GNOTOBIOTIC CULTURE AND PHYSIOLOGICAL ECOLOGY OF THE MARINE NEMATODE *RHABDITIS MARINA* BASTIAN

BY J. H. TIETJEN, J. J. LEE, J. RULLMAN, A. GREENGART, AND J. TROMPETER

GNOTOBIOTIC CULTURE AND PHYSIOLOGICAL
ECOLOGY OF THE MARINE NEMATODE
RHABDITIS MARINA BASTIAN

BY J. H. TIETJEN, J. J. LEE, J. RULLMAN, A. GREENGART, AND J. TROMPETER

GNOTOBIOTIC CULTURE AND PHYSIOLOGICAL ECOLOGY OF THE MARINE NEMATODE *RHABDITIS MARINA* BASTIAN[1]

J. H. Tietjen,[2] *J. J. Lee, J. Rullman, A. Greengart, and J. Trompeter*
Department of Biology, City College of City University of New York, New York 10031

ABSTRACT

The marine nematode *Rhabditis marina* Bastian has been isolated in gnotobiotic culture from collections of *Zostera marina* made in North Sea Harbor, Southampton, New York. A strain of *R. marina* has been maintained with three species of bacteria (*Pseudomonas* sp., *Flavobacterium marinum,* and *Micrococcus* sp.) for over 120 generations. A monoxenic strain with *Pseudomonas* sp. has been maintained for over 25 generations. The life cycle is completed in 5 days under "normal" laboratory growth conditions. Reproduction occurs over a wide (0–80‰) salinity range (the highest salinity tested) and a wide (10–38C) temperature range; optimum growth occurs at 45–55‰ and 25–30C. Normal development is oviparous, but ovoviviparous development is seen in older females. Tracer feeding and synxenic culture experiments have shown that *R. marina* has rather specific nutritional requirements, which seem to be completely satisfied by a relatively restricted group of microorganisms. Selected species of bacteria comprise the normal diet of this organism. Microscopic observations of intact algae in the digestive tract of the worms and failure of ^{32}P to transfer to the worms from the ingested algae indicate that the worms are not able to digest all ingested potential food organisms.

The geographical distribution of the species in relation to its physiological ecology is discussed.

INTRODUCTION

Despite the fact that marine nematodes generally abound (millions/m^2) in intertidal and shallow subtidal areas, relatively little is known about their life histories and physiological ecology. Their proposed role in the ecology of the shallow marine ecosystem ranges from serving as a source of nutrition for larger animals (Rees 1940; Mare 1942; Perkins 1958; McIntyre 1964; Tietjen 1969) to playing an important role in benthic nutrient regeneration (Johannes 1965; Tietjen 1967; McIntyre 1969). In view of their probable versatility in the marine environment the lack of information on their basic biology and physiological ecology is particularly unfortunate.

Data on life histories exist for only a few marine species and suggest a varied pattern of life cycles. The life cycles range from annual (*Enoplus communis*, Wieser

and Kanwisher 1960) to less than 1 yr (*Metoncholaimus scissus*, Hopper and Meyers 1966a) for relatively large species (4.00 mm or more long). Smaller species have been reported to have life cycles of about a month (*Monhystera disjuncta* and *Diplolaimella schneiderei*, Chitwood and Murphy 1964; *Monhystera filicaudata*, Tietjen 1967; miscellaneous chromadorids, monhysterids, and oncholaimoids, Hopper and Meyers 1966b).

Detailed information on the feeding habits and nutrition of marine nematodes is also scarce. While some information has been gathered from gut analyses of field samples (Perkins 1958; Tietjen 1969), it must be pointed out that these are inadequate because food materials of known origin are rarely found in the gut. Laboratory studies of nematode feeding are few. Webb (1956) noted that nematodes fed on small diatoms and clumps of bacteria but not ciliates. *Monhystera disjuncta* and *D. schneiderei* were observed feeding on bacteria by Chitwood and Murphy (1964). Hopper and Meyers (1966a, b) reported having raised *Metoncholaimus scissus* on

bacteria, yeasts, and ciliates and *Monhystera parelagantula* on yeast cells. Tietjen (1967) reported successful maintenance and reproduction of *M. filicaudata* on bacteria and small flagellates and also its successful maintenance (but not reproduction) on the feces and pseudofeces of the bay scallop *Aequipecten irradians*.

The role of the nematodes is being investigated as part of a larger study of the physiological ecology of marine aufwuchs communities. This paper deals with the cultivation and various biological characteristics of one species of nematode, *Rhabditis marina* Bastian, 1865, which has been in continuous culture since May 1968.

METHODS

Cultivation

The area of investigation is North Sea Harbor, Southampton, New York, a shallow, unpolluted embayment on eastern Long Island surrounded by an extensive salt marsh. Small samples (0.2 g dry wt) of *Zostera marina*, *Enteromorpha intestinalis*, and other marine macrophytes plus their aufwuchs are removed with sterile forceps, inoculated into 25- × 150-mm screw-cap test tubes containing 30 ml of sterile seawater, and refrigerated as soon as possible in an insulated ice chest. At a nearby field laboratory, those samples containing more than 50 organisms are selected for further analysis. Small portions of the sample are inoculated into a series of liquid differential growth media (Lee et al. 1966, 1970) and also streaked out on solidified agar plates of the same media. They are then refrigerated and brought to our permanent laboratory where they are incubated at 15 or 25C either in an environmental chamber (Sherer Model Cel 4-4) or in front of a light bank. Portions of the sample are also placed in finger bowls and incubated in front of a light bank at 20–25C. The temperature of the room is maintained by room air conditioning. The agnotobiotic samples are examined periodically for the development of nematode "blooms." When blooms of nematodes occur, the worms are subcultured in tissue culture flasks, petri dishes, or on agar slants. The nematodes in the inoculum are aseptically washed in 9-hole spot plates containing sterile seawater, sometimes with antibiotic mixtures, and inoculated into fresh media with potential food organisms isolated axenically from the same environment (Lee et al. 1970).

It was in this manner that *R. marina* was isolated from the epiphytic community of *Z. marina* in May 1968. Empirically we found that this organism requires a very thin agar layer for successful cultivation. In test tubes, the agar slopes had to be practically parallel to the walls of the tubes. In conventional agar slopes and petri plates thicker than 4 mm, the worms tunneled deeply and eventually died. Survival in liquid media is poor. Stock cultures of this organism are now being maintained in 20- × 125-mm screw-cap test tubes with a long slope of agar and 2–5 ml of liquid overlay. The tubes are incubated horizontally in test-tube racks turned on their sides.

By means of aseptic washing and antibiotics, synxenic cultures of *R. marina* were established with three species of bacteria: *Micrococcus* sp., *Pseudomonas* sp., and *Flavobacterium marinum*. Attempts to grow the worm in unialgal culture were unsuccessful. Three media supported excellent growth: marine nutrient agar and media No. 9 and 10 (Lee et al. 1970). Fungal mycelia were present in the original subcultures from agnotobiotic stocks. To eliminate these, both Mycostatin (50 μg–50 mg/ml) and Fungizone (50 μg–50 mg/ml) were tested. After four serial transfers through Fungizone-containing media, no mycelia were recovered. Concentration of the fungicides did not seem important, perhaps because neither is very soluble in the media. Mycelia were eliminated at both high and low concentration, and the nematodes survived well even at the highest concentration of fungicide. Current stock cultures are being maintained on media No. 2, 7, 9, and 10 (Table 1; Lee et al. 1970).

From these trixenic bacterized cultures, monoxenic cultures were successfully iso-

TABLE 1. *Media used in these experiments*

Base (1 liter)		
NaCl	2.5%	25 g
MgSO₄·7H₂O	0.9%	9 g
KCl	0.07%	700 mg
Ca (as Cl⁻)	0.05%	300 mg
NH₄NO₃	25.0 mg%	250 mg
Na glycerol·PO₄	10.0 mg%	100 mg
NaSiO₃·9H₂O	0.007%	70 mg
P II metals	3 ml/100	30 ml
Tris	0.1 g/100	1 g
B₁₂	10.0 μg	0.1 mg
NTA	0.007	70 mg
NaH₂CO₃	0.010	100 mg

Additions (per 100 ml)

1.	Base alone		
2.	B₁₂	5.0 μg%	
	Vitamins 8A	1	ml/100
3.	Soil extract	2	ml/100
4.	NH₄NO₃	10	mg%
5.	Alanine	50	mg%
	Glutamic acid	50	mg%
	Glycine	50	mg%
6.	Na glycerol·PO₄	10	mg%
	NaH₂PO₄	10	mg%
7.	Na acetate	50	mg%
	Na lactate	50	mg%
	Glucose	100	mg%
8.	2 + 4		
9.	2 + 3 + 4 + 5 + 6 + 7		
	P II metals	3	ml/100
10.	4 + 6 + 7		

Marine nutrient agar

Marine nutrient agar was made as follows: Nutrient agar (Difco 0001-01) 23 g, 500 ml of distilled water, 500 ml of seawater (Towd Point, North Sea Harbor, L.I.). It was sterilized for 15 min at 1.02 atm in an autoclave.

lated. Tracer-feeding experiments (Lee et al. 1970) indicated that the *Pseudomonas* sp. and *F. marinum* were ingested in large numbers. Several antibiotics (Erythromycin, Novobiocin, Polymyxin B, Chloramphenicol, and triple sulfa) were tried in various concentrations to eliminate one or more of the bacteria. A monoxenic culture of *R. marina* was established with *Pseudomonas* sp. with the aid of 200 μg/ml Erythromycin; this culture has been maintained for more than 80 generations as of this writing.

Three serial generations of worms were grown in axenic culture in marine nutrient agar and medium No. 10 +0.05% Bacto-Peptone, using aseptic washing techniques and 200–333 μg/ml Erythromycin. Although such crude materials as casein hydrolysate, BBL Trypticase, yeast extract, Bacto-Peptone serum fractions, amino acid mixtures, and so forth have been tested as bacterial substitutes, living bacteria have not yet been replaced as a nutrient source for continuous culture.

Life history studies

The life histories of individual organisms were studied in the wells of 9-hole Pyrex spot plates. Each well was filled with about 0.1 ml of solid medium (medium No. 2, Lee et al. 1970), overlaid with a thin film of sterile seawater. Individual gravid females were isolated from the stock cultures and inoculated into each well. A small inoculum of *Pseudomonas* sp. was also transferred to each well to be used as food. The plates were sealed by covering them with glass plates moistened with stopcock grease, which formed an effective seal against evaporation and the agar remained moist for 3–4 weeks. The females were observed almost continuously until egg deposition in the medium took place; individual eggs were then washed in sterile seawater and transferred to new media in new spot plates. Thus individual worms could be examined microscopically to observe growth rate, gonad maturation, molting times, and length at molting. After reaching sexual maturity, single males and single females were paired to study copulation, fate of the adults after copulation, fecundity, egg deposition, survival of offspring and sex ratios of offspring. Hundreds of individual worms were thus examined with relatively little difficulty. Length was measured with a calibrated ocular micrometer.

Effects of salinity and temperature on R. marina

The average salinity at North Sea Harbor is 25–30‰. We decided to observe the effect of salinity on reproduction and other aspects of the life cycle over a 0–80‰ range

at 5‰ intervals. Worms grown on agar slants at 25‰ salinity and pH 7.8–8.2 served as stock cultures. For each experiment 25–35 adult nematodes were inoculated into each of 17 test tubes over the test range. Sterile seawater (2 ml) of equal salinity was added to each experimental tube at the time of inoculation. Six replicates at each salinity were used in each of two sets of experiments. The tubes were incubated at 20C in environmental chambers in test-tube racks turned on their sides.

Nematodes were harvested after 2, 4, 6, 8, 10, and 11 days, by serially washing them with sterile seawater followed by 2 ml of 5% formalin containing 1 g/l Rose Bengal to kill them. After allowing 24 hr for penetration of the stain, the worms were counted. Direct counts of all worms on the slant and in the overlay were possible for 2-day-old cultures; after 4, 6, and 8 days of incubation it was necessary to dilute the overlay and wash by 1 : 3; after day 10, by 1 : 9; and after day 11, by 1 : 9 and 1 : 18. Replicate counts were found to be within 5% of the mean.

The effect of temperature on reproduction and other aspects of the life history was studied over a range of 10–40C. Eight replicate tubes were inoculated with 25–35 worms each and incubated in environmental chambers at 5C intervals in the range tested. Experiments were conducted with adults and first-, second-, and third-stage juveniles. After 4 days the worms were harvested and counted. Replicate counts were found to be within 10% of the mean.

In all instances the standard inocula of 25–35 individuals were checked for uniformity of size. It was not always possible to check sex distribution in these inocula; this could have produced large variations in the counts, but apparently failed to do so. Medium No. 2 was used in the temperature and salinity studies. In all studies the food source was *Pseudomonas* sp. (concn 10⁶ cells/ml).

Tracer-feeding studies

The basic technique outlined by Lee et al. (1966) was followed with some mod-

ifications. Experimental animals were harvested from stock cultures by gentle agitation, transferred with Pasteur pipettes to 9-hole spot plates, washed under a dissecting microscope by serial transfer in sterile seawater, and transferred to experimental test tubes (20- × 125-mm borosilicate glass). Care was taken to select organisms of the same size. From 10–25 nematodes were inoculated per experimental test tube, which contained 10 ml of sterile Millipore-filtered (HA 0.45 μ) seawater. Experimental cultures were incubated for 24 hr under conditions identical to those of the stock cultures, to starve the nematodes before feeding.

The microorganisms to be tested as food were grown in appropriate media with ^{14}C or ^{32}P added as label. After an appropriate incubation period, the potential food organisms were harvested by centrifugation, aseptically washed to remove extraneous label and waste products, and diluted to the desired concentration (usually 1×10^6 or 10^7 organisms/ml). The food organisms were labeled with ~ 0.1–5 dpm/organism and placed in culture with aseptically washed nematodes. After 24 hr when bacteria were used, or 72 hr when algae were the food, the nematodes were harvested, washed, and transferred to scintillation vials where they were suspended in a PPO-POP-POP-Cab-O-Sil thixotropic counting mixture. Dead or autoclaved nematodes were used as controls. After measuring the uptake of labeled foods, the number of food organisms ingested per nematode per day and the weight (μg) of food organisms ingested per nematode per day were calculated.

RESULTS

Life history

Growth is relatively fast; full sexual maturity is generally reached within 5 days after hatching (Fig. 1). At time of deposition, the eggs have an average measurement of 52×28 μm. Hatching generally occurs within 24 hr after deposition. An average female deposits 70–100 eggs, either in clusters of 5–10 or singly. At time of

hatching, the first-stage juvenile measures 102–127 μm. After 24 hr the first molt occurs; three molts occur after the first one at intervals of about 24 hr. At the time of the final molt, the females measure about 645 μm and the males about 610 μm. Egg production occurs generally 4.5 days after hatching at a length of 935–960 μm and sperm production 4.5–5.0 days after hatching at 920–945 μm. Females continue to grow to about 1,400 μm and males to about 1,120 μm; these lengths are attained usually within 6–8 days after hatching.

Copulation may occur immediately after attainment of sexual maturity, although the worms will continue to grow even after copulation. After copulation, a period of 2–4 days is generally required before the female deposits the first of her eggs in the medium. Egg deposition continues for 3–4 days after the appearance of the first eggs in the medium. Thus, the total time elapsed from copulation to conclusion of egg deposition may be as long as 1 week. About 60% of the females died after completing egg deposition; of those that did not, several produced a second group of eggs which were successfully fertilized, in some instances by the same male that had fertilized the first group. Most males continued to live after copulation until the termination of the experiments at 18 days after hatching.

The sex ratios of the offspring were about 2 : 1 or 3 : 1 in favor of the females. Survival among the offspring was quite dependent on the condition of the medium. If the animals were transferred to fresh medium, there was about 50% survival to sexual maturity. If they were left in the original medium, however, survival to sexual maturity was only about 10–20%. When mortality rates were high, they were highest for the early juvenile stages (first and second stages). Third-stage juveniles appeared to be relatively resistant to environmental stresses, as will be seen below.

An interesting phenomenon occurred among several older females. If they were left unfertilized until a relatively old age (14 days), although copulation was nor-
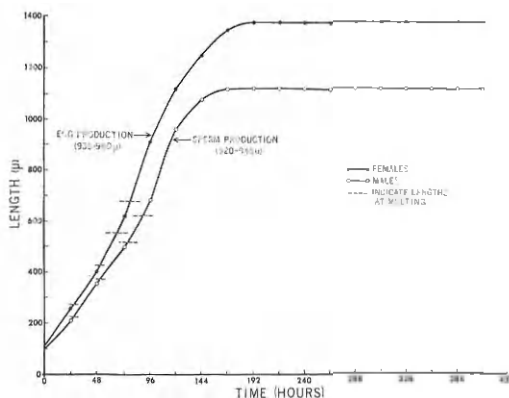

Fig. 1. Growth rate of *Rhabditis marina* at 25C and 25‰ salinity. Each point represents the mean of 50 individuals.

mal, the eggs hatched within the female rather than being deposited outside. The time from copulation to egg hatching was about the same as that occurring in normal females (2–4 days). After hatching, the juvenile nematodes began to crawl within the body of the mother, killing her in the process (Fig. 2, right). Usually, one or two juveniles resulted from this ovoviviparous development although in one worm four were seen at once. Development was much slower than in normal juveniles. Six days were required before the juveniles were able to break through the body wall; these were always third-stage juveniles. After emergence from the dead female, the worm grew at a normal rate and a normal adult was formed. The sex ratios of the ovoviviparously developed individuals were about the same as the sex ratios of the normal individuals; that is about 65–76% of the juveniles were females.

Effects of salinity and temperature on R. marina

The population growth curves of *R. marina* at salinities of 0, 25, 50, and 75‰ are shown in Fig. 3. Although *R. marina* grew at all salinities from 0–80‰, maximum population increases occurred between 45 and 55‰. At the extremes of the salinity range, the length of the life cycle was increased and the reproductive potential decreased. In addition to surviving and
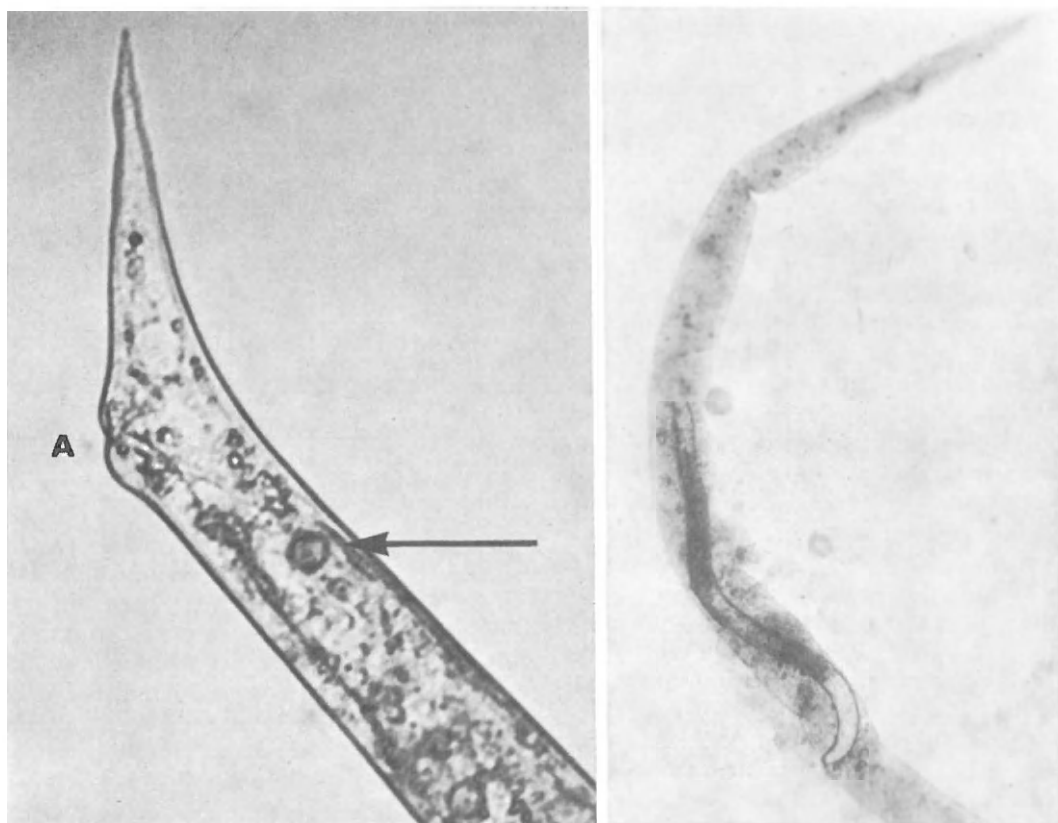
FIG. 2.   **Left:** An undigested chlorophyte in the digestive tract near the anus. Right: An ovoviviparous female with a juvenile within her body wall.

reproducing at 0‰, *R. marina* could survive for up to 3 days in de-ionized water.

Population increases at 25 and 30C were significantly greater than at any other temperature between 10–40C (Fig. 4). Death of the nematodes always occurred at 38–40C. At temperatures above 35C, mortality among adults and first- and second-stage juveniles was high; the third-stage juveniles were quite resistant to these higher temperatures, except that they too died at 38–40C. Separate studies showed that death was not due to starvation, because the bacteria on which the worms were feeding were capable of growing at temperatures up to 42C.

Feeding habits and nutrition

As stated above, synxenic cultures of *R. marina* were established with *Micrococcus*

sp., *F. marinum*, and *Pseudomonas* sp. The uptake by *R. marina* of these and other species of bacteria, as well as various species of algae, is shown in Table 2. *Pseudomonas* sp. was clearly the most likely food for a monoxenic culture, since *R. marina* ingested about 10× its own weight of *Pseudomonas* sp. per day. With the aid of antibiotics, *R. marina* was isolated in such a monoxenic culture. Attempts to culture *R. marina* on *Micrococcus* sp. or *F. marinum* have thus far been unsuccessful. The relatively low uptake of the other bacteria appears to preclude their use as food by *R. marina*.

Tracer-feeding experiments indicated that chlorophyte No. 55 (a small *Nannochloris* sp.) and *Dunaliella parva* were ingested in greater numbers than other species of algae. Attempts to culture *R. marina* on
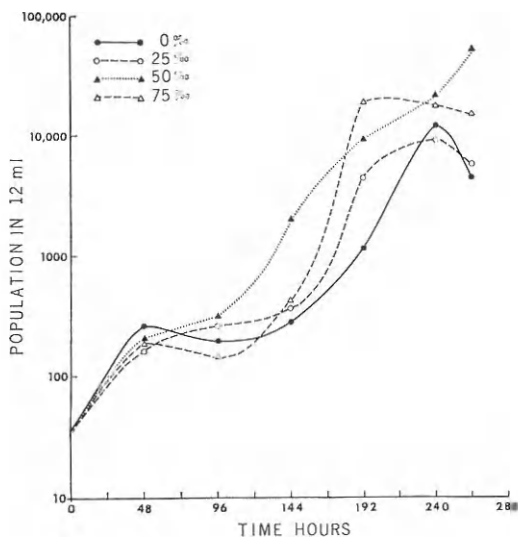
Fig. 3. Effect of salinity on the growth of *Rhabditis marina*. Each point represents the mean of six replicate experiments.

algae have thus far been unsuccessful. This at first seemed surprising, since under appropriate culture conditions we found worms with green guts. Examination of the worms under phase contrast has perhaps revealed a partial answer. One of the algae (chlorophyte No. 55), although ingested
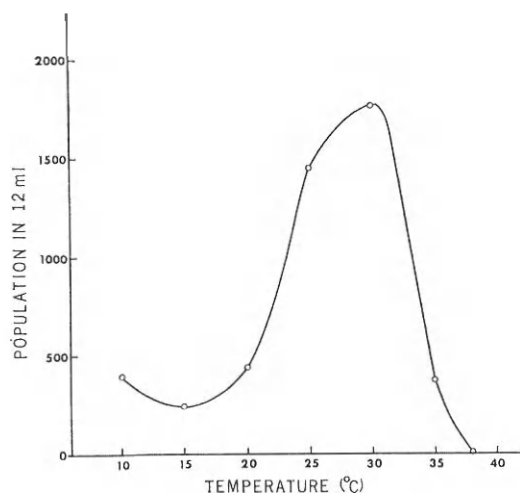

Fig. 4. Effect of temperature on the growth of *Rhabditis marina*. Each point represents the mean of eight replicate experiments.

Table 2. *Consumption of food by* Rhabditis marina

Food organism	No. organisms nematode^{-1} day^{-1}	μg nematode^{-1} day^{-1}
Micrococcus sp.	2.9×10^3	5.9×10^{-4}
Flavobacterium sp.	5.3×10^5	1.5×10^{-1}
Pseudomonas sp.	4.3×10^7	1.5×10^1
P. fluorescens	1.1×10^3	3.4×10^{-4}
E. coli	6.8×10^3	1.6×10^{-2}
Alcaligenes faecalis	7.9×10^3	3.2×10^{-6}
Proteus sp.	9.7×10^3	4.0×10^{-3}
Serratia marcescens	3.1×10^4	6.5×10^{-3}
Sarcina lutea	4.5×10^3	2.3×10^{-3}
Klebsiella sp.	3.2×10^4	1.3×10^{-2}
Bacillus megatherium	2.0×10^4	1.1×10^{-1}
Mycobacterium phlei	background	——————
Streptococcus faecalis	background	——————
Salmonella pullorum	2.2×10^3	2.0×10^{-3}
Cylindrotheca closterium	——————	——————
BL-38	2.2×10^2	1.4×10^{-1}
Amphora sp.	9.7×10^2	5.7×10^{-1}
Nannochloris sp.	——————	——————
BL-1	——————	——————
55	9.0×10^3	1.6×10^0
SH-1 (1)	2.2×10^2	2.1×10^{-1}
(2)	6.1×10^1	5.7×10^{-2}
Dunaliella salina	5.0×10^2	4.7×10^{-1}
D. parva	1.2×10^3	1.1×10^0
Archais symbiont	3.9×10^2	3.7×10^{-1}
50	8.2×10^1	7.7×10^{-2}
93	2.6×10^1	1.7×10^{-3}
94	4.4×10^1	4.1×10^{-2}
95	4.4×10^2	4.1×10^{-1}
96	1.8×10^2	1.7×10^{-1}
98	4.2×10^1	3.9×10^{-2}
105	5.6×10^1	5.3×10^{-2}

in quantity, appears to pass through the gut completely undigested (Fig. 2, left).

DISCUSSION

The reproductive rate and heartiness of some organisms newly isolated in gnotobiotic culture never ceases to amaze even experienced workers. *Rhabditis marina*, like its close relative *Caenorhabditis briggsae* (Tomlinson and Rothstein 1962), a hermaphroditic soil nematode grown axenically, completes its life cycle in as little as 5 days under favorable culture conditions. Reports on other marine nematodes suggest minimum life cycles of about 1 month.

Rhabditis marina is cosmopolitan. Our

laboratory observations agree with the field data (Gerlach 1956; Chitwood 1951; Meyl 1955; and others) and show that *R. marina* has the potential for surviving in a wide variety of habitats. Although it has a definite salinity optimum of 45–55‰, growth and reproduction occur within a wide range. It is quite eurythermal with an optimum of 25–30C. Both temperature and salinity optima suggest that this species is well adapted to conditions encountered in the upper littoral zone. Large numbers of *R. marina* have been recovered from seaweeds stranded between tides in the upper littoral zone (Inglis and Coles 1961). A number of foraminifera recovered from the same environment (*Trochammina inflata*, *Allogromia* sp., *Allogromia laticollaris*, and *Quinqueloculina lata*) are similarly adapted to the higher salinities and temperatures produced during intertidal evaporation (Freudenthal, Lee, and Pierce 1963; Lee and Pierce 1963; Muller and Lee 1969). Although the epiphytic communities and stranded seaweeds where *R. marina* is most common abound with bacteria and microscopic algae, tracer-feeding and synxenic-culture experiments have shown that *R. marina* has rather specific nutritional requirements, which seem to be completely satisfied by a relatively restricted group of microorganisms. Of the three bacteria with which it was isolated, it picked up the ^{32}P label from only two in tracer-feeding experiments. In continuous monoxenic culture it grew and reproduced only in association with the *Pseudomonas* sp., not with *F. marinum*. Monoxenic cultures of *R. marina* with *F. marinum* were viable for three transfers, so we suspect that this bacterium is lacking in some nutrient required at vitamin levels by the nematode. Perhaps for similar reasons we were unable to establish monoxenic cultures of *R. marina* with *Nannochloris* sp. (Bl-55), the alga taken up in the largest quantity and which is good food for several species of *Chromadora*. The morphology of the buccal apparatus does not suggest a mechanism for feeding on selected species of bacteria although tracer-feeding experi-

ments strongly suggest a high degree of selectivity. Microscopic observations on intact algae in the digestive tract of the worms suggest restriction in the diet based on the inability to digest all ingested potential food organisms. Thus the wide range in ^{32}P transfer to the worms from various species of bacteria might be explained by a lack of an array of the necessary enzymes to digest many bacteria.

Ovoviviparous development is fairly common in rhabditid nematodes. As was noted by Hirschmann (1960), this phenomenon is restricted to old virgin females.

The tremendous reproductive potential of *R. marina* observed in the laboratory never seems to be attained in the field. In common with two easily cultured and prolific species of foraminifera from the same environment (*A. laticollaris* and *Spiroloculina hyalina*), *R. marina* is usually a minor component of the epiphytic and littoral fauna. Only once in the midst of a severe drought was one of these species (*A. laticollaris*) observed in a bloom (Lee et al. 1969). Though we are well aware that salt marsh environments such as the one from which we isolated *R. marina* present a dynamically changing multivariate environment (Bradshaw 1968), which impresses some integrated effect on the growth and reproduction of individual species, we find it hard to believe that physical factors in the field are severely limiting for this worm. Our laboratory studies suggest much wider physical tolerance than is usually encountered in the environment. We suspect that biotic factors, predation, lack of sufficient quantities of appropriate food organisms, antibiotics, and so forth must be more important in limiting growth and reproduction of this nematode species in the field.

REFERENCES

BRADSHAW, J. S. 1968. Environmental parameters and marsh foraminifera. Limnol. Oceanogr. **13**: 26–38.

CHITWOOD, B. G. 1951. North American marine nematodes. Texas J. Sci. **4**: 617–672.

———, AND D. G. MURPHY. 1964. Observations on two marine Monhysterids—their classification, cultivation, and behavior. Trans Amer. Microsc. Soc. **83**: 311–329.

FREUDENTHAL, H. D., J. J. LEE, AND S. PIERCE. 1963. Growth and physiology of foraminifera in the laboratory, Part 2—Collection and maintenance: a tidal system for laboratory studies on eulittoral foraminifera. Micropaleontology **9**: 442–448.

GERLACH, S. A. 1956. Die Nematodenbesiedlund des tropischen Brandungsstrandes von Pernambuco. Brasilianische Meeres-Nematoden II. Kiel. Meeresforsch. **12**: 202–218.

HIRSCHMANN, H. 1960. Reproduction of nematodes, p. 140–167. *In* J. N. Sasser and W. R. Jenkins [eds.], Nematology. Univ. North Carolina Press, Chapel Hill.

HOPPER, B. E., AND S. P. MEYERS. 1966a. Observations on the bionomics of the marine nematode, *Metoncholaimus* sp. Nature **209**: 899–900.

————, AND ————. 1966b. Aspects of the life cycle of marine nematodes. Helgolaender Wiss. Meeresunters. **13**: 444–449.

INGLIS, W. G., AND J. W. COLES. 1961. The species of *Rhabditis* (Nematoda) found in rotting seaweed on British beaches. Bull. Brit. Mus. (Natur. Hist.) **7**(6): 320–333.

JOHANNES, R. E. 1965. Influence of marine protozoa on nutrient regeneration. Limnol. Oceanogr. **10**: 434–442.

LEE, J. J., M. McENERY, S. PIERCE, H. D. FREUDENTHAL, AND W. MULLER. 1966. Tracer experiments in feeding littoral foraminifera. J. Protozool. **13**: 659–670.

————, W. A. MULLER, R. J. STONE, M. McENERY, AND W. ZUCKER. 1969. Standing crop of foraminifera in sublittoral epiphytic communities of a Long Island salt marsh. Mar. Biol. **4**: 44–61.

————, AND S. PIERCE. 1963. Growth and physiology of foraminifera in the laboratory: Part 4—monoxenic culture of an allogromiid with notes on its morphology. J. Protozool. **10**: 404–411.

————, J. H. TIETJEN, M. McENERY, R. J. STONE, J. RULLMAN, AND W. A. MULLER. 1970. Cultivation and physiological ecology of members of salt marsh epiphytic communities. Helgolaender Wiss. Meeresunters. **20**: 136–156.

MARE, M. F. 1942. A study of a marine benthic community, with special reference to the microorganisms. J. Mar. Biol. Ass. U.K. **25**: 517–574.

McINTYRE, A. D. 1964. Meiobenthos of sublittoral muds. J. Mar. Biol. Ass. U.K. **44**: 665–674.

————. 1969. Ecology of marine meiobenthos. Biol. Rev. Cambridge Phil. Soc. **44**: 245–290.

MEYL, N. H. 1955. Freilebende Nematoden aus binnenland Salzbiotopen zwischen Braunschweig Magdeburg. Arch. Hydrobiol. **50**: 569–614.

MULLER, W. A., AND J. J. LEE. 1969. Apparent indispensibility of bacteria in foraminiferan nutrition. J. Protozool. **16**: 471–478.

PERKINS, E. J. 1958. The food relationships of the microbenthos, with particular reference to that found at Whitstable, Kent. Annu. Mag. Natur. Hist. Ser. **13**: 64–77.

REES, C. B. 1940. A preliminary study of the ecology of a mud flat. J. Mar. Biol. Ass. U.K. **24**: 185–199.

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

————. 1969. The ecology of shallow water meiofauna in two New England estuaries. Oecologia **2**: 251–291.

TOMLINSON, G. A., AND M. ROTHSTEIN. 1962. Nematode biochemistry. I. Culture methods. Biochim. Biophys. Acta **63**: 465–470.

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

WIESER, W., AND J. KANWISHER. 1960. Growth and metabolism in a marine nematode, *Enoplus communis* Bastian. Z. Vergl. Physiol. **43**: 29–36.