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Life history of a free-living marine nematode *Daptonema normandicum* reared in laboratory

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Abstract

Life history of a free-living meiobenthic nematode *Daptonema normandicum* (DeMan, 1890) was studied in the laboratory. Live specimens were primarily collected from the sewage outlet site near the mouth of the Mandovi estuary, Goa. This species was the most dominant (>67%) among the meiobenthic nematodes. Vertically, nematode abundance was highest at the surface sediment and correlated with the organic carbon and sediment chlorophyll-a. Considering their dominance in the meiofauna, attempts were made to rear *D. normandicum* in laboratory. Salinity of the culture medium was maintained at 14 to 17 PSU (same as the collection site). All the culture experiments were conducted in semisolid nutrient agar media at 27 ±2°C temperature for 12 hr dark: 12 hr light conditions. The food consists primarily of an unidentified bacterium and mixed algae, but diatom and ciliates were also observed in culture. Females produced first batch of eggs at the age of 23 days. Gravid female normally carry 8-10 eggs. Embryonic development is completed in ~72 hr and entire life cycle (egg to adult) was completed in 22-24 days. Average size of juveniles at the hatching was 0.189 mm. Young individuals attains a maximum size of 1.23 mm (male) and 1.04 mm (female) in ~21-23 days. Growth, in terms of length was augmented upto 23rd day and ceased thereafter. The daily growth increment for the first 5 days was 0.01-0.04 mm which increased upto 0.05-0.08 mm d^{-1} during the maturation (10-18 days). Male : female ratio was 1:2. In this laboratory study, we provided information on the embryonic development, the life cycle and ecology. Our results demonstrated that *D. normandicum* can be reared successfully under the controlled conditions, suggesting possible use of this species in toxicological and aquaculture studies. The culture method described is very handy and can be applicable for rearing other meiobenthic species particularly the nematodes with comparable feeding habits.

Key words

Nematode, *Daptonema normandicum*, Life history

Introduction

Free-living nematodes are the most numerous animal group in the marine benthos. Their density includes several million m^{-2} of the sea floor in many shallow subtidal environments and represents a biomass of 0.2-0.5 g cm^{-2} (Heip *et al*., 1982). The importance of nematode in terms of food for macrofauna and fish is still a matter of research but their role in stimulating bacterial productivity and thus mineralization of detritus, decomposition and nutrient regeneration has been well studied (Tenore *et al*., 1977). According to De Mesel *et al*. (2004) nematodes can have a significant influence on bacterial density. Meiobenthic nematodes have been recognized as environmental indicators in many temperate water studies (Boyd *et al*., 2000). However, in tropical and subtropical waters, information on free-living marine nematode communities is very scarce. Most of the researchers suggested (Mohmoudi *et al*., 2005; Hedfi *et al*., 2007) that species having very short life cycle are well suited for pollution studies, provided accurate knowledge on several aspects of their life history traits *e.g.* the duration of embryonic development, life cycle, daily growth and mortality are obtained through laboratory rearing. Nevertheless, field data is also vital in understanding the natural environmental condition of the habitant. A cosmopolitan *D.normandicum* has been reported from widely scattered localities around the world *viz*., Adriatic Sea (Travisi and Vidakovic, 1997) Mediterranean Sea (Gerlach and Riemann, 1973), North Atlantic (Warwick *et al*., 1998), Westerschelde estuary of Netherland (Soetaert *et al*., 1994), from Oostende beach (Schuurmans, 1942), Belgian exclusive economic-zones (Coomans, 1989), Yellow Sea (Huang *et al*., 2005) and North Sea (Vincx, 1989). Even though

Mahmoudi *et al*. (2005) and Hedfi *et al*. (2007) used *D. normandicum* in their laboratory microcosm experiments, but information on life history and reproductive biology of this species is lacking. The high dominance of *D. normandicum* in the sandy beach meiofauna impelled us to study the several aspects of ecology under the laboratory and field conditions.

Materials and Methods

Field sampling: The study area lies in the vicinity of Panjim market (Lat.15°30'02.30"N; Long.73°49'10.30"E; Fig. 1). A long stretch of sandy beach (1-2 km) along the Mandovi estuary, which opens into Arabian Sea, was selected for sampling. It receives ~1300 million litters of urban runoff daily (Anonymous, 1978). A single station in the mouth of *'nullah'* was sampled in July 2009 for nematode biodiversity. Sediment samples were collected with a corer (Govaere and Thielemans, 1979). Three replicates were taken for nematodes with a core of 5.7 diameter (area 25.5 cm^2) and 10 cm deep for organic carbon, grain size, and chlorophyll analysis. Sediment columns were cut into five depth fractions (0-2, 2-4, 4-6, 6-8, 8-10 cm). Temperature was measured with a hand held mercury thermometer and salinity using a refractometer. All the nematodes were separated from the sediment samples by sieving through a 63 µm mesh and identified upto genus/species level using the pictorial key of Platt and Warwick (1983). Specimens of *D.normadicum* were sorted, identified as male, female, and juvenile categories. Sediment grain size was determined by dry-sieving, using a set of sieves with decreasing mesh size of 2000 to 63 µm. The grains were desegregated by drying in an oven at 100°C for 24 hr and the samples were then passed through the sieves and shaken by an automatic shaker for 10 min (Buchanan, 1984). Sediment chlorophyll-a (Chl-a) was determined according to Lorenzen and Jeffrey (1980). Organic carbon (OC) was estimated with wet oxidation method of El Wakeel and Riley (1957).

Laboratory cultures: Surface sediment was collected separately and transported alongwith the estuarine water to the laboratory where all the material was sieved through a 45 µm sieve using filtered seawater of same salinity (*i.e.* 14). All the live organisms were transferred into petri dishes (14 cm diameter) containing filtered sea water. Specimens of *D. normandicum* were then sorted under inverted binocular microscope and maintained in filtered seawater as per culture method of Vranken *et al.* (1981) and Singh *et al*. (2009). The algal debris provides place for egg deposition and hiding for juveniles, thus increasing the overall survival rate of juvenile nematode population. The food consisted primarily of unidentified bacteria and mixed algae. The diatoms *Cosinodiscus* sp and ciliates *Euplotes* sp were also observed in the culture plates but their actual consumption by *D. normandicum* could not be confirmed. Following this procedure, we could maintain the nematodes for 30 days at room-temperature (27±2°C). Thus, the mono-specific subcultures of *D. normandicum* were established by transferring gravid female into individual cavity block (3.7 cm diameter and 2.3 cm height) with enriched 0.8% sterile bacto-nutrient agar with following constituents (peptic digest of animal tissue 5 g, sodium chloride 5 g, beef extract 1.5 g, yeast extract 1.5 g, agar (Himedia®) 20 g and detritus).

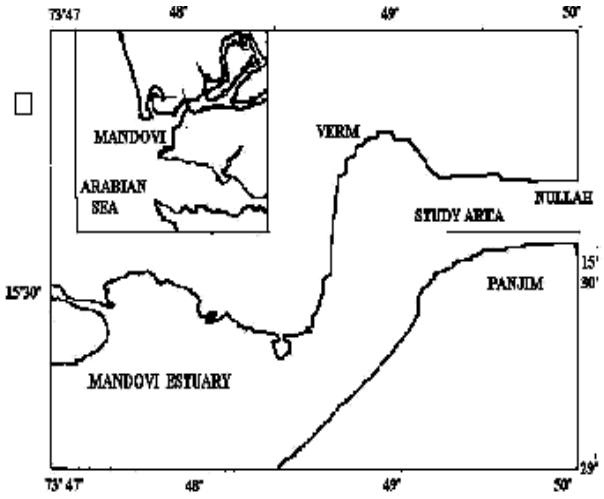

Fig. 1: Location of the study area, near Panjim

The salinity of nematode culture medium was maintained between 14 to 17 PSU.

Results and Discussion

Sediment characteristics of the sampling site: Sediment of the sampling site was mainly of sandy in nature with fine sand being the major constituent (98.72%). Organic carbon (OC) was generally low and values varied from 0.32 to 1.0% with a maximum value in the surface (0.99%) and decreased with increasing sediment depth down to 6-8 cm. Marginal increase was observed in OC (0.39%) at deeper sediment section of 8-10 cm. The trend was similar for sediment Chl-*a*, *i.e.* higher values at surface (2.32 µg g^{-1} in 0-2 cm) and decreasing progressively at deeper sections.

Nematode abundance: A total of 12 genera and 4 nematode species belonging to 5 families were recorded in the study area.

The mean nematode abundance was 285 ind 10 cm^{-2}. Among the nematodes, *D. normandicum* was the most dominant constituting 67% (193 nos 10 cm^{-2}, Fig. 2a) of the total nematode population. Nematodes, *D. hirstum* (5%) and *D. invagiferoum* (4%) were the second and third in order of dominance (Fig. 2b). Other species recorded in low number and together comprised 24% of the nematofauna (Fig. 2b). Maximum abundance of *D.normandicum* (71%) was in the top 0-2 cm sediment section. The density value showed a decrease with increasing sediment depth (Fig. 2a). The natural population of *D. normandicum* mainly comprised of adult forms in which females were highly predominant (79%). Males and juvenile stages constituted 15 and 6% respectively (Fig. 2c).

Embryonic, post embryonic development and growth rate: The development of 47 eggs of *D. normandicum* was followed in the laboratory at 27±2°C temperature and 14 salinity under 12 hr day and night regime.

The eggs are generally deposited at the single-cell stage with nucleus clearly visible inside (Fig. 3a). The deposited eggs have spherical ovoid shape with a mean diameter of 22 µm. 2 hr

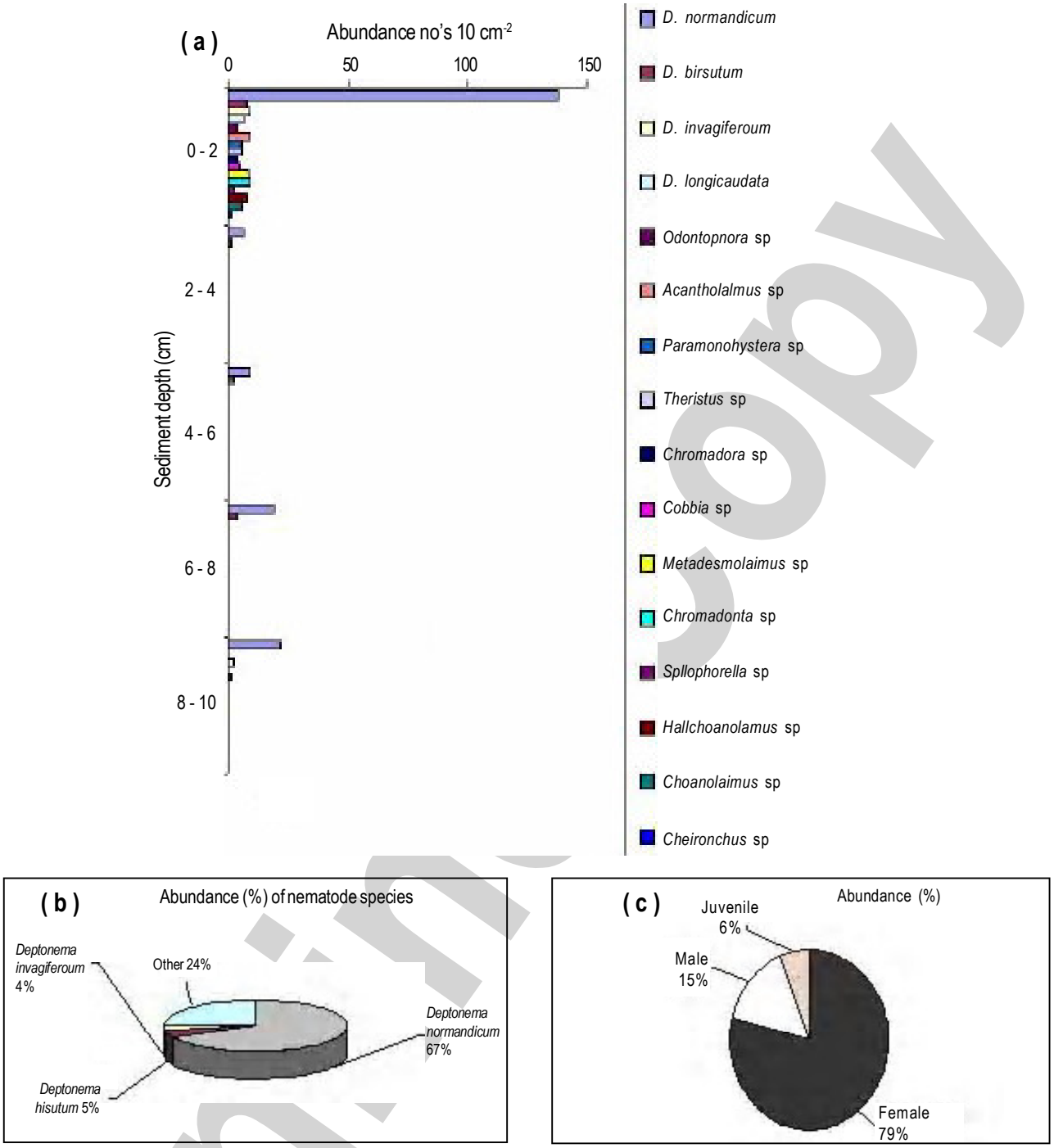

Fig. 2: Vertical distribution of nematode species (a); Contribution (%) of dominant nematode species (b); Contribution (%) of male, female and juvenile *D. normandicum* in natural population (c)

after deposition, the first cleavage occurs with two unequal blastomeres (Fig. 3b). The blastomere divides and three and four cell stage is reached (Fig. 3c,d). After fourth stage, the blastomere is formed in an irregular manner, which remains unaltered till sixth stage (Fig. 3e,f).The colour of the developing egg changes from dark-brown at the single-cell stage to light brown at embryonic stage. After 8 hr, gastrulation begins (Fig. 3g) and in additional 9 hr, the embryo was observed with constant movement (Fig. 3h). This stage can be recognized easily by the active movements of embryo. Embryo was seen to acquire different positions in the egg shell and it takes about 20 hr to hatch into first juvenile. The embryonic development was competed in ≈ 78 hr. Daily increase in growth was observed in 20 juveniles till they reach adult stage (male with well developed organs and female carry eggs).After hatching, the

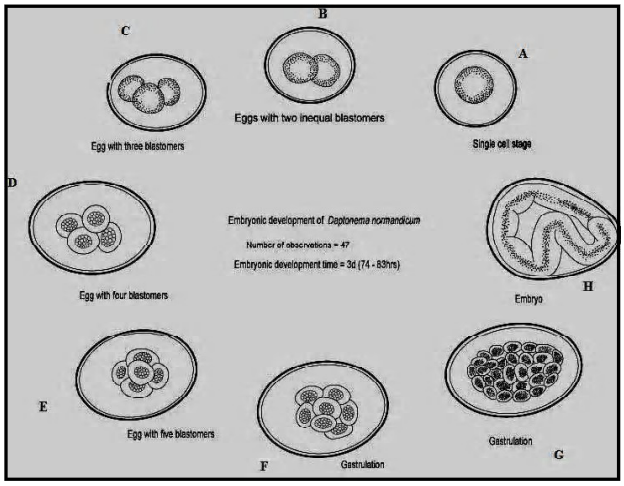

Fig. 3: Schematic diagram showing the embryonic development in *D. normandicum*

juvenile was transparent, pale in colour with a mean length of 0.189 mm. The time required for development of each life stage is presented in Fig. 4a. Growth, in terms of length continued to increase upto 23rd day and remained constant thereafter (Fig. 4b). Rate of growth increment was calculated on a daily basis. During initial 5 days, the increment in growth was 0.01-0.04 mm day^{-1} which increased to 0.05-0.07 mm day^{-1} during the maturation phase (10-18 d) and reduced thereafter (Fig. 4c). The mean length of matured male was 1.04 mm and ovigerous female was 1.23 mm (Fig. 5a,b). The first gravid female appeared 23 days after hatching, which indicates that under the laboratory condition, female *D. normandicum* takes about 23 days for maturation. Gravid females normally carry 8-10 eggs (Fig. 5b). Thus, the life cycle, *i.e.* from egg to adult stage is competed in 22-24 days (n=20). The different developmental stages of *D. normandicum* are presented in Fig. 5a-h.

The field data suggests that there are various factors that effect the distribution of *D.normandicum* like organic carbon, chlorophyll-*a*, dissolved oxygen and sediment composition in the present study area. It prefers to live in surface sedimentary layer as their maximum value was recorded in the uppermost 0-2 cm sediment section which supports the earlier finding of Soetaert *et al*. (1995) as *D. normandicum* prefers coarser particles rather than silt or clay. Higher abundance of *D.normandicum* in upper 0-2 cm sediment section and lower in the deeper sediment layer was probably related to the decreasing trend of dissolved oxygen, sediment organic carbon and Chl-*a* with depth. While studying the tidal migration in nematodes, Steyaert *et al*. (2001) reported upward movement of another related species *Daptonema setosum* with incoming tides and downward movement when the flat become exposed. According to various workers, such type of behavior is a part of survival strategy in nematodes, mainly to utilize the epipelic diatoms which are the main food source for deposit feeders (Hopkins,1963; Joint *et al*.,1982; Pinckney *et al.*,1994) as well as to avoid predators (Steyaert *et al*.,

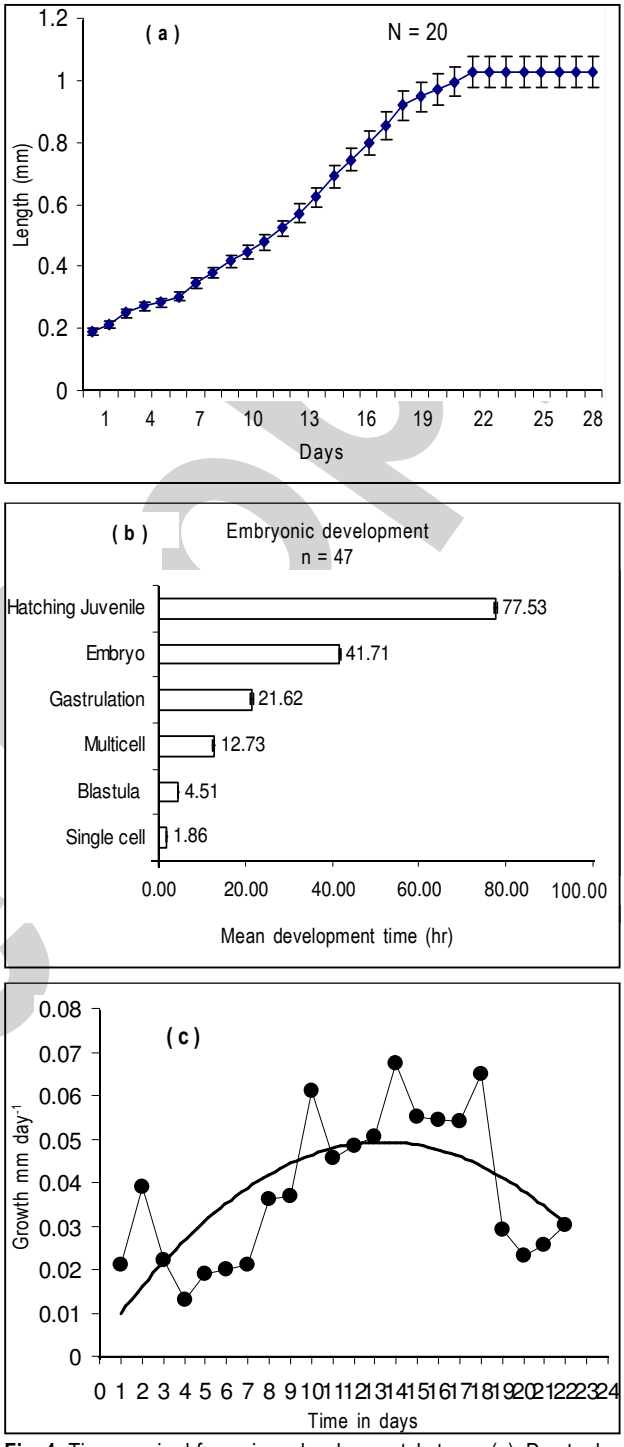

Fig. 4: Time required for various developmental stages (a); Day-to-day growth rate (b); Growth increment in *D. normandicum* (c)

2001). While confirming the above findings, we believe that several abiotic and biotic factors as well as their combinations may explain the better picture of the depth-wise distribution of *D. normandicum.* The higher aggregation of *D. normandicum* at surface sediment was perhaps due to the availability of food material and feeding preference. The sampling area is located in the vicinity of municipal

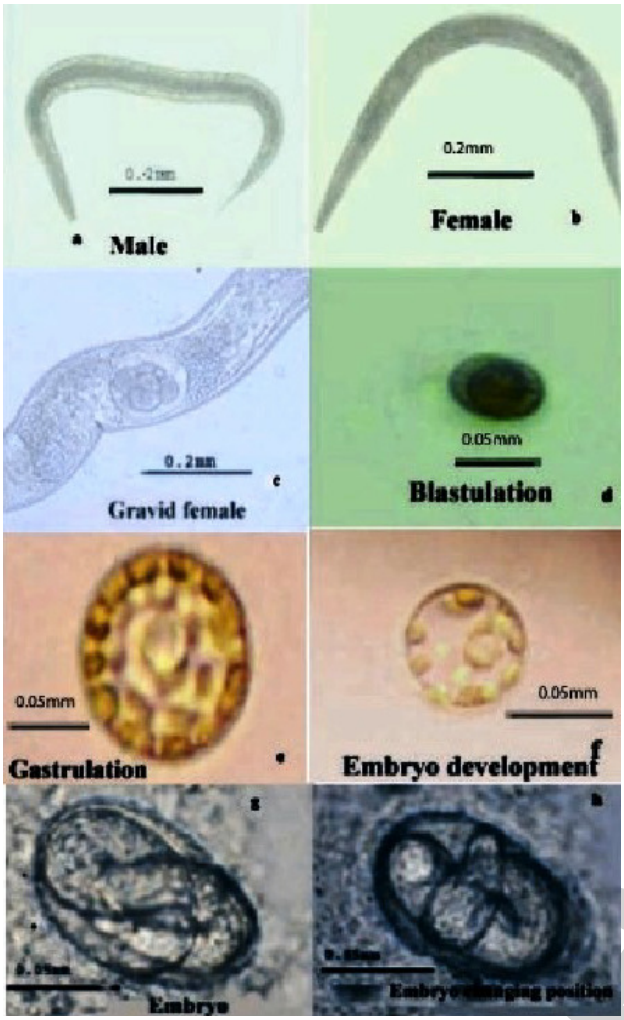

Fig. 5: Adult *D.normandicum male* (a) and female (b), Gravid female (c), Blastulation (d), Gastrulation (e), Embryo development (f), Embryo (g), Another position of embryo (h).

sewage dumping site, where food in the form of organic carbon was abundant (Nanajkar and Ingole, 2010). According to Maria *et al*. (2008) sewage input promotes the increase of bacteria and protozoans in the sediment, which forms the main food source for deposit-feeders. The ciliate protozoans and benthic diatoms, form important food items for deposit feeders. Even diatoms frustules have been reported in the intestines of *D. setosum (*Moens and Vincx, 1997). Although, gut content of *D. normandicum* was not examined, but diatom (*Cosinodiscus* sp.) and ciliate (*Euplotes* sp.) were abundantly available in the estuarine water and in our laboratory stock cultures, indicating their importance as a food source for this species. Our experimental results revealed that, culturing of the free-living nematodes is difficult, since individual species may have specific environmental requirements and can be realized with a few trials. Some species of nematodes can grow on agar, while others may require preferable medium with different feeding combination (Moens and Vincx, 1998). We found that *D.normandicum* favor a semi-solid medium because in liquid medium this nematode actively swims, probably searching for a substratum; which results in a higher mortality for juveniles and adults. *D. normandicum* is a slow swimmer and shows speedy movement by coiling, when light is directed to the culture and then immediately hides in detritus. The semisolid nutrient medium act as artificial habitat under laboratory condition and also provide nutrient for bacterial growth. Preference for semi-solid media (nutrient agar) by nematode under laboratory conditions is also reported for *Monhystera parelegantula* (Vranken *et al*.,1981) and *I. tentabunda* (Singh *et al*., 2009). We provided artificial conditions (salinity 14 and temperature 27±2°C) to *D. normandicum*. The semi-solid nutrient agar (0.8%) and diatom (*Cosinodicus* sp.) was found to be suitable feeding combination by *D. normandicum.* Overall, the females of *D. normandicum* were highly dominant in the laboratory culture with the ratio of 1:2. Even in field data, females (79%) outnumber the males and the contribution of juvenile to the natural population was only 8%. However, we believe that some juveniles may have escaped through the 63 µm mesh, used for sieving the sediment samples, resulting in this low juvenile density. Nevertheless, depressed sex ratio in the field data, in favor of female is a matter of further investigation. In our laboratory culture most of the males died after copulation resulting in lower sex ratio. Vranken *et al*. (1981) also observed very few males in laboratory studies of *M. pareleqantula.* Similar behaviour is also reported during the cultivation of *I. tentabunda (*Singh *et al*., 2009). However, a combined laboratory and field study is required to confirm this aspect. Under the laboratory conditions, females of *D. normandicum* deposited eggs singly or in pairs and the number varied from 8 to 10. According to Tietjen and Lee (1973) females of *Chromadora macrolaimoides* produced 10-12 eggs and 14-19 eggs are reported for *Prochromadorella* sp. and *Spiliphera* sp. (Mutsumi *et al*., 1998). *M.denticulata* deposit 18-23 eggs after a single copulation with male in optimum condition (Tietjen and Lee,1972). Singh *et al*. (2009) observed 10-20 eggs in *I. tentabunda*. In comparison to *M. pareleqantula* which require 48 hr for embryonic development (Vranken *et al*.,1981) *D.normandicum* needs 78 hr.

The higher growth rate recorded during the maturity stage in *D. normandicum* and in *M. pareleqantula* during post embryonic stage (Vranken *et al*.,1981) was possibly due to the accumulation of more feed. Females of *D. normandicum* carry 8-10 eggs, whereas ovigerous female of *M. pareleqantula* carries 1 egg but at the end of life there may be 2-3 eggs in the uterus (Vranken *et al*.,1981). *D. normandicum* takes 22-24 days to complete the entire life cycle (egg to adult). In comparison, Hopper and Meyers (1966) observed life cycles of approximately one month for several chromadorid species maintained in a laboratory culture and Mutsumi *et al*. (1998) reported 40 days life cycle for two chromadorids, *Prochromadorella* sp. and *Spiliphera* sp. According to Vranken *et al*. (1981) *Monhystera parelegantula* can be used for mass cultivation due to its short life cycle. Likewise, *D. normandicum* is also suited for small scale laboratory experiments, particularly owing to its short generation time, life cycle and survival in sewage polluted area. The culture method described here is very handy and can be applicable to other nematode species with comparable feeding habits.

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