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Evaluating Patterns of Nitrogen Supply Using Macroalgal Tissue Content and Stable Isotopic Signatures in Tomales Bay, CA

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Through bioassay techniques and field collections of red macroalgae (Gracilariopsis sp.) and eelgrass (Zostera marina), we evaluated growth, tissue %Nitrogen (N) and N stable isotopic signatures as bioindicators of potential changes in N supply to Tomales Bay, CA (USA). Gracilariopsis sp. collected, cultured, and outplanted across a spatial gradient in Tomales Bay showed pronounced changes in N patterns from past studies in this system, and was superior to field collections in detecting spatial N signals. Rather than a single peak in N concentration near the oceanic source found in previous work, we detected two N peaks, one near the bay head and one near the bay mouth. This spatial pattern suggests two sources account for these discrete regions of increased N supply. The temporal N patterns showed marked seasonality, with greater tissue N concentration during the wet season and reduced N concentrations during the dry season. The spatial patterns presented here suggest shifting nutrient dynamics within Tomales Bay, with increased N supply detected near the major watershed inflow. Nitrogen isotopic values suggest an enriched wastewater source, but additional work is needed to confirm the source of this newly reported N signal.

Keywords nutrients, nitrogen, macroalgal bioindicator

Introduction

Nearshore estuarine systems can receive nutrients from oceanic and terrestrial sources (Valiela 1995), including coastal upwelling and watershed delivery (Fry et al. 2003). Irrespective of source, nutrients often arrive in pulses and are spatially and temporally variable (Fong and Zedler 2000; Fry et al. 2003). Macroalgae commonly associated with increased nutrient loading (Valiela et al. 1997; Kamer et al. 2001) can rapidly take up pulsed nutrients from the water column, often before these pulses can be reliably detected by conventional water sampling methods (Wilson 1994). Consequently, marine scientists have developed alternative water sampling methodologies, employing macroalgae as biological indicators to determine nutrient supply (Fong et al. 1998; Costanzo et al. 2000; Umezawa et al. 2002; Cohen and Fong 2005) based on their ability to integrate dissolved nitrogen (N) and phosphorus (P) over time within their tissues (Fujita 1985; Björnsäter and Wheeler 1990). In particular, red macroalgae (e.g., *Gracilaria,Gracilariopsis spp.*) have been used in bioassay

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studies (Horrocks et al. 1995; Costanzo et al. 2000) because they can tolerate wide fluctuations in light and temperature (Bird et al. 1979) and show a strong tissue nutrient response to changes in water nutrient concentrations (McLachlan and Bird 1986; Lapointe 1987).

Tomales Bay, California has been considered a pristine, temperate estuary with low watershed nutrient delivery and minimal coastal development. However, recent changes in watershed nutrient loading have been suspected in Tomales Bay, fueling water quality concerns (Smith 2002). The few studies recently conducted within Tomales Bay only secondarily address spatial nutrient patterns and do not investigate seasonal variations (Judah 2002). Consequently, our understanding of the current nutrient supply and patterns in the estuary is limited (Smith 2002).

In addition to potentially altered nutrient loading, changes in the macrophyte community composition within the Bay have been observed. Recent surveys of the eelgrass (*Zostera marina)* populations in Tomales Bay conducted by the California Department of Fish and Game noted dramatic increases in abundance of the native macroalga, *Gracilariopsis* sp. (Gurgel et al. 2003) in the inner bay (Tom Moore pers. comm). Similar shifts from seagrasses to macroalgae have been linked to increased nutrient supply in temperate bays in the eastern United States (Valiela et al. 1997; McGlathery 2001). A re-evaluation of the nutrient supply in Tomales Bay, spatially and seasonally, is needed to determine if increased macroalgae may be attributed to changes in magnitude and source of nutrients.

The aim of this study was to use macroalgal bioassays to identify current nutrient patterns in Tomales Bay. We had three specific objectives. First, a dose-response experiment was conducted to validate the use of the macroalga *Gracilariopsis* sp. as a bioindicator of nutrient supply. Second, *in situ* bioassays using outplanted arrays of *Gracilariopsis* sp. were conducted from May to September 2005 to investigate temporal and spatial patterns of nutrient supply. Lastly, N sources to Tomales Bay were investigated using stable isotope values in *Gracilariopsis* sp. and eelgrass from field collections. $\delta^{15}N$ values in macrophyte tissues have been successfully applied to identifying the source of nitrogen supplies to coastal waters (Umezawa et al. 2002; Cohen and Fong 2005; Fry 2006), thus isotopic values across sample sites could indicate differences in dissolved inorganic nitrogen (DIN) sources.

Methods

Tomales Bay is a temperate estuary located 50 km northwest of San Francisco, California (Fig. 1). Two major streams flow into Tomales Bay: Walker Creek near the bay mouth and Lagunitas Creek at the head. The Mediterranean-like climate consists of wet and dry seasons. Previous studies have shown the bay to be well mixed during the wet season (~Oct.-April), with short water residence time (1–3 d), relatively high DIN and no detectable spatial nutrient patterns (Smith et al. 1987; Fourqurean et al. 1997). In the dry season (~May-Sept.) oceanic nutrients are brought into the bay through advection during coastal upwelling events, mixing is reduced, and water residence time increases to several months (Smith et al. 1987; Hollibaugh et al. 1988). A dry season N gradient, with maximum DIN concentration near the oceanic opening and decreasing towards the bay head, was detected by Smith et al. (1987). This gradient was confirmed by Fourqurean et al. (1997) a decade later using eelgrass tissue as a bioindicator of nutrient availability.

Dose-Response Nutrient Experiment

To establish whether *Gracilariopsis* sp. assimilates nutrients proportionally to the availability of nutrients in the water column, a dose-response experiment was conducted. Previous

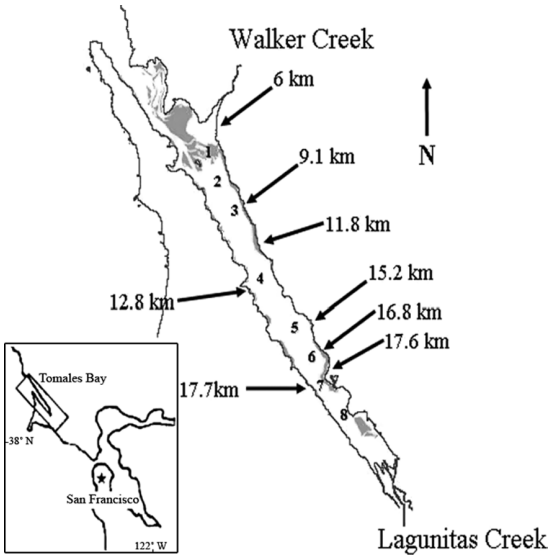

Figure 1. Map of Tomales Bay, CA showing bioassay sites (1–8). Arrows indicating stable isotope collection sites are followed by distance from the bay mouth in km. Shaded regions are the areas of existing eelgrass cover according to 2002 mapping conducted by the California Department of Fish and Game.

studies reported seasonal fluctuations in Tomales Bay ranging from 2–25 μM for DIN and 1–4 μM for dissolved inorganic phosphorus (DIP) (Fourqurean et al. 1997; Judah 2002). P is thought to co-vary with N in Tomales Bay macroalgal tissue at an approximate 10:1 ratio (P. Fong, unpublished data). Using this ratio, five nutrient enrichment treatments were selected to represent and exceed the reported natural variation of N to P (μM N: μM P = 0:0, 10:1; 20:2; 30:3; 40:4). Each treatment was replicated 8 times for a total of 40 experimental units.

Gracilariopsis sp. thalli were collected from Tomasini Cove on the east side of Tomales Bay (Fig.1), cleaned of epiphytes, and cultured in aerated tanks containing ambient water from the collection site for five days to reduce variability in initial tissue nutrient content (Fong et al. 1998). Additional seawater collected from the same site was enriched with $NaNO_3$ and $NaPO_4$ to determined treatment levels above ambient. The 0:0 treatment consisted of ambient seawater (0.33 ± 0.28 μM NO_3 and 1.05 ± 0.03 μM PO_4) with no addition of nutrients. Each experimental unit (clear plastic jar) was filled with 600 ml of a treatment solution and 5.0 g (wet wt) of algae. Wet weight was determined by placing thalli in a nylon mesh bag, spinning for 1 minute in a salad spinner to remove excess water and then weighing with a balance. Experimental jars were randomly arranged at the same height in an outdoor flow-through seawater system to maintain ambient temperature and covered with window screening to reduce light and evaporation yet not restrict air flow (Fong et al. 1998). Every 48 hrs, treatment water was replaced to ensure that algae had access to new nutrients for uptake and assimilation. The experiment lasted 7 days.

Initial and final water samples from each experimental unit were filtered through Whatman GF/C glass fiber filters and analyzed for NO_3, and PO_4 according to Whitledge et al. (1981) and NH_4 according to Solorzano (1969). A single factor ANOVA, followed by Fishers PLSD post hoc test, identified significant differences among treatments. Levene's test of homogeneity was used to test for equality of variances; no transformations were needed.

Tissue nutrients were measured at the end of the experiment. Macroalgal samples were rinsed in deionized water to remove salts, dried to a constant weight at 60ºC, ground with a mortar and pestle, and analyzed for tissue N content (% dry wt) using a CHN autoanalyzer at the Moss Landing Marine Laboratory or University of California, Santa Barbara Marine Science Institute. A single factor ANOVA, followed by Fisher's PLSD post hoc test, identified significant differences in tissue %N between treatments. Levene's test of homogeneity was used to test for equality of variances; no transformations were needed.

Bioassay of Nutrient Supply

To evaluate nutrient availability seasonally and spatially in Tomales Bay, four bioassay experiments were conducted between April and September 2005 along eight sites spanning the length of the inner Bay (Fig. 1). Bioassays conducted in April, May, and July 2005 immediately followed or coincided with rain events due to an unusually wet spring and summer, while the September bioassay did not (Fig. 2). All bioassays overlapped to some degree with periods of local coastal upwelling (Fig. 2). Bioassay deployment was not intentionally timed with upwelling or rain events.

As in the dose response experiment, *Gracilariopsis* sp. tissue was collected, cleaned, and cultured for 5 days. Thalli were then weighed into 6.0 g wet weight subsamples using previously described methods. Subsamples were sewn into mesh bags constructed of fiberglass window screening and stored in a cooler of low nutrient water until deployment in Tomales Bay later that day.

Each bioassay array consisted of six replicate mesh bags suspended at the water surface off a buoyed nylon line which was anchored to the bottom. At each of the 8 sites, an array

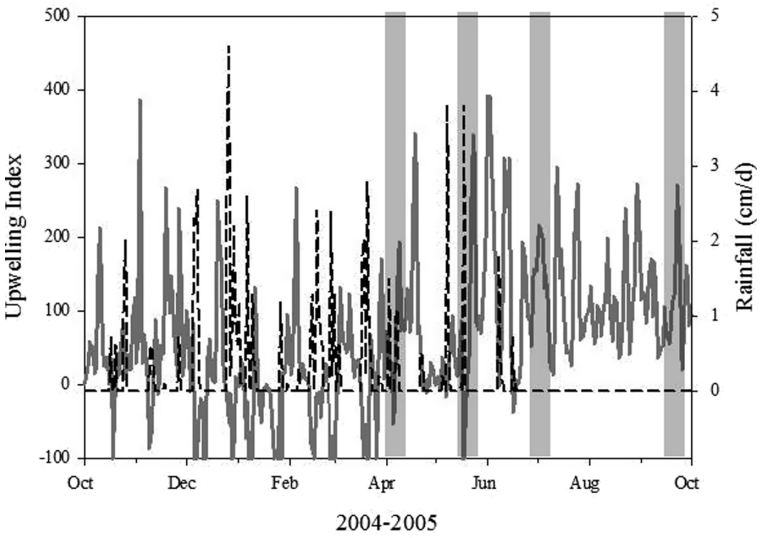

Figure 2. Daily upwelling index (solid grey line) and rainfall (cm/d; dashed line) for Tomales Bay region from April to October 2005. Shaded grey bars depict dates of *in situ* bioassay experiments. Data from Data Access in Real Time (www.cbr.washington.edu/dart) Pacific Coastal Upwelling Index at 39°N, 125°W and California Department of Water Resources (www.water.ca.gov).

was deployed near the middle of the Bay. Each array remained *in situ* for 7 days. All bags (n = 48) were collected on the seventh day, transported back to the laboratory in a cooler, total macroalgal subsample wet weighed, dried and reweighed, and subsample was prepared for tissue N and C content as described earlier.

N content of macroalgal tissue was calculated on a per experimental unit basis as tissue %N to facilitate comparison across spatial and temporal gradients. A two-factor ANOVA (month x site) was used to determine significance of %N results. Factors generating significant F values were further analyzed with Fisher's PLSD post hoc analyses to determine overall temporal (month) and spatial (site) patterns. To further explore differences in spatial patterns within a sampling month, one-factor ANOVA (factor: site) was conducted on each bioassay, followed by Fisher's PLSD post hoc analysis if significant. Levene's test of homogeneity of variances concluded no transformations were necessary.

Field Collection of Macrophyte Tissue for Stable Isotope Analysis

Nitrogen stable isotope ratios in macroalgae and seagrasses were used to identify possible N sources to Tomales Bay and to explore whether these two morphologically different macrophytes use the same nutrient pools. Seagrasses can efficiently access sediment N stores through their root system (Evrard et al. 2005) that are not easily accessed by the overlying macroalgae in the water column. Hence, these two classes of macrophytes may be drawing on different nutrient sources: *Gracilariopsis* sp. primarily from DIN in the water column and *Z. marina* primarily from mineralized nutrients within the sediments. At each of 8 intertidal sites along the latitudinal gradient of the bay (Fig. 1), 5 tissue samples of *Gracilariopsis* sp. and *Z. marina* were collected in early June 2005. Epiphyte-free material was targeted in *Z. marina* and *Gracilariopsis* sp. collection samples to avoid epiphyte signals cofounding results. Sites differed from the bioassay sites as they were selected for occurrence of *Gracilariopsis* sp. within shallow *Z. marina* habitat, included both the eastern and western shore of Tomales Bay, and overlapped with stable isotope sites selected by Fourqurean et al. (1997) to foster data comparison. Collected samples were cleaned, rinsed, dried, ground and weighed into tin cups for stable isotope analysis. Samples were sent to University of California, Davis Stable Isotope Facility for determination of natural abundances of ^{15}N as well as %N using a continuous flow isotope ratio mass spectrometer (Europa Scientific Hydra 20/20). Isotopic ratios for each sample were calculated using the formula:

$$\delta X(‰ = \quad [(R_{sample} - R_{standard})/R_{standard})] \times 1000,$$

where $\delta X = \delta^{15}N$ and $R = {}^{15}N: {}^{14}N$. The nitrogen standard was atmospheric N_2.

Tissue $\delta^{15}N$ and %N were evaluated with a one-factor ANOVA to detect among site differences. Fisher's PLSD post hoc test determined where significant differences occurred. Linear relationships between tissue $\delta^{15}N$ and %N and distance from the bay mouth were evaluated using correlation analysis.

Results

Dose-Response Experiment

Initial water samples showed that the intended treatment levels were each statistically distinct (ANOVA; $p < 0.001$) and in the proper approximate concentrations above ambient for both NO_3 and PO_4 (Table 1). Residual nutrients measured in the media after 48 hrs suggest

Table 1
Mean NO_3 and PO_4 values (±1 SE; n = 8) of initial and final water enrichments for dose response experiment (ANOVA; $p < 0.000$). *indicates that nutrient levels are different than all others (PLSD; $p < 0.0001$)

	Initial water		Final water	
	NO_3 µM	PO_4, µM	NO_3 µM	PO_4, µM
0:0	0.33 (0.28)*	1.05 (0.030)*	1.08 (0.27)	0.29 (0.20)*
10:1	9.98 (0.20)*	2.05 (0.025)*	0.45 (0.10)	0.58 (0.17)
20:2	19.84 (0.16)*	2.6 (0.132)*	0.99 (0.16)	0.91 (0.07)
30:3	29.09 (0.23)*	3.51 (0.070)*	0.70 (0.22)	0.74 (0.07)
40:4	37.21 (0.28)*	4.42 (0.017)*	0.84 (0.20)	1.31 (0.18)

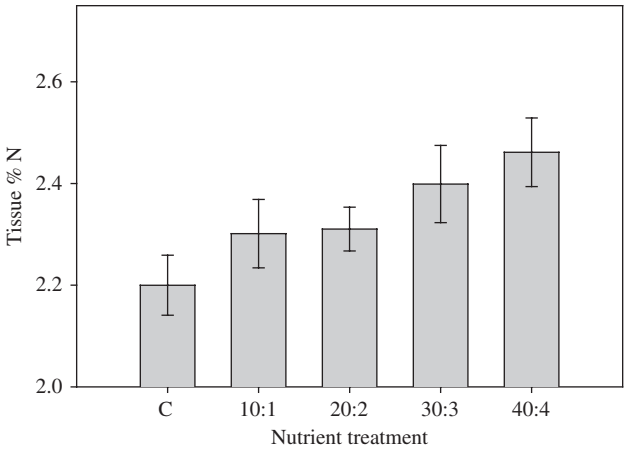

Figure 3. Dose response results as tissue nutrient concentration of N (% dry wt) across increasing enrichment treatments (µM N: µM P) in Tomales Bay, CA. Means are shown (n = 8) with error bars (± 1 SE).

complete draw down of the available nutrients by the macroalgae (Table 1). Final tissue %N results confirmed the ability of *Gracilariopsis* sp. to take up available NO_3 in proportion to supply. Tissue %N compared among the treatments showed increasing tissue enrichment with increasing nutrient concentration (Fig. 3; ANOVA; $p = 0.015$).

Bioassay Experiments

Final tissue %N varied temporally among and spatially within bioassays (two–factor ANOVA Month: $p < 0.001$; Site: $p < 0.001$; Fig. 4). Two-factor ANOVA results also showed a significant interaction ($p < 0.001$) between factors of month and site, underscoring the importance of considering seasonal variability in conjunction with site variability when interpreting nutrient patterns for Tomales Bay. Temporal differences supported previously documented patterns where N supply is significantly elevated, with minimal between-site variability, in the wet season (i.e., April) relative to the summer season (Month: $p < 0.001$; PLSD, $p < 0.05$ for each sampling month). April mean tissue %N was the highest

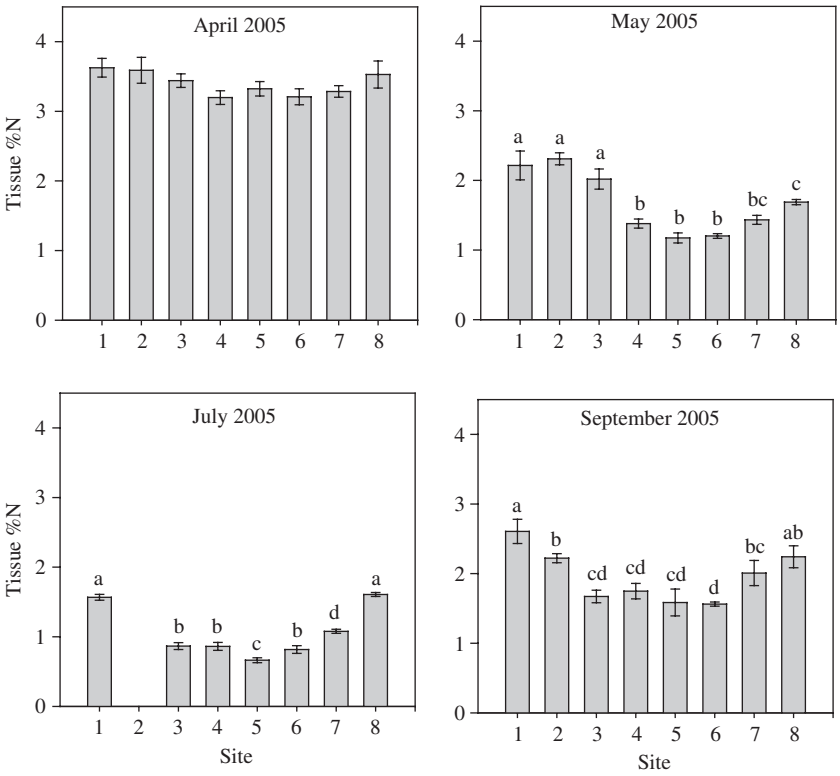

Figure 4. Temporal and spatial variation of bioassay tissue %N content from April to September 2005. Means are shown (n = 6) with error bars (±1 SE). *In situ* array from July 2005 site 2 was lost. Bars sharing the same letter do not differ (PLSD; $p > 0.05$).

(3.399 ± 0.16 %N) and decreased significantly by May (mean = 3.130 ± 0.23 %N). Tissue %N was lowest in the July bioassay (mean = 1.066 ± 0.13 %N). In the September bioassay %N began to increase (mean = 1.950 ± 0.22 %N).

Significant spatial variation of tissue %N was detected in the May, June and September bioassays (Table 2; Fig. 4). In May, sites 1, 2 and 3, located nearest the estuary mouth, had significantly greater N content than all other sites up estuary (PLSD, $p < 0.05$). Though not as strong as the signal near the bay mouth, an additional increase in tissue %N was detected near the bay head (site 8) which was significantly higher than the mid-bay signal (PLSD, $p < 0.05$). This two peak pattern at the bay mouth and bay head emerged as early as May, which was still in the rainy season for this sampling year. In July, a significant peak in %N was again detected nearest the bay mouth as well as nearest the bay head (PLSD, $p < 0.05$). The pronounced peak detected at site 8 was the greatest value detected during the July bioassay, registering two fold greater in %N compared to mid-bay sites. The final bioassay, conducted in September, also had significantly increased %N values near the bay mouth and bay head relative to the mid-bay sites (PLSD, $p < 0.05$).

Field Collections of Macrophyte Tissue

The percent N of *Gracilariopsis* sp. and *Z. marina* tissues did not differ significantly between species (mean = 2.0 ± 0.2% N and 1.9 ± 0.1% N, respectively), though both species

Table 2
ANOVA table for analysis of site differences in tissue %N across different bioassay sampling months

Month	Source of variance	df	MS	F	*p*
April	Site	7	0.173	1.638	0.153
	Error	40	0.105		
May	Site	7	1.122	16.949	<0.001
	Error	37	0.066		
July	Site	6	0.849	73.359	<0.001
	Error	35	0.012		
September	Site	7	0.841	7.705	<0.001
	Error	39	0.109		

exhibited significant spatial patterns (Fig. 5; ANOVA, $p < 0.0001$). Similarly, spatial differences in $\delta^{15}N$ values were detected in both species (ANOVA, $p < 0.0001$), but neither species exhibited a linear spatial pattern with distance from the bay mouth (correlation analysis, $p > 0.05$). The mean value for $\delta^{15}N$ in *Gracilariopsis* sp. was 11.3 ± 0.4‰, with a range of 3.4‰. The mean value for $\delta^{15}N$ in *Z. marina* was 9.8 ± 0.5‰, with a range of 4.4‰. The highest values in $\delta^{15}N$ for both macrophytes occurred at the same collection site, located 9.1 km from the bay mouth on the eastern shore. In contrast, the lowest $\delta^{15}N$ values for both species were found at the two western shore sites, located 12.8km and 17.7km from the bay mouth, respectively (Fig. 5). Planned contrast ANOVAs of tissue

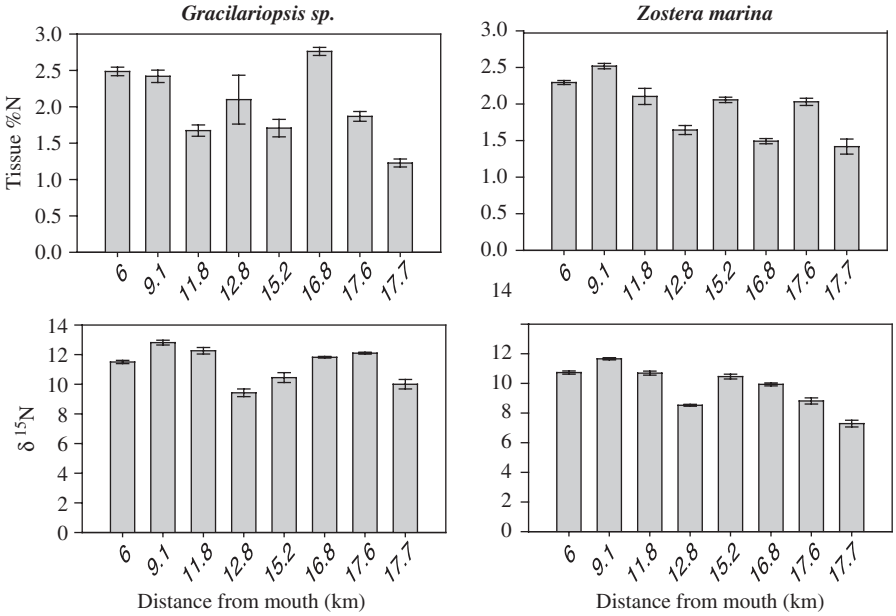

Figure 5. Tissue %N and $\delta^{15}N$ isotopic composition of *Gracilariopsis* sp. and *Zostera marina* collected from 8 locations along Tomales Bay, CA. Means are shown (n = 5) with error bars (±1 SE).

$\delta^{15}N$ between eastern and western shore sites showed significantly different values for both *Gracilariopsis* sp. and *Z. marina* ($p < 0.0001$ for both macrophytes). The west bank values in *Z. marina* averaged $9.7 \pm 0.2‰$ compared to $11.8 \pm 0.2‰$ on the east bank. West bank values averaged $7.9 \pm 0.2‰$ in *Gracilariopsis* sp. compared to east bank values of $10.4 \pm 0.2‰$.

Discussion

Spatial patterns of N supply showed pronounced changes from previous studies conducted in Tomales Bay. The linear gradient in N concentration reported by Fourqurean et al. (1997) and Smith et al. (1987, 1989) in the dry season was not detected in this study. Rather than a single N peak near the oceanic source, we detected two discrete, pronounced peaks in macroalgal tissue N in summer. These peaks correspond to the previously described peak near the bay mouth and an additional peak at the bay head, suggesting greater N supply at these locations in Tomales Bay. This previously undocumented supply of bioavailable N at the bay head near Lagunitas Creek was the largest peak detected during the July bioassay, indicating the relative strength of this new N source compared to other sources along the length of Tomales Bay in summer months.

The lack of a linear pattern in the *Z. marina* $\delta^{15}N$ values sampled along the inner bay also contrasts with the spatial patterns in water column concentration and seagrass tissue described by Fourqurean et al. (1997). During an August sampling, Fourqurean et al. (1997) detected higher $\delta^{15}N$ up estuary, suggesting denitrification in the sediments was responsible for this enriched signal. While our results showed similar values (~12‰) to the Fourqurean et al. study (1997) up estuary, we also found continually elevated $\delta^{15}N$ along the length of the inner bay where the previous study did not. A variety of mechanisms could account for these heavy $\delta^{15}N$ values including increased denitrification rates (Fourqurean et al. 2005), decreased fractionation of the DIN pool in response to summer declines in N availability (Anderson and Fourqurean 2003) or altered nutrient sources to the system.

Using the bioassay approach, the elevated tissue N found near Lagunitas Creek at the bay head suggests that the terrestrial watershed may be functioning as nutrient source into the bay. In contrast, the estuary mouth has two potential sources of N: oceanic upwelling and stream input from Walker Creek. The elevated macroalgal tissue N found nearest the oceanic opening of the bay in the September bioassay (when watershed inputs are reduced and no rain events were recorded), suggests an oceanic source in this region. Reduced water mixing, month-long water residence times, and weak ocean exchange during the summer months hinders the movement of upwelled nutrients towards the bay head (Hollibaugh et al. 1988). Hence, it is unlikely that the oceanic nutrients upwelled into Tomales Bay in the summer were advected 20km to the bay head and detected by our bioassay approach at site 8. Rather, a second nutrient source in this region is probable.

Terrestrial wastewater sources may account for the high N detected near the Lagunitas Creek inflow. Numerous point and non-point wastewater sources occur in the Lagunitas Creek watershed to accommodate increasing human populations, including eight small sewage treatment systems, leach fields, holding tanks, and seepage pits (Smith 2002). These systems are most likely to fail during heavy rainfall events (Smith 2002). A 1980 study by the Food and Drug Administration sanitation survey demonstrated that elevated streamflow from rainfall events led to bacterial contamination in two regions of the bay: the head of the bay and the east side of the bay near Walker Creek (cited in Smith 2002). These two regions correspond to the same two regions of elevated N concentration we

detected. Given that rain events coincided with or preceded the summer bioassays and the isotope collection sampling, a similar response to elevated creek inflows may have carried wastewater nutrients into the system during our sampling. Alternatively, atmospheric wet deposition within the watershed could be of quantitative importance as an N loading source (Rudek et al. 1991), potentially masking coastal upwelling and wastewater runoff N signals. However, Ayer and Gao (2007) studying a US east coast temperate estuary, found only a fraction of the atmospheric N deposited in the watershed will actually reach the estuary itself. Furthermore, Smith et al. (1996) found atmospheric nutrient influx within Tomales Bay to be in approximate equilibrium with dissolved nutrient efflux. Lastly, the September bioassay, while not preceded by any rainfall event, still showed evidence of elevated nutrients at the bay head and near Walker Creek, further suggesting a low likelihood of atmospheric wet deposition as an important nutrient source. Our data show that watershed N inputs to Tomales Bay are not restricted to the rainy season but occur in the dry season as well.

The isotopic N value of wastewater becomes increasingly enriched in ^{15}N during the treatment process and undergoes denitrification and ammonium volatilization, reaching values between +10–20‰ $\delta^{15}N$ as it enters the groundwater (McClelland and Valiela 1998). Mean $\delta^{15}N$ values detected toward the bay head fell within this reported range for *Gracilariopsis* sp. but not *Z. marina*. Wastewater discharge from Lagunitas Creek watershed, coupled with groundwater discharge, could have served as an enriched $\delta^{15}N$ source. This pulse of isotopically heavy N would be most readily integrated in the short-term by macroalgae compared to seagrasses (Valiela et al. 1997). Clonal plants like *Z. marina* integrate resources throughout the clone, so even by selecting for new growth, signals would be integrated over a much longer period of time than *Gracilariopsis* sp.

The anomalously lighter $\delta^{15}N$ values along the western bank of the inner bay, detected in both *Z. marina* and *Gracilariopsis* sp., lend further evidence to localized terrestrial inputs. Both of the western bank sites exhibit low tissue %N concentrations and low $\delta^{15}N$ values. These values are well below average oceanic upwelling signatures (~10–13‰) (Altabet et al. 1999), or an enriched wastewater signature (~10–20‰). Given that the western bank drainage is not part of the Lagunitas Creek watershed, it is probable that these two different drainages could reflect differing N sources. Furthermore, the two collection points farthest up estuary (at 17.6km and 17.7km; Fig. 1) are separated by only 100m along the length of Tomales Bay but are located on opposing shorelines of the estuary. These sites exhibited different *Graciliariopsis* sp. and *Z. marina* tissue %N and $\delta^{15}N$ values, further evidence of an east/west difference in nutrient sources/supply.

While the observed spatial nutrient patterns within the dry season differed from past studies in Tomales Bay, the seasonal nutrient patterns detected lie within previously reported observation for this system. Tissue N content was uniformly high in the wet season (e.g., April sampling) when mixing is rapid and N availability is elevated. As rainfall declined and then ceased, nutrients became increasingly limiting, corresponding to the decreased N content during May, July and September sampling. Both bioassay out-planting and *in situ* collection data confirmed this temporal pattern of decreasing N availability, with similar ranges of tissue %N in the May bioassay to the collection data collected one week later in early June.

While the bioassay technique was sensitive to patterns of N availability in both a laboratory controlled dose-response experiment and in the water column, the *in situ* collections were highly variable, poorly corresponding to bioassay spatial patterns despite using the same macroalgal species as a bioindicator. Given the location of the collection sites along the shallow intertidal rather than the water column deployment of bioassay rigs, collection results suggest localized factors that are not detectable at the bay-wide scale may be

important to site-specific nutrient availability. A variety of factors influence biota over time in the field, including light, temperature, disturbance, and herbivory (Bird et al. 1979, Valentine and Heck, 1999). This emphasizes the need to outplant material on shorter time scales to detect nutrient signals. Hence, a bioassay approach, which attempts to control within-tissue nutrient variation and can be deployed *in situ* for a limited time period, is a preferable bioindicator technique. Furthermore, our dose-response experiment prior to *in situ* out-planting validated the ability of *Gracilariopsis* sp. to uptake N in proportion to the water column supply. We did not attempt to use our dose-response results to establish standard curves between algal tissue %N and water column supply and relate these to bioassay and collection data sets due to the narrow range of nutrient treatments in our dose-response experiment. However, it is plausible that repeated dose-response experiments over a wider nutrient gradient could generate such curves and be related to *in situ* tissue %N in future applications.

Using macroalgal bioindicators, this study documented different spatial nutrient patterns in Tomales Bay that appear closely connected to upwelling, rain events, and watershed nutrient sources. The geographic distance and watershed differences between the N peaks, suggest that two different sources account for the elevated N concentrations at the bay head and mouth. Thorough monitoring should continue to validate the role of terrestrial versus oceanic sources along the length of Tomales Bay and improve our understanding of anthropogenic wastewater impacts on nutrient supply in this system.

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