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Effects of the density of an invasive crayfish (*Procambarus clarkii*) on pelagic and surface microalgae in a Mediterranean wetland

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With 2 figures and 3 tables

Abstract: To understand the relationship between the density of an introduced crayfish species (*P. clarkii*) and the abundance and composition of pelagic and surface microalgae (hereafter referred to as phytoplankton and phytoneuston, respectively) we ran an in situ experiment in a Mediterranean wetland. In May 2004, we delimited six 10×7 m areas along a channel in the "Padule di Fucecchio" (Italy). Each area was randomly chosen to host crayfish populations at either low (1 crayfish/m^2) or high densities (14 crayfish/m^2). Phytoplankton and phytoneuston samplings were conducted in August and in September 2004, corresponding to the periods in which the highest and the lowest numbers of free-moving crayfish were found, respectively, during the lentic phase of the water regime. Results showed that (1) phytoneuston biomass, composed of cyanobacteria and, to a lesser extent, of euglenoids, was strongly affected by the presence of dense populations of *P. clarkii* in August; (2) crayfish seemed to modify the composition of microalgal communities; (3) these effects were not accompanied by significant differences between experimental areas in water chemistry and temperature; (4) physico-chemical measures highly varied with sampling periods along with a change in phytoplankton and phytoneuston abundance; and (5) high crayfish densities did not to exert any measurable effect on phytoplankton abundance. The decrease of phytoneuston may be the result of top-down effects of crayfish removing invertebrate grazers or, most likely, of direct grazing of crayfish. Indeed, crayfish were often observed climbing on macrophytes and feeding on the floating film.

Key words: Invasive species, phytoplankton, phytoneuston, eutrophication, cyanobacterial bloom, wetlands, *Procambarus clarkii*.

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Introduction

Notwithstanding the large consensus existing today about the potential threats that alien species pose to recipient communities (GHERARDI 2000), still little attention has been placed on quantifying their effects on biodiversity and eco-system functioning (PARKER et al. 1999). Indeed, both information and predictions of impacts are needed, first, to clarify the biological traits that confer invasiveness and, second, to improve strategies for preventing and mitigating the ecological and economic damage produced (BYERS et al. 2002).

There are many examples of alien crayfish being stocked throughout the world (HOBBS et al. 1989). Because of their high commercial value, the introduction and cultivation in Europe of several species have increased during the last few decades (e.g. PÉREZ et al. 1997). Today, most European countries have at least one introduced crayfish (GHERARDI & HOLDICH 1999), *Procambarus clarkii* contributing more than 90 % of the total crayfish production (ACKEFORS 1999). These introductions have created obvious conflicts between the need for crayfish production and the maintenance of biodiversity in freshwater ecosystems (NYSTRÖM 2002). In their review of global crayfish introductions, HOBBS et al. (1989) concluded that "once an exotic crayfish is introduced into a lake or stream, it may pose considerable environmental stress on the system". That "environmental stress" is a direct consequence of the primary role that many crayfish play in the inhabited systems. Indeed, crayfish are the largest and relatively long-lived invertebrate organisms in temperate areas, and often dominate the invertebrate biomass (MOMOT 1995). Most of them are keystone consumers (NYSTRÖM et al. 1996), acting as selective omnivores and detritivores (e.g. LODGE & LORMAN 1987, CHAMBERS et al. 1990, GHERARDI et al. 2001, 2004) and feeding on a wide variety of material including macrophytes, macroinvertebrates, periphytic algae, detritus, seeds, leaves, and dying, or incapacitated fish (e.g. ALEXANDER & COVICH 1991, LODGE et al., 1994, USIO 2000, SCHOFIELD et al. 2001). They are prey for several species, including otter (SLATER & RAYNER 1993), fish (MATHER & STEIN 1993, GARVEY et al. 1994, BLAKE 1995), and birds (RODRIGUEZ et al. 2005). Because of their interactions at all trophic levels in the food web (MOMOT et al. 1978, NYSTRÖM et al. 1996, NYSTRÖM 2002), crayfish are expected to exert direct and indirect effects on the communities.

Where potentially invasive crayfish colonize, the combination of their rapid population increase and omnivorous feeding habits may have dramatic consequences on the structure of freshwater food webs (LODGE et al. 1998). In the short term, invasive crayfish may reduce biomass and species richness of macroinvertebrates, macrophytes, and periphyton (e.g. LODGE & LORMAN 1987, CREED 1994, CHARLEBOIS & LAMBERTI 1996, GUTIÉRREZ-YURRITA et al. 1998, NYSTRÖM et al. 2001, CORREIA 2002, CRONIN et al. 2002). This behav-

iour may also lead to direct economic damage, due, for instance, to the reduced recruitment of commercially valuable fish stocks, such as trout (NYSTRÖM 1999), or to the destruction of rice plants (e. g. ANASTÁCIO et al. 2005). In the long term, invasive crayfish may induce drastic habitat changes with the consequent decline of several invertebrate taxa, amphibians, and fish (e. g. GUAN & WILES 1997).

Notwithstanding the long list of studies cited above, the effects that introduced crayfish potentially exert on the pelagic and surface microalgae (hereafter referred to as phytoplankton and phytoneuston, respectively) have not been much acknowledged. Yet, intense feeding activity and bioturbation may induce sediment resuspension and increased nutrient release and may lead to the change from a macrophyte dominated, transparent water equilibrium to a turbid system, dominated by planktonic microalgae (ANGELER et al. 2001). In the present study, we conducted an in situ experiment to understand the relationship between the density of an introduced crayfish species (*P. clarkii*) and the relative abundance and composition of the phytoplankton and phytoneuston communities in a Mediterranean inland wetland.

This research is part of a more extensive study aimed at understanding the factors responsible of the drastic loss of biodiversity recorded in the study area during the last decade. A similar trend has been observed in several other wetlands threatened by both biological invasions and anthropogenic alterations. In the same habitats, cultural eutrophication and global warming are also leading to range extention and increased duration of potentially toxic algal and cyanobacterial blooms (SKULBERG et al. 1984). Nevertheless, wetlands are second only to rainforests as reservoirs of biodiversity and productivity and are ranked second to estuaries in terms of ecosystem services provided to the human welfare (COSTANZA et al. 1997).

Methods

The study animal

The red swamp crayfish, *P. clarkii*, is native of north-eastern Mexico and south-central USA. Since the 1950's, it has been introduced, mostly for aquaculture, into 25 other countries (HOBBS et al. 1989), Italy included (GHERARDI et al. 1999). In the areas of introduction, either because they escaped from cultivation ponds or because they were purposively introduced, crayfish have colonized natural water bodies giving rise to reproductive populations. Together with other sources of anthropogenic disturbances, the spread of this invasive species has been considered one of the major causes of biodiversity loss in freshwater systems of south-central Europe (LODGE et al. 2000).

The study area

The "Padule di Fucecchio" in Tuscany (43° 48′ 0″ N, 10° 47′ 38″ E; 13–16 m above sea level) is the largest Italian wetland (1800 ha), in part (230 ha) protected as a nature reserve. It consists of a net of channels connected to the River Arno. Water depth varies among seasons, ranging between 0 and 3 m, and the water regime changes from lotic during the rainy period (October–January) to lentic in summer. Water temperature varies between 15–28 °C and pH 6–7.

Situated on the boundary between the Mediterranean and continental climates of the Italian peninsula, the Padule hosts plants adapted to cold (*Sphagnum* spp.) and to warm wet climates (e. g. the Frogbit, *Hydrocharis morsus-ranae* and the Royal Fern, *Osmunda regalis*). It offers refuge for species, now regarded as extremely rare: the Southern Bladderwort (*Utricularia australis*), the Fringed Water-lily (*Nymphoides peltata*), and the Water Floating Fern *Salvinia natans*. Other more abundant floral entities are: *Azzolla caroliniana, Carex elata, C. riparia, Phragmites australis, P. communis, Lemna minor, L. gibba, Lythrum salicaria, Scirpetum maritimum*, and *Sparganium erectum*. It also forms a basic area for migratory birds; over 190 species of birds can be seen during the year; at least 70 nest here, among which Ardeidae (Night Heron, Little Egret, Squacco Heron, and Cattle Egret).

Pre-sampling, sampling, and pigment analyses

We sampled on August 9 and on September 20, 2004 during the lentic phase of the water regime. As shown in a companion study (A. CACCHIANI, unpubl. data), early August and late September corresponded to the periods in which the number of freely-moving *P. clarkii* was the highest and the lowest, respectively, during the lentic phase. This was related to the intensity of foraging activity.

Approximately a year before sampling started, we selected a 110 × 7 m reach of a channel (about 50–100 cm water height). There, macrophytes (*Carex elata, C. riparia*, and *Phragmites australis*) were homogeneously distributed, substrate was uniform (composed of sand and mud, interspersed with cobbles and gravel), and a few macro-invertebrates (Ephemeroptera and Diptera larvae) were present. The selected reach was delimited using a 4 mm wire mesh fence (1.20 m high) and divided into six 10 × 7 m non-adjacent areas using a similar fence. Fences were buried 15 cm in the sediments to impede the passage of crayfish. Each experimental area was randomly designed to host crayfish populations at either low (LDA) or high density (HDA), so that we obtained three LDA and three HDA whose sequence along the channel was random.

During the pre-sampling phase (between May and June 2004), we first assessed the natural density of the study population (14 crayfish/m^2) by intensively capturing crayfish from the whole experimental area using 12 baited traps. A week before the first sampling started, approximately the same number of crayfish as those removed during the pre-sampling phase was added to HDA. The resulting crayfish density was 1 and 14 crayfish/m^2 in LDA and HDA, respectively.

During each period, we collected four phytoplankton and four phytoneuston samples per experimental area at random, reaching a total of 48 samples. Samples (about

$200\,cm^3$ of water) were taken at 20 cm depth using a plastic bottle to collect phyto-plankton and from a $320\,cm^2$ wide surface using a plastic flat container to collect sur-face biofilm for phytoneuston analysis. A preliminary qualitative assessment of the mi-croalgal components of phytoplankton showed the occurrence of several species of Chlorophyta, Dinophyta, and Chrysophyta in August, while a shift to the dominance of chlorophytes was recorded in the second sampling in September. Phytoneuston was composed of cyanobacteria with prevalence of *Microcystis* spp. and, to a lower extent, of euglenoids.

In each experimental area, we also measured in the two sampling periods: air and water temperatures with a mercury thermometer; water depth with a graduated stick; dissolved oxygen concentration with an Ati Orion 810 Dissolved Oxygen meter; am-monium, pH, nitrites, nitrates, and calcium concentration using colorimetric methods (Aquamerk®, Darmstadt, Germany); and water clarity using a Secchi disk.

Samples were analyzed in the laboratory to obtain the concentration of: chloro-phylls-a, -b, and -c, and phaeopigments in dm^3 as estimates of microalgal biomass, quality, and status, respectively (LAZZARA et al. 1990). First, water was filtered through glass fibre filters. Then, after mechanical homogenization and centrifugation, the spectral absorbance of acetone extracts (90 % v/v) was measured (Beckman, DU–64) for the quantification of chlorophylls-a, -b, and -c (JEFFREY & HUMPHREY 1975). We also used the acidification procedure, as modified by RIEMANN (1978), to sepa-rately estimate chlorophyll-a and phaeopigments concentrations (LORENZEN 1967).

Statistical analyses

All data were checked for normality and homogeneity of variance using Cochran's C-test and, when necessary, were $\ln(x+1)$ transformed to remove heteroscedasticity. The effects of density (LDA vs. HDA) on the concentration of chlorophylls-a, -b, and -c, and phaeopigments in both phytoplankton and phytoneuston were examined by nested ANOVAs (statistic: F) with experimental areas and sampling units as nesting factors (UNDERWOOD 1997). The probability of error was adjusted to compensate for the number of tests (4) using the Bonferroni correction. Sampling periods were com-pared using paired Student's t-test (statistic: t) on mean values per experimental area. Water parameters were analyzed for differences between LDA/HDA and sampling units using a two-way repeated measures ANOVA (statistic: F). P values of less than 0.05 (0.0125 after Bonferroni correction) were considered statistically significant.

Results

Phytoplankton

In August, no significant differences were found between LDA and HDA in any of the analyzed pigments (Table 1, Fig. 1). In September, the only signifi-cant difference was the higher relative concentration of chlorophyll-b in LDA

Table 1. Comparisons between low density areas (LDA) and high density areas (HDA) in the two sampling periods using nested ANOVAs for the concentrations of chlorophylls (Chl)-a, -b, -c, and phaeopigments (Phaeo) in phytoplankton samples. Significant differences after Bonferroni correction in bold.

	August					September			
	df	MS	F	p			df	MS	F	p
Chl-a ($\mu g/dm^3$)						**Chl-a ($\mu g/dm^3$)**				
LDA/HDA	1	0.310	0.025	0.875		LDA/HDA	1	398.590	1.394	0.303
Replicates	4	16.450	1.451	0.258		Replicates	4	285.880	1.971	0.142
Error	18	11.337				Error	18	145.070		
Chl-b/Chl-a						**Chl-b/Chl-a**				
LDA/HDA	1	0.047	0.012	0.9131		LDA/HDA	1	0.019	10.842	**0.004**
Replicates	4	3.652	0.943	0.462		Replicates	4	0.004	2.197	0.110
Error	18	3.874				Error	18	0.002		
Chl-c/Chl-a						**Chl-c/Chl-a**				
LDA/HDA	1	0.041	2.026	0.185		LDA/HDA	1	0.000	0.034	0.854
Replicates	4	0.014	0.666	0.624		Replicates	4	0.001	1.784	0.176
Error	18	0.021				Error	18	0.001		
Phaeo/(Chl-a + Phaeo)						**Phaeo/(Chl-a + Phaeo)**				
LDA/HDA	1	0.011	0.380	0.545		LDA/HDA	1	0.024	0.252	0.642
Replicates	4	0.108	3.714	0.022		Replicates	4	0.097	2.123	0.120
Error	18	0.029				Error	18	0.045		

than in HDA (Table 1, Fig. 1). Chlorophyll-a was relatively more abundant in September rather than in August (t = –7.5952, df = 5, p = 0.0006), while no differences between periods were found for chlorophyll-b (t = –2.3824, df = 5, p = 0.063), chlorophyll-c (t = 2.1355, df = 5, p = 0.0858), and phaeopigments (t = –0.3654, df = 5, p = 0.7298).

Phytoneuston

In August, chlorophyll-a was significantly less in HDA than in LDA, whereas chlorophylls-b, -c, and phaeopigments were significantly (or nearly significantly) higher (Table 2, Fig. 2). In September, a significant difference was only found for the relative concentration of chlorophyll-b, which was higher in HDA than in LDA (Table 2, Fig. 2). Except for chlorophyll-c (t = 11.5914, df = 5, p = 0.0001), the analyzed pigments were more abundant in September than in August (chlorophyll-a: t = –3.8623, df = 5, p = 0.0119; chlorophyll-b: t = –5.5081, df = 5, p = 0.0027; phaeopigments: t = –6.9367, df = 5, p = 0.001).

Fig. 1. Phytoplankton: comparisons between experimental areas and between sampling periods in the concentration of chlorophylls (Chl)-a (**a**), -b (**b**), -c (**c**), and phaeopigments (Phaeo) (**d**). The asterisk denotes a significant difference of at least $P < 0.01$ after nested ANOVA.
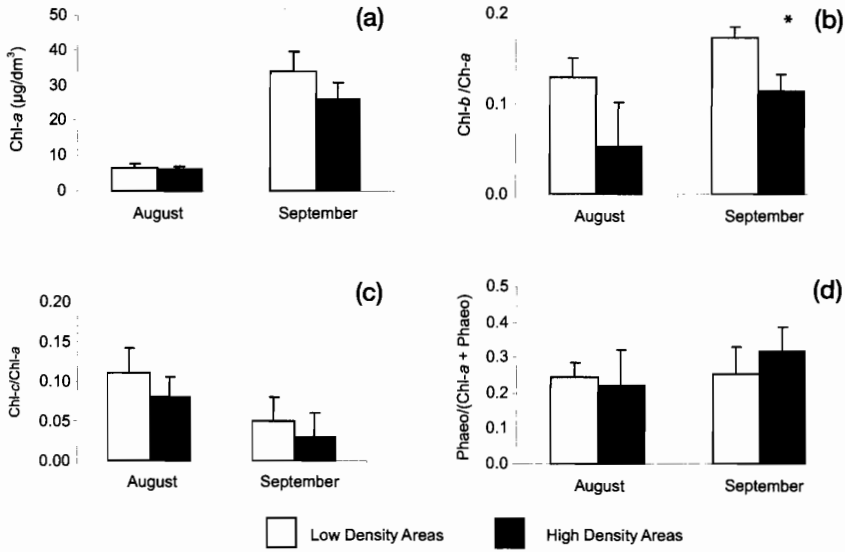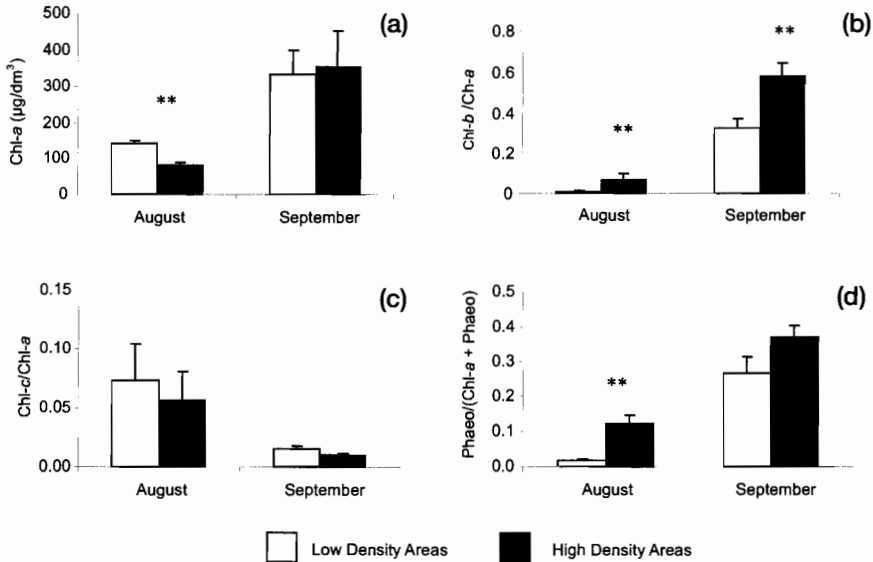

Fig. 2. Phytoneuston: comparisons between experimental areas and between sampling periods in the concentration of chlorophylls (Chl)-a (**a**), -b (**b**), -c (**c**), and phaeopigments (Phaeo) (**d**). Two asterisks denote a significant difference of at least $P < 0.001$ after nested ANOVA.

Table 2. Comparisons between low density areas (LDA) and high density areas (HDA) in the two sampling periods using nested ANOVAs for the concentrations of chlorophylls (Chl)-a, -b, -c, and phaeopigments (Phaeo) in phytoneuston samples. Significant differences after Bonferroni correction in bold.

		August					September		
	df	MS	F	p		df	MS	F	p
Chl-a (μg/dm^3)					**Chl-a (μg/dm^3)**				
LDA/HDA	1	15220.0	15.432	**<0.001**	LDA/HDA	1	2493.0	0.023	0.887
Replicates	4	820.8	0.802	0.539	Replicates	4	108211.0	16.264	**<0.001**
Error	18	1023.1			Error	18	6654.0		
Chl-b/Chl-a					**Chl-b/Chl-a**				
LDA/HDA	1	0.039	26.495	**<0.001**	LDA/HDA	1	0.4	20.451	**<0.001**
Replicates	4	0.012	8.228	**<0.001**	Replicates	4	0.1	3.461	0.029
Error	18	0.001			Error	18			
Chl-c/Chl-a					**Chl-c/Chl-a**				
LDA/HDA	1	0.002	4.915	0.054	LDA/HDA	1	0.000	26.380	0.125
Replicates	4	0.000	0.236	0.914	Replicates	4	0.000	0.911	0.478
Error	18	0.000			Error	18	0.000		
Phaeo/(Chl-a + Phaeo)					**Phaeo/(Chl-a + Phaeo)**				
LDA/HDA	1	18.114	17.798	**<0.001**	LDA/HDA	1	0.1	2.303	0.204
Replicates	4	1.030	1.015	0.426	Replicates	4	0.0	1.865	0.159
Error	18	1.015			Error	18	0.0		

Table 3. Physico-chemical data (mean and SE) of water taken in each experimental area and in the two sampling periods.

	August		September	
	Mean	SE	Mean	SE
Water temperature (˚C)				
LDA	24.2	0.2	19.9	0.2
HDA	24.2	0.1	19.5	0.1
Water depth (cm)				
LDA	56.7	6.7	45.0	5
HDA	48.3	1.7	38.3	1.7
Dissolved oxygen (%)				
LDA	50.1	4.1	16.0	1
HDA	52.1	2.6	13.8	1
Ammonia (PPM)				
LDA	1.6	0	1.1	0.1
HDA	1.6	0	1.5	0.1
Secchi depth (cm)				
LDA	21.7	1.7	10.0	0
HDA	18.3	3.3	10.0	0

Water parameters

Physico-chemical data of water are given in Table 3. Mean air temperature was 31 °C in August and 25 °C in September; pH, nitrites, nitrates, and calcium averaged 6.5, 0.05 PPM, 10 PPM, 80 mg/l in August and 6.0 PPM, 13 PPM, 90 mg/l in September, respectively. Except for a slight difference in water depth ($F = 6$, $df = 1$, $p = 0.0441$), LDA and HDA did not differ in any period (F values were between 0.0025 and 4.3448, p between 0.9614 and 0.076). Water clarity was higher ($F = 26.1818$, $p = 0.0014$) and water temperature, depth, dissolved oxygen concentration, and ammonium concentration were lower in September than in August (F values between 12.068 and 190.805, p between 0.01 and 0.00). No significant interactions in the patterns of difference between densities vs. the two periods were found for any parameter (F between 0.0741 and 4.3448, p between 0.7933 and 0.0756).

Discussion

Results from the present study reveal unexpected effects of *P. clarkii* on the invaded community. First, they show that the biomass of surface microalgae is strongly affected by the presence of dense populations of this crayfish species, at least during the period of its more intense foraging activity. In fact, chlorophyll-a and phaeopigments (the products of chlorophyll degradation) showed a significantly lower and higher concentration, respectively, in areas hosting a high, rather than a low, crayfish density. This is however a short term effect: chlorophyll-a and phaeopigments increased and decreased their concentration, respectively, with the lowering of crayfish activity during the second sampling period. Second, crayfish were also found to modify the composition of microalgal communities, as suggested by the different relative concentration of chlorophyll-b recorded in both sampling periods of phytoneuston (and in the second sampling period of phytoplankton). The observed effects on both abundance and composition of phytoneuston were not accompanied by significant differences between areas in water chemistry and temperature. These measures, on the contrary, varied highly between sampling periods. A parallel change was observed in phytoplankton and phytoneuston abundance that increased over three times from the first to the second sampling period. Finally, high crayfish densities seemed not to exert any measurable effect on phytoplankton abundance.

Overall, these findings suggest that crayfish (independently of water parameters and of other habitat features) induce a sharp decrease in the abundance of phytoneuston (but not of phytoplankton) and a change in its composition. Indeed, an understanding of the crayfish effects on phytoneuston might be of importance for the management of eutrophic water bodies with extensive cyano-

bacterial blooms. The consequences of these blooms on human health, water-based industries, recreation, and wildlife are of concern worldwide. Several cyanobacteria, in fact, release a wide range of toxins and BMAA (β-N-methyl-amino-L-alanine) that may produce lethal animal and human intoxications (e. g. CARMICHAEL 1988, COX et al. 2005). Among the few organisms so far investigated, crayfish were found to accumulate toxins in their tissues (LIRÅS et al. 1988, VASCONCELOS et al. 2001), being therefore able to transfer them to more sensitive organisms, man included.

Obvious questions arise regarding the mechanisms that lie behind the observed crayfish-induced decline of surface microalgae. It seems highly improbable that this is the result of bottom-up effects of crayfish inhibiting the growth of phytoneuston by, e. g., reducing the availability of nutrients (MUY-LAERT et al. 2003). In fact, physico-chemical variables did not differ between low and high crayfish treatments. Instead, it is more likely that the decrease of phytoneuston, and specifically of cyanobacteria, is the result of cascading top-down effects. Crayfish may have removed invertebrate grazers or may have directly grazed on phytoneuston. However, crayfish feeding on zooplankton has been never documented. Neither are the effects of zooplankton on cyano-bacteria certain (e. g. LAMPERT 1987), whole-basin and enclosure studies having reported positive, negative, and no correlation between cyanobacterial abundance and zooplankton density (e. g. BURNS 1987). Although zooplank-ters (especially *Daphnia*) are known to consume cyanobacteria, some species fed on pure cultures usually suffered decreased survival, growth, and fecundity (LAMPERT 1987, GLIWICZ 1990). Indirect effects of zooplankton can be their selective grazing on microalgae that are better competitors for nutrients (STERNER 1989) or their changing the relative availability of different nutrients (MACKAY & ELSER 1998) or other unknown mechanisms (PATERSON et al. 2002).

The potential of a direct consumption of cyanobacteria and of other phyto-neuston components by *P. clarkii* has been suggested by our observations of crayfish climbed upon macrophytes and immersed at 5–10 cm from the surface feeding on the floating film. In this form of surface feeding, crayfish roll back and protrude their appendages through the surface layer. The pereopods move anteriorly, and in conjunction with the chelipeds bring the food to a point over the maxillipeds. Indeed, the reduction in the number of freely-moving crayfish was correlated with a significant increase of phytoneuston abundance in September. A few studies have suggested that this (VASCONCELOS et al. 2001) and other crayfish species (*Pacifastacus leniusculus,* LIRÅS et al. 1988) do ingest cyanobacteria, and other studies have suggested that crayfish may even be selective cyanobacteria consumers (STEINMAN et al. 1987, TUCHMAN & STE-VENSON 1991, HART 1992). Moreover, crayfish consumption of the microalgal component of periphyton has been repeatedly documented from the analysis

of stomach contents (WHITELEDGE & RABENI 1997), as well as in field (KEL-LER & RUMAN 1998) and laboratory studies (FULLER et al. 1998).

In conclusion, our results indicate that the abundance and composition of surface microalgae is most likely affected by direct top-down effects of crayfish consuming the organically-enriched film at the water surface. This phenomenon might be of importance in littoral zones of lentic water bodies and/or in shallow lakes and ponds with emergent vegetation, whereas it is probably insignificant in deep and lotic waters. Crayfish are able to accumulate potential toxins in their tissues (VASCONCELOS et al. 2001) and can potentially transfer them to their predators. Hence, more investigations are needed that examine the mechanisms behind *P. clarkii*'s and other crayfish's consumption of the cyanobacterial component of phytoneuston.

Acknowledgements

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