Experimental comparison of periphyton removal by chironomid larvae and *Daphnia magna*

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Abstract

*Daphnia magna* is a large pelagic cladoceran known to feed on phytoplankton. Our laboratory experiments demonstrate that it can also remove periphyton at rates similar to or higher than chironomid larvae, which are typical periphyton grazers. After a 2-week laboratory exposure at 20 °C, periphyton biomass (dominated by green algae) was significantly reduced by *D. magna* (38%). Similar periphyton removal was observed for a naturally associated invertebrate community dominated by chironomid larvae (33%) and chironomid larvae alone (37–62%). Periphyton removal rates of all tested grazers were comparable at the community level (360–540 mg dry weight [DW] m⁻² d⁻¹). The larger chironomid larvae had higher individual periphyton removal rates (0.12–0.17 mg DW ind.⁻¹ d⁻¹) than *D. magna* (0.03 mg DW ind.⁻¹ d⁻¹). Body mass-specific periphyton removal rates of *D. magna* (0.96 mg DW mg grazer DW⁻¹ d⁻¹) were 55% higher than those of chironomids. We suggest that the impact of *D. magna* on periphyton may be significant when phytoplankton concentrations are low, such as during the clear-water phase or in macrophyte beds where daphnids seek refuge from fish predation.

Key words: chironomids, *Daphnia magna*, periphyton removal

Introduction

Periphyton can contribute >80% to total primary production in shallow lakes and can thus be a significant energy source for higher trophic levels by transferring nutrients between the benthic and pelagic zones (Vadeboncoeur et al. 2003) and shading submerged macrophytes. As such, periphyton can be responsible for changes in macrophyte abundance (Jones and Sayer 2003). There is a considerable body of evidence for top-down effects by different invertebrate grazers on periphyton (Feminella and Hawkins 1995).

Epiphytic chironomid larvae are an important invertebrate group in shallow lakes (Tarkowska-Kukuryk 2013) and are often a dominant group of plant-associated macroinvertebrates (Körner et al. 2002, Boll et al. 2012). Their grazing is known to have marked effects on periphyton standing crop (e.g., Mason and Bryant 1975, Cattaneo 1983) due to high grazing and rapid growth rates (Lalonde and Downing 1991, Pinder 1992). Plant-associated cladocerans such as *Sida crystallina* (O. F. Müller), *Chydorus sphaericus* (O. F. Müller), and *Alona affinis* (Leydig) have also been shown to feed on periphyton. Their periphyton ingestion rates were related to periphyton concentration, presence of phytoplankton as an alternative food, and body size (Downing 1981). Few studies, however, have examined periphyton as a food source for herbivorous pelagic zooplankton.

Stable isotope analyses showed that some *Daphnia* species might use periphyton as a food source in both eutrophic and oligotrophic lakes (Jones and Waldron 2003, Rautio and Vincent 2006). Siehoff et al. (2009) were the first to show the ability of *Daphnia* to establish a stable population when fed solely on periphyton and to switch to periphyton when phytoplankton availability was low. They assumed that *D. magna* removes small
periphyton particles with the first trunk limbs. These particles are probably accumulated in the filter apparatus, which keeps working while *D. magna* is feeding on periphyton (Siehoff et al. 2009).

*Daphnia magna* (Straus) and other pelagic filter feeders of phytoplankton often undergo diel horizontal migration (DHM) into macrophyte beds of shallow lakes (Burks et al. 2002). Phytoplankton abundance can be low in these zones due to the direct and indirect negative effects of macrophytes (e.g., Schriver et al. 1995, Hilt and Gross 2008), which may force pelagic filter feeders to use periphyton as an alternative food source. It has not yet been shown, however, whether this feeding activity of *D. magna* also has a significant impact on the removal of periphyton. In this study we compared periphyton removal by *D. magna* to that by a naturally periphyton-associated invertebrate community and by chironomid larvae alone. Specifically, we compared community-, individual-, and body mass-specific periphyton removal rates.

**Methods**

We performed 3 subsequent laboratory experiments to compare periphyton removal by a natural grazer community (NGC; exp. 1), 2 different size classes of chironomid larvae (exp. 2 and 3), and *Daphnia magna* (exp. 3; Fig. 1). The initial periphyton biomass was developed during a 4-week exposure period (Table 1) on polypropylene strips (16 × 2 cm, IBICO, Germany) in mesocosms that contained a mixture of phytoplankton and zooplankton collected from 5 different German lakes, as well as sediments, macrophytes, and sticklebacks (for further details see Landkildehus et al. 2014; for nutrient concentrations see Table 1).

Periphyton was dominated by green algae determined by high-performance liquid chromatography (HPLC) following Fietz and Nicklisch (2004). For all experiments, periphyton strips were removed from mesocosms and divided, with half placed into carbonated water for 3 minutes to remove all grazers, thus serving as a control (Fig. 1). Subsequently, strips were placed horizontally in 0.5 L glass beakers filled with GF/F filtered mesocosm water for 2 weeks. Laboratory conditions were maintained at saturating light (166 µE m⁻² s⁻¹, 12:12 h light:dark) and 20 °C. Water losses via evaporation were replenished with filtered mesocosm water.

To prevent nutrient limitation, we added daily 5 mL of a nutrient solution containing CaSO₄ 0.5 mM, CaCl₂ 0.5 mM, MgSO₄ 0.25 mM, NaNO₃ 0.5 mM, KH₂PO₄ 0.05 mM, KCl 0.1 mM, Na₂SiO₃ 0.4 mM, HCl 0.75 mM, NaHCO₃ 2 mM, FeCl₃ 0.010 mM, Na₂EDTA 0.020 mM; and trace elements H₃BO₃ 4 µM, MnSO₄ 0.8 µM, ZnSO₄ 0.08 µM, Na₂MoO₄ 0.04 µM, CuSO₄ 0.04 µM, AlK(SO₄)₂ 0.08 µM, CoCl₂ 0.04 µM, NiSO₄ 0.04 µM, KBr 0.08 µM, KJ 0.04 µM, and H₂SeO₃ 0.06 µM (Körner and Nicklisch 2002). For exp. 1, the treatment strips contained the NGC of the mesocosms. For exp. 2 and 3, all strips were treated with carbonated water for 3 minutes to eliminate grazers and were subsequently stocked with 5 chironomid larvae (mean length: 5.1 ± 0.2 mm in exp. 2; 4.0 ± 0.2 mm in exp. 3) collected from mesocosms and adapted to laboratory conditions for 3 days. In exp. 3, an additional treatment contained 10 adult *D. magna* (Fig. 1). Two-day-old *D. magna*, average length 1.4 ± 0.05 mm, were collected from a laboratory culture originating from a single female isolated from Müggelsee, adapted to experimental conditions for 5

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**Fig. 1.** Experimental set-up: Periphyton with its associated grazer community was developed on plastic strips (grey) in lake mesocosms for 4 weeks and transferred to the laboratory. CO₂ treatment removed all grazers for controls and for exp. 2 and 3. Periphyton biomass development was measured after 2 weeks in treatments with no grazers (controls), the natural periphyton-associated grazer community (exp. 1), large chironomids (exp. 2), small chironomids (exp. 3) and *Daphnia magna* (exp. 3).
days, and fed daily with green algae (Scenedesmus sp.) cultured in Z8 medium (Kotai 1972). Initial densities were 0.3 chironomid larvae cm$^{-2}$ (10 chironomid larvae L$^{-1}$) and 0.6 D. magna cm$^{-2}$ (20 D. magna L$^{-1}$), respectively (dry weights in Table 2). All treatments in the 3 experiments as well as the controls were run with 4 replicates.

After 2 weeks, all strips were treated with carbonated water for 3 minutes to remove grazers. Periphyton was scrubbed from the strips using a toothbrush and suspended in GF/F-filtered mesocosm water. After homogenization by vigorous shaking, subsamples were filtered (GF/C) to determine dry weight (DW) and ash-free dry weight (AFDW). Organic carbon (C) and nitrogen (N) were determined using a Vario EL analyser (Elementar) after removing the carbonates (Köhler et al. 2010). Total phosphorus (TP) concentrations in suspensions were measured using standard methods (Wasserchemische Gesellschaft 2005). For chlorophyll $a$ (Chl-$a$) determination, subsamples of the homogenate were filtered (GF/F) and quantified by HPLC (Waters, USA) as described by Fietz and Nicklisch (2004). The same treatment was performed with additional strips at the beginning of each experiment to calculate periphyton removal rates for DW, AFDW, and Chl-$a$.

The collected grazers were fixed in formaldehyde. The chironomid community, identified after raising to maturity in exp. 1 (Langton and Pinder 2007), was composed of Glyptotendipes pallens (Meigen), Paratanytarsus spp., Farachironomus spp., and Cricotopus reversus (Hirvenoja). These chironomids are free-living scrapers or grazers (Paratanytarsus spp., C. reversus) or collector-gatherers, eating fine deposits (G. pallens). The cladocerans in exp. 1 were initially dominated by C. sphaericus, while at the end some Diaphanosoma brachyurum (Lievin) and Bosmina longirostris (O.F. Müller) were also found. Bosmina longirostris contributed <0.4% to the total cladoceran biomass and were neglected as grazers.

Initial and final grazer DW were calculated using length–DW regressions of D. magna, D. brachyurum, and C. sphaericus (Bottrell et al. 1976); B. longirostris (Michaloudi 2005); and chironomids (Maren Mährlein, IGB Berlin, 2013, unpubl. data). Length measurements were performed using a Nikon (SMZ1500) stereomicroscope with a digital camera and NIS-Elements D software.

Because daphnids need to be fixed for length measurements, their initial biomass was estimated as the average biomass of a subsample of 21 individuals from the same laboratory culture and age. Daphnids reproduced during the experiment and reached an average population size of 32.8 ± 0.6 individuals per replicate at the end of the experiment. Mean population size (required to calculate individual periphyton removal rates) was estimated at 1.3 ± 0.02 ind. cm$^{-2}$ (43 ± 0.6 ind. L$^{-1}$) assuming linear population growth. In addition, mean total biomass for chironomids and D. magna in exp. 2 and 3 (required to calculate body mass-specific periphyton removal rates) was determined based on the same linear growth model.

All experimental data were tested for normal distribution using Kolmogorov-Smirnov tests and log(x+1) transformations when needed. In cases of 2-sample comparisons, the Student’s t-test was used. In cases with >2 factor levels, one-way analyses of variance (ANOVA) with subsequent Tukeys HSD post-hoc tests were applied. All analyses were carried out using the statistical package SPSS 17 (SPSS Inc., Chicago, IL, USA).

### Results

In the presence of the natural grazer community, which consisted mainly of chironomids (78% of total initial grazer biomass) and the cladoceran C. sphaericus, periphyton DW was significantly reduced by 33% relative to controls in 2 weeks. Larger chironomid larvae in exp. 2 grazed roughly 62%, and smaller chironomid larvae in exp. 3 roughly 37% of the initial periphyton DW (Table 2). Periphyton biomass was also significantly reduced by D. magna, which removed 38% of the initial periphyton DW in exp. 3 (Table 2). Reductions of initial AFDW and Chl-$a$ were comparable to those of periphyton DW (Table 2). Total periphyton

### Table 1. Exposure periods of periphyton strips in outdoor mesocosms and mean nutrient concentrations in mesocosm water during exposure (±SE).

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>Exposure period</th>
<th>Total phosphorus (μg L$^{-1}$)</th>
<th>Total nitrogen (mg L$^{-1}$)</th>
<th>Dissolved silica (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>15 Jul–16 Aug</td>
<td>44.0 ± 4.0</td>
<td>3.0 ± 1.5</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>16 Aug–13 Sep</td>
<td>50.0 ± 11.0</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.05</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>14 Sep–12 Oct</td>
<td>57.0 ± 18.0</td>
<td>4.0 ± 1.0</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>

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removal varied between 5.1 and 7.6 g DW m\(^{-2}\), and between 2.8 and 4.3 mg Chl-a m\(^{-2}\) (Table 2).

Mean chironomid larvae biomasses were estimated at 843 ± 12 mg DW m\(^{-2}\) and 617 ± 12 mg DW m\(^{-2}\) in exp. 2 and 3, respectively, and the mean biomass of \textit{D. magna} in exp. 3 was estimated at 390 ± 10 mg DW m\(^{-2}\). Community periphyton removal rates of NGC, both large and small chironomid larvae and \textit{D. magna} were in the same order of magnitude for DW, AFDW, and Chl-a (Fig. 2A and B; one-way ANOVA, \(p > 0.05\), data for AFDW not shown). Periphyton accumulation rates (measured as changes in biomass in controls without grazers) were highest in exp. 1 and comparable in exp. 2 and 3 (Table 2). Periphyton removal rates reached 35, 63, and 39% of periphyton accumulation in exp. 1, 2, and 3, respectively. Individual periphyton removal rates were lower for \textit{D. magna} than those of chironomids (one-way ANOVA, DW: \(F = 21.4, \ p < 0.001\) [Fig. 2C]; Chl-a: \(F = 10.7, \ p = 0.004\) [Fig. 2D]). Body mass-specific periphyton removal rates of \textit{D. magna} were significantly higher than those of chironomid larvae in exp. 2 and 3 for DW and AFDW (one-way ANOVA, DW: \(F = 5.9, \ p = 0.022\) [Fig. 2E]; AFDW: \(F = 13.10, \ p = 0.002\)); as well as for Chl-a (one-way ANOVA, \(F = 9.1, \ p = 0.007\) [Fig. 2F]).

Final TP concentrations and C:N ratios in periphyton did not differ between controls and grazer treatments (except for C:N ratios in exp. 3; Table 2).

**Discussion**

Our experiments revealed that community removal rates of periphyton by the pelagic cladoceran \textit{D. magna} were similar to those of chironomid larvae. In accordance with allometric theory (Cyr and Pace 1993), the larger chironomid larvae had higher individual periphyton removal rates than \textit{D. magna}, whereas body mass-specific periphyton removal rates (DW) of \textit{D. magna} were 55% higher than those of chironomids. Periphyton removal rates of \textit{D. magna} at 20 °C reached 39% of periphyton accumulation rates, indicating their potential importance for a periphyton standing crop.

Community periphyton removal rates measured under laboratory conditions at 20 °C showed a striking similarity between NGC and chironomid larvae (Fig. 2A and B) in exp. 1 and 2 despite differences in initial grazer biomasses of an order of magnitude. The NGC reached similar removal rates despite lower grazer biomasses, potentially due to higher initial periphyton biomass (Cattaneo and Mousseau 1995) or due to a
### Table 2. Average initial and final grazer and periphyton biomass over the 4 replicates: DW (dry weight), AFDW (ash-free dry weight), Chl-α (chlorophyll α), periphyton accumulation rates, total periphyton removal, and periphyton nutrient composition (±SE). * indicates significant differences between control and treatment at p ≤ 0.05 (t-tests).

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grazers</td>
</tr>
<tr>
<td></td>
<td>DW (µg DW cm(^{-2}))</td>
</tr>
<tr>
<td>1</td>
<td>Initial control</td>
</tr>
<tr>
<td></td>
<td>Chironomids + C. sphaericus</td>
</tr>
<tr>
<td></td>
<td>Final Control</td>
</tr>
<tr>
<td>2</td>
<td>Initial Control</td>
</tr>
<tr>
<td></td>
<td>Chironomids</td>
</tr>
<tr>
<td></td>
<td>Final Control</td>
</tr>
<tr>
<td>3</td>
<td>Initial Control</td>
</tr>
<tr>
<td></td>
<td>Chironomids</td>
</tr>
<tr>
<td></td>
<td>D. magna</td>
</tr>
<tr>
<td></td>
<td>Final Control</td>
</tr>
</tbody>
</table>
higher food quality of the periphyton (e.g., higher initial TP concentrations and thus lower C:P ratios [Table 2]; Burks et al. 2002).

Overcrowding effects as suggested for many laboratory experiments (Hillebrand 2009) can also reduce the effect size of grazers on periphyton, but our grazer densities were lower or comparable to natural densities. In exp. 3, chironomid larvae and D. magna were tested at the same initial periphyton biomass, and their periphyton removal rates at the community level were comparable (Fig. 2A). Both chironomid larvae (0.3 ind. cm\(^{-2}\), average total biomass 617–843 mg DW m\(^{-2}\)) and D. magna (average individuals 1.3 ind. cm\(^{-2}\) or 43 ind. L\(^{-1}\), average total biomass 1.2 mg DW L\(^{-1}\)) were tested at naturally occurring densities. Other studies reported equal or even higher densities of chironomid larvae (Mason and Bryant 1975: 0.7 ind. cm\(^{-2}\) on Typha stems; Menzie 1981: 4.5 ind. cm\(^{-2}\) on Myriophyllum leaves; Cattaneo 1983: up to 6 ind. cm\(^{-2}\) on artificial plants; Grese and Lowe 1994: 1.04 ind. cm\(^{-2}\) on Potamogeton leaves) and of D. magna (Lauridsen and Buenk 1996: 40 ind. L\(^{-1}\) in macrophyte stands; Östmann 2011: up to 100 ind. L\(^{-1}\) in rock pools) in their natural environment.

Our measured periphyton removal rates by chironomid larvae (Fig. 2A) were about 3–7 times higher than those reported from field studies. Mason and Bryant (1975) measured periphyton removal rates by chironomids of 74 mg DW m\(^{-2}\) d\(^{-1}\) on Typha stems, and Kesler (1981) found maximum periphyton removal rates by chironomids of 107 mg DW m\(^{-2}\) d\(^{-1}\). Periphyton removal rates of chironomids at the individual level, however, were on the order of those predicted by Cattaneo and Moussseau (1995) for grazers with a body mass of 200–300 µg (in our experiments mean individual biomasses of chironomids were 270 ± 15 µg and 197 ± 9 µg in exp. 2 and 3, respectively). Their multiple regression on periphyton removal rate data by grazers showed a significant dependence of individual rates on grazer body mass, while crowding and food availability only explained a minor part of the variation (Cattaneo and Moussseau 1995). In their study, chironomids and oligochaetes were the smallest grazers tested. Mean individual periphyton ingestion rates of the plant-associated cladocerans S. cristallina, C. sphaericus, and A. affinis (18.4, 7.6, and 8.5 µg DW ind.\(^{-1}\) d\(^{-1}\), respectively) were smaller than those determined for D. magna in our study (60 µg DW ind.\(^{-1}\) d\(^{-1}\)). Variability can be high, however; maximum values of up to 923 µg DW ind.\(^{-1}\) d\(^{-1}\) have been reported for the largest species, S. cristallina (Downing 1981).

The diet composition of periphyton grazers is size-class–specific and may affect the community composition of periphyton (Cattaneo and Kalff 1986, Tarkowska-Kukuryk 2013), which was not the subject of our study. We observed, however, that D. magna with a highly developed filtering apparatus detached small periphyton particles by turbulence and subsequently accumulated them. A similar behaviour was previously reported for Chydoridae (Fryer 1968) and D. magna (Siehoff et al. 2009). Such physical damage of periphyton due to locomotion and other activities have already been reported for chironomids and explained the grazing rates up to 6 times lower than removal rates (Cattaneo and Moussseau 1995).

We conclude that the planktonic cladoceran D. magna, which is known to exert a strong control on seston components (Gliwicz 1990), may also significantly affect periphyton standing crop by grazing when phytoplankton concentrations are low. Previous laboratory studies indicated that periphyton is only used below certain threshold levels (0.05 mg L\(^{-1}\) C) of phytoplankton densities (Siehoff et al. 2009). Such low phytoplankton concentrations have been reported, for instance, inside of macrophyte beds with >15–20% plant volume infested (Schriver et al. 1995) or in allelopathically active macrophyte stands (Jasser 1995).

Phytoplankton concentrations can also be low during clear-water phases in eutrophic lakes (e.g., Hilt et al. 2013); thus, we contend that the effects of large planktonic cladocerans on periphyton have been underestimated. Changes in zooplankton abundance, for instance by top-down control of fish (Jeppesen et al. 1997), would then not only affect phytoplankton but also periphyton biomass, which may have cascading effects down to submerged macrophytes, as suggested by Jones and Sayer (2003). Additional in situ tests considering different phytoplankton qualities and quantities as alternative food sources are needed to confirm the ecological relevance of periphyton removal by D. magna and other larger daphnids.

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