### Title

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# INVERTEBRATE-MEDIATED NUTRIENT LOADING INCREASES GROWTH OF AN INTERTIDAL MACROALGA[1]

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**Even in nitrogen-replete ecosystems, microhabitats exist where local-scale nutrient limitation occurs. For example, coastal waters of the northeastern Pacific Ocean are characterized by high nitrate concentrations associated with upwelling. However, macroalgae living in high-zone tide pools on adjacent rocky shores are isolated from this upwelled nitrate for extended periods of time, leading to nutrient limitation. When high-intertidal pools are isolated during low tide, invertebrate-excreted ammonium accumulates, providing a potential nitrogen source for macroalgae. I quantified the influence of mussels (*Mytilus californianus* Conrad) on ammonium accumulation rates in tide pools. I then evaluated the effects of ammonium loading by mussels on nitrogen assimilation and growth rates of *Odonthalia floccosa* (Esp.) Falkenb., a common red algal inhabitant of pools on northeastern Pacific rocky shores. *Odonthalia* was grown in artificial tide pool mesocosms in the presence and absence of mussels. Mesocosms were subjected to a simulated tidal cycle mimicking emersion and immersion patterns of high-intertidal pools on the central Oregon coast. In the presence of mussels, ammonium accumulated more quickly in the mesocosms, resulting in increased rates of nitrogen assimilation into algal tissues. These increased nitrogen assimilation rates were primarily associated with higher growth rates. In mesocosms containing mussels, *Odonthalia* individuals added 41% more biomass than in mesocosms without mussels. This direct positive effect of mussels on macroalgal biomass represents an often overlooked interaction between macroalgae and invertebrates. In nutrient-limited microhabitats, such as high-intertidal pools, invertebrate-excreted ammonium is likely an important local-scale contributor to macroalgal productivity.**

*Key index words:* **ammonium; excretion; facilitation; macroalgae; *Mytilus*; nutrients; *Odonthalia*; Oregon; rocky intertidal; uptake**

*Abbreviations:* **ANOVA, analysis of variance; dm, dry mass**

Bottom-up factors, such as nutrient availability, have received comparatively little attention from benthic marine ecologists (Menge 1992). However, by influencing macroalgal abundances, nutrients can have important effects on the structure and dynamics of near-shore and intertidal marine communities (Bosman et al. 1987, Wootton et al. 1996, Worm et al. 2002, Nielsen 2003). Understanding the sources and variability of nutrients is crucial for understanding the distributions and abundances of macroalgae, which can account for most primary productivity in temperate coastal ecosystems (Newell 1984).

Inorganic nitrogen, a primary growth-limiting nutrient for algae in near-shore marine systems (Ryther and Dunstan 1971, Howarth 1988), exists in two forms: nitrate and ammonium. Most studies addressing the influence of nitrogen on open-coast macroalgae have focused on nitrate because it is the form of nitrogen associated with oceanographic processes, such as upwelling, that transport nutrient-rich water above the nutricline and make it available to algae in the photic zone. However, especially in oligotrophic waters, ammonium, excreted as a waste product by heterotrophs, is also an important nitrogen source for autotrophs (Eppley et al. 1973).

Biological oceanographers differentiate between "new production," primary production associated with nitrate upwelled or advected from beneath the nutricline, and "regenerated production," primary production that uses locally recycled nitrogen in the form of ammonium (Dugdale and Goering 1967). Although most phytoplankton production in the world's oceanic and coastal ecosystems is fueled by regenerated nitrogen (Eppley and Peterson 1979), it is only recently that benthic ecologists have addressed the influence of local-scale ammonium excretion on macroalgal productivity. Emerging evidence suggests that ammonium is an important nitrogen resource for temperate open-coast macroalgae (Fujita et al. 1989, Wheeler and Björnsäter 1992) and that sessile invertebrates, which often live in close association with macroalgae, can influence algal C:N ratios and growth (Hurd et al. 1994, Williamson and Rees 1994). However, many of the studies that link invertebrate-excreted ammonium and macroalgal growth have been conducted in oligotrophic regions, where nitrogen concentrations are low and ammonium makes up a large fraction of the available nitrogen. The question therefore remains: Is there an effect of invertebrate ammonium

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excretion on macroalgal growth in systems where nitrogen concentrations are not generally considered limiting?

In regions characterized by seasonal upwelling, including the northeastern Pacific's California Current system, the growth season of some macroalgal species occurs during the nitrate-limited winter and spring, not during the nitrate-replete May-to-October upwelling season (Lubchenco and Cubit 1980, Ruesink 1998). Even on the Oregon coast, where seasonal upwelling periodically elevates summer nitrate levels to $>20$ $\mu mol \cdot L^{-1}$ (Fujita et al. 1989, Menge et al. 1997), macroalgal growth can be nitrogen limited (Fujita et al. 1989, Wheeler and Björnsäter 1992), and nitrate can be severely depleted from the water column ($<2\,\mu mol \cdot L^{-1}$) for periods of several days (K. J. Nielsen and B. A. Menge, unpublished data).

Additionally, within the larger context of an upwelling ecosystem like the Oregon near-shore environment, there are nitrate-limited microhabitats. For example, macroalgae inhabiting high-intertidal pools are isolated from oceanic nitrate inputs for extensive periods of time, which may result in local-scale nutrient depletion. By experimentally adding nutrients (nitrogen and phosphorus) to Oregon-coast tide pools, Nielsen (2001, 2003) effected increases in macroalgal biomass and diversity, indicating that algae in those pools may have been nutrient limited. By excreting ammonium into tide pools, invertebrates may ameliorate this nutrient limitation. High biomasses of both macroalgae and sessile invertebrates inhabit high-intertidal pools on the Oregon coast, and invertebrate-excreted ammonium may be an important nitrogen source for the algae in these pools (Bracken and Nielsen 2004).

A common association in Oregon's high-intertidal pools consists of the branching red alga *Odonthalia floccosa* (Esp.) Falkenb. (Rhodophyta) (hereafter *Odonthalia*) growing epizoically on shells of mussels, *Mytilus californianus* Conrad. This alga–invertebrate pairing provided a convenient unit for experimental evaluation of ammonium excretion by invertebrates and its assimilation by macroalgae. By manipulating the presence and absence of mussels in laboratory mesocosms, I quantified the influence of invertebrate-excreted ammonium on nitrogen assimilation and growth of *Odonthalia*.

## MATERIALS AND METHODS

*Laboratory mesocosms.* I constructed an array of 32 tide pool mesocosms in four running seawater tanks (91 cm wide × 96 cm long × 15 cm deep) using eight 2.5-L plastic tubs (10 cm deep × 27 cm diameter) per tank. The seawater was pumped from Yaquina Bay, Oregon and passed through a 40-μm filter before entering the tanks. To mimic the low-tide accumulation and high-tide flushing of ammonium in high-intertidal pools on the Oregon coast, I simulated a tidal cycle in the running seawater tables (Fig. 1). This was accomplished by using an overflow outlet just below the top of each tank and a controllable drain valve (model 252–203.8-cm pin-type hydraulic valve, The Toro Co., Riverside, CA, USA) in the bottom of each tank. The valves were attached to a vacuum pump via a hydraulic line. Power to the vacuum pump was controlled by a digital timer programed to mimic daily tidal fluctuations experienced at 2.25 m above mean lower low water on the central Oregon coast. The digital timer stored a 1-week simulated tidal cycle and was programed weekly according to the tidal model in Tides & Currents for Windows™ v. 2.5b (Nautical Software, Inc., Beaverton, OR, USA).

Water flow was maintained at a sufficient level to flush the mesocosms and prevent invertebrate-excreted ammonium from influencing adjacent treatments. This was verified by sampling the ammonium concentrations in the + mussel mesocosms at intervals after the simulated rising tide flooded the tubs. Mean ammonium concentrations in the mesocosms were indistinguishable from concentrations in the water flowing into the tanks 10 min after the tubs were flooded by the rising

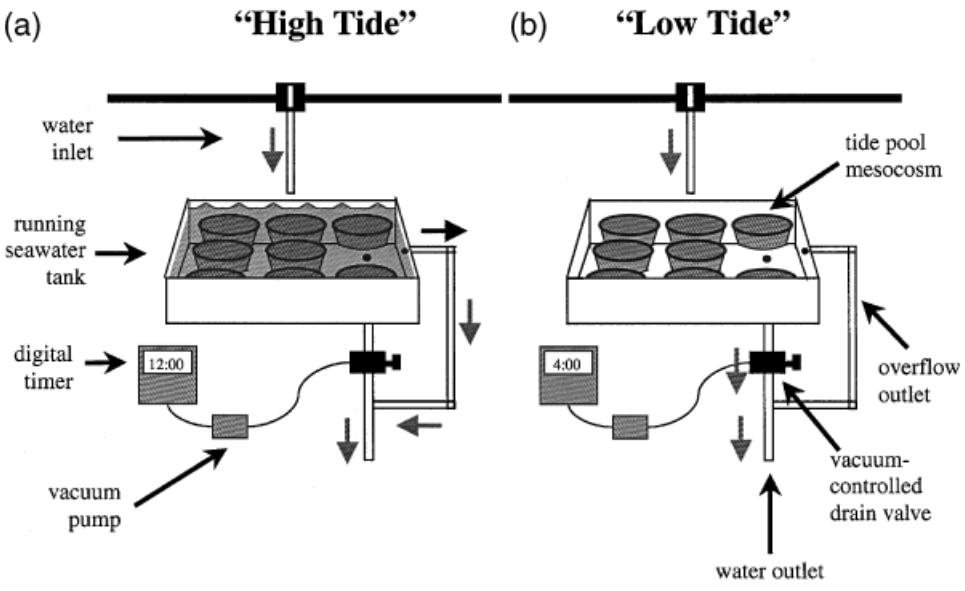


FIG. 1. Simulating a tidal cycle in a running seawater tank. Filtered seawater enters the tank via the water inlet. (a) When the vacuum-controlled drain valve is closed (vacuum off), water fills the tank and exits through the overflow outlet, covering the tide pool mesocosms and simulating high tide. (b) When the drain valve is open (vacuum on), water drains out of the tank, simulating low tide.

water (analysis of variance [ANOVA]: Bonferroni-adjusted $P = 0.1366$).

Each seawater tank was illuminated by eight 1.22-m full-spectrum fluorescent lamps. A combination of Hagen Power-Glo high-intensity aquarium lamps (Rolf C. Hagen Corp., Mansfield, MA, USA) and GE wide-spectrum plant and aquarium lamps (General Electric Co., Cleveland, OH, USA) provided $77.4 \pm 1.2$ (mean $\pm$ SE) μmol photons $\cdot m^{-2} \cdot s^{-1}$ PAR inside the mesocosms. Although these light intensities were low relative to full sunlight, they were more similar to overcast April weather on the Oregon coast, when maximum light intensities at solar noon are often less than 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ PAR and mean sunrise to sunset light intensities can be as low as 130 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (National Renewable Energy Laboratory 1992). *Odonthalia*'s photosynthetic rate saturates at relatively low light intensities (100–200 μmol photons $\cdot m^{-2} \cdot s^{-1}$), so that individuals in the mesocosms received approximately 78% of saturating irradiance (Ruesink 1998). The entire experimental apparatus (tanks and light fixtures) was shrouded in black plastic lined with reflective Mylar to exclude outside light and decrease light loss. Lights were attached to a timer set to approximate a natural sunrise and sunset cycle, because growth of *Odonthalia* is highly dependent on season (Ruesink 1998).

*Experimental treatments.* To evaluate the influence of mussel-excreted ammonium on growth and nitrogen assimilation of *Odonthalia*, mussels (*M. californianus*) with substantial epizoic biomasses of *Odonthalia* ($2.6 \pm 0.2$ SE g dry algal tissue mass per mussel) were collected from tide pools approximately 2.25 m above mean lower low water at Neptune State Park on the central Oregon coast (44°15′41″ N, 124°03′57″ W). Algae and mussels were rinsed with seawater to remove sediment and epiphytes and were maintained in the laboratory in a 40-μm filtered running seawater system on a simulated natural sunrise and sunset cycle before experimental manipulations.

Three mussels were randomly assigned to each pool, and pools were randomly assigned (within each tank) to the following treatments: 1) + mussels, + phytoplankton; 2) + mussels, − phytoplankton; 3) − mussels, + phytoplankton; and 4) − mussels, − phytoplankton. *Odonthalia* was present in all treatments, attached to either live mussels or empty mussel shells. Mean *Odonthalia* biomasses (ash-free dry mass) were $1.88 \pm 0.08$ (SE) g $\cdot L^{-1}$, and mean mussel biomasses (ash-free dry mass in + mussel treatments) were $11.39 \pm 0.47$ (SE) g $\cdot L^{-1}$. These fit within the range of algal and invertebrate biomasses harvested from 20 Oregon-coast tide pools, where macroalgal biomasses ranged from 1.02 to 8.97 g $\cdot L^{-1}$ and invertebrate biomasses ranged from 0.80 to 28.06 g $\cdot L^{-1}$ (Bracken and Nielsen 2004). Treatments without mussels ( − mussels) were prepared by dissecting the mussel tissue out of each shell.

Phytoplankton were added to + phytoplankton treatments to test the effects of feeding on mussel ammonium excretion and macroalgal growth. The phytoplankton consisted of cultured *Chaetoceros calcitrans* (Paulsen) Takano (Heterokontophyta, Bacillariophyceae) raised on high-nitrate (approximately 60 μmol $\cdot L^{-1}$) f/2 culture medium. *Chaetoceros* was courtesy of C. Langdon, Hatfield Marine Science Center, Newport, Oregon.

Introduction of nitrate from the culture medium into the experimental treatments was avoided by centrifuging phytoplankton in the culture medium, discarding the supernatant, and resuspending the pellet in filtered seawater. *Chaetoceros* abundances in the resuspended pellet were then quantified using a Coulter Counter and Coulter Channelyzer 256 (Coulter Electronics, Hialeah, FL, USA). Phytoplankton were added once each day to + phytoplankton treatments to bring the phytoplankton concentration to $10^5$ cells $\cdot mL^{-1}$ in each mesocosm.

*Nitrogen concentrations and fluxes.* Nitrate concentrations in the tanks were determined at simulated "high tide" on two occasions during the experiment. Three water samples (5 mL each) were collected, and the total concentration of nitrate was determined on a spectrophotometer after reduction with spongy cadmium and addition of sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride to form an azo dye (Jones 1984). In both cases, nitrate concentrations were low; mean nitrate levels never exceeded 1.8 μmol $\cdot L^{-1}$.

Ammonium fluxes in each experimental treatment were quantified at the experiment's beginning and end. Water samples (1 mL) were taken from each mesocosm at 0, 1, 2, and 4 h after emersion by the simulated receding tide, and the concentration of ammonium was determined using the phenol-hypochlorite method (Solórzano 1969). Ammonium loading into the mesocosms, defined as the rate of ammonium accumulation (μmol $\cdot L^{-1} \cdot h^{-1}$) in the absence of *Odonthalia*, was determined after removing the macroalgae from each pool at the end of the experiment. First, I measured ammonium concentrations in + *Odonthalia* pools, as described above. Then I removed the *Odonthalia* from each pool. On the following day, I measured changes in ammonium concentrations after pool emersion. I then removed the live mussels (from + mussel treatments) and the empty mussel shells (from − mussel treatments). Finally, on the third day, I measured changes in ammonium concentration in the absence of both mussels and macroalgae. The mussel and macroalgal tissues from each mesocosm were dried to constant mass at 50° C for calculation of mass-normalized ammonium excretion and uptake rates based on the measurements described above.

These sequential manipulations did not allow me to evaluate the influence of *Odonthalia* on ammonium fluxes in the absence of mussels. Therefore, patterns of ammonium uptake and excretion were verified by collecting additional *Odonthalia* growing on mussels (as above) and preparing fully factorial manipulations of seaweeds and invertebrates in the mesocosms. Mesocosms within each running seawater tank were randomly assigned to the following treatments, for a total of $n = 8$ mesocosms per treatment: 1) + *Odonthalia*, + mussels; 2) + *Odonthalia*, − mussels; 3) − *Odonthalia*, + mussels; and 4) − *Odonthalia*, − mussels. As in the sequential removals, water samples were taken at 0, 1, 2, and 4 h after emersion by the simulated receding tide, and the ammonium concentration of each sample was determined.

*Measurement of algal growth.* The experiment was initiated on 10 April 2002 to coincide with *Odonthalia*'s natural growth cycle; thalli grow from January to June on northeastern Pacific shores (Ruesink 1998). At the beginning of the experiment, I removed two *Odonthalia* thalli from each mesocosm. One of these was randomly selected for determination of initial nitrogen content and frozen at − 20° C. The other was weighed (blotted wet tissue mass), labeled by placing its base in a numbered clamp, and returned to the mesocosm. Blotted wet tissue masses of all labeled *Odonthalia* individuals were determined every 2 days for 24 days. At the termination of the experiment, both thalli from each pool (the one frozen on day 0 and the one monitored throughout) were dried to constant mass at 50° C. After the dry tissue mass of each thallus had been measured, samples were frozen in liquid nitrogen and powdered using a mortar and pestle. They were then analyzed for percent tissue nitrogen using a CHN Organic Elemental Analyzer (Exeter Analytical, North Chelmsford, MA, USA) at the Analytical Laboratory, Marine Science Institute, University of California, Santa Barbara.

*Nitrogen assimilation by Odonthalia.* Nitrogen accumulation in the algal tissues was calculated based on the percent tissue nitrogen values from each treatment (days 0 and 24) and the dry masses (dm) of each labeled individual on days 0 and 24. A regression relating blotted wet tissue mass and dry tissue

mass for day 24 (dry tissue mass = 0.1646 × wet tissue mass; $r^2 = 0.98$) was used to back calculate the dry tissue mass of each individual at day 0. The per-mass nitrogen assimilation rate ($\mu g \cdot h^{-1} \cdot g\ dm^{-1}$) was calculated according to Eq. 1:

$$\text{N assimilation rate} = (10^6\ \mu g \cdot g^{-1}) \frac{\left(\left(M_{24} \frac{\%N_{24}}{100}\right) - \left(M_0 \frac{\%N_0}{100}\right)\right)}{576\ h} (M_{24})^{-1} \quad (1)$$

where $M_0$ and $M_{24}$ are the initial and final biomass (g dm), respectively, and $\%N_0$ and $\%N_{24}$ are the initial and final values for percent tissue nitrogen.

*Statistical analyses*. Analyses were performed using the SAS System for Windows v. 8 (SAS Institute, Inc., Cary, NC, USA). Data were analyzed using general linear models, including ANOVAs and repeated-measures ANOVAs. The ANOVA assumptions of normality and homogeneity of variances were verified for each model by visually examining residual plots and normal probability plots of the residuals. In all analyses, "tank" was included as a factor, because treatments were randomly assigned within the running seawater tanks.

Five labeled *Odonthalia* individuals lost biomass during the course of the experiment and were statistical outliers, based on a regression of algal tissue nitrogen assimilation rates on rates of ammonium loading by mussels. These individuals were therefore excluded from all analyses, giving final sample sizes of $n = 8$ for the + mussels, + phytoplankton treatment; $n = 7$ for the + mussels, − phytoplankton treatment; $n = 6$ for the − mussels, + phytoplankton treatment; and $n = 6$ for the − mussels, − phytoplankton treatment. Because 4 of 5 algae that lost biomass were from − mussel treatments, excluding those samples actually provided a more conservative evaluation of the influence of mussels on *Odonthalia* growth.

## RESULTS

*Experimental treatments and ammonium fluxes*. The presence of mussels was associated with increased ammonium accumulation rates in mesocosms, both at the beginning (day 0) and the end (day 24) of the experiment (repeated-measures ANOVA: "time × mussels" interaction; $F_{3,60} = 60.76$, $P < 0.0001$ for day 0 and $F_{3,60} = 7.56$, $P = 0.0002$ for day 24; Fig. 2). Ammonium accumulation rates at the beginning of the experiment were $3.96 \pm 0.44$ (SE) $\mu mol \cdot L^{-1} \cdot h^{-1}$ higher when mussels were present, and accumulation rates at the end of the experiment were $1.21 \pm 0.65$ (SE) $\mu mol \cdot L^{-1} \cdot h^{-1}$ higher when mussels were present. The slight increase in ammonium concentrations in the − mussels treatments (Fig. 2) was likely associated with the presence of sessile invertebrate epifauna (e.g. sea anemones, polychaetes, and sponges) on the mussels' shells.

The influence of phytoplankton on ammonium concentrations and accumulation rates depended on the presence of mussels (Fig. 3). At the experiment's end, significant "mussels × phytoplankton" ($F_{1,20} = 5.97$, $P = 0.0239$) and "time × mussels × phytoplankton" ($F_{3,60} = 2.52$, $P = 0.0662$) interaction terms suggest that when mussels were present, ammonium concentrations and accumulation rates were higher when phytoplankton were also present. Conversely, when mussels were absent from mesocosms, ammonium concentrations and accumulation rates were lower when phytoplankton were present.

Sequential removals of *Odonthalia* and mussels in + mussel mesocosms revealed differences in ammonium accumulation rates associated with the presence and absence of macroalgae and invertebrates (ANOVA: $F_{2,39} = 115.41$, $P < 0.0001$; Fig. 4a). Removal of *Odonthalia* increased the mean ± SE ammonium accumulation rate by $2.38 \pm 0.31$ $\mu mol \cdot L^{-1} \cdot h^{-1}$ (ANOVA: Bonferroni-adjusted $P < 0.0001$), and subsequent removal of mussels reduced the ammonium accumulation rate by $4.16 \pm 0.31$ $\mu mol \cdot L^{-1} \cdot h^{-1}$ (ANOVA: Bonferroni-adjusted $P < 0.0001$). These differences in ammonium concentrations were associated with the removal of known quantities of *Odonthalia* and mussel biomass. Therefore, it was possible to calculate mass-specific uptake and excretion rates based on changes in the ammonium fluxes ($\mu mol \cdot h^{-1}$) and on the dry tissue masses of *Odonthalia* and mussels. Thus, at the end of the experiment, mussels excreted ammonium into + mussel mesocosms at $0.31 \pm 0.02$ (SE) $\mu mol \cdot h^{-1} \cdot g\ dm^{-1}$ (one-sample $t$-test: $t = 13.53$, $P < 0.0001$, df = 14), and *Odonthalia* reduced the ammonium in those mesocosms via uptake at $-0.76 \pm 0.07$ (SE) $\mu mol \cdot h^{-1} \cdot g\ dm^{-1}$ (one-sample $t$-test: $t = -10.19$, $P < 0.0001$, df = 14).

Simultaneous manipulations of seaweeds and invertebrates revealed similar patterns to the sequential removals (Fig. 4b). Ammonium accumulation rates were affected by the presence and absence of macroalgae and mussels (repeated-measures ANOVA: "time × manipulation" interaction; $F_{9,75} = 68.31$, $P < 0.0001$). Highest accumulation rates occurred in the − algae, + mussels treatment ($3.94 \pm 0.19$ [SE] $\mu mol \cdot L^{-1} \cdot h^{-1}$), followed by the + algae, + mussels treatment ($2.40 \pm 0.19$ [SE] $\mu mol \cdot L^{-1} \cdot h^{-1}$). These treatments differed from each other and from every other treatment (ANOVA: Bonferroni-adjusted $P < 0.0001$ for all comparisons with both treatments). However, the other two treatments differed neither from each other nor from zero (− algae, − mussels: $P = 0.6602$; + algae, − mussels: $P = 0.1897$).

*Ammonium loading and algal N assimilation*. Ammonium loading rates, defined as the change in the ammonium concentration over time ($\mu mol \cdot L^{-1} \cdot h^{-1}$) in the absence of *Odonthalia*, were primarily associated with the presence of mussels in mesocosms: The mean ammonium loading rate was $3.69 \pm 0.42$ (SE) $\mu mol \cdot L^{-1} \cdot h^{-1}$ higher when mussels were present (ANOVA: $F_{1,21} = 77.90$, $P < 0.0001$).

Using changes in the biomass (g dm) of labeled *Odonthalia* individuals and the nitrogen content of *Odonthalia* tissue, I calculated the rate of macroalgal nitrogen assimilation ($\mu g \cdot h^{-1} \cdot g\ dm^{-1}$) for each mesocosm. Mussels primarily influenced algal biomasses: Over the course of the experiment, labeled thalli added $123.7 \pm 16.3$ (SE) mg dm in + mussel mesocosms and only $42.6 \pm 18.4$ (SE) mg dm in − mussel mesocosms (ANOVA: $F_{1,22} = 10.90$, $P = 0.0032$). Tissue nitrogen content was not influenced by the presence of

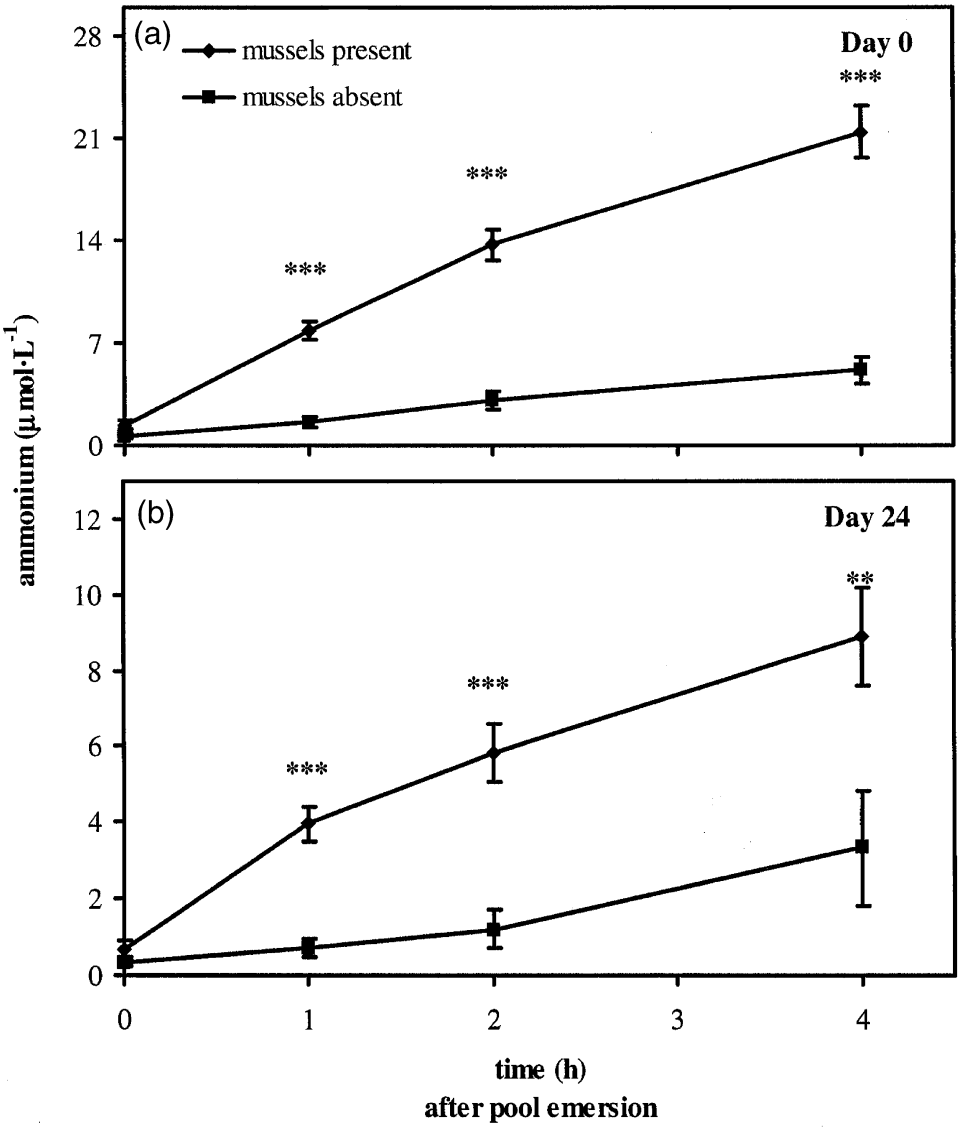


FIG. 2. Influence of mussels on ammonium concentrations in mesocosms. Although ammonium concentrations were higher at the beginning of the experiment (a) than at the end of the experiment (b), in both cases the presence of mussels resulted in a significant increase in ammonium concentrations. Values are means ± SE, and asterisks indicate significant differences in ammonium concentrations at $P < 0.01$ (**) and $P < 0.001$ (***).

mussels (ANOVA: $F_{1,22} = 1.39$, $P = 0.2502$) and did not change over the course of the experiment (paired $t$-test: $t = 1.26$, $P = 0.2181$, df = 26), though tissue nitrogen levels tended to be higher and C:N ratios lower in + mussel mesocosms at the end of the experiment. Initial percentages of nitrogen in dried algal tissue were 4.0 ± 0.1 (SE) in + mussel mesocosms and 3.9 ± 0.1 (SE) in − mussel mesocosms; at the end of the experiment they were 4.2 ± 0.1 (SE) in + mussel mesocosms and 4.0 ± 0.1 (SE) in − mussel mesocosms. Mean C:N ratios in dried *Odonthalia* tissue were 6.4 ± 0.1 (SE) in both + mussel and − mussel mesocosms at the start of the experiment. C:N ratios increased over the 24-day growth period, so that at the end of the experiment, mean C:N ratios were 7.4 ± 0.1 (SE) in + mussel mesocosms and 7.7 ± 0.1 (SE) in − mussel mesocosms.

Thus, primarily due to changes in biomass associated with nitrogen loading by mussels, nitrogen assimilation rates were higher when mussels were present (ANOVA: $F_{1,22} = 17.66$, $P = 0.0004$). Similarly, nitrogen assimilation by *Odonthalia* reflected rates of ammonium loading into the mesocosms (ANOVA: $F_{1,22} = 40.38$, $P < 0.0001$; Fig. 5).

*Ammonium loading and algal growth.* Although mussels increased the percent growth of macroalgae (ANOVA: $F_{1,20} = 8.19$, $P = 0.0097$), neither phytoplankton ($F_{1,20} = 0.06$, $P = 0.8019$) nor the interaction between mussels and phytoplankton ($F_{1,20} = 0.45$, $P = 0.5105$) explained significant variation in growth. When mussels were present, growth was slightly higher when phytoplankton were present, and when mussels were absent, growth was slightly higher when phytoplankton were absent. This corresponded to the between-subjects "mussels × phytoplankton" interaction terms associated with ammonium concentrations and fluxes at the end of the experiment (Fig. 3).

In evaluating growth of *Odonthalia* over time, I used a reduced model, which addressed the influence of

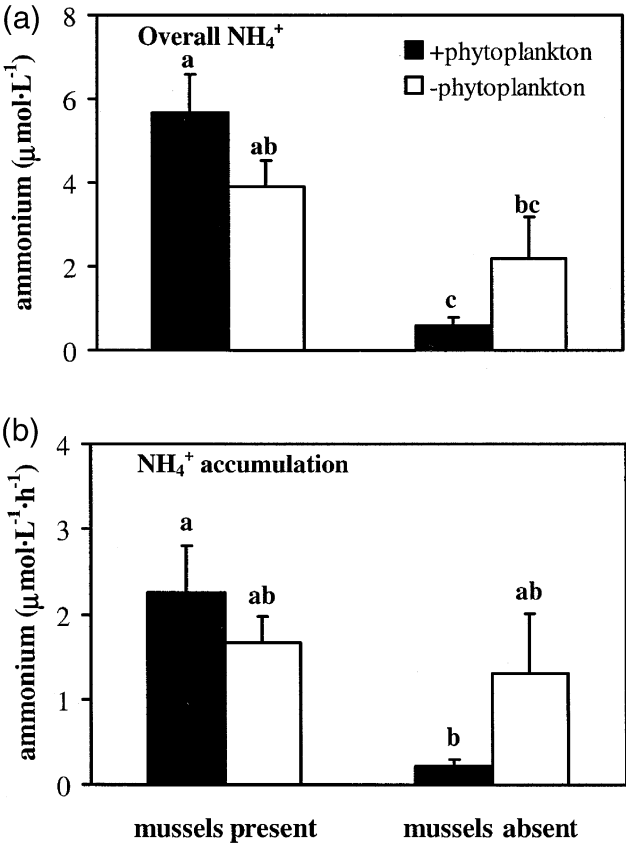


FIG. 3. Influence of phytoplankton and mussels on ammonium concentrations and fluxes in mesocosms. Values are mean SE ammonium (a) concentrations and (b) accumulation rates measured at the end of the experiment. Letters indicate significant differences ($P < 0.05$) after Bonferroni adjustment.

mussels on percent thallus growth. Macroalgae grew in both + mussel and − mussel treatments, adding $74.9 \pm 9.3$ (SE) ($P < 0.0001$) percent wet tissue mass when mussels were present and $33.6 \pm 10.5$ (SE) ($P = 0.0041$) percent when mussels were absent. After 24 days, *Odonthalia* in treatments where mussels were present grew $41.3 \pm 14.0$ (SE) percent more than those in treatments without mussels (ANOVA: $F_{1,22} = 8.66$, $P = 0.0075$; Fig. 6). Similarly, repeated-measures ANOVA indicated that the change in percent growth of *Odonthalia* over time was higher when mussels were present (repeated-measures ANOVA: "time × mussels" interaction; $F = 7.13$, $P < 0.0001$).

## DISCUSSION

*Ammonium fluxes in mesocosms.* Ammonium uptake rates of macroalgae in still water are very low; *Odonthalia* in this study reduced the concentration of ammonium in tide pool mesocosms by only $0.76\ \mu mol \cdot h^{-1} \cdot g\ dm^{-1}$. Most studies of macroalgal nutrient uptake are conducted in moving water, where maximum nonsurge ammonium uptake rates range from $22\ \mu mol \cdot h^{-1} \cdot g\ dm^{-1}$ (*Fucus vesiculosis*) to $72\ \mu mol \cdot h^{-1} \cdot g\ dm^{-1}$ (*Ulva lactuca*) (Pedersen and Borum 1997). Furthermore, in still water, the rate of ammonium uptake by *Odonthalia* increases slowly and linearly with increasing ammonium concentration (Bracken 2003). The lack of saturation and the low overall uptake rates suggest that diffusion is the likely mechanism of ammonium acquisition by *Odonthalia* when pools are emersed, probably due to the formation of boundary layers in the still water (Hurd 2000). Especially high in the intertidal zone, where tide pool macroalgae are isolated from oceanic nitrate and subject to still water for extensive periods of time, nitrogen limitation is a potential stress. Because of the direct relationship between ammonium concentration and uptake by *Odonthalia*, increasing the ammonium concentration in tide pools would increase uptake and could ameliorate this stress. Invertebrates excrete substantial amounts of ammonium into tide pools and may therefore help alleviate the nutrient limitation characteristic of high-intertidal pools.

In this experiment, I quantified the influence of a dominant intertidal invertebrate, the mussel *Mytilus californianus*, on ammonium fluxes in tide pool mesocosms. At the experiment's end, when I sequentially removed *Odonthalia* and mussels to quantify excretion by *Mytilus* and uptake by *Odonthalia*, mussels excreted $0.31\ \mu mol \cdot h^{-1} \cdot g\ dm^{-1}$ ammonium into the mesocosms, raising mean ammonium concentrations to $> 8.9\ \mu mol \cdot L^{-1}$ when algae were present and $> 18.3\ \mu mol \cdot L^{-1}$ when algae were absent. The contribution of mussels was even more pronounced at the beginning of the experiment, when ammonium concentrations increased to $> 21.4\ \mu mol \cdot L^{-1}$ in mesocosms where both *Odonthalia* and mussels were present. It is likely that macroalgae and invertebrates have similar effects on ammonium loading in natural tide pools. Sequential manipulations similar to those described above were conducted in 20 high-intertidal pools on the central Oregon coast (Bracken and Nielsen 2004). Removal of macroalgae from pools increased ammonium accumulation rates in those pools by $3.8\ \mu mol \cdot L^{-1} \cdot h^{-1}$ and subsequent removal of invertebrates decreased ammonium accumulation rates by $3.4\ \mu mol \cdot L^{-1} \cdot h^{-1}$.

Why did mussel ammonium-excretion rates decrease over the 24-day experimental period, even when mussels in + phytoplankton treatments were being fed? Although $10^5$ cells $\cdot$ mL$^{-1}$ was experimentally determined to be the ration that maximized ammonium excretion rates (M. E. S. Bracken unpublished data), feeding the mussels only once a day may not have been sufficient for them to maintain natural excretion rates. Furthermore, evidence shows that starved mussels may actually excrete at higher rates than fed mussels because of catabolism of protein (Bayne and Scullard 1977). The factors influencing the assimilation of ingested particulate organic nitrogen into new growth versus its excretion as ammonium are complex and involve both the quantity and quality of food (Bracken 2003). Overall, mussels were the primary factor influencing ammonium fluxes in the

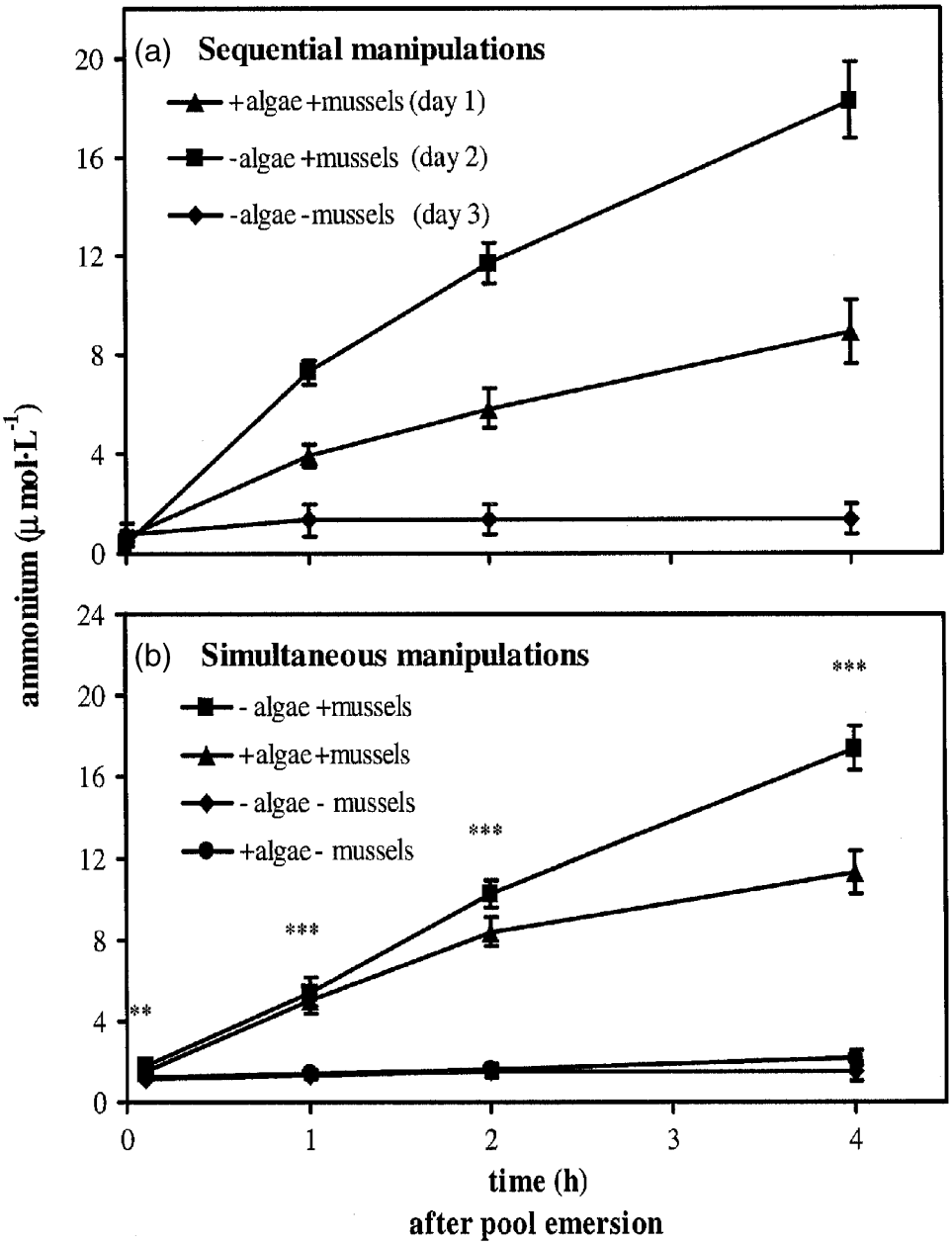


FIG. 4. Ammonium fluxes in mescosms based on manipulations of *Odonthalia* and mussels. Values are mean ± SE ammonium concentrations ($n = 8$ mesocosms), determined 0, 1, 2, and 4 h after pool emersion. (a) In the sequential manipulations, removals were conducted after each day's trial and treatments consisted of + algae, + mussels (day 1); − algae, + mussels (day 2); and − algae, − mussels (day 3). (b) Simultaneous manipulations consisted of the following four treatments: − algae, + mussels; + algae, + mussels; − algae, − mussels; and + algae, − mussels. Asterisks indicate significant differences in ammonium concentrations at $P < 0.01$ (**) and $P < 0.001$ (***).

experimental mesocosms, especially at the beginning of the experiment.

The only notable effect of phytoplankton on ammonium concentrations involved their interaction with the presence of mussels at the end of the experiment ("mussels × phytoplankton"). Although there was not a corresponding effect on *Odonthalia* growth ("mussels × phytoplankton" interaction; $F = 0.45$, $P = 0.5105$), these data suggest that mussels may play a dual role in their positive interaction with macroalgae. It is clear that mussels' excretion of ammonium increases macroalgal abundance. Mussels also remove phytoplankton, which can reach high abundances in tide pools (Metaxas and Scheibling 1994) and may therefore compete with macroalgae for inorganic nitrogen. Thus, mussels may influence macroalgal growth both directly, by excreting a nutrient necessary for growth, and indirectly, by ingesting microalgal competitors.

*Ammonium loading and algal N assimilation.* The long-term rate of nitrogen assimilation (its incorporation into algal tissue) is a function of changes both in the nitrogen content of the tissue and in the mass of the algal thallus. Rates of nitrogen assimilation into the thalli of labeled individuals ($\mu g \cdot h^{-1} \cdot g\ dm^{-1}$) increased with the rates of ammonium accumulation in their respective mesocosms. This increase in nitrogen assimilation was primarily associated with addition of new biomass. Mussels did not affect tissue nitrogen content nor did tissue nitrogen content change over the course of the experiment. Tissue

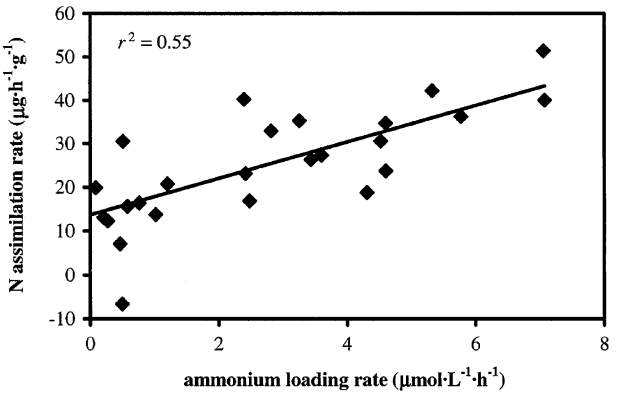


FIG. 5. Nitrogen assimilation rates, based on growth of individual *Odonthalia* thalli and elemental analysis of tissue nitrogen, increased with ammonium loading by mussels in tide pool mesocosms.

nitrogen percentages in algal thalli were never less than 3.3%.

*Odonthalia* individuals never appeared to be nutrient limited, based on tissue nitrogen levels. The C:N ratios ranged from 5.9 to 9.1, all of which were less than the approximate ratio of 10 that D'Elia and DeBoer (1978) associated with red algal nutrient limitation. Thus, the potential for *Odonthalia* to grow may not be a simple function of the nitrogen stored in their tissues. Although most macroalgal species deplete stores of tissue nitrogen to support additional growth (Hanisak 1983, Pedersen and Borum 1997), *Odonthalia* appears to maintain a high constant level of tissue nitrogen, transforming external nitrogen inputs directly into new growth instead of depleting internal stores.

An alternative explanation is worth considering. It is possible that the apparent lack of nitrogen limitation—the relatively high tissue nitrogen levels and low C:N ratios—in the absence of mussels indicates that nitrogen is not the limiting nutrient in this system. For example, phosphate is also excreted by mussels as a waste product (Asmus et al. 1995), and there is growing evidence for seasonal phosphorus limitation in temperate macroalgae (Wheeler and Björnsäter 1992). However, even in the nitrate-replete California Current upwelling system, nitrogen appears to be the primary limiting nutrient (Kudela and Dugdale 2000). When nitrate is depleted from Oregon-coast waters, there is still a significant excess (approximately 0.3 µmol · L$^{-1}$) of soluble reactive phosphorus available (Corwith and Wheeler 2002).

Most of *Odonthalia*'s growth occurs in winter and spring (Ruesink 1998) during periods of low nitrate availability. Pulses of elevated nitrate associated with coastal upwelling begin in mid-May (Fujita et al. 1989), and "high-tide" nitrate levels measured in the experimental tanks were low, never exceeding 1.8 µmol · L$^{-1}$. The combination of low nitrogen (nitrate and ammonium) availability in the − mussels treatments and the link between ammonium loading and nitrogen assimilation suggest that invertebrate-excreted ammonium contributed to the observed differences in macroalgal growth.

*Ammonium loading and algal growth.* By excreting ammonium into the tide pool mesocosms, mussels increased *Odonthalia* growth: When mussels were present, algal thalli added 41% more mass than when mussels were absent. It is likely that a similar interaction occurs in natural tide pools. Nielsen

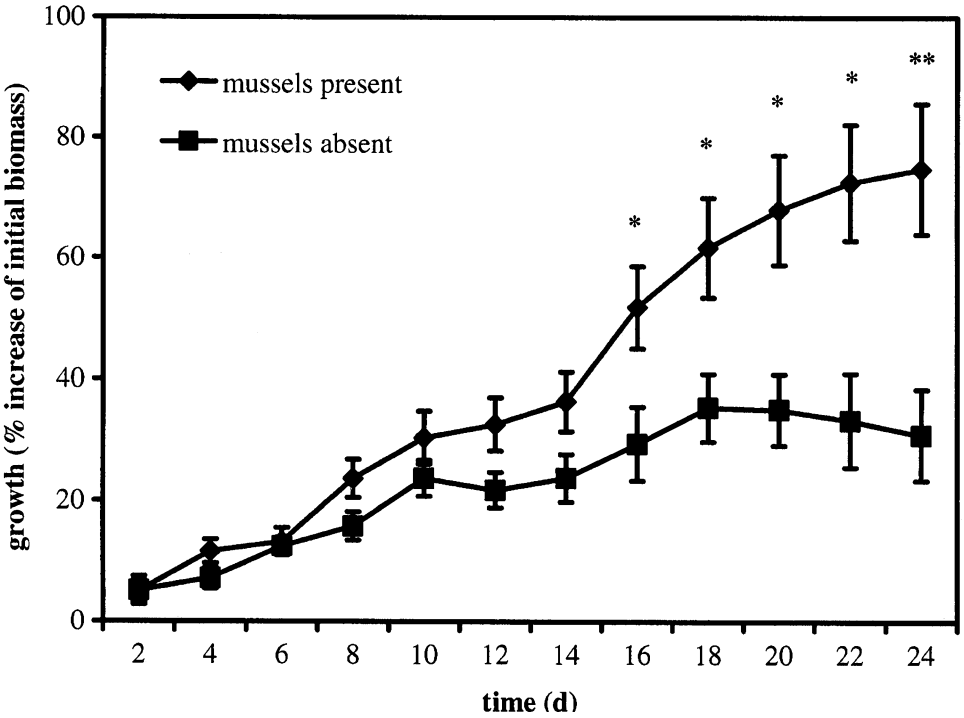


FIG. 6. Growth of *Odonthalia* in the presence and absence of mussels. Values are mean ± SE percent increase in initial biomass in the presence and absence of mussels. Asterisks indicate significant differences in growth between treatments at $P < 0.05$ (*) and $P < 0.01$ (**). By the experiment's end (day 24), *Odonthalia* in mesocosms where mussels were present had grown 41% more than when mussels were absent.

(2001) demonstrated that artificially adding nitrogen and phosphorus to Oregon-coast tide pools increased both macroalgal biomass and productivity. However, a positive influence of nutrients was apparent only in pools where herbivore abundances were experimentally reduced. If herbivore consumption rates equal or exceed macroalgal growth rates, the bottom-up influence of nutrients on tide pool community structure may be masked by top-down regulation by herbivores. Wootton et al. (1996) conducted a similar nutrient-enrichment study on rocky intertidal reefs. During an El Niño period of low nitrate availability, their addition of slow-release nitrate and phosphorus to intertidal plots increased herbivore biomasses but had no influence on algae. Thus, although nutrient additions have demonstrable effects on macroalgal growth, the magnitude and fate of those increases in primary productivity and the consequent influences on community structure and dynamics depend on the relative strengths of both bottom-up nutrient loading and top-down herbivory.

*Summary.* Regenerated nitrogen increased the growth of *Odonthalia*, an abundant macroalga in high-intertidal pools. Even within the nitrate-replete upwelling ecosystem of the northeastern Pacific, there are nutrient-limited microhabitats, like tide pools, where local depletion of nitrate during pool emersion can result in nutrient stress. This stress is ameliorated by the excretion of ammonium by invertebrates. Based on the results of this study, I suggest that local-scale nutrient availability, mediated by processes such as localized nitrogen limitation and regeneration, can play an important and previously overlooked role in determining the structure and dynamics of intertidal communities.

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Asmus, H., Asmus, R. M. & Zubillaga, G. F. 1995. Do mussel beds intensify the phosphorus exchange between the sediment and tidal waters? *Ophelia* 41:37–55.

Bayne, B. L. & Scullard, C. 1977. Rates of nitrogen excretion by species of *Mytilus* (Bivalvia: Mollusca). *J. Mar. Biol. Assoc. UK* 57:355–69.

Bosman, A. L., Hockey, P. A. R. & Siegfried, W. R. 1987. The influence of coastal upwelling on the functional structure of rocky intertidal communities. *Oecologia* 72:226–32.

Bracken, M. E. 2003. *Linking Marine Communities and Ecosystems: Invertebrates Mediate Nutrient Availability in Intertidal Communities*. Ph.D. dissertation, Oregon State University, Corvallis, 103 pp.

Bracken, M. E. S. & Nielsen, K. J. 2004. Diversity of intertidal macroalgae increases with nitrogen loading by invertebrates. *Ecology* 85:2828–36.

Corwith, H. L. & Wheeler, P. 2002. El Niño related variations in nutrient and chlorophyll distributions off Oregon. *Prog. Oceanogr.* 54:361–80.

D'Elia, C. F. & DeBoer, J. A. 1978. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *J. Phycol.* 14:266–72.

Dugdale, R. C. & Goering, J. J. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* 12:196–206.

Eppley, R. W. & Peterson, B. J. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* 282:677–80.

Eppley, R. W., Renger, E. H., Venrick, E. L. & Mullin, M. M. 1973. A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean. *Limnol. Oceanogr.* 18:534–51.

Fujita, R. M., Wheeler, P. A. & Edwards, R. L. 1989. Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. *Mar. Ecol. Prog. Ser.* 53:293–303.

Hanisak, M. D. 1983. The nitrogen relationships of marine macroalgae. *In* Carpenter, E. J. & Capone, D. G. [Eds.] *Nitrogen in the Marine Environment*. Academic Press, New York, pp. 699–730.

Howarth, R. W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Annu. Rev. Ecol. Syst.* 19:89–110.

Hurd, C. L. 2000. Water motion, marine macroalgal physiology, and production. *J. Phycol.* 36:453–72.

Hurd, C. L., Durante, K. M., Chia, F. S. & Harrison, P. J. 1994. Effect of bryozoan colonization on inorganic nitrogen acquisition by the kelps *Agarum fimbriatum* and *Macrocystis integrifolia*. *Mar. Biol.* 121:167–73.

Jones, M. N. 1984. Nitrate reduction by shaking with cadmium: alternative to cadmium columns. *Water Res.* 18:643–6.

Kudela, R. M. & Dugdale, R. C. 2000. Nutrient regulation of phytoplankton productivity in Monterey Bay, California. *Deep Sea Res. I* 47:1023–53.

Lubchenco, J. & Cubit, J. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology* 61:676–87.

Menge, B. A. 1992. Community regulation: under what conditions are bottom-up factors important on rocky shores? *Ecology* 73:755–65.

Menge, B. A., Daley, B. A., Wheeler, P. A., Dahlhoff, E., Sanford, E. & Strub, P. T. 1997. Benthic-pelagic links and rocky intertidal communities: bottom-up effects on top-down control? *Proc. Natl. Acad. Sci. USA* 94:14530–5.

Metaxas, A. & Scheibling, R. E. 1994. Changes in phytoplankton abundance in tidepools over the period of tidal isolation. *Bot. Mar.* 37:301–14.

National Renewable Energy Laboratory. 1992. *National Solar Radiation Data Base (1961–1990)*. National Climatic Data Center, Asheville, NC, 243 pp.

Newell, R. C. 1984. The biological role of detritus in the marine environment. *In* Fasham, M. R. J. [Ed.] *Flows of Energy and Materials in Marine Ecosystems: Theory and Practice*. Plenum Press, New York, pp. 317–43.

Nielsen, K. J. 2001. Bottom-up and top-down forces in tide pools: test of a food chain model in an intertidal community. *Ecol. Monogr.* 71:187–217.

Nielsen, K. J. 2003. Nutrient loading and consumers: agents of change in open-coast macrophyte assemblages. *Proc. Natl. Acad. Sci. USA* 100:7660–5.

Pedersen, M. F. & Borum, J. 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. *Mar. Ecol. Prog. Ser.* 161:155–63.

Ruesink, J. L. 1998. Diatom epiphytes on *Odonthalia floccosa*: the importance of extent and timing. *J. Phycol.* 34:29–38.

Ryther, J. H. & Dunstan, W. M. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 171:1008–13.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14:799–801.

Wheeler, P. A. & Björnsäter, B. R. 1992. Seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific northwest coast. *J. Phycol.* 28:1–6.

Williamson, J. E. & Rees, T. A. V. 1994. Nutritional interaction in an alga-barnacle association. *Oecologia* 99:16–20.

Wootton, J. T., Power, M. E., Paine, R. T. & Pfister, C. A. 1996. Effects of productivity, consumers, competitors, and El Niño events on food chain patterns in a rocky intertidal community. *Proc. Natl. Acad. Sci. USA* 93:13855–8.

Worm, B., Lotze, H. K., Hillebrand, H. & Sommer, U. 2002. Consumer versus resource control of species diversity and ecosystem functioning. *Nature* 417:848–51.