

MACROBENTHIC NEMATODES INHABITING MUSSEL BEDS: SEASONAL VARIABILITY IN THE POPULATION STRUCTURE OF *ENOPLUS QUADRIDENTATUS* BERLIN, 1853 AROUND SINOP PENINSULA (SOUTHERN BLACK SEA)

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Abstract:

Spatio-temporal distribution and population structure of *Enoplus quadridentatus* Berlin, 1853 associated with mussel beds were investigated seasonally (October 2013, January 2014, May 2014 and July 2014) along the coasts of Sinop Peninsula, the northernmost point of Turkey located at the Black Sea. A 20x20 cm quadrat was used by scuba divers to collect samples at five stations, of which four of them were located on rocky substrata and one on a concrete pier pile. Nematode densities ranged between 0 – 996 ind.m⁻² and found high in October and May. A total of 1100 individuals and 9 species belonging to 8 families were recruited. *E. quadridentatus* Berlin, 1853 was the dominant species in most of the nematode assemblages inhabiting mussel beds and had a contribution of 74% to the total fauna. The density of the species varied among the seasons with peaks in May and October, consistent with the general nematode densities. Population composition revealed seasonal changes. In general, females were dominant in the population and ovigerous females were recorded only in January and May. Juveniles were found during all seasons, but formed dense populations in July and May. It seems that the reproduction of the species is continuous throughout the year.

Keywords: Black Sea, *Enoplus quadridentatus*, Freeliving marine nematodes, Population dynamics, Mussel beds

Introduction

Free-living marine nematodes are known to be the most diverse and most abundant group of metazoans living in the benthic zone of marine environments (Heip *et al.*, 1982). They can be found both in macrobenthic and in meiobenthic samples, although their diversity is so much low in macrobenthic samples with a general dominance of larger taxa such as the members of the order Enoplida.

The genus *Enoplus* Dujardin, 1845 is a large group of the marine family Enoplidae which has 36 valid species (Lee *et al.*, 2015). It is one of the dominant genera at the intertidal zone with a cosmopolitan distribution in the world seas. Species identification can be made based on several characteristics such as the shape of the tail, spicule, gubernaculum and morphometric ratios.

Enoplus quadridentatus Berlin, 1853 was originally described from the Mediterranean Sea. The species has three synonyms in literature (Gerlach *et al.*, 1974). A few years after its description, it was reported again as a new species under the name *Enoplostoma hirtum* Marion, 1870 from the Mediterranean Sea.

It has been described from Black Sea as a new species to science under the name *Enoplus euxinus* Filipjev, 1918. After that, several other authors (Paladian, 1965; Kisseleva and Slavina, 1964; Sergeeva, 1974) followed Filipjev and reported the species as *E. euxinus* Filipjev, 1918 from the Black Sea. The species has also been found in the Sea of Marmara (Turkey) (Allgen, 1941), however given under the name *Enoplus hirtus* (Marion 1870).

Only three species, *Enoplus quadridentatus* Berlin, 1853, *Enoplus littoralis* Filipjev, 1918 and *Enoplus maeoticus* Filipjev, 1916 have been reported up to now from the Black Sea (Gerlach and Riemann, 1974; Zaitsev and Alexandrov, 1998). In Turkish seas, two species of the genus *Enoplus* have been recorded so far. *E. quadridentatus* was found in the Sea of Marmara, Bosphorus and Black Sea coasts of Turkey (Allgen, 1941; Sergeeva, 1973; Ürkmez, 2015) and *E. meridionalis* was reported from the Aegean coast of Turkey (Çınar *et al.*, 2006).

Mussel beds may have influence on biodiversity by inhibiting or assisting the occurrence of some species by changing the environmental properties (Ragnarsson and Raffaelli, 1999). Mussels also clear the water by filter feeding in contrast to the

other organisms that change habitats, such as seagrasses (Kautsky and Wallentinus, 1980). Bio-deposition is also high in mussel beds providing food for deposit-feeders (Norkko *et al.*, 2001). Furthermore, the presence of mussels cause hydrodynamic changes at the bottom surface causing decrease in oxygen and increase in sulphide levels in the sediment (Ragnarsson and Raffaelli, 1999).

In the Black Sea, the free-living nematode fauna of the *M. galloprovincialis* and *M. phaseolina* facies have been previously studied in southern area of the Crimean Peninsula (Sergeeva, 1974). The role of *Mytilus galloprovincialis* in the formation of meiobenthic communities in the Black Sea with a special emphasis on free-living nematodes as the dominant group of free-living nematodes has been described for the first time in Sevastopol (Crimea, Russia) on the results of experimental studies in natural conditions (Sergeeva, 1985a, b). It has been established that this mussel acts as an ecological factor determining the development of the species composition, abundance and trophic structure of the nematode assemblages. The biodiversity and ecology of mussel beds have also been studied by scientists in Turkey (Topaloglu and Kihara, 1993; Çınar and Gönlügür-Demirci, 2005; Çınar *et al.*, 2008). However the species lists generally cover macrofauna and free-living nematode assemblages have never been examined.

The aim of this paper is to describe nematode assemblages and the spatial and temporal patterns of *Enoplus quadridentatus* Berlin, 1853 associated with mussel beds located along the coasts of Sinop Peninsula, Southern Black Sea.

Materials and Methods

Sinop Peninsula is a coastal region located at the northernmost point of Anatolia (Turkey) projecting towards the Black Sea. It lies in the middle of the eastern and western Black Sea basins of the Turkish coast. The peninsula is surrounded by the sea at its three sites. Four field studies were conducted at five stations in October 2013 and January, May and July 2014 around the peninsula. Stations M2, M3, M4 and M5 were natural rocky habitats whereas station M1 was a concrete pier pile at the city harbour (Figure 1). Mussel samples (*Mytilus galloprovincialis* Lamarck, 1819 and *Mytilaster lineatus* Gmelin, 1791) facies) were collected as three replicates at all the sampling stations using a 20x20 cm metal frame (quadrat) equipped with a bag made of a plankton net. On

board, samples were wet sieved through 0.5 mm mesh and obtained material was fixed in separate jars containing 4% seawater formaldehyde solution.

Temperature, salinity, pH, dissolved oxygen concentration, total dissolved solids (TDS) and conductivity were recorded in situ using a YSI 6600 V2 probe.

The material was wet sieved under fresh water in the laboratory and sorted into taxonomic groups under a stereo microscope, and preserved in 70% ethanol. Nematodes were transferred to anhydrous glycerol with slow evaporation method and mounted on glycerine slides. Specimens of *Enoplus* were examined and photographed using a LEICA

research microscope equipped with a software platform LAS V.4 (Leica Application Suite) and a NIKON Eclipse Ni-U research microscope with Nomarski differential interference contrast (DIC) optics. Specimens were also categorized as males, females, ovigerous females or juveniles. The material was deposited at the Sinop University.

Community parameters such as number of species, number of individuals, density (ind.m^{-2}), quantitative dominance index ($D\%$), Margalef species richness (R), Pielou's evenness index (J') and Shannon diversity index (H') were estimated for each station in each sampling period. PRIMER software was used for the calculation of diversity indices (Clarke and Warwick, 2001).

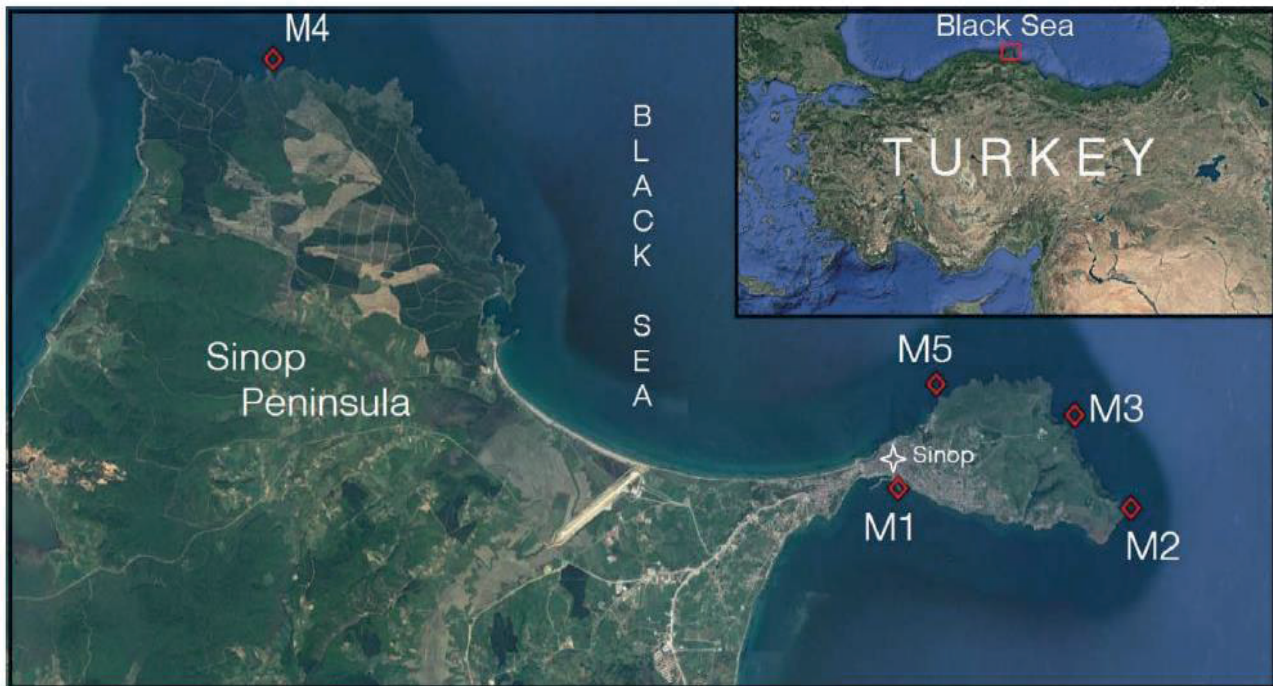


Figure 1. Map of the study area

Results and Discussion

Temperature showed variation during the study period. The lowest temperature was recorded in January 2014 (9.79 °C) and the highest in July 2014 (22.08 °C). Dissolved oxygen (DO) ranged between 7.9 and 10.4 mgL⁻¹. Salinity did not show high variation and recorded between 18.22 and 18.49. Total dissolved solids and pH revealed consistent levels throughout the study period (Table 1).

A total of 1100 individuals of free-living marine nematodes were counted and analysed from the 60 samples collected at five stations and four sampling seasons along the coasts of Sinop Peninsula. Nematode communities were present at all the months sampled. Highest mean densities were recorded at station M2, in May (996 ind.m⁻²) and October (626 ind. m⁻²). No nematodes were recruited at station M5 in January and at station M4 in July.

Identifications yielded 9 free-living marine nematode species (*Anticoma acuminata* (Eberth, 1863) Bastian, 1865; *Chromaspirina* sp., *Enoplus quadridentatus* Berlin, 1853; *Euchromadora* sp., *Linhomoeus* sp., *Oncholaimus dujardini* de Man, 1876; *Thoracostoma* sp., *Symplocostoma tenuicolle* (Eberth, 1863) Wieser, 1953; Unidentified species A) belonging to 8 families (Anticomidae, Desmodoridae, Enoplidae, Chromadoridae, Linhomoeidae, Oncholaimidae, Leptosomatidae, Enchelidiidae) and 4 orders (Enoplida, Desmodorida, Chromadorida, Monhysterida). All the identified genera were monospecific. In total, *Enoplus quadridentatus* Berlin, 1853 was the dominant species in mussel beds (74 % of total specimens). Its contribution to nematode assemblages was high at all stations except St. M1 (January and May) and St. M4 (January) (Figure 2).

Table 1. Depth, coordinates and seasonal environmental parameters of the sampling stations (S: station, D: depth (m), AD: abiotic data, T: temperature (°C), TDS: total dissolved solids, S: salinity (‰), DO: dissolved oxygen (mgL⁻¹)).

| S | D | Coordinates | AD | Oct/13 | Jan/14 | May/14 | Jul/14 |
|---|---|---------------|-----|--------|--------|--------|--------|
| | | | T | 17.2 | 9.8 | 15.4 | 22.1 |
| | | | TDS | 19.1 | 19.5 | 19.1 | 19.2 |
| 5 | | 42°1'19.2"N | S | 18.3 | 18.5 | 18.2 | 18.3 |
| | | 35°9'4.57"E | pH | 6.7 | 6.6 | 6.8 | 7.0 |
| | | | DO | 8.7 | 9.8 | 9.5 | 8.2 |
| | | | T | 17.0 | 9.9 | 12.4 | 21.9 |
| | | | TDS | 19.2 | 19.5 | 19.3 | 19.2 |
| 4 | | 42°1'18.26"N | S | 18.3 | 18.5 | 18.4 | 18.3 |
| | | 35°12'54.43"E | pH | 6.9 | 6.8 | 6.9 | 7.0 |
| | | | DO | 8.7 | 9.7 | 9.5 | 8.1 |
| | | | T | 17.4 | 10.0 | 12.3 | 21.5 |
| | | | TDS | 19.1 | 19.4 | 19.3 | 19.2 |
| 5 | | 42° 2'2.78"N | S | 18.2 | 18.5 | 18.4 | 18.3 |
| | | 35°11'50.05"E | pH | 6.9 | 6.8 | 6.9 | 7.0 |
| | | | DO | 8.6 | 9.7 | 9.6 | 8.4 |
| | | | T | 17.0 | 10.0 | 12.4 | 23.3 |
| | | | TDS | 19.1 | 19.4 | 19.3 | 19.2 |
| 4 | | 42°5'36.6"N | S | 18.2 | 18.5 | 18.4 | 18.3 |
| | | 34°58'57.79"E | pH | 6.9 | 6.8 | 6.9 | 6.9 |
| | | | DO | 8.7 | 10.1 | 9.9 | 7.9 |
| | | | T | 16.9 | 9.9 | 12.1 | 23.5 |
| | | | TDS | 19.1 | 19.4 | 19.4 | 19.1 |
| 5 | | 42°2'15.97"N | S | 18.2 | 18.5 | 18.5 | 18.2 |
| | | 35°9'49.57"E | pH | 6.9 | 6.8 | 6.9 | 7.0 |
| | | | DO | 8.6 | 9.8 | 10.4 | 8.2 |

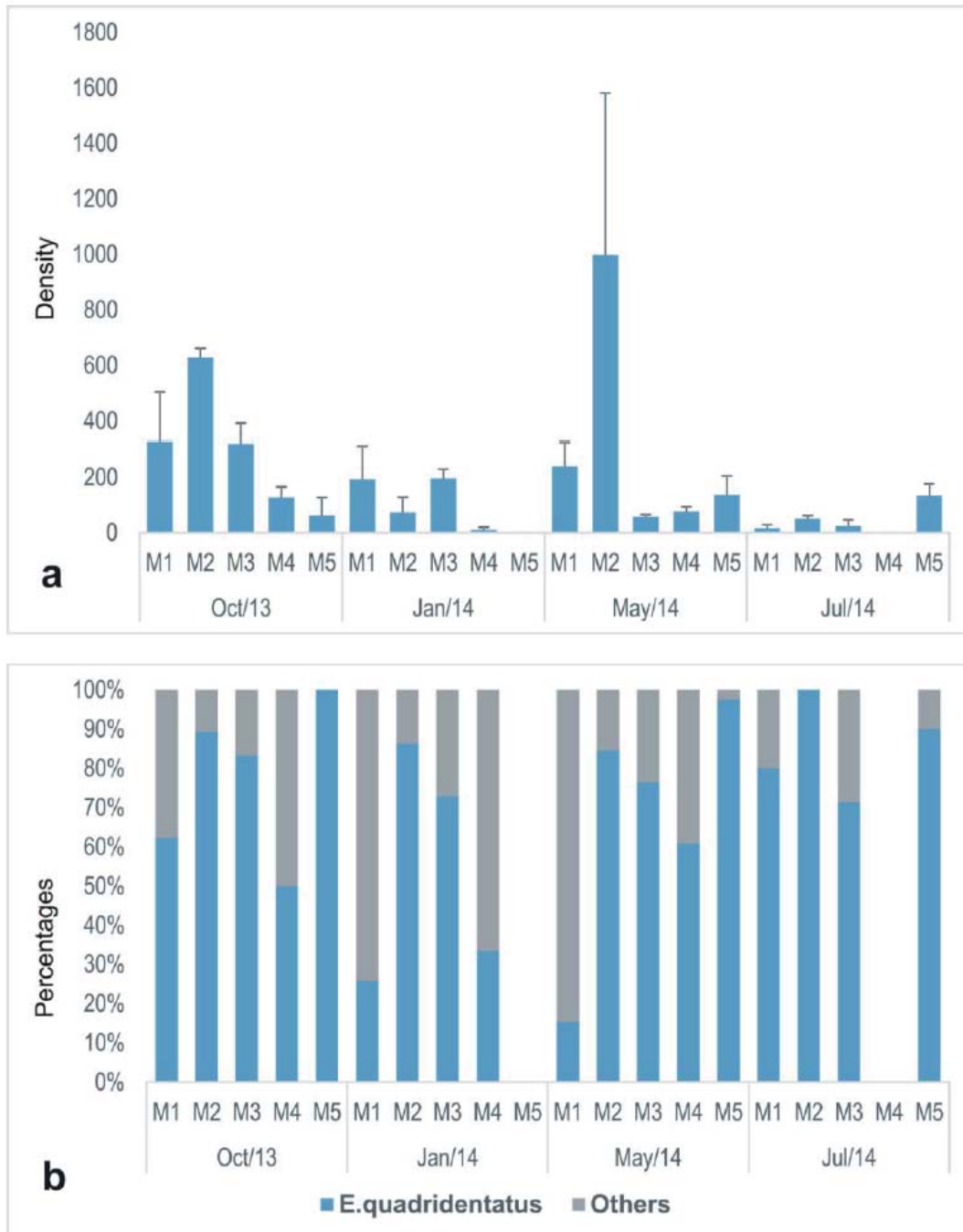


Figure 2. a) Spatio-temporal distribution of mean nematode densities (ind. m⁻²) with ±standard errors
 b) Contribution of *E. quadridentatus* to nematode assemblages at stations in each sampling month

According to Filipjev (1918) and Platt and Warwick (1983), general features of the species include a total body length of 3.1-4.8 mm with a maximum diameter of 110-60 μm ($a=23-35$), jaw length equal to $\frac{1}{2}$ head width (Figure 3a), tail a little over 3 anal body diameter, spicules 1.7 anal body diameter, precloacal supplement half the spicule length, with a characteristic tube lining the distal third (Figure 3b). Scales on spicules variable up to eight (Figure 3c). Body tapers very little anteriorly and seems truncated. The middle of the body is swollen, more in the females. The body tapers to the anus in the male very slightly. The body width increases with growth. *E. quadridentatus* Berlin, 1853 is very similar to *E. communis* Bastian, 1865 but it is smaller and has a relatively longer tail and a shorter spicule.

Systematic accounts:

Class Enoplea Inglis, 1983

Order Enoplida Filipjev, 1929

Family Enoplidae Dujardin, 1845

Genus Dujardin, 1845

Enoplus quadridentatus Berlin, 1853

Table 2 shows the total number of species and individuals, diversity and evenness indices as well as the dominant species at each occasion. *E. quadridentatus* had the highest percentage at most of the cases except two occasions: one with the dominance of *Oncholaimus dujardinii* de Man, 1876 at station M1 in May (39%) and in the other case *E. quadridentatus* shared the same percentage (33%) with *Symplocostoma tenuicolle* (Eberth, 1863) Wieser, 1953 and *O. dujardinii* de Man, 1876 at station M4 in January. Highest species diversity was found at station M1 in January 2014 (2.78) and the lowest at station M4 in July 2014 and station M5 in January 2014. Table 2 presents the other community parameters of the stations.

Temporal fluctuations were recorded in the mean densities of the species. In total, October and May revealed the highest values (1156 and 1103 ind.m^{-2}). Mean densities were considerably low in January and July (260 and 200 ind.m^{-2}). Spatial distribution showed that the highest number of individuals (1516 ind.m^{-2}) was found at station M2 when the total densities of four seasons were taken into consideration.

The population pattern of *E. quadridentatus* was consistent during the study period and individuals

were recorded during all the four sampling seasons in mussel beds. The density of the species varied among the seasons. Dense populations were recorded at station M2 in May 2014 (843 ind.m^{-2}) and October 2013 (560 ind.m^{-2}), showing a prominent seasonal fluctuation (Figure 4). No individuals were found at station M5 in January and at station M4 in July 2014.

When all the samples considered, 209 males (26%), 267 females (34%), 82 ovigerous females (10%) and 237 juveniles (30%) were analysed (Figure 5). In total, females shared the largest part in the population (44%) and the males had the smallest part (26%) during the study period. In general, male to female ratio was 0.59:1. Ovigerous females made up 76.5% of all females in the population examined and they were recorded only in January and May (Figure 6) at all the stations.

Consistent with the population density trends, highest number of adults were found in October and they more abundantly found at station M2 compared to the other stations. Juveniles were recorded during the four sampling months but they were dominant particularly in July (79%) and May (56%). Ovigerous females disappeared as the temperature rises from May towards July and they were not found in October samples. Seasonal fluctuations in the population structure of the species are presented in Figure 6.

The nematode diversity of the mussel beds around Sinop Peninsula was low with a total of 9 free-living nematode species. *Enoplus quadridentatus* Berlin, 1853 was found to be the dominant species at most cases. This species was also reported from mussel beds of Sevastopol, Crimea, under the name *E. euxinus* Filipjev, 1918. In recent years, it was found to be dominant in *Mytilus* spp. beds at Romanian coasts (Mihaela Muresan, pers. comm.). Muresan (2012) also reported it under the name *E. euxinus* Filipjev, 1918 and stated it to be one of the most common species below 50 m at the Romanian coasts and also reported it from periazotic waters (Muresan, 2014). *E. quadridentatus* was listed among the species of Ukrainian waters as well (Zaitsev and Alexandrov, 1998).

The species has been previously reported from soft bottoms of Turkish waters from the Bosphorus (Sergeeva, 1973) and from Sinop, central Black Sea coast (Ürkmez, 2015). Allgen (1941) reported it from algae samples he collected at shallow rocky shores of Bandırma (Sea of Marmara).

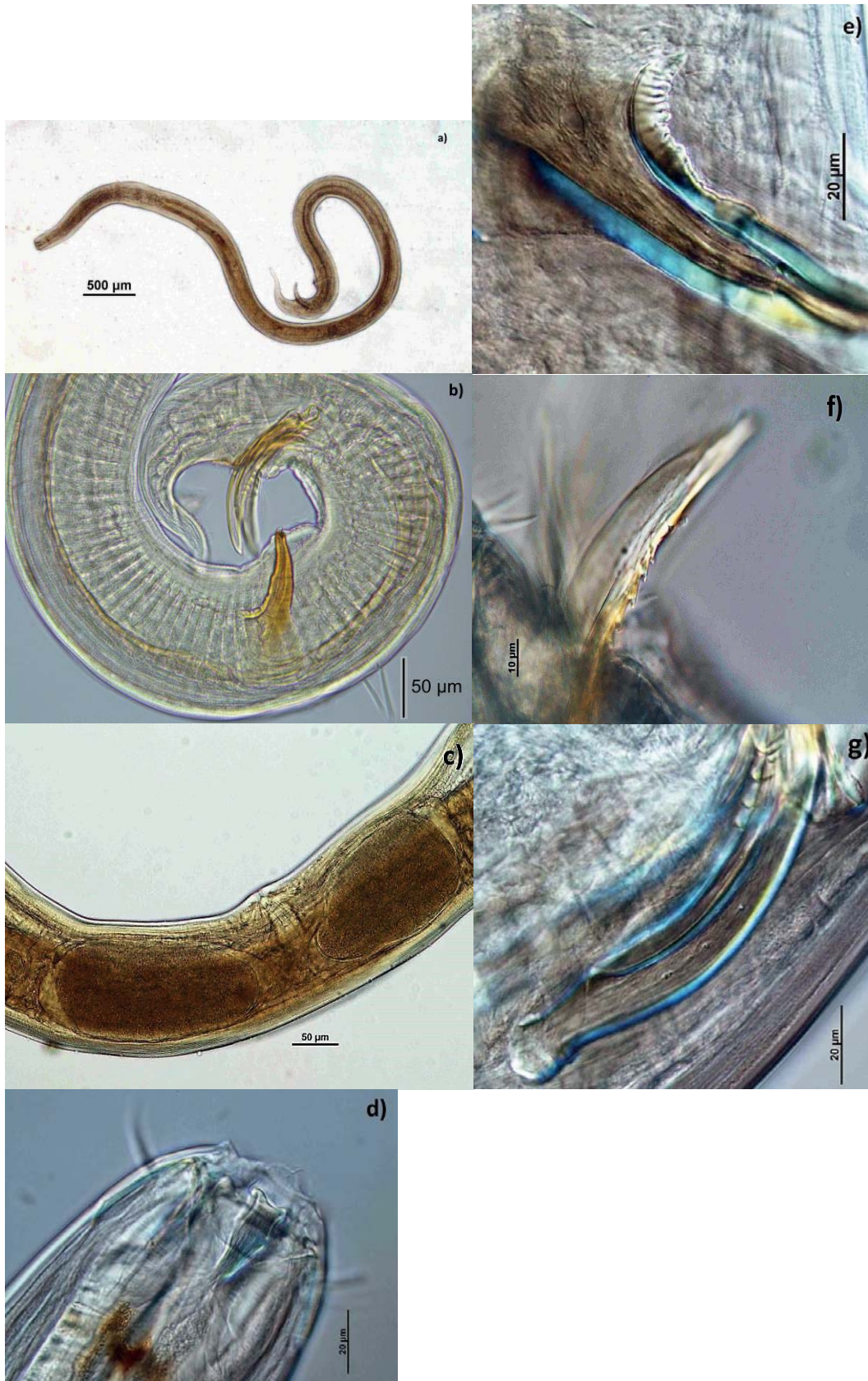


Figure 3. *Enoplus quadridentatus*, light microscopy photographs (a-c), DIC photographs (d-g) a. Total body of male b. Posterior region of male showing tail, spicules, gubernaculum and precloacal supplement c. Vulva region of female with eggs d. Head region e. Precloacal supplement in male f. Spicule plates in male g. Spicule of male

Table 2. Number of species (Sp), Density (D, ind.m⁻²), Margalef species richness (R), Pielou's evenness (J') and Shannon's diversity (H') indices of each station and season showing the dominant species (S: station).

| S | Months | Sp | D | R | J' | H' | Dominant species (%) |
|---|--------|----|-----|------|------|------|--|
| | Oct/13 | 6 | 325 | 0.86 | 0.58 | 1.50 | <i>Enoplus quadridentatus</i> (62) |
| | Jan/14 | 8 | 194 | 1.33 | 0.93 | 2.78 | <i>E. quadridentatus</i> (26) |
| | May/14 | 7 | 241 | 1.09 | 0.88 | 2.47 | <i>Oncholaimus dujardinii</i> (39) |
| | Jul/14 | 2 | 16 | 0.36 | 0.70 | 0.70 | <i>E. quadridentatus</i> (80) |
| | Oct/13 | 7 | 627 | 0.93 | 0.26 | 0.74 | <i>E. quadridentatus</i> (89) |
| | Jan/14 | 3 | 73 | 0.47 | 0.44 | 0.70 | <i>E. quadridentatus</i> (86) |
| | May/14 | 4 | 997 | 0.43 | 0.40 | 0.80 | <i>E. quadridentatus</i> (85) |
| | Jul/14 | 1 | 50 | 0.00 | 0.00 | 0.00 | <i>E. quadridentatus</i> (100) |
| | Oct/13 | 6 | 321 | 0.87 | 0.40 | 1.02 | <i>E. quadridentatus</i> (83) |
| | Jan/14 | 5 | 196 | 0.76 | 0.50 | 1.16 | <i>E. quadridentatus</i> (72) |
| | May/14 | 4 | 56 | 0.75 | 0.56 | 1.12 | <i>E. quadridentatus</i> (76) |
| | Jul/14 | 3 | 23 | 0.64 | 0.69 | 1.09 | <i>E. quadridentatus</i> (71) |
| | Oct/13 | 3 | 127 | 0.41 | 0.90 | 1.42 | <i>E. quadridentatus</i> (50) |
| | Jan/14 | 3 | 9 | 0.91 | 1.00 | 1.58 | <i>E. quadridentatus</i> (33), <i>S. tenuicolle</i> (33) |
| | May/14 | 4 | 77 | 0.69 | 0.74 | 1.48 | |
| | Jul/14 | 0 | 0 | 0.00 | 0.00 | 0.00 | <i>E. quadridentatus</i> (60) |
| | Oct/13 | 1 | 63 | 0.00 | 0.00 | 0.00 | <i>E. quadridentatus</i> (100) |
| | Jan/14 | 0 | 0 | 0.00 | 0.00 | 0.00 | |
| | May/14 | 2 | 136 | 0.20 | 0.15 | 0.15 | <i>E. quadridentatus</i> (98) |
| | Jul/14 | 3 | 133 | 0.41 | 0.34 | 0.54 | <i>E. quadridentatus</i> (90) |

Although *E. quadridentatus* was mostly the dominant species, station M1 showed different compositions and it was replaced by *Oncholaimus dujardinii* in May and by the second dominant species, *Thoracostoma* sp. in January. The difference may be due to the fact that station M1 was the only sampling area on a concrete pier pile with mussels attached as fouling organisms, while the other stations were mussel beds on natural rocky habitats. Another point was that, the size of the adults seemed to vary depending on the season and in females, depending on the number of eggs in the genital tract. This observation was also mentioned by Filipjev (1918) in the Crimean population of *E. quadridentatus*.

Seasonal dynamics and different spatial distribution patterns were observed in the density of this species associated with mussel beds along the coasts of Sinop Peninsula. *E. quadridentatus* seems to make denser populations in October and May in mussel beds. Oviparous females were recorded at three seasons from January and declining

towards July. Juveniles were found during each season with an increasing share beginning from October to July. These may indicate that the species have a continuous reproduction during the year.

Several other members of the order Enoplida have previously been studied in terms of their population structure at tropical sandy beaches. Venekey et al. (2011) found that *Mesacanthion hirsutum* Gerlach, 1953 may have a continuous reproduction and, certain species of the genus *Oncholaimus* have also been studied by Skoolmun and Gerlach (1971) and Smol et al. (1981). They reported one or two generations per year. It was also stated that the annual number of generations is more than one or two, with a continuous reproduction in many species (Heip et al., 1982). However seasonal sampling is a limiting factor in the present study to tell more about the life cycle of a species since many species complete their life cycle in very short periods (Tietjen and Lee, 1972; Moens and Vincx, 2000).

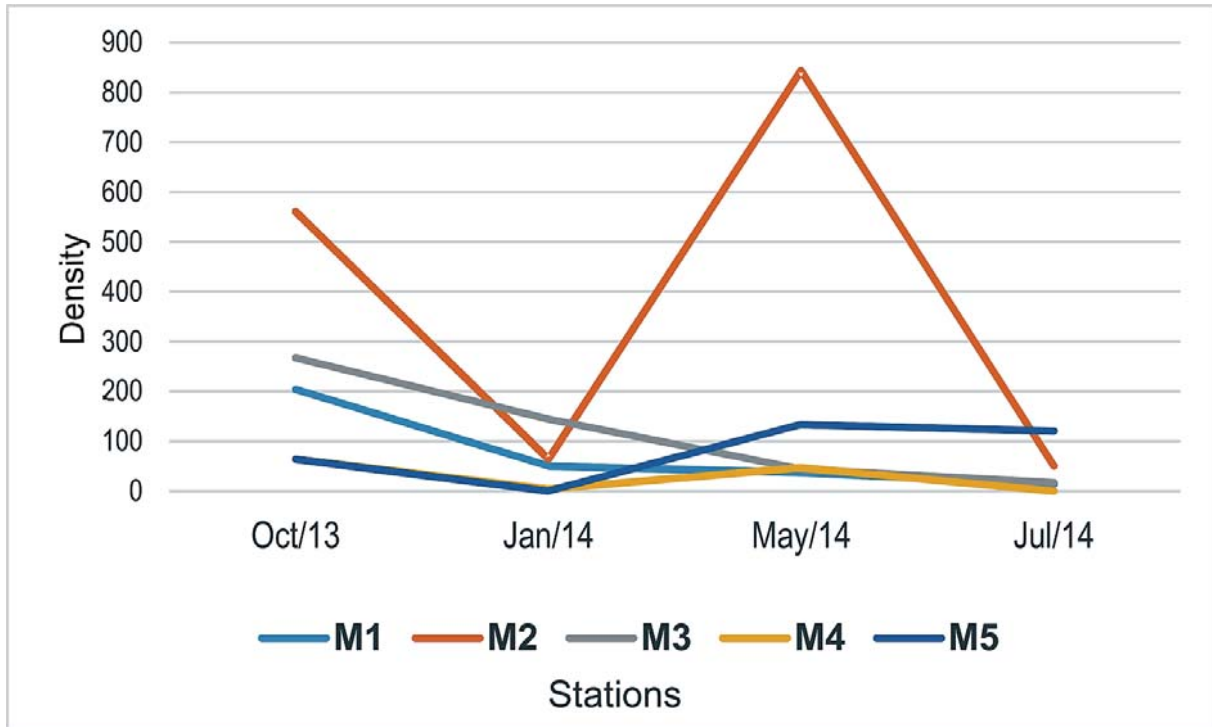


Figure 4. Variation in the seasonal densities (ind. m⁻²) of *Enoplus quadridentatus* at the sampling stations

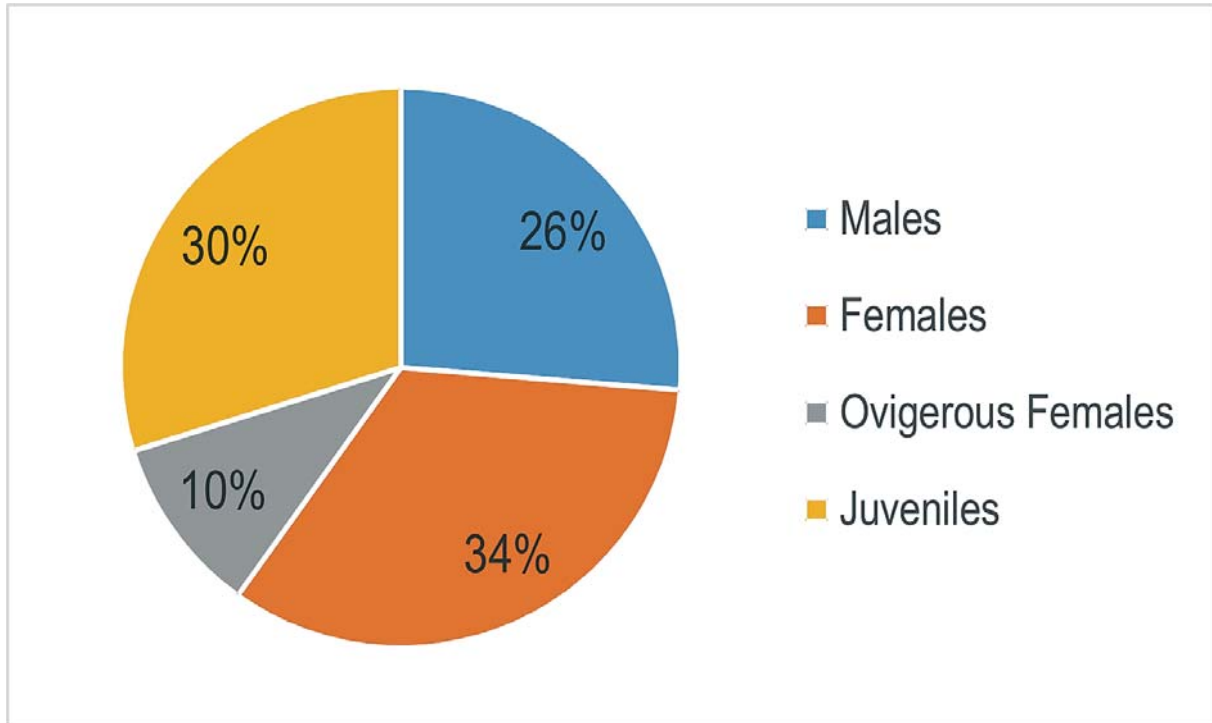


Figure 5. Percentage of population categories during the whole study period

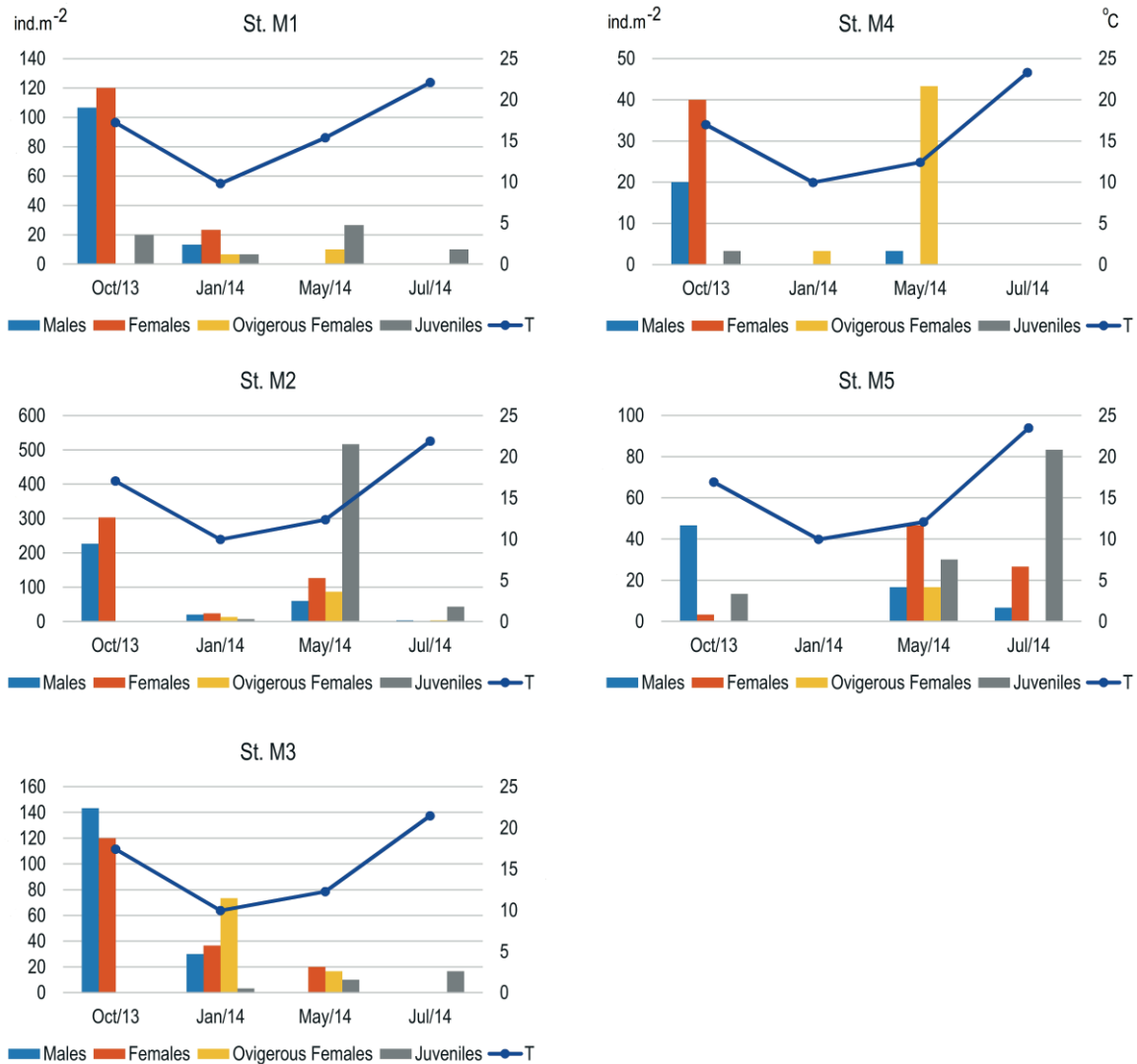


Figure 6. Seasonal population structure at sampling stations.

Conclusions

As a conclusion, the present paper contributes to the study of free-living marine nematodes in Turkey focusing on the mussel beds from the Turkish Black Sea coast. Although it may give an idea about the nematode composition of *Mytilus galloprovincialis* and *Mytilaster lineatus* facies, the macrobenthic sampling design using 0.5 mm mesh size is a limiting factor to examine the smaller meiobenthic nematodes and this may result in underestimation of nematode density and diversity of the habitat in focus. However, this study stands as the first paper about population structure of a marine nematode in Turkish Seas. *E. quadridentatus* Berlin, 1853 and it seems to have a continuous reproduction cycle throughout the year. On the other hand, it is not possible to obtain all the necessary information on reproductive cycles of free-living

nematodes by taking samples from their natural environment. Laboratory experiments are also needed to clearly specify the reproduction cycle of a marine nematode species.

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