Population dynamics of the calanoid copepod *Acartia italica* Steuer in a small saline lake

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The calanoid copepod *Acartia italica* is the only metazoan plankton species surviving and reaching high abundance in the middle Adriatic saline coastal Lake Rogoznica. Seasonal variations in the abundance of nauplii, copepodites, adults and their faecal pellets were assessed in the period January 2000 to December 2004. Development and behaviour of *A. italica* were regulated by thermohaline conditions, biology (phytoplankton, bacteria, ciliates), and chemistry (organic matter, dissolved oxygen, reduced sulphur species) of the lake. The diatom *Chaetoceros curvisetus* could provide important food for *A. italica* adult specimens; however, great diatom abundances could reduce copepod reproductive ability. This study show that top-down control which characterized the lake ecosystem before the disastrous anoxia event in 1997 were re-established again.

Key words: *Acartia italica*, population dynamics, extreme environments

INTRODUCTION

The calanoid copepod *Acartia italica* Steuer is reported to be endemic to the Mediterranean Sea distributed in very limited coastal areas and is among the least known species of the genus *Acartia* (HURE & KRŠINIĆ, 1998). Distribution status of *Acartia* species has been recently modified to Atlanto-Mediterranean species (BELMONTE & POTENZA, 2001). *Acartia italica* forms an abundant, single copepod community in the small saline Lake Rogoznica, on the Dalmatian coast in the Adriatic Sea. The population is acclimatized to low salinity, hypoxia/anoxia and euxinic conditions with relatively high presence of hydrogen sulphide (mM concentrations) and occasionally very high concentrations of ammonium (up to 150 µM) (KRŠINIĆ et al., 2000). Survival strategies for many coastal planktonic copepods rely on the production of resting eggs during unfavourable conditions which sink to the sea bottom and then disappear from the plankton (MARCUS, 1996). In favourable conditions, they appear along with *A. italica* population growth. *A. italica* produces both subitaneous and resting eggs with variable morphology (BELMONTE, 1997, 1998), as well as delayed-hatching eggs (CHEN & MARCUS, 1997).

Due to its relatively small size, shape and isolation from open sea water, the Lake Rogoznica
can be considered as a natural laboratory ideal for the research of population dynamics on one side and monitoring of different bio-geochemical processes on the other. The major characteristic of the lake is seasonal thermohaline and redox conditions stratification. Usually during spring/summer months the surface water is well oxygenated, and anoxic conditions appear below 8–10 m depth. Anoxic water is characterized by high concentrations of RSS, nutrients and DOC as a result of the pronounced remineralization of autochthonous organic matter produced in the surface water (CIGLENEČKI et al., 2005). As a result of fall/winter mixing which is under direct influence of meteorological conditions (CIGLENEČKI et al., 2011; ŽIC et al., 2013 in press) bottom water rich in nutrients comes to the surface, supporting new phytoplankton and oxygen production. Due to the very specific and extreme conditions prevailing in the lake, phytoplankton population is represented by relatively small number of species. The micro plankton was composed of about 30 species among which the most frequent and abundant were micro flagellates (Prorocentrum arcuatum and Hermesinum adriaticum) and diatoms (Chaetoceros curvisetus and Eunotia sp.) (CIGLENEČKI et al., 2005; BURIĆ et al., 2009). Arcatia italica is the only planktonic metazoan species in the lake (KRŠINIĆ et al., 2000).

Investigations in Lake Rogoznica have been particularly intensive after the anoxic event on September 27, 1997 when presence of hydrogen sulphide in the whole water column was detected. This event followed by massive mortality of all organisms was taken as a zero point from which ecological recovery of the lake was monitored (CIGLENEČKI et al., 2005; BARIĆ et al., 2003; ŠESTANOVIĆ et al., 2005; SVENSEN et al., 2008; BURIĆ et al., 2009; ŽIC et al., 2010). In this paper, we present inter- and intra-annual variability and structure of the Acartia italica population in relation to biological i.e. phytoplankton and physico-chemical conditions in the lake. Our study shows that A. italica aclimatizes relatively quickly to changes in the environmental conditions, such as changes in hydrography, redox and chemical conditions (particularly the presence of sulphur compounds), diet conditions and the composition and abundance of phytoplankton. There is also a strategy for survival under extreme conditions here mainly represented by anoxic stress, as well as population renewal following natural excesses.

**MATERIAL AND METHODS**

Investigations were performed during 35 field campaigns carried out in Lake Rogoznica between January 2000 and December 2004. The sampling site was in correspondence of the deepest point (about 13 m) in the Lake situated on the eastern Adriatic coast (Fig. 1). 12 samples at 1 m intervals from the surface to the bottom were collected with 5 L Niskin bottles (1L sub-samples for zooplankton and 250 mL for phytoplankton). All samples were taken during the day. Samples were preserved in 2% neutralised formaldehyde. Phytoplankton cells were counted using a Zeiss Axiovert 200 inverted microscope, following the UTERMÖHL (1958) method. Cells were counted at a magnification of 400x (1 transect) and 200x (transects along the rest of the counting chamber base plate). The error of the counting method is ± 10% (LUND et al., 1958). Samples collected below 12 m depths were characterised by many re-suspended particles, and could not be analysed microscopically.

Zooplankton samples were reduced to a subsample volume of 30 ml from the original volume of 1000 ml by sedimentation in the laboratory, a process taking 72 h (KRŠINIĆ, 1980). A glass cell of 7x4.5x0.5 cm was used for counting. Nauplii (NI-NVI), copepodites (CI-CV) and adult females and males of A. italic were identified and counted using an Olympus inverted microscope at a magnification of 100x, with an examination of the entire sub-samples. The rest of metazoan zooplankton was not present at all. Ciliates and copepod faecal pellets in subsamples of a quarter of the original sample were counted at a magnification of 400x. For identification of A. italic eggs and for faecal pellets see (BELMONTE, 1997, 1998; KRŠINIĆ et al., 2000).

Temperature and salinity were measured in situ with an Hg thermometer and refractometer.
(Atago, Japan), and oxygen was measured using standard titration methods. Samples for reduced sulphur species (RSS) analysis which were taken under atmosphere of nitrogen to prevent possible oxidation, were measured unfiltered and fresh, within 24 h in the electrochemical cell of 50ml.

Electrochemical methods (CIGLENEČKI & ĆOSOVIĆ, 1997) were used for the determination of RSS which is based on the interaction between the mercury electrode and different, sulphur species.

The non-parametric Spearman correlation coefficient (Rs) was used for the correlation between variables and the t-test was used to determine significant changes in the population during the research period. To obtain a normal distribution, all data were log transformed. Statistica 6.0 software was used for statistical analyses.

RESULTS

Hydrography

The salinity ranged between 24.0 and 38.0 at the surface and between 34.0 and 40.0 near the bottom (Fig. 2A). The average salinity in the bottom layer was 36.2, before 2001, while it was 38.2 after 2001. The temperature in surface waters varied from 10°C (during winter and spring) up to 30°C (summer), while in bottom waters, these changes ranged between 9°C and 24°C (Fig. 2B). Mixing of water layers is influenced by meteorological changes and was missing and/or incomplete in 2001, 2002 and 2004. The concentrations of RSS and oxygen in the bottom water layer were inversely proportional and varied between $10^{-9}$ M and $10^{-1}$ M, and between 0 mM and 0.6 mM, respectively. Dur-
ing the period of very low oxygen concentrations (below 0.05 mM), RSS was very high (between $10^{-4}$ M and $10^{-1}$ M). Small peaks in oxygen concentrations recorded in the fall of 2000 and 2003 and in the spring of 2002 and 2004 coincided with a decrease in RSS concentration (Figs. 2C, D), as well as weak thermohaline stratification, and indicated water layer mixing.

In May 2002, the whole water column was exceptionally well saturated with dissolved oxygen with a maximum over-saturation (up to 180%) at an 8 m depth (Fig. 3). Weak thermohaline stratification, together with oxygen saturation, had a direct influence on the spreading of anoxic conditions and sulphide distribution, which were detected only at the lake bottom (13 m depth). In August 2003 (Fig. 3), a strong thermocline at a depth of 9 m prevented the mixing of water layers, which enabled the accumulation of anoxic conditions with a high concentration of sulphide (up to $10^{-1}$ M) below a depth of 10 m. Contrarily, over-saturation with oxygen (up to 160%) was detected in the surface water layer, above a depth of 8 m.

(Fig. 4A). Phytoplankton had two maxima, in early spring ($1.9 \times 10^6$ cells L$^{-1}$) and in summer ($2.3 \times 10^6$ cells L$^{-1}$). The exception was 2001, when the summer maximum failed to develop.

The dominant phytoplankton species in the lake were the diatoms *Chaetoceros curvisetus* (maximum abundance $7.5 \times 10^5$ cells L$^{-1}$, frequency of appearance 72%) and *Dactyliosolen fragilissimus* (maximum abundance $9.7 \times 10^5$ cells L$^{-1}$, frequency of appearance 34%). *C. curvisetus* dominated the phytoplankton assemblage until May 2002 at which point *Dactyliosolen fragilissimus* took over the dominant position. It remained as a dominant species in the phytoplankton assemblage until the end of 2004, when the usual dominance of *C. curvisetus* was re-established.

**Fig. 4.** (A) Temporal distribution of diatoms and dinoflagellates in Rogoznica lake in the January 2000 to December 2004 period

**Ciliates**

The planktonic ciliate (20-40 µm in size) abundances were extremely variable during the research period. Benthonic ciliates (>100 µm) were present only above the sea bottom, and were not considered in this study. As a rule, ciliate abundance decreased very soon from the plankton after establishing their maximum abundances. The lowest abundance was found in late autumn and winter, while higher abundance occurred later in spring or at the beginning of summer. The highest abundances were in April-June 2003 and in April-May 2004 (Fig. 5B). High abundances of $6 \times 10^3$ ind. L$^{-1}$ were found in August 2000 at a depth of 9 m, and in April 2004, at a depth of 1 m.

**Fig. 3.** Vertical profiles of oxygen saturation, temperature, salinity and sulfide species in the Rogoznica Lake in May 2002 (A) and August 2003 (B)

**Phytoplankton**

The phytoplankton composition changed over the research period. After 2001, diatoms took over the domination of phytoplankton assemblages, contributing on average up to 94%
Acartia italica

The abundance of *Acartia italica* followed the temporal distribution of *Chaetoceros curvisetus* (Fig. 4B). According to the vertical distribution of nauplii (NI-NVI), copepodites (CI-CV) and adult females and males of *Acartia italica*, two periods can be separated. The first period, to March 2002, and the second one, running to December 2004 (Figs. 6A, B).

**Nauplii (NI-NVI)**

During frequent investigations in 2000, two typical annual maxima of nauplii abundance were noted, the first in March-April, and the second in July-August. In most cases, nauplii were most abundant in the middle 6-10 m layer; however, the first maximum average abundance was found in the surface 0-5 m layer. In 2001, one peak of abundance of nauplii occurred in September, with higher average values in the whole column and a maximum of 1x10^3 ind. L^{-1} at a depth of 6 m. During 2002, nauplii abundance was atypically very low in the entire water column. A re-established naupliar population was registered during 2003, however, with very low abundance in deeper layers. In August 2003, the maximum average abundance was recorded in the 1-5 m layer, while extreme values of 2x10^3 ind. L^{-1} at 1 m depths were registered.

**Copepodes (CI-CV) and adults females and males**

Two typical annual abundance maxima of copepodes and adults were registered during frequent investigations carried out in 2000, the first in March and April, and the second in July and August (Fig. 6B). In May 2002, copepodes and adults *Acartia* were absent from the plankton assemblage for a short time. After July 2002, the core of the population was at 1 to 5 m depths, while average maximum values were
only two times higher than 100 ind. L⁻¹. In the layer below 6 m, abundance was low. Subitaneous egg abundance in the entire water column was low with average values usually lower than 10 eggs L⁻¹. Exceptionally in July 2002, a maximum egg abundance was registered in plankton, when resting eggs dominated with over 90% of the total egg density (Fig. 7). In the first period of investigation, A. italica males doubled the number of females. In May 2002, there was a low abundance of females, while males were not found in the plankton. In July and September 2002, the male/female ratio was the same with the dominance of resting eggs (Fig. 8). A significant positive correlation was found between copepodites and adults (Rₛ=0.584; n=73; p<0.001) and between adults and Chaetoceros curvisetus (Rₛ=0.242; n=73; p<0.05). A similar relationship of sexes remained, except in August 2004. A statistically significant difference was found in the average abundance of postnaupliar Acartia before May 2001 and after May (t-test, p<0.01, n=17). Significant positive correlations between nauplii and copepodites (Rₛ=0.3194; n=343; p<0.001), and between nauplii and adults (Rₛ=0.2727; n=343; p<0.001) were found. A statistically significant difference between naupliar abundances before and after May 2001(t-test) was not found.

**Faecal pellets**

Faecal pellets of copepodites and adults were permanently present throughout the water column. A significant positive correlation between the distribution of faecal pellets and copepodites (Rₛ=0.2016; n=343; p<0.001) and adults (Rₛ=0.2023; n=343; p<0.001) was noted.

Faecal pellets were mainly more abundant in the bottom 6-12 m layer (Fig. 5A). However, the highest average abundance was found in the surface 1 to 5 m layer in the beginning of March 2000, with a maximum abundance of 6-7 x 10³ pELL. L⁻¹. Relatively higher average abundances were found in September 2001 and in April 2004; the lowest in the period May 2002 to April 2004. Naupliar minipellets (length 20.4 ± 2.9 µm, diameter 13.7 ± 1.5 µm, 3.0 x 10³ µm³; n=20) were usually present in low numbers, and the highest numbers were recorded only in August 2003 (6x10² to 1.6x10³ minipellets L⁻¹).

**DISCUSSION**

The lake was permanently thermally stratified with thermocline mainly following the position of the halocline, enhancing the stability of the water column stratification. Since, rainfall and surface runoff are the only sources of freshwater inflow in the lake the results indicate a higher amount of precipitation in the period after 2001, and at the same time, a more stable stratification of the water layers, since there was no influence of rainfall on the bottom water as noted before 2001 (CIGLENČKI et al., 2011).

Following the post-anoxia period from September 1997 to May 2002, a new population of A. italica was constantly present in the lake, due to continuous reproduction of the population. Annual maximum abundances usually appear in March-April. However, during 2000 and 2001, a peak abundance of copepodites and adults
was clearly noted in the summer/autumn period. Extremely low rainfall in 2002 caused a change in the hydrography of the lake in spring 2002. Until May, stratification was weak, enabling diffusive transport from the sediment in the whole water column. In May 2002, the entire water column was exceptionally well saturated, with a maxima at a depth of 8 m, probably as a consequence of the accumulation of *C. curvisetus*. Anoxic conditions, sulphide and possible trace metals (spread from the bottom layer and sediment (CIGLENEČKI et al., 2006; HELZ et al., 2011) probably caused the mortality of the diatom *C. curvisetus*. After this event, stratification was restored, making it possible for phytoplankton to develop. The diatom *D. fragilissimus* took over the dominant position, probably due to the absence of *C. curvisetus*. Temperatures were too high for the mass development of *C. curvisetus*, because of its preference for lower temperatures (BURIĆ et al., 2009).

A break in the continuity of older copepod populations also occurred in May 2002. The entire year of 2002 was characterised by very low abundances at all development stages. A break and changes in *A. italica* populations occurred probably due to significant changes in the hydrographic features of the lake, as well as structural changes in phytoplankton populations. A maximum abundance of adult copepods specimens recorded almost at the same time as the maximum diatom density as well as the positive significant correlation between their distribution suggest that the diatom *C. curvisetus* is probably comprised in the diet of adult specimens. Before the anoxia event in 1997, the lake was top-down controlled, while in the post-anoxic period to the year 2000, due to the absence of predation pressure on nauplii or post-naupliar copepods, bottom-up control become more important (CIGLENEČKI et al., 2005). The results from this study show top-down control during the entire research period, indicating that the conditions from before the disastrous anoxia event were re-established in approximately two years.

Temperature and microzooplankton, rather than phytoplankton abundance, may indicate the *A. tonsa* reproductive potential in Chesapeake Bay (WHITE & ROMAN, 1992). Ciliates are important in the diet of copepods (PAFFENHÖFER, 1998). Ciliates are certainly important in the diet of nauplii and copepodites in Lake Rogoznica. Following the high abundance of ciliates in June 2003, a maximum average abundance of nauplii was recorded in August in the 1-5 m layer, and with simultaneously high mini-pellet density values. Therefore we assumed that in the period to 2001 ciliates can provide important food for this estuarine species.

In May 2004, based on one week of monitoring of Lake Rogoznica, the contribution of faecal pellets was only 4-5% of the vertical particulate organic carbon flux. Therefore, in this productive lake, organic material is rapidly transformed to detritus (SVENSEN et al., 2008). Also in May 2004, bacteria were less abundant in the surface layers, but had very high production per cell (ŠESTANOVIĆ et al., 2005). At the same time, on May 11, a very high abundance of ciliates was found indicating intensive breakdown of organic matter and grazing of ciliates. According to our data, a relatively low abundance of postnaupliar copepods was noted at the beginning of May 2004, in comparison to the period 2000/2001, but with relatively high faecal pellet abundance. Ten days later, a significant decrease in the abundance of ciliates, postnaupliar copepods, as well as faecal pellets was noted, followed by a period of increased abundance of copepodites and adult specimens of *A. italica*. Our results confirm that organic matter, bacteria and ciliates in Lake Rogoznica are strongly correlated with the dynamics of the copepod population.

These eggs have been previously noted in Lake Rogoznica (KRŠINIĆ et al., 2000). During long-term research, we could not determine the periodicity of the production of resting eggs. Moreover the annual periodicity for the recruitment of nauplii into the plankton from resting eggs has not been determined. In the lake, the bottom anoxic layer was characterised by a high concentration of sulphide (CIGLENEČKI et al., 2005; BURA-NAKIĆ et al., 2009). In such conditions, the hatching of eggs did not occur either in anoxic water or within mud (GRICE & MAR-
CUS, 1981). During vertical mixing, resting eggs arrived in the oxygenated layer, where hatching followed rapidly. A recruitment of the population from resting eggs or delayed-hatching eggs was found after lake anoxia in October 1997 (KRŠINIĆ et al., 2000). In May 2002, an almost complete absence of all stages of copepods was recorded, with exceptionally low abundance of adult females and subitaneous eggs. During July and September 2002, over 90% of resting eggs were found in the water column. We can assume that a new population developed from the hatching of resting eggs. Subitaneous egg production was not calculated; however, it is evident that their number in the plankton is always relatively low. The reason for this could be a possible negative influence of the feeding behaviour of A. italica on the diatom C. curvisetus. C. curvisetus has a strong effect on the hatching success of Temora stylifera (IANORA et al., 1995). Feeding on this diatom resulted in poor egg quality, with a hatching success as low as 20% of the total egg production. For this reason, we assume that the abundance of 1.8 10^6 cells L^-1 in February 2002 affected the reproductive abilities of the species.

In the first period, and in the greater part of the second period of the research, the number of subitaneous eggs during the day was lower than that of adult females. Acartia tonsa assimilates sufficient energy to produce about two eggs d^-1 (KIøRBOE et al., 1985). Egg production for the same species in a Mediterranean coastal lagoon showed 2.3−40.7 eggs female^-1 day^-1, depending on ambient food concentrations. Maximum egg production is known to occur at night (CERVETTO et al., 1993). In Lake Rogoznica, hatching of subitaneous eggs occurs quickly, as those that fall to the anoxic bottom are lost. Exceptionally, in August 2003, in a well-oxygenated layer at a depth of 1-5 m, a high average number of subitaneous eggs was recorded. In the same layer, an extremely high abundance of nauplii was recorded, with a predominance of NIII–NV. Usually, the abundance of mini-pellets is low, except in April and July 1998 (KRŠINIĆ et al., 2000), and in August 2003. Following this exceptional predominance of nauplii, their continued development was interrupted by increasing mortality. Copepodite abundances continued to decrease, more than expected, and they did not achieve the abundance recorded in 2000. Namely, in September 2003, during a turnover of water, suspended material from the sediment occurred in the lake surface (SVENSEN et al., 2008), which resulted in a significant drop in oxygen and an increase in total sulphur species throughout the entire water column (ZIC et al., 2013 in press), and an increase in nauplii mortality.

A characteristic feature of the A. italica population in Lake of Rogoznica is the almost constant predomination of male over female numbers. In the first period up to May 2002, the males were on average twice as abundant compared to females, which varied significantly from other published data (KIøRBOE & SABATINI, 1994; HIRST et al., 2010). The overall abundance of adult specimens increased simultaneously with the predominance of males. Contrarily, in May 2002, males were not recorded during a drop in population numbers. Population renewal started with an equal number of specimens from both sexes. The pronounced predomination of males over females in open waters is not common. The mating behaviour of A. italica in Lake Rogoznica is not known. A few spermatophores are usually attached to the female body. Multiple placement in Acartia tonsa is cited by HAMMER (1978). If we consider males living for a shorter period of time than females, we can assume that the pronounced predomination of males is important for the survival of A. italica populations, the only type of predominant copepods that can rule under the unsatisfactory conditions of Lake Rogoznica.

The A. italica nauplii abundance, copepods and adults appeared as a monoculture in Lake Rogoznica. Their abundances are significantly higher in Lake Rogoznica than those recorded in in the northern and western Adriatic Sea, enriched with nutrients from the biggest Adriatic river, the Po (DEGOBBIS et al., 2000). Occasionally, the abundance of nauplii, copepods and adult specimens achieved extreme maxima, which are rare for natural saline ecosystems. As the very numerous planktonic species in the lake, A. italica has a key role in
the lake’s food web. Our investigations indicate that *A. italica* aclimatizes relatively quickly to various changes in the environment, such as hydrographic conditions, hypoxia/anoxia conditions, the presence of sulphur compounds, diet conditions and the composition and abundance of phytoplankton. There is also a strategy for survival under extreme conditions, as well as population renewal following natural excesses. The above indicates a need for further research on this natural laboratory.

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Dinamika populacije kalanoidnog kopepoda Acartia italica Steurer u malom slanom jezeru

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SAŽETAK

Kalanoidni kopepod Acartia italica je jedini predstavnik mezozooplanktona koji se uspješno razvija u slanom jezeru Rogoznica u obalnom dijelu srednjeg Jadrana. Sezonalnost nauplija, juvenilnih i odraslih jedinki kopepoda A. italica, kao i njihovih fěkalnih peleta reguliraju termohalini odnosi, biološki parametri (fitoplankton, bakterije, cilijati) i kemijski parametri (organska tvar, otopljeni kisik, reducirani sumporni spojevi) u jezeru. Dijatomeja Chaetoceros curvisetus važna je za ishranu odraslih jedinki A. italica, međutim njene velike abundancije mogu negativno utjecati na reprodukciju vrste. „Top down“ kontrolu u jezeru narušili su anoksični događaji iz 1997. godine.

Ključne riječi: Acartia italica, dinamika populacije, ekstremni okoliš