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C. L. Hurd · P. J. Harrison · L. D. Druehl

Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave-sheltered and exposed sites

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Abstract In conditions of low water motion ($<0.06\ m\ s^{-1}$), the availability of essential nutrients to macroalgae, and thus their potential productivity, may be limited by thick diffusion boundary-layers at the thallus surface. The ability of macroalgae to take up nutrients in slow moving water may be related to how their blade morphology affects diffusion boundary-layer thickness. For the giant kelp, *Macrocystis integrifolia* Bory, morphological measurements indicate that blades of plants from a site exposed to wave action are thick, narrow and have a heavily corrugated surface. In contrast, blades from a site with a low degree of water motion are relatively thin, with few surface corrugations and large undulations along their edges. The aim of our work was to test the hypothesis that morphological features of *M. integrifolia* blades from a sheltered site allow enhanced inorganic nitrogen uptake at low seawater velocities compared to blades with a wave-exposed morphology. The rate of nitrate and ammonium uptake by morphologically distinct blades of *M. integrifolia*, from sites that were sheltered from and exposed to wave action, were measured in the laboratory at a range of seawater velocities (0.01 to $0.16\ m\ s^{-1}$), between March and May 1993. For both sheltered and exposed blade morphologies, nitrate and ammonium uptake rates increased with increasing seawater velocity, reaching a maximum rate at 0.04 to $0.06\ m\ s^{-1}$. Uptake parameters V_{max} (maximum uptake rate) and $U_{0.37}$ (the velocity at which the uptake rate is 37% of the maximum rate) were estimated using an exponential decay formula. These parameters were similar for both blade morphologies, at all seawater velocities tested. Additional measurements suggest that the nitrogen status of *M. integrifolia* blades from wave-sheltered and exposed sites were similar throughout the experimental period, and thus nitrogen status did not affect the rate of nitrogen uptake in these experiments. On the basis of these results, we conclude that blade morphology does not enhance nitrogen uptake by *M. integrifolia* in conditions of low water motion. Potential effects of diffusion boundary-layers on kelp productivity are discussed.

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C. L. Hurd[1] (✉) · P. J. Harrison
Department of Oceanography, University of British Columbia, Vancouver, V6T 1Z4, Canada

L. D. Druehl
Bamfield Marine Station, Bamfield, British Columbia, V0R 1B0, Canada

Present address:
[1] Department of Botany, University of Otago, P.O. Box 56, Dunedin, New Zealand

Introduction

The importance of water motion as a factor controlling the structure, dynamics and productivity of marine macroalgal communities is well documented (Neushul 1972; Norton et al. 1982; Leigh et al. 1987; Wheeler 1988). In regions subjected to a high degree of water motion, for example in wave swept environments (up to $14\ m\ s^{-1}$) or currents (0.5 to $2.5\ m\ s^{-1}$; Hiscock 1983), drag and acceleration forces may limit macroalgal productivity by physically removing seaweeds from their substratum (Koehl 1984) or limiting the size to which they may grow (Gaylord et al. 1994). In contrast, in areas that are sheltered from wave action, currents and strong tidal effects, and experience water motion $< 0.10\ m\ s^{-1}$, macroalgal productivity may be reduced by thick diffusion boundary-layers at the thallus surface (Wheeler 1980, 1988).

Diffusion boundary-layers are well described in the literature and have been estimated experimentally and by mathematical modeling for aquatic plants

(e.g. Wheeler 1980; Jørgensen and Revsbech 1985; Borchardt et al. 1994). For nutrient uptake, the diffusion boundary-layer may be considered as a concentration gradient of a particular ion, for example nitrate, that is theoretically zero at the thallus surface if the alga acts as a perfect "sink" for that ion and is maximal some distance away from the thallus surface (Dromgoole 1978; Wheeler 1988; Borchardt et al. 1994). The thicker the concentration gradient, the greater the distance over which ions must travel. It has been shown experimentally that the rate of photosynthesis and inorganic nitrogen uptake of seaweeds increases with increasing outer seawater velocity, reaching a maximum rate at velocities of 0.02 to 0.06 $m\,s^{-1}$ (Wheeler 1980, 1982; Gerard 1982). These observed increases in uptake rates with increasing water velocities are due to the progressive decrease in diffusion boundary-layer thickness until it reaches a minimum of about 0.3 mm (Jørgensen and Revsbech 1985; Wheeler 1988). At this minimum thickness some other factor, for example enzyme activity, becomes rate-limiting (Wheeler 1988).

Seawater velocities of less than 0.06 $m\,s^{-1}$ occur in estuaries and embayments that are sheltered from wave action and regions within large kelp beds where the kelp themselves reduce the ambient seawater velocity (Wheeler 1980; Jackson and Winant 1983). It has been suggested that in these regions of low water motion, diffusion boundary-layers may become thick enough to significantly reduce nutrient availability at the seaweed thallus surface, and hence reduce macroalgal productivity (Wheeler 1980, 1988; Raven 1984).

The giant kelp, *Macrocystis integrifolia* Bory, inhabits the northeast Pacific coastline, forming dense beds from Kodiak Island, Alaska, to the Monterey peninsula (Druehl 1970). The blade morphology of *M. integrifolia* varies with the degree of water motion in which it grows (Pace 1972; Druehl 1978; Druehl and Kemp 1982). Blades of *M. integrifolia* from wave-exposed sites are narrow, with a highly corrugated surface and marginal spines that are flush with the blade edge, while blades from sheltered sites are wider, with few surface corrugations and marginal spines that arise at irregular angles to the blade (Pace 1972). When plants from wave-exposed and sheltered sites were transplanted to a common site of intermediate wave exposure, morphologies of new blades were similar, regardless of the site of origin, which suggests that blade morphology was controlled by factors associated with water motion (Pace 1972; Druehl and Kemp 1982).

Morphological variations in *Macrocystis* spp. may represent adaptations to enhance the uptake of essential inorganic nutrients in areas of slow moving water (Neushul 1972; Wheeler 1980; Norton et al. 1982). The aim of our work was to test the hypothesis that the morphology of *M. integrifolia* blades from a sheltered site enhance nitrate and ammonium uptake at low seawater velocities relative to blades from an exposed site. Uptake experiments were conducted in a low volume (46 litre) recirculating flow tank in which seawater flow was virtually laminar and allowed any differences in uptake rate to be attributed to differences in blade morphology (Hurd et al. 1994a).

Materials and methods

Collection sites

Two collection sites at Dixon Island, Barkely Sound, British Columbia, Canada (48° 51.24′N; 125° 7′W), which were within 500 m of each other, were chosen so that the sites were exposed to and sheltered from wave action, but were close enough to each other that levels of irradiance and temperature were expected to be similar (Fig. 1). *Macrocystis integrifolia* Bory from the wave-sheltered and exposed sites possessed morphologies typical of those water motion conditions (Fig. 2).

Seawater samples were collected at both wave-sheltered and exposed sites 1 wk prior to experiments, and throughout the experimental period (18 March to 5 May 1993). Samples were filtered through a Whatman GF/C filter and analysed for nitrate and ammonium concentrations using a Technicon AutoAnalyser II.

Collection of *Macrocystis integrifolia*

The top 1.5 m of individual *Macrocystis integrifolia* fronds were collected from both sheltered and exposed sites the evening before

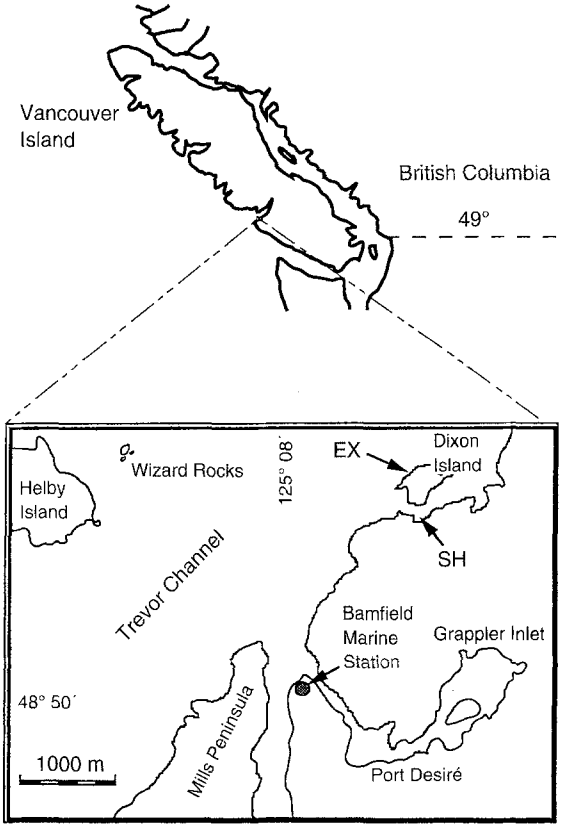

Fig. 1 Collection sites for sheltered (*SH*) and exposed (*EX*) blade morphologies of *Macrocystis integrifolia* at Dixon Island, British Columbia, Canada

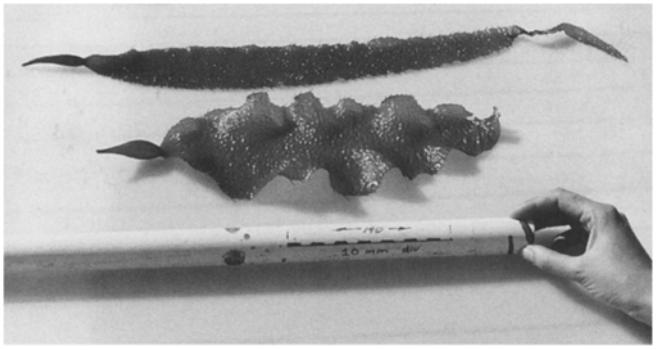

Fig. 2 *Macrocystis integrifolia.* Photograph of blades typical of those from sheltered (*lower*) and exposed (*upper*) sites at Dixon Island. Main differences: blades from the sheltered site are wide, have few surface corrugations and large undulations along each blade edge; blades from exposed sites are narrow, have a densely corrugated surface and no edge undulations

an experiment and returned to Bamfield Marine Station within 15 min. Two adjacent blades that were healthy in appearance and had no visible epiphytes were selected from both fronds and were gently wiped with tissue to remove silt and micro-organisms. One blade from each of the two fronds (pretreatment blade) was used to determine the soluble tissue nitrate and total tissue carbon and nitrogen for blades collected directly from the field, and to assess the effect of pretreatment on the soluble tissue nitrate content. The other two blades (experimental blades) were used in uptake experiments, and their nitrogen status determined at the end of the experiments.

Blade pretreatment

Two pieces of tissue (2.5 cm^2) were cut from each of the two pretreatment blades, 10 and 20 cm distal to the bulb for each blade. The soluble tissue nitrate pool was estimated for each cut piece. Another tissue piece (5 cm^2) was removed 22 cm distal to the bulb and used to assess total tissue carbon and nitrogen.

The four blades (pretreatment and experimental blades from sheltered and exposed sites) were placed in four 4 litre Pyrex beakers containing 4 litre of ultraviolet-sterilized (Behrick Enterprises), filtered (1 and 0.5 µm Cuno Microwynd II filters in series) seawater containing 15 to 20 µ*M* nitrate and undetectable levels of ammonium. Beakers were placed in a constant temperature room (12 °C) overnight with an overhead irradiance of 170 µmol $m^{-2} s^{-1}$ provided by 4 Vitalite fluorescent tubes, set to a 16 h light: 8 h dark photoperiod (see Hurd et al. 1994a for details). Immediately prior to the start of an uptake experiment, two more tissue pieces (2.5 cm^2) were cut from the pretreatment blades, analysed for soluble tissue nitrate and the blades discarded. Experimental blades were used in the uptake experiments, conducted between 18 March and 5 May 1993.

Nitrate and ammonium uptake experiments

Uptake experiments were conducted in a 46 litre recirculating flow tank, the design and operation of which are detailed by Hurd et al. (1994a). Seawater was obtained from Bamfield Marine Station seawater system (pumped from 20 m) and contained 15 to 20 µ*M* nitrate and 0 to 0.5 µ*M* ammonium at the time of the experiments. Seawater nitrate concentration was reduced to below that of the starting concentration of the experiment (15 µ*M*) by filling an outdoor aquarium with 100 litre seawater and placing a single 1.5 m apical region of a *Macrocystis integrifolia* frond in the tank overnight, with vigorous water motion provided by a submersible pump.

On the morning of an experiment, two flow tanks were placed in a constant temperature room at 12 °C, with 170 µmol $m^{-2} s^{-1}$ irradiance provided by four Vitalite fluorescent tubes overhead. Tanks were filled with 46 litre of ultraviolet sterilized, filtered seawater. Nitrate and ammonium were added to the seawater to initial concentrations of 15 µ*M*, and seawater was then allowed to recirculate for ca. 2 h until it reached the experimental temperature of 12 °C. Experiments commenced between 0930 and 1030 hrs each day.

For each uptake experiment, one sheltered blade was placed in one flow tank, and one exposed blade placed in the second flow tank. Because each experiment was 10 h in duration, replicate experiments were conducted on different days. The origin of the blade (sheltered or exposed site) in each tank was alternated between experiments to avoid any bias associated with the different tanks. Nitrate and ammonium uptake rates were estimated from the difference between the initial and final concentrations and expressed on a surface area (µmol $m^{-2} s^{-1}$) basis. In all cases, surface area was estimated taking corrugation height and density into account (see "Surface area estimation").

Initial concentrations of nitrate and ammonium in uptake experiments in the present study were 15 µ*M*. For nitrate, this concentration is typical of those found in Barkley Sound during winter (Smith et al. 1983; see also Fig. 4 present study). For ammonium, however, concentrations used in uptake experiments were higher than those naturally found in this region. These higher ammonium levels were used to avoid the potential interactive effects between nitrate and ammonium, previously recorded for members of the Laminariales (Harrison et al. 1986).

Time-course of uptake

Nutrient uptake by seaweeds is often non-linear over time; periods of initially high (surge uptake), low or no (lag phase) uptake can lead to the over- or under-estimation of rates (e.g. Hurd and Dring 1990 and references therein). To determine if uptake rate varied with time on the time-scale of our experiments, the removal of nitrate and ammonium by individual *Macrocystis integrifolia* blades was followed over 10 h at three seawater velocities (0.02, 0.04, 0.16 m s^{-1}). Four replicate experiments were conducted for each velocity used. Seawater samples for nutrient analysis were taken at time = 0 h and every 2 h thereafter. The mean uptake rate was calculated for each 2 h interval.

Uptake rate at a range of flow velocities

Nitrate and ammonium uptake rates were measured sequentially, for each of six replicate blades, at six seawater velocities: 0.01, 0.02, 0.04, 0.06, 0.08 and 0.16 m s^{-1}. For each blade, uptake rates were measured at the highest velocity (0.16 m s^{-1}) first, and then at progressively lower velocities with 0.01 m s^{-1} being the final velocity used. For each velocity, an initial seawater sample was taken, and then one experimental blade was placed in each flow tank and left for 1 to 2 h. A second (final) seawater sample was then taken. To ensure that the nitrate and ammonium concentrations at the start of each flow velocity were the same (15 µ*M*), the blades were removed from the tanks after each final sample was taken and placed in the 4 litre beaker containing the pretreatment seawater. The water in the tank was then vigorously mixed, and a seawater sample analysed for nitrate and ammonium. The amount of nitrate and ammonium taken up by the kelp was calculated, and this amount was added back to the seawater to return the initial concentration to 15 µ*M*. The next initial sample was then taken, and the blade returned to the tank at the new seawater velocity. This process took 20 min for each velocity.

For each blade used, nitrate and ammonium uptake rates were plotted against flow velocity and the uptake parameters V_{max} (maximum uptake rate under the given experimental conditions) and $U_{0.37}$ (the velocity at which the uptake rate is 37% of the maximum rate) estimated using a non-linear fitting routine (Sigma Plot, Jandell Scientific) from the exponential decay formula:

$$V = V_0 + (V_{max} - V_0)\,[1 - \mathrm{EXP}(-U/U_{0.37})],$$

where V = uptake rate (μmol m^{-2} s^{-1}), $V_0 = 0.7 \times 10^{-2}$ μmol m^{-2} s^{-1} which is the estimated uptake rate at zero velocity if the diffusion boundary-layer is allowed to form over a 1 to 2 h period, and U = flow velocity (m s^{-1}). Mean values ($\pm$ 1 SE) of V_{max} and $U_{0.37}$ were then calculated, and differences between means tested using a Student's t-test ($p < 0.05$).

At the end of each uptake experiment, blade wet weight was estimated and tissue disks cut 10 and 20 cm distal to the bulb, for soluble tissue nitrate analysis. Blades were then photocopied to produce an outline that was used to calculate blade area, and morphological parameters determined. Finally, blades were oven dried at 80 °C overnight, their dry weight determined, and then stored on silica gel until analysed for total tissue carbon and nitrogen.

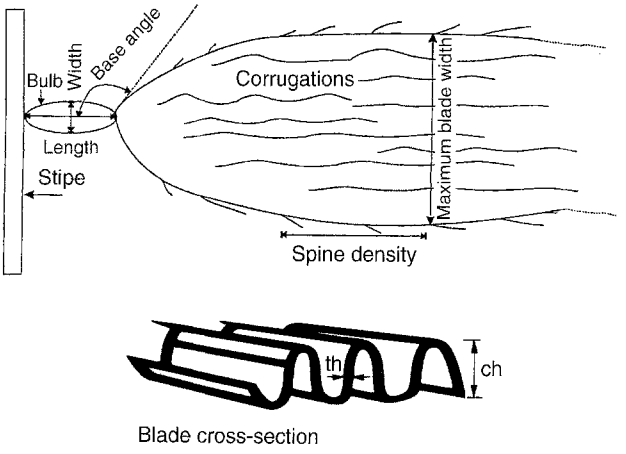

Fig. 3 *Macrocystis integrifolia.* Morphological parameters measured for each blade used in uptake experiments (not to scale) (*ch* corrugation height; *th* blade thickness)

Soluble tissue nitrate, total tissue carbon and nitrogen

Soluble tissue nitrate was estimated using a boiling-water extraction (Thomas 1983). The 2.5 cm^2 tissue disks were placed in a 50 ml boiling-tube containing 40 ml of distilled water: no increase in yield was observed for pieces that were ground or chopped into small pieces prior to extraction. Boiling-tubes were placed in a boiling water bath for 20 min, cooled and the liquid filtered though a 0.45 µm Whatman GF/C filter prior to nitrate analysis. No further nitrate was released when second and third extractions were conducted on the same algal disk.

The 5 cm^2 blade samples taken for total tissue carbon and nitrogen were dried overnight in an oven at 80 °C, and stored in a desiccator with silica gel for up to 3 mo. Tissue was then ground into a fine powder using a mortar and pestle, and a 2 to 8 mg subsample analysed for total carbon and nitrogen using a Perkin-Elmer Model 240 Elemental Analyser.

Morphological measurements

At the end of each experiment, the following morphological parameters were obtained for each blade: maximum blade width and bulb length were measured to the nearest millimeter using a rule, and bulb width, corrugation height and blade thickness were measured to the nearest 0.1 mm using calipers (Fig. 3). In addition, photographs were taken of each blade on a light table using a 35 mm SLR camera, and the following information obtained at a later date: spine density (spines cm^{-1}), base angle (°) and corrugation density (corrugations cm^{-1}; Fig. 3).

Surface area estimation

In preliminary experiments, uptake rates of *Macrocystis integrifolia* blades from the sheltered site appeared higher than those of exposed blades (Hurd et al. 1994b), but this was subsequently found to be a function purely of differences in blade dry weight. As nutrient uptake occurs across the blade surface, surface area is probably the standard that best reflects the rate at which nutrients are taken up across the cell membrane (Harrison and Druehl 1982).

The surface area of the photocopied blade was estimated using MacMeasure (Version II, Macintosh). The increase in surface area due to surface corrugations was estimated from the thickness of the blade, the corrugation height and the mean number of corrugations per unit blade width (Fig. 3). The number of corrugations was estimated at four regions along the blade by drawing transverse lines across each photograph at distances that were 20, 40, 60 and 80% of the total length of the blade. The number of peaks or dips crossing each line was counted, providing the number of corrugations per cm (wavelength). The corrugations were assumed to be sinusoidal, with an amplitude that was the corrugation height (ch) minus the blade thickness (th; Fig. 3). Knowing the length of a sine wave of known amplitude (ch − th) and wavelength (corrugations cm^{-1}) a new surface area that took the corrugations into account was estimated.

Results

Seawater nitrogen and nitrogen status of the blades

Between 14 and 28 March, the concentration of nitrate in surface waters ranged from 0.24 to 13.66 µM at the exposed site and from undetectable to 6.43 µM at the sheltered site (Fig. 4A, B). Ammonium concentrations were below the limits of detection at the exposed site throughout the experimental period, while at the sheltered site, ammonium (< 0.6 µM) was recorded in surface waters on two occasions (data not shown). After 28 March, concentrations of inorganic nitrogen were undetectable at both sites.

Soluble tissue nitrate concentrations reflected the seawater nitrate concentration; concentrations dropped from maximal values of 48 µmol g^{-1} wet wt (wave-exposed site) and 30 µmol g^{-1} wet wt (wave-sheltered site) recorded between 14 and 28 March to below the limits of detection (Fig. 4C, D).

Total tissue carbon of both exposed and sheltered blades fluctuated around a mean value of 28% dry wt (Fig. 4E, F). At the start of the experimental period, total tissue nitrogen was 3% dry wt for both exposed and sheltered blades (Fig. 4G, H). By 17 April, % total

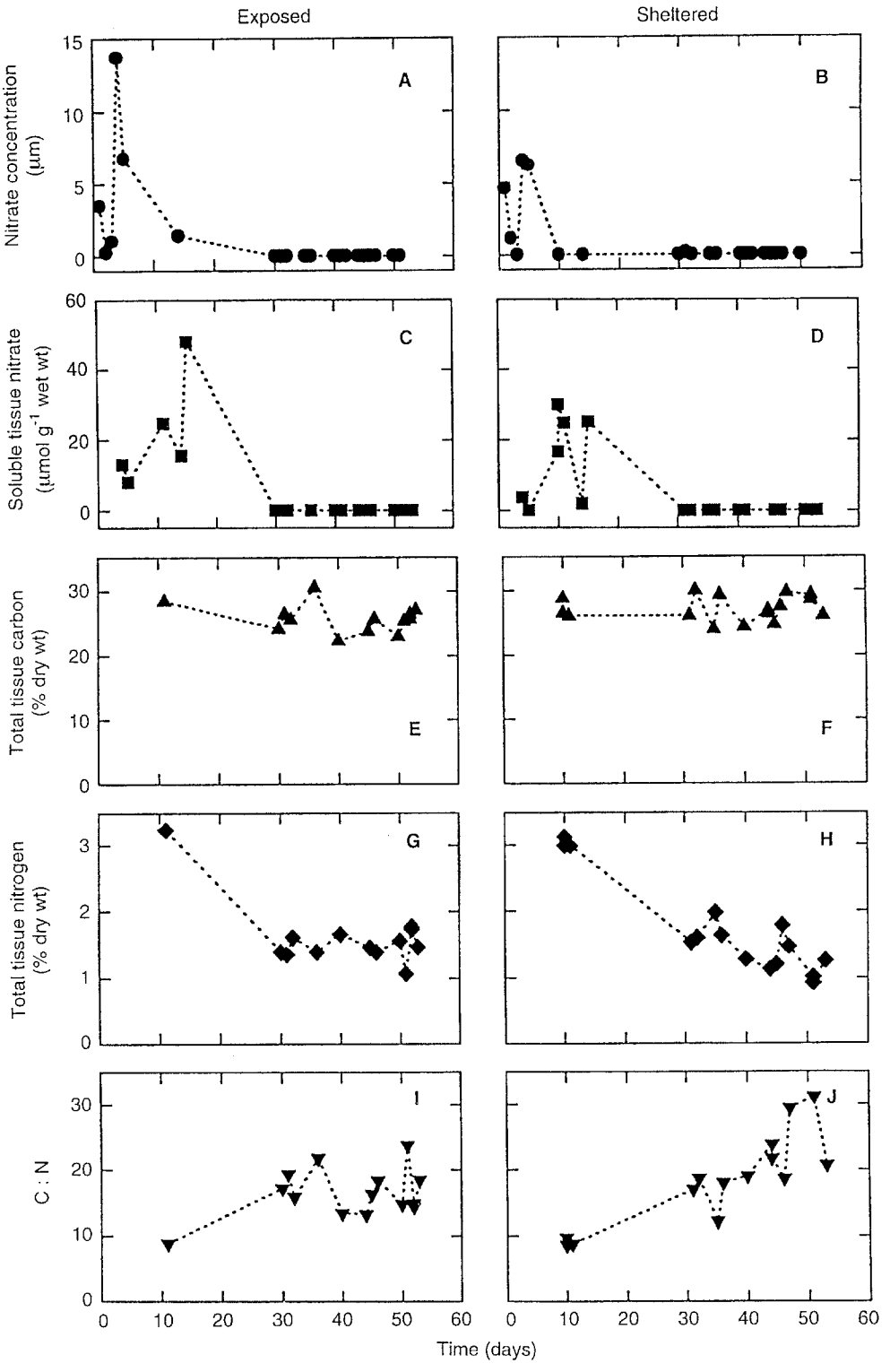

Fig. 4 *Macrocystis integrifolia.* Surface seawater nitrate concentration at exposed and sheltered Dixon Island sites (**A**, **B**). Soluble tissue nitrate (**C**, **D**), total tissue carbon (expressed as % dry wt; **E**, **F**) and nitrogen (expressed as % dry wt; **G**, **H**) and C:N molar ratio (**I**, **J**) for blades collected from exposed and sheltered sites between 14 March (Day 1) and 5 May 1993

tissue N had dropped to around 1.5% for both exposed and sheltered blades, and remained at this level until the end of the experimental period. Variations in tissue N were reflected by the C:N ratio, which increased from 9 to ca. 20 between 14 March and 17 April (Fig. 4I, J).

Effect of pretreatment on blade nitrogen status and uptake rates

There was no difference between soluble tissue nitrate content of freshly collected blades from both exposed and sheltered sites and the same blades following

pretreatment (data not shown). Similarly, there was no difference between tissue nitrate content of freshly collected (pretreatment) blades and the experimental blades following the uptake experiment (data not shown).

Soluble tissue nitrate and total tissue nitrogen of blades used in uptake experiments showed no correlation with maximum nitrate or ammonium uptake rates (data not shown). There was no change in maximum nitrate or ammonium uptake rates at seawater velocities of 0.16 m s^{-1} over the entire experimental period (data not shown).

Morphological parameters and surface area

Macrocystis integrifolia blades from the sheltered site were wider and had a smaller base angle than blades from exposed sites, while exposed blades had wider, longer bulbs and twice as many corrugations as sheltered blades (Table 1). The number of spines per cm along the edge of the blade, corrugation height and blade thickness were not significantly different for sheltered and exposed blades ($p > 0.05$; Student's t-test; Table 1). Blades from the sheltered site possessed large undulations (ca. 5 cm wide) along each edge (Fig. 2).

When surface corrugations were taken into account, the mean increase in surface area of exposed blades compared to a flat blade was 7.2% with a maximum increase of 19%. By comparison, the mean increase in surface area of sheltered blades was 1.4%, with a maximum increase of 6.4%. For blades of the same wet weight, the surface area of blades from sheltered sites was around 1.4 times greater than that of blades from the exposed site (Fig. 5). Slopes of regression lines were significantly different at $p < 0.05$ (Student's t-test; Zar 1984). The ratio of wet wt:dry wt for blades from sheltered and exposed sites was 8.55 and 9.74, respectively.

Table 1 *Macrocystis integrifolia.* Morphological parameters of blades used in uptake experiments (± 1 SD, $n = 16$; for corrugation height, $n = 10$). Differences between means were tested using a Student's t-test and asterisks indicate the level of significance (*$p > 0.05$; **$p > 0.001$; *NS* = not significant at $p = 0.05$)

Blade parameters	Sheltered	Exposed	p
Max. blade width (cm)	13.41 ± 1.3	8.9 ± 1.1	***
Corrugation height (cm)	0.077 ± 0.044	0.12 ± 0.029	NS
Blade thicknes (cm)	0.032 ± 0.004	0.039 ± 0.007	NS
Bulb width (cm)	1.31 ± 0.18	1.45 ± 0.13	*
Bulb length (cm)	4.10 ± 0.71	5.5 ± 0.89	***
Base angle[a] (°)	124 ± 14.3	136 ± 10.4	*
Spine density[a] (spines cm^{-1})	1.29 ± 0.30	1.37 ± 0.23	NS
Corrugation density[a] (corrugations cm^{-1})	1.02 ± 0.39	2.19 ± 0.39	*

[a]See Fig. 3 for illustration of these terms

Time-course of nitrate and ammonium uptake

Uptake rates estimated for each 2 h sampling period were generally constant over time at the three outer velocities tested (2, 4, 16 cm s^{-1}); there was no evidence for initially rapid or slow uptake (data not shown).

Nitrate and ammonium uptake rate at a range of flow velocities

At all flow velocities tested, nitrate and ammonium uptake rates of sheltered and exposed blades were similar (Fig. 6). No differences were found between V_{max} or $U_{0.37}$ for either sheltered or exposed blades ($p > 0.05$, Student's t-test; Fig. 7). For nitrate and ammonium, uptake rates reached a maximum at between 0.04 and 0.06 m s^{-1}. For ammonium, uptake rate increased rapidly up to 0.04–0.06 m s^{-1}, but then increased slowly to 0.16 m s^{-1} (Fig. 6).

Estimated diffusion boundary-layer thickness

The thickness of the diffusion boundary-layer was estimated from Fick's first law:

$$J = \frac{D \times C}{L},$$

where J is a flux, or in this case the nitrate or ammonium uptake rate of *Macrocystis integrifolia* blades (10^{-2} μmol m^{-2} s^{-1}; values obtained from Fig. 6), D is the diffusion coefficient for nitrate or ammonium in seawater (14.11×10^{-10} m^{-2} s^{-1} at 12 °C; Li and

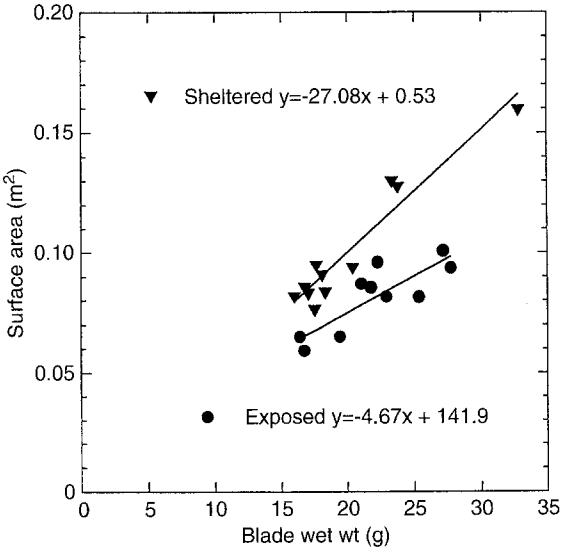

Fig. 5 *Macrocystis integrifolia.* Estimated surface area against wet weight for *M. integrifolia* collected from sheltered and exposed sites at Dixon Island between 14 March and 5 May 1993. Slopes of regression lines were significantly different ($p < 0.05$, Student's t-test)

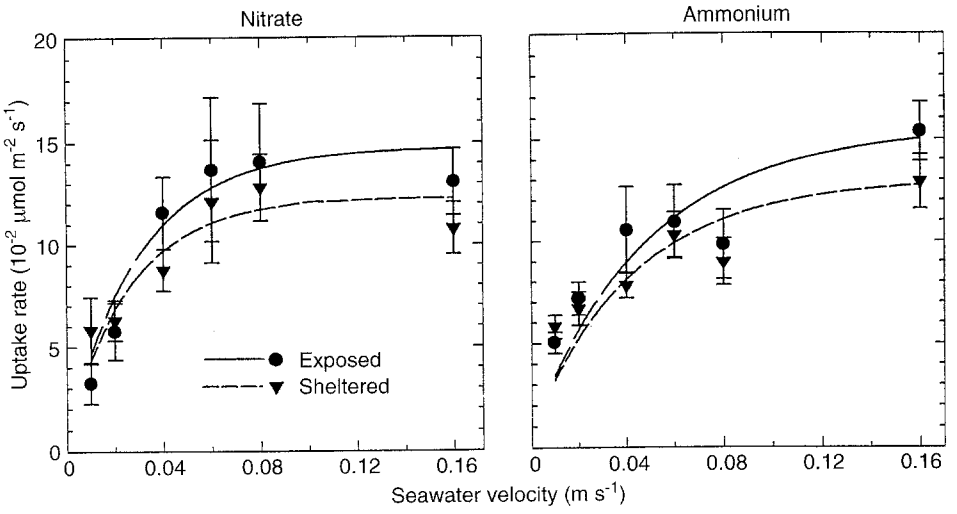

Fig. 6 *Macrocystis integrifolia.* Mean nitrate and ammonium uptake rates as a function of seawater velocity for sheltered and exposed blade morphologies (± 1 SE, $n = 6$). Curves fitted by substituting values of V_{max} and $U_{0.37}$ averaged from results of each individual uptake experiment into the exponential decay formula (see "Methods – Uptake rate at a range of flow velocities")

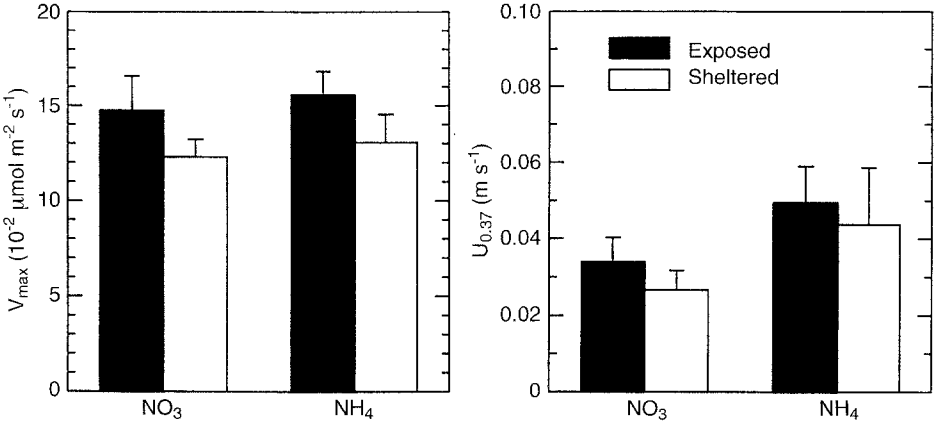

Fig. 7 *Macrocystis integrifolia.* Mean values of V_{max} and $U_{0.37}$ (the flow velocity at which $V = 0.37\ V_{max}$; ± 1 SE, $n = 6$) for nitrate and ammonium uptake by sheltered and exposed morphologies. Uptake parameters were not statistically different ($p = 0.05$, Student's t-test)

Gregory 1974), C is the change in concentration between the outer flow (15 µM) and the blade surface (0 µM) and L is the thickness of the diffusion boundary-layer (m). Two assumptions were made in this calculation: (1) the concentration of nutrients at the blade surface was zero, i.e. the blade was assumed to be a perfect "sink" (Wheeler 1980; Raven 1984), and (2) the flux across the diffusion boundary-layer was in steady state (Vogel 1981). Diffusion boundary-layer thickness was similar for blades from sheltered and exposed sites (Fig. 8).

Discussion

Nitrogen status of *Macrocystis integrifolia*

The nitrogen status of seaweeds, in particular the soluble tissue nitrate content, is considered an important factor controlling the rate of nitrate and ammonium uptake (see Wheeler and Srivastava 1984; Fujita 1985; Kopczak 1994). Before discussing the possible effects of blade morphology on inorganic nitrogen uptake, it is first important to consider the confounding influence of blade nitrogen status on uptake rate.

Macrocystis integrifolia populations in Barkley Sound have been well studied with regard to seasonal variations in growth (Lobban 1978; Druehl and Wheeler 1986; Wheeler and Druehl 1986), photosynthesis (Smith et al. 1983) and nitrogen physiology (Wheeler and Srivastava 1984; Rosell and Srivastava 1985). This large data base allows predictions of when blades located along particular regions of the frond will exhibit a similar physiological status for extended periods. For these experiments, a 6 wk period was identified when the soluble tissue nitrate in blades between the 3rd and 12th positions behind the apical scimitar were expected to be depleted, but nitrogen requirement was high implying maximal nitrate and ammonium uptake rates (Wheeler and Srivastava 1984). This approach proved successful, as soluble tissue nitrate of blades sampled was zero for the majority of experiments, and there was no relationship between tissue nitrate content following the experiment and nitrate or ammonium uptake rate. Blade total tissue nitrogen was around 1.5% of dry weight for the majority of the experimental period and C:N remained around 20, indicative of mild nitrogen limitation (Rosell and Srivastava 1985). Further, the maximum observed uptake

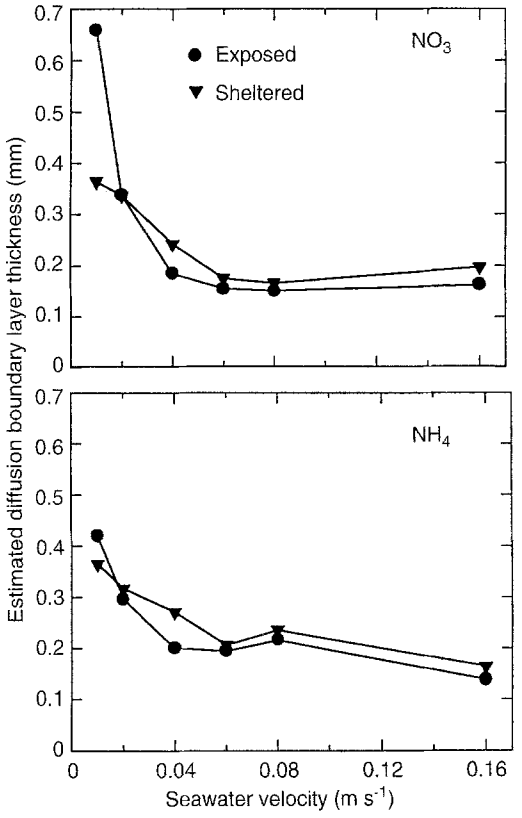

Fig. 8 *Macrocystis integrifolia.* Estimated thickness of the diffusion boundary-layer at a range of flow velocities for sheltered and exposed blade morphologies. Boundary-layer thickness was calculated from the results of uptake experiments (Fig. 6) using Fick's first law (see "Results – Estimated diffusion boundary-layer thickness

rates (V_{max}) were similar for sheltered and exposed blades, again indicating that blades were physiologically similar with respect to inorganic nitrogen uptake (Hanisak 1983; Kopczak 1994). Based on this evidence, we suggest that the nitrogen status of blades from the sheltered and exposed sites were similar over the experimental period, and thus any differences between uptake rate must be attributed to differences in blade morphology.

Blade morphology and diffusion boundary-layer thickness

Despite clear between-site differences in blade morphology (Fig. 2; Table 1), nitrate and ammonium uptake rates and estimated diffusion boundary-layer thickness were similar for sheltered and exposed blades of *Macrocystis integrifolia* at all seawater velocities tested. Based on this result, we must conclude that contrary to previous hypotheses, morphological variations of *M. integrifolia* do not represent adaptations to enhance the uptake of inorganic nitrogen in slow moving water. In flow visualization experiments, conducted to complement the uptake experiments described in this paper, distinct differences were observed between the structure of the velocity boundary-layers around the sheltered and exposed blade morphologies of *M. integrifolia*; the large edge undulations of the sheltered blades generate recirculating eddies, while seawater flow over exposed blades is similar to that over a flat plate (Hurd et al. 1994a; Hurd et al. 1996). However, the transition from a laminar to a turbulent velocity boundary-layer occurred at the same outer seawater velocities (2 cm s^{-1}) for both blade morphologies (Hurd et al. 1996). This finding supports our conclusion that the sheltered blade morphology of *M. integrifolia* does not enhance the transport of inorganic nitrogen across boundary-layers at low seawater velocities.

Diffusion boundary-layers and *Macrocystis integrifolia* productivity

As blade morphology had no apparent effect on inorganic nitrogen uptake by *Macrocystis integrifolia*, then nitrogen availability and hence the productivity of *M. integrifolia* will be reduced if seawater velocities are frequently less than those required to saturate uptake. While there is no doubt that nutrient uptake, photosynthesis and growth are reduced in slowly moving water in laboratory conditions (e.g. Whitford and Kim 1967; Wheeler 1980, 1982; Fujita and Goldman 1985), the extent to which diffusion boundary-layers reduce productivity of subtidal kelp growing in situ is unclear.

The degree to which diffusion boundary-layers reduce nitrogen availability to *Macrocystis integrifolia* in situ depends on the occurrence of periods when water motion is less than that required for maximum uptake (0.04 to 0.06 m s^{-1}; Fig. 6). Outer velocities of 0.03 m s^{-1} were sufficient to saturate nitrate uptake by *M. pyrifera* (Gerard 1982; Wheeler 1982). Gerard (1982) measured seawater velocity at the blade surface of *M. pyrifera* and concluded that even under the calmest conditions, in situ water movement would be saturating for uptake. S4 (Inter-Ocean Systems, Inc, CA, USA) electromagnetic current meters deployed at our wave-sheltered site during April 1994 showed that flow velocities < 0.06 m s^{-1} occur 30% of the time (Isachsen 1995). However, the growth of the diffusion boundary-layer within this time period (30%) is reduced markedly as velocity pulses > 0.06 m s^{-1} occur frequently, meaning that the diffusion boundary-layer never grows significantly (Stevens and Hurd in preparation).

If diffusion boundary-layers significantly reduced nitrogen availability (or the availability of other essential nutrients, e.g. bicarbonate) to *Macrocystis integrifolia* then lower growth rates at the wave-sheltered site compared to the wave-exposed site could be predicted. Frond elongation rates (an estimate of growth rate) of a wave-sheltered *M. integrifolia* population (also at

Dixon Island) were consistently lower than those of a wave-exposed site at Wizard Rocks (Pace 1972; see Fig. 1 present study for location). However, seasonal growth and productivity of *M. integrifolia* from nearby wave-sheltered (Grappler Inlet) and exposed sites (Helby Island) were not significantly different, but were highly variable between years (Wheeler and Druehl 1986; see Fig. 1 for locations). Other workers report inconsistent differences between growth rates for subtidal kelp at wave-sheltered and exposed sites. Annual growth rates of wave-sheltered *Laminaria longicruris* were higher than those of wave-exposed plants for $8\,mo\,yr^{-1}$ (Gerard and Mann 1979). In contrast, a Chilean population of *M. pyrifera* from a wave-sheltered site was described as pale, brittle, frequently non-reproductive and lacking blades in lower regions of the frond compared to healthy wave-exposed plants (Dayton 1985). Other confounding factors associated with sites of low water motion that might reduce kelp productivity include: (1) reduced irradiance at the blade surface due to siltation (Norton et al. 1982; Dayton 1985), (2) little blade movement leading to reduced light transmittance to understorey blades (Dayton 1985), (3) lower nutrient availability due to reduced mixing in the entire water column (Norton et al. 1982).

Reduced productivity due to boundary layers has been demonstrated for small turf-forming algae (Carpenter et al. 1991; Carpenter and Williams 1993). These algae grow within the benthic boundary-layer, a region of reduced velocity that extends about 20 cm above the seafloor (Denny 1988). Thus, seawater flow can be described as having a velocity boundary-layer within a velocity boundary-layer; the velocity boundary-layer at their thallus surface within the benthic boundary-layer (Carpenter and Williams 1993). In contrast, a 20 m long adult *Macrocystis integrifolia* sporophyte extends beyond the benthic boundary-layer, and the majority of its blades lie within surface waters where velocities are relatively enhanced (Phillips 1977, Sections 4.2, 6.6).

Macrocystis integrifolia from the wave-sheltered site had a 40% greater surface area per unit wet weight than that of the wave-exposed blades (Fig. 5), and would therefore require a lower uptake rate to fulfill the blades' nitrogen requirement for growth. So, even if diffusion boundary-layers did reduce nutrient availability to *M. integrifolia* at the wave-sheltered site, this would be alleviated by the higher surface area:volume ratio. This is further evidence for our conclusion that diffusion limitation is unlikely to reduce nitrogen availability to the wave-sheltered *M. integrifolia* population of this study.

At our wave-exposed site, mean seawater velocities were rarely below those required to saturate inorganic nitrogen uptake (Isachsen 1995). However, the densely corrugated surface of wave-exposed blades may have an important role in nitrogen uptake by maintaining a high surface area:volume ratio. Other kelp species have relatively smooth, flat blades, with a narrow and thick morphology when exposed to wave action (e.g. Gerard and Mann 1979). *Laminaria longicruris* serves as an example in which a three-fold increase in blade thickness and decreased blade width in wave-exposed plants translates into a three- to four-fold decrease in the surface area:wet wt ratio (analogous to the surface area:volume ratio) compared to plants from a sheltered site (Gerard and Mann 1979). A narrow, thick morphology will reduce drag forces that would otherwise damage or remove blades, and increase blade strength. However, the concomitant reduction in the surface area:volume ratio offsets the diffusional advantage of growing in fast moving water (Vogel 1981, p 151). In contrast, blades of *Macrocystis integrifolia* from the exposed site were not significantly thicker than those from the sheltered site (Pace 1972; Table 1 present study) and the surface area:wet wt ratio of exposed blades was only 1.4 times lower than that of sheltered blades (compared to the three- to four-fold decrease for *L. longicruris*; Gerard and Mann 1979). This supports the hypothesis of Norton et al. (1982) that surface corrugations of *M. integrifolia* increase blade strength while allowing a relatively low blade thickness and a high surface area:volume ratio, and do not serve to enhance turbulence and nutrient transport at the blade surface as previously suggested by Wheeler (1980).

In conclusion, blade morphology of *Macrocystis integrifolia* had no effect on inorganic nitrogen uptake or diffusion boundary-layer thickness. We suggest that at the wave-sheltered site of this study, the combined effects of frequent pulses of high velocity seawater, the location of blades in surface waters away from the benthic boundary-layer, and the higher surface area:wet wt ratio of sheltered blades means that diffusion boundary-layers have little effect on inorganic nutrient availability to *M. integrifolia*. However, other factors associated with low water motion, e.g. siltation, could lead to lower productivity in wave-sheltered sites. Surface corrugations of wave-exposed blades may maintain a high surface area:wet wt ratio, while increasing structural strength.

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