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NOTE

AN EXPERIMENTAL ASSESSMENT OF THE EFFECTS OF NUTRIENT ENHANCEMENT ON THE INTERTIDAL KELP *HEDOPHYLLUM SESSILE* (LAMINARIALES, PHAEOPHYCEAE)[1]

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We tested whether experimentally enhancing nutrients around the kelp *Hedophyllum sessile* would increase growth, tissue nitrogen, or allocation to phenolic compounds. Packets of time-released fertilizer were anchored adjacent to fronds in the field, and algae were monitored for several months. Although fertilizer packets increased the concentration of ammonium, nitrate, and phosphorus adjacent to treatment algae by an order of magnitude, there was little evidence that this increased frond growth or size. *Hedophyllum* individuals showed no tendency to alter allocation patterns in response to nutrient addition. Tissue carbon and nitrogen was unchanged by the nutrient manipulation; most *H. sessile* had tissue nitrogen concentrations in excess of 2.0% of dry mass. Additionally, the concentration of phloroglucinol equivalents was also unaffected by the presence of increased water column nutrients. Although nutrient concentrations in the water column surrounding the study site show relatively high mean values for ammonium, nitrate, and phosphorus, they are characterized by high spatial and temporal variation. Nonetheless, these data suggest that this intertidal kelp is not limited by nitrogen or phosphorus in wave-exposed areas in the northeast Pacific Ocean.

Key index words: **kelp; nutrient enhancement; nutrient limitation; phenolics; tissue nitrogen**

Coastal eutrophication, El Niño and La Niña events, global climate change, upwelling, and variability in local currents can alter nutrient concentrations in coastal environments. Thus, it is likely that kelp living in these environments experience wide spatial and temporal variation in nutrient availability. However, relatively little is known about how kelp respond to changes in water column nutrients. Although there are several documented instances of kelp decline in response to relaxation of upwelling events during El Niño events (Dayton and Tegner 1984, Dean and Jacobsen 1986), the relative importance of nutrients in causing these declines, relative to other factors such as temperature, wave disturbance, and herbivore fluctuations (Leigh et al. 1987, Tegner and Dayton 1991, Dayton et al. 1992), is uncertain.

Kelp might respond in several ways to enhanced nutrients if nutrients are limiting algal fitness. First, if nutrients limit algal growth rates, then nutrient enhancement should increase growth (Chapman and Craigie 1977, Fujita et al. 1989, Schaffelke and Klumpp 1998), resulting in an increase in alga size and reproduction if other factors (such as loss due to herbivores and wave action) are minimal. Second, algae might increase internal stores of carbon and nitrogen. This response would be especially advantageous to a perennial or long-lived alga that could use these stores at a later date. Nutrient pulses can leave a signal in the tissue concentration of nitrogen, as demonstrated by the red alga, *Gracilaria edulis* (Costanzo et al. 2000). Nutrient storage from previous supplies of nitrogen might explain why tissue nitrogen levels in the kelp *Pleurophycus gardneri* did not reflect changes in water nutrient concentrations (Germann et al. 1987), a phenomenon also noted by Fujita et al. (1989). Finally, nutrient enhancement might alter the secondary metabolite concentration in kelp tissues, either decreasing the amount of phlorotannin secondary metabolites if resources are allocated to growth or reproductive functions instead or increasing phlorotannins if their production is nutrient limited. Although phlorotannins do not contain nitrogen, their synthesis depends on nitrogen-containing compounds, such as enzymes (Herbert 1989).

In this study we experimentally enhanced nutrients in the waters surrounding *Hedophyllum sessile* individuals in the field. Because wave action and ambient light are important determinants of nutrient limitation in a natural setting, it is important that nutrient limitation is tested *in situ*. Few studies have experimentally tested the role of nutrients in limiting kelp growth in the field, with some notable exceptions (Chapman and Craigie 1977, Dean and Jacobsen 1986).

Hedophyllum is an abundant kelp of rocky intertidal habitats from Alaska to California (Abbott and Hol-

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lenberg 1976). Although coastal waters of the northeast Pacific have been shown to be relatively nutrient rich (Fujita et al. 1989, Menge et al. 1997) and algal productivity is high (Leigh et al. 1987), there are several reasons to expect that intertidal kelp may not always have plentiful nutrients. First, our estimates of water column nutrients (see below) show high temporal and spatial variation. Second, the relatively long emersion periods and high light levels in the intertidal zone might make intertidal kelp more susceptible to nutrient limitation than their subtidal counterparts. Thus, we asked whether intertidal kelp were limited by nutrient availability at any time during their primary growing season, by continuously fertilizing focal individuals during the spring and summer months.

To determine if increased nutrients would be used in any way by the alga, we assayed a number of indicators. We asked if experimental increases in the ambient nitrogen and phosphorus surrounding individual *Hedophyllum* would 1) increase frond growth or size, 2) increase the allocation to nitrogen and carbon content, or 3) alter the allocation to secondary metabolites (phlorotannins). We also probed whether allocation patterns might be altered by nutrient enhancement by estimating the relationships among growth, nutrient storage, and phlorotannins.

MATERIALS AND METHODS

All *H. sessile* used in our analyses occurred in the rocky intertidal of Tatoosh Island (48°24′N, 124°44′W). The island is separated by a 0.6-km water gap from the tip of the Olympic Peninsula and is exposed to the predominantly westerly oceanic swells. To ensure that our results were general to *Hedophyllum* around the island, we used four different locales on the island (Hedophyllum Cove, The Fingernail, Ladd's Finger, and Strawberry Draw; see map in Paine and Levin 1981). Although all sites are exposed to oceanic swells, they are oriented to different compass directions and represent a range of physical conditions. The experimental and control algae were 0.5 m above mean low water.

We experimentally assessed nutrient limitation in *H. sessile* by placing nutrient diffusing or control substrates adjacent to young-of-the-year *Hedophyllum in situ*. Between June and July 1999, we identified 52 newly recruited *Hedophyllum* that were located at least 3 m apart. We focused on these small young individuals because we hypothesized that nutrient enrichment would be most beneficial at this stage and because these young algae have had little opportunity to accumulate stored nutrients. Additionally, their small size, compared with adults, might place them in the benthic boundary layer with decreased water movement and nutrient exchange. Individuals were randomly assigned to either a control or nutrient supplementation treatment. Two mesh bags containing 65 g of Osmocote fertilizer (Scotts-Sierra, Marysville, OH, USA) were enclosed in window screen material and anchored to the rock with stainless steel hardware on either side and within 5 cm of each *Hedophyllum*. The same procedure was used for controls, except that the Osmocote was omitted. We used Osmocote containing a ratio 18% N, 6% P, and 12% K, thus enhancing both nitrogen and phosphorus. Nitrogen was released as 9.7% NH_4^+ and 8.3% NO_3^-, whereas phosphorus was released as P_2O_5. Potassium was derived from potash (K_2O). We chose this method of applying fertilizer because it has been shown to be an effective means of increasing nutrient concentrations for several weeks (Worm et al. 2000, Nielsen 2001) and has successfully increased water nutrients surrounding kelp (Dean and Jacobsen 1986). The mesh bags (constructed of nylon hosiery) were replaced every 2 weeks in control and treatments to replenish the Osmocote supply. *Hedophyllum* surrounding the experimental algae were trimmed every 2 weeks to minimize shading effects.

We monitored size, growth, and survivorship of the experimental algae until 28 August 1999 and thus encompassed the primary growing season of this species. We terminated the experiment at the end of August to ensure that we were able to get the necessary tissue samples before the complete or partial removal of frond tissue that often occurs with fall storms.

The area of each frond was estimated as the product of the maximum length and maximum width. *Hedophyllum* grows from a basal meristem with additional diffuse growth throughout the frond. Thus, growth was estimated by punching two 2.5-mm holes (using an office hole punch) near the base of the alga and adjacent to each other at every 2-week census. The exact distance from the base of the frond to the two holes was measured; they were usually 4–5 cm from the base of the frond and approximately 3 cm from each other. Growth, expressed as area, was estimated from the vertical and horizontal movement of these two holes every 2 weeks. Although most *Hedophyllum* add more tissue in an interval than is indicated by this estimator, this dual-hole punch technique provides a relative estimate of growth for each alga. To test if individuals differed in thickness among treatments, bores of known area (2.02 cm^2) were taken from the basal meristem of each alga at the end of the experiment, dried to a constant mass at 70° C, and weighed. These mass per unit area estimates were also used to determine if growth in the final interval of the experiment (as an estimate of dry mass) was increased by nutrient supplementation. None of our focal *Hedophyllum* were reproductive by the end of the summer, a result consistent with other young-of-the-year individuals of this perennial species. Because we used newly recruited *Hedophyllum*, not all individuals had the same start date. Instead, we added individuals to our experiment until July, as they became large enough to identify and hole punch (approximately 50 cm^2). Thus, our statistical analysis uses a *t*-test for each 2-week interval during the course of the experiment. Because of initiating new *Hedophyllum* and some loss ($n = 6$ controls and $n = 5$ nutrient addition algae), the sample size often differed throughout the summer.

We hypothesized that increasing nutrient concentrations might lead to increased tissue nitrogen and increased phlorotannin content. At the termination of the experiment, tissue was taken from each individual for CHN analysis and measurements of phlorotannin concentrations. CHN analyses were done with a CEC 440-SHA Elemental Analyzer (Exeter Analytic, North Chelmsford, MA, USA) at the Marine Chemistry Laboratory at the University of Washington. Phlorotannin analyses were conducted at the Shannon Point Marine Center in Anacortes, Washington. The algal tissues were frozen in liquid nitrogen, lyophilized, and ground by hand with a mortar and pestle to a fine powder. The ground tissues were then extracted in 80% aqueous methanol, and phlorotannin concentrations were measured using a Folin-Ciocalteu method as described in Van Alstyne (1995). Phloroglucinol-dihydrate was used as a standard.

Using the information collected on individuals, we probed whether nutrient content within *Hedophyllum* was correlated with frond size, growth, or investment in phloroglucinol-based phenolic compounds. This allowed us to ask whether there were any factors independent of nutrient enhancement that might explain individual differences in performance. We used control and treatment algae both separately and combined in Spearman correlation tests among variables.

To test that Osmocote was effective at increasing the nutrient concentrations in the surrounding waters, we sampled water 5 cm above both control and Osmocote-containing mesh bags and adjacent to experimental algae on an incoming tide. Because the Osmocote mesh bags were immediately adjacent to the experimental algae and because fronds move in water flow, our sampling ensured that water that could potentially contact the focal algae was collected. On 1 July 1999, a 60-mL syringe

was used to draw water from 15 sites with nutrient addition and 14 controls sites. The water was filtered through a GF/C filter into acid-washed HDPE plastic bottles. Samples were immediately put on ice and then frozen within 4 h of collection. Water nutrient concentrations (PO_4^{3-}, $Si(OH)_4$, NO_3^-, NO_2^-, NH_4^+) were analyzed by the Marine Chemistry Laboratory at the University of Washington. The water chemistry methods followed those published by UNESCO (1994).

We estimated the levels of nutrients at the four island locales where the experiment was performed during the spring and summer months from 1998 until 2001. Using the water collection and analysis methods already described, we collected water samples on the same day at approximately the same time (within 1 h) at each of the four island locales. Samples were always collected on the incoming tide during daylight hours.

RESULTS

Over the 4 years that water nutrients were estimated on Tatoosh Island, we found relatively high mean values of ammonium, nitrate, and phosphate (1.84, 16.5, 1.75 μM, respectively) but high spatial and temporal variability. Ammonium varied over 9-fold (0.58–5.39), nitrate almost 18-fold (1.77–31.5), and phosphate almost 4-fold (0.97–3.80).

Our fertilization manipulation significantly increased the nutrients surrounding experimental algae. Nitrate, ammonium, and phosphorus concentrations were all an order of magnitude higher adjacent to Osmocote-filled mesh bags and experimental algae (162, 111, and 14.8, respectively) when compared with control sites (15.5, 2.45, and 1.80, Mann-Whitney U tests, $P < 0.001$). There was no significant difference in nitrite (0.35 vs. 0.31 in controls), a result we expected because the fertilizer did not contain nitrite. Importantly, our sampling also indicated that nutrients diffusing from experimental sites did not contaminate control sites; nutrients at control sites were consistently lower and similar to nutrient concentrations estimated at other times around Tatoosh Island. Thus, the nutrient enhancement appeared effective and localized to our target kelp.

Despite the significant elevation in water nutrients adjacent to experimental *Hedophyllum*, there was no evidence for nutrient limitation. The mean frond area increased throughout the experiment, but not in relation to enhanced nutrient levels (Fig. 1). *t*-Tests at every census date indicated no difference in the area of control versus treatment algae.

Growth rates, estimated by hole punching, also showed little evidence of a treatment effect throughout the experiment (Fig. 2). As the growing season advanced and size increased, the estimated growth rate of *Hedophyllum* increased to over 50 cm^2 every 2 weeks. The only interval where there was any suggestion of a difference between the two treatments was the 2-week interval starting at 13 June. A P value of 0.064 with an unpaired *t*-test suggested that nutrient-enhanced algae may have grown slightly more than controls. For all other intervals during the summer, the estimated P values strongly rejected the hypothesis that added nutrients could enhance growth. When we estimated the statistical power of our analyses, we found that our replication on 13 June provided only 56% certainty in detecting the small difference we observed in growth rate ($\alpha = 0.05$). However, because our replication was 20 for some intervals, our certainty exceeded 80% at other dates during the experiment (13 July and 28 July).

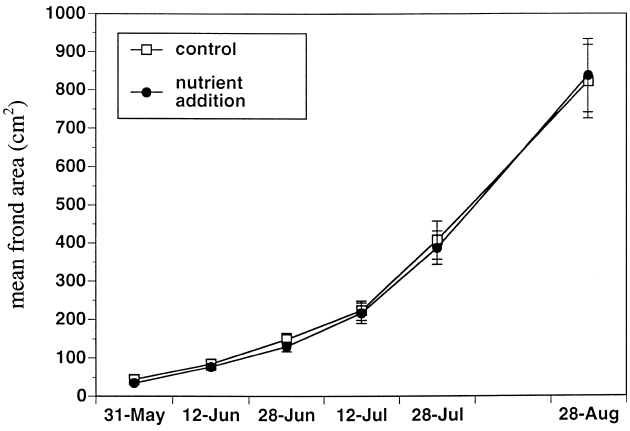

FIG. 1. The mean frond area (in cm^2) of *Hedophyllum sessile* at each of the census dates. At no time did algae with enhanced nutrients differ in size from controls with unpaired *t*-tests. Error bars are SE. Sample sizes ranged from 13 to 34 per treatment.

Repeated-measures analysis of variance is another appropriate method for analyzing growth differences on our focal algae. However, because tissue loss could result in the loss of the hole for tracking growth, not all individuals had growth estimates for all intervals. For individuals where growth was estimated for at least three consecutive intervals ($n = 11$ for controls, $n = 13$ for nutrient enhanced), we used repeated-measure analysis of variance and found no difference among controls and algae where nutrients were enhanced (Wilks lambda = 0.771, $P = 0.386$). Addition-

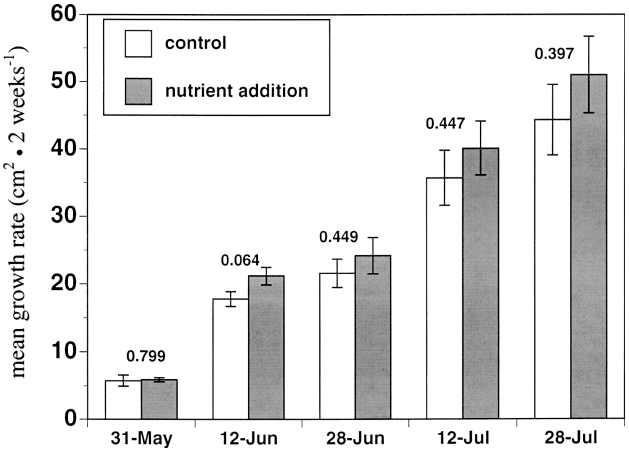

FIG. 2. The mean growth rate (in cm^2) over a 2-week period, estimated from the movement of two holes punched in the basal meristem. Numbers over each pair of histograms represent the P value from an unpaired *t*-test. The dates at the beginning of the interval are given. Sample sizes varied from 7 to 20 per treatment.

ally, the treatment effect remained nonsignificant when we analyzed only those experimental algae with the earliest start date and the longest exposure to supplemental nutrients. In sum, none of our analyses of growth provided strong evidence of a nutrient effect on *Hedophyllum* growth.

Cork bore samples for mass per unit area at the end of the experiment provided us with an estimate of frond thickness and allowed us to assess whether *Hedophyllum* differed in their growth rates when frond thickness was considered. When we used these estimates of mass per area and multiplied it by the area of growth, there was no significant difference in the estimated growth in mass (unpaired *t*-test, t = 0.340, $df = 30$, $P = 0.737$). Controls added an average of 2.32 g of dry mass (±0.29 SE) in the final month of the experiment, whereas nutrient-enhanced algae added 2.58 g (±0.28 SE).

In addition to not affecting demography, nutrient enhancement did not affect the nitrogen and carbon content in the tissue of *Hedophyllum*. Nitrogen stores did not differ between treatments (control mean = 2.29 ± 0.04% [SE], nutrient-enhanced mean = 2.32 ± 0.045 [SE], t = 0.446, $P = 0.658$, $df = 38$), and carbon stores were also statistically indistinguishable (28.49 ± 0.60% vs. 28.09 ± 0.40% [SE], t = 0.539, $P = 0.539$, $df = 38$).

The manipulation of nutrients also had no effect on the concentration of phlorotannins in *Hedophyllum*. Controls had phlorotannin concentrations of 0.85% by dry mass (±0.05 SE; given as phloroglucinol equivalents), whereas the addition of nutrients resulted in a concentration of 0.95% dry mass (±0.06 SE) in *Hedophyllum* tissue. These estimates were statistically indistinguishable with an unpaired *t*-test (t = 1.264, $P = 0.214$, $df = 38$). The amount of tissue nitrogen in an individual *Hedophyllum* was uncorrelated with either the frond area or the relative growth rate, either when individuals from both treatments were combined or analyzed separately (Table 1). However, tissue nitrogen was correlated with phlorotannin concentrations (Fig. 3), but only for algae in the nutrient addition treatment ($r = 0.658$, $P < 0.001$, $n = 21$).

TABLE 1. Correlations between the percent of tissue nitrogen by dry mass and growth, frond area, and phlorotannin concentrations.

	Tissue nitrogen vs.		
	Growth	Frond area	Phlorotannins
Controls	−0.009	−0.049	0.110
	$P = 0.973$	$P = 0.842$	$P = 0.653$
	$n = 15$	$n = 19$	$n = 19$
Nutrient addition	0.213	−0.241	**0.658**
	$P = 0.412$	$P = 0.292$	$P < 0.001$
	$n = 17$	$n = 19$	$n = 19$
All algae combined	0.129	−0.096	**0.451**
	$P = 0.482$	$P = 0.554$	$P = 0.003$

Correlations were estimated using either control and treatment algae separately or together. Spearman correlation coefficients and their significance values are shown; significant correlations are shown in bold type.

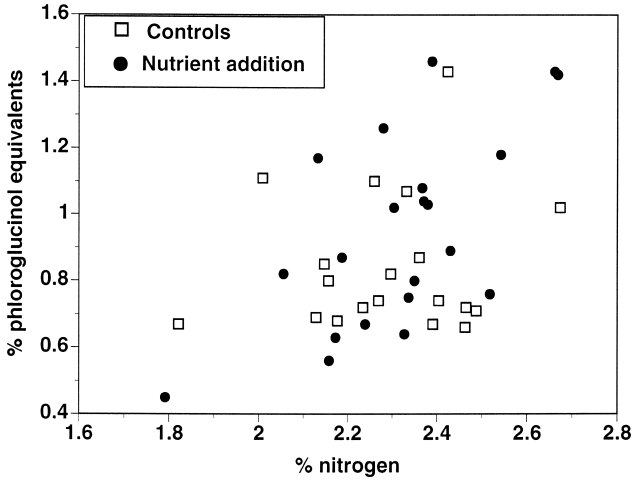

FIG. 3. The relationship between the percent of nitrogen in *Hedophyllum* tissue and phlorotannin concentration. The two variables were uncorrelated for controls but significantly positively correlated in nutrient addition algae (Table 1).

Controls showed no such correlation ($r = 0.110$, $P = 0.653$, $n = 19$). Note that this correlation between nitrogen levels and phlorotannins occurred despite the finding that nutrient additions did not change either the nitrogen or the phlorotannin levels relative to controls.

DISCUSSION

There was little evidence that *Hedophyllum* was limited by nitrogen or phosphorus concentrations in the waters off Tatoosh Island. Frond area, tissue nitrogen and carbon, and phlorotannin concentrations were all unaffected by nutrient enhancement from fertilizer that greatly increased water column nutrients surrounding the treatment algae. There was only a brief suggestion that algal growth rate might be increased with added nutrients during one interval in June, based on a *P* value of 0.064. Otherwise, this kelp was remarkably unaffected by the experimental treatment. There was no evidence that epiphytes or grazers differentially affected the two treatments; conspicuous epiphytes were nonexistent and grazing on this kelp was minimal during the study.

Internal nitrogen content has been advocated as an indicator of nitrogen limitation (Hanisak 1983, Fujita et al. 1989, Wheeler and Bjornsater 1992), with 1.9%–2.0% dry mass as a threshold value below which nitrogen limitation might be expected in green and red algae (Hanisak 1983). The mean tissue nitrogen of *Hedophyllum* individuals was usually in excess of 2.0% in our study, and these values were unchanged with enhanced water column nutrients, suggesting that critical tissue nitrogen levels are at or below 2.0% in *Hedophyllum*. In comparison, during El Niño conditions, juvenile *Macrocystis pyrifera* had tissue nitrogen levels at or below 1.20% (Dean and Jacobsen 1986). Experimental fertilization increased these values only slightly. In the kelp, *Ecklonia maxima*, nitrogen con-

tent was 2.12% during periods of upwelling, declining to 1.79% during relaxation events (Probyn and McQuaid 1985). However, it is unknown whether this species is limited by nutrients at any time. The brown alga *Sargassum baccularia* had relatively low values of tissue nitrogen (approximately 0.6%–1.4%), and laboratory experiments and field censuses gave strong indications that nitrogen limitation was important (Schaffelke and Klumpp 1998). Although there is still relatively little field evidence that tissue nitrogen levels will be an informative indicator of water column events, observations indicate that tissue nitrogen can vary in accordance with the nitrogen content of the water (Wheeler and North 1981, Björnsäter and Wheeler 1990, 1992, Horrocks et al. 1995, Naldi and Wheeler 1999, Costanzo et al. 2000, Hurd et al. 2000, but see Germann et al. 1987, Fong et al. 1998). Tissue nutrient concentrations have been suggested to be a useful indicator of water column nutrients in some algae (Wheeler and Björnsäter 1992, Fong et al. 1998); however, the utility of this approach depends on a demonstrated relationship between nitrogen limitation and internal N content.

The strong positive correlation between tissue nitrogen and phlorotannin concentrations in fertilized but not in unfertilized algae is enigmatic. If nitrogen was limiting the production of phlorotannins, then we would have expected the nutrient-enhanced algae to have greater phlorotannin levels overall, a result that was not borne out by the data. However, the positive relationship between tissue nitrogen and phlorotannin concentration does suggest a link between nitrogen stores and phlorotannin production. Physiological mechanisms underlying the allocation of phlorotannins are poorly understood, and nutrient enrichment studies can produce results that are difficult to interpret. For example, enhanced nutrient levels can affect phlorotannin production in *Fucus gardneri*, even when the enhanced nutrient is not a limiting one (Van Alstyne and Pelletreau 2000). Furthermore, mixtures of nutrients, such as the Osmocote used in this study, may have synergistic effects on secondary metabolite production that can not be anticipated by studying the effects of single nutrients in isolation (Van Alstyne and Pelletreau 2000). These results suggest that the effects of nutrients on macroalgal secondary metabolite production may be physiologically complex.

An absence of a nutrient effect on *Hedophyllum* could be due to sufficient ambient nutrients in the waters surrounding Tatoosh Island. Mean levels of different nutrients at Tatoosh Island are higher than other studies documenting nitrogen limitation in brown algae. For example, our ammonium estimates correspond only with the high nitrogen site reported by Yates and Peckol (1993), and our estimated water column nutrients were usually greater than those reported by Chapman and Craigie (1977) for nitrogen-limited *Laminaria longicruris* in the northwest Atlantic Ocean. Water nitrogen estimates at Tatoosh Island were also comparable with the nutrient concentrations in the fertilized areas surrounding *Macrocystis pyrifera* in the study by Dean and Jacobsen (1986) and two orders of magnitude higher than the estimates during El Niño conditions in this same study. Although the above studies suggest that the mean nutrient concentrations around Tatoosh Island are relatively high, they are comparable with other coastal areas in Oregon (Fujita et al. 1989, Menge et al. 1997, Nielsen 2001). Nutrient limitation of algal growth was demonstrated by Nielsen (2001), although the experiments were done in tide pools where nutrient dynamics may be different from those reported here. In our study, nutrient levels can be relatively low (e.g. ammonium estimates often fell below 1 μM), but our experimental results suggest that low ammonium or nitrate availability may be brief.

Of course, even more extreme temporal fluctuations in nutrients associated with El Niño events or seasonal variation in the strength of upwelling events might change these conclusions. Previous experiments in this system indicate that during times of nutrient stress associated with El Niño conditions, nutrient limitation can affect the food web at Tatoosh Island. Wootton et al. (1996) found that although nutrients did not increase the amount of benthic microalgae, micrograzer abundance was increased. In the autumn months of an El Niño or in "normal" years, there were no effects of nutrient supplements. Conversely, Paine (1986) found little effect of the 1982–1983 El Niño event on the size or survivorship of the intertidal kelp *Postelsia palmaeformis*. Our study with *Hedophyllum* did not occur during an El Niño year; our results might be different if it had. Nonetheless, this experiment provides valuable baseline information on kelp growth and allocation responses to compare with subsequent intervals where local oceanographic conditions might differ.

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