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Microcystin-LR toxicity on dominant copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* of the upper San Francisco Estuary

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A B S T R A C T

This study investigates the toxicity and post-exposure effects of dissolved microcystin (MC-LR) on the dominant copepods of the upper San Francisco Estuary (SFE), where blooms of the toxic cyanobacteria *Microcystis aeruginosa* coincide with record low levels in the abundance of pelagic organisms including phytoplankton, zooplankton, and fish. The potential negative impact of *Microcystis* on the copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* has raised concern for further depletion of high quality fish food. Response of copepods to MC-LR (MC) was determined using a 48-h standard static renewal method for acute toxicity testing. Following exposure, a life table test was performed to quantify any post-exposure impacts on survival and reproduction. The 48-h LC-50 and LC-10 values for MC were 1.55 and 0.14 mg/L for *E. affinis*; and 0.52 and 0.21 mg/L for *P. forbesi*. Copepod populations recovered once dissolved MC was removed and cultures returned to optimal conditions, suggesting no post-exposure effects of MC on copepod populations. Dissolved microcystin above 0.14 mg/L proved likely to have chronic effects on the survival of copepods in the SFE. Since such high concentrations are unlikely, toxicity from dissolved microcystin is not a direct threat to zooplankton of the SFE, and other mechanisms such as dietary exposure to *Microcystis* constitute a more severe risk.

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1. Introduction

Cyanobacterial toxins are a key issue in aquatic management for a variety of reasons that stem from toxin exposure to the foodweb and changes in trophic transfer of carbon (Chorus and Bartram, 1999; Hansson et al., 2007). *Microcystis aeruginosa* is one of the most common species of freshwater cyanobacteria in eutrophic waters and produces several secondary metabolites that are toxic to aquatic organisms including zooplankton (Rohrlack et al., 2003; Wilson et al., 2006).

In recent years there has been an increase in the abundance and distribution of toxic *Microcystis* blooms in the upstream portion of the San Francisco Estuary (SFE), where abundance of pelagic organisms have reached historically low levels (Lehman et al., 2005; Sommer et al., 2007). The calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* are among the dominant prey species for endangered fish in this system (Kimmerer, 2004). Despite trends of declining abundance, these copepods are the seasonally dominant zooplankton of the upper SFE (*E. affinis* — spring and *P. forbesi* — summer) (Kimmerer, 2004; Sommer et al., 2007). Among the management actions under consideration for reversing the declining fish populations are actions aimed at improving their food

supply and especially the seasonal abundance of *E. affinis* and *P. forbesi* (CA Resources Agency, 2007). Thus, potential mechanisms through which *Microcystis* can impact copepod abundance are of both management and scientific concern in the SFE.

Although *Microcystis* produces a variety of secondary metabolites toxic to aquatic organisms, research has mainly focused on microcystins (MCs), which covalently bind to and inhibit protein phosphatases 1 and 2A, causing damage in a wide variety of animals, and can also negatively affect plants, algae, and protozoa (Dawson, 1998; Watanabe et al., 1996; Rohrlack et al., 2003; Zurawell et al., 2005; Gkelis et al., 2006). Of the 70+ different structural variants of MCs identified, MC-LR is one of the most toxic and common forms (WHO, Chorus and Bartram 1999; Best et al. 2003; Zurawell et al., 2005). MCs can have severe impacts on aquatic ecosystems ranging from acute toxicity to the disruption of trophic transfer of energy (Christoffersen, 1996). While MCs can transfer through the foodweb, their fate in the foodweb is not well understood, and it is not clear if MCs actually "bio-accumulate" in the proper sense of the term (Thostrup and Christoffersen, 1999; Ibelings et al., 2005; Xie et al., 2005; Ibelings and Chorus 2007). However, in a recent study MCs were found to transfer from lake water and aquatic animals to a chronically exposed human population with indication of hepato-cellular damage (Chen et al., 2009). As an endotoxin, MC remains within healthy cells until they rupture at death when the toxin

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dissolves and degrades relatively quickly, in a matter of days to several weeks, though factors determining degradation rates are variable (Perez and Aga, 2005; Zurawell et al., 2005).

In general, *Microcystis* impacts on zooplankton are caused by exposure to toxic metabolites either from dissolved extracellular concentrations or from ingested algal cells (Rohrlack et al., 1999, Zurawell et al., 2005, Wilson et al., 2006). While most of the *Microcystis*–zooplankton literature uses daphnids, copepods are known to be more sensitive to dissolved MC exposure (DeMott et al., 1991; Reinikainen et al., 2002). Reinikainen (2002) found reduced survival of *E. affinis* at elevated levels of MC's but no effect on egg hatching, suggesting that at least some reproductive processes are not affected once the toxin is removed. However, because lethal concentrations of MC are several orders of magnitude higher than typical environmental concentrations, natural cases of acute zooplankton toxicity from dissolved MC are probably very rare (DeMott et al., 1991; Christoffersen, 1996; Park et al., 1998; Song et al., 2007). While the significance and toxicity of non-MC metabolites such as microviridin and other cyanopeptides have become increasingly obvious, the focus of this paper is limited to MC toxicity (Rohrlack et al., 2003; Welker and von Döhren, 2006).

The purpose of this paper is to determine: 1) the species sensitivity of copepods (*E. affinis* and *P. forbesi*) to MC-LR, and 2) whether a short term (48 h) exposure to dissolved MC-LR has any long term impacts on copepod populations. To our knowledge, there is no information regarding post-exposure effects of MCs on copepods. We hypothesize that 1) copepods will have a species specific response to dissolved MC-LR, 2) that lethal concentrations will be significantly above common environmental concentrations, and 3) that possible effects on copepod population growth following exposure to sub-lethal MC-LR concentrations will be detected.

2. Materials and methods

2.1. Copepod cultures

P. forbesi was collected in June 2006, and *E. affinis* in March 2006 from various locations between Rio Vista and Suisun Marsh in the San Francisco Estuary. These periods reflect dominance of these species in the zooplankton community. Double filtered Delta water (35 mm mesh followed by GF/C, Millipore Corp) collected at Rio Vista was used as a culture medium, and cultures were maintained in aerated 4-L beakers placed in an environmental water bath at 24 ± 1 °C for *P. forbesi*, 18 ± 1 °C for *E. affinis* reflecting temperatures during their respective dominance in zooplankton community. Water quality in beakers including dissolved oxygen (>8 mg/L; YSI instruments), pH (8.3 ± 0.1), water hardness (100 mg/L; Hach, USA), salinity (5.0 ppt) and ammonia (<1 mg/L Hach, USA) was monitored weekly. Copepods were given 500 µg C L^{-1} day^{-1} equivalent of an equal mixture of highly nutritious algae *Nannochloropsis* and *Pavlova* as the food source (Reed Mariculture, San Jose, CA). Algal carbon content was determined by filtering known bio-volumes of diluted algae and analyzing for total carbon using a Gas Chromatogram (Stable Isotope facility, UC Davis). 60% of the total culture medium was replaced with freshly filtered medium weekly. Copepod culture density was monitored weekly, maintained between 50 and 100 adults/L, and diluted as necessary in order to ensure exponential population growth and minimize overcrowding. The systems were maintained under a natural photoperiod (16L:8D) and covered with a semi transparent black tarp to reduce contact with outside disturbance. Actively swimming healthy adult copepods were randomly collected from the brood cultures, which were acclimated to laboratory conditions for a minimum of three months prior to experiment.

2.2. Acute toxicity test (LC-50, TL-50)

To determine mean lethal concentrations of MC-LR after 48 h of exposure, three replicates with 15 copepods each in a 20-mL glass tube were exposed to control (0 mg/L MC), vehicle control (methanol), and seven nominal treatment concentrations of purified MC-LR toxin (0.1, 0.25, 0.50, 0.75, 1.0, 1.25, and 1.5 mg/L for *P. forbesi*, and 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, and 1.75 mg/L for *E. affinis*). These concentration ranges were chosen following a pilot study which indicated that *E. affinis* was more tolerant to MC-LR than *P. forbesi*. To prepare a 500-mg/L stock solution, 0.5 mg of MC-LR (Calbiochem, USA) was dissolved in 1 mL of 50% methanol:50% water solution, as MC-LR is slightly hydrophobic and methanol is commonly used as a solvent (Watanabe et al., 1996; Perez and Aga, 2005). Treatment solutions were then prepared by adding appropriate volumes of stock solution to the 15-mL culture medium immediately prior to adding the copepods. Healthy adult copepods were obtained by gently pouring the brood cultures through a 147-µm mesh and rinsing them to a petri dish. From here, 15 active adult copepods were randomly selected and placed in a small beaker with the 5-mL culture medium and then gently added to the previously prepared treatment solutions to bring the total volume to 20 mL for each replicate vessel. Copepods were starved for 24 h prior to the experiment. Vehicle control included the same concentration of methanol found in the highest treatment group of each experiment to ensure observation of effects due to trace additions of methanol only (0.175% by volume). Methanol concentration varied between 0.01 and 0.175% (by volume) among treatments, as added from the MC-LR stock solution, and 0% for the true control.

Two steps were taken to minimize the loss of MC-LR during the 48-h exposure period. First, inert glass tubes were presoaked overnight with the appropriate corresponding treatment concentration to minimize loss of dissolved MC-LR through glass surface binding during the exposure (Hyenstrand et al., 2001). Second, treatment solutions were replaced after 24 h with freshly prepared MC-LR spiked medium by gently siphoning through a 30-µm mesh with minimal disturbance to copepods.

Copepods were monitored between 4 and 6 h intervals for mortality by examining each 20-mL tube under a dissecting microscope. Dead copepods, defined as completely motionless after repeated disturbance (any twitching was still considered alive), were removed immediately. Mortality values were then used to calculate mean time to lethality (TL-50) and the 48-h LC-50. Percent mortality was calculated as the number of dead relative to total (15) in each replicate at a given time.

2.3. Post-exposure life table test

At the end of the 48-h exposure, all survivors were cleaned with three changes of culture medium and were transferred to optimal culture conditions for the observation of survival and reproduction.
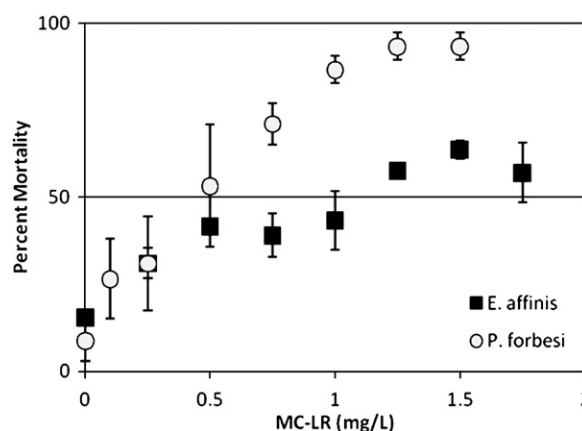

Fig. 1. Dose response of copepods to a 48-h exposure to increasing MC-LR concentrations. Mortality is significantly different between controls and all treatments for both species. Error bars = standard errors.

Table 1
LC values of dissolved MC-LR (mg/L) for *P. forbesi* and *E. affinis* obtained by PROBIT.

Copepod	LC-50 (95% CI)	LC-10 (95% CI)	Slope (95% CI)
P. forbesi	0.52 (0.38, 0.63)	0.21 (0.11, 0.31)	3.21 (2.19, 4.24)
E. affinis	1.55 (0.93, 3.59)	0.14 (0.03, 0.36)	1.24 (0.45, 2.03)

Table 2
Population growth rate (r) of *P. forbesi* and *E. affinis* post 48-h exposure to MC-LR.

($n = 15$)	N_o range, mean, (SD)	N_{29} range, mean, (SD)	r range, mean, (SD)
E. affinis	3–6, 4.40 (0.82)	0–47, 22.4 (13.39)	0–0.09, 0.05 (0.02)
P. forbesi	2–7, 4.25 (2.15)	0–85, 29.87 (23.8)	0–0.09, 0.05 (0.03)

Range and mean values for number of copepods per replicate at day 4 (N_o) and final day (N_{29}). Values include all 15 replicates for each species, including those replicates with zero copepods at N_{29} (SD = standard deviation).

Immediately after the exposure (at $t = 48$ h; end of LC-50), this is defined as day 0. Because of high latent mortality between day 0 and day 4, population growth rates were calculated starting from day 4, when the mortality subsided in all treatments. Surviving copepods were transferred to fresh, MC-free filtered medium in 1-L aerated beakers and fed immediately following culture conditions above. A number of individual life stages (N_{1-5}, C_{1-6}) were monitored and recorded daily (by visual inspection and dissecting microscope as necessary), and maintained under brood culture conditions to evaluate post-exposure population growth rate and average development time for each treatment/replicate. Chronic effects were studied in treatments that were at or below the calculated LC-50 concentration because treatments above the LC-50 concentration did not have adequate copepods surviving for a reliable study. Population growth rate was quantified by population growth of adults over time (adult reproduction) for each treatment replicate (3) and averaged per treatment. The post-exposure experiment continued for 33 days after the 48-h LC-50 exposure ended. The following formula was used for net population growth rate (r).

$$r = ln(N_t / N_o) / t$$

where

N_t	number of adults at the end of experiment
N_o	number of adults at day 4 after the 48-h LC-50 ends
t	duration of experiment
r	per day population growth rate.

2.4. Data analysis

LC-50 results were analyzed using PROBIT software, where mortality is plotted against MC concentration and the log transformed dose response curve estimates percent mortality at a given toxin concentration. LC-50 concentration is by definition the estimated concentration that causes 50% mortality at a given time. LC-10 is calculated similarly, but for the concentration tha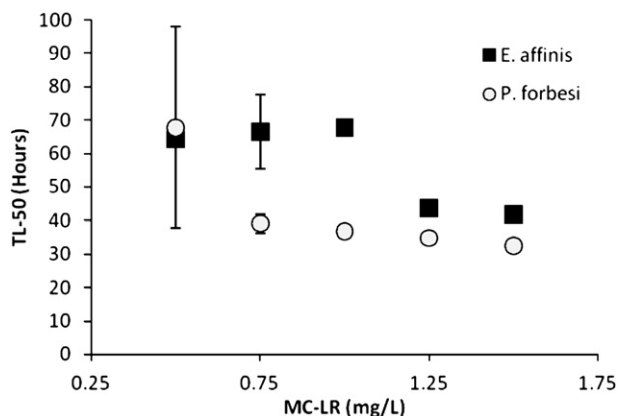t results in 10% mortality at a given time. TL-50 was estimated using the linear regression for individual replicate mortality as a function of time. Each replicate was assigned a linear regression and the time to reach 50% mortality was calculated. The average of all three replicates was used to determine the TL-50 for a given treatment. Differences in the effect of MC-LR exposure and copepod species on the post-exposure population growth rate, as well as differences in each species response to MC-LR (interaction term) were analyzed with a factorial ANOVA. The growth rate for each treatment is based on the average from all replicates in the treatment.

3. Results

3.1. Acute and latent mortality

Both species of copepods showed a clear dose response to increased concentrations of dissolved MC-LR over the 48-h exposure period. Mortality was significantly higher than controls in all treatments including the lowest MC-LR concentrations (Fig. 1). Vehicle control (w/ methanol) mortalities were 8.8% for *P. forbesi*, and 15.5% for *E. affinis*, respectively, during the 48-h exposure to MC-LR (Fig. 1). Comparison between the control and the methanol spiked control showed no significant difference (data not shown), and control hereafter refers to the vehicle (methanol) control. Species differences in sensitivity were observed in 48-h LC-50 values for MC-LR where *P. forbesi* (0.52 mg/L) was 3 folds more sensitive to MC-LR than *E. affinis* (1.55 mg/L) (Table 1). Mortality increased with treatment concentration for both species, though the slope of their response was significantly different, with *P. forbesi* having a more pronounced dose response. On average, *E. affinis* was less sensitive to MC-LR exposure, and its response to increasing concentrations was less pronounced, having almost a third of the slope compared to *P. forbesi* (Table 1, Fig. 1). The difference in slope resulted from the two copepods having similar mortality at MC-LR concentrations <0.5 mg/L, as their LC-10 concentrations were comparable (Table 1).

3.2. Mean survival time (TL-50)

TL-50 indicates how fast the toxin begins to cause mortality and is a temporal measure of the dose response by showing how long it takes a population to reach 50% mortality. While TL-50 of both copepods declined similarly to increasing MC-LR concentration, species specific mean survival time was consistently higher for *E. affinis* compared to *P. forbesi*

Fig. 2. Mean survival time for copepods exposed to MC-LR, as measured by TL-50, i.e. time to reach 50% mortality for both species. Error bars = standard errors.

Table 3
Factorial ANOVA testing the effect of treatment variables on post-exposure population growth rates.

Source	DF	F ratio	p
Species	1	<0.001	0.98
MC-LR	1	0.14	0.71
Species × MC-LR	1	0.77	0.39

Variables are copepods species (species), MC-LR exposure concentration (MC-LR), and their interaction term (species × MC-LR: how previous MC-LR exposure affects each species population growth rate).

Table 4
Comparison of measured LC-50 concentrations for various zooplanktons exposed to MC-LR.

Zooplankton	48-h LC-50[a]	95% confidence interval	Source
Tetrahymena pyriformis (ciliate)	252[b]	(200–316)	Ward and Codd (1999)
Thamnocephalus platyurus nauplii (fairy shrimp)	0.46[c]	(0.38–0.54)	Keil et al. (2002)
Artemia salina (brine shrimp)	15–17[d]	–	Lahti et al. (1995)
Daphnia pulicaria	21.4	(16.8–29.2)	DeMott et al. (1991)
Daphnia hyalina	11.6	(7.0–15.6)	DeMott et al. (1991)
Daphnia pulex	9.6	(7.8–11.7)	DeMott et al. (1991)
Diaptomus birgei (copepod)	0.45	(0.29–0.58)	DeMott et al. (1991)
E. affinis (copepod)	0.27	(0.197–1.446)	Reinikainen et al. (2002)
E. affinis (copepod)	1.55	(0.93–3.59)	This study
P. forbesi (copepod)	0.52	(0.38–0.63)	This study

Values are in mg/L MC-LR and for a 48-h exposure unless otherwise noted.
[a] Unless otherwise noted.
[b] 24-h dissolved MC-LR exposure.
[c] Various congeners of MC.
[d] MC-LR and MC-RR mixture.

when MC-LR concentration >0.5 mg/L (Fig. 2). At lower concentrations, two species behaved similarly.

3.3. Post-exposure effects on copepod population growth

Once removed from the acute toxicity experiment, both species of copepods showed high mortality (up to 0.18 per day) for 4 days of the post-exposure period, regardless of MC-LR exposure. At the end of this 4-day period following exposure, the number of surviving copepods varied between 2 and 7 in each vessel, and did not depend on exposure history. Thereafter, mortality rate subsided for both species, and population growth started with the appearance of nauplii on day 4 for *E. affinis* and on day 7 for *P. forbesi* (data not shown). Gravid females were observed across all treatments within a few days following post-exposure conditions. More than 50% of the *E. affinis* treatments had gravid females by day 1, and *P. forbesi* by day 6. More than 50% of treatments in both species had copepodites by day 11, and more than 50% had new adults by day 21.

Post-exposure population growth rate ranged between 0 and 0.09 per day (Table 2), and was independent of both MC exposure history and copepod species (Table 3). Initial number of adult copepods at day 4 (N_o) predicting final number of adult copepods (N_{29}) was not related for *E. affinis*, and very weakly linear with *P. forbesi* ($R^2 = 0.03$ and 0.25 respectively).

While a few treatments had no population growth following exposure, the majority of vessels (80% for *P. forbesi* and 93% for *E. affinis*) showed positive growth rates at the end of the experiment with the emergence of new adults. A total of 3 vessels (out of 15) for *P. forbesi* and 1 vessel (out of 15) for *E. affinis* had a zero or negative population growth rate. We assumed these vessels had no population growth because they lacked both sexes due to latent mortality in the post-exposure period. These no growth vessels were included in the calculation of population growth rate for their respective treatments, though their exclusion does not change the results and lack of relationship (data not shown). Overall, there was no difference in average population growth between the control and exposed copepods, no effect of MC-LR exposure history, and no difference in post-exposure population growth between species (Table 3).

4. Discussion

The LC-50 values found for MC-LR in this study agree with the general consensus that calanoid copepods are more sensitive than cladocerans, by an order of magnitude (DeMott et al., 1991). Results

demonstrate that calanoid copepods are some of the most sensitive zooplankton species to dissolved MC exposure (Table 4). While MC-LR is known to degrade relatively rapidly, the measures taken to minimize MC-LR loss such as presoaking and static renewal of treatment solutions should have prevented any MC-LR degradation strong enough to significantly change treatment concentrations (Perez and Aga, 2005). In a similar experiment Reinikainen et al. (2002) found no significant degradation of MC-LR over 48 h even though no measure was taken to prevent degradation.

MC-LR toxicity was similar for the two copepod species when concentrations were <0.5 mg/L. Above this, *P. forbesi* was more sensitive and its response to increased MC-LR was more pronounced than *E. affinis*. The difference at higher concentrations between two species could be due to species specific uptake via the integument and by inherent physiological tolerance and enzymatic detoxification, which may be controlled at larger scales through evolutionary adaptations (DeMott et al., 1991; Blaxter and Ten Hallers-Tjabbes, 1992; Chen et al., 2005).

In order to prevent protein phosphatase (PP) inhibition, *Daphnia* use enzymes of the anti-oxidant system (GST and glutathione) to detoxify MC (Pflugmacher et al., 1998; Chen et al., 2005). At low or initial doses of MC exposure, there is a decline in the anti-oxidant enzymes with no inhibition of PP enzymes; under extended MC exposure, the continuous detoxification can cause oxidative stress such as increased LDH, which eventually overwhelms detoxification and *Daphnia* die from PP inhibition (Chen et al., 2005). *E. affinis* is able to detoxify nodularin, another cyanobacterial hepatotoxin, showing that copepods have adapted to algal toxins and have detoxification mechanisms (Karjalainen et al., 2006).

Another factor that could explain why copepods here showed differences in sensitivity at higher concentrations is the different exposure temperature (18 °C for *E. affinis* versus 24 °C for *P. forbesi*), as higher temperature could increase sensitivity due to a faster metabolism. In another study for MC-LR, Reinikainen et al. (2002) found the 48-h LC-50 for *E. affinis* from the Baltic Sea to be significantly lower than the current study even at a lower exposure temperature (Table 4). However, comparing results of the two studies may not be that useful because unlike the current study, Reinikainen et al. (2002) fed copepods during MC exposure tests. Higher sensitivity in that study could be from both ingested and dissolved MC, since feeding during the exposure could increase ingestion of MC via adsorption to the food particles. Temperature effects on sensitivity within the context of this study may not be as vital as other factors such as geographical differentiation and previous exposure to MC.

Evidence is accumulating for geographically distinct populations of copepods from the same species having genetically distinct life history traits and tolerance to stress (Wyngaard, 1986). *E. affinis* is a common invasive species in Northern temperate estuaries and some lakes, and its success may be due to its ability to respond to natural selection (i.e. evolve tolerance), as opposed to an inherent broad range of plasticity and high tolerance to a wide range of conditions (Lee, 2002). Differences in stress tolerance among distinct populations of copepods, including MC toxicity for *E. affinis*, may be due to geographically isolated populations that have been adapting to local selective regimes (Lee, 2002).

Increased tolerance may also be related to previous exposure to *Microcystis*, which may improve dietary tolerance in some zooplankton, and could also mean decreased sensitivity to dissolved MC (Gustafsson and Hansson, 2004; Sarnelle and Wilson, 2005). Since copepods in this study were cultured with no contact to *Microcystis* or MC for several months, the possibility of any tolerance to MC from previous exposure can be omitted.

The difference in MC tolerance reported here could be due to gender biased effects as female copepods have higher tolerance to dissolved MC than males (Ojaveer et al., 2003). In this study, however, the chances for MC-LR toxic effects based on gender should be minor because 45 adult copepods per treatment were randomly selected from a pool of >200 copepods in a 4-L beaker and divided to 15 copepods per replicate.

The LC-10 concentrations, which represent the threshold for chronic effects, are at the highest of naturally observed MC concentrations (Song et al., 2007). This supports previous work showing that acute toxicity from dissolved MC-LR to copepods requires abnormally high concentrations to be a significant threat (McDermott et al., 1995; Park et al., 1998). Since their LC-10 concentration was comparable, MC-LR toxicity for *E. affinis* and *P. forbesi* should be similar at environmentally relevant concentrations. With regards to their sensitivity, calanoid copepods of the SFE are similar to other calanoids, and are among the most sensitive zooplankton to dissolved MC (Table 4).

While acute toxicity is not a direct threat expected to occur in the field, extended periods of low level dissolved MC can still present a potential impact to zooplankton through sub-lethal effects such as oxidative stress (Chen et al., 2005). Effects of longer MC exposure (>48 h) on copepods at sub-lethal concentrations are not known and may be significant in food limited systems such as the SFE. Monitoring of dissolved MC concentrations from the SFE was not available at the time of this study.

Results indicate no post-exposure effects on reproduction, and predict that short term (<48 h) spikes of sub-lethal MC will not have significant consequences on a population scale. Copepods can repopulate quickly if provided with good conditions such as clean water, high food quality, and optimal temperature under laboratory conditions. However such conditions do not usually exist in the SFE, where zooplanktons are limited by food, predation by invasive filter feeding bivalves, and possibly contaminants (Mueller-Solger et al., 2002; Kimmerer, 2004).

If exposure to sub-lethal MC concentrations had a significant effect on copepod fecundity and population growth, we would expect to see a negative relationship between MC exposure treatments and population growth rate. The number of initial females at N_o along with the individual fitness of each copepod will be a major determinant of the final number of copepods in each vessel population, and the current study would have benefited from knowing the number of females at the start of the post-exposure experiment. However, despite the lacking record for the initial female population per replicate, differences between individual population growth rates of replicates were completely random and not related to the number of initial copepods (N_o) or MC exposure concentration. Similarly, high latent mortality between the end of the exposure and beginning of the life table test (N_o) was not related to exposure history and was probably due to handling stress, including the 48-h starvation during the exposure period. The variation of population growth rate within each treatment was high, such that any potential effect of number of females is overwhelmed by the variation among replicates. For instance, *P. forbesi* replicates in the control, 0.25, and 0.5 mg/L MC-LR treatments each having only 2 copepods at N_o all had population growth rates that were among the highest (>0.08 per day). Thus, despite the difference in previous MC exposure, and regardless of the initial number of females, copepods can repopulate at variable but similar rates. Accordingly, the conclusion that a sub-lethal 48-h MC exposure does not affect post-exposure population growth in these copepods may still be extracted from the results.

While exposure to sub-lethal concentrations of dissolved MC had no effect on post-exposure reproduction in the laboratory, other stressors in the SFE could increase sensitivity of copepods to MC in the field. The ecosystem scale impact of dissolved MC to the SFE's zooplankton community is likely a function of its persistence, the extent and variability of dissolved MC, zooplankton adaptations, sub-lethal effects and other stressors in the environment. Taking a better look at several non-MC compounds which *Microcystis* produces has become necessary to realize the full impact of *Microcystis* on zooplankton (Rohrlack et al., 2003). More importantly, the potential threat from *Microcystis* to the dominant zooplankton of the SFE is probably regulated via ingestion and food quality related trophic interactions rather than dissolved MC. Research on copepod–*Microcystis* interactions should be directed to feeding studies, which have overwhelmingly focused on daphnids (Wilson et al., 2006).

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