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Enhancement of marine phytoplankton blooms by appendicularian grazers

Diego Fernández and José Luis Acuña¹

Área de Ecología, Departamento de Biología de Organismos y Sistemas, Facultad de Biología, Universidad de Oviedo, Oviedo 33071, Spain

Abstract

Short-lived blooms of large phytoplankton are the main vector in the atmosphere–ocean–sediment carbon flux and are associated with high fish production. A common assumption is that grazing zooplankton can only have a negative effect on the growth of these blooms. We have found that appendicularians, the most abundant microphagous metazoans, strongly stimulate the magnitude of experimentally induced blooms and that this effect can be scaled to any appendicularian density. Moreover, during a postspring bloom cruise in May 2000 off the central Cantabrian coast, we found a high positive correlation between the biomass of large phytoplankton and the abundance of appendicularians. These results suggest that these marine microphagous zooplankton actively catalyze the biological CO₂ pump by shifting the size structure of phytoplankton blooms toward large, rapidly sinking particles.

Bursts of large phytoplankton chains and colonies (or net phytoplankton) associated with transient physical processes represent the main contribution to atmospheric CO₂ sequestration in ocean sediments and to fish production because large particles sink fast and are readily consumed by large predators (Cushing 1989; Legendre 1990; Kiorboe 1993; Falkowski et al. 1998). It is a commonly accepted notion that grazing zooplankton can only have a negative effect on the growth of phytoplankton blooms (Cushing 1989; Legendre 1990; Kiorboe 1993; Falkowski et al. 1998). However, a long line of studies of the indirect effects in freshwater ecosystems, initiated by Sterner (1986) with cladoceran grazers, has shown that herbivores might selectively favor the growth of some algal species by a combination of selective grazing and nutrient regeneration. When the herbivore preys only on phytoplankton, its net effect on phytoplankton net growth ranges from negative to absent (Sterner 1986). In

contrast, grazers of small bacteria and colloids could mediate a net flux of nutrients from the nonphytoplanktonic to the phytoplankton fraction, thus resulting in a net enhancement of phytoplankton growth (Sterner 1986), although this possibility has never been tested.

Appendicularians, the most abundant marine microphage metazoans, are capable of capturing particles in the colloidal and bacterial size fractions (King et al. 1980; Deibel and Lee 1992; Flood et al. 1992; D. Fernández, A. López-Urrutia, A. Fernández, J. L. Acuña, and R. P. Harris unpubl. data). Appendicularians are highly productive zooplankton (Hopcroft and Roff 1995) that use an external mucous filter house to remove small particles from the ocean (Flood and Deibel 1998). We have observed that the natural seawater used to nurture cultured appendicularians consistently develops dense blooms of large diatom chains and *Phaeocystis* colonies. In addition, dense populations—or even “red tides” (sensu Seki 1973)—of appendicularians usually coincide with high phytoplankton standing stocks and productivities (Acuña 1992; Ashjian et al. 1995; Dagg et al. 1996), blooms of diatom chains (Seki 1973; Madhupratap et al. 1981; Prézelin and Alldredge 1983; Nakamura 1998) or *Phaeocystis* colonies (Pesant et al. 1998), and red tides of dinoflagellates (Nakamura 1998). Here, we report on experimental results and field observations that suggest that the coincidence of dense appendicularian populations and algal blooms could be due, at least partially, to the ability of these grazers to stimulate the growth of net phytoplankton.

¹ Corresponding author (acuna@correo.uniovi.es).

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Materials and methods

Grazer exclusion experiment—To test whether appendicularians are capable of stimulating phytoplankton blooms,

we performed a microcosm grazer exclusion experiment during an artificially induced bloom using natural, light-limited winter phytoplankton containing a dense appendicularian population (36 individuals L^{-1} *Oikopleura dioica*) as inocula. For this experiment, surface water at the El Musel harbor, Gijón (N Spain), was inspected daily for appendicularian abundance. On 22 February 1999, we found optimal conditions for a grazer exclusion experiment, with high-nutrient, low-chlorophyll concentrations and a dense (36 individuals L^{-1}) appendicularian population. Surface water was collected gently using two 50-liter wide-mouth plastic vessels, carried to the laboratory, thoroughly mixed to provide homogeneity of inocula, and distributed among 10, 5-liter glass beakers. We used five replicates per treatment because one preliminary experiment with three replicates per treatment resulted in differences that were not significant because of the low power of the statistical tests. The temperature of the cold room was set to 15°C, which coincides approximately with the temperature of the water at the site of collection. The beakers were closed with a polyvinyl chloride lid and continuously stirred with paddles of the kind used to culture appendicularians (Fenaux and Gorsky 1985). All appendicularians, along with a certain amount of water, were removed from five of the beakers using a wide-bore pipette (ungrazed treatment); an equivalent amount of water, but no appendicularians, was removed from the remaining five beakers (grazed treatment). The beakers were then placed under controlled temperature and mixing and exposed to controlled, unlimited light conditions (35 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 8:16 light:dark photoperiod) to induce a phytoplankton bloom. We then followed the time course of chemical and biological parameters within the grazed and ungrazed beakers. Comparison of the number of appendicularians retrieved from the ungrazed treatment (36 ± 3 , individuals L^{-1} , mean \pm SD) with the number of appendicularians retrieved after the experiment from the grazed treatment (16 ± 4 individuals L^{-1}) suggests negative growth of the enclosed populations.

Every day we fractionated one 200-ml water sample from each beaker through 47-mm diameter, 0.2- and 30- μm pore size Millipore membrane filters to measure the chlorophyll concentration of the 0.2–30- and >30- μm particles fluorometrically (Strickland and Parsons 1972). A size cut at 30 μm was chosen because *O. dioica* have 30- μm prescreening filters in their filter houses (Flood and Deibel 1998). Four 12-ml and one 250-ml water samples were collected from each beaker for the analysis of nutrient concentrations and phytoplankton composition, respectively (Strickland and Parsons 1972). Phytoplankton samples corresponding to the day of maximum phytoplankton biomass, as determined from the chlorophyll concentration, were analyzed, and carbon concentration in diatom chains was calculated using the appropriate relationships for the estimation of carbon from volume (Hillebrand et al. 1999; Menden-Deuer and Lessard 2000). In addition, we allowed the *Phaeocystis* colonies contained in a sample of 200 ml of water from each beaker to sediment in a round glass beaker for 1 d. The colonies were then photographed, and images were archived in digital format. These pictures were later analyzed for colony carbon using image analysis software and volume-to-carbon trans-

formations (Rousseau et al. 1990). Carbon concentrations were based on microscopic counts to guarantee that the enhancement effects detected on chlorophyll in large particles were due to growth of net plankton and not to aggregation of the small particles on which appendicularians prey into the large filter houses or to the fecal pellets that they produce. Also, we wanted to determine which of the algal species responded more strongly to the presence of appendicularians. Because of our low sample size, we tested differences between treatments by a randomization test (Manly 1997).

Grazer gradient experiment—Appendicularian densities in the previous experiment (36 individuals L^{-1}) are among the highest that can be found in nature (≤ 26 individuals L^{-1} found by Seki [1973] and >100 individuals L^{-1} found by Nakamura [1998] and Dagg et al. [1996]). Thus, it is possible that the enhancement effect is limited to appendicularian densities above a certain threshold, in which case it would seldom take place in nature. To test for thresholds and to confirm the result of the previous experiment, we imposed a gradient of appendicularian (*O. dioica*) densities on 12, 5-liter microcosms containing natural seawater. For this experiment, the water at the Cudillero Harbor, Cudillero (N Spain), was inspected daily for appendicularian abundance. On 19 May 2000, we found optimal conditions for a grazer gradient experiment, with high-nutrient, low-chlorophyll surface water and no appendicularians. Surface water was collected in two wide-mouth 50-liter plastic vessels, brought to the cold room, thoroughly mixed to provide homogeneity of inocula, distributed among 12, 5-liter glass beakers, and incubated as in the previous experiment. Next, we combined cultured appendicularians of different sizes (1,200, 750, 475, and 240 individuals with trunk lengths of 750, 550, 350, and 150 μm , respectively) to simulate the size structure of a natural population similar to that used in the previous experiment. This appendicularian inoculum was gently distributed among the 12 beakers to reach a final appendicularian density of 36 individuals L^{-1} in each. Next, appendicularians were randomly retrieved from each of the beakers along with 25 ml of water, killed using microwaves, and brought back to the beaker. The number of appendicularians retrieved in each beaker was adjusted to obtain a final gradient of appendicularian density from 0 to 36 individuals L^{-1} . Data presented correspond to the fourth day, when we observed peak chlorophyll concentrations.

Field observations—During the cruise SARDINA 2000 in the southern Bay of Biscay, we had the chance to sample the density of appendicularians and the biomass of two phytoplankton size fractions, >5 and $<0.2\text{--}5$ μm , concurrently during the senescent phase of the spring phytoplankton bloom at 11 stations (Fig. 1). At each location, we towed a triple, 30-cm mouth diameter, 200- μm pore size WP2 net from 100 m depth to the surface or from the bottom to the surface in shallow stations. An aliquot of the cod end contents was stored in a plastic test tube and flash frozen in liquid nitrogen for later analysis of appendicularian abundance under a binocular microscope. Water samples (100 ml) were collected in the photic layer at depths of 0, 10, 20, 30,

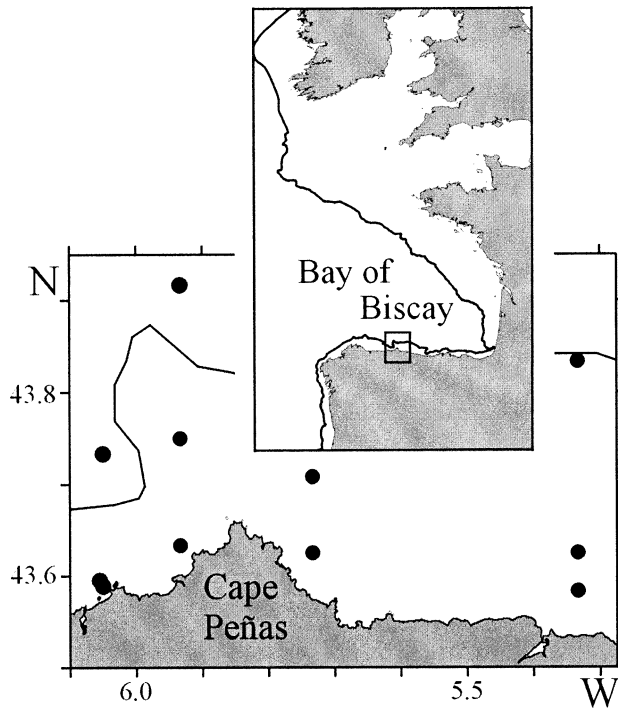


Fig. 1. Geographical location of the stations in the southern Bay of Biscay visited during SARDINA 2000 cruise, 2 to 11 May 2000. The shelf slope is delimited by the 200-m isobath.

40, 50, and 60 m by CTD casts with 5-liter Niskin bottles; size fractionated through 0.2- and 5- μm Nuclepore membrane filters; and analyzed fluorometrically for chlorophyll concentration (Strickland and Parsons 1972). Chlorophyll concentrations in large, $>5\text{-}\mu\text{m}$ particles were then transformed to carbon units using a conversion coefficient. To develop this coefficient, we collected water samples at the surface (upper mixed layer), at the chlorophyll maximum, and at the depth receiving 1% of surface light; preserved these samples using Lugol's iodine; and analyzed them for carbon concentration of net plankton as above. Plots of carbon versus chlorophyll concentration in particles of $>5\text{ }\mu\text{m}$ revealed a positive, significant relationship ($n = 35$, $r^2 = 0.77$, $F_{1,33} = 108.45$, $P < 0.001$) that we applied to our chlorophyll measurements to estimate carbon concentrations. Carbon concentration data at point depths were then integrated with polygons to obtain areal carbon concentrations.

Results

Grazer exclusion experiment—When the bloom was fully developed (i.e., peak total chlorophyll concentrations, fourth day, Fig. 2a), chlorophyll concentration in large, $>30\text{-}\mu\text{m}$ particles was significantly ($P = 0.008$, Manly's 1997 randomization test for differences between means) higher in the grazed ($11.39 \pm 3.44\text{ }\mu\text{g Chl } a\text{ L}^{-1}$) than in the ungrazed ($3.70 \pm 0.87\text{ }\mu\text{g Chl } a\text{ L}^{-1}$) treatment (Fig. 2b). In contrast, the chlorophyll concentration in small, $<0.2\text{-}30\text{-}\mu\text{m}$ particles was significantly ($P = 0.016$) lower in the grazed ($1.50 \pm 0.17\text{ }\mu\text{g Chl } a\text{ L}^{-1}$) than in the ungrazed ($2.04 \pm 0.54\text{ }\mu\text{g Chl } a\text{ L}^{-1}$) treatment (Fig. 2c), which

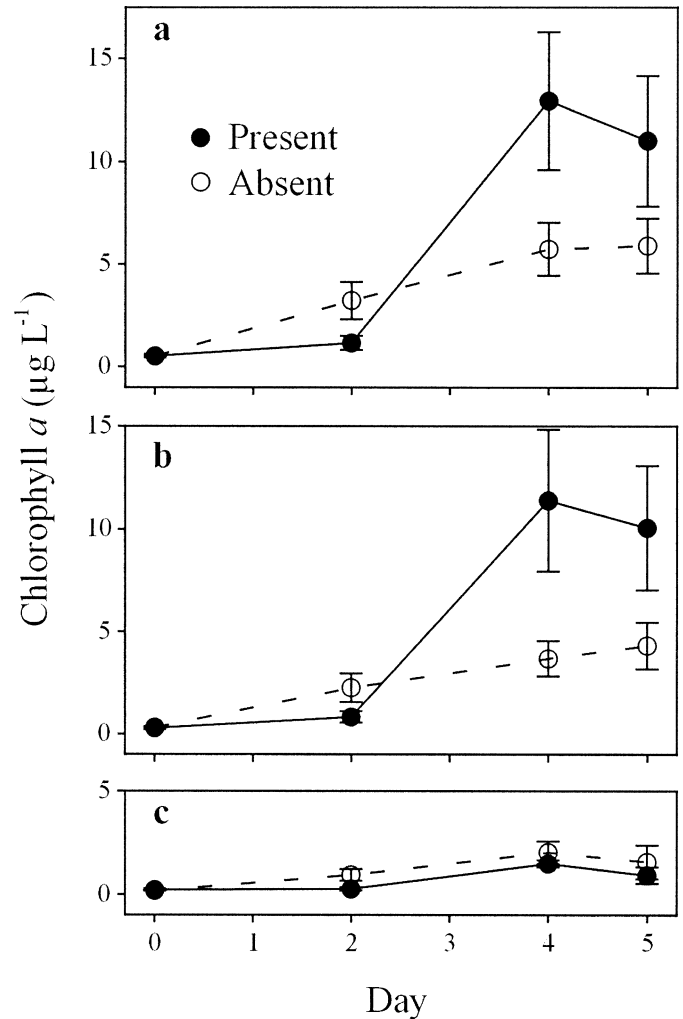


Fig. 2. Time evolution of the response of chlorophyll size fractions to the presence of appendicularians during a laboratory phytoplankton bloom. (a) total ($>0.2\text{ }\mu\text{m}$) chlorophyll, (b) chlorophyll in large ($>30\text{ }\mu\text{m}$) particles, and (c) chlorophyll in small ($0.2\text{-}30\text{ }\mu\text{m}$) particles. Solid dots indicate appendicularians present and empty dots appendicularians absent ($\pm\text{SD}$).

is in agreement with the selective removal by appendicularians of small flagellates and bacteria. Water without appendicularians had a typically hazy aspect, contrasting with extreme clarity in the grazed treatment. The presence of appendicularians had a strong, fourfold stimulating effect on the carbon concentration of chain diatoms and *Phaeocystis* colonies (Table 1). Wider diatom chains responded more strongly to the presence of appendicularians (Table 1), which suggests that the initial inocula of smaller diatoms were amenable to ingestion by appendicularians or that the nutrient environment in our enclosed populations favored larger diatoms.

Grazer gradient experiment—In the resulting monospecific bloom, the chlorophyll concentration in large ($>30\text{ }\mu\text{m}$) and in total ($>0.2\text{ }\mu\text{m}$) particles increased linearly (both $P = 0.001$, Fig. 3a,b), whereas there was a slight, nonsignificant ($P = 0.557$) decrease in the chlorophyll concentration

Table 1. Size range and carbon concentration (mean \pm SD) of chain diatoms and *Pheocystis* colonies and significance of differences in carbon content between grazed and ungrazed treatments during a fully developed bloom in the grazer exclusion experiment.

Net phytoplankton species	Minimum width observed (μm)	Concentration with appendicularians ($\mu\text{g C L}^{-1}$)	Concentration without appendicularians ($\mu\text{g C L}^{-1}$)
<i>Pheocystis</i> colonies***	225	198 \pm 63	41.7 \pm 6.1
<i>Chaetoceros decipiens</i> *	66	14.8 \pm 3.8	7.0 \pm 5.0
<i>Chaetoceros curvisetus</i> ***	60	1.7 \pm 1.2	0.39 \pm 0.34
<i>Lauderia annulata</i> **	34	19.0 \pm 8.0	4.5 \pm 6.5
<i>Thalassiosira</i> sp.***	16	1.14 \pm 0.55	0.030 \pm 0.050
<i>Asterionellopsis glacialis</i> ***	10	0.104 \pm 0.094	0
<i>Guinardia delicatula</i>	10	0.056 \pm 0.092	0.023 \pm 0.052
<i>Nitzschia longissima</i> ***	7	0.75 \pm 0.55	0.190 \pm 0.078
<i>Thalassionema nitzschioides</i>	4	0.31 \pm 0.38	0.11 \pm 0.15
<i>Pseudo-nitzschia pungens</i>	4	0.099 \pm 0.046	0.064 \pm 0.067
Total***		236 \pm 74	54 \pm 14

Values are significant at the * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$ levels; other values are not significant.

of small, <0.2 – 30 - μm particles with appendicularian density (Fig. 3c). Carbon concentration in chains of the diatom *Chaetoceros socialis* increased linearly with appendicularian density (F -test for H_0 : slope = 0, $P < 0.001$, Fig. 4). This suggests that there are no thresholds and that the effect can be scaled to any appendicularian density.

Field observations—During SARDINA 2000, chlorophyll in the >5 - μm size fraction increased significantly with appendicularian density ($P < 0.001$, Fig. 5b) but had a slight and nonsignificant decrease in the <0.2 – 5 - μm size fraction ($P = 0.905$, Fig. 5c). Interestingly, appendicularians were nearly absent at some of the stations with the highest total chlorophyll concentrations (Fig. 5a); almost all of that chlorophyll pertained to the <0.2 – 5 - μm particle size fraction (Fig. 5c). Carbon concentration in particles >5 μm also increased significantly with appendicularian density ($n = 11$, $r^2 = 0.79$, $F_{1,9} = 33.14$, $P < 0.001$, Fig. 6). We did not find similar trends when plotting our chlorophyll data versus the densities of other microphages, such as ciliates ($r^2 = 0.131$; $P = 0.274$ for the regression) or copepod nauplii ($r^2 = 0.012$; $P = 0.800$), which were also abundant during the survey ($43,060 \pm 54,393$ and $9,523 \pm 5,562$ individuals m^{-2} , respectively, $n = 11$), and there were no significant correlations between the densities of appendicularians and those of nauplii ($r^2 = 0.124$; $P = 0.391$) or ciliates ($r^2 = 0.277$; $P = 0.096$). Diatom and appendicularian species compositions differed considerably between stations; therefore, this ecosystem process seems remarkably robust to variations of the species composition.

Discussion

During our microcosm bloom experiments with natural, light-limited phytoplankton, the presence of appendicularians enhanced the growth of net phytoplankton (Table 1; Figs. 2–4). These results are consistent with repeated observations on the co-occurrence of dense appendicularian populations with net phytoplankton blooms (e.g., Seki 1973; Madhupratap et al. 1981; Prézelin and Alldredge 1983; Acuña 1992;

Ashjian et al. 1995; Dagg et al. 1996; Nakamura 1998; Pesant et al. 1998) and with our field data showing marked trends in the abundance of phytoplankton size fractions with appendicularian density during the postspring bloom phase in the Cantabrian Sea (Figs. 5, 6). A key issue is the extent to which our microcosm results mimic processes taking place in the field. In our stirred microcosms, phytoplankton do not sink; grazer populations and their by-products (ammonia, fecal pellets, and shed filter houses) are confined; turbulence levels, and therefore the light regime, are probably unrealistic; and nutrient concentrations of the inshore water (≤ 8 $\mu\text{mol L}^{-1}$ nitrate concentration) are higher than in the open ocean (≤ 4 $\mu\text{mol L}^{-1}$ at the surface during winter mixing). A first condition that must be met in the ocean for the enhancement process described here is the presence of dense appendicularian populations prior to a phytoplankton bloom. The water used in our grazer exclusion experiment, sampled prior to the spring bloom and containing a dense appendicularian population, suggests that this is indeed possible. The water used in our grazing gradient experiment shows that the reverse is also possible (i.e., nutrient-laden water potentially leading to a phytoplankton bloom could contain no appendicularians). A second condition is a net plankton inoculum to generate a bloom. In the Cantabrian Sea during the spring, high chlorophyll concentrations are observed, based on either small flagellates (Fernández et al. 1993) or net plankton (Fernández et al. 1991), which suggests that the potential for net plankton blooms varies greatly during this season. Yet another condition for a net plankton enhancement effect by appendicularians is that grazers and phytoplankton populations must coexist for a sufficient amount of time and at sufficient densities for the process to take place, as in our experimental beakers, where they coexisted for 5–6 d. We ignore enough appendicularian biology to remain cautious on this regard, although appendicularians have a tendency to aggregate in the upper layers of the ocean, sometimes in extremely high densities (e.g., Dagg et al. 1996). Because phytoplankton blooms are often triggered by increased stratification or reduced turbulence in the upper layers of the ocean, the potential for such a spatiotemporal

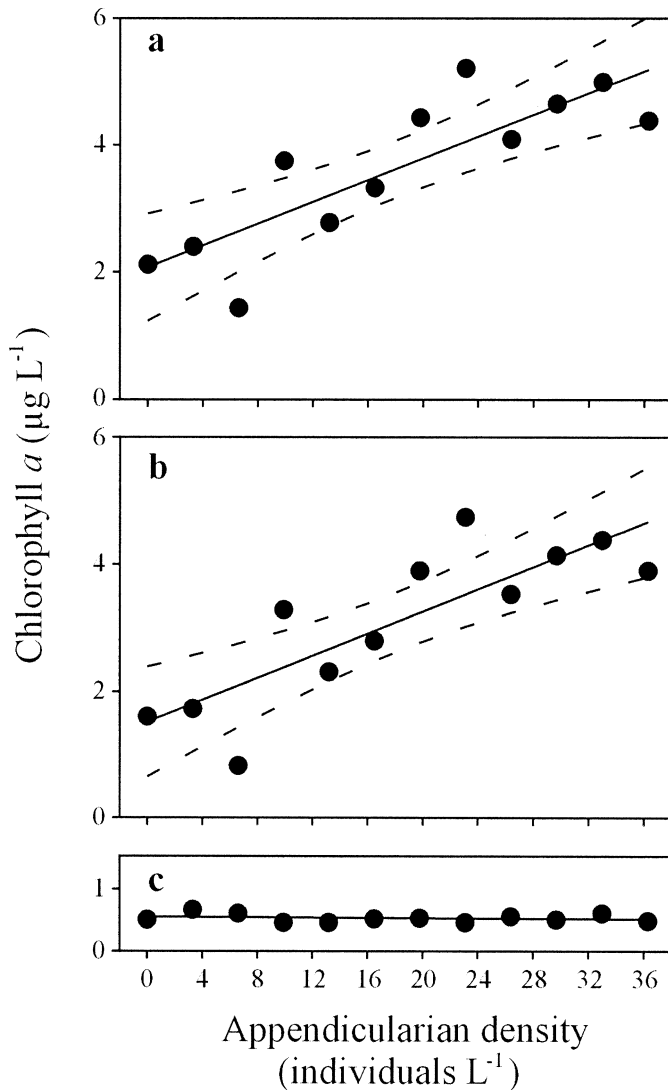


Fig. 3. Response of chlorophyll size fractions to a gradient of appendicularian density (app) in a fully developed laboratory phytoplankton bloom. Chlorophyll in (a) total ($>0.2 \mu\text{m}$), (b) large ($>30 \mu\text{m}$), and (c) small ($0.2\text{--}30 \mu\text{m}$) particles. Solid lines indicate least squares type I regressions, whose equations were $\text{Chl } a = (0.086 \pm 0.018)\text{app} + (2.08 \pm 0.38)$ (parameter estimates \pm SE, $n = 12$, $r^2 = 0.70$, $F_{1,10} = 23.64$, $p < 0.001$), for panel a, and $\text{Chl } a = (0.087 \pm 0.018)\text{app} + (1.52 \pm 0.39)$ ($n = 12$, $r^2 = 0.69$, $F_{1,10} = 22.55$, $p < 0.001$), for panel b. Dashed lines indicate 95% confidence intervals for the regressions.

coincidence of phytoplankton blooms and appendicularians is not negligible. In any case, our intention was not to develop a model resembling the situation in the field, but to show that appendicularians can enhance the growth of net phytoplankton in a bottle. Only through observation at the relevant scales will we be able to determine the extent and magnitude of this process.

In their study of appendicularian distributions across the Mississippi River plume, Dagg et al. (1996) suggested that appendicularians could be responsible in part for the high phytoplankton biomass observed at the plume borders because of their selective removal of fine lithogenic particles

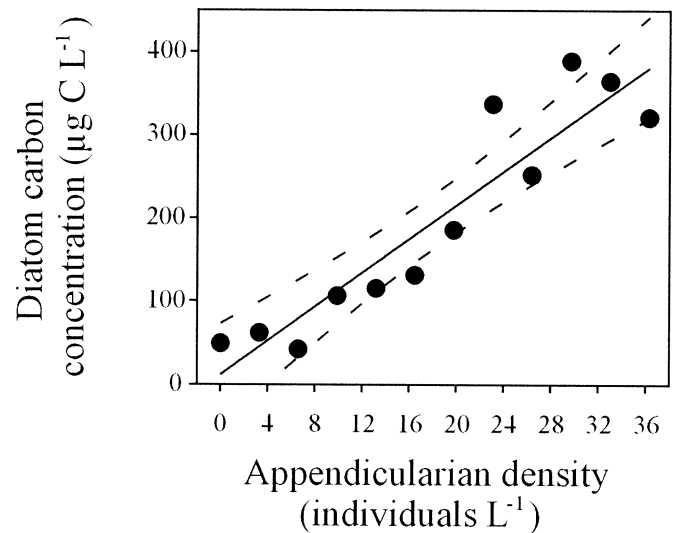


Fig. 4. Response of the carbon concentration in large diatom chains to the density of appendicularians during a fully developed laboratory phytoplankton bloom. The type I least squares regression (solid line) between appendicularian density (app) and carbon concentration (CC) is: $\text{CC} = (10.16 \pm 1.28)\text{app} + (12.04 \pm 27.46)$, (parameter estimates \pm SE, $n = 12$, $r^2 = 0.86$, $F_{1,10} = 62.85$, $p < 0.001$). Values between brackets represent the standard error of parameter estimates. Dashed lines indicate 95% confidence intervals for the regression.

that would significantly reduce water turbidity. Although this possibility cannot be ruled out in particular places (*see below*), the optical path within our beakers was not long enough to reduce photon flux significantly, which leads us to hypothesize that appendicularians stimulate the growth of net phytoplankton by a combination of selective grazing on small colloids, bacteria, and picoplankton and by the regeneration of nitrogenous nutrients. Similar indirect effects are known to trigger competitive displacements among freshwater phytoplankton species (Sterner 1986) and to explain the spring “clear-water” phase in lacustrine systems, where the presence of dense cladoceran populations leads to the competitive dominance by large, blue-green filaments (Lampert et al. 1986). Moreover, the water transparency during the clear-water phase is strongly reminiscent of the sharp increase in the depth of Secchi disk visibility observed by Nakamura (1998) after an appendicularian/diatom bloom in the sea. This situation is also consistent with several reported observations of dense appendicularian populations co-occurring with blooms of large phytoplankton (e.g., Seki 1973; Madhupratap et al. 1981; Prézélin and Alldredge 1983; Acuña 1992; Ashjian et al. 1995; Dagg et al. 1996; Nakamura 1998; Pesant et al. 1998). Additionally, all but one surface warm-water species of oikopleurid appendicularian (*O. longicauda*) have evolved coarse ($30\text{-}\mu\text{m}$ pore size in most cases) meshes on their filter houses to prescreen large particles (Flood and Deibel 1998), just like man-made purification systems in which the operation of fine meshed filters is improved by prescreening through coarse nets. All this evidence indicates that blooms of large diatoms that are capable of inhibiting the feeding rates of appendicularians (Knoechel and Steel-Flynn 1989; Acuña et al. 1999) could be a recur-

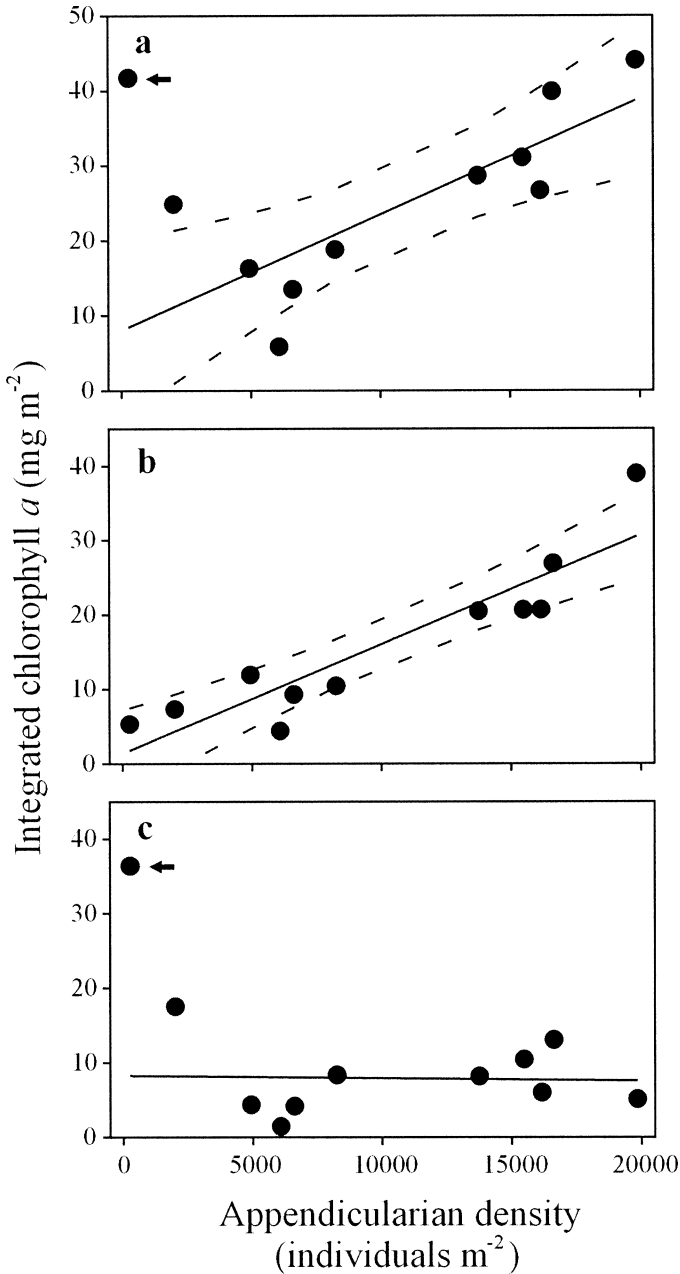


Fig. 5. Relationship between chlorophyll size fractions and appendicularian abundance during a senescent spring phytoplankton bloom in the sea. Plots of (a) total Chl *a* concentrations, (b) Chl *a* concentrations of large (>5 μm) particles, and (c) Chl *a* concentrations of small (0.2–5 μm) particles versus the areal density (AD) of appendicularians. Solid lines indicate least squares type I regressions, whose equations were $\text{Chl } a = (0.00155 \pm 0.00041)\text{AD} + (8.02 \pm 5.14)$ (parameter estimates \pm SE, $n = 10$, $r^2 = 0.63$, $F_{1,8} = 13.91$, $p = 0.006$), for panel a, and $\text{Chl } a = (0.00147 \pm 0.00022)\text{AD} + (1.38 \pm 2.57)$ ($n = 11$, $r^2 = 0.83$, $F_{1,9} = 45.56$, $p < 0.001$), for panel b. Dashed lines indicate 95% confidence intervals for the regressions. The arrow indicates a point that was not included in the statistical analysis.

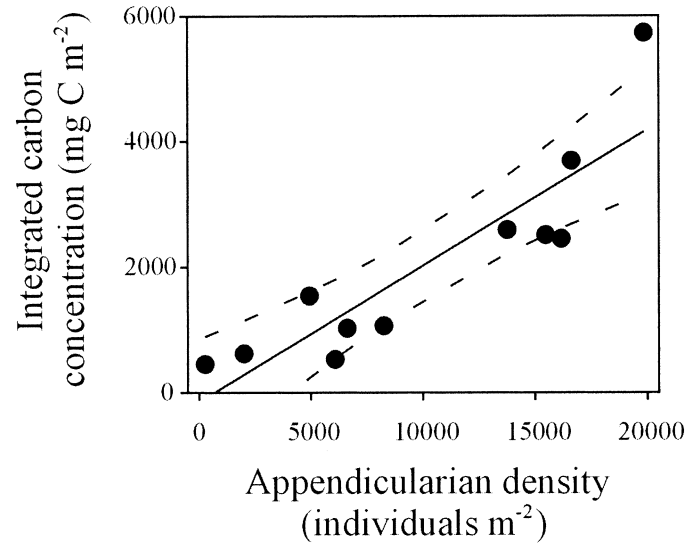


Fig. 6. Relationship between carbon concentration and appendicularian abundance during a fully developed spring phytoplankton bloom in the sea. The type I least squares regression (solid line) between the areal density of appendicularians (AD) and carbon concentration (CC) is $\text{CC} = (0.217 \pm 0.037)\text{AD} + (-145.36 \pm 443.36)$ (parameter estimates \pm SE, $n = 11$, $r^2 = 0.79$, $F_{1,9} = 33.39$, $p < 0.001$). Dashed lines indicate 95% confidence intervals for the regression.

rent feature in their life cycle. Under these circumstances, intense vertical fluxes of diatom chains and *Phaeocystis* colonies can be expected. Therefore, appendicularians can act as strong catalysts of the downward flux of photosynthetically fixed CO_2 .

Our microcosm results can only be a crude caricature of the processes that might be taking place in the ocean (Carpenter 1997), although they do confirm a previous suspicion about a particular mechanism that was raised by numerous observations and supported by theoretical arguments. Extrapolation of this process to realistic conditions and assessment of its spatial and temporal dimensions and of its frequency and ecological significance will only be attained through a careful research program spanning a range of scales—from the experimental mesocosm approach to purely observational oceanographic surveys—and based on due consideration to our limnological precedents (e.g., Lampert et al. 1986). Because protozoans fall in the ingestible size range for appendicularians and because some of the more common copepods prey on appendicularians (e.g., *Candacia bipinnata*, Ohtsuka and Onbé 1989), the potential for complicating food web effects and their experimental study is enormous. Our findings prompt further investigation on the role of grazers of small particles in marine pelagic ecosystems and on their indirect effects on the size structure of phytoplankton communities, and they suggest that the growth of microalgal blooms should not necessarily vary as an inverse function of grazing pressure in the ocean.

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