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Primary Research Paper

Colony structure and seasonal differences in light and nitrogen modify the impact of sessile epifauna on the giant kelp *Macrocystis pyrifera* (L.) C Agardh

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Abstract

The presence of the stoloniferous hydroid *Obelia geniculata* (L.) had no effect on the pigment concentration or nitrogen status of underlying blade tissue of the giant kelp *Macrocystis pyrifera* (L.) C. Agardh. The sheet-like colonies of the bryozoan *Membranipora membranacea* (L.) markedly reduced the pigment concentration of colonized blade tissue, but only during winter. Reductions in pigment concentration are most likely a result of damage to underlying tissue due to some factor related to the presence of bryozoan colonies on blade surfaces. Blade tissue colonized by *M. membranacea* also had higher $\delta^{15}N$ signatures than surrounding bryozoan-free tissue, possibly indicating the provision of nitrogen to *M. pyrifera* by bryozoan colonies. Results show that seasonal changes in nitrogen and colony size can strongly modify the effect of epifauna on macroalgae they colonize. Unlike bryozoans, hydroid colonies provided no barrier to nitrogen uptake by colonized *M. pyrifera* tissue and enhanced ammonium uptake was observed for tissue colonized by *O. geniculata* during nitrogen limitation. Epifauna with stoloniferous growth forms such as hydroids are more likely to have benign or even mutualistic relationships with macroalgae they colonize than the sheet-like colonies of bryozoans.

Introduction

Macroalgae are important primary producers in coastal seas (Mann, 1988; Duggins et al., 1989 Bustamante et al., 1995), fulfil important roles in nutrient regeneration (Hanisak, 1993; Gatusso et al., 1998), and provide habitats and sites for recruitment of marine invertebrates and fish (Foster & Schiel, 1985; Duggins et al., 1990; Steneck et al., 2002). Macroalgal surfaces also provide a substratum for a diverse community of sessile and mobile fauna, particularly the surfaces of large brown macroalgae (Phaeophyceae) or kelp (Hagermann, 1966; Seed & O'Connor, 1981; Taylor & Cole, 1994; Christie et al., 2003). Some of the most common inhabitants of kelp surfaces are sessile filter-feeding bryozoans and microcarnivorous hydrozoans (Bernstein & Jung, 1979; Dixon et al., 1981; Yoshioka, 1982; Lambert et al., 1992; Jennings & Steinberg, 1997; Hepburn & Hurd, 2005). These animals possess flexible, semi-transparent colonies that are most likely adaptations to allow survival on the surfaces of macroalgae. Sessile epifauna gain many advantages from their association with macroalgae including; supplemental carbon from kelp exudates (DeBurgh & Fankboner, 1978), increases in feeding efficiency due to extension form the benthic boundary layer (Duggins et al., 1990), refugia from the competitive benthos (Oswald & Seed, 1986), and a vector for colonization of new habitats (Highsmith, 1985). However, relatively little

is known about how these animals affect their living substratum. Due to their close relationship with their substratum and their ubiquitous nature, sessile epifauna are likely to strongly influence the physiology and the productivity of macroalgae they colonize.

To date, studies investigating the influence of colonization by sessile epifauna on macroalgae have concentrated on relationships involving sheet-forming fauna, specifically the bryozoan genus *Membranipora*. Negative consequences of the colonization of algal surfaces by *Membranipora* spp. include; shading of colonized algal tissue (Oswald et al., 1984; Cancino et al., 1987; Muñoz et al., 1991), the formation of a barrier to nutrient uptake (Hurd et al., 1994) and spore release (Kain, 1975; Saier & Chapman, 2004), increased susceptibility to tissue loss as a result of wave or current action (Dixon et al., 1981; Lambert et al., 1992; Schiebling et al., 1999), and damage as a result of feeding by carnivorous fish (Bernstein & Jung, 1979; Yoshioka, 1982, pers. obs.). The emphasis on studies that determine how *Membranipora* spp. effect macroalgal tissue directly beneath their colonies is perhaps responsible for the prevailing view that specialized macroalgal-dwelling sessile epifauna have a negative influence on the growth and survivorship of macroalgae of they colonize. There is however no evidence in natural macroalgal/epifaunal associations to support this claim (Cancino et al., 1987; Hepburn & Hurd, 2005). In fact, epifauna including *Membranipora* spp., have the potential to provide benefits to host macroalgae by the provision nitrogen via excretion of ammonium as a waste product (Gerard & Mann, 1979; Probyn & Chapman, 1983; Hurd et al., 1994; Taylor & Rees, 1998, Hepburn & Hurd, 2005) and carbon through production of carbon dioxide due to their respiration (Mercado, 1998).

The canopy forming giant kelp *Macrocystis pyrifera* (L. C. Agardh) is a dominant component of shallow subtidal ecosystems in the Northwest Pacific and in temperate areas of the southern hemisphere (reviewed in Dayton, 1985; Foster & Schiel, 1985; Steneck et al., 2002). The sheet-forming bryozoan *Membranipora membranacea* (L.) and the stoloniferous hydroid *Obelia geniculata* (L.) co-exist on the surfaces of *M. pyrifera* and other macroalgae in both northern and southern hemispheres (e.g., Barrales & Lobban, 1975; Bernstein & Yung, 1979; Seed & O'Connor, 1981; Yoshioka, 1982; Hepburn & Hurd, 2005).

While the effect of colonization by *Membranipora membranacea* on underlying macroalgal tissue is reasonably well known the impact of stoloniferous epifauna like *Obelia geniculata* on colonized tissue has yet to be quantified. Hydroid colonies are more diffuse than those of bryozoans and consist of narrow hydrorhiza (stolons) that run along the macroalga's surface. These differences suggest the two groups could have a markedly different influence on the macroalgae they colonize. The barrier provided by *Membranipora* spp. colonies to light (Cancino et al., 1987) and nutrient uptake (Hurd et al., 1994) was predicted to have a stronger affect on the nitrogen status and pigment pigments concentrations of underlying kelp tissue than that of *O. geniculata* colonies which are in direct contact with a smaller proportion of blade surfaces. The influence of hydroid colonies on nitrate and ammonium uptake by *Macrocystis pyrifera* was also determined. It was also predicted that the more diffuse *O. geniculata* colonies would provide less of a barrier to uptake of nutrients by colonized tissue than *M. membranacea* colonies that have been shown to reduce uptake by up to 50% in *Macrocystis integrifolia* from British Columbia (Hurd et al., 1994, 2000).

Macrocystis pyrifera and other kelps from temperate locations typically exhibit light limited growth during winter and nitrogen limited growth during summer (e.g. Chapman & Craigie, 1977; Dieckmann, 1980; van Tussenbroek, 1989; Hepburn & Hurd, 2005). During the summer ammonium excreted by sessile epifauna provides an important nitrogen source for *M. pyrifera* and high summer light levels may reduce any negative affects of shading by sessile epifauna on growth (Hepburn & Hurd, 2005). During winter shading by colonies could exacerbate the affects of low light on the growth of *M. pyrifera* while high seawater nitrogen concentrations reduce the benefits of ammonium excretion by epifauna. Therefore, comparisons were made between the affects of colonization by *Membranipora membranacea* and *Obelia geniculata* on *M. pyrifera* blade tissue during summer and winter. Provision of nitrogen by epifauna during the summer was predicted to ameliorate the effects of nitrogen limitation providing benefits to underlying blade tissue by

enhancing nitrogen status and concentrations of nitrogen-containing pigments beneath colonies. During winter, significant nitrogen provision to the kelp is predicted to be less likely due to high ambient and internal nitrogen concentrations. This is the first study to determine how seasonal changes in light and nitrogen could modify the impact of sessile epifauna on macroalgae they colonize.

Materials and methods

Study site

The study site was a current-dominated *Macrocystis pyrifera* bed at Harington Point, located near the entrance to Otago Harbour on the east coast of the South Island of New Zealand, S. W. Pacific (45° 47′ S, 170° 43′ E). The blades of *M. pyrifera* at Harington Point are often heavily colonized by sessile suspension feeding epifauna, primarily *Membranipora membranace*a and *Obelia geniculata* (Hepburn & Hurd, 2005).

Environmental parameters

Monthly averages of 'global' radiation (MJ m^{-2} d^{-1}), a measure of all light that reaches a pyranometer were obtained courtesy of the National Institute of Water and Atmospheric Research from a weather station 20 km from the study site. Triplicate water samples were taken from the waters' surface and at 4 m depth from within the kelp bed at Harington Point. Samples were filtered (Whatman™ GF/C 0.45 μM filters) before determinations of nitrate and ammonium concentration using a Quickchem® 8000 automated ion analyzer (Lachat Instruments Inc., Milwaukee, WI, USA).

Blade collection

Using information gained from determinations of seasonal growth rates and nutrient status of *Macrocystis pyrifera* at Harington Point (Hepburn & Hurd, 2005) appropriate times of year were selected for collection of blade tissue. The first was during the austral winter when nitrogen levels in seawater and blade tissue were maximal and the second during summer when nitrogen concentrations were minimal. One blade colonized by *Membranipora membranacea* was collected from each of 10 separate *M. pyrifera* individuals using SCUBA during summer 1999 (January) and winter 1999 (August). Similarly, ten blades colonized by *Obelia geniculata* colonies were collected during the winter 2000 (August) and summer 2001 (January). Blades were collected from mature, subcanopy blades at 2–4 m depth where hydroid and bryozoan colonies were common. Blades were placed in covered plastic bins for transport to the laboratory one hour away.

Nitrogen status and pigment concentration

From each of the ten blades six 1.8 cm diameter discs were cut using a leaf corer. Two discs were taken from blade tissue with epifaunal colonies covering both sides (100% colonized group), two from tissue with a colony covering one side (50% colonized group) and a further two from tissue free from epifaunal colonization adjacent to the colony (clean group) using a similar sampling plan to Hurd et al. (1994). Blade tissue discs were always taken from blade tissue of the same age (i.e. parallel to each other) to remove the possibility of any physiological differences in blade tissue age confounding results. All discs were carefully scraped to remove colonies (colonized discs) or to control for the effect of scraping (clean discs) and then weighed. One group of discs from each blade (i.e., one clean, 50 and 100% colonized) was dried at 80 °C until discs reached a constant weight and then wet to dry ratios were calculated ($n = 10$); these ratios were used to estimate dry weight values for adjacent blade tissue discs used in pigment assays. Five groups of discs were then randomly selected from those used in determinations of dry weights and used in assays for total carbon and nitrogen. Each blade disc was ground separately in a mortar and pestle before total carbon and nitrogen was determined using a CHNS-O Elemental Analyser (CENA110 Carlo-Erba Instruments, Milan, Italy, $n = 5$).

Blade tissue samples were collected for determinations of the natural nitrogen stable isotope signatures ($\delta^{15}N$) of *Macrocystis pyrifera* blades colonized by *Membranipora membranacea* and *Obelia geniculata*. Samples were taken from 50%

colonized blade and clean blade tissue (adjacent to the colonized tissue sample) on the 7th of June 2001 ($n = 5$) and the 9th of December 2002 ($n = 10$). All blade tissue samples were carefully scraped to remove the attached colony or to control for its removal and then washed to remove any remnants of the epifaunal colony. Samples were dried at 80 °C in separate 10 ml plastic centrifuge tubes before being ground in a 10% HCl washed mortar and pestle (washed with purified water between samples) and placed in sealed 2 ml eppendorf tubes for storage prior to determinations of $\delta^{15}N$. Subsamples of dried, ground *M. pyrifera* tissue (1–2 mg) were combusted in a CENA1500 Elemental Analyzer (Carlo-Erba® instruments) interfaced to a Europa Scientific® 20–20 update continuous flow mass spectrometer (Sercon Australia Pty Ltd., Fulham Gardens, Australia). Corrections for drift were made automatically from a standard (EDTA) with a known isotopic ratio every five samples. Ratios of $^{15}N/^{14}N$ are expressed in standard $\delta^{15}N$ (‰) notation = $([R_{sample}/R_{standard}])-1)\times 1000)$ where $R = {}^{15}N/{}^{14}N$ and the standard is the $^{15}N/^{14}N$ of N_2 of the atmosphere.

Chlorophyll *a* and the accessory pigments chlorophyll *c* and fucoxanthin were estimated per gram dry weight of seaweed with the other group of discs ($n = 10$) using a modified method of Seely et al. (1972).

Nitrogen uptake

The effect of *Obelia geniculata* on nitrogen uptake by *Macrocystis pyrifera* was determined following Hurd et al. (1994). One blade colonized by *O. geniculata* each from eight separate kelp individuals were collected using SCUBA at Harington Point on the 19th of July 2000 and again on the 26th of January 2001. After collection blades were placed in plastic bins and covered for transport to the laboratory. Three 4.4 cm diameter blade discs with varying degrees of hydroid colonization (clean, 50, 100% colonized) were cut from each blade ($n = 8$). Blade tissue discs were then placed in aerated seawater over night (approximately 15 h) in a constant-temperature growth cabinet (Conviron Model E15, Controlled Environments, Winnipeg, Canada) in the dark at 12 °C to facilitate wound healing and allow blade tissue and attached hydroid colonies to acclimatize to experimental conditions. 270 ml of filtered seawater (1 μm) was placed in 28, 10% HCl washed, 600 ml beakers and also left overnight in the growth cabinet. Eight beakers were assigned to each of the colonization treatments and four beakers containing only filtered seawater were used as controls. Beakers were then placed in random order onto three orbital shaker tables (Model SS70, Chiltern Scientific, Auckland, New Zealand). Nitrate and ammonium uptake rates were determined over a 2 h period at 12 °C at a photon flux density of 250–280 μmol photons m^{-2} s^{-1} supplied by cool white fluorescent tubes (F72T12-CW-VHO, Sylvania, Ontario, Canada) while water motion was provided by orbital shaker tables at 125 rpm.

At the start of each uptake experiment, nitrate as $NaNO_3$ and ammonium as NH_4Cl solution was added to the 270 ml of filtered seawater in each beaker via pipette to give initial concentrations of 6 μM nitrate and 4 μM ammonium. After thorough mixing, blade discs were added to each of the treatment beakers. 20 ml water sample were removed from all beakers at $t = 0$, $t = 1$ h, and $t = 2$ h using a 10 ml pipette. Concentrations of nitrate and ammonium were determined following uptake experiments using the ion analyzer. Discs used in experiments were blotted dry before hydroid colonies were carefully scraped off using a plastic card. Wet and dry weights were then determined for both tissue discs and hydroid colonies. Uptake rates were calculated from the following:

$$V = \frac{(S_i - S_f) \times \text{vol}}{w \times t}$$

V = uptake rate (nmol g^{-1} dry wt s^{-1}), S_i = substrate concentration at start of time interval, S_f = substrate concentration following time interval, vol = volume at start of time interval, w = dry weight (g), and t = time (s).

Uptake rates were adjusted to include ammonium excretion by *Obelia geniculata* colonies (see below) by subtracting the estimated amount of ammonium excreted over the 2-h incubation period from the final ammonium concentration. This amount was standardized using the wet weight of colonies attached to blade discs used in uptake experiments.

Ammonium excretion

Rates of ammonium excretion were determined for *Membranipora membranacea* and *Obelia geniculata* colonies independent of seaweed tissue as it was impossible to remove bryozoan and hydroid colonies from *M. pyrifera* blades without causing significant damage to the colonies. Independent colonies were obtained from settlement plates deployed within the Harington Point kelp bed. Four 0.4 m^2 black perspex plates were fixed in a horizontal position, approximately 2 m above the substratum, to an aluminium pole anchored at its base by a concrete-filled car tyre. Settlement plates were deployed from January to July 2000. Several sets of perspex plates had to be replaced due to fouling by algae before a significant settlement event occurred during early winter.

Colonized plates were collected by divers during July 2000 and carefully transported to the laboratory. Perspex plates were cut into pieces, so that each perspex section contained one bryozoan or hydroid colony. Ammonium excretion rates were determined using six replicate bryozoan and hydroid colonies and six other similar sized sections of perspex, scraped clean of any encrusting organisms as controls. Colonized and control sections of perpex were pre-treated in the same manner as that used for blade tissue and attached hydroid colonies in uptake experiments.

Ammonium excretion rates were determined over 2 h using identical experimental conditions to uptake experiments described above. Following experiments the outline of bryozoan and hydroid colonies were traced before being scraped from the perspex, blotted dry and their wet weights determined. The surface area of the irregularly shaped colonies was determined by cutting out the shape of the colonies in white copy paper and then calculating the relationship between the weight and area of the paper. Ammonium excretion rates were determined using the following:

$$E = \frac{(S_f - S_i) \times \text{vol}}{w \times t}$$

$E = NH_4^+$ excretion rate (nmol g^{-1} dry weight s^{-1}), $S_i = NH_4^+$ concentration at start of time interval, $S_f = NH_4^+$ concentration following time interval, vol = volume at start of time interval, w = wet weight (g) or area (cm^2) of the colony, and t = time (s).

Statistical analyses

Differences in nitrogen status, pigment concentration, and nitrogen uptake rates between colonization groups were determined using One-way ANOVA Differences between means were determined using Tukey's HSD *post hoc* test. Significance was set at the 5% level ($\alpha = 0.05$). Paired and unpaired *t*-tests were used for statistical analysis where appropriate. Tests for normality (Kolmogorov–Smirnov test with Lilliefor's correction) and equal variance (Levene median test) were carried out on all data to see if the criteria to perform parametric ANOVA were fulfilled. All statistical analyses were carried out using the software package Sigma Stat® 2.03 (SPSS).

Results

Light and seawater nitrogen concentration

Average monthly global radiation levels were similar within winter (6.6 MJ m^{-2}, 1999; 6.3 MJ m^{-2}, 2000) and summer (20.9 MJ m^{-2}, 2000; 19.9 MJ m^{-2}, 2001) sampling dates. Seawater nitrate and ammonium concentrations were within the same range during summer both (<1 μM NO_3^-, 1–2 μM NH_4^+) and winter (3.5–4.5 μM NO_3^-, 1–3 μM NH_4^+) sampling dates.

Nitrogen status and pigment concentration

Colonization by neither *Membranipora membranacea* nor *Obelia geniculata* had any influence on the nitrogen content or C:N of underlying *Macrocystis pyrifera* blade tissue during summer or winter sampling dates (Fig. 1).

The $\delta^{15}N$ value of *Macrocystis pyrifera* blade tissue colonized by *Membranipora membranacea* was 4‰ lower than adjacent blade tissue free from colonization during both sampling dates (Fig. 2). This difference was significant during summer (paired *t*-test $t = 4.54$; $p = 0.001$), however, the $\delta^{15}N$ value of colonized blade tissue was more variable during the winter and no significant difference was observed (paired *t*-test $t = 1.94$; $p = 0.12$). $\delta^{15}N$ of kelp colonized by *Obelia*

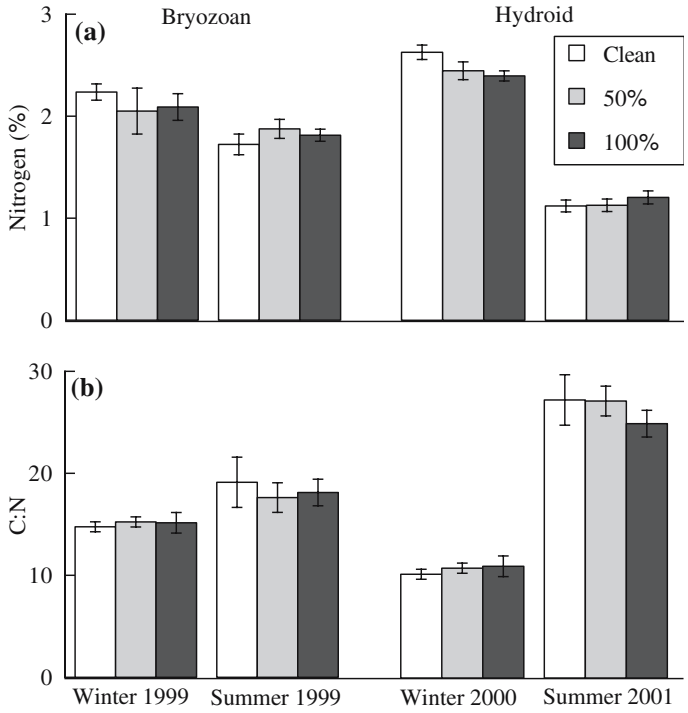

Figure 1. % Nitrogen and C:N of *Macrocystis pyrifera* blade tissue with varying degrees of colonization by the bryozoan *Membranipora membranacea* during summer (1999) and winter (1999) and the hydroid *Obelia geniculata* during winter (2000) and summer (2001). Values represent means ± 1 S.E ($n = 5$).

geniculata was similar to that of surrounding blade tissue during both the winter and summer.

There were significant reductions in the concentration of chlorophyll *a* (one-way $F_{2,27} = 9.086$ ANOVA $p = 0.001$) and the accessory pigments chlorophyll *c* (one-way $F_{2,27} = 5.015$ ANOVA $p = 0.014$) and fucoxanthin (one-way $F_{2,27} = 10.592$ ANOVA $p = 0.001$) in *Macrocystis pyrifera* tissue beneath *Membranipora membranacea* colonies compared to surrounding blade tissue free from colonization during winter (Fig. 3). During the summer this pattern was not evident and pigment concentrations were the same in both colonized and clean blade tissue. Pigment concentrations of blade tissue beneath *Obelia geniculata* colonies were not significantly different from surrounding clean blade tissue during both winter and summer.

Nitrogen uptake

There was no significant influence of hydroid colonies on uptake of nitrate by *Macrocystis pyrifera* blade tissue either during summer or winter (Fig. 4a). Nitrate uptake rates of all blade tissue groups were between four and five times higher during summer than uptake rates observed for blade tissue collected during winter.

During winter 2001, 100% colonized *Macrocystis pyrifera* blade tissue discs exhibited negative ammonium uptake rates, i.e., more ammonium was excreted by hydroids than was taken up by *M. pyrifera* (Fig. 4b). These rates were significantly lower than those observed for discs taken from clean blade tissue but not different from uptake rates observed for 50% colonized discs (one-way ANOVA $F_{2,18} = 3.514$, $p = 0.049$). During summer, ammonium uptake rates were 25–33% higher for colonized blade tissue discs (50 and 100% groups) when compared to clean discs, although no significant differences were detected between colonization groups (one-way ANOVA $F_{2,18} = 2.634$, $p = 0.099$).

When the uptake rates were adjusted to include estimates of ammonium excretion by *Obelia geniculata* colonies attached to blade discs on a wet weight basis, winter uptake rates of ammonium by colonized and clean blade tissue became almost identical (Fig. 4c). During summer, adjusted 100%

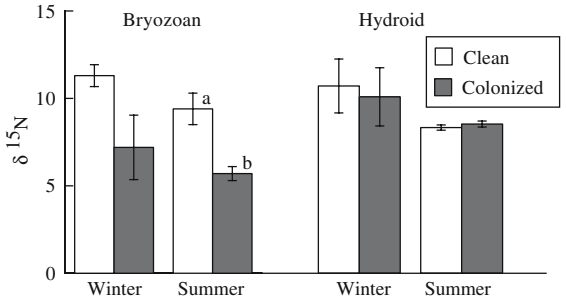

Figure 2. $\delta^{15}N$ values of *Macrocystis pyrifera* blade tissue colonized by *Membranipora membranacea* and *Obelia geniculata* and adjacent tissue free from colonization during winter 2001 ($n=5$) and summer 2002 ($n=10$). Values represent means ± 1 S.E. Significant differences between colonization groups ($p<0.05$) for paired *t*-tests are indicated by different letters above bars.

tissue disc uptake rates were significantly higher than clean blade tissue (one-way ANOVA $F_{2,18}=4.819$, $p=0.021$). No significant difference of ammonium uptake rates was detected between 50% colonized discs and clean discs although there was a clear trend of increasing uptake rates as hydroid colonization levels increased.

As observed for nitrate there was a strong influence of season on the uptake of ammonium by both colonized and non-colonized discs. Ammonium uptake rates were approximately seven times higher during summer for blade discs 100% colonized by hydroids while summer uptake rates of non-colonized and 50% colonized discs were four and four and a half times higher than winter values respectively.

Ammonium excretion

Ammonium excretion rates of *Membranipora membranacea* colonies averaged 3.33 ± 0.64 nmol g^{-1} wet weight s^{-1} and 0.045 ± 0.006 nmol g^{-1} cm^2 s^{-1} (mean ± S.E.; $n=6$) while for *Obelia geniculata* colonies excretion rates averaged 6.61 ± 1.01 nmol g^{-1} wet weight $s^{-1} \times 10^{-4}$ and 0.0357 ± 0.007 nmol cm^{-2} s^{-1} (mean ± S.E.; $n=6$). Ammonium excretion rates by *O. geniculata* were significantly higher on a wet weight basis than those of *M. membranacea* (*t*-test, $t=-2.74$, $p=0.020$). On an area basis, however, excretion rates were not significantly different (*t*-test, $t=1.01$, $p=0.335$).

Discussion

Nitrogen status and pigment concentration

In winter, nitrogen provision by attached epifauna in the form of ammonium was not expected to enhance the nitrogen status of colonized blade tissue as nitrogen pools are already likely to be full due to high seawater nitrate concentrations. This was the case as evidenced by high seawater nitrate concentrations, low C:N, low nitrogen uptake rates, and by nitrogen concentrations of >2% of dry mass which are near the top of the seasonal scale observed for *M. pyrifera* at Harington Point. High blade tissue nitrogen during this time was a result of high seawater nitrate concentrations and low growth rates due to low winter light levels (Hepburn & Hurd, 2005).

In summer, contrary to our predictions there was no enhancement of the nitrogen status of *Macrocystis pyrifera* blade tissue colonized by *Membranipora membranacea* or *Obelia geniculata*. This was despite conditions thought to be favourable for the utilization of epifaunal ammonium by *M. pyrifera* including low seawater nitrogen concentration, low blade tissue N concentrations, C:N above the proposed threshold for nitrogen limitation in kelp (Hanisak, 1983) and high ammonium uptake rates.

Macrocystis pyrifera blade tissue colonized by *Membranipora membranacea* had a natural $\delta^{15}N$ signature that was 4‰ less ^{15}N enriched than adjacent blade tissue free from colonization. This shows that blade tissue colonized by *M. membranacea* has a different nitrogen source to the surrounding bryozoan-free blade tissue, most likely ammonium excreted by the bryozoan colony. There was, however, no indication of any difference in the natural $\delta^{15}N$ values of blade tissue colonized by *Obelia geniculata* and adjacent non-colonized tissue during summer or winter. Unlike *M. membranacea*, *O. geniculata* provides no barrier to the uptake of nitrogen from the water column and colonized blade tissue has unrestrained access to nitrogen sources including nitrate and other sources of ammonium. Uptake of these nitrogen sources that probably have distinct $\delta^{15}N$ signatures by colonized blade tissue could mask any nitrogen inputs from hydroid colonies.

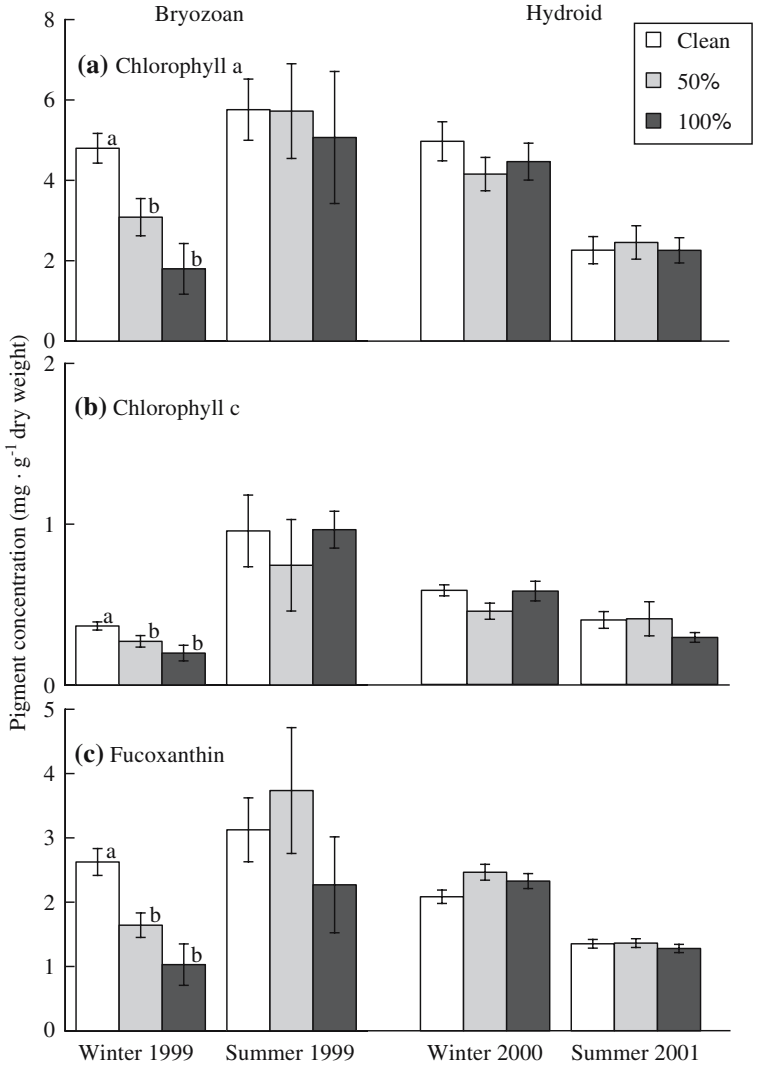

Figure 3. Chlorophyll *a*, *c*, and fucoxanthin concentration of *Macrocystis pyrifera* blade tissue with differing degrees of colonization by the bryozoan *Membranipora membranacea* during summer (1999) and winter (1999) and the hydroid *Obelia geniculata* during winter (2000) and summer (2001). Values represent means ± 1 S.E. ($n = 10$). Significant differences between colonization groups ($p < 0.05$) for Tukey's Tests are indicated by different letters above bars.

Despite an identical 4‰ difference between the mean $\delta^{15}N$ signature of *Macrocystis pyrifera* blade tissue colonized by *Membranipora membranacea* and that of surrounding tissue free from colonization during winter and summer, the difference was only statistically significant during the summer. The lack of a significant difference during winter was primarily a result of the $\delta^{15}N$ values of colonized blade tissue being more variable during this time of year. This may be due to the greater importance of nitrate as a nitrogen source for *M. pyrifera* during winter. While bryozoan-derived nitrogen is still being taken up by colonized tissue its isotopic signature is probably diluted by the signature of nitrate found at high concentrations in the seawater and within *M. pyrifera* blade tissue during the winter (Hepburn & Hurd, 2005). Low seawater and tissue nitrate concentrations observed during summer and the barrier to uptake provided by *M. membranacea* (Hurd et al., 1994, 2000) means that it is likely that bryozoan derived ammonium is the main nitrogen source for colonized blade tissue during this time.

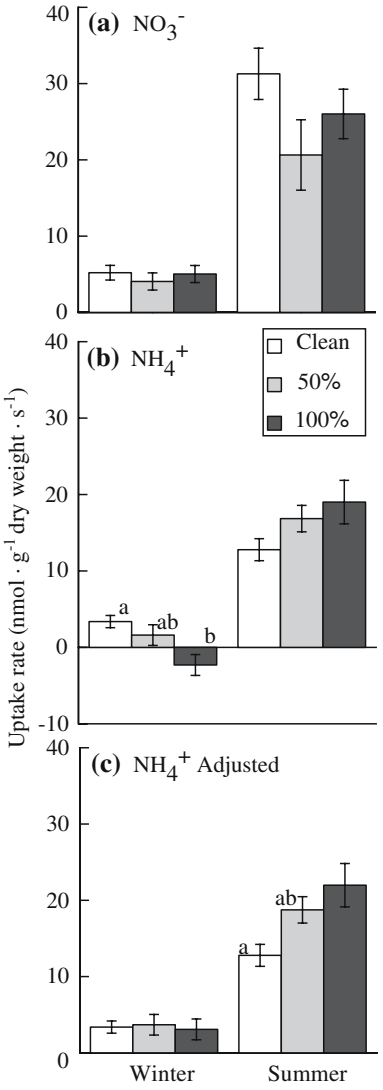

Figure 4. Nitrate (a) and ammonium (b) uptake by *Macrocystis pyrifera* blade tissue with varying degrees of colonization by *Obelia geniculata* during the winter and summer. Values represent means ± 1 S.E. ($n = 8$). Adjusted ammonium uptake rates (c) include estimates for ammonium excretion by *O. geniculata* on a wet weight basis obtained from colonies independent of kelp tissue. Significant differences between colonization groups ($p < 0.05$) for Tukey's Tests are indicated by different letters above bars.

During winter *Macrocystis pyrifera* blade tissue chlorophyll *a*, *c* and fucoxanthin concentrations were lower beneath *Membranipora membranacea* colonies. The large light harvesting area of *Macrocystis* spp. and its fast growing nature is considered to make shade acclimation as a result of bryozoan colonization observed in smaller macroalgae unnecessary (Hurd et al., 2000). Further, at Harington Point *M. membranacea* colonies are rarely found on blades near the frond apex (Hepburn & Hurd, 2005) and are most common on older blade tissue in the relatively low light environment beneath the kelp bed canopy where irradiances can be up to 99% lower than those at the surface (Foster, 1975; Gerard, 1984; Reed & Foster, 1984). This, combined with the fact that photosynthetic rates and efficiencies are higher in younger than more mature blades (Towle & Pearse, 1973; Wheeler, 1980; Arnold & Manley, 1985; Gerard, 1986; Chin, 1989), makes it unlikely that photosynthetic compensation beneath bryozoan colonies would provide any substantial photosynthetic advantage to *M. pyrifera* during winter.

The clear pattern of reduced pigment concentration in blade tissue colonized by *Membranipora membranacea* in winter is most likely a result of direct damage to pigments due to bryozoan colonization. Pigment reductions may be symptomatic in an overall breakdown of blade tissue beneath *M. membranacea* colonies. A significant reduction in the structural integrity of blade tissue beneath *M. membranacea* colonies was observed in biomechanical testing of *Macrocystis pyrifera* from Harington Point (Hepburn, 2003). What caused this damage is unclear, but substances either excreted by bryozoan colonies or used to adhere to the seaweed surface may be responsible. The breakdown of pigments and blade tissue could be initiated by the physical presence of the colony alone or as a result of shading. It may even be a defensive response by *M. pyrifera* to shed heavily colonized blades to prevent being "overrun" by bryozoan colonies. A similar response has been suggested for the siphonalean green algae *Avrainvillea longicaulis* when colonized by epiphytes (Littler & Littler, 1999).

In contrast, there was no effect of *Membranipora membranacea* colonization on the pigment concentration of underlying *Macrocystis pyrifera* blade tissue during the summer. This appears to primarily be a result of the size and age of colonies at this time of year rather than environmental conditions during summer. During the summer *M. membranacea* colonies were small and are likely to be recent recruits (Hepburn, 2003) and the short

period between the initial colonization of blade tissue and its collection for pigment assays may not allow enough time for damage to pigments or a response by blade tissue to occur. The idea that colony size and age rather than season is important in determining the influence of bryozoan colonies on underlying algal tissue is also supported by the results of Hurd et al. (2000) who found clear reductions in pigment concentration beneath *M. membranacea* colonies during summer at a time when colonies were very large and covered much of colonized blades at their study site.

The absence of lower pigment concentrations for *Macrocystis pyrifera* blade tissue beneath *Obelia geniculata* colonies compared to the clear reductions in pigment content observed for blade tissue colonized by *Membranipora membranacea* are probably a result of morphological differences between the two colonies. The more diffuse form of hydroid colonies will not shade blade tissue to the same extent as sheet-forming epifauna such as bryozoans. Due to their stoloniferous nature *O. geniculata* colonies are in contact with less of the seaweed surface, which may lessen any negative impacts of colonization on underlying seaweed tissue.

Influence of hydroid colonies on nitrogen uptake

Hydroid colonies did not negatively influence the uptake of ammonium and nitrate by *Macrocystis pyrifera* blade tissue. This contrasts with the physical barrier provided by *Membranipora membranacea* colonies that reduced uptake of nitrate and ammonium by *Macrocystis integrifolia* up to 50% (Hurd et al., 1994). Although hydroid colonies can cover up to 85% of the blade surfaces of *M. pyrifera* during certain times of year (Hepburn & Hurd, 2005) their stoloniferous nature means that they are in contact with little of the blade surface and are thus unlikely to affect the acquisition of nutrients to a significant degree by providing a physical barrier.

During winter, ammonium was excreted by *Obelia geniculata* colonies at a greater rate than colonized *Macrocystis pyrifera* blade tissue could take it up, but in summer ammonium uptake rates, adjusted to account for ammonium excretion by attached hydroid colonies, were 30–43% higher for colonized than adjacent non-colonized discs. It is probable that higher concentrations of ammonium maintained between the hair-like hydrocauli of hydroid colonies during summer stimulate the higher uptake rates observed for colonized blade tissue allowing *M. pyrifera* to utilize as much ammonium provided by hydroids as possible during nitrogen limitation. The rapid uptake observed in colonized tissue may be facilitated by a more efficient ammonium assimilation pathway beneath hydroid colonies which may prevent the accumulation of intracellular pools of ammonium that are thought to exert control over ammonium uptake (Pederson, 1994; McGlathery et al., 1996).

Nitrate and ammonium uptake rates of all *Macrocystis pyrifera* blade tissue discs were markedly higher during summer than winter in Otago Harbour. Like many macroalgae *M. pyrifera* blade tissue exhibits heightened uptake of nitrate and ammonium after growth in conditions of low ambient nitrogen (Haines & Wheeler, 1978; Kopzak, 1994). This may allow utilization of spatial and temporal pulses of higher ammonium (including that from epifauna) and nitrate concentrations at Harington Point (Hepburn, 2003) and apparent in many near shore environments (e.g. Zimmerman & Kremer, 1984; Ramus & Venable, 1987).

Despite evidence that suggested colonized blade tissue took up ammonium provided by attached epifaunal colonies no evidence of nitrogen enrichment was observed. The most likely reason for this was that ammonium derived from epifauna was rapidly exported from colonized blade tissue to sinks both within the blade and beyond. Evidence from experiments using heavy isotope labelled ammonium tracers suggests mature blades, most commonly colonized by sessile epifauna, can export nitrogen to actively growing sections of the frond (Hepburn, 2003).

Conclusions

This study shows that epifauna with stoloniferous growth forms such as *Obelia geniculata* are less likely to negatively affect underlying algal tissue by causing a barrier to nutrient uptake and damaging pigments than sheet-forming epifauna such as *Membranipora membranacea*. The flexible and translucent nature of both colony types are likely to be adaptations that allow these organisms to

live on seaweed surfaces and is probably a result of strong selective to reduce damage to their living substratum. Sheet-forming epifauna, however, have a greater negative influence on colonized algal tissue due to the greater proportion of the blade surface they are in contact with compared to epifauna with stoloniferous growth forms. Heavy colonization by sessile epifauna may actually be a positive for macroalgae particularly in oligotrophic regions or in times of nitrogen limitation.

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