able to find *Metoncholaimus* either in the sediment or on fungal mats in this locale. A regular, although comparatively small, population of *Metoncholaimus* still is present at Area B. The majority of the specimens are gravid females.

Metoncholaimus sp. activity showed some correlation with changes in water temperature. The species was first isolated in large concentrations in October 1964 (28°C), and was found regularly on the fungal mats during the subsequent period of decreasing water temperature (28°-18°C). Maximal number of gravid females coincided with the period of minimal water temperature, i.e., 18°C, during January 1965, when a striking drop in water temperature of more than 5°C occurred within a short period, followed by an equally rapid rise. The possible effect of water temperature on the periodicity of the animals, particularly in view of the total absence of *Metoncholaimus* from Area A, is now being examined.

The response of *Metoncholaimus* sp. to the fungal mats does not appear to be one of feeding in view of the predominance of gravid females noted. If mere feeding were involved, a similar number of males and females would be expected. Furthermore, the attractive effect is not solely a substrate response, for, except for occasional tests, the cellulose and glass disc controls gave negative results. The attraction is more likely a response to substance(s) emitted from the fungal cellulose complex as it undergoes degradation, for the fungal mycelium alone appeared ineffective. The

attractant(s) may originate directly from the fungal-cellulose complex or from the associated microbiota that rapidly colonize the substrata following submergence. Further tests will include a greater number of fungi species, of both marine and terrestrial origin, especially a comparison of cellulolytic and non-cellulolytic species. Studies are in progress to characterize more definitively the attractive effect reported in this paper.

Sumario

Atracción del Nemátodo Marino, *Metoncholaimus* sp., Por los Substratos Fungosos

Investigaciones nematológicas en Biscayne Bay, Florida, han demostrado extraordinaria abundancia del nemátodo marino *Metoncholaimus* sp., tanto en el sedimento como dentro de la estera micelio-celulósica de hongos marinos sumergida en el área. Los animales encontrados en el substrato fungoso fueron predominantemente hembras grávidas, encontrándose machos y juveniles sólo ocasionalmente. Sin embargo, los machos de la especie eran más comunes en las poblaciones dentro de las zonas más altas del sedimento, adyacentes a la "trampa" fungosa, igual que otras especies de nemátodos no encontradas en las esteras. Se comprobó una reacción de poderosa atracción de las hembras grávidas de la especie por el substrato fungo-celuloso. Se notó una gran irregularidad entre las concentraciones de nemátodos en las áreas de estudio situadas muy próximas.

Se sugiere una correlación de la actividad de *Metoncholaimus* sp. con la baja en la temperatura del agua. Algunos hongos, especialmente el Ascomyseto marino, *Halosphaeria mediosetigera* Cribb y Cribb, son especialmente efectivos como atrayentes mientras otros substratos fungosos dan resultados negativos. Pruebas con muestras controladas indican que el efecto de atracción no es sólo una respuesta al substrato. La atracción parece ser una reacción a substancias que se originan directamente del complejo fungo-celuloso o a una microbiota asociada que coloniza rápidamente los substratos una vez sumergida.

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