



In situ enclosure experiments on the influence of cultured mussels (*Perna canaliculus*) on phytoplankton at times of high and low ambient nitrogen

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Abstract

The influence of the cultured mussel *Perna canaliculus* (Gmelin 1791) on the abundance of phytoplankton was investigated in Pelorus Sound, New Zealand. Four in situ enclosure experiments were undertaken, two in summer when ambient nitrogen was low, and two in winter when it was high. Each experiment had four manipulation types: added mussels; added nitrogen; both mussels and nitrogen added; and control (no additions). In summer, there was a significant increase of chlorophyll *a* in response to added nitrogen, indicating that the phytoplankton were nitrogen-limited. At this time, mussels caused an increase (11–17%) in phytoplankton biomass, possibly by converting particulate nitrogen to ammonium, making the nitrogen available for phytoplankton utilisation. The highest ambient chlorophyll *a* concentrations coincided with high ambient nitrogen in the winter. At this time, mussel grazing caused a significant decrease (5–14%) in phytoplankton concentration, indicating that within-farm depletion of phytoplankton is most likely to occur in winter. On an annual time scale, the mussels had a stabilising influence on phytoplankton biomass, reducing high ambient levels in winter and slightly increasing low levels in summer.

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1. Introduction

Cultivation of the mussel *Perna canaliculus* has been practised in New Zealand for over 30 years. The majority of farms are situated in the Marlborough Sounds, at the northern end of South Island. The longline method of cultivation is used, whereby mussels are grown on ropes that hang down into the water column from surface floats (Hickman, 1989). Filter-feeders such as mussels can have a significant reducing impact on primary production in aquatic systems (Officer et al., 1982; Frechette et al., 1989; Reeders and Bij de Vaate, 1990; Alpine and Cloern, 1992; MacIsaac et al., 1992). The majority of studies on the relationship between bivalves and primary production have been undertaken with bivalves in a benthic environment. Such studies have shown that bivalves can cause a reduction of phytoplankton biomass (e.g. Dame et al., 1989). However, some studies have also shown that bivalves can promote primary production (e.g. Asmus and Asmus, 1991), perhaps by converting particulate nitrogen into dissolved inorganic nitrogen, and hence making it available for phytoplankton utilisation.

Cultivation of mussels on longlines moves the mussels from the benthic environment to the pelagic environment. Hence, there are potentially quantitative differences in mussel–primary producer interactions due to the pelagic environment having: (1) a more open 3-dimensional structure which ought to result in less tendency for boundary-layer depletion of phytoplankton; (2) more scope for phytoplankton being nutrient limited because the culture system, at the surface of a 30-m water column, is more likely to be isolated from sediment nutrients; and (3) greater potential for particle removal due to settling into deeper waters and lower re-suspension.

Ogilvie et al. (2000) have shown that chlorophyll concentrations are reduced inside mussel farm longlines during winter; whereas in summer, chlorophyll levels are slightly enhanced inside the longline. This suggests that there could be an interaction of mussels, nutrients, and phytoplankton, which changes according to seasonal cycles of factors affecting phytoplankton growth. Previous work on Pelorus Sound has shown that the abundance of dissolved inorganic nitrogen has a seasonal cycle, with high levels occurring in winter months, decreasing through late winter and spring, down to low levels in summer because of phytoplankton uptake (Gall et al., 2000). Pelagic primary production in this system is limited by nitrogen in summer (Gibbs and Vant, 1997). Through autumn and winter, the shorter daylight periods result in reduced primary productivity, and a reduction in nitrogen uptake, which allows nitrogen concentrations to increase to high levels in winter (Gibbs and Vant, 1997). This clear transition from light- to nitrogen-limited phytoplankton growth makes this system suitable for investigating the interactions between light, nitrogen, phytoplankton, and suspended mussel culture.

In this work, our aim was to quantify the relative effects of grazing and of excretion of the mussel *Perna canaliculus*, on the abundance of phytoplankton, in the controlled environment afforded by in situ experimental enclosures. Enclosures are a useful experimental tool for investigating trophic relationships since they allow the repeated sampling of the same body of water, with replicated experimental treatments allowing interactions of these treatments to be isolated.

2. Methods

2.1. Study site

This study was done at a mussel farm at Wilson Bay ($41^{\circ}04'S$, $173^{\circ}55'E$), in Pelorus Sound, a drowned river valley approximately 50 km long, in the Northern part of South Island, New Zealand. The head of Pelorus Sound has a width of about 1.5 km and mean depth of 30 m, the mouth of the sound has a width of about 2.4 km, and mean depth 70 m. Circulation in Pelorus Sound is mainly tidal and the residence time of water is about 21 days (Heath, 1974).

2.2. Enclosure design

Experimental manipulations were undertaken using enclosures consisting of clear polyethylene (200- μm thickness) tubing of 60-cm diameter (Fig. 1). The open end was folded over a galvanised steel hoop (60-cm diameter) and attached using a stainless steel hose clip. The hoop was kept about 25 cm above the surface of the water with a polystyrene float. The lower end of the polyethylene tubing was sealed at a length of 3 m, allowing the enclosure to extend 3 m down into the water column, and enclose

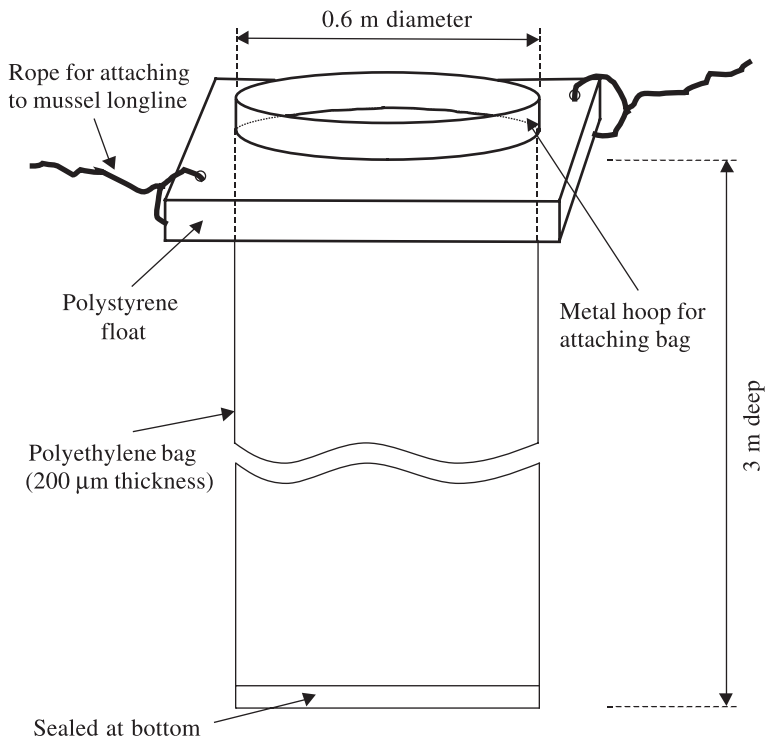


Fig. 1. Enclosure design used in the present experiments. Not drawn to scale.

approximately 850 l of water. Each enclosure was deployed between the headlines of a mussel longline (for a description of mussel longline structure, see [Hickman, 1989](#)). The enclosures were filled using an impeller pump, at a rate of 200 l per minute and were orientated vertically in the water column once full. The design of the enclosures and the spacing between mussel floats precluded fouling by seabirds, which was confirmed by the absence of unexpected nutrient increases in any enclosures.

Four experiments were undertaken: two at times of low ambient nitrogen concentration (December 1998 and January 1999), and two at times of high ambient nitrogen (May and July 1999). Twelve enclosures were deployed in each experiment, allowing three replicates within each of four manipulation types. The four manipulation types were: added mussels (seven harvestable-sized mussels per enclosure); added nitrogen (sufficient nitrate and ammonium to increase the concentration of each by around $50 \mu\text{g l}^{-1}$); added nitrogen and mussels; and a control of seawater with no additions. For the mussel enclosures, seven mussels of size ~ 70 mm were used. This number was based on the assumption that each mussel cleared 5 l per hour ([Hawkins et al., 1999](#); [James et al., in press](#)) giving a total clearance of ~ 840 l per day—i.e. a volume approximately equivalent to the enclosure volume was cleared by the mussels per day, imparting a strong grazing pressure. The mussels were placed in a nylon mesh bag (2-cm mesh size) that was secured at 1.5 m depth inside the enclosures. The amount of nitrogen added was sufficient to raise the concentration to that observed in winter months ([Gibbs and Vant, 1997](#)). The nitrogen was added as a solution during the filling of the enclosures, to allow complete mixing.

2.3. Stirring and sampling

A preliminary enclosure experiment was done in October 1998 to estimate phytoplankton sinking rates. Three enclosures were deployed and chlorophyll *a* was measured in each enclosure every day for 3 days. The assumption was made that changes in chlorophyll were caused only by particle sinking, and therefore sinking rate estimated from measured chlorophyll decreases. The mean sinking rate was 1.3 m day^{-1} (S.E. = 0.24). This is within the range of phytoplankton sinking rates believed to be representative of temperate coastal populations ([Bienfang, 1982](#)). These sinking rates would cause phytoplankton to collect at the bottom of the enclosures, so it was decided to mix each enclosure once every 24 h. An electric impeller pump (Rule industries, 500 GPH) was used to bring the bottom water to the surface. Settled material at the bottom of each enclosure was visible to the naked eye, and was resuspended into the water column after no more than 20 s of pumping.

Each enclosure was therefore pumped for 2 min to be certain of equal mixing. Water samples (2 l) were taken immediately after mixing. Two aliquots (500 ml) were each filtered through a Whatman[®] GF/F filter and the filters stored frozen for analysis of chlorophyll *a*. A 250-ml sub-sample of filtrate was stored frozen for analysis of nitrate and ammonium concentration. In the December and May experiments, the remaining water was preserved with acidified Lugols iodine ([APHA, 1992](#)) and stored in the dark for later phytoplankton species cell counts. The enclosures were sampled once a day for 5 days, and a sample was also taken from outside the enclosures.

2.4. Chemical analyses and phytoplankton species cell counts

Chlorophyll *a* concentrations were determined with a Perkin-Elmer fluorometer, following extraction in 90% acetone (Strickland and Parsons, 1968). Nitrate-nitrogen and ammoniacal nitrogen were analysed using an Alpkem auto-analyser. Nitrate was measured as the sum of nitrate and nitrite nitrogen by the cadmium reduction method (Grasshof, 1970) and ammonium by the phenol-hypochlorite method (Ivancic and Degobbis, 1984).

For phytoplankton cell counts 250 ml of preserved sample was settled for 24 h, decanted to 25 ml, placed in an Utermöhl chamber, and allowed to settle for a further 24 h. Samples were observed under a Wild M40-82720 inverted microscope, as described by Utermöhl (1958). Phytoplankton were identified to species or genus level and enumerated. Identification of phytoplankton was undertaken according to Lebour (1978), Dodge (1980), Sourmia (1986), Ricard (1987), Chretiennot-Dinet (1990), Round et al. (1990), Larsen and Moestrup (1992), UNESCO (1995), and Tomas (1997). Cell counts for each genus and species were converted to biovolumes using conversion factors from the above literature, and from measurements made during the counting process.

2.5. Statistical analysis

For each dependent variable (chlorophyll *a*, ammonium, and nitrate), a three-way ANOVA was used (Underwood, 1981). The independent variables in each ANOVA were ambient nitrogen concentration ($n=2$: high vs. low), months (random factor, $n=2$, 2 months nested within each ambient nitrogen level), and experimental manipulation type ($n=4$, 3 replicates each of, added mussels, added mussels and nitrogen, added nitrogen and controls). Only initial and final concentrations were used. Results of each experiment were standardised by converting final concentration values into a proportion of initial concentration. The proportion values were log transformed, and the data sets satisfied the ANOVA assumptions of normality (Kolmogorov-Smirnov) and homogeneity of variances (Cochran C). To further analyse the effects of time during the experiments, repeated measures ANOVA was used to test for significant differences between enclosure manipulation means over all five sampling days. Where significances were found, additional two-way ANOVA and Tukey's post hoc comparisons were used to compare manipulation type means within each sampling time (Sokal and Rohlf, 1981). All statistical analyses used Statistica™ 5.1 (StatSoft, Tulsa, USA).

3. Results

3.1. Ambient nitrogen concentrations

Ambient surface nitrate concentrations in the summer months near the enclosures were around $3 \mu\text{g l}^{-1}$, increasing during winter to around $25 \mu\text{g l}^{-1}$ in May, and $60 \mu\text{g l}^{-1}$ in July (Table 1). Ammonium was not as variable, staying within the range of $5\text{--}8 \mu\text{g l}^{-1}$ over the entire study period (Table 1). In the enclosures with added nitrogen, the nitrate

Table 1

Mean initial nitrate and ammonium concentrations ($\mu\text{g l}^{-1}$) for each enclosure experiment

Experiment	Nitrate		Ammonium	
	Ambient	Raised	Ambient	Raised
December 1998	3.1 (0.6)	47.3 (3.3)	5.1 (1.3)	59.6 (2.4)
January 1999	2.8 (0.2)	62.3 (4.3)	6.5 (1.4)	63.9 (5.1)
May 1999	25.2 (1.0)	111.0 (1.6)	7.0 (1.3)	61.6 (1.3)
July 1999	59.8 (1.8)	67.7 (0.5)	7.7 (0.7)	19.2 (0.9)

Standard errors are in parentheses. Ambient values are means of five samples taken outside the enclosures during each experiment. Raised values are means of six enclosures with experimentally added nitrate and ammonium, at time 0.

concentrations from December to May were increased from ambient by between 44 and 85 $\mu\text{g l}^{-1}$, while ammonium was increased by between 54 and 58 $\mu\text{g l}^{-1}$. In July, nitrate and ammonium were only increased by 8 and 12 $\mu\text{g l}^{-1}$, respectively, because after analysis of the May experiment data it was decided that there would already be ample nitrogen present.

3.2. Chlorophyll *a*

Ambient nitrogen concentration was a significant factor ($P=0.043$) influencing chlorophyll *a* concentration (Table 2). Month was also a significant factor ($P=0.006$), and there was a highly significant interaction ($P<0.001$) between month and the experimental manipulation type. During December and January, when ambient nitrogen was low, chlorophyll *a* concentrations in enclosures with added nitrogen showed a highly significant ($P<0.001$) increase compared to the controls (Fig. 2a and b). This response indicated that phytoplankton productivity at this time of year was limited by nitrogen. In the enclosures with mussels and nitrogen added, chlorophyll *a* levels also showed an increase by day 4, but only to about half of the concentrations reached in the absence of mussels (Fig. 2a and b). During the months with low ambient nitrogen, there was

Table 2

Summary of 3-way ANOVA

Source	DF	Chlorophyll <i>a</i>			Ammonium			Nitrate		
		MS	F	<i>p</i>	MS	F	<i>p</i>	MS	F	<i>p</i>
Nitrogen	1	2.774	22.004	0.043*	1.081	11.789	0.075	0.007	0.117	0.765
Month	2	0.126	5.980	0.006*	0.092	1.062	0.358	0.061	3.444	0.044*
Manipulation	3	0.236	1.968	0.220	1.678	5.812	0.033*	0.409	3.214	0.104
Nitrogen*Manipulation	3	0.231	1.929	0.226	0.469	1.623	0.281	0.012	0.093	0.961
Month*Manipulation	6	0.120	5.675	0.000*	0.289	3.334	0.011*	0.127	7.224	0.000*
Residual	32	0.021			0.086			0.018		
Total	47									

Independent factors are ambient nitrogen level, month, and experimental treatment. Dependent factors are chlorophyll *a*, ammonium, and nitrate.

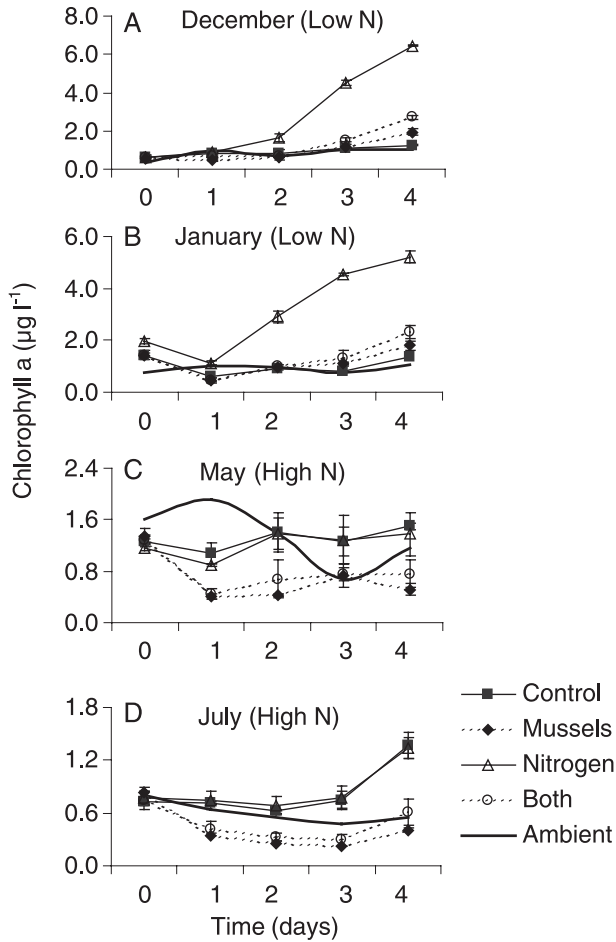


Fig. 2. Mean chlorophyll *a* concentrations for each of the four experiments, for the four experimental manipulations. Ambient values are a single sample taken outside the enclosures each sampling time, the ambient lines have been smoothed to allow easier visualisation. Error bars are ± 1 SE.

significantly greater chlorophyll *a* ($P < 0.05$ in both cases) in enclosures with added mussels compared to the controls, by the end of the experiment (Fig. 2a and b).

In May and July (times of high ambient nitrogen), there was no significant difference in chlorophyll *a* in the enclosures with added nitrogen compared to controls (Fig. 2c and d). This lack of a response to added nutrients suggests that phytoplankton are not nitrogen limited during these winter months. At this time, ambient nitrogen accumulates because phytoplankton are light limited. There was, however, a significant decrease in chlorophyll *a* concentration in enclosures with mussels. Repeated measures ANOVA showed that on the second day of the May experiment there was less chlorophyll in the mussel enclosures compared to controls ($P < 0.005$). On day 4, there was significantly less chlorophyll *a* in the enclosures with only mussels added ($P = 0.04$). The mean

values for the May data show lower chlorophyll in the mussel enclosures, the fact that statistical significance was only seen on days 2 and 4 is explained by the large degree of variation between replicate enclosures, illustrated by the large standard error bars in Fig. 2c. There was also a large range of ambient chlorophyll *a* values measured in this month.

In July, the pattern of lower chlorophyll *a* in mussel enclosures was more obvious, with repeated measures ANOVA showing all chlorophyll *a* concentrations after day 0 being significantly lower in the presence of mussels ($P < 0.05$ in all cases) irrespective of added nitrogen (Fig. 2d). The repeated measures ANOVA also showed a significant increase in chlorophyll *a* in enclosures without mussels over the duration of the experiment ($P < 0.001$).

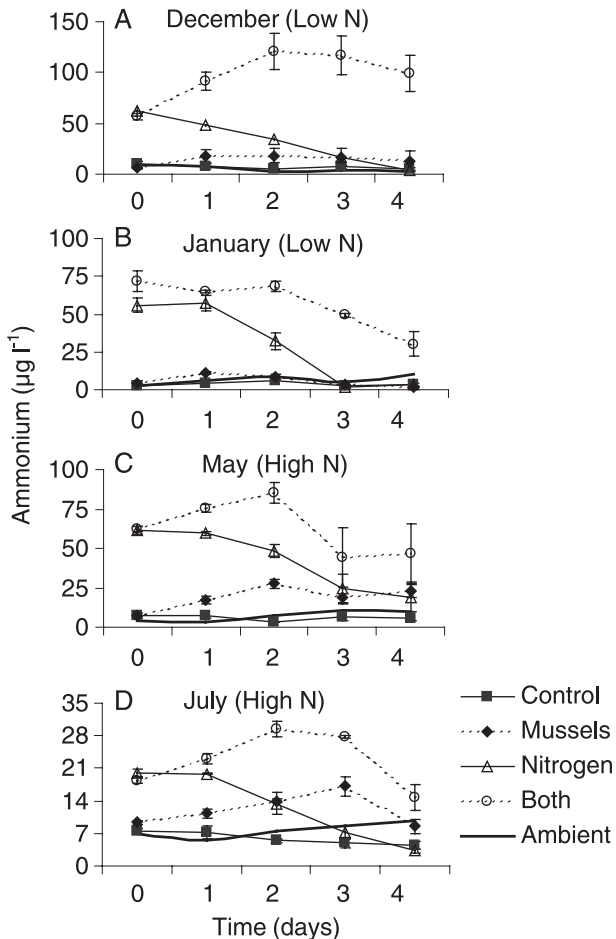


Fig. 3. Mean ammonium concentrations for each of the four experiments for the four experimental manipulations. Error bars are ± 1 SE.

3.3. Ammonium

The factors causing significant variability in ammonium concentrations were manipulations ($P=0.033$) and an interaction between month and manipulation type ($P=0.011$) (Table 2). As expected, in all 4 months, the initial concentration of ammonium was higher in the enclosures with added nitrogen (Fig. 3). In December and January (times of low ambient nitrogen), there were no significant differences in ammonium concentrations in enclosures with mussels only, compared to controls, on any of the sampling days. In both of these months, the ammonium that was added to enclosures decreased rapidly, becoming

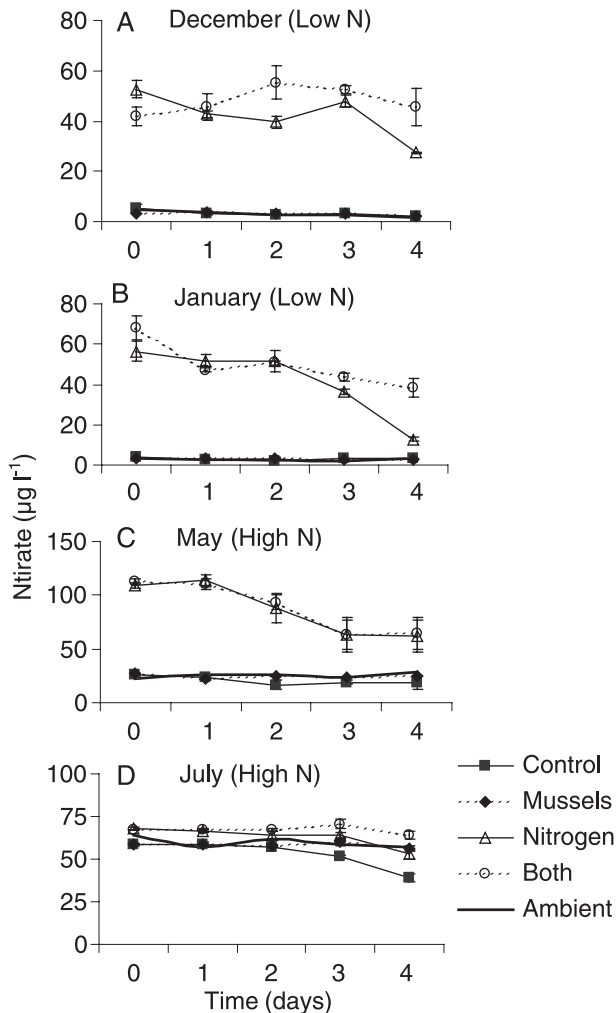


Fig. 4. Mean nitrate concentrations for each of the four experiments for the four experimental manipulations. Error bars are ± 1 SE.

similar to ambient concentrations after a maximum of 3 days (Fig. 3a and b). In enclosures with nitrogen and mussels added, the ammonium concentrations were significantly higher than all other enclosures on the final sampling day in both months ($P < 0.001$ in both cases).

In May and July, when ambient nitrogen levels were high, all enclosures with mussels showed an increase in ammonium to levels significantly higher than the controls on day 1 and 2 ($P < 0.05$ in all cases). Only in July did this difference persist through to the end of the experiment (Fig. 3c and d).

3.4. Nitrate

The significant sources of variability in nitrate concentrations were month ($P = 0.044$) and an interaction between month and manipulation ($P < 0.001$) (Table 2). As with

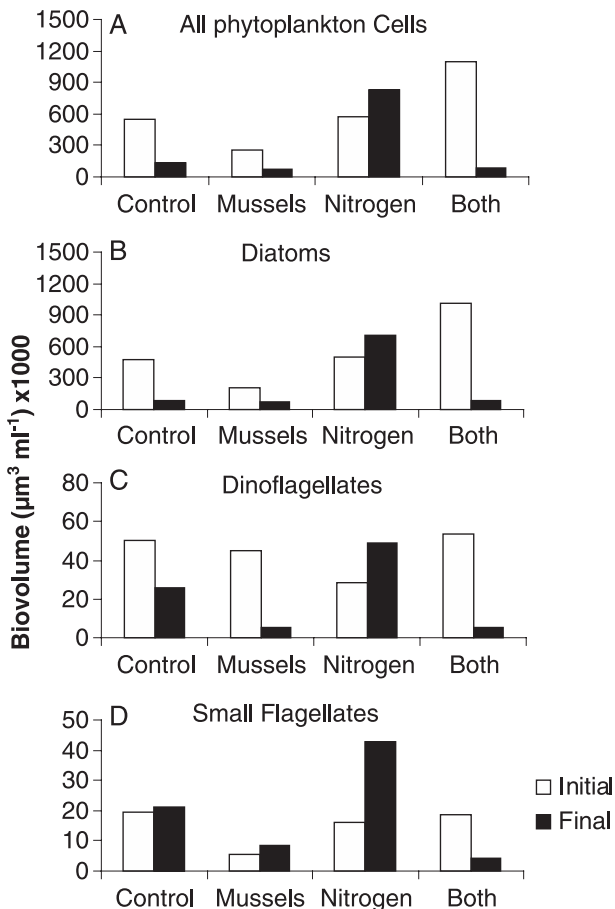


Fig. 5. Initial (day 0) and final (day 4) biovolumes of all phytoplankton cells, diatoms, dinoflagellates, and small flagellates ($< 20 \mu\text{m}$) in each experimental manipulation type in January. Samples from the three replicate enclosures of each manipulation were pooled.

ammonium, there were the expected higher initial concentrations of nitrate in those enclosures where nitrogen was added (Fig. 4). In December (time of low ambient nitrogen), on the final sampling day, there was significantly less ($P=0.04$) nitrate in the enclosures with added nitrogen, compared to those with both nitrogen and mussels (Fig. 4a). The same occurred in January, but it happened a day earlier ($P<0.05$) (Fig. 4b). This was linked to lower chlorophyll *a* concentrations in the enclosures with added nitrogen and mussels, indicating that the mussel grazing reduced phytoplankton utilisation of added nitrate. By the end of the January experiment, there was no significant difference in nitrate concentration in the enclosures with added nitrate, compared to the controls (Fig. 4b).

By the final day of the May experiment, there were no significant differences ($P>0.06$) in nitrate concentration between any of the enclosures (Fig. 4c), indicating that the added nitrate was removed from the enclosure water column. A similar pattern was seen in July, with no significant differences between enclosures by the third day, except that nitrate remained higher in the enclosures with both nitrogen and mussels (Fig. 4d).

3.5. Phytoplankton biovolumes

In January, there was a substantial increase in phytoplankton biovolume when nitrogen was added (Fig. 5a). All the main phytoplankton groups showed an increase with added nitrogen (Fig. 5b–d) which was even larger when considered that controls decreased. The diatom species that showed increases over the experiment, in order of highest to lowest increase, were *Skeletonema* sp., *Chaetoceros convolutus*, *Eucampia zodiacus*, *Nitzschia closterium*, and *Thalassothrix* sp. The main dinoflagellates that showed an increase with added nitrogen were *Gymnodinium* sp. The small flagellates were the most numerically important of the phytoplankton groups (Table 3), making up over 95% of the cells counted in the January samples, but they only contribute 4% of the biovolume. In enclosures with mussels, there appeared to be a decrease in biovolume of all phytoplankton groups except small flagellates (Fig. 5).

Table 3
Small flagellate and total phytoplankton cell counts (cells ml⁻¹) on the initial and final sampling days of the January and May experiments

	Small flagellates		Total phytoplankton cells	
	Initial	Final	Initial	Final
<i>January</i>				
Control	582	632	623	650
Mussels	164	256	192	262
Nitrogen	478	1270	505	1328
Both	554	131	599	139
<i>May</i>				
Control	172	144	251	239
Mussels	163	13	255	24
Nitrogen	134	72	201	130
Both	89	23	208	51

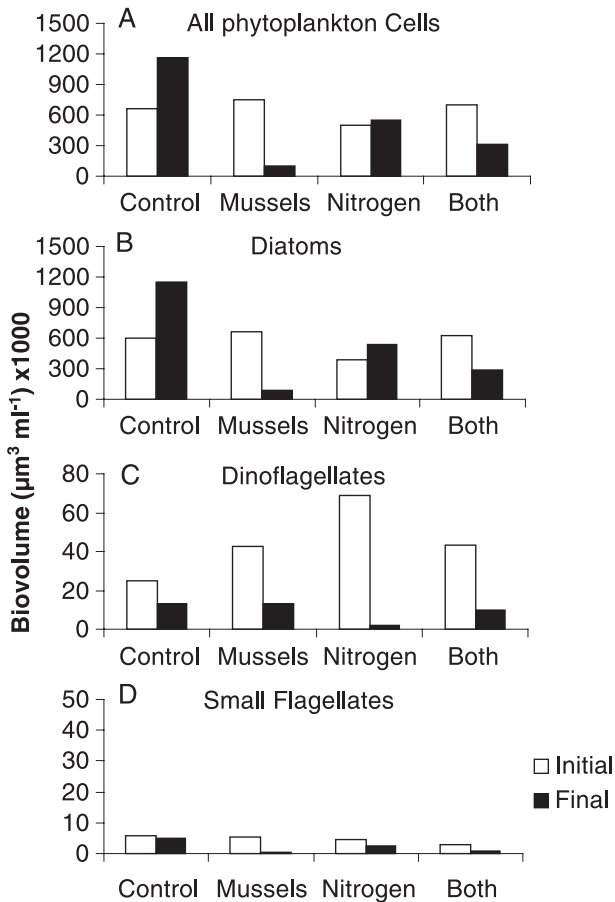


Fig. 6. Initial (day 0) and final (day 4) biovolumes of all phytoplankton cells, diatoms, dinoflagellates, and small flagellates ($<20\ \mu\text{m}$) in each experimental manipulation in May. Samples from the three replicate enclosures of each manipulation were pooled.

In May, there were fewer phytoplankton cells, with numbers in control enclosures being around $250\ \text{cells ml}^{-1}$, compared to around $600\ \text{cells ml}^{-1}$ in January (Table 3). Small flagellate cells were again numerically dominant, making up 65% of the cells counted. The most prominent trend in the May data was lower biovolumes in the presence of mussels (Fig. 6). The diatoms that decreased in biovolume most in the presence of mussels were *Chaetoceros* and *Skeletonema* sp.

4. Discussion

Ogilvie et al. (2000) showed that mussel farms in Pelorus Sound could both reduce and enhance phytoplankton abundance (as estimated by chlorophyll *a* concentration), depend-

ing on circumstances. Reductions occurred in winter when the phytoplankton were predominantly light limited, and enhancement occurred in summer when the phytoplankton were typically nitrogen limited (Gibbs and Vant, 1997). The present study set out to quantify the interactions between mussels, phytoplankton, and nutrients in a controlled environment of enclosures, to determine the mechanisms for these effects of mussels on phytoplankton.

The ambient nitrogen concentrations in the present study showed a similar pattern to that reported previously by Gibbs and Vant (1997) and Ogilvie et al. (2000), with highest concentrations occurring in the winter months, and decreasing to low levels in spring and summer. As with earlier studies, the seasonality of the dissolved inorganic nitrogen concentration was largely mediated by variation of nitrate (Table 1). It is of interest that low levels of ammonium were always present, even during the summer period of intense nitrate limitation. The large increase of chlorophyll *a* in enclosures with added nitrogen in December and January strongly suggested that phytoplankton productivity at this time of year is limited by nitrogen. However, the strong summer phytoplankton growth response to added ammonium, and the subsequent reduction of this ammonium to pre-addition levels, suggests that the low amounts of ammonium recorded in the analyses may not have been readily available for phytoplankton consumption and may have resulted from the breakdown of small cells during the filtering and freezing of samples (Gibbs and Vant, 1997). The ammonium levels recorded are also at the low end of estimates of half saturation levels for DIN (Eppley et al., 1969) and this alone could explain the nitrogen limitation.

When both nitrogen and mussels were added to an enclosure, there was an increase in chlorophyll *a* but to only about half of that measured in the absence of mussels. This indicated that the mussels consumed about half of the new phytoplankton biomass produced in response to the added nitrogen. A further consideration is the potential influence that high concentrations of ammonium could have had on the mussels. In the December experiments, ammonium levels increased to more than $100 \mu\text{g l}^{-1}$ in the enclosures with both nitrogen and mussels added. This concentration of ammonium is not lethal to shellfish (Abraham et al., 1996). There is scant published evidence of sub-lethal influences of ammonium on shellfish, but the fact that mussels in these enclosures were filtering (as evidenced by reduced chlorophyll concentrations) suggests that the ammonium did not have a major impact.

In winter mussels had a clear grazing effect on chlorophyll *a*. In months with low ambient nitrogen there was a significant but small increase in chlorophyll *a* in enclosures with mussels and no added nitrogen. Previous studies have shown that the production of regenerated inorganic nutrients by bivalves can stimulate primary production (Bertness, 1984; Prins and Smaal, 1990; Prins et al., 1995; Asmus and Asmus, 1991; Ogilvie et al., 2000), suggesting that grazing was counteracted by growth. Mussels excrete metabolic ammonium (Smaal and Prins, 1993; Prins et al., 1995; Pitta et al., 1999), supporting the hypothesis that regeneration of inorganic nutrients by mussels can stimulate primary production. There was, however, no statistically significant increase in ammonium in December and January, but at these times of severe nitrogen limitation, the phytoplankton may assimilate the ammonium as soon as it is produced. Ammonium production by mussels was measurable in the winter (Fig. 3c

and d), when phytoplankton were not nutrient limited and so did not rapidly assimilate the regenerated nitrogen.

In May, the higher ambient nitrogen concentrations (well above published values for maximal growth rate, Eppley et al., 1969), and the lack of a phytoplankton response to added nutrients is further evidence that phytoplankton in Pelorus Sound are not nitrogen limited at this time. Instead, they are limited by light, as suggested by Gibbs and Vant (1997) and Gall et al. (2000). Studies on the influence of mussels on this system therefore need to consider these contrasting seasonal light and nitrogen limitation cycles.

In July, there was an increase in phytoplankton in controls and DIN additions. A factor for consideration with the present experimental system is that the enclosures were at the surface of the water, enclosing the top 3 m of the water column. This would increase the light for phytoplankton in the enclosures relative to outside, where the mixing depth is likely to be deeper (Ross et al., in press). This mixing depth/light effect will be most pronounced in winter because this is the time of year that phytoplankton production is more likely to be limited by light, and ambient mixing depth is deepest. The most significant changes in phytoplankton concentrations in the winter experiments were observed in the enclosures with mussels. During both winter experiments, and in all six mussel enclosures of each experiment, the mussels caused a decrease of phytoplankton (Fig. 2d), compared to the enclosures without mussels.

An unexpected result occurred in May, where added nitrate and ammonium both decreased by a substantial amount over the duration of the experiment. This decrease was not coupled with an increase in chlorophyll *a* concentration, so the added nitrogen was not assimilated into phytoplankton biomass via photosynthesis or intracellular sequestration. What happened to the added DIN? The first possibility is that the nitrogen leaked out of the system due to damage to the enclosures. The decrease in nitrate and ammonium was, however, observed in all six added-nitrogen replicates, and the possibility of all six enclosures being damaged is highly unlikely, and certainly would have been noticed.

A more likely possibility is that the DIN was taken up by periphyton on the enclosure walls. The introduction of plastic walls dramatically increases the substratum available for periphyton, which may subsequently increase in biomass and utilise available nutrients. Wall effects are a general problem with enclosure experiments (Ogilvie, 1993) and one approach to minimising these effects is to have experiments of relevant duration to observe the ecological interactions of interest (Uehlinger et al., 1984). The present experiments were of sufficient duration to observe mussel effects on phytoplankton and phytoplankton responses to added nutrients. If the experiments were any longer, then periphyton influences may have been even more pronounced.

In January, in the presence of mussels, phytoplankton species biovolumes did not appear to follow the chlorophyll *a* measurements. This experiment was unusual compared to the other three in that there was a decrease in chlorophyll *a* concentration in all enclosures between the first and second sampling (Fig. 2b). It is possible that this was a result of an incomplete mixing of the enclosures, so a proportion of the original phytoplankton sedimented to the bottom of the enclosures during the first day of the experiment. The observed changes in phytoplankton biomass in each enclosure therefore occurred after the second sampling. Unfortunately, the strategy of water sampling for the

phytoplankton species analysis, where samples were taken only on the initial and final sampling days, is likely to have resulted in the observed cell counts showing decreases in all but the nitrogen-only manipulation (Fig. 5), despite the likelihood that increases occurred in the other manipulations from the second sampling. The sampling did, however, show that the addition of nitrogen had a large impact on phytoplankton biovolume and species composition. In the absence of mussels, all taxa increased with added DIN especially the small flagellates. The phytoplankton species that showed the highest increases were those that are able to grow quickly in response to new nitrogen. These were small flagellates, and the diatoms, *Skeletonema* sp. and *Chaetoceros* sp. In this situation, these phytoplankton are likely to have a competitive advantage over other slower-growing species (Carlsson and Graneli, 1999), and may have suffered lower grazing mortality due to their small size.

The slight increase in phytoplankton levels mediated by mussels in low nitrogen conditions suggests that there might be a critical nutrient level and consequent phytoplankton concentration (for any given density of mussels) below which the grazing impact of mussels is counteracted by phytoplankton growth. So, when phytoplankton concentration gets down to this level inside mussel farms, the increased level of excreted nutrients may allow phytoplankton to increase in biomass compared to outside the farms. A useful future exercise would be to construct models that will allow the prediction of these critical concentrations. These models would be useful for determining when phytoplankton productivity is most likely to be limited by nitrogen.

In summary, the ANOVA showed that the over-arching factors influencing phytoplankton biomass in this system are the seasonal cycling of nitrogen (mostly nitrate) in the water column, and light. The effect of mussels is nested within these seasonal cycles. In the winter, when nitrogen is plentiful but light is not, the mussels reduce phytoplankton concentrations. This would be the time that within-farm food depletion is most likely to occur. Conversely, in summer nitrogen concentrations are low, resulting in low phytoplankton concentrations. However, mussel grazing can slightly compensate for nutrient limitation by producing ammonium, resulting in a net increase in phytoplankton biomass. These processes are more likely to occur in close proximity to mussel farms. A logical next step might be to investigate how these mussel-mediated processes influence phytoplankton on a wider spatial scale, such as for the whole of Pelorus Sound.

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