Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link)

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Abstract

We explored the use of an opportunistic green alga, *Enteromorpha intestinalis* (L. Link), as an indicator of N enrichment in a southern California salt marsh. In conjunction with N additions to cordgrass (*Spartina foliosa*, Trin) in April, June and August 1995, mesh bags containing N-starved algal tissue were placed within cordgrass patches, at their edges along islands, and in adjacent channels. After 1 week in the field, recovered algal tissue was used to test detection of two levels of total N supply (one twice as high as the other), as well as no added N (control). Tissue N concentration, calculated as the percentage change in N, was the best of several algal measures at discerning differences in N availability in any month. In both April and June, tissue N declined from the marsh plain to the channels, reflecting declining N supply. Tissue N concentration also reflected differences in the total quantity of N added. Within the channels adjacent to fertilized areas, algal tissue N was similar to control areas, suggesting that N additions to cordgrass are not resulting in eutrophication of open waters. In August, the algae detected N additions on the marsh plain, but survivorship was poor; other algal species may be better indicators of enrichment in late-summer. With further investigation, the technique presented in this paper has the potential to be developed into a useful bioassay for detecting eutrophication of coastal salt marshes and lagoons. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Coastal eutrophication, especially of semi-enclosed bays (Cambridge and McComb, 1984; Cambridge et al., 1986; Silberstein et al., 1986; Lapointe, 1987, 1989), estuaries (Gordon et al., 1981; Day et al., 1989) and coastal water (Lapointe and O’Connell, 1989), is a major problem in many parts of the world. For many years, scientists have been trying to develop an effective indicator of nutrient enrichment that provides early warning and predicts the response of the ecosystem with a high level of accuracy (Shubert, 1984). Successful techniques have been developed for freshwater lakes in temperate zones (Wetzel, 1975). However, in marine or estuarine systems there has been little success. One useful technique was developed for some temperate zone estuaries characterized by deep water and a year-round freshwater inflow; in this case, water-column phosphorus (P) concentration in inflowing waters has been used to predict the phytoplankton abundance ‘downstream’ in the estuary (Lee and Jones, 1981).

Monitoring of water-column nutrient concentrations is the historical method used to indicate nutrient enrichment in marine environments. However, in warm-temperate zone (Fong et al., 1987, 1993a,b) and subtropical (Fong and Jacobson unpubl. data) coastal ecosystems there is little correlation between water-column nitrogen (N) or P concentrations and either productivity or abundance of primary producers. In southern California lagoons and estuaries, nutrients are often supplied in pulses that are spatially and temporally variable (Peters et al., 1986). The frequency and duration of nutrient pulses depend on seasonal rainfall. Another source of nutrient pulses is episodic, but increasingly frequent influxes of untreated wastewater. Although pulses of high nutrients produce transient peaks of water-column nutrients (Peters et al., 1986), they do not accumulate in the water column unless loading rates are very high. Because of the rapid uptake abilities of macroalgae (Fong et al., 1993b,c), traditional methods of monitoring water-column nutrients to predict changing community structure may not be useful in coastal systems subjected to pulsed inflows.

The concentration of nutrients in the tissue of macroalgae may be a useful indicator of enrichment or eutrophication potential (Bjoernsaeter and Wheeler, 1990; Wheeler and Bjoernsaeter, 1992; Fong et al., 1994a). Macroalgae respond to nutrient enrichment by taking up nutrients, growing, and storing ‘excess’ nutrients for future growth (Hanisak, 1979; Fujita, 1985; Bjoernsaeter and Wheeler, 1990). Thus, unlike water column nutrient concentrations, nutrient content of algal tissue integrates nutrient regime over a period of time (Wheeler and Bjoernsaeter, 1992). Thus, tissue nutrients may record a nutrient pulse that may be missed by periodic sampling of the water column.

Many laboratory studies that link nutrient uptake, tissue nutrient concentration, and growth for different species of macroalgae (Hanisak, 1979; Wheeler and North, 1980; Rosenburg and Ramus, 1982; Lapointe and Duke, 1984; Fujita, 1985; Neori, 1996) suggest foliose green macroalgae may be an especially useful indicator for assessing nutrient enrichment as they are opportunists with very fast nutrient uptake and growth rates, and have a large internal storage capacity for nutrients (Waite and Mitchell, 1972; Birch et al., 1981; Gordon et al., 1981; Lapointe and Tenore, 1981; Duke et al., 1986, 1989).

In this paper, we explore the feasibility of using the N content in the tissue of the
macroalga *Enteromorpha intestinalis* (L. Link) as an indicator for nutrient enrichment. *E. intestinalis* has several life-history characteristics that enable it to dominate in a variable environment; it is euryhaline (Rudnicki, 1986), eurythermal (Fong and Zedler, 1993), tolerant of desiccation (Fong et al., in review), with low light saturation of photosynthesis (Kentula, unpubl. data). Thus, this species is able not only to survive, but to proliferate in a wide range of physical environments. In addition, it has rapid N uptake and growth rates, as well as a large internal N storage capacity (Gordon et al., 1981; Rosenberg and Ramus, 1982; Fujita, 1985; Fujita et al., 1988; Duke et al., 1989). These characteristics make it an ideal candidate as a bioindicator of nutrient enrichment, especially in systems where nutrients are supplied in pulses, as they enable the alga to respond rapidly to enrichment, and then record the nutrient signal through time as storage in tissues (Fong et al., 1994). We started our investigation of algae as a nutrient indicator with a field test in order to take advantage of an ongoing experiment where we were adding N to vascular plants in a salt marsh adjacent to San Diego Bay, CA. We used the enrichment experiment as a known N source, and placed previously starved samples of *E. intestinalis* in an array around this source to determine whether, after a 1-wk exposure, tissue N content would reflect N supply.

2. Methods

2.1. Experimental design and site location

The objective of our research was to determine if the tissue N content of nutrient-starved *Enteromorpha* cultured in the laboratory and then placed in the field for 1 wk reflected the supply of N to that field location. The field experiments were designed around two ongoing enrichment experiments at Sweetwater Marsh National Wildlife Refuge in San Diego County, California, USA (Boyer et al., 1996; Fig. 1). South Connector Marsh is a created salt marsh that consists of 3 islands surrounded by tidal channels (Fig. 2). Our observations of tidal flow suggest that the tide comes in down the deeper, western channel, and slows as it circles around to the eastern channel. The islands were planted with *Spartina foliosa* (Trin) in 1985. In spring of 1995, fertilization of 2 of the islands with urea (46% N by mass) was initiated. Urea was used as it is inexpensive, readily available commercially, and had been used successfully in earlier salt marsh fertilization experiments. From north to south, the three islands received: high N addition (6 kg island$^{-1}$ every 2 wk), no fertilization, and low N addition (3 kg island$^{-1}$ every 2 wk).

The first indicator experiment was conducted in April (fertilized islands only), and repeated in June and August (all three islands) to test for robustness of the indicator during seasonal variation in environmental conditions and the associated biotic communities (during this period, the *Spartina foliosa* went from low to maximum biomass). We assumed that N supply was highest in fertilized areas and decreased with distance from this known N source. Thus, for each enriched island, nutrient-starved (see below) algal tissue was outplanted within five randomly chosen locations within fertilized and unfertilized *S. foliosa* marsh, five sites along the east and west edges of the islands about
Fig. 1. Sweetwater Marsh National Wildlife Refuge in San Diego County, CA.

3 m from the fertilized plots, and five sites within both east and west channel, about 3 m offshore of the edge sites. For the unenriched island, there were no fertilized sites; otherwise the array of sites was identical. Unenriched control plots in the *S. foliosa* marsh for the low N addition island were interspersed with enriched plots while for the high N addition island, control sites were at a similar distance from the enriched areas as the channel sites. Thus, we predicted that tissue N content in the low N addition experiment would rank as enriched > control > edge > channel. In contrast, in the high N addition site we predicted that not only would the amount of tissue N be higher, but the ranking would be enriched > edge > channel ≥ control.

2.2. Collection, preconditioning, deployment, and recovery

*Enteromorpha intestinalis* was collected from low intertidal to subtidal areas around the North Islands of Connector Marsh, just north of the flood control channel (Fig. 1). Each of the three collection dates was 3 to 4 wk prior to the beginning of the outplanting experiments. Algal tissue was removed from the mud substrate where it was loosely attached. Algae were rinsed in the channel to remove mud and sorted to remove large invertebrates. Cleaned tissue was placed into a large cooler and transported to the Pacific Estuarine Research Laboratory’s greenhouse at San Diego State University within 2 h of collection.

In the greenhouse, the algae were removed from the cooler and placed into shallow
Fig. 2. Locations of the high and low N addition experiments and the unfertilized island within South Connector Marsh. The high addition island had a large area of marsh fertilized, indicated by the black square. On the low addition island, fertilized areas were 1 m² plots interspersed with no addition plots, indicated by the grid.

Pans filled with unfiltered seawater from the pier at Scripps Institution of Oceanography. Pans were shallow to minimize shading and to mimic the shallow habitat in which the algae are typically found. Seawater with lower N (mean = 14.7 μM N as NO₃⁻ + NH₄⁺, SE = 0.5) compared to estuarine concentrations (mean = 224.8 μM as NO₃⁻ + NH₄⁺, SE = 56.3, for Sweetwater Marsh, Sept to March 1995) was used for culturing the algae because we wanted to reduce and equalize tissue N content during culture. Water was not changed during the 3 or 4 wk of culture to eliminate new sources of N; deionized water was added daily to counter evaporation.

The day before each of the indicator experiments was initiated, algae were removed from the culture pans, spun for 1 minute in a lettuce spinner to attain a uniform wet wt, and divided into 3 g subsamples. Five replicate 3 g subsamples were briefly rinsed in freshwater, dried in a forced air oven until constant weight (~24 h), re-weighed, ground in a Wiley Mill, and analyzed for tissue N content (Quik Chem Method 13-107-06-2-D, revision 28 July 1988) on a Lachat nutrient autoanalyzer (model #2100-000). Initial tissue N of the algae that were placed in the field was low; mean tissue N as % dry wt was 1.35 (SE = 0.10), 0.74 (SE = 0.02) and 0.61 (SE = 0.03) after the culturing period for experiments in April, June, and August, respectively.

The remaining subsamples were placed into mesh bags (dimensions are 10x15 cm; mesh size 1 cm when stretched) sewn from knotted nylon netting normally used in aquaculture; netting was tubular, so algae were placed in the tube and the two open ends
hand stitched with polyester thread. Bags with algae were replaced into the culture pans overnight, transported back to the estuary at low tide on 13 April, 8 and 9 June, and 3 and 4 August 1995, and placed in the arrays within and around islands as described above. Bags were attached with fishing line to bamboo stakes; stakes were pushed into the mud substrate to a depth of 20 to 40 cm, depending on whether they were placed in the salt marsh (shallowest depth) or channel (deepest depth). Bags were placed at similar heights above the water to ensure that they were covered approximately the same amount of time each tidal cycle.

2.3. Retrieval and tissue N

After 1 wk, bags were retrieved from the estuary (20 April, 15 and 16 June, and 10 and 11 August). In April and June there was 100% recovery of the algae and bags; in August, one bag was lost. During the August experiment there were unequal sample sizes because 44 of 84 bags recovered were either empty or the tissue was too badly decomposed to weigh or analyze for nitrogen. Algal tissues in the 40 bags with algae remaining were analyzed using a Carlo-Erba CHN analyzer due to the small amount of biomass. A CHN analyzer measures all forms of N in tissue while the auto analyzer method does not include NO₃. However, comparisons were made within seasons, when methods are the same.

Algal tissue was removed from each bag, and wet wt, dry wt, and tissue N were quantified as described for initial samples. N content of the tissue from each bag (N bag⁻¹) was calculated by multiplying the tissue N content by the total dry wt of algae in each bag. Tissue N content was also reported as percentage change in N from the initial N content.

2.4. Data analysis

The two estimates of algal biomass (wet wt, dry wt) as well as three measures of tissue N (N as % dry wt, N bag⁻¹, and % change in N) were compared to determine which of these response variables may be of greatest use as an indicator of enrichment. Thus we seek to identify the variable that has the clearest relationship with nitrogen supply (as distance from the site of N addition), with the least variability. We used a single factor ANOVA to determine if there were significant differences among mean wet wt, dry wt, N as % dry wt, N bag⁻¹, and % change in N, from each of the five sites for each of the experimental islands. Prior to running an ANOVA, data were tested to ensure that assumptions were not violated. After a significant ANOVA, a Protected Least Significant Difference (PLSD) test was performed to establish where differences in means occurred.

3. Results

3.1. April

Tissue N content calculated as % change in N (Fig. 3) and N as % dry wt (Fig. 4)
Fig. 3. Percentage change in the amount of nitrogen in the tissue of algae in experimental bags in April. Bars are ±1 SE.

Fig. 4. Tissue N content as % dry weight of algae in experimental bags in April. Bars are ±1 SE.
Fig. 5. Wet weight of algae in experimental bags in April. Initial wet weight was 3 g; bars are ±1 SE.

Fig. 6. Dry weight of algae in experimental bags in April. Bars are ±1 SE.
Fig. 7. N content bag$^{-1}$ of algae in experimental bags in April. Bars are ±1 SE.

were good bioindicators for nitrogen supply in April. For both measures, there was a significant difference among sites for both the low and high N addition islands (ANOVA, $P = 0.0001$ and 0.0039; results of statistical analyses are identical for both variables as one is calculated directly from the other). Around the low N addition island, tissue N and change in tissue N were significantly greater in the + N site and lowest in the channel sites (PLSD, $P < 0.05$). There were no differences among the control and edge sites, but there was a declining trend from the N source to edges and channels sites. In and around the high N addition island, both variables were highest in the + N site (PLSD, $P < 0.05$); there were no significant differences among other sites. However, as hypothesized, there was a decline with distance from the N source, from edge to channel and control sites.

Changes in wet wt were not a good indicator of nitrogen supply to algal tissue placed in or around either enriched island in April (Fig. 5). There was no statistically significant pattern of greater growth within or near the nitrogen source. There were significant differences in wet wt among sites for both the low (ANOVA, $P = 0.005$) and high (ANOVA, $P = 0.002$) N islands. However, these differences were not related to distance from N-supply. Similarly, dry wt was not a good indicator of nitrogen supply in either of the fertilized islands in April (Fig. 6). There was a significant difference in mean dry wt among sites around the low N island (ANOVA, $P = 0.0001$), but this difference was due to the higher dry wt in the west channel (PLSD, $P < 0.05$) rather than any pattern with nitrogen supply. In the high N addition island, there were no differences among sites in the mean dry wt of algae (ANOVA, $P = 0.141$).
In the April experiment, N bag$^{-1}$ was also a fairly good indicator of nitrogen supply for both enriched islands (Fig. 7; ANOVA, $P = 0.0006$ and 0.0031, respectively). However, this effect was largely due to the response of tissue N; using dry wt in the calculation lowered its usefulness as an indicator.

### 3.2. June

In June, as in April, both percentage change in N and N as % dry wt provided good indicators of nitrogen supply during the course of the week-long experiment (Figs. 8 and 9). In both the low (ANOVA, $P = 0.0286$) and high (ANOVA, $P = 0.0001$) N addition
islands, there were significant differences among means. In both areas, for both measures of tissue N, the N content was greater in the + N sites (PLSD, $P < 0.05$); as hypothesized, tissue N was also elevated in the control sites of the low N addition island (PLSD, $P < 0.05$), but not the control sites of the high addition island. Although there were differences among means in the areas of the unfertilized island (ANOVA, $P = 0.0028$), these differences were unrelated to N supply. However, there was no increase in tissue N in algae placed into the *S. foliosa* marsh on the unfertilized island, confirming that the elevated N measured in the other two experiments was not simply an effect of being within the island salt marsh vegetation.

As in April, none of the response variables based on weight were good indicators, so data were not presented here.
3.3. August

In August, *E. intestinalis* had poor survivorship in the experimental bags. We also observed little biomass in the field at this time.

Despite the limited number of samples, the data indicate that change in tissue N appeared to reflect the N supply (Fig. 10). On the low N addition island, tissue N increased by over 100% during the week long experiment; there were no differences between the + N and control sites. In the high N addition samples, tissue N increased 100% in the fertilized areas, and over 50% in the control areas. Tissue N decreased
Fig. 11. Tissue N content as % dry weight of algae in experimental bags in August. Bars are ±1 SE.

during the experimental period in all sites away from the N source. Algae placed within the unfertilized island slightly increased in tissue N, while tissue N of algae placed around the island decreased. Unfortunately, few bags retained algae (n = 1 for control and east channel, n = 3 for east edge) in this area. N as % dry wt showed similar patterns (Fig. 11).

4. Discussion

The technique presented in this paper has potential for development into a useful indicator of nitrogen supply. In areas where N supply is pulsed, repeated week-long
deployments of algal tissue using this technique would integrate the pulses over time and enable us to quantify complex temporal patterns of supply. Alternatively, this technique may be useful for identifying specific sources of nutrients, e.g., point and non-point sources such as specific creeks and canals, or groundwater, by placing algal samples in a spatial array around suspected sources.

In an attempt to develop an in situ bioindicator of nitrogen supply, Hanisak (pers. comm.) quantified the tissue N content of many species of macroalgae collected along onshore-offshore transects in the Florida Keys; although variability was high, he found decreasing tissue N with increased distance from shore. It is possible that variability in the history of N supply of these field-collected algae may have led to the variable results, and that culture under identical conditions and then placement in the field, as done in this experiment, may help to reduce variability.

Clearly, tissue N of the algae is a better indicator of N supply than any estimate of growth or biomass accumulation. Measures of biomass may not be useful for several reasons. First, we may not have measured biomass accumulation adequately, because the relatively large mesh size of the experimental bags did not prohibit removal of algal biomass. There is a trade-off when choosing mesh size. A large mesh allows for more natural light levels and patterns of water flow. Water flow may influence nutrient supply to algal tissue by reducing boundary layers (Larned and Stimson, 1996). However, the large mesh also may confound measures of algal growth due to significant removal of biomass by water motion or herbivory. Second, earlier studies indicate that *E. intestinalis* may exhibit a considerable lag between N uptake and growth, when nitrogen supply is altered rapidly (Fong et al., 1994). Thus, tissue N may be a more rapid response variable than growth rate.

Of the three response variables that include measures of tissue N, the percentage change in N appears to be the most likely candidate to develop as an indicator. Simple measures of tissue N do not take into consideration initial conditions, and do not allow assessment of whether there was a net increase or decrease in tissue N. Knowing the direction of change in tissue N will enable the indicator to detect N supplies both higher and lower than the culture conditions. The second response variable, calculating N bag⁻¹, relies on determination of final biomass, a response variable that we measured with unknown certainty, as discussed above. However, the percentage change in N is insensitive to any estimate of biomass, yet very sensitive to initial conditions.

Although our indicator technique worked well in April and June, the success was limited in August because the algae had such poor survivorship. It is possible that the algae were outcompeted by *Spartina foliosa* in the field. By August, *S. foliosa* biomass has reached its seasonal maximum (Boyer and Zedler, 1998), and formed dense stands in each of the experimental areas. Thus, competition for either N or light may have been occurring. Alternatively, the algae may not be eurythermal enough to survive the high temperatures that occur in the marsh and channels in August. Earlier work established that the optimal water temperature for growth of *E. intestinalis* ranged from 18 to 22°C (Fong and Zedler, 1993); this is considerably lower than temperatures in the field in August. If this is the case, another alga with a higher temperature tolerance may be more appropriate as an indicator during summer. One option that should be investigated is
Ulva expansa, an alga that often proliferates in these systems in summer (Fong and Boyer, pers. obs). Another possibility is the red alga, Gracilaria spp, which is more abundant in late summer than Enteromorpha (Boyer, pers. obs.). In a field trial using similar methods to the current study, Gracilaria maintained more of its initial mass and was better able to detect N inputs than Enteromorpha (Boyer and Fong, unpub. data from August 1996). Enteromorpha is generally more widely distributed spatially and temporally, but Gracilaria may prove a useful late-summer substitute.

Further work is needed to develop an indicator for nitrogen supply. One question that needs to be addressed is the inability of the indicator to consistently distinguish among areas with lower nutrient supplies (e.g., distant from the N source). It is possible that this indicator is only accurate at higher N supplies. Alternatively, at lower N sites the signal of the addition may have been very weak, and other N sources such as flux from the benthos and different water column supplies from tidal water may have swamped the signal. A second area for future work is to develop a quantitative rather than relative scale for nutrient supply. At present, we are only able to use the indicator to determine supply to an area relative to another area measured simultaneously. Extensive laboratory or microcosm experiments would enable us to hindcast the actual nitrogen supply to tissue from tissue N concentrations. These experiments will need to investigate the effect of various physical and chemical conditions, including variation in temperature, light, and water flow rate, on tissue nitrogen concentration. However, once these relationships are established, these techniques may be developed into a very useful indicator for nitrogen enrichment.

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