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NOTE

Cascading effect of three-spined stickleback *Gasterosteus aculeatus* on community composition, size, biomass and diversity of phytoplankton in shallow, eutrophic brackish lagoons

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ABSTRACT: We conducted a 4 mo mesocosm experiment to elucidate the cascading impact of the zooplanktivorous three-spined stickleback *Gasterosteus aculeatus* on phytoplankton in slightly brackish lakes. The mesocosms were nutrient-enriched with low salinity (2 psu) and contrasting densities of sticklebacks (0 to 10 fish m^{-2}). Total phytoplankton biovolume increased by 2 orders of magnitude when stickleback density increased from 0 to 3 to 6 to 10 fish m^{-2}, most likely reflecting a parallel change in zooplankton biomass and size distribution. Phytoplankton size distribution was affected in that large forms dominated at high fish density, which most likely reflects a parallel reduction in size of zooplankton. A few taxa (especially Cryptophyceae) dominated at fish densities above 3 to 6 fish m^{-2}, whereas higher diversity (characterised by taxa of Bacillariophyceae and Chlorophyceae) was recorded at fish densities below 3 to 6 m^{-2}. However, genera richness showed no significant relationship with fish density. The threshold fish density for a transition from low to high phytoplankton biovolume at these high nutrient levels was 3 to 6 fish m^{-2}. This level lies well below stickleback densities found in many eutrophic, low-salinity lagoons, implying that the intensity of the cascading top-down effect exerted by planktivorous fish on the phytoplankton abundance and community structure is of importance.

KEY WORDS: Sticklebacks · Phytoplankton diversity · Top-down control · Trophic cascade · Saline · Brackish lakes

INTRODUCTION

The cascading trophic interactions hypothesis suggests that nutrient supply determines the potential productivity of an aquatic ecosystem, whereas the deviation of the actual from the potential productivity is caused by variability in predator–prey interactions and their influence on community structure (Carpenter et al. 1987). According to this hypothesis, alterations at the top of the food web cascade to the lower trophic levels, the major process involved being the concept of selective predation by consumers on prey type and size, which shapes the structure of each lower trophic level (top-down control) as demonstrated in many freshwater lakes (Kitchell & Carpenter 1993).

Trophic structure and dynamics of eutrophic brackish lakes differ substantially, however, from eutrophic freshwater lakes (Jeppesen et al. 1994). They are typically species-poor and generally lack large-sized mussels (Moss 1994), and the zooplankton:phytoplankton

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ratio is markedly lower, indicating lower grazing pressure on phytoplankton. Yet, how phytoplankton responds to changes in fish densities has received little attention. The salinity of most Danish brackish lakes is low (0.5 to 8 psu) and the lakes are characterised by low water depth, high nutrient levels, a low zooplankton:phytoplankton biomass ratio and low water transparency (Jeppesen et al. 1997a, Jensen et al. 2000). Planktivorous fish, mainly sticklebacks, constitute the major part of the fish community and apparently maintain a high predation pressure on zooplankton (Jeppesen et al. 1997b). To further elucidate the structuring role of planktivorous fish in eutrophic-hypereutrophic, shallow, brackish lakes, a mesocosm experiment adjusted to low salinity (2 psu) and a high nutrient level was conducted at contrasting densities of three-spined sticklebacks. In an earlier paper (Jakobsen et al. 2003) we reported the effects on the zooplankton community; here, we describe changes in phytoplankton biovolume, composition and size.

MATERIALS AND METHODS

Experimental set-up and design. The experiments were carried out from 3 May to 20 September 2000 in the slightly brackish (0.5 psu) Lake Kogleaks, located in the northern part of the nature reserve Vejlerne, Denmark. Eleven cylindrical, polyethylene enclosures (surface area ≈ 1 m^2) were fixed in and kept open to the sediment at a water depth between 70 and 80 cm (Jakobsen et al. 2003).

Each enclosure was stocked with three-spined sticklebacks (average weight 1.7 g ± 0.3, average length 5.8 cm ± 0.4) in 6 different densities (0, 1, 2, 4, 8, 16 m^{-2}).

Upon establishment, the salinity of the enclosures was adjusted to 2 psu by adding a solution of NaCl, $MgSO_4$ and $NaHCO_3$. The enclosures were then inoculated with additional sediments (200 ml) and water samples (100 or 200 ml from each location) from brackish and marine waters covering a salinity gradient from 1 to 22 psu. Total nitrogen and total phosphorus concentrations were adjusted to a high level of 3.5 and 0.4 mg l^{-1}, respectively, to avoid nutrient limitation in the experiment (Jakobsen et al. 2003). Throughout the sampling period salinity was adjusted and nutrients, equivalent to the initial solutions, were added monthly to the enclosures. The enclosures were kept free of macrophytes (mainly *Myriophyllum spicatum* L. and *Lemna minor* L.) by harvesting.

Sampling methods and processing of samples. Samplings were conducted weekly from 16 May to 14 June and then bi-weekly until 20 September. Samples for chemical analyses and phytoplankton were collected with a tube sampler (length = 1.85 m, depth = 7 cm). For analysis of chemical and physical variables, see Jakobsen et al. (2003). For identification of phytoplankton, a 50 ml sample was preserved with 1 ml Lugol's solution immediately after sampling.

Phytoplankton was enumerated for 6 samplings between 7 June and 20 September. The counting was carried out using an inverted microscope at 400× magnification. Depending on the sample concentration, 5, 10 or 20 ml sedimentation chambers were used and the samples were diluted if necessary. Algae identification was conducted to genera or, if possible, to species level. Cell volumes were calculated from linear measurements using the appropriate geometric formulae (Olrik 1991). Genera/species biovolume (mm^3 ww l^{-1}) was calculated by multiplying average cell volume by cell population density. Average greatest axial linear dimension (GALD) was recorded, omitting the large colonies of Cyanophyceae because of inaccuracy in measurement. Phytoplankton diversity or abundance was estimated as Shannon diversity ($H' = -\Sigma\, p_i \log_2 p_i$, where p_i is the relative contribution to the biovolume of genera. Phytoplankton richness was recorded as number of genera present during the experimental period. Fish density was recorded 4 times throughout the experimental period, and adjusted if fish kill had occurred. Based on these recordings a time-weighted average of stickleback density was calculated for each enclosure (0, 0, 1, 1, 1.3, 1.6, 2.9, 6.9, 7.6, 9.4, 10.2) (Jakobsen et al. 2003).

Statistical analyses. The effects of fish and zooplankton on phytoplankton size and biovolume were assessed by regression analyses. Time-weighed averages of phytoplankton, zooplankton and fish data for each mesocosm were used. Dependent variables were $\log_e$-transformed to stabilise variance. As data from some dependent variables fall in 2 blocks (low: 0 to 3 fish m^{-2}, high: 6 to 10 fish m^{-2} fish density) rather than expressing a linear relationship, we also used Mann-Whitney's nonparametric *U*-test to evaluate differences between the 2 blocks.

RESULTS

Total phytoplankton biovolume correlated positively with fish density, increasing by 2 orders of magnitude from 0–3 fish m^{-2} to 6–10 fish m^{-2} (Fig. 1A). Equally, average phytoplankton GALD correlated positively, as relatively large-sized phytoplankton (Cryptophyceae) dominated at high fish densities, whereas smaller phytoplankton (various species of Bacillariophyceae and Chlorophyceae) dominated at low fish densities. Average cladoceran size correlated negatively with fish density (Fig. 1B) and average GALD of phytoplankton was negatively related to average cladoceran

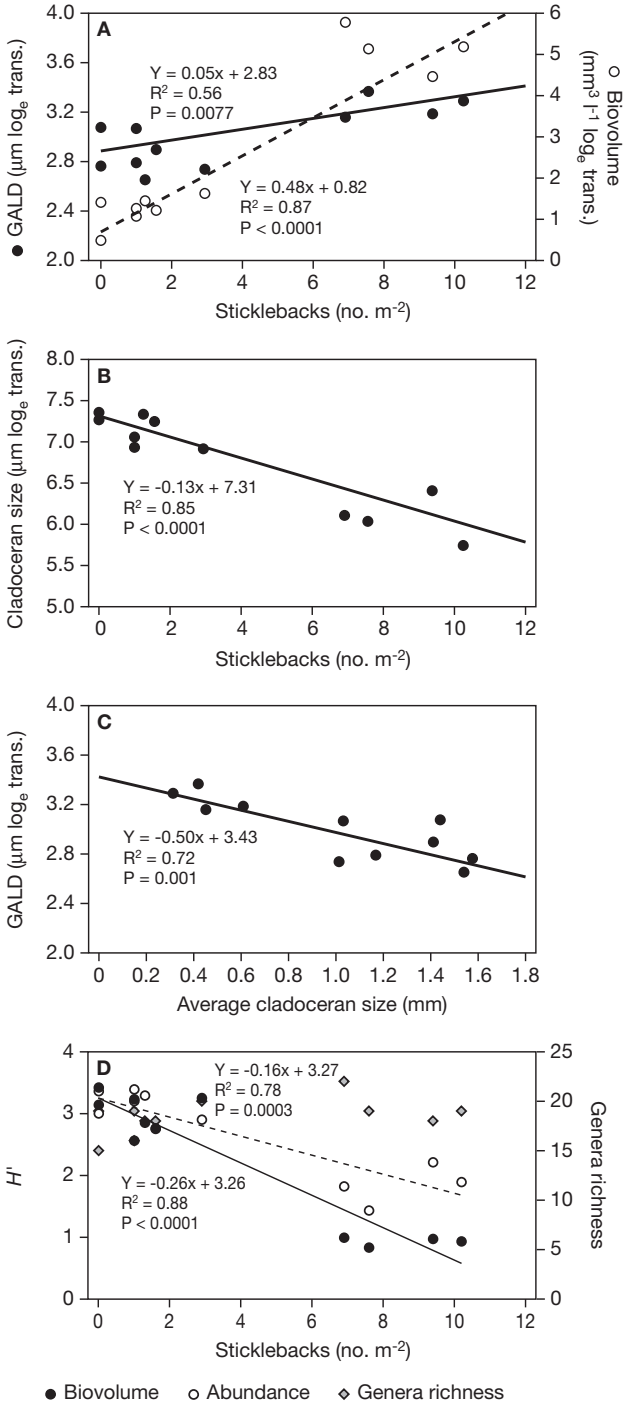

Fig. 1. Phytoplankton, *Gasterosteus aculeatus* and cladocerans. (A) Greatest axial linear dimension (GALD) (full regression line) and biovolume of phytoplankton (broken regression line) versus stickleback densities in the enclosures. (B) Average cladoceran size versus stickleback densities in the enclosures. (C) Average phytoplankton GALD versus average cladoceran size. (For A–C, dependent variables are log$_e$-transformed.) (D) Genera richness and phytoplankton diversity (*H'*) based on biovolume (full regression line) and abundance (broken regression line) versus stickleback densities in the enclosures

size (Fig. 1C). Phytoplankton diversity (Shannon index), based on both biovolume and abundance, was negatively related to fish density, whereas genera richness showed no clear relationship with fish density (Fig. 1D).

The phytoplankton community consisted of many different taxa (especially Bacillariophyceae and Chlorophyceae) at low fish densities, whereas *Cryptomonas reflexa*, *Cryptomonas* sp. and *Gonium pectorale* dominated above 3 to 6 sticklebacks m^{-2} (Fig. 2). Hence, the biovolume of Cryptophyceae and Chlorophyceae differed between the 2 blocks (U = 0, $p < 0.05$), both correlating positively (R^2 = 0.86, $p < 0.05$ and R^2 = 0.96, $p < 0.05$, respectively) to fish density. Biovolume of 3 other phytoplankton groups also differed between blocks, as Cyanophyceae (U = 2, $p < 0.05$) and *Prochlorothrix* (U = 1, $p < 0.05$) increased with fish density, whereas Euglenophyceae (U = 2, $p < 0.05$) decreased. Biovolume of Bacillariophyceae (U = 8, ns), Dinophyceae (U = 6, ns) and unidentified flagellates (U = 8, ns) showed no difference between blocks.

DISCUSSION

Our experiment showed a clear effect of sticklebacks on phytoplankton community, abundance and size structure in slightly brackish water. In accordance with investigations from freshwater lakes (e.g. Andersson et al. 1978, Søndergaard et al. 1997) increased fish

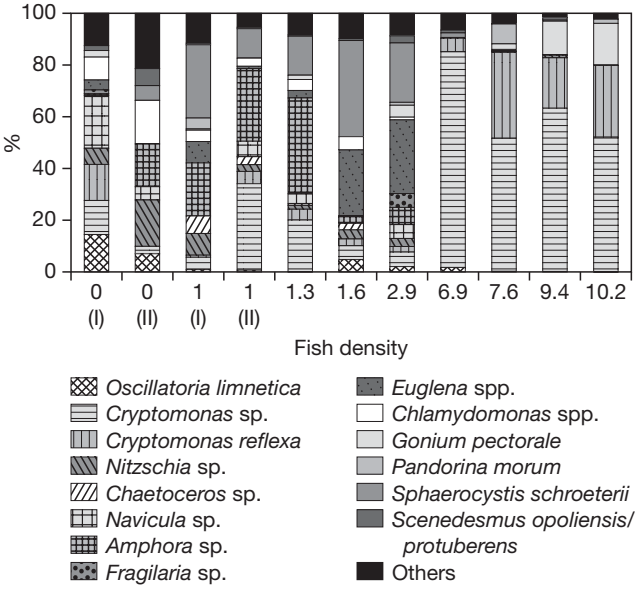

Fig. 2. Relative contribution (%) of major phytoplankton taxa at different average stickleback densities. Only species contributing more than 5% of the total biovolume at any sampling date are depicted, the rest are categorised as 'Others'. Note 2 enclosures with 0 and 1 stickleback m^{-2} (I and II)

density (here >3 to 6 sticklebacks m^{-2}) led to increased biovolume of phytoplankton. The observed disappearance of large-bodied zooplankton (*Daphnia*) above 3 to 6 fish m^{-2} (Jakobsen et al. 2003) likely reduced zooplankton grazing pressure and allowed phytoplankton biovolume to increase.

Phytoplankton size (expressed as GALD) correlated negatively with average cladoceran size. This contrasts with several studies in freshwater lakes that indicate a positive relationship between phytoplankton mean size and cladoceran biomass and mean size (e.g. Burns 1968, Bergquist et al. 1985, Cottingham 1999), which is explained by a shift towards large grazing-resistant species at high grazing pressure. Yet, in enclosure experiments conducted in shallow freshwater lakes, small, fast-growing algae were found to predominate at high grazing pressure (Schriver et al. 1995, Jeppesen et al. 2002). A high growth rate can be an advantage at high grazing pressure, as fast growth enables the algal population to replace algae lost by grazing.

In our experiment higher fish densities resulted in lower phytoplankton diversity. The combination of low grazing pressure and excess nitrogen and phosphorus facilitated the dominance of a few small, fast-growing species, as also seen in an enclosure experiment in Lake Flakensee, Germany (Weithoff et al. 2000). However, the presence of *Daphnia* may counteract this effect (Flöder & Sommer 1999), leaving, as in our experiment, phytoplankton diversity at a high level at fish densities below 4 to 6 m^{-2}. That changes in top-down control affect phytoplankton diversity was equally found in a hypertrophic pond in Hungary, where an increase in phytoplankton diversity occurred after a fish kill incident (Borics et al. 2000). However, others have found an increase in diversity of primary producers following reduced grazing pressure in both aquatic and terrestrial ecosystems (e.g. Schmitz 2003). In contrast to the Shannon diversity, genera richness of phytoplankton was not affected by fish density in our experiment. In shallow lakes, however, enhanced grazing leading to enhanced water clarity may facilitate growth, temporarily or permanently, of species attached to substrates (sediment, plant surfaces or, as in our experiment, enclosure walls). Accordingly, the contribution of such benthic forms (e.g. *Nitzschia*, *Navicula*, *Amphora*) to the biomass was relatively high at low fish density (Fig. 2).

The phytoplankton community structure changed from a diverse assembly, as described above, to dominance by *Cryptomonas* spp. at fish densities above 3 to 6 m^{-2}. This flagellate is often numerous in slightly brackish areas, such as the Bothnian Bay (Alasaarela 1979). *Cryptomonas* is generally highly nutritious for zooplankton (Schindler 1971), but in our study their size exceeded the upper food size of the dominant rotifers (*Keratella* sp. and *Brachionus* sp., Jakobsen et al. 2003). The colony forming *Gonium pectorale* and *Pandorina morum* also responded positively to higher fish densities.

A high grazing pressure by *Daphnia* on small or readily edible algal taxa has often been shown to result in dominance of less edible, particular gelatinous, spiny or colonial forms (Gulati et al. 1982). In freshwater and slightly brackish lakes with high nutrient levels, grazing resistant algae are sometimes represented by large, colonial cyanobacteria (Cyanophyceae) (Elser & Goldman 1991, Moss 1994). However, in our experiment *Sphaerocystis schroteii* was the only grazing-resistant alga that became abundant in enclosures with high grazing pressure and its dominance was recorded on only one sampling date in the beginning of August. The lack of dominance by large cyanobacteria cannot be explained by absence from the species pool, since *Oscillatoria limnetica* and *Spirulina major* individuals were occasionally observed. More likely, these species could not escape the intense grazing pressure by *Daphnia* sp. It has been suggested that as long as the initial density of large cyanobacteria is low and *Daphnia* abundance high, *Daphnia* is able to prevent cyanobacteria blooms (Gulati et al. 2001).

In brackish lakes and lagoons, the abundance of sticklebacks is commonly high, not least in eutrophic lakes (Pont et al. 1991, Jeppesen et al. 1994). The substantial changes in phytoplankton biovolume, composition and diversity around the interval of 3 to 6 sticklebacks m^{-2} recorded in our study provide experimental evidence for the empirically based conclusion of a strong cascading top-down control of planktivorous fish on phytoplankton in eutrophic brackish lakes (Jeppesen et al. 1994, 1997b). However, the results also show that in slightly brackish lakes phytoplankton can, despite high nutrient levels, be grazed down to low concentrations if the fish population declines below a certain density; in our study below 3 to 6 sticklebacks m^{-2}.

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