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Taxonomical and ecological characteristics of *Centropages ponticus* (Copepoda, Calanoida) from the saline Lake Mir (Dugi Otok, Adriatic Sea)

Frano KRŠINIĆ

Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split

****Corresponding author; e-mail: fkrsinic@izor.hr***

Calanoid copepod Centropages ponticus, *endemic to the Mediterranean and Black Sea, is the only pelagic copepod species which was found in the closed ecosystem of the saline Lake Mir on Dugi Otok, eastern Adriatic Sea. This paper presents some important morphological characteristics and the annual population structure investigated from November 1999 to October 2000 at one station in the deepest part of Lake Mir. Investigation topics included the temporal distribution and abundances of nauplii, copepodites and adult specimens, as well as the density of their faecal pellets. It would appear that only* C. ponticus *is adapted to the extreme conditions of the lake, low winter and high summer temperatures, as well as long period of hypersaline conditions. During the summer-autumn period, the abundances of nauplii, copepodites and adult specimens reach extreme values. Lake Mir is ecologically very interesting and generally among very rare habitats. Our results suggest the necessity for further complex research of this natural laboratory for the purpose of its full protection.*

Key words: Calanoids, copepods, Lake Mir, Adriatic Sea

INTRODUCTION

Saline lakes are widespread on the mainland, being almost as common as freshwater lakes. These lakes vary considerably in size, depth, salt concentration and other environmental factors. The fauna is poor, freshwater origin (TIFFANY *et al*., 2002; DAWSON & HAMNER, 2005; HUMPHREYS *et al*. 2009). On the other hand, marine lakes has considerably less, which are smaller in size and located in the narrow coastal areas. The lakes are formed in the Holocene at points in time when there was an increase in the sea level and flooding of coastal valleys (HODGKIN & HESP, 1998). Fauna in marine lakes is from the surrounding sea, but the number of species is small.

Along the coast of the eastern Adriatic Sea, there are several examples of marine lakes. The most studied are two interconnected lakes on the island of Mljet, characterized with poor faunal composition encompassing about 25 holoplanktonic species in the Veliko Jezero which connects to the open sea, and by 50% fewer species in the more isolated Malo Jezero (VUČETIĆ, 1957). There are also two small permanently closed marine lakes; Lake Rogoznica near the city of Šibenik on the mainland and Lake Mir located on the island of Dugi Otok. Lake Rogoznica is naturally eutrophicated, with permanent hypoxia/anoxia in the bottom layer, and periodical presence of hydrogen sulfide and high values of ammonium. The only planktonic copepod species adapted to occasional extreme conditions in the Rogoznica Lake is *Acartia italica* (KRŠINIĆ et al., 2000). In the Lake Mir, the only pelagic copepod species is *Centropages ponticus*.

Lake Mir, with wide temperature range and hypersaline conditions, is one of the few rare habitats in which only one species of planktonic copepods can exist and thrive. Due to significantly smaller size of both sexes of *C. ponticus* specimens from this Lake compared to those in the surrounding open sea, a more detailed investigation of its morphology is required in order to contribute to correct taxonomical determination of *Centropages* species. This paper examines the reasons why the species is identified as *C. ponticus*. It also deals with the population structure observed during a one-year period, which included the abundances of nauplii, copepodites and adult specimens, as well as the density of their faecal pellets.

Study area

Saline lake Mir is located in the south-western part of the Nature Park Telašćica on island Dugi Otok, central part of the eastern Adriatic Sea. (Figure 1). The length of the lake is approximately 900 m, width 280 m and the maximum depth 7 m. The lake is situated in a narrow area of land between the Bay Telašćica and the open sea. The lake is separated from the open sea by karstic rock about 90 m wide and has no direct link with the open sea. Nevertheless, because of the porosity of karstic rocks, very low tidal activity can be observed in the lake only about 2 cm. The lake bottom in shallow areas is rocky and in deeper it is covered with clay. Because of that, the lake is warmer than the coastal sea in the summer 27.5 ^{o}C in June and colder in winter 3.60 ^{o}C in January due to the shallowness of the lake. The salinity of the lake is over average due to evaporation, ranged from 37.9 in March to 44.36 in September (ČALIĆ *et al*. 2007). These extreme conditions are the cause of biological diversity poverty of the lake. In the benthos there are only a few species of shellfish and crabs. Detailed analysis of hydrographic condition and chemical characteristics of the lake is presented by CARIĆ *et al*. 2010; ŽIC *et al*., 2012; TRUESDALE *et al*., 2012.

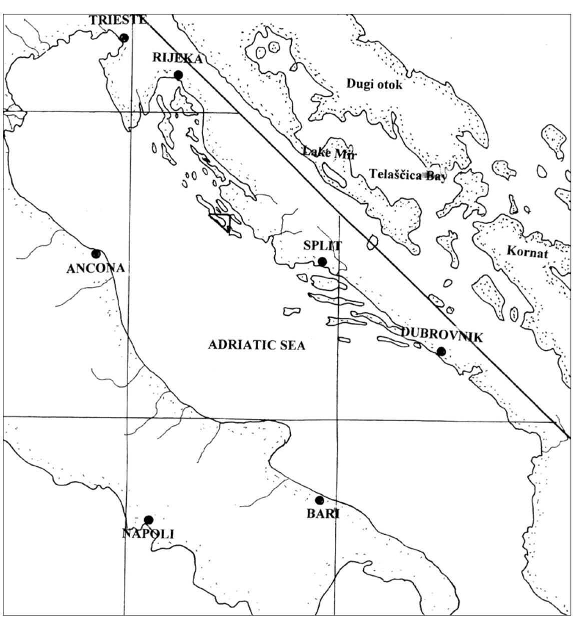

Fig. 1. Location of sampling station in the Lake Mir.

MATERIALS AND METHODS

Samples for zooplankton analysis were obtained from November 1999 to October 2000 at one station in the deepest part of Lake Mir (Figure 1). Samplings were conducted once a month. Water samples were taken with 5-L Niskin sampler at depth intervals of 1 m to 7 m from the surface. Samples were preserved in neutralized formaldehyde with $CaCO_3$ with a 2.5% concentration after sedimentation . Materials were sedimented for 24 h in plastic containers in the laboratory, from which about 3/4 of the water was decanted. The remainder was poured into a glass cylinder (10 cm diameter) and was sedimented for a further 24 h after which the superfluous volume was decanted. This process reduced the original volume of 5 l to 30 ml in 72 hours. Decanting was accomplished using a vacuum pump and a slightly curved pipette (KRŠINIĆ, 1980). The organisms were analised by an Olympus IMT-2 inverted microscope at magnifications of 20 and 100x. Total samples were counted in a glass cell, dimensions 7 x 4.5 x 0.5 cm. Drawings were made with the aid of a camera lucida on an Olympus BX51 differential interference contrast microscope. Specimens were measured using an ocular micrometer.

RESULTS

Taxonomic consideration

Species *Centropages ponticus* KARAVAEV, 1895 from the Lake Mir was determined on the basis of comparison with description of congeneric and morphologically very similar species *Centropages kroyeri* GIESBRECHT 1893 [„1892“], and the new samples materijal from the Bay of Naples (which were kindly made available by M.G. Mazzocchi). Also original description of *C. ponticus* KARAVAEV 1895 and a revision with a new description (SOLER *et al.*, 1988).

The total length of female specimens *C. ponticus* from the Lake Mir was 740 – 820 µm; average 786.3 ± 27.2 µm; n=8 and male 680 – 800 µm; average 757.5 ± 38.0 µm; n=8. Genital double-somite is swollen more conspicuosus on

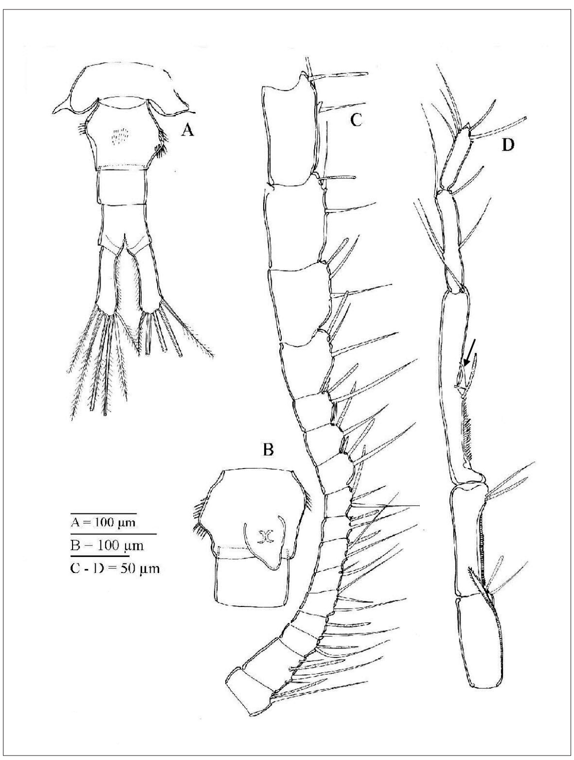

Fig. 2. Centropages ponticus; *A, female urosome and caudal rami, dorsal view; B. genital double-somite, ventral view; C,D, male antennule.*

the right side, with numerous spinules placed more dorso-laterally than on the left side, and with a very small spinular area on the middorsal surface (Figure 2A). External genital area is latero-ventral, with paired gonopores positioned approximately at 1/6 distance from posterior margin, and is covered by an operculum triangular placed more on the left side. Operculum’s posterior part, at approximately 1/3 of length, overlaps second somite (Figure 2B). Intermediate somite is short. Caudal rami are slightly curved and divergent, setose along the inner margin. Each ramus is armed with 6 setae, 5 are distal and one is a short appendicular seta arising from the inner corner.

Male right antennule (Figures 2C,D) armature and fusion pattern of segments are as follows: segment 1 (ancestral I) 1 + ae; segment 2 (II – III) 3 + ae; segment 3 (IV) 1 + ae; segment 4 (V) 1 + ae; segment 5 (VI) 2 setae + ae; segment 6 (VII) 1 + ae; segment 7 (VIII-IX) 2 setae + ae; segment 8 (X) 1 + ae; segment 9 (XI) 2 setae + ae; segments 10-12 (XII-XIV) 1 seta + 1 spina + ae each; segments 13-16 (XV-XVIII) 2 setae + ae each; segments 17 – 18 (XIX – XX) 1 seta + ae each; segment 19 (XXI-XXII) 2 setae + ae; segment 20 (XXIII-XXIV) 4 setae; segment 21 (XXV-XXVIII) 4 setae + ae. Segment 16 (ancestral XVIII) has an hyaline horn on outer border. Geniculation is between ancestral segments XX and XXI. Ancestral segment XIX with denticulate lamella that exceeds 1/3 of the ancestral segment XX and terminating in a thorn with denticles on both margins. The following segment XXI also with uniformly denticulate lamella reaching 2/3 of the segment. Longest segment XXI –XXIII with smaller denticles and in the middle position a hyaline spur (see arrow in the Figure 2D), an aestetask and a small seta.

Female fifth legs are symetrical, biramous. Coxa and basis are unarmed. First and second exopodal segments fused, on inner distal corner the setae are transformed into strong spine, setal formula is I-II, 4-I,II. First endopodal segment seta is transformed into spine, setal formula is I-0, 1-0, 2-2-2 (Figure 3A). Second exopodal segments of male forth leg with a relatively long curved spine (Figure 3B). Male fifth legs are

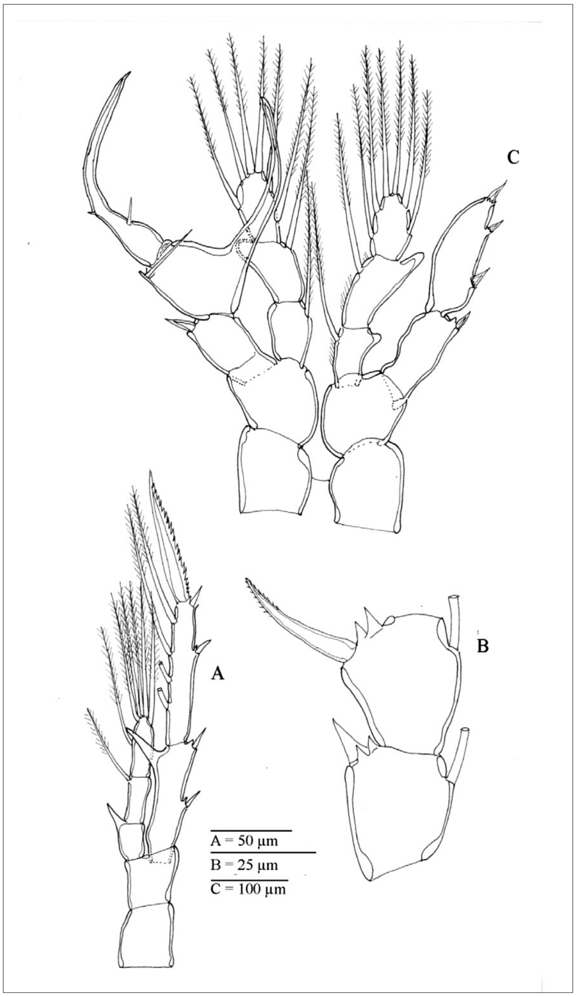

Fig. 3. Centropages ponticus, *A, female fifth leg; B, male second exopodal segment of forth leg; C, male fifth legs.*

asymetrical, biramous; coxa and basis are unarmed (Figure 3C). Endopodites are 3-segmented with on similar setation both sides, with a large swelling on the outer distal corner of second segment of both legs, and a smaller swelling of first segment of the left leg. Left exopodite is 2-segmented, the terminal segment has three spines, with a small proximal tooth. Right exopodite is 3-segmented; the first segment has a spine on the distal external corner, the second segment is in form of a huge claw, with a fine spine at the distal external corner, in horizontal position exceeds inner segment margin. Third segment is curved with small seta at the internal margin and very small spine on external margin (Figure 3C).

Distribution and abundance

Nauplii were present throughout the year and the range of abundance was 1 ind.L^{-1} to a maximum of 123 ind.L^{-1} in June at 2 m depth (Figure 4A). Their abundance was very low in January and it slightly increased gradually till May. An abrupt increase was observed June, followed by a drop in July. Then it increased again till September, it dropped again in October followed by a small increment in November and finally declined in December. (25.6 ± 25.7 ind.L^{-1} n=78). Vertical stratification of abundances was found only in June, with the highest values in upper 2 m layer.

Copepodites (Fig. 4B) and adults (Fig. 4C) have similar annual distribution, therefore a significant correlation was found between their abundance values (R_s=0.717; n=78). High values were detected in November 1999, followed by a decrease until May, when very low values were noted. The abundance of copepodites and adults increased gradually from June till August (adults) or September (copepodites) and it declined in October. Maximum of copepodites abundance was observed in September at 7 m depth (175 ind.L^{-1}) and that of adults in August at 6 m depth (31 ind.L^{-1}) (Figure 4 C). Ratio of annual average abundance between nauplii, copopodites and adults was 5.7 : 4.4 :1. Opposite to nauplii, the vertical stratification of copepodites and adults was very pronounced with highest abundance values above the sea bottom. The sex ratio was equal to 1.

Faecal pellets of copepodites and adults are permanently present from 2 m depth to the bottom. A significant positive correlation was found between abundance values distribution of faecal pellets and copepodites (R_s=0.693; $p<0.005$) and adults (R_s=0.379). The abundance of feacal pellets varied between 0 and 590 pell. L^{-1} (maximum at 7 m depth in August) and it was more important in the 5-7 m layer, 112 ± 109 pell. L^{-1}. The lowest abundance of faecal pellets was found in the period of January/March 2000 (Figure 4D).

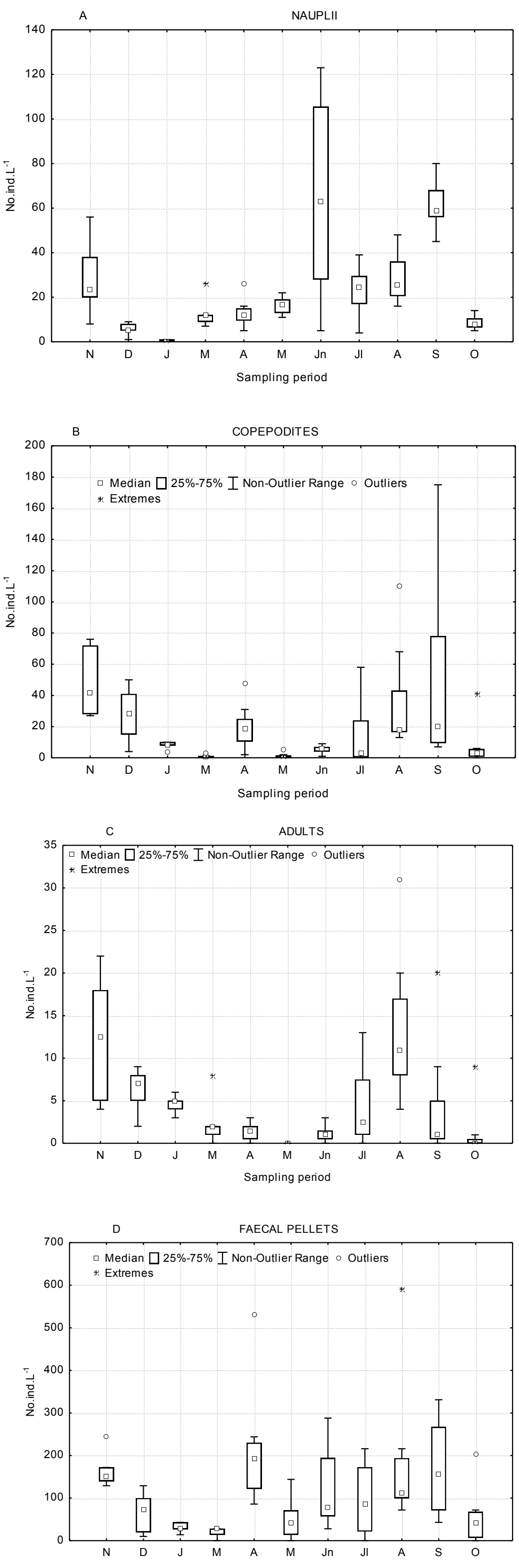

Fig. 4. abundance of nauplii (A), copepodites (B), adults (C) and faecal pellets (D), during sampling period in the Lake Mir.

DISCUSSION

Taxonomical consideration

In the monograph on copepods from the Bay of Naples, GIESBRECHT 1893 [„1892“] described the new species *Centropages kroyeri*. A few years later, on the basis of material from the Black Sea, Karavaev described *Centopages kroyeri* var. *pontica* (KARAVAEV, 1895). However, while Giesbrecht gave a very detailed description with drawings of most of the morphological characteristics, Karavajev described the above variety with few and relatively unclear illustrations. Later on GURNEY (1927) proposed the separation of this variety as a species, namely *Centropages ponticus* (KARAVAEV 1895), based on morphological differences in the fifth leg of males between specimens collected in the Suez Canal and the Bay of Naples. Morphological characteristics of *Centropages ponticus* have been investigated by KOVALEV (1967) and GARCIA-RODRIGUEZ (1985), while SOLER *et al*. (1988) made a revision *C. ponticus* with a new description of the species. The drawings of KARAVAEV (1895), KOVALEV (1967) and SOLER *et al*. (1988) are given in the description of the species in database of marine planktonic copepods (RAZOULS *et al*. 2005-2017).

C. hamatus, *C. kroyeri* and *C. ponticus* were included in the “*hamatus*” group due to their great similarities (VERVOORT, 1964). SOLER *et al*. (1988) presented the morphological differences of these species. Unfortunately, all descriptions of *C. kroyeri* and *C. ponticus* are missing the same details, which is the main reason for confusion in their determination. Notable morphological differences were found between our individuals and *C. kroyeri.* Also, similar differences were registered in the population of *C. ponticus* from Lake Mir in comparison with material from Culliera Bay, on the coast of Spain (SOLER *et al*.,1988). The average total length of males was very similar, while the total length of females of the Lake Mir population was 7% less. On the contrary, individuals of both sexes from the Black Sea and Azov Sea were significantly longer (KOVALEV, 1967) than the specimens of the present study. However several morphological differences were found between individuals of these populations. Female genital double-somite lateral swellings and an area on the dorsal surface position covered with much less spinules are present. The proportional length of intermediate somite of the female is shorter in the Lake Mir population than in those from the Black and Azov seas. First and second exopodal segments of fifth legs are fused as in individuals from Étang de Thau (SOLER *et al*. 1988). In the males found in Lake Mir, the right antennule has small hyaline horn on the outer border exclusively at segment 16, and the second exopodal segment of the forth leg has a relatively long curved spine. It should be noted that the majority of authors do not mention a fine spine at the distal external corner of the second right exopodite of male fifth legs of both species *C. kroyeri* and *C. ponticus*. In our individuals, the second segment of both endopodites of male fifth legs exhibit large swellings on the outer distal corner, while those from the Culliera Bay, SOLER *et al*. (1988) had small swellings.

Ecology of *C. ponticus* from the Lake Mir

C. ponticus is widespread in the Mediterranean Sea and Black Sea (KOVALEV & SHMELEVA, 1982; SIOKOU-FRANGOU, 1999; MAZZOCCHI & Di CAPUA, 2010; BELMONTE *et al*. 2013). In the Adriatic Sea, 7 species of genus *Centropages* were noted: *C. hamatus*, *C. aucklandicus*, *C. chierchiae*, *C. violaceus*, *C. typicus*, *C. kroyeri* and *C. ponticus*. Many authors mention that the most common and dominant species are *C. typicus* and *C. kroyeri* (GAMULIN, 1939; HURE *et al*. 1979; HURE & KRŠINIĆ, 1998; MOLINERO *et al*. 2008; VIDJAK *et al*. 2009). Only CAR (1884) recorded numerous individuals of *C. hamatus* in the area of the Gulf of Trieste, while CARAZZI & GRANDORI (1912) found *C. aucklandicus* in Brindisi and in Venice Lagoon. GAMULIN (1939) mentioned several individuals of *C. violaceus* in the coastal area of Split and *C. ponticus* is recorded in the coastal area of Albania (KOVALEV, 1967) and along the western part of the Strait of Otranto (HAIDERI *et al*., 1994). The findings of *C. chierchiae* by PESTA (1920) and MUŽINIĆ (1936) are doubtful. Distribution of *C. kroyeri* in the Adriatic Sea is well known. It is present in the bays and channels of the eastern coast, throughout the north and coastal area of the western part of the Adriatic Sea. Only VUKANIĆ (1971) cited *C. kroyeri* var. *pontica* in Kotor Bay, which involved 26.5% of the total number of *C. kroyeri*. The largest abundance in the Bay of Kotor was present during December and January.

Distribution of *C. ponticus* in Lake Mir is specific with differences in winter-spring and summer-autumn population. In November, a relatively small abundance of nauplii and high abundance of copepodites and adults was recorded. The annual minimum of nauplii was found in January, copepodites in March and adults in May. A new cycle begins in April when temperature significantly increases. The seasonal cycle is characterized by low population density in winter-spring, a major peak in June-September, followed by a decline in October and a minor peak in November. In the April-September period salinity varied between 39 and 44.36, while temperature was very low from December to February (3-10 ^{o}C), and more than 25 ^{o}C from April to October. It may be assumed that these conditions are limiting for other species. In the neighboring central Adriatic 155 species of planktonic copepods were found (HURE & KRŠINIĆ, 1998) *C. ponticus* was found in the Suez Canal where salinity range is 45-47 as well as in the Azov Sea with very low salinity values (6-7) (KOVALEV, 1967). Very low winter temperatures in Lake Mir most probably reduce significantly the population abundance of *C. ponticus*. In Cullera Bay this species was very scarce in temperatures between 13.5 and 17.7 ^{o}C (SOLER *et al*.,1988). After the presence of a maximum number of nonloricate ciliates in May and the prevalence of small dinoflagellates, the population of *C.ponticus* starts increasing as shown by the maximum abundance of nauplii in June. According to PAFFENHÖFER (1998), ciliates are important in the diet of copepods. Therefore *C. ponticus*, as the only copepod species in Lake Mir, is very important for a top-down regulation of the lake ecosystem. During present investigations, copepod eggs were not found in plankton samples. However, in the Black Sea, both subitaneous and resting eggs were found (SAZHINA, 1968). The absence of eggs in the plankton is due to the rapid hatching of eggs and the sinking of eggs to the lake bottom (TANG et al., 1998).

During present investigations, high significant correlation between copepodites and faecal pellets were found. According to CARIĆ *et al*. (2010), in Lake Mir nanophytoplanktona peaked in November, April and August 2000, while microphytoplankton peaked in November, April and July. From November to April, diatoms prevailed, with dominant species being *Actinocyclus* sp., and from May to October dinoflagellates, made up mostly of *Scrippsiella trochoidea*. It seems that food availability should account for the increased abundance of copepodites, adults and feacal pellets in November and in the period July-September. The same authors noted that during the summer there was no depletion of nutrients. ŽIC et al. (2012) mentioned that ammonium and nitrate concentrations in Lake Mir are 15-16 times greater than those found in sea water, and they were attributed to atmospheric input and not *in-situ* nitrification. However, the contribution of *C. ponticus* to the nutrient cycle during summer and autumn could not be excluded, considering its abundance in the lake. Indeed, the abundance of copepodites and adults is one order of magnitude higher in Lake Mir than in the neighboring area of Telašćica Bay (max=37 ind.L^{-1} copepodites and 8.8 ind.L^{-1} adults), (KRŠINIĆ non-published data). Consequently, the intensive pellet disintegration through bacterial activity coupled with the excretion of ammonia by copepods, increase nitrification in the lake.

Among other planktonic metazoans, bivalve larvae were recorded with extreme values only in June, which were several times higher than those found in the Bay of Mali Ston (VILIČIĆ et al. 1994). Neverthelless the Bay is very rich in both cultivated and natural edible shellfish. Decapods larvae in June in the lake have less quantitative importance. By all accounts predation pressure on copepods population is low. Potential predators are mainly shellfish.

CONCLUSION

Saline Lake Mir, probably due to the extreme conditions, low winter and high summer temperatures, as well as a long period of hypersaline conditions, is not suitable for the existence of typical neritic-estuarine species of planktonic copepods, except for *C. ponticus*. Lake Mir is an ecologically interesting and generally rare habitat. Our results suggest that further research of this natural laboratory is needed for the purpose of its full protection.

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Taksonomska i ekološka istraživanja vrste *Centropages ponticus* (Copepoda, Calanoida) u slanom jezeru Mir (Dugi Otok, Jadransko more)

Frano KRŠINIĆ

Kontakt e-adresa: fkrsinic@izor.hr

SAŽETAK

Kalanoidni kopepod *Centropages ponticus*, endem Sredozemnog i Crnog mora, jedina je pelagična vrsta kopepoda prisutna u zatvorenom ekosustavu slanog jezera Mir na Dugom otoku u istočnom Jadranu. U radu su prikazane važne morfološke karakteristike vrste, kao i podaci o strukturi populacije za razdoblje od jedne godine od studenog 1999. do listopada 2000. godine, na jednoj postaji u najdubljem dijelu jezera Mir. Istraživanja uključuju vremensku raspodjelu i abundancije nauplija, kopepodita i odraslih jedinki, kao i gustoću fekalnih peleta. Čini se da je samo vrsta *C. ponticus* prilagođena ekstremnim uvjetima u jezeru, niskim zimskim i visokim ljetnim temperaturama, kao i dugom razdoblju hipersalinih uvjeta. Tijekom ljeta/jeseni, naupliji, kopepoditi i odrasle jedinke postižu ekstremne vrijednosti abundancije. Jezero Mir je ekološki vrlo zanimljivo i općenito vrlo rijetko stanište. Naši rezultati potiču daljnja multidisciplinarna istraživanja ovog prirodnog laboratorija radi njegove trajne zaštite.

Ključne riječi: Kalanoidi, veslonošci, jezero Mir, Jadransko more