Aspects of the life cycle of marine nematodes

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KURZFASSUNG: **Aspekte des Lebenszyklus mariner Nematoden.** Bei einigen marinen Nematoden, die in Biscayne Bay (Florida) gesammelt und im Labor gezüchtet worden sind, vollzieht sich der gesamte Lebenszyklus ungefähr innerhalb eines Monats. Bei *Acanthonchus cobbi, Chromadora macrolaimoides* und *Euchromadora gaulica* ist zweigeschlechtliche Fortpflanzung festgestellt worden. *Monohystera parelegantula* vermehrt sich parthenogenetisch. Ein möglicherweise durch Umwelteinflüsse bedingter Wechsel von bisexueller und parthenogenetischer Fortpflanzung tritt bei *Chromadora epidemos* und *Viscosia macramphida* auf.

INTRODUCTION

In spite of the abundance of marine nematodes, data on their life cycles are comparatively rare. One of the few exceptions is the report on the life history and metabolism of *Enoplus communis* BASTIAN by WIESER & KANWISHER (1960). These investigators calculated an annual life cycle for the species at Woods Hole, Massachusetts, with spawning taking place in early spring. Among the smaller-sized marine nematodes, *Monhystera disjuncta* BASTIAN (1865) has been observed to complete its life cycle within 30 days, and *Diplolaimella schneideri* TIMM (1952) in approximately 40 days, (CHITWOOD & MURPHY 1964).

Methodology using a fungus-infested cellulose matrix has recently been developed for investigation of nematode populations in the field. Prolonged periodic examinations are made possible by incubation of the exposed mats under specific cultural conditions in sea water vessels. Aspects of these procedures are discussed elsewhere (MEYERS, FEDER & TSUE 1964, MEYERS & HOPPER 1966, 1967).

Development of various species of nematodes during incubation of the mats has shown that eggs had been deposited on these substrates in the field. While *Monhystera* spp. were especially abundant, other nematodes that developed in various concentrations at different sequential periods after incubation included species of *Prochromadorella, Diplolaimella, Viscosia, Acanthonchus,* and *Araeolaimus.* As many as four or five different taxa may be active in the same culture vessel.

Based on these observations, more detailed studies were conducted on selected species of *Acanthonchus, Chromadora, Chromadorina, Euchromadora, Monhystera,* and *Viscosia.*

RESULTS AND DISCUSSION

Acanthonchus cobbi CHITWOOD (1951) is a moderately-sized species approximately 1.2 mm in length with a head diameter of 23 μ. The cyathiform stoma is armed with a sharp dorsal tooth. However, it is not known how this structure is employed by the nematode in obtaining nourishment.

A. cobbi was isolated from a fungal mat submerged for six days in a seagrass community (*Thalassia testudinum* KÖNIG) in Biscayne Bay (Miami, Florida). Nematodes were not observed initially, but after a 28-day incubation period the development of small populations of *A. cobbi* and *Monhystera* spp. were noted, along with an associated fauna of ciliates, colorless flagellates, copepods, and polychaetes. A small portion of the mycelial mat containing about 20 *A. cobbi* and an approximately equal number of *Monhystera* spp. was surface-inoculated on 0.5 % sea water agar fortified with washed cells of a marine yeast (MY-111, *Kluyveromyces aestuarii*). By 19 days only a few *A. cobbi* appeared viable, but by 48 days a heavy population of adult specimens of both genera was evident. Approximately 100 adult and 75 juvenile *A. cobbi* and several hundred *Monhystera* spp. were noted.

It is difficult to determine the actual generation time of *A. cobbi* since various stages of growth, as well as eggs, could have been originally in the mycelial mat. Nevertheless, based on the terminal population of *A. cobbi,* with a five-fold increase in population of adult animals as well as a large number of juveniles present, it appears that at least one generation of the nematode developed in the 29-day incubation period.

Males and females occur in equal numbers; thus *A. cobbi* most likely is an amphimictic species.

Chromadora macrolaimoides STEINER (1915) is 0.75 to 0.88 mm long with a head diameter of 12 to 14 μ. The 16 to 17 μ long stoma is armed at the anterior extremity by three small movable teeth. The latter are thought to be used in a scraping or rasping manner in the acquisition of food.

C. macrolaimoides is a common inhabitant of the *Thalassia* epigrowth in the Bay and is one of the most prevalent foliicolous species initially colonizing fungal mats. The latter, upon submergence for as short a period as six days, have shown large numbers of this species. At times *C. macrolaimoides* is the only nematode present. In one test, initial observation on a fungal mat exposed in the field for 28 days revealed only a few animals. Incubation of the mat for 24 days in sea water flasks produced numbers in excess of 400 sexually mature specimens suggesting that eggs of *C. macrolaimoides* had been deposited on the fungal substrate during submergence.

As all field and laboratory studies have shown the presence of relatively proportionate numbers of males and females, *C. macrolaimoides* is considered to be an amphimictic species.

Chromadorina epidemos HOPPER & MEYERS (1967) is a fairly small species, 520 to 780 μ in length with a head diameter of 8 to 9 μ. The 11 to 12 μ long stoma is armed in a manner comparable to that described for the preceding species. Likewise, the method of feeding would be similar for the two species although the larger dimensions of *C. macrolaimoides* may permit it to utilize a greater variety of food sources.

Scrapings from *Thalassia* leaves and rhizomes were incubated on a fungal substrate in sea water culture for 30 days. From an original inoculum of 11 animals, a population of over 100 individuals was tabulated. In other laboratory tests doubling of the population has occurred within two weeks, i. e., from 8 to 15 animals in 12 days to 30 specimens after subsequent incubation for an additional 2-week period. Small, static populations have been maintained in an active state for up to two months, with only a slight increase in number of specimens (9 to 15).

It is interesting to note that males were not present under these cultural conditions although they have been described from field collections. It would seem that this species can reproduce by both parthenogenesis and amphimixis.

Euchromadora gaulica INGLIS (1962) is a large animal, 1.35 to 1.52 mm long with a head diameter of 22 μ. The stoma is armed with a large, movable, dorsal tooth and opposing, fixed denticles. The species is widely distributed and has been isolated repeatedly from the epigrowth on *Thalassia* leaves and mangrove prop 'roots' in the Bay. The genus *Euchromadora* is reported to feed "especially on algae – unicellular and filamentous" (CHITWOOD & TIMM 1954).

Initial examination of fungal mats, submerged for one week, showed a small population (about 50 animals) of this species. After incubation for two weeks, about 150 nematodes were present. At the end of another two-week period, a large number of gravid females were noted with subsequent release of eggs, hatching and maturation of females and redevelopment of gravid females, the latter within 16 to 28 days. Specimens have been observed encopulate, with males attached to both gravid and non-gravid females, as well as to other males. The latter were grasped at approximately the same somatic area, where the vulva occurs in the female.

The development of *E. gaulica* on mycelial pellets of the deuteromycetous marine fungus, *Dendryphiella arenaria* NICOT, is shown in Table 1. The nematode population developed in fungal culture for 26 to 28 days prior to inoculation of the individual test flasks.

In one series of subcultures of *E. gaulica* over a period of several months, specimens were observed which were significantly shorter than those of the parent

Table 1

Development of *Euchromadora gaulica* INGLIS on *Dendryphiella arenaria* pellets

Flask No.	Initial population				Terminal population				Time in culture (in days)
	♀	♂	juv.	total	♀	♂	juv.	total	
1	2	–	1	3	5	3	8	16	18
2	2	2	–	4	(– 11* –)		5	16	18
3	–	–	7	7	–	4	1	5	16
4	2	–	–	2	2	–	2	4	16
5	1	1	–	2	(– 12* ** –)		7	19	28
6	3	2	5	10	(– 12* ** –)		5	17	28
7	2	1	1	4	(– – – 63* – – –)			63	28

* Population undifferentiated as to sex.
** One gravid female present.

population and those from field collections, i. e., 0.91 to 1.06 mm vs. 1.35 to 1.52 mm. It is probable that factors such as temperature, age of culture, overcrowding, etc., may account for this size difference.

In another culture test with specimens of *E. gaulica,* an initial population of 18 animals developed to numbers in excess of 150 within 44 days. Comparable tests have showed other generation times of between 30 to 40 days.

From the latter flasks, a total of 75 juveniles were collected and attempts made to support continued growth of the animals on fungal pellets in sea water. While the animals were active and appeared to feed for at least 14 days, within 30 days all the animals had died. Other attempts to raise the organism from gravid females were unsuccessful due to specimen mortality prior to egg deposition. Maintenance of *E. gaulica* specimens on sea water media fortified with washed yeast and bacterial cells also has been unsuccessful.

Observations of actively copulating individuals of *E. gaulica* suggest that amphimixis is the principle method of reproduction in this gonochoristic species.

Monhystera parelegantula De Coninck (1943) is a small species 330 to 410 μ in length with a head diameter of 4 μ and a stomatal length of approximately the same distance. The minuteness of the anterior extremity would require that the species be selective in obtaining food.

M. parelegantula De Coninck (1943) develops readily on sea water agar containing yeast cells with reproductive cycles less than 30 days. At the end of one test period of 23 days, an original inoculum of 15 specimens had produced a total of 75 animals (females and juveniles). The lack of males in our cultures suggests that this species is parthenogenetic. However, it is not known if this is the sole mode of reproduction.

Viscosia macramphida Chitwood (1951) attains a maximal length of nearly 2.0 mm with a head diameter of 12 to 16 μ. The stoma is 17 to 22 μ long and is armed with three slender elongated fixed teeth. Previously, species possessing such stomatal configuration have been regarded as predacious. Recent findings indicate that some species with such stomatal armature, while being able to utilize captured prey, tend to feed indiscriminately, and are perhaps best regarded as omnivorous.

Gravid females of this species usually have two eggs, one in each uterus. Occasional specimens are found containing three to four eggs, one to two in each uterus. This nematode differs from another large, omnivorous species, *Metoncholaimus scissus* Wieser & Hopper (Wieser & Hopper 1967) (reported as *Metoncholaimus* sp. Hopper & Meyers 1966; Meyers & Hopper 1966), in that the eggs are not "stored" and deposited in batches. In *V. macramphida* egg laying appears to be a continual process, with each egg deposited when mature and only one to two eggs (per uterus) maturing at a time. Our investigation suggest that *M. scissus* females die after oviposition. Possible correlations may exist between the number of ovaries and the egg-laying habit of oncholaimids in general.

An interesting hypothesis can be formulated on the basis of observations conducted on cultural material when compared with naturally occurring populations within various Biscayne Bay habitats (Meyers & Hopper 1967). In the field, males and females of *V. macramphida* are found with relatively equal commonness. However,

under laboratory conditions within a fungal-infested cotton cellulose filter, after a period of 10 days all sexually mature individuals have been females. Weekly collections over a two-month period gave similar results. The question of an alternation between amphimitic and parthenogenetic generations arises similar to that which occurs in some other Metazoa, i. e., rotifers. It appears that under certain conditions in the Bay, reproduction is amphimictic. In contrast, under laboratory conditions a parthenogenetic cycle is established, which, according to our observations, is capable of maintaining the population for several generations. To date, females of *V. macramphida* have been maintaining the laboratory population for a period of over three months. A life cycle of several weeks, or less, is indicated.

We have not been able to characterize the food requirements of these animals although different types of intestinal contents have been recorded. As noted by OVERGAARD NIELSEN (1949), a well-packed lumen is not direct evidence that substances in the culture medium necessarily constitute the diet of the animal in the field. In all likelihood, xenic maintenance and reproduction of nematodes may be realized with substances, or organisms, as food other than those ordinarily used by the animal in nature. Nevertheless, the studies reported here have supplied useful information on the comparatively short life cycle of marine nematodes in warmer oceanic waters.

Within the various sized groups and feeding types discussed, the modes of reproduction vary between amphimixis and parthenogenesis. Alternation of the reproductive cycle under laboratory conditions from amphimixis to solely parthenogenesis is especially striking with *Chromadorina epidemos* and *Viscosia macramphida.* To our knowledge, this has not been reported for marine nematodes. While comparable situations occur in certain soil nematodes, the latter are basically hermaphroditic and occasionally produce the odd male. It is not known if the male is functional.

Tests are in progress to evaluate more fully the growth requirements of certain of the dominant foliicolous and benthic nematode species in Biscayne Bay, Florida, and to characterize cultural conditions for maintenance of the animals over extended periods. Particular attention is being given to the effect of physical and biological factors, or stress conditions, on the sexual behavior and morphological characteristics of certain species.

SUMMARY

1. Life cycles of approximately one month are recorded for laboratory maintained cultures of chromadorid, monhysterid and oncholaimid nematodes.
2. Amphimictic and parthenogenetic reproduction occurs in the species investigated. Reproduction in *Monhystera parelegantula* is by parthenogenesis.
3. The chromadorid *Chromadorina epidemos* and the oncholaimid *Viscosia macramphida* are able to reproduce by either amphimixis or parthenogenesis.
4. *Acanthonchus cobbi, Chromadora macrolaimoides* and *Euchromadora gaulica* are amphimitically reproducing species.

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