The importance of phytoplankton size in mediating trophic interactions within the plankton of a southern African estuary

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Abstract

The influence of the phytoplankton size composition in mediating the trophic interactions between the bacteria, phytoplankton, microheterotrophs (<200 μm) and mesozooplankton (>200 μm) was investigated on three occasions in a warm temperate, temporarily open/closed estuary situated along the southern African coastline. Results of the investigation indicated that the microheterotrophs represented the most important consumers of bacteria and chlorophyll (chl)-*a* <5.0 μm. The low impact of the mesozooplankton on the bacteria and chl-*a* <5.0 μm during the study appeared to be related to the inability of the larger zooplankton to feed efficiently on small particles. During those periods when total chl-*a* concentration was dominated by picophytoplankton (<2.0 μm) and microphytoplankton (>20 μm), mesozooplankton were unable to feed efficiently on the chl-*a* due to feeding constraints. In response to the unfavorable size structure of the phytoplankton assemblages, mesozooplankton appeared to consume the microheterotrophs. The negative impact of the mesozooplankton on the microheterotrophs resulted in a decrease in the impact of these organisms on the bacteria and the chl-*a* <5.0 μm. This result is consistent with the predator-prey cascades. On the other hand, when the total chl-*a* was dominated by nanophytoplankton (2–20 μm), mesozooplankton were able to feed directly on the phytoplankton. Results of the study indicate that size structure of the phytoplankton assemblages within estuaries plays an important role in mediating the trophic interactions between the various components of the plankton food web.

1. Introduction

The coupling between the different trophic levels can via trophic cascading profoundly influence the plankton community structure and the rates of primary and secondary production within aquatic systems (Menge and Sutherland, 1976, Power, 1990, Lavrentyev et al., 1997, Pace et al., 1998, Jurgens and Jeppesen, 2000, Froneman, 2002c and Froneman and Bernard, 2004). For example, by feeding on protozooplankton, metazoans (particularly copepods) decrease in the feeding impact of these organisms on the small phytoplankton and bacteria (Calbet and Landry, 1999 and Froneman, 2002c). The metazoans may further benefit the growth of the bacteria and phytoplankton through nutrient excretion (Calbet and Landry, 1999). In those regions where the phytoplankton are too small to be directly utilized by the crustacean dominated metazoans (e.g. oligotrophic regions of the worlds oceans), omnivory prevails (Stoecker and Capuzzo, 1990, Gifford, 1991, Gifford and Dagg, 1991 and Froneman, 2002a). Here, the effects of trophic cascading on the lower trophic levels are expected to be substantial. On the other hand, where the phytoplankton are readily available to the crustaceans (e.g. eutrophic regions), the extent of omnivory is reduced with the subsequent decrease in the trophic cascading. The phytoplankton size composition thus plays an important role in determining the trophic interactions within aquatic food webs.

Increased population growth coupled with industrialization has coincided with a decrease in the magnitude of freshwater flowing into southern African estuaries. The influence of the reduced freshwater inflow on the biology of southern African estuaries is now well documented (Adams et al., 1999 and Wooldridge, 1999). Reduced freshwater inflow into estuaries has been linked to a decrease in the phytoplankton size composition and daily production rates largely because freshwater inflow represents the primary source of nutrients necessary to sustain the growth of the phytoplankton, particularly diatoms (Allanson and Read, 1987, Allanson and Read, 1995, Campbell et al., 1993 and Adams et al., 1999). In the absence of continuous freshwater inflow into estuaries, total

daily phytoplankton production is low and is typically dominated by small (<20 μm) phytoplankton cells (Adams et al., 1999 and Froneman, 2002b). Among the heterotrophic components of the estuarine food web, the alteration in the riverine inflow into estuaries has been linked to changes in the recruitment, biomass, species composition and distribution patterns of both invertebrates (Hodgson, 1987 and Wooldridge, 1999) and vertebrates (Vorwerk et al., 2003).

It is now well established that the size composition of the phytoplankton community plays an important role in the feeding ecology of the dominant zooplankton (mainly copepods of the genera *Acartia* and *Pseudodiaptomus*) in southern African estuaries (Froneman, 2000 and Froneman, 2004). Under conditions of sustained freshwater inflow, phytoplankton derived carbon is often sufficient to meet the basic metabolic requirements of the zooplankton (Froneman, 2000 and Froneman, 2004). Conversely in freshwater deprived systems, the phytoplankton are largely unavailable to direct utilization because of size constraints of feeding (Froneman, 2000, Froneman, 2002a and Froneman, 2004). Nonetheless, production within the phytoplankton is made available to the larger zooplankton through trophic intermediates represented by microheterotrophs (<200 μm) (Froneman, 2002b).

In a study conducted in the freshwater deprived Kariega Estuary (south-east coast of southern Africa), Froneman (2002b) demonstrated that by feeding on microheterotrophs, the mesozooplankton promoted the growth of both the bacteria and the small phytoplankton (<2.0 μm). This result was consistent with the expectations of predator-prey cascades (Pace et al., 1998). To the author's knowledge, there have been no further studies on the impact of the phytoplankton size community structure on the trophic interactions among the plankton in southern African estuaries. The absence of these studies is surprising as future developments within the region is likely to exacerbate the decline in freshwater inflow into estuaries which concurrent effects on the biology within these systems. This study was designed to investigate the influence of varying phytoplankton sizes on the trophic interactions among the various components of the plankton assemblage in a southern African estuary.

2. Materials and methods

2.1. Study site

The Kasouga Estuary (33°39′S; 26°44′E) is classified as a small temporarily open/closed estuary (Whitfield, 1992; Fig. 1). The estuary has a surface area of approximately 0.22 km^2 excluding the shallow salt marsh areas, which are only inundated during periods of high water levels. The estuary is navigable for approximately 2.5 km and the widest portion is about 150 m (Froneman, 2002b). The system is mostly shallow, with the main channel depth varying between 0.5 and 1.5 m. The catchment area of the Kasouga Estuary is estimated at 39 km^2. Most of the catchment area is used primarily for cattle farming. The nearby stream and river valleys within the catchment area are, however, relatively undisturbed and covered by Valley Bushveld vegetation. Depending on the amount of rainfall that has occurred within the catchment region, average monthly water temperatures and salinities in the Kasouga Estuary range from 10 °C to 30 °C and from 0 to 40 (salinity practical units), respectively. Mouth opening events occur during or shortly after periods of high rainfall (usually in months with sustained rainfall exceeding 100 mm). The estuary mouth rapidly closes off from the sea due to extensive sandbar development caused by long-shore drift. During the subsequent closed period, seawater inflow into the estuary occurs only during peak spring tides and during severe storms.

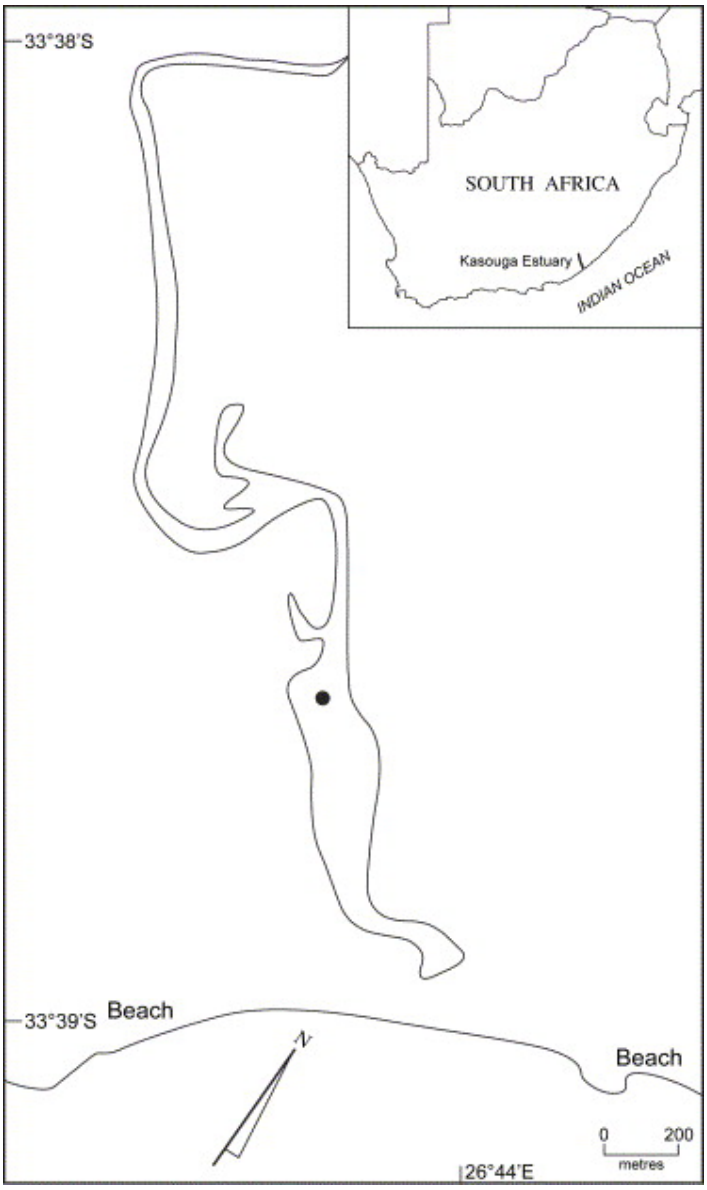

Fig. 1. Position of biological station occupied within the middle reaches of the temporarily open/closed Kasouga estuary.

The trophic interactions between the bacteria, phytoplankton, microheterotrophs (<200 μm) and mesozooplankton (>200 μm) were investigated during three surveys according to the method of Calbet and Landry (1999). The study was conducted in summer to negate possible seasonal effects.

2.2. Determination of in situ chlorophyll-a (chl-a) concentration

The size fractionated chl-*a* concentration was determined on each occasion from a 250 ml seawater sample obtained from the surface (depth ± 0.5 m). Only surface samples were employed as previous studies have shown that the water column of the estuary is well mixed with no clear stratification present (Froneman, 2002a, Froneman, 2002b and Froneman, 2004). The sample was serially filtered (vacuum <5 cmHg) through 20.0 μm, 2.0 μm and GF/F filters for the determination of the micro-, nano and picophytoplankton chl-*a* concentrations. Filters were placed in 8 ml of 90% acetone and stored at −20 ° C in the dark for 24 h (Froneman, 2002a). After centrifugation (5000 rpm) the chl-*a* concentration was measured using a Turner Designs 10AU Fluorometer according to the method of Holm-Hansen and Riemann (1978). Three replicates were prepared for each size fraction on each occasion.

2.3. Bacterivory by heterotrophs

Surface water (depth 0.5 m) was collected at night using a 5-L Niskin bottle. For the grazing experiments, 5-L water samples were gently prescreened through a 2000-μm filter to isolate the mesozooplankton, microheterotrophs and bacteria. A further three 5-L water samples were prescreened through a 200-μm filter to isolate the microheterotrophs and bacteria. For the control ($n = 3$) water collected was gently (<1 cmHg) passed through a 2.0-μm filter to exclude the majority of grazers. Three replicates were prepared for each treatment. The flasks were then incubated in situ for 24 h at depth of collection. Before and after incubation, a 2-ml water sample was taken from each flask and preserved in 2.5% glutaraldehyde (final concentration 2.5%). In the laboratory, samples were stained with Acridine Orange (AO) then gently filtered through a 0.2-μm Irgalan Black Nucleopore filter. Bacterial densities were then estimated using a Zeiss epifluorescent microscope fitted with an exciter filter (BP 490), a blue excitation filter combination (Dichronic mirror with a built in barrier filter 0–515) and an additional filter (0–530) and operated at 1000× magnification (Turley, 1993). A minimum of 50 fields or 1000 cells was counted for each sample. Bacterial numbers were then estimated using the equation of Turley (1993).

2.4. Direct feeding interactions

To assess direct feeding relationships, water was collected at night and stored in a 100-L polyethylene carboy. Mesozooplankton were collected from the surface waters (depth 1 m) using a WP-2 net (mesh size 60 μm; nominal mouth area 0.2 m^2) fitted with a 5-L cod end. The zooplankton collected were carefully transferred into a 10-L container containing natural seawater. A one tenth subsample was then transferred to the 100-L polyethylene carboy. Densities of the mesozooplankton within the carboy were within one order of magnitude of natural densities (Froneman, unpublished data). Samples of water from the 100-L carboy were sieved through 200 μm, and 2000 μm mesh to obtain fractions that contained phytoplankton and microheterotrophs (<200 μm), phytoplankton, microheterotrophs and mesozooplankton. Samples ($n = 3$ for each treatment) were then incubated in 5-L polyethylene containers. Densities of the various size classes of plankton during the three surveys are shown in Table 1. Finally, additional three-polyethylene containers were filled with water that had been gently (vacuum <2 cmHg) passed through a 30-μm mesh to assess the growth of phytoplankton without grazers (control). Preliminary study indicated that the filtering of the water through a 30-μm mesh removed <75% of all heterotrophic organisms and <20% of the total chl-*a* (unpublished data). Treatments and controls were then incubated in situ for between 20 and 24 h in an incubator cooled with running surface water. Samples (200 ml) for the determination of total chl-*a* concentration and the concentration of the <5.0 μm chl-*a* fraction were taken from each bottle at the beginning and end of the incubations. Chlorophyll-*a* concentrations were determined fluorometrically as described above. Net instantaneous growth rates of the chl-*a* in the various treatments were estimated from the initial and final concentrations assuming exponential change during the incubations (Calbet and Landry, 1999).

Table 1.

Summary of the environmental and biological variables in the Kasouga estuary during the three trophic cascading experiments. Values in parentheses are standard deviations

Experiment	Temperature (°C)	Salinity	Depth (m)	Bacterial densities ($\times 10^5$ cells ml^{-1})	Microheterotroph densities ($\times 10^3$ cells L^{-1})	Mesozooplankton densities ($\times 10^3$ ind m^{-3})
September	21.5	15	1.5	1.72 (±0.21)	6.97 (±0.97)	9.38 (±0.58)
October	24.6	25.5	1.4	1.29 (±0.37)	5.84 (±0.58)	6.04 (±0.39)
November	26.2	32.5	1.5	1.29 (±0.59)	6.08 (±0.49)	6.89 (±0.29)

2.5. Cascading effects of zooplankton grazing

To assess the cascading effects of mesozooplankton grazing on the lower levels of the food web, the net growth response of the bacteria, <5.0 μm chl-*a* fraction, total chl-*a* and the protozooplankton to increasing concentrations of mesozooplankton was investigated. The experimental setup was similar to that described above. Aliquots of mesozooplankton were added to three replicate bottles per treatment; in addition, three controls were prepared without animals. For the determination of mesozooplankton biomass, a 250-ml water sample was taken from each bottle for later assessment of dry weight of the added zooplankton. Incubations were conducted over a period of 19–24 h in situ at depth of collection. At the beginning and end of the experiment, aliquots of water were taken from each bottle for bacterial counts and for the determination of the concentrations of the two chl-*a* fractions and the microheterotroph densities. Bacterial counts and chl-*a* concentrations were determined as described above. For the determination of protozooplankton densities, 50-ml samples were fixed in glutaraldehyde and stained with Proflavine (40 μl ml^{-1} for 3 min) and gently filtered through a 0.2-μm pre-stained Irgalan Black Nucleopore filter (Froneman and Perissinotto, 1996). Permanent slides were then prepared according to the method of Booth (1978). Slides were then examined within 1 month of preparation using a Zeiss Fluorescent microscope equipped with a 450–490 excitation filter, a FT 510 chromatic beam splitter and a long-pass 528 barrier filter (Haas, 1982). Phototrophic plankton were distinguished from heterotrophic forms by the red auto-florescence of chl-*a*. A minimum of 500 cells was counted for each sample.

Net instantaneous growth rates of the bacteria, chl-*a*, and microheterotrophs were estimated from the initial and final concentrations assuming exponential change during the incubations. The net growth rates of the various components of the plankton versus the changing biomass of mesozooplankton were then fit to linear regression using the Statistical Graphics computer program, Version 5.0.

2.6. Statistical analyses

A Kruskal–Wallis one-way analysis of variance by ranks test was employed to test for differences in the growth rates of the bacteria and size fractionated chl-*a* in the various treatments during the study. Where significant differences were detected, a Mann–Whitney *U*-test was used to identify the sources of variation. The analyses were conducted using the software package Statistica, version 6.0.

3. Results

3.1. Trophic environment

Summary of the environmental variables during the study is shown in Table 1. Water temperatures during the three experiments ranged between 21.5 and 26.2 °C and salinity (salinity practical units) between 15 and 32.5. A clear temporal pattern in salinity and water temperature was observed with the lowest values recorded during the first experiment conducted in September and the highest during November (Table 1). Intermediate values were recorded during the survey conducted in October (Table 1).

3.2. In situ chl-a concentrations

Mean total chl-*a* concentration during the three experiments ranged between 1.49 and 7.72 mg chl-*a* m^{-3} (Fig. 2). In September 2002, total chl-*a* concentration was dominated by the large microphytoplankton (>20 μm), which comprised on average 63% (SD ±11%) of the total pigment. The nanophytoplankton fraction (2–20 μm) was identified as the second largest contributor to total pigment (mean contribution = 26%, SD ±16% of the total), followed by the picophytoplankton. The contribution of the picophytoplankton to the total pigment was always <10% (Fig. 2).

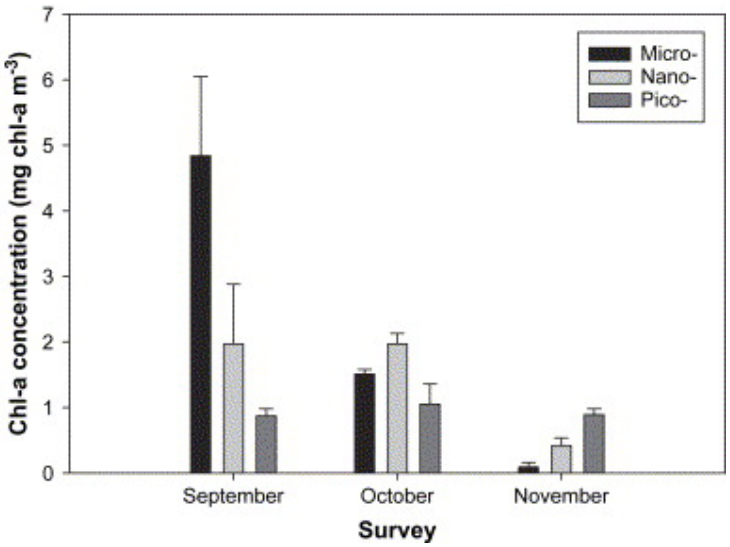

Fig. 2. Size fractionated chlorophyll-*a* concentrations during the three trophic cascading experiments conducted in the temporarily open/closed Kasouga estuary.

During the second survey conducted in October 2002, the nanophytoplankton and the microphytoplankton were identified as the largest and second largest contributors to total chl-*a* concentration. Average concentration of nanophytoplankton was estimated at 1.98 (SD ±0.16) mg chl-*a* m^{-3} and the microphytoplankton concentration, 1.51 (SD ±0.07) mg chl-*a* m^{-3}. The contribution of picophytoplankton to total chl-*a* was always <25% (Fig. 2). In November 2002, total chl-*a* concentration was dominated by the picophytoplankton that comprised on average 64% (SD ±13%) of the total pigment concentration. Concentration of the nanophytoplankton during the survey ranged between 0.30 and 0.53 mg chl-*a* m^{-3} or between 21% and 39% of the total pigment. The contribution of the microphytoplankton was always <10% of the total pigment.

The total chl-*a* concentration in September was significantly higher than the values obtained during the October and November surveys ($P < 0.05$ in both cases). Total chl-*a* concentrations between October and November surveys were not significantly different from one another ($P > 0.05$).

3.3. Bactivory by heterotrophs

In the absence of any grazers (water prescreened through 2.0 μm filter), net growth of the bacteria was always positive and ranged between 0.69 and 1.32 d^{-1} (Fig. 3). There were no significant differences in the growth rates of the bacteria in the absence of grazers during the three surveys ($H = 6.16$, $P > 0.05$). In the microheterotroph treatment (<200 μm), net growth of the bacteria ranged between 0.42 and 0.83 d^{-1} and between 0.78 and 1.36 d^{-1} in the mesozooplankton (>200 μm) treatment (Fig. 3). There were no significant differences in the estimated growth rates of the bacteria in the three treatments during the study ($H = 3.45$; $H = 2.22$; $H = 5.06$; $P > 0.05$ in all cases).

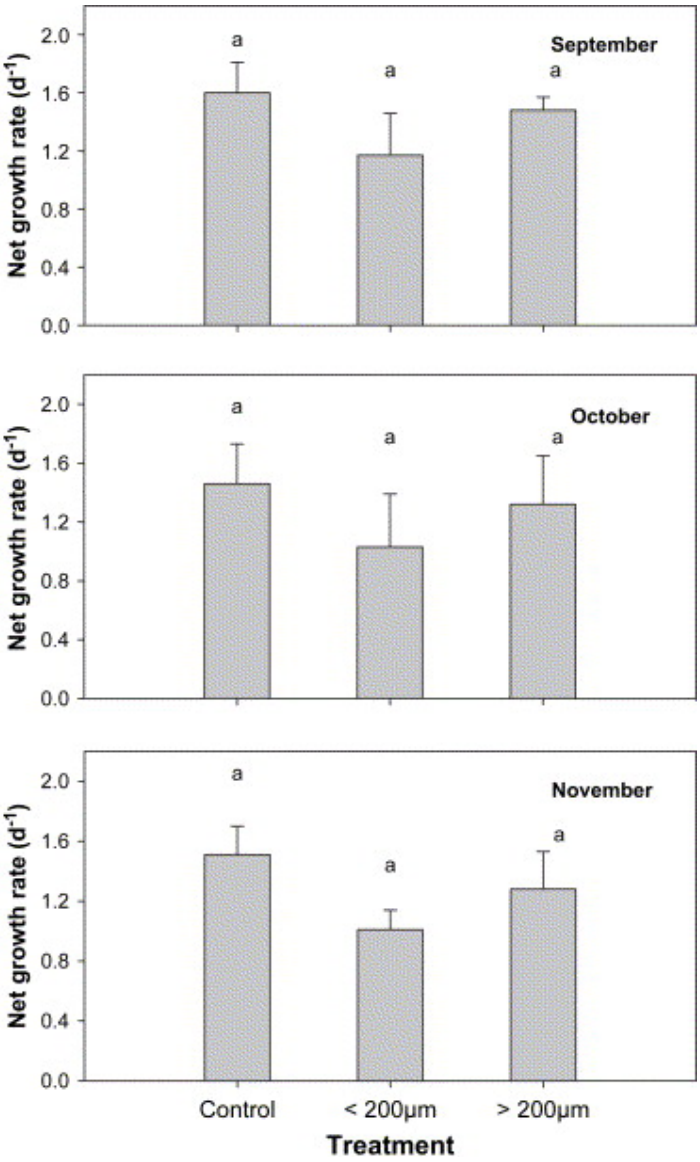

Fig. 3. Net growth of the bacteria in various treatments conducted during the three surveys. Vertical bars show standard deviation of three replicates for each treatment. Different letters indicate significant difference (Mann–Whitney *U*-test performed after a Kruskal–Wallis one-way analysis of variance by ranks test; $P < 0.05$ in all cases).

3.4. Direct feeding experiments

The initial grazing experiments conducted with the microheterotrophs (<200 μm) and mesozooplankton are shown in Fig. 4. In all control experiments (water prescreened through 30 μm screen) where the majority of the grazers had been excluded, the net growth of the chl-*a* <5.0 μm and total chl-*a* was positive and ranged between 0.29 and 0.32 d^{-1} and between 0.19 and 0.48 d^{-1}, respectively (Fig. 4). Differences in the estimated growth rates of the of <5.0 μm and total chl-*a* fractions during the three surveys were not significant ($P > 0.05$ in all cases). The estimated growth rate of total chl-*a* in September was significantly higher than the values obtained during October and November ($P < 0.05$ in both cases). The growth rates of total chl-*a* were not significant different between the October and November surveys ($P > 0.05$). The response of the <5.0 μm and total chl-*a* fractions did, however vary according to survey and treatment. In the microheterotroph treatment, the net growth of the <5.0 μm chl-*a* fraction although variable, was always negative and varied between −0.01 and −0.10 d^{-1}. In the experiment conducted in September, the net growth of total chl-*a* was positive (0.30 d^{-1}) while in experiments conducted in October and November, the net growth of the total chl-*a* was negative, estimated at −0.18 and

−0.09 d^{-1}, respectively (Fig. 4). The growth rates of <5.0 μm chl-*a* fraction and total chl-*a* in the microheterotroph treatment were significantly different from one another during the September survey ($P < 0.05$). There were no significant differences in growth rates of the two chl-*a* fractions in the <200 μm treatment during the October and November surveys ($P > 0.05$ in both cases).

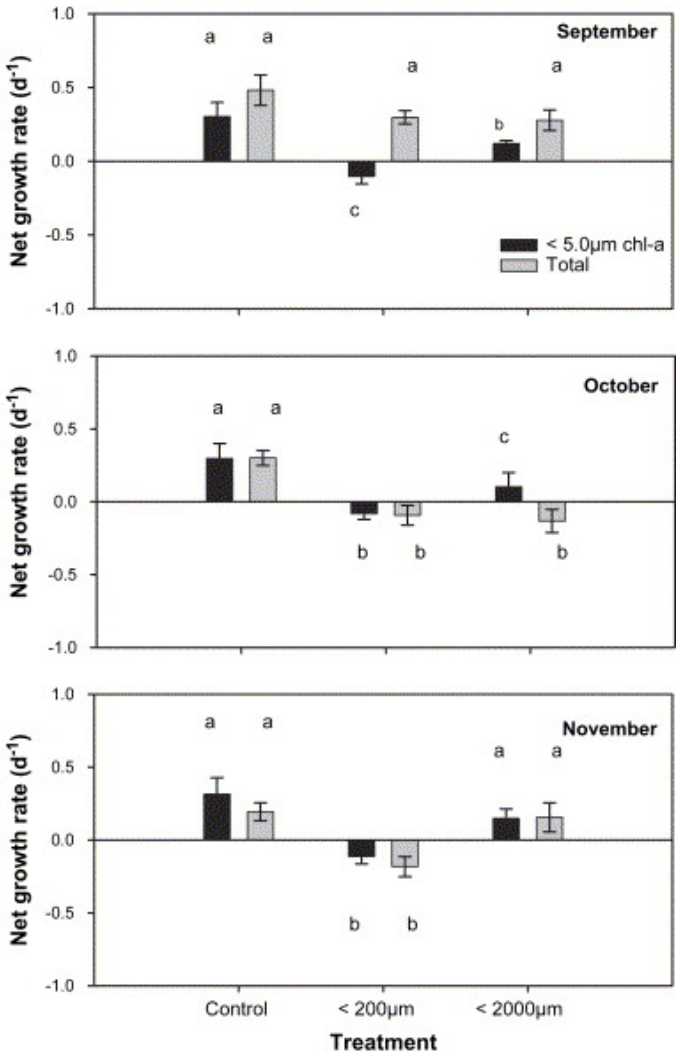

Fig. 4. Impacts of the protozooplankton and mesozooplankton on the net growth rate of the chlorophyll-*a* <5.0 μm and total chlorophyll concentration during the three surveys. Vertical bars show standard deviation of three replicates for each treatment. Different letters indicate significant difference (Mann–Whitney *U*-test performed after a Kruskal–Wallis one-way analysis of variance by ranks test; $P < 0.05$ in all cases).

In all cases, the net growth rate of the <5.0 μm chl-*a* fraction and total chl-*a* in the mesozooplankton treatment was positive An exception was recorded in October, where the net growth of the total chl-*a* was negative, estimated at −0.13 d^{-1} (Fig. 4).

3.5. Cascading experiments

The results of the trophic cascading experiments and the regression coefficients of the linear regression analyses during the three experiments are shown in Table 2. The projected growth rates of the bacteria, the chl-*a* <5.0 μm, total chl-*a* and protozooplankton in the absence of mesozooplankton (i.e. the *Y* intercept of the regression equations) although variable, were always positive (Table 2). The addition of mesozooplankton during all three experiments resulted in a net increase in the growth rate of the bacteria and the chl-*a* <5.0 μm (Table 2). In contrast, the net growth rate of both the total chl-*a* and protozooplankton decreased in response to an increase in the mesozooplankton biomass (Table 2). Net growth of the total chl-*a* during the experiments ranged between −0.08 and −0.11 d^{-1} and the protozooplankton between −0.06 and −0.16 d^{-1}, respectively (Table 2).

Table 2.

Linear regression parameters and coefficients of determination (r^2) of experiments conducted to assess trophic cascades in the temporarily open/closed Kasouga estuary during three phases

Parameter	Intercept	Slope	r^2	*P* value
September				
Bacteria	1.05	0.13	0.67	<0.05
Chl-*a* <5.0 μm	0.24	0.003	0.50	<0.05
Total chl-*a*	0.33	−0.10	0.87	<0.001
Microheterotrophs	0.37	−0.06	0.85	<0.05
October				
Bacteria	0.89	0.14	0.79	<0.01
Chl-*a* <5.0 μm	0.22	0.02	0.51	<0.05
Total chl-*a*	0.19	−0.08	0.91	<0.001
Microheterotrophs	0.19	−0.07	0.89	<0.05
November				
Bacteria	1.03	0.08	0.81	<0.05
Chl-*a* <5.0μm	0.31	0.04	0.10	<0.05
Total chl-*a*	0.20	−0.11	0.81	<0.05
Microheterotrophs	0.35	−0.16	0.96	<0.001

4. Discussion

In agreement with a number of previous studies conducted in a variety of southern African estuaries (Allanson and Read, 1987, Allanson and Read, 1995, Campbell et al., 1993, Adams et al., 1999 and Froneman, 2002b), the phytoplankton growth rates and community structure within the Kasouga estuary was strongly linked to the inflow of freshwater into the system. The estimated net growth rates of the total chl-*a* in September following freshwater inflow into the estuary (as evident from the reduced salinity values) were significantly higher ($P < 0.05$) that those recorded during the experiments where little or no freshwater inflow into the estuary occurred. The elevated growth rates during September could largely be attributed to the high contribution of the larger microphytoplankton to total chl-*a* concentration (Fig. 2) The reduction in the freshwater inflow into the estuary coincided with a decrease in growth rates of the phytoplankton and an increase in the contribution of the small phytoplankton cells (<20 μm) to the total phytoplankton biomass. Indeed, in the absence of freshwater inflow into the estuary, total phytoplankton biomass was almost entirely dominated by small picophytoplankton (Fig. 2). The observed pattern can likely be linked to decreased availability of macronutrients reflecting varying freshwater inflow into the estuary (Campbell et al., 1993, Adams et al., 1999 and Froneman, 2002b).

It is now well established that microheterotrophs (<200 μm) can be a significant source of mortality for suspended bacteria in both marine and freshwater ecosystems (see review by Sherr and Sherr, 2002). The decrease in the net growth of the bacteria in the <200 μm treatment is thus, not unexpected (Fig. 3). The primary bacterivores in aquatic systems are small heterotrophic nanoflagellates, generally <5.0 μm in size (Sherr and Sherr, 2002). During the present study, no distinction was made between the heterotrophic nanoflagellates (2–20 μm) and the larger microheterotrophs (20–200 μm) within the protozooplankton community. The presence of the larger microheterotrophs within the <200 μm treatment may account for the lack of any significant difference in the estimated growth rate of the bacteria between the controls and the <200 μm treatments ($P > 0.05$ in all cases). Alternatively, the lack of a significant difference between the control and <200 μm treatment may reflect the presence of small quantities of small heterotrophs that may have squeezed through the 2 μm filter during the preparation of the controls. Although a study conducted in the oligotrophic Pacific Ocean suggested that nutrient excretion by mesozooplankton might stimulate the growth of bacteria (Calbet and Landry, 1999), no such response was observed during the present study (Fig. 3). These data suggest that the growth of the bacteria was not substrate limited in the Kasouga estuary during the present study. Finally, it is apparent that the mesozooplankton were able to release the bacteria from the predation impact of the microheterotrophs as evident from the elevated growths rates within the treatment (Fig. 3).

The net growth of the phytoplankton <5.0 μm in the microheterotroph treatment was negative during the three experiments (Fig. 4). A number of studies have shown that microheterotrophs (<200 μm) represent the most important consumers of phytoplankton production in regions where small phytoplankton cells dominated total phytoplankton biomass (Froneman and Perissinotto, 1996, Sherr and Sherr, 2002 and Froneman, 2004). The high impact of these organisms can be related to the predominance of small phytoplankton cells (mainly picophytoplankton) within the region, which are too small to be grazed efficiently by the larger metazoans (copepods) (Fortier et al., 1994 and Hansen et al., 1994). Laboratory and field studies of microheterotroph grazing dynamics suggest that they preferentially graze on particles <20 μm (Hansen et al., 1994). The decrease in the net growth rates of total chl-*a* during the October and November experiments thus reflects the availability of the optimum size particle for the microheterotrophs (Fig. 4B,C). Conversely, the increase in the net growth rate of the total chl-*a* treatment during the survey conducted in September can be attributed to the inability of microheterotrophs to feed on large microphytoplankton which dominated total phytoplankton biomass (Fig. 4A).

Analysis of the literature indicates that copepods preferentially consume particles in the size range 5–20 μm (Fortier et al., 1994). The increase in the net growth of total chl-*a* during September can thus be attributed to the inability of copepods to consume large microphytoplankton, which dominated the total chl-*a* concentration (Fig. 4). Similarly during the November experiment when total chl-*a* concentration was dominated by the picophytoplankton, much of the total chl-*a* was unavailable for direct utilization by the copepods. Not surprising, therefore, was that when the nanophytoplankton dominated the total chl-*a*, the copepods were able to consume the available water column chl-*a*. This resulted in a net decrease in the growth rate of the total chl-*a*.

The increase in the mesozooplankton biomass during the trophic cascading experiments resulted in a net increase in the growth rates of the bacteria and the chl-*a* <5 μm coupled with a net decrease in the growth rates of the total chl-*a* and microheterotrophs (Table 2). Carnivory by metazoans is most prevalent when the phytoplankton are largely unavailable to direct utilization (Stoecker and Capuzzo, 1990). The increase in the net growth rate of the bacteria and the chl-*a* <5.0 μm can likely be attributed to the predation impact of larger mesozooplankton on the microheterotrophs. This would have the net effect of reducing the grazing impact of the protozooplankton on the bacteria and the smaller chl-*a* fraction. Alternatively, the increase in the growth rate of the smaller phytoplankton may result from nutrient excretion by the metazoans during the incubations, which would stimulate the growth of the phytoplankton (Calbet and Landry, 1999). It is worth noting that results of the trophic cascading experiments suggest that the mesozooplankton readily consume microheterotrophs even in instances where the available chl-*a* is within the size range readily consumed by mesozooplankton (e.g. during the October experiment). Analysis of the literature suggests that protozooplankton may be a particularly rich source of proteins, amino acids and essential lipids when compared to phytoplankton and detritus (see reviews by Stoecker and Capuzzo, 1990 and Klein Breteler et al., 1999). The mesozooplankton may therefore preferentially consume microheterotrophs to gain metabolic or reproductive benefits (Klein Breteler et al., 1999).

Results of trophic cascading experiments indicate that the size structure of the phytoplankton assemblage has important implications for the trophic interactions within the plankton food webs of southern African estuaries. In those systems where small phytoplankton cells dominate total production (i.e. freshwater deprived estuaries), complex foods web prevail largely as a result of much of the phytoplankton production not being available to the larger grazers. Nonetheless, the production is made available to the larger zooplankton through trophic intermediates represented by the protozooplankton. The complex food webs that persist within these systems will probably be coupled with a reduction in the trophic efficiency. On the other hand, in those systems characterized by freshwater inflow, the phytoplankton are readily consumed by copepods. These short food webs would be able to sustain high levels of secondary and tertiary production.

Acknowledgments

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References

Adams et al., 1999 J. Adams, G. Bate and M. O'Callagan, Estuarine microalgae. In: B.R. Allanson and D. Baird, Editors, *Estuaries of South Africa*, Cambridge University Press, Cambridge, UK (1999), pp. 91–100.

Allanson and Read, 1987 B.R. Allanson and G.H.L. Read, The response of estuaries along the southeastern coast of South Africa to marked variation in freshwater inflow, Institute for Water Research, report # 2/87, Rhodes University (1987) 67 pp.

Allanson and Read, 1995 B.R. Allanson and G.H.L. Read, Further comment on the response of Eastern Cape province estuaries to variable freshwater inflows, *South African Journal of Aquatic Science* 21 (1995), pp. 56–70.

Booth, 1978 B.C. Booth, The use of epifluoresence for analysing oceanic phytoplankton communities, *Botanica Marina* 30 (1978), pp. 101–108.

Calbet and Landry, 1999 A. Calbet and M.R. Landry, Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean, *Limnology and Oceanography* 44 (1999) (6), pp. 1370–1380.

Campbell et al., 1993 E.E. Campbell, W.T. Knoop and C.G. Bate, A comparison of phytoplankton biomass and primary production in three eastern Cape estuaries, *South African Journal of Aquatic Science* 87 (1993), pp. 259–264.

Froneman, 2000 P.W. Froneman, Feeding studies of selected zooplankton in a temperate estuary, South Africa, *Estuarine, Coastal and Shelf Science* 51 (2000), pp. 543–552.

Froneman, 2002a P.W. Froneman, Trophic cascading in an oligotrophic temperate estuary, South Africa, *Journal of Plankton Research* 24 (2002), pp. 807–816.

Froneman, 2002b P.W. Froneman, Response of the biology to three different hydrological phases in the temporarily open/closed Kariega estuary, *Estuarine, Coastal and Shelf Science* 55 (2002) (4), pp. 535–546.

Froneman, 2002c P.W. Froneman, Seasonal variations in selected physico-chemical and biological variables in the temporarily open/closed Kasouga estuary (South Africa), *African Journal of Aquatic Science* 27 (2002), pp. 117–123.

Froneman, 2004 P.W. Froneman, Food web dynamics in a temperate temporarily open/closed estuary (South Africa), *Estuarine, Coastal and Shelf Science* 59 (2004), pp. 87–95.

Froneman and Bernard, 2004 P.W. Froneman and K.S. Bernard, Trophic cascading in the Polar Frontal Zone of the Southern Ocean in austral autumn 2002, *Polar Biology* 27 (2004), pp. 112–118.

Froneman and Perissinotto, 1996 P.W. Froneman and R. Perissinotto, Microzooplankton grazing and protozooplankton community structure in the South Atlantic and in the Atlantic sector of the Southern Ocean, *Deep-Sea Research I* 43 (1996) (5), pp. 703–721.

Fortier et al., 1994 L. Fortier, J. Le Fevre and L. Legendre, Export of biogenic carbon to fish and the deep ocean: the role of large planktonic microphages, *Journal of Plankton Research* 16 (1994), pp. 809–839.

Gifford, 1991 D.J. Gifford, The protozoan-metazoan trophic link in pelagic ecosystems, *Journal of. Protzooplankton* 38 (1991) (1), pp. 81–86.

Gifford and Dagg, 1991 D.J. Gifford and M.J. Dagg, The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchurus* Murukawa, *Marine Microbial Food Webs* 5 (1991) (1), pp. 161–177.

Haas, 1982 L.W. Haas, Improved epifluorescent microscopy for observing planktonic microorganisms, *Annales de l'Institute Oceanographique* 58 (1982), pp. 261–266.

Hansen et al., 1994 B. Hansen, P.K. Bjornsen and P.J. Hansen, The size ratio between planktonic predators and their prey, *Limnology and Oceanography* 39 (1994), pp. 395–403.

Hodgson, 1987 A.N. Hodgson, Distribution of macrobenthic fauna of the Kariega Estuary, *South African Journal of Zoology* 22 (1987), pp. 153–162.

Holm-Hansen and Riemann, 1978 O. Holm-Hansen and B. Riemann, Chlorophyll-*a* determination: improvements in methodology, *Oikos* 30 (1978), pp. 438–447.

Jurgens and Jeppesen, 2000 K. Jurgens and E. Jeppesen, The impact of metazooplankton on the structure of the microbial food web in a shallow hypertrophic lake, *Journal of Plankton Research* 22 (2000), pp. 1047–1070.

Klein Breteler et al., 1999 W.C.M. Klein Breteler, N. Schogt, M. Bass, S. Schouten and G.W. Kraay, Trophic upgrading of food quality by protozoans enhancing growth: role of essential lipids, *Marine Biology* 135 (1999), pp. 191–198.

Lavrentyev et al., 1997 P.J. Lavrentyev, W.S. Gardner and J.R. Johnson, Cascading trophic effects on aquatic nitrification: experimental evidence and potential implications, *Aquatic Microbial Ecology* 13 (1997), pp. 161–175.

Menge and Sutherland, 1976 B.A. Menge and J.P. Sutherland, Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity, *American Naturalist* 110 (1976), pp. 351–369.

Pace et al., 1998 M.L. Pace, J.J. Cole and S.R. Carpenter, Trophic cascades and compensation: differential responses of microzooplankton in whole-lake experiments, *Ecology* 79 (1998) (1), pp. 138–152.

Power, 1990 M.E. Power, Effects of fish in river food webs, *Science* 250 (1990), pp. 811–814.

Sherr and Sherr, 2002 B. Sherr and E. Sherr, Significance of predation by protests in aquatic microbial food webs, *Antonie van Leeuwenhoek* 81 (2002), pp. 293–308.

Stoecker and Capuzzo, 1990 D.K. Stoecker and J.M. Capuzzo, Predation on protozoa: its importance to zooplankton, *Journal of Plankton Research* 1 (1990), pp. 891–908.

Turley, 1993 C.M. Turley, Direct estimates of bacterial numbers in seawater samples without incurring cell loss due to storage. In: P.E. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole, Editors, *Handbook of Methods in Aquatic Microbial Ecology*, Lewis publishers, Boca Raton, FL (1993), pp. 143–147.

Vorwerk et al., 2003 P.D. Vorwerk, A.K. Whitfield, P.D. Cowley and A.W. Paterson, The influence of selected environmental variables on fish assemblage structure in a range of southeast African estuaries, *Environmental Biology of Fishes* 66 (2003), pp. 237–247.

Whitfield, 1992 A.K. Whitfield, A characterization of southern African estuarine systems, *South African Journal of Science* 18 (1992), pp. 89–103.

Wooldridge, 1999 T. Wooldridge, Estuarine zooplankton community structure and dynamics. In: B.R. Allanson and D. Baird, Editors, *Estuaries of South Africa*, Cambridge University Press, Cambridge, UK (1999), pp. 141–166.