Research

Oikos 000: 000–000, 2009
doi: 10.1111/j.1600-0706.2009.17352.x,

Subject Editor: Martin Solan, Accepted 30 December 2008

Vertical distribution of *Chlamydomonas* changes in response to grazer and predator kairomones

Leigh C. Latta IV, Ryan P. O'Donnell and Michael E. Pfrender

L. C. Latta IV (leigh@biology.usu.edu), R. P. O'Donnell and M. E. Pfrender, Dept of Biology, Utah State Univ., 5305 Old Main Hill, Logan, UT 84322-5305, USA.

Individuals in aquatic communities frequently assess their biotic environment through infochemicals. In particular, kairomones are commonly involved in interactions between predator and prey. However, the relationship between individuals and chemicals produced by other organisms that are not direct predators, but may indicate the presence of a predator, is not well characterized. We used experimental microcosms to test whether the unicellular green alga *Chlamydomonas reinhardtii* alters vertical migration patterns in response to kairomones produced by zooplankton (*Daphnia*) and planktivores (fish). Our results suggested that phototaxis in *C. reinhardtii* was strongly affected by the type of kairomone present, the concentration of the kairomone, and the duration of exposure to the kairomone. Kairomones generally increased phototaxis in *C. reinhardtii*. The adaptive significance of such behavioral changes in natural settings would depend largely on local community composition. The similarity in phototactic responses of *C. reinhardtii* to *Daphnia* and fish kairomone suggest that, in at least this species of phytoplankton, the underlying genetic elements responsible for kairomone detection may be responsive to a broad range of chemical stimuli, allowing this species to adjust its phototaxis in response to not only the presence of its grazers, but also to predators of its grazers.

In many communities, infochemicals provide a means for individuals to assess the biotic environment. Kairomones are a class of infochemicals that benefit the receiver but do not benefit the sender. Kairomones have received extensive attention because the changes in behavior, morphology, and life history they elicit are often easily characterized (Lass and Spaak 2003, Pohnert et al. 2007, van Donk 2007).

Kairomones frequently manifest in predator–prey interactions whereby prey chemically detect the presence of potential predators. A well characterized kairomone-mediated interaction between predator and prey is that between *Daphnia* and their vertebrate and invertebrate predators. For example, in response to fish kairomone *Daphnia* reduce body size and increase fecundity (Stibor 1992, Reede 1995, Latta et al. 2007), display increased escape ability (Brewer et al. 1999), and reduce the amplitude of diurnal vertical migrations (De Meester 1993).

Kairomones produced by zooplankton such as *Daphnia* elicit adaptive responses in phytoplankton. Unicellular green algae belonging to the genera *Scenedesmus* and *Desmodesmus* exposed to kairomones produced by numerous zooplankton species form colonies and, in some species, long rigid spines, both of which increase resistance to grazing by zooplankton (Hessen and Van Donk 1993, Lürling 2003). *Daphnia* kairomones also induce behavioral changes in some phytoplankton species. *Gonyostomum semen* and *Peridinium* sp. exhibit lower rates of recruitment into the water column in the presence of *Daphnia* (Hansson 2000).

The vertical distribution of phytoplankton in naturally occurring freshwater lakes also varies in response to the resident zooplankton community (Arvola et al. 1992), suggesting behavioral responses to zooplankton kairomones. However, studies in natural lakes are difficult to interpret because the distribution of phytoplankton is both indirectly affected by kairomones and directly affected by grazing. Thus, the vertical distribution of phytoplankton may be a reflection of zooplankton consumption and not a direct response to zooplankton kairomones.

Largely ignored in kairomone research is the response of individuals to kairomones produced by organisms that are not direct consumers, but that may indicate the presence of a consumer, such as the relationship between fish kairomones and phytoplankton. There is evidence that the cryptomonad *Plagioselmis prolonga* var. *nordica* produce longer tails in the presence of the silver carp *Hypophthalmichthys molitrix* (Kim et al. 2003). However, these experiments were conducted in mesocosms that also contained zooplankton, and thus the morphological response in *P. prolonga* may have been a direct effect of changes in zooplankton density and not related to the presence of fish kairomone.

The unicellular green alga *Chlamydomonas reinhardtii* provides an ideal organism with which to investigate kairomone-mediated changes in vertical distribution. They

are distributed worldwide in freshwater ecosystems and are a common food source for naturally occurring zooplankton populations. They are approximately 10 μm in length and swim using two flagella. Wild-type strains display positive phototaxis during the day to maximize photosynthesis (Bruce 1970) and use chemotaxis at night to acquire nitrogen sources (Byrne et al. 1992). In environments with ideal temperatures (20–25°C), constant light, and sufficient nitrogen availability haploid vegetative cells reproduce mitotically to produce clonal haploid daughter cells every 5–8 h. Cultures of *C. reinhardtii* can be entrained, using regular light:dark photoperiods, to liberate daughter cells once every 24 h by exploiting the underlying circadian control of the cell division cycle (Goto and Johnson 1995).

To investigate the effects of kairomones produced by grazers and predators we examined the response of *C. reinhardtii* to kairomones produced by zooplankton (grazers) and zooplanktivores (predators). In the lab we constructed microcosms containing a population of *C. reinhardtii* in water aged with grazers (*Daphnia*), predators (fish), and both. We then measured the response of the phytoplankton by assessing their vertical distribution after 3 and 24 h of exposure to kairomones. We were particularly interested in assessing whether or not phytoplankton can respond to kairomones produced by organisms that have direct effects on phytoplankton population dynamics (zooplankton), as well as kairomones produced by organisms that have indirect effects on phytoplankton population dynamics (fish). We discuss our results in the context of the potential advantages changes in behavior may offer natural populations. We also offer ideas on the chemical nature of kairomones suggested by our results.

Material and methods

Organisms

The subject of this study was strain CC-1928 of *Chlamydomonas reinhardtii*, acquired from the Chlamydomonas Culture Collection (<www.chlamy.org>). The strain was semi-continuously cultured in an aerated 5-litre carboy containing 4 l of modified Bold's Basal Medium (BBM; Stein 1973). Every 2–3 days 2 l of fluid were removed from the carboy and replaced with fresh BBM. Algal cultures were maintained in an 18L:6D photoperiod at 20°C in order to entrain our cultures to liberate mitotically-produced daughter cells once every 24 h. Because we clonally propagated a single strain of *C. reinhardtii* with normal phototactic responses, there is essentially no genetic variation among our treatments. This lack of genetic variation is convenient for the primary purpose of our investigation, because it eliminates the potential confounding effects of genetic variation among treatments.

Grazer kairomone water was created by isolating several hundred individual *Daphnia pulex*, whose diet consisted of the unicellular green alga *Scenedesmus obliquus*, and placing them in 4 l of filtered well-water for 24 h. Predator kairomone water was obtained from a 110-l aquarium containing two tinfoil barbs (*Barbonymus schwanenfeldii*; 10–12 cm length), a potential predator of *Daphnia*. The diet of the fish was comprised of fish flakes and fish pellets that did not contain *Daphnia*. Prior to use in microcosms the kairomone water was filtered through 165 μm nitex mesh to remove particulate matter, or in the case of grazer kairomone water, the *Daphnia*.

Experimental design

The experiment consisted of five treatments: 1) a control treatment of pure filtered well-water, 2) a 100% grazer kairomone water treatment, 3) a 50% grazer kairomone treatment consisting of half grazer kairomone water and half filtered well-water, 4) a 100% predator kairomone treatment, and 5) a grazer/predator kairomone treatment consisting of 50% grazer kairomone water and 50% predator kairomone water. To prepare treatments, we centrifuged 2 l of our *C. reinhardtii* culture at 3000 rpm for 3 min and 15 s. This centrifugation concentrates the algae into a slurry at the bottom of the sample. Based on previous experiments in which we centrifuged cells and then exposed them to top-lighting to promote phototaxis we determined that centrifugation does not cause significant mortality or injure *C. reinhardtii* flagella substantially enough to cause a noticeable reduction in motility at the population level (unpubl.). We removed the supernatant and added 100 ml of distilled water to this slurry and resuspended the algal cells by gentle mixing. We then added 10 ml of this concentrated *C. reinhardtii* into 500 ml of each kairomone treatment or control water.

Microcosms were established in 25 ml Falcon serological pipettes filled with 25 ml of control or treatment water mixed with *C. reinhardtii*, and sealed at the bottom with parafilm. Initially, the density of *C. reinhardtii* in each microcosm was equal and individuals were evenly distributed throughout the water column. Over the course of the experiment the microcosms were maintained at 18°C in a top-lit controlled temperature room.

Ten replicates for each treatment were divided evenly into two sampling periods. After 3 h of exposure to top lighting, five replicates were randomly chosen and destructively sampled by placing the bottom, middle, and top 2 ml of fluid from each microcosm in eppendorf tubes using a pipette pump. The remaining replicates were exposed to a 10L:8D:6L photoperiod. The dark period reset phototactic responses in each microcosm sample. After 24 h (6 h after the lights turned on in the morning) these five replicates for each treatment were destructively sampled in the same manner as the first five.

We froze samples immediately after collection to kill the individual cells and prevent *C. reinhardtii* from a normal phototactic response towards a spectrophotometer beam. This protocol ensures accurate estimates of cell density in a spectrophotometer. We thawed and mixed each sample and used a spectrophotometer to measure % light transmittance. An index of *C. reinhardtii* density was estimated as 1 – (% transmittance). We also measured % light transmittance in treatment water samples prior to seeding with *C. reinhardtii* and used these values to correct for differences in baseline transmittance due to water aged with live organisms.

Statistical analyses

For each group of samples corresponding to the top, middle, or bottom layer in the microcosms we used two-factor ANOVA, with treatment and time as main effects, for analysis (SAS Inst. 2004). We performed ANOVA on the entire dataset corresponding to each microcosm level. We then compared the control group to the 100% grazer treatment to test whether *C. reinhardtii* responds directly to kairomones produced by a grazer. We also compared the 50% grazer treatment to the 100% grazer treatment to determine whether the behavioral response is concentration dependent.

We tested whether *C. reinhardtii* responds directly to kairomones produced by predators, and whether the presence of predator kairomones alters *C. reinhardtii* response to grazer kairomones. We compared the control group to the predator treatment to test for a direct response to predator kairomones. We also compared the 50% grazer treatment to the grazer/predator treatment, both of which had equal concentrations of grazer kairomone.

Results

Cell density at the surface

Cell density of *Chlamydomonas reinhardtii* in the top 2 ml of fluid in the microcosms was significantly affected by the type of kairomone and the duration of exposure to the kairomone (ANOVA; $p < 0.0001$). Specifically, grazer kairomones induced phototactic movement that resulted in higher cell density at the surface than in untreated water (Fig. 1A); however, this response did not differ over a doubling of concentration (Fig. 2A, Table 1). Predator kairomone did not directly affect cell density, but predator and grazer kairomone combined significantly reduced cell density relative to grazer kairomone alone (Fig. 2A, Table 1). This effect of multiple kairomones was only manifest in the 6 h sampling period.

Cell density in the middle

Density of *C. reinhardtii* in the middle 2 ml of fluid in the microcosms was significantly affected by the type of kairomone present (ANOVA; $p = 0.0002$). *Chlamydomonas reinhardtii* density in the presence of grazer kairomones was higher than untreated water (Fig. 1B) and was independent of time and kairomone concentration (Fig. 2B, Table 1). Cell density in the middle sample was not significantly changed by predator kairomone or a combination of grazer and predator kairomone (Fig. 2B, Table 1).

Cell density at the bottom

Cell density in the bottom 2 ml of fluid in the microcosm was significantly affected by the type of kairomone present

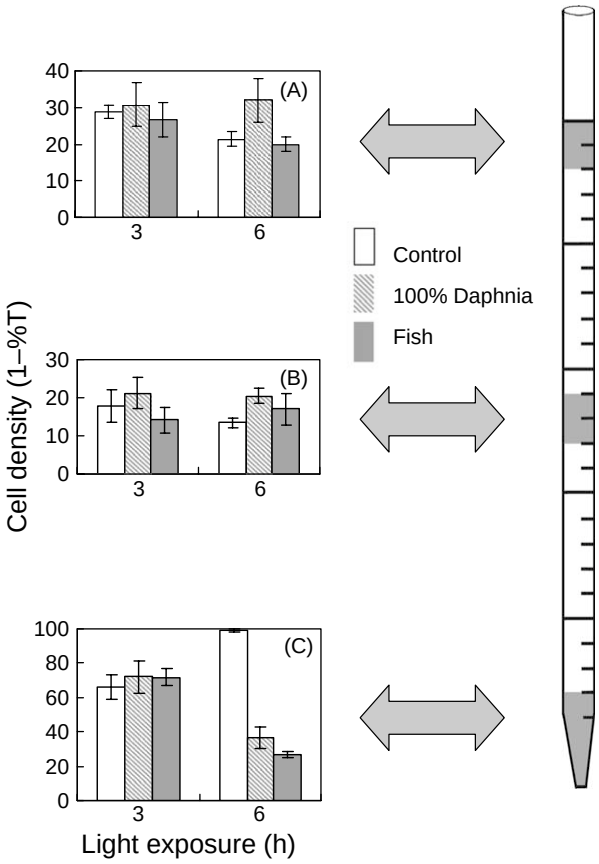

Figure 1. Cell density estimates for the (A) top, (B) middle, and (C) bottom of the experimental microcosms depicting the direct response to *Daphnia* and fish kairomone. Error bars are ±2 SE.

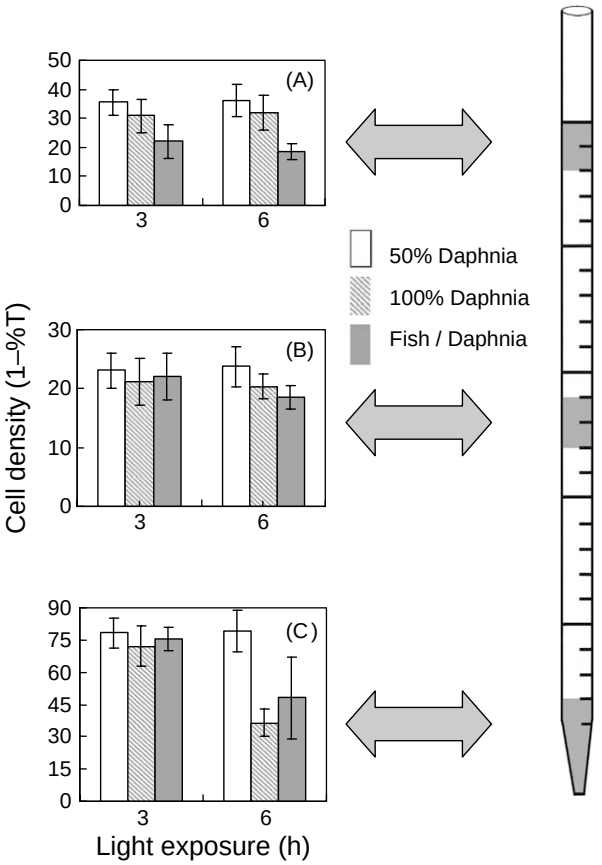

Figure 2. Cell density estimates for the (A) top, (B) middle, and (C) bottom of the experimental microcosms depicting concentration dependence and the effect of simultaneous exposure to multiple kairomones. Error bars are ± 2 SE.

and the duration of time exposed to the kairomone (ANOVA; $p < 0.0001$). In response to grazer kairomone, density estimates decreased relative to controls and the difference in density between control and grazer kairomone treatments was highest after 24 h of exposure (Fig. 1C, Table 1). Density was also significantly reduced as grazer kairomone concentration increased and the difference was most pronounced after 24 h of exposure (Fig. 2C, Table 1). Density was also significantly reduced by predator kairomone and a combination of grazer and predator kairomone. As in the surface sample, these differences were most pronounced after prolonged exposure to kairomone (Fig. 2C, Table 1).

Discussion

The vertical distribution of *Chlamydomonas reinhardtii* in our microcosms was strongly affected by the type of kairomone(s) present, the concentration of the kairomone, and the duration of exposure to the kairomone. Overall, the general response of *C. reinhardtii* to kairomones was increased cell density in the water column or near the surface as evidenced by significantly high density estimates at the middle and top of microcosms containing kairomone and/or significantly low density estimates at the bottom of microcosms containing kairomone. Although we did not specifically measure the rate of movement of individual cells, the estimated swimming speed of *Chlamydomonas* cells in response to light is approximately 0.5 m h^{-1} (Berthold et al. 2008). Thus, our results suggest that natural populations of *Chlamydomonas* may undergo vertical shifts of several meters due to kairomone-dependent phototaxis.

Our interpretation of these results is that kairomones induce a stronger phototactic response in *C. reinhardtii* than water that does not contain kairomones. Phototaxis in *C. reinhardtii* is controlled through an underlying circadian rhythm (Bruce 1970). During the day, individuals swim maximally towards light sources in order to optimize photosynthesis. Kairomones produced by a potential grazer, in this case *Daphnia*, have the effect of increasing phototaxis resulting in more individuals in the water column or at the surface. The response to *Daphnia* kairomone also showed concentration dependence in the lowest level of our microcosms with higher concentrations of kairomone inducing a stronger phototactic response. Such a response may appear adaptive in that more individuals would be exposed to light sources for use during photosynthesis. However, the response is only adaptive in specific ecological settings.

For example, in lake communities that contain only zooplankton and phytoplankton, increased phototaxis in phytoplankton may be maladaptive. In the absence of visually-feeding predators, zooplankton frequently do not exhibit diel vertical migration and are able to exert continuous grazing pressure which can reduce the growth rate of phytoplankton populations (Reichwaldt et al. 2004). Alternatively, in lake communities with populations of

Table 1. Results from two-factor ANOVA. Degrees of freedom (DF), F-values, and level of significance (p) for type III sums of squares for each source of variation corresponding to specific comparisons for the top (T), middle (M), and bottom (B) sample collected from the microcosms.

Comparison	Source of variation								
	Treatment			Time			Interaction		
	DF	F-value	p	DF	F-value	p	DF	F-value	p
All (T)	4	10.3	<0.0001	1	12.0	0.0013	4	3.1	0.0254
All (M)	4	9.0	<0.0001	1	1.0	0.3361	4	1.7	0.1794
All (B)	4	23.3	<0.0001	1	30.0	<0.0001	4	27.2	<0.0001
Control vs 100% *Daphnia* (T)	1	7.9	0.0128	1	2.2	0.1583	1	4.1	0.0601
Control vs 100% *Daphnia* (M)	1	11.0	0.0044	1	2.7	0.1227	1	1.3	0.2763
Control vs 100% *Daphnia* (B)	1	72.4	<0.0001	1	0.2	0.7014	1	106.6	<0.0001
50% *Daphnia* vs 100% *Daphnia* (T)	1	2.6	0.1241	1	0.1	0.7443	1	0.0	0.9171
50% *Daphnia* vs 100% *Daphnia* (M)	1	2.9	0.1100	1	0.0	0.9900	1	0.2	0.6353
50% *Daphnia* vs 100% *Daphnia* (B)	1	35.1	<0.0001	1	18.3	0.0006	1	19.4	0.0004
Control vs Fish (T)	1	1.6	0.2194	1	25.6	0.0001	1	0.1	0.7534
Control vs Fish (M)	1	0.0	0.9628	1	0.2	0.6756	1	4.4	0.0512
Control vs Fish (B)	1	231.7	<0.0001	1	7.3	0.0155	1	319.2	<0.0001
50% *Daphnia* vs Fish/*Daphnia* (T)	1	9.4	0.0074	1	5.1	0.0380	1	6.4	0.0225
50% *Daphnia* vs Fish/*Daphnia* (M)	1	4.2	0.0586	1	0.8	0.3937	1	1.8	0.1979
50% *Daphnia* vs Fish/*Daphnia* (B)	1	8.5	0.0103	1	5.5	0.0324	1	5.9	0.0271

planktivorous fish, zooplankton, and phytoplankton, increased phototaxis in phytoplankton may be an adaptive strategy because fish induce diel vertical migration in zooplankton such that during the day, when visual predators are active, zooplankton reside near the bottom (De Meester 1993). Thus, daytime phytoplankton movement into the water column and away from resident zooplankton populations should act to reduce individual mortality because of the discontinuous grazing pressure that results from the daily migration of phytoplankton away from zooplankton.

Phototaxis in *C. reinhardtii* also increased when exposed to predator kairomones. For natural populations, this result suggests that phytoplankton may be able to detect the presence of their grazers indirectly through predators of their grazers. This behavior could be of great utility when zooplankton population density varies seasonally as individuals could still detect the presence of grazers even when grazer density is low.

The photosensory and chemosensory pathways in *C. reinhardtii* share common elements as the addition of specific chemoeffectors can inhibit phototaxis (Ermilova et al. 1997, Govorunova and Sineshchekov 2003). These results bear on two aspects of our study. First, a caveat to our study is that we cannot rule out that differences in nutrient concentrations and ratios among treatments may have contributed to the different phototactic responses we observed. Water used in our experimental treatments had been previously inhabited by live animals and thus kairomones as well as nutrients, such as nitrogen and phosphorous compounds, may have differed among treatments. If the phototactic response in *C. reinhardtii* is influenced by nutrient levels then the responses we observed in our experiment may not solely reflect differences in the type of kairomone.

Second, our conclusion that the similarity in phototactic response to *Daphnia* and fish is due to kairomones suggests a few possibilities on the nature of the chemoreceptor and photosensory systems in *C. reinhardtii*. *Daphnia* and fish kairomones may have a similar chemical structure that can be detected by the same chemoreceptor, or different chemoreceptors are involved in detection, but the transduction pathways converge at some junction and result in the same response. The chemical nature of *Daphnia* and fish kairomones is not well resolved, but aliphatic sulfates have been identified as a candidate class of *Daphnia* chemicals known to induce morphological defenses in phytoplankton (Yasumoto et al. 2005, 2006). Given the vast array of genomic tools and complete genome sequence available for *C. reinhardtii*, a functional genomic approach using microarray experiments could be utilized to address the effect of nutrients on phototaxis as well as the nature of the signal transduction cascade that arises from exposure to different kairomones.

In conclusion, phototaxis in *C. reinhardtii* is responsive to kairomones produced by both grazers and predators. However, the adaptive significance of the behavioral change would be context dependent varying with the community composition in natural settings. The similarity in response to kairomones produced by different organisms may also lend insight into the characterization of the specific chemicals that induce morphological, life history, and

behavioral changes in other taxa known to respond to kairomones. Furthermore, the similarity in phototactic response to *Daphnia* and fish kairomone suggest that, in at least this species of phytoplankton, the underlying genetic elements responsible for kairomone detection may be responsive to a broad range of chemical stimuli and endow the individual with a broader knowledge of the prevailing biotic environment, allowing this species to adjust its phototaxis in response to not only the presence of its grazers, but also to predators of its grazers.

Acknowledgements – The USU Evolution Group provided helpful comments on this manuscript. Funding for this research was provided by the National Science Foundation (DEB-021212487 to MEP), National Institutes of Health (GM078274 to MEP) and Utah State Univ. Center for Integrated Biosystems (Research Grant to MEP).

References

Arvola, L. et al. 1992. Vertical distributions of bacteria and algae in a steeply stratified humic lake under high grazing pressure from *Daphnia longispina*. – Hydrobiologia 229: 253–269.

Berthold, P. et al. 2008. Channelrhodopsin-1 initiates phototaxis and photophobic responses in *Chlamydomonas* by immediate light-induced depolarization. – Plant Cell 20: 1665–1677.

Brewer, M. et al. 1999. Interactive effects of fish kairomone and light on *Daphnia* escape behavior. – J. Plankton Res. 21: 1317–1335.

Bruce, V. G. 1970. The biological clock in *Chlamydomonas reinhardi*. – J. Protozool. 17: 328–334.

Byrne, T. E. et al. 1992. Circadian rhythms of chemotaxis to ammonium and of methylammonium uptake in *Chlamydomonas*. – Plant Physiol. 98: 879–886.

De Meester, L. 1993. Genotype, fish-mediated chemicals, and phototactic behavior in *Daphnia magna*. – Ecology 74: 1467–1474.

Ermilova, E. V. 1997. Chemotaxis and its correlation with photoresponse in the *Chlamydomonas reinhardtii* strain with negative phototaxis. – Biol. Bull. 24: 411–413.

Goto, K. and Johnson, C. H. 1995. Is the cell division cycle gated by a circadian clock? The case of *Chlamydomonas reinhardtii*. – J. Cell Biol. 129: 1061–1069.

Govorunova, E. G. and Sineshchekov, O. A. 2003. Integration of photo- and chemosensory pathways in *Chlamydomonas*. – Planta 216: 535–540.

Hansson, L.-A. 2000. Synergistic effects of food chain dynamics and induced behavioral responses in aquatic systems. – Ecology 81: 842–851.

Hessen, D. O. and Van Donk, E. 1993. Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. – Arch. Hydrobiol. 127: 129–140.

Kim, B. H. et al. 2003. Effects of fish introduction on the length of the tail of cryptomonads in mesocosm experiments. – Oecologia 136: 73–79.

Lass, S. and Spaak, P. 2003. Chemically induced anti-predator defences in plankton: a review. – Hydrobiologia 491: 221–239.

Latta, L. C. IV et al. 2007. Rapid evolution in response to introduced predators II: the contribution of adaptive plasticity. – BMC Evol. Biol. 7: 21.

Lürling, M. 2003. Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology. – Ann. Limnol. - Int. J. Limnol. 39: 85–101.

Pohnert, G. et al. 2007. Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. – Trends Ecol. Evol. 22: 198–204.

Reede, T. 1995. Life history shifts in response to different levels of fish kairomones in *Daphnia*. – J. Plankton Res. 17: 1661–1667.

Reichwaldt, E. S. et al. 2004. The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment. – Oecologia 141: 411–419.

Stein, J. R. 1973. Handbook of phycological methods: culture methods and growth measurements. – Cambridge Univ. Press.

Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. – Oecologia 92: 162–165.

Van Donk, E. 2007. Chemical information transfer in freshwater plankton. – Ecol Inf. 2: 112–120.

Yasumoto, K. et al. 2005. Aliphatic sulfates released from *Daphnia* induce morphological defense of phytoplankton: isolation and synthesis of kairomones. – Tetrahedron Lett. 46: 4765–4767.

Yasumoto, K. et al. 2006. Isolation and absolute configuration determination of aliphatic sulfates as the *Daphnia* kairomones inducing morphological defense of a phytoplankton. – Chem. Pharm. Bull. 54: 271–274.