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CLADOCERA

Microcystis extracts and single cells have differential impacts on the demography of cladocerans: a case study on *Moina* cf. *micrura* isolated from the Mediterranean coastal shallow lake (L'Albufera, Spain)

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Abstract Cyanobacteria often have a deleterious effect on zooplankton. We hypothesized that the presence of either *M. aeruginosa* cells or microcystin extracts from *M. aeruginosa* would have a significant impact on the population growth rate, survivorship, and fecundity of *Moina* cf. *micrura* isolated from L'Albufera, Valencia, Spain. The cladocerans were exposed to different concentrations of *Microcystis* extracts on a diet of *Nannochloris oculata* (Chlorococcales) as well as different proportions of *Microcystis* single cells and *N. oculata*. Cyanotoxins were extracted from a *Microcystis* bloom by its repeated freezing, thawing, and sonication. Total microcystin concentration was 138.2 μg l^{-1}. *M. aeruginosa* single cells were obtained by sonication. We used five microcystin concentrations (from 4.3 to 69.1 μg l^{-1} and controls without microcystins) with non-toxic *Nannochloris oculata* at 0.5 × 10^6 cells ml^{-1} as diet or on different proportions of *M. aeruginosa* single cells and *N. oculata*. Microcystin reduced generation time and longevity of *M.* cf. *micrura* but increased daily production of offspring. Single-celled *Microcystis* (100%) diet for *Moina* cf. *micrura* decreased growth rates compared with treatments using cyanotoxin extracts. We suggest routine testing of cyanobacterial crude extracts using plankton to estimate harmful impacts of blooms especially for drinking water reservoirs.

Keywords Cyanobacteria extract · Cladocera · Demography · Microcystins

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Introduction

Cyanobacterial blooms are widespread and on the rise, in temperate and tropical water bodies, especially due to global warming and accelerated rates of anthropogenic eutrophication (Paul, 2008). In the 1970s, it was shown that changes in the availability and ratios of limiting nutrients, particularly nitrogen and phosphorus, were the main cause of cyanobacterial blooms (Schindler, 1977). These blooms often consist of toxic

cyanobacteria, such as those of *Microcystis, Dolichospermum, Planktothrix,* and *Cylindrospermopsis* (Paerl & Otten, 2013). Toxicity of cyanobacteria varies among strains depending on the presence of genes capable of coding for the synthesis of toxins (Rouhiainen et al., 2004); it also depends on environmental factors, such as temperature (Gilbert, 1996), grazing pressure by zooplankton (Lampert, 1987), and other characteristics of the water body (Jacoby et al., 2000). Several kinds of hepatotoxins, neurotoxins, and cytotoxins produced by different cyanobacterial species have been identified (Carmichael, 1997). Most agencies responsible for water quality set limits on the concentrations of microcystins, saxitoxins, and cylindrospermopsins, depending on whether the water is for drinking purpose or for recreation; the maximal permitted values for these toxins range from 1 to 15 $\mu g\ l^{-1}$ (Chorus, 2005).

The toxicity of cyanobacteria has been estimated by culturing them separately, extracting and purifying the toxins and then conducting bioassays (Duy et al., 2000). However, it has been realized that extracts from natural blooms are easier to obtain and provide a more realistic assessment about their toxic effects (Dow & Swoboda, 2002). Pietsch et al. (2001) show that there could be synergistic or antagonistic effects of multiple cyanotoxins depending on the dominant cyanobacterial taxa in the bloom. The bioassay organisms used to test the toxicity of cyanobacterial extracts cover a wide range of phyla from bacteria to mammals (Ferrão-Filho & Kozlowsky-Suzuki, 2011). For crustaceans, there are only a few studies (<30 to date) on cladocerans, which include species of *Daphnia, Ceriodaphnia*, and *Moina* (Olvera Ramírez et al., 2010; Pineda Mendoza et al., 2012; Zamora-Barrios et al., 2015). Cladocerans have been subjected to extracts from common cyanobacterial genera such as *Cylindrospermopsis, Pseudanabaena, Dolichospermum, Microcystis*, and *Aphanizomenon* (Nogueira et al., 2004); Olvera Ramírez et al., 2010; Pineda Mendoza et al., 2012; Zamora-Barrios et al., 2015). Most studies focus on enzymatic tests to evaluate activities of phosphatases and proteases in the presence of cyanotoxins (Agrawal et al., 2005a, b; Freitas et al., 2014a) and acute toxicity tests to compare LC_{50} data (Okumura et al., 2007; Zhang et al., 2007; Sotero Santos et al., 2008; Freitas et al., 2014b). A few studies have examined the effects of physiological changes such as thoracic beats, ingestion rates, and swimming speed in *Daphnia* (Haney et al., 1995; Rohrlack et al., 1999; Müller et al., 2013; Pérez-Morales et al., 2014). Duy et al. (2000) reviewed different approaches to evaluate ecotoxicology of cyanobacteria. Among these, analyzing the population growth and life table demography are particularly relevant in short-lived zooplankton. The effect of cyanotoxins on survivorship and reproduction-related traits often differ depending on the test species (Ferrão-Filho & Kozlowsky-Suzuki, 2011). *Moina* is especially useful in such tests since it has a short life span and is easy to culture in large numbers; it is also commonly found in warm waters where there is a higher tendency for the occurrence of cyanobacterial blooms (Dodson & Frey, 2010).

Zooplankton may coexist with toxic cyanobacteria and could even be abundant during blooms; however, this does not always imply that cyanobacterial toxicity is weak (Ferrão-Filho & Azevedo, 2003). At times cladocerans may not ingest the toxic colonies or filaments, especially if they are too large and with a patchy distribution. Thus, the effect of toxins could be important but difficult to evaluate from field data. We studied the potentially deleterious effects of a bloom of *Microcystis aeruginosa,* in the shallow lake L'Albufera of Valencia (Iberian Peninsula, Mediterranean Coast), on *Moina* cf. *micrura,* one of the dominant cladoceran taxa inhabiting the lake during the cyanobacterial bloom. We tested both, the effect of *M. aeruginosa* cells and of the dissolved microcystin extracts from *M. aeruginosa* collected during the bloom. We hypothesized that the presence of *M. aeruginosa* cells or microcystin extracts from *M. aeruginosa* has a significant impact on the population growth rate, survivorship, and fecundity of *Moina* cf. *micrura*. For testing this hypothesis, we investigated survivorship and population growth of *Moina* cf. *micrura* using the microcystin extracts at different concentrations. Then we evaluated the effect of toxic cells when ingested as the only source of food or as diet combined with the non-toxic green algae, *Nannochloris oculata*.

For ecotoxicological tests, laboratory clones are usually used (Zamora Barrios et al., 2015); however, some studies show that several cladocerans when freshly collected from the field are more sensitive to toxins than the laboratory selected clones (Bossuyt & Janssen, 2005). Since there are only a few studies on the sensitivity of field-collected cladocerans to cyanotoxins (Lopes et al., 2011), we chose to work on

Moina cf. *micrura* collected recently from the Lake L'Albufera.

Materials and methods

Study site

L'Albufera (location: 39.33°N, 0.35°W; lake area: 24 km^2; mean depth: 1 m) is a large shallow lake that has been used since historical times for fishing. Nowadays, fish community in this lake is impoverished and dominated by mullets (Mugilidae), which are commercially exploited. Since the mid-1970s, this lake has become turbid due to a an increase in the density of phytoplankton as a result of increased nutrient inputs from the fertilizers used in the rice fields in the lake's catchment area. The lake lost its macrophyte cover after it became hypertrophic, dominated by planktonic filamentous cyanobacteria (Vicente & Miracle, 1992). The composition of cyanobacterial species has also changed over the past decade; the proportion of filamentous Oscillatoriales decreased, and that of colonial Chroococcales has increased (Romo et al., 2005). *Microcystis* blooms are at present common in this lake, especially during the summer, and microcystins in water, seston and fish have also been detected (Romo et al., 2012, 2013). In spite of the frequent blooms of cyanobacteria in L'Albufera, the lake still is a source of fish for the local fishermen. Fish depend on zooplankton during their early life stages, yet, there are no studies concerning the adverse effects of these blooms on the lake zooplankton.

Culture of *Moina* species

Moina individuals were randomly isolated from the zooplankton of L'Albufera, collected on the 20th of July 2014, during a *Microcystis aeruginosa* bloom. Comparison of sequences of two mitochondrial genes of this *Moina* and *M. micrura* sensu stricto from the vicinity of its type locality indicated that the Spanish population is a cryptic species closely related to but not conspecific with *Moina micrura* (Miracle, unpublished data). For the present study, we mark this *Moina* species as *Moina* cf. *micrura*. Physical conditions of the lake water at the time of collection were water temperature 25°C, conductivity 2 mS cm^{-1}, and pH 8.6. The samples analyzed a few days earlier indicated chlorophyll *a* concentration of about 140 μg l^{-1}, and *M. aeruginosa*, the dominant taxon in the phytoplankton, comprised about 60% of phytoplankton total biomass, density of about 1×10^6 cells ml^{-1}. The other main phytoplankton constituents were filamentous cyanobacteria *Pseudanabaena* and *Jaaginema* (28% biomass), colonial cyanobacteria such as *Merismopedia* and *Aphanocapsa* (8%), with a small proportion of green algae and dinoflagellates.

We cultured *Moina* cf. *micrura* in the laboratory in moderately hard artificial medium similar to L' Albufera water (0.9 g of $NaHCO_3$, 0.6 g of $CaSO_4$, 0.6 g of $MgSO_4$, and 0.04 g of KCl dissolved in 1 L of distilled water) (Weber, 1993). The animals were fed the green alga *Nannochloris oculata* (Chlorococcales) at 0.5×10^6 cells ml^{-1} until the initiation of the experiments. The cladoceran populations, started with several randomly selected individuals, were maintained in 500 ml glass beakers at densities less than 0.5 ind. ml^{-1}. *Nannochloris oculata* was cultured axenically using standard Bold's medium (Borowitzka & Borowitzka, 1988). Single cells of *Microcystis aeruginosa* were obtained by sonication of a refrigerated suspension of *M. aeruginosa* colonies for four min at 20 kHz. *Microcystis* colonies were collected from the lake every two days. To ensure cell ingestion, *Moina* were fed single cells of *M. aeruginosa* detached from natural colonies. They were mixed at different proportions with *Nannochloris oculata* as the control alga, to test the sensitivity of this cladoceran to the cyanotoxin in terms of survivorship and fecundity.

All experiments were conducted in a temperature-controlled chamber set at 25 ± 1°C and 15:9 photoperiod with diffuse fluorescent illumination, to approximate lake conditions.

Preparation of the microcystin crude extract

For obtaining a sufficient quantity of cyanobacteria to extract the cyanotoxin, we filtered 100 l of surface lake water through a mesh (pore size 45 μm) on 1st July 2014 and concentrated it to 1.4 l. The phytoplankton community of this concentrated suspension was quantified by the sedimentation method in 1 ml Lugol fixed samples; its microscopy analysis revealed that *Microcystis aeruginosa* made up more than 97% of the suspension biovolume (the rest consisted of other cyanobacteria, mainly *Pseudanabaena galeata*, with a 2% biovolume, and very small proportions of

assorted non-cyanobacteria nanoplankton). This concentrate was divided into 5 equal parts (280 ml each) in glass bottles. These were then frozen at −70°C, thawed, and sonicated for 4 min. at 150 W and 20 kHz; this process was repeated five times to ensure complete cell lysis. After the last cycle, we filtered the extract using a GF/C Whatman filter to get a clear, transparent extract. The extract was maintained at −70°C until its use in the assays (Pietsch et al., 2001). The extract and the Whatman filters were sent to IPROMA Research and Environmental Projects (UNE-EN ISO/IEC 17025) (Castellon, Spain) for HPLC analysis of the different fractions of microcystin concentrations. Based on these estimates, concentrations in the experiments were chosen and calculated. Microcystins are persistent in the medium without changes for more than 30 days after they are extracted and frozen (Lahti et al., 1997). For estimating the microcystin concentrations in the lake water, we filtered whole lake water onto GF/F glass fiber filters and kept the filter papers and the filtrate at −70°C until sent to IPROMA for HPLC analyses.

Population growth study

Ecotoxicological tests using zooplankton are often conducted in volumes less than 20 ml in order to minimize the use of toxins, and we followed the same here. The experiments were started by introducing 5 individuals from *Moina* cf. *micrura,* 2-5 days old, from our cultures in 10 ml of medium and with appropriate food concentrations. All live individuals in each test jar were counted daily and transferred to 10 ml of fresh medium with appropriate *Microcystis* or microcystin concentration and food level. The experiment was continued until the *Moina* populations began to decline. This period was about 15 days in the treatment containing *N. oculata* alone and *N. oculata* : *M. aeruginosa* 50%–50% treatment. On a diet of pure *M. aeruginosa*, the population died out completely by day 6, while in the treatment *N. oculata* : *M. aeruginosa* 25%–75%, the experiment was discontinued after day 8, when the first decline in the population was observed. Four replicates for each treatment were set up.

Population growth rates were calculated using the formula: $r = (\ln N_t - \ln N_0)/t$ where N_0 = initial population density and N_t = final density at time t in days (Krebs, 2009). We selected t for the first peak density in each curve to calculate the r value. We calculated the r value for each replicate and then took the mean. Data were compared using one-way ANOVA and post hoc Tukey tests with the statistical package of Sigma Plot 11.

Life table experiments

The life table experiments were initiated using 5 neonates (<24 h old) per cohort in 10 ml medium. Previous studies indicate that maternal effect on parthenogenetic reproduction in other species of *Moina* is insignificant (Zadereev, 2003). We used four cohorts per treatment. Daily, the living individuals of the original cohort were transferred to fresh medium, while the neonates and the dead adults, when observed, were counted and removed. After all members of the original cohort died, we calculated the demographic traits using Krebs (2009) formulae:

l_x is the Proportion surviving to start of age x

m_x is the Offspring produced per female at age x

Life expectancy at the start of age x: $e_x = \dfrac{T_x}{n_x}$

where T_x is the cumulative number of individuals from age x and n_x is the number of live individuals at age x (days)

Gross reproductive rates $= \sum m_x$

Net reproductive rate $R_o = \sum_0^\infty l_x \cdot m_x$

Generation time $T = \dfrac{\sum l_x \cdot m_x \cdot x}{R_o}$

Chronic toxicity tests

We performed (a) population growth and (b) life table demography studies. For each, we conducted two types of experiments: The first was to test different proportions of *N. oculata* and *M. aeruginosa* live single-cell suspensions. Cells of both phytoplankton species (spheres of 3–4 μm diameters) had a similar size, shape and organic carbon content. We used suspensions with concentrations between 0.5×10^6 and 0.6×10^6 cells ml^{-1} of each of the two phytoplankton species (particulate organic carbon (POC)) and particulate total nitrogen (PN) measured with Shimadzu TOC analyzer gave similar results for these

two algal suspensions: POC/PN ratio about 7 with POC concentrations about 2.7 mgC l^{-1}, well over the limiting conditions when daily renewed). The proportions used were as follows: *N. oculata* 100%, *N. oculata* + *M. aeruginosa* 50% + 50%, *N. oculata* + *M. aeruginosa* 25% + 75%, and *M. aeruginosas* 100%.

The second experiment to test the effect of the following concentrations of the cyanotoxin extract in the medium: 0% (control), 50, 25, 12.5, 6.25, and 3.125% (corresponding to 0, 69, 34.5, 17.25, 8.625, and 4.3125 μg l^{-1}, respectively, of microcystins). We selected these concentrations because we found that our test organisms were resistant to the impact of cyanotoxins in the short-term LC_{50} tests, even at 138 μg l^{-1}, the extract concentration without dilution. These microcystin concentrations are all within the range reported from different parts of the world (Davis et al., 2009, Bigham et al., 2009, Alillo-Sanchez et al., 2014) as well as in Lake L'Albufera (Romo et al., 2012). In all treatments, *N. oculata* (0.5×10^{-6} cells ml^{-1}) was the only diet used. All experiments were run for 4 replicates per toxicant/food treatment.

For deriving the rate of population increase, Euler equation ($\Sigma\, e^{-rx} l_x m_x = 1$) was solved iteratively. Data were analyzed using ANOVA or Kruskal–Wallis tests depending on the normality of distribution of the data, followed by post hoc Tukey's test using Sigma Plot 11.

Results

Microcystin concentrations

Concentration of microcystins in the extract from the concentrate (see methods) obtained during the bloom was 138 μg l^{-1} (microcystins RR: 50.5 μg l^{-1}, LR: 26.7 μg l^{-1}, YR: 60.8 μg l^{-1}, demethylated RR: <0.2 μg l^{-1}). The level of sestonic microcystins in Lake L'Albufera (on 1st July 2014) was about 1.94 μg l^{-1}. On 30 July 2014, some days after cladoceran collection, microcystin concentration in the lake was 0.8 μg l^{-1} (below the method of detection limit in dissolved form).

Population growth

Population growth rates of *Moina* cf. *micrura* (Fig. 1) differed on mixed diets of *Nannochloris* and *Microcystis* single cells. On *N. oculata* alone, the population increased to a plateau around day 7, and the growth rate was 0.24 day^{-1}. On the other hand, with 50% *Microcystis*, there was a delay in the population growth, decrease in the growth rate, and the maximum density reached was well below that in controls. With 75% *Microcystis*, the reproductive output was greatly hastened. On *Microcystis* alone, *M.* cf. *micrura* growth rates were negative. Population growth rates significantly differed among the different treatments ($P < 0.05$, one-way ANOVA).

Survivorship and fecundity

Survivorship of *Moina* cf. *micrura* decreased with increasing proportions of *Microcystis* in the diet (Fig. 2), and there was a clear shortening of the maximum life span. From Fig. 2, we cannot see any adverse effect on fecundity; on the contrary, it was somewhat enhanced. This increase in fecundity was coupled with a significant shortening of generation time (Fig. 3) especially in diets with 75% and 100% *Microcystis*. The decreases in the average life span and life expectancy at birth with increasing concentrations of *Microcystis* were statistically significant ($P < 0.05$, Kruskal–Wallis test), and the population growth rates were significantly lower ($P < 0.05$, one-way ANOVA) (Fig. 3). The post hoc Tukey tests showed that the differences were significant only on diets with exclusively or varying proportions of *Nannochloris* versus the exclusive *Microcystis* diet. On the other hand, there were no significant differences in the variation of reproductive parameters ($P > 0.05$, one-way ANOVA), because they were derived from two traits on which *Microcystis* had opposite effects, i.e., it increased fecundity but also caused a decrease in the life span.

The effect of microcystin extracts on the survivorship and fecundity of *Moina* cf. *micrura* is shown in Fig. 4. Results agreed with those of the previous experiment. The survivorship tended to decrease with increasing percentages of the crude extract in the medium. The clearest result was again a decrease in maximum life span, which dropped to almost 50% at the higher microcystin concentrations (25% and 50%) compared with the controls (life span 8–10 days) (Fig. 4). At the same time, fecundity per day was higher at the higher microcystin concentrations. Moreover, reproduction started earlier in animals

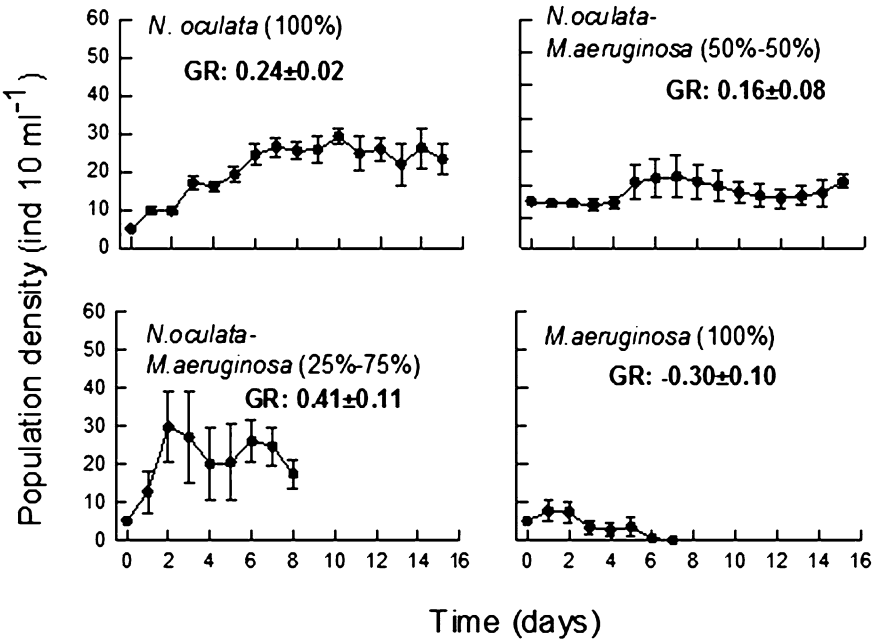

Fig. 1 Population growth curves of *Moina* cf. *micrura* fed different proportions of *Nannochloris oculata* and *Microcystis* unicells. *GR* Growth rates per day. Shown are the mean ± standard error based on four replicates

maintained at 34 and 69 μg l^{-1} of microcystin; at these concentrations of *Microcystins*, the animals started reproducing within 48 h after birth, had a short life span, and died soon after reproducing.

Survivorship and reproduction-related parameters in relation to increasing concentrations of microcystins are indicated in Fig. 5. Compared with controls, the average life span and life expectancy at birth were significantly lower only at microcystin concentrations exceeding 8.6 μg l^{-1} ($P < 0.005$, one-way ANOVA), but at the lowest toxin concentration (4.3125 μg l^{-1}), *M*. cf. *micrura* survived better during the first days than at higher concentrations of the toxin. Reproduction-related traits (Fig. 5) did not significantly differ from controls, even at the highest (17–60 μg l^{-1}) microcystin concentration ($P > 0.05$, one-way ANOVA). In fact, our results indicated a positive influence of the higher microcystin concentrations on gross reproduction.

Discussion

Moina *cf.* micrura *responses to* Microcystis *toxicity*

Cyanotoxins are endotoxins and are usually more effective when cells containing these toxins are consumed by filter-feeding zooplankton (Lampert, 1987). Microcystins affect zooplankton and other organisms by inhibiting the activity of phosphatases (Dow & Swoboda, 2002). We observed higher adverse effects of the consumption of whole *Microcystis* cells by *Moina* cf. *micrura* compared with exposure to microcystins; *Moina* was almost unable to reproduce on 100% *Microcystis* diet.

The most important response of *M*. cf. *micrura* when exposed to microcystins was the speeding up of development time. We observed a diminution of generation time and longevity, as well as an increase in the number of offspring per day. This outcome is similar to the well-studied temperature effects on zooplankton (Miracle & Serra, 1989; Benider et al., 2002). Usually, coupled with this result, there is an increase in the population growth rate, because the number of offspring per individual may be the same, but these are produced in less time. In our case, we only obtained an increase of growth rate in the experiments with dissolved microcystin, especially evident at the highest concentration (50%) tested. When *Microcystis* cells are given directly as food, their deleterious effect is greater, and the net outcome is a diminution of longevity as well as that of the growth rate.

The two most important traits that determine the population growth rate are age at first reproduction and

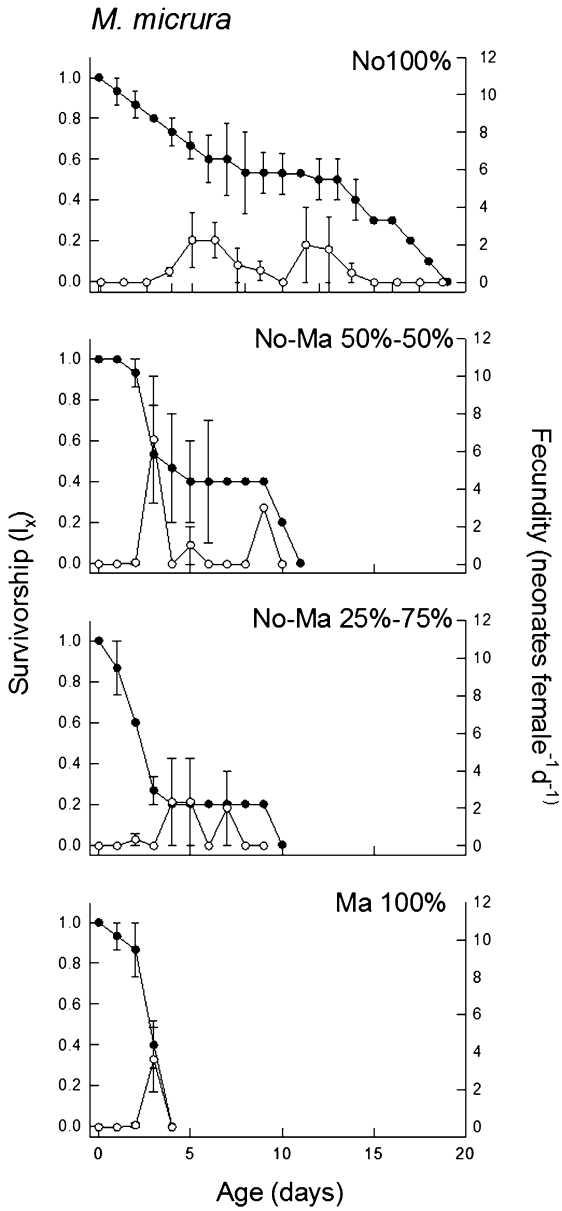

Fig. 2 Age specific survivorship and fecundity of *Moina* cf. *micrura* fed different proportions of *N. oculata* and *Microcystis*. Shown are the mean ± standard error based on four replicates (cohorts). *Filled symbols* indicate survivorship, and *open symbols* indicate fecundity

peak population density. We observed that *Moina* cf. *micrura* reached peak densities earlier if fed *Microcystis* cells or if exposed to the microcystins. This perhaps is a strategy to hasten offspring production when faced with the imminent danger of poor quality diet during *Microcystis* blooms. Under the pressure of different stress factors such as excess food availability, poor food quality, the presence of predators, or toxicants, several zooplankton taxa tend to mature faster and thus reproduce earlier (Boersma et al., 1998). Our results show that life history traits are to be carefully evaluated since high growth rates, as a result of an early and large clutch, do not *per se* signify more favorable environmental conditions.

Ebert & Jacobs (1991) suggest that in order to extrapolate the experimental results with greater reliability, tests should be conducted on various clones. Since the aim of this study was to analyze the effects of microcystins at the population level, in our study, we used individual animals randomly picked from a the filtered zooplankton and their descendants, which may have differed in clonal history. Our focus was to work on natural populations, so we selected *Moina* individuals from the same lake and site, and maintained them in the laboratory for a very short time, just to acclimate them to experimental conditions intended to broadly mimic natural conditions. Since we collected the cladocerans on the same day and same site, we did not expect great differences in experimental individuals. However, we cannot assume that some of the variability in our experiments could be attributed to clonal differences in sensibility to the toxicant. Results from other studies are not conclusive about this issue; Baird et al. (1991) found that the response of *Daphnia magna* to toxicity stress is not different among clones, whereas other authors (Hietala et al., 1997; Bednarska et al., 2014) reported significant differences between clones with respect to life history traits in *Daphnia* fed cyanobacteria. Moreover, Hairston et al. (1999) showed temporal shifts in the frequency of clones more or less resistant to cyanobacteria in terms of growth rates, when hatched from resting eggs from sediments corresponding, respectively, to periods of more or less eutrophication.

Other factors influencing demographic parameters such as food availability were maintained constant, and the controls were subject to the same test (volume and food level) conditions. The volume in the experimental beakers was substantially lower compared with several other studies (Hardy & Duncan, 1994; Nandini & Sarma, 2003) yet we observed that the small-bodied pelagic *Moina* attained densities in these experiments as in previous studies conducted in greater volumes (>1200 ind. L^{-1}: Pagano et al., 2000; Sipaúba-Tavares & Bachion, 2002). Also, based on

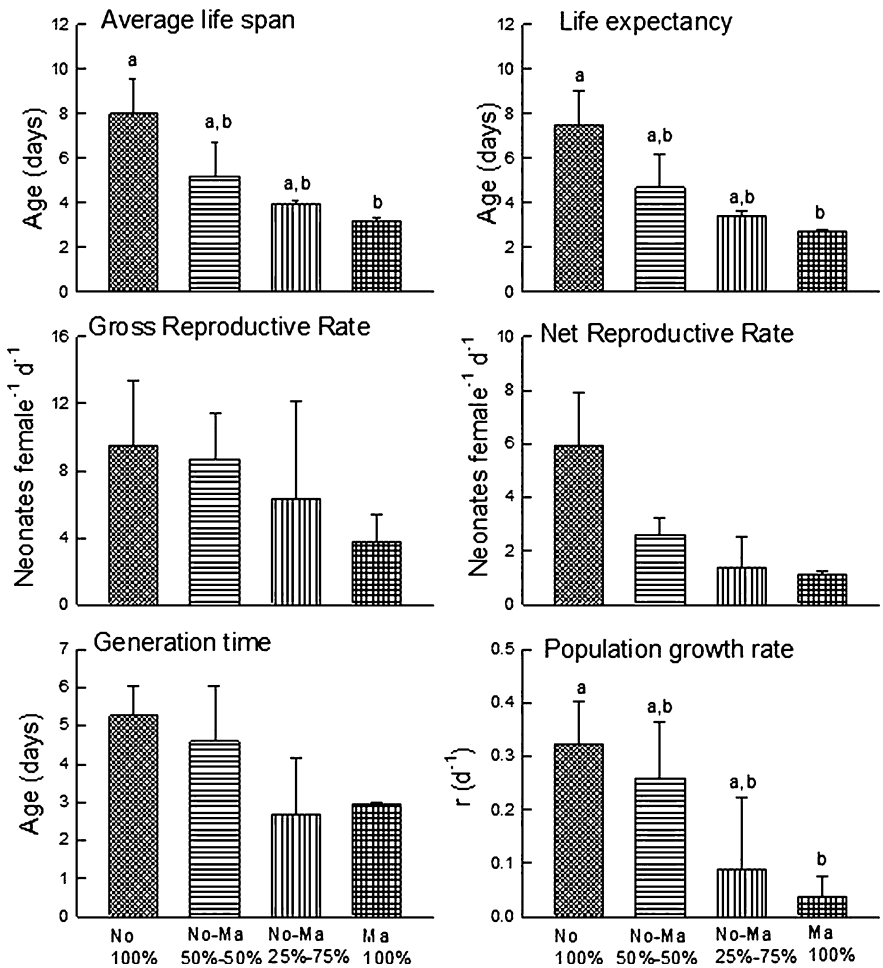

Fig. 3 Selected life history traits (average lifespan, life expectancy at birth, gross and net reproductive rates, generation time and the rate of population increase) of *Moina* cf. *micrura* fed different proportions of *N. oculata* and *Microcystis*. Shown are the mean ± standard error based on four replicates (cohorts)

the threshold food concentration for *Moina* (Hardy & Duncan, 1994), the dry weight and carbon content of *N. oculata* and *M. aeruginosa* (Pal et al., 2011; Li et al., 2014), and the fact that the culture medium was changed daily, the food available to the cladocerans was well above the limiting conditions.

Impacts of cyanobacterial blooms

We have observed that microcystin crude extracts do have adverse effect on *M. cf. micrura*. Although studies such as ours are both cost and time effective, they have not been conducted extensively around the world despite the rising temperatures and increased anthropogenic effects on freshwater systems, resulting in more persistent or perennial cyanobacterial blooms. In this regard, it may be mentioned that similar conclusions have been drawn from some other recent studies, which indicate that zooplanktonic species are sensitive in their demography to water from lakes collected after the peak in cyanobacterial blooms (Harper, 1992; Okumura et al., 2007; Zamora Barrios et al., 2015). Cladocerans often do not grow well on cyanobacteria alone due to several reasons such as their manageability and/or the presence of toxins (Bednarska et al., 2014). In nature, however, despite the toxic blooms and the subsequent release of toxins (when the blooms collapse), small zooplankton can survive since they often avoid or are unable to feed on the large filaments or colonies of toxic cyanobacteria (Work & Havens, 2003). Moreover, peak concentrations of cyanobacteria are frequently found for short periods of time only, and selected zooplankton taxa may be adapted to withstand lower concentrations before or after the peak. Our observations on the ability of *Moina* cf. *micrura* to survive and reproduce, in spite of high concentrations of microcystins, are similar to those of Chen et al. (2013) who have shown

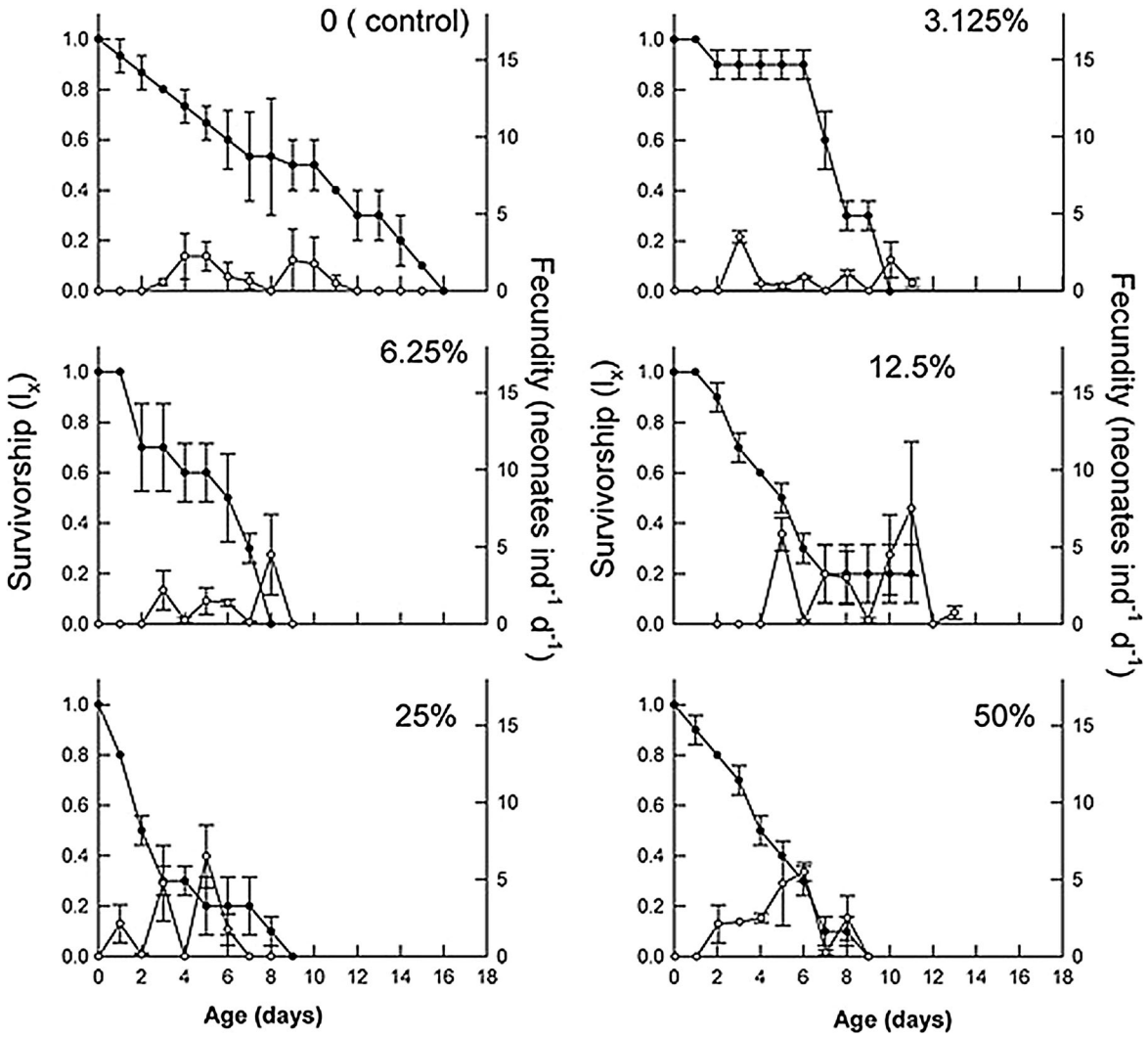

Fig. 4 Age specific survivorship and fecundity of *Moina* cf. *micrura* exposed to different concentrations of microcystin extracts. Shown are the mean ± standard error based on four replicates. *Filled symbols* indicate survivorship, and *open symbols* indicate fecundity

that some cladocerans, e.g., *Bosmina longirostris* and *Ceriodaphnia cornuta*, withstand the effect of microcystins from lysed *Microcystis*. That the response of various species of *Moina* and *Ceriodaphnia* to cyanotoxins is species specific is well documented (Wilson et al., 2006; Okumura et al., 2007). A recent study in Brazil by Herrera et al. (2015) also shows that *Moina micrura* is highly resistant to cyanotoxins. However, it is quite likely that the species studied here may not be the same taxon considering that *Moina micrura* is not one species but a species complex with wide distribution (Petrusek et al., 2004). We have still very little information about the level of microcystins that the different zooplankton species can tolerate, and we suggest that conducting such studies on water with decomposing cyanobacterial blooms could provide new data on the effect of dissolved cyanotoxins

Microcystis aeruginosa dominated the phytoplankton in Lake L'Albufera during our study in July 2014. The strain of *Microcystis* from this lake, is quite toxic. Romo et al. (2012) have also reported the presence of microcystin in this lake in previous years; they found occasional peaks (up to 16 and 120 μg l^{-1} in water and seston, respectively) that are even higher than the concentrations reported in the present work. These authors highlighted that *M. aeruginosa* blooms in this

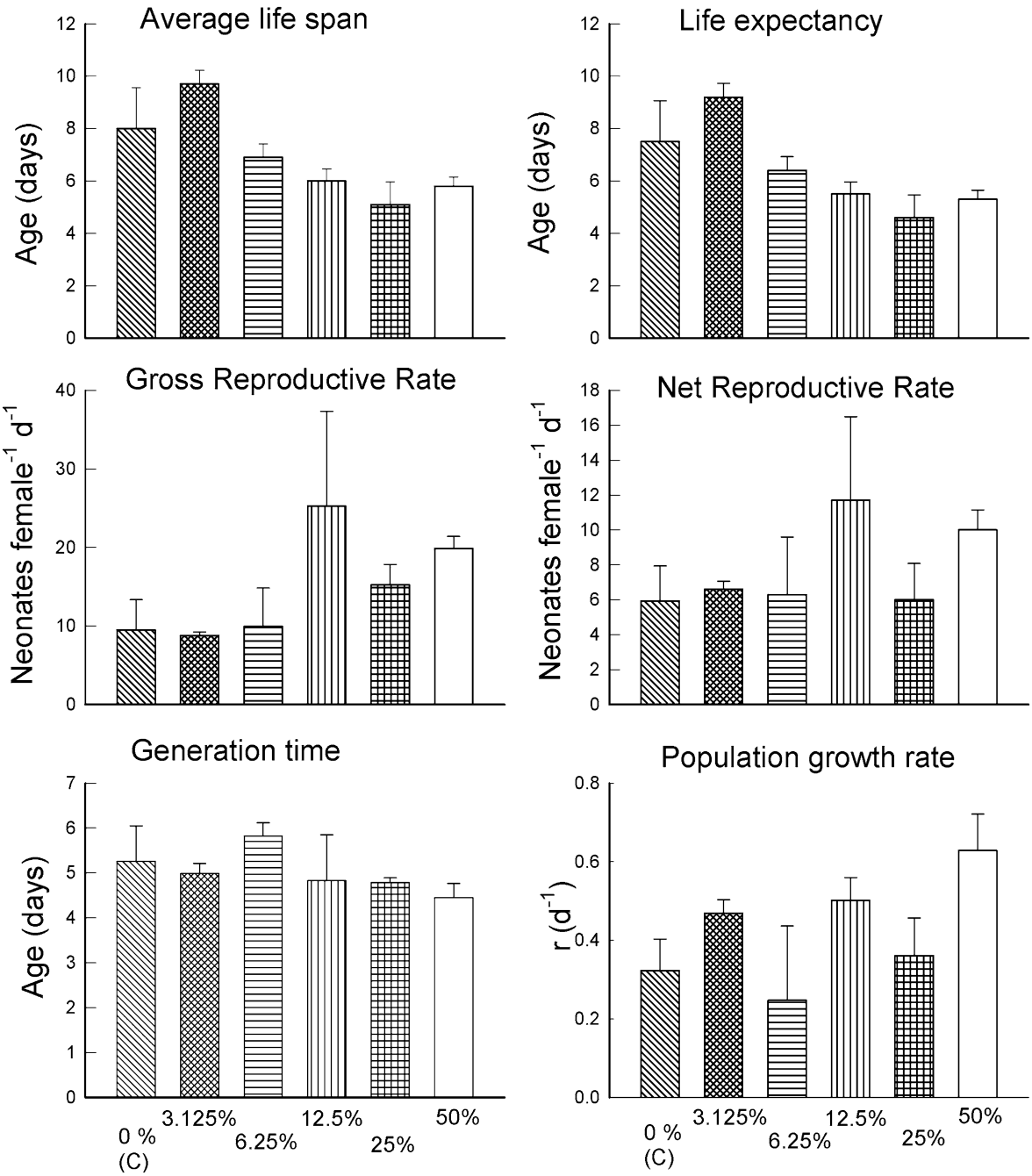

Fig. 5 Selected life history traits (average lifespan, life expectancy at birth, gross and net reproductive rates, generation time and the rate of population increase) of *Moina* cf. *micrura* exposed to different concentrations of microcystin extracts. Shown are the mean ± standard error based on four replicates

lake are associated with bioaccumulation of microcystins above the permissible levels in fish, mainly mullets extracted from this lake. This could be a source of high risk because these fish are consumed by human beings, by migratory birds, etc. Similar complaints have been made for other lakes with high densities of cyanobacteria, in which fish are frequently caught for human consumption and could pose a health problem (Berry & Lind, 2010; Berry et al., 2011).

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