

Spatial and temporal variation in C:N:P ratios, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA

James W. Fourqurean^{1,*}, Thomas O. Moore², Brian Fry¹, James T. Hollibaugh³

¹Department of Biological Sciences and Southeast Environmental Research Program, Florida International University, Miami, Florida 33199, USA

²California State Department of Fish and Game, Marine Resources Division, PO Box 1560, Bodega Bay, California 94923, USA

³Department of Marine Science, University of Georgia, Athens, Georgia 30602, USA

ABSTRACT: The nutrient content, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of seagrasses in Tomales Bay (California, USA) vary in both space and time. The variations in these measurements were not random, but followed spatial and seasonal patterns that provide insight into the sources and processing of nutrients in the Tomales Bay ecosystem. Wide ranges of carbon, nitrogen, and phosphorus content of green leaves of *Zostera marina* were found in 72 samples collected over the 2 yr of sampling. Carbon content varied from 29.0 to 40.9% of dry weight, nitrogen from 1.13 to 3.79%, and phosphorus from 0.11 to 0.90%. Stable isotopic composition was variable as well: the mean $\delta^{15}\text{N}$ for all samples collected was $9.7 \pm 0.3\text{‰}$ with a range of 6.2‰. Carbon isotopic content had a range of 7.5‰, with a mean $\delta^{13}\text{C}$ of $-9.6 \pm 0.2\text{‰}$. There was a strong spatial trend in the N content, but not the P content, of *Z. marina* leaves from Tomales Bay. The C:N ratio was around 15 near the mouth of the bay and increased linearly with distance into the bay to ~25 near the head of the bay; there was a concomitant pattern of increasing $\delta^{15}\text{N}$ of seagrass leaves, from 7‰ near the mouth of the bay to near 12‰ at the head of the bay. The spatial pattern in N content was only present during summer months, but the spatial pattern in stable isotopic composition was present in both summer and winter. The patterns in the N and $\delta^{15}\text{N}$ content of the seagrasses indicate the importance of denitrification in the C and N cycles in Tomales Bay. The analysis of patterns in variation of elemental content and isotopic composition of seagrasses is a powerful tool for investigating ecosystem-scale processes in coastal marine systems. Similar analyses of seagrasses from less well studied ecosystems should provide data to generate hypotheses about spatial and temporal variation in processes in these other systems.

KEY WORDS: Seagrass · Ecosystems · Nitrogen · Phosphorus · Stable isotopes

INTRODUCTION

Seagrasses form extensive beds in many coastal embayments worldwide. Unfortunately, seagrass-dominated ecosystems have fared poorly in anthropogenically influenced coastal regions because of the sensitivity of seagrasses to changes in water quality

(e.g. Orth & Moore 1983, Giesen et al. 1990, Larkum & West 1990). Owing to the sensitivity of seagrasses to environmental conditions, the long life of individual seagrass organisms, and the sessile habit of these rooted plants, seagrasses can be used as indicators of conditions in the environment. In effect, the seagrasses continually 'sample' their environment; in their tissues, growth form, and distribution they record environmental conditions under which they have been growing. For example, depth penetration of seagrasses in a

*E-mail: fourqure@fiu.edu

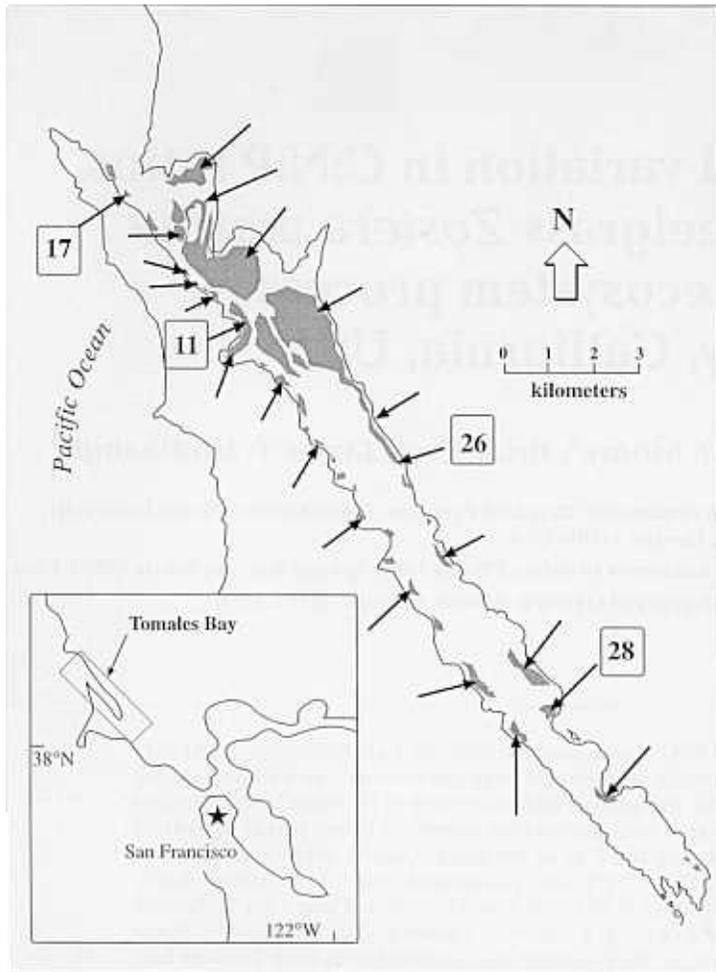


Fig. 1. Tomales Bay, California, USA. Shaded areas indicate extent of *Zostera marina* beds. Arrows point to August 1992 sampling locations. Station numbers in boxes were sampled repeatedly between August 1992 and July 1994

water body may be used as an integrating biological light meter' to assess the light regime in an estuary on long temporal and spatial scales without the effort of an extensive sampling program (Dennison et al. 1993). In a similar fashion, the elemental content of seagrass tissues can be used as a long-term integrator of nutrient availability (Gerloff & Krombholz 1966, Atkinson & Smith 1983, Duarte 1990, Fourqurean et al. 1992a), allowing comparisons of nutrient availability over large spatial scales in an ecosystem (Fourqurean et al. 1992b).

In addition to indicating the quantity of a resource over long temporal and spatial scales, qualitative information about elemental sources can also be obtained from seagrasses. Natural abundances of stable isotopes that comprise seagrass biomass have been used as indicators of the source of C and N for seagrass growth (e.g. Zieman et al. 1985, Fry et al. 1987, Lin et al. 1991). The

natural abundances of stable isotopes vary due to sources and processes in an ecosystem, so variation in stable isotope composition of seagrass tissue can be indicative of ecosystem-scale processing of elements (e.g. Macko et al. 1984, Cooper 1989, Grice et al. 1996). Biotic processes in ecosystems can lead to large variations in the stable isotopic composition of the nitrogen pool (see Peterson & Fry 1987 for review). In particular, denitrification leads to the loss of isotopically light ^{14}N from the nitrate pool, causing the remaining nitrate pool to be enriched in ^{15}N (Cline & Kaplan 1975). The loss of light N from the dissolved inorganic nitrogen (DIN) pool of an estuary can lead to increases in the $\delta^{15}\text{N}$ of the dissolved inorganic pool as the distance from the source of the DIN increases (Horrigan et al. 1990). Plants utilizing the DIN pool should reflect the stable isotopic composition of the pool.

The elemental and isotopic content of plants can vary in a seasonal pattern, as supply rates, relative nutrient availability, microbial processes, and plant growth rates respond to the seasonal pattern of temperature and light availability. There is a general seasonal trend of higher tissue concentrations of N and P of seagrasses in winter, with minimum concentrations in summer (Harrison & Mann 1975, Pirc 1985, Pirc & Wollenweber 1988, Alcoverro et al. 1995). This seasonality of tissue N and P content is a consequence of the seasonality of growth rate of seagrasses; generally only during times of rapid growth and maximum biomass does nutrient demand of seagrasses outstrip

the local supply (Pedersen & Borum 1992). Stable isotope ratios of seagrasses and macroalgae also have been shown to vary seasonally, owing to seasonality in source stable isotope ratios, irradiance, and temperature (Stephenson et al. 1984, Fry et al. 1987, Hemminga & Mateo 1996). In order to use spatial pattern in seagrass elemental and isotopic content as indicators of ecosystem-scale pattern in nutrient availability, the seasonal pattern must be documented.

Tomales Bay, California, USA (Fig. 1) supports between $2.6 \times 10^5 \text{ m}^2$ and $3.9 \times 10^6 \text{ m}^2$ of seagrass beds along the entire 20 km length of the bay (Spratt 1989 and unpublished report from Ecoscan Resource Data, submitted to California Department of Fish and Game in June 1992). These beds are predominantly *Zostera marina*, although *Z. asiatica* (Phillips & Echeverria 1990) and *Phyllospadix* sp. (pers. obs.) are also found in the bay. The seagrass beds are denser and more

expansive near the mouth of the bay and are generally restricted to a narrow band along the shores of most of the bay at depths less than 4 m below mean low water (Spratt 1989).

Experimental evidence suggests that N is limiting to primary production, at least over short time scales, in Tomales Bay (Smith et al. 1987). The major source of N to support the primary production of the ecosystem is DIN in water advected into the bay from the Pacific Ocean; N from freshwater runoff and N fixation are relatively minor sources of N for Tomales Bay (Smith et al. 1987, 1989, 1991, 1996). The apparent N limitation of plant growth rate is a result of denitrification within the bay, and Smith et al. (1989, 1991) argue that the rate of denitrification is controlled by the rate of organic matter oxidation. A substantial fraction of the N brought into the bay is lost from the system through denitrification, which accounts for about 30% of the heterotrophic activity in the bay (Smith et al. 1991). The net result of the oceanic source of nitrogen and the loss of nitrogen through denitrification is a pronounced gradient in the concentration of dissolved N in the waters of the bay, with maximum concentrations near the mouth of the bay and minimums near the head of the bay (Smith et al. 1987, 1989). If this concentration gradient reflects a gradient in N availability for benthic plants, there should exist a gradient in the N content of seagrasses along the axis of the bay.

In this paper, we investigate ecosystem-scale patterns in nutrient sources and processes in Tomales Bay using seagrasses as indicators. We test for the existence of variation in elemental content and isotopic composition of C and N from seagrass leaves, both in time (seasonal variation) and in space (regional variation). We tested several specific predictions: (1) there would be seasonal patterns in N and P content of the seagrass tissue, with minimum N content corresponding to the summer peak biomass and productivity; (2) seagrass N content would decrease as the distance from the oceanic N source increased; (3) because substantial loss of N from the ecosystem occurs via denitrification, there should be a relationship between distance from the N source and the $\delta^{15}\text{N}$ of seagrass tissues; and (4) there would be no regional variation in the content of C or P, owing to the general nitrogen limitation of primary production of the bay.

METHODS

Site description. Tomales Bay is a 20 km long and 1.4 km wide embayment formed at the intersection of the rift valley formed by the San Andreas fault and the Pacific Ocean (Fig. 1). Average depth of the bay is 3.1 m, with a maximum depth of 19 m. The bay's water-

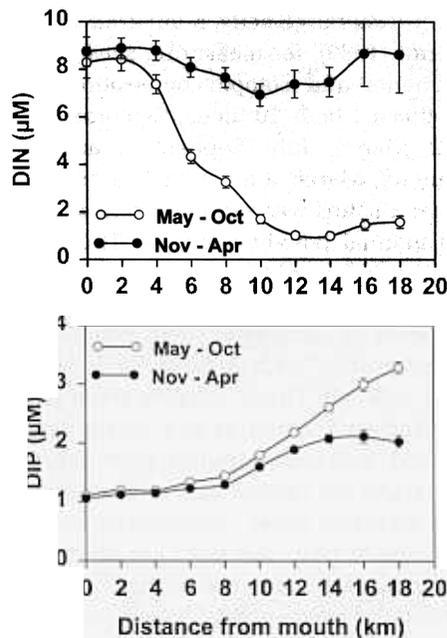


Fig. 2. Tomales Bay. Average water column concentrations of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) by location in the bay for summer upwelling season (May to October) and the winter rainy season (November to April). Data from the Tomales Bay Land Margin Ecosystem Research project, 1987 to 1994 (S. V. Smith & J. T. Hollibaugh unpubl. data). Error bars are ± 1 SE, each point is the mean of 100 to 200 discrete measurements

shed is 561 km², with a human population of only ca 11000. Since 1985, Tomales Bay has been the subject of an intensive study into the biogeochemistry of estuaries (e.g. Smith et al. 1987, 1989, 1991, 1996, Hollibaugh et al. 1988, 1991). The bay has a Mediterranean climate with 2 distinct seasons of nutrient inputs. Rainfall and surface water runoff are restricted to the winter months (November to April). In the summer (May to October), streamflow is greatly reduced, and oceanic upwelling delivers nutrient-rich water to the mouth of the bay. During winter, DIN concentrations show little variation across the bay, but during summer there is a strong DIN gradient, with high DIN at the mouth of the bay and low DIN at the head of the bay (Fig. 2, unpublished data from S. V. Smith and J. T. Hollibaugh). During both summer and winter, dissolved inorganic phosphorus (DIP) increases from the mouth to the head of the bay, but the increase is greater in summer (Fig. 2).

To describe the spatial variation in seagrass elemental and isotopic composition, we sampled 23 of the 28 eelgrass beds identified by Spratt (1989) in August 1992, near the seasonal peak in seagrass biomass (Fig. 1). These 23 beds were distributed from the head of the bay to the mouth. We arbitrarily

chose 4 representative beds, numbered 11, 17, 26 and 28 by Spratt (1989), for measuring seasonality in seagrass elemental and isotopic composition (Fig. 1). We sampled these 4 beds 10 times (September and October 1992, March, July, September, and November 1993, January, March, June, and July 1994). Seagrass tissue from all sites was sampled from a boat using a modified manual post-hole digger. The first 5 intact seagrass shoots brought into the boat constituted the sample. The leaves from all 5 short shoots in a sample were cleaned of epiphytes and adhered sediment by gentle scraping with a razor blade, then pooled and dried (<80°C). Dried samples were ground and homogenized with a mortar and pestle. Additionally, we collected sediment samples from the top cm of sediment along the central axis of Tomales Bay using a 2.5 cm diameter corer. Unfortunately, we did not collect sediment from the seagrass beds themselves. Samples were not acidified prior to $\delta^{13}\text{C}$ analysis. We also collected representative plants and sediments from the watershed for bulk stable isotope analysis.

Carbon and nitrogen content of the ground plant material was measured using a CHN analyzer. Phosphorus content was measured using a dry oxidation-acid hydrolysis procedure (Fourqurean et al. 1992a). C, N and P content was determined in duplicate for all samples. All elemental ratios were calculated on a mole:mole basis. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ measurements were made on a stable isotope mass spectrometer. Sample preparation for the mass spectrometer was done using a CHN elemental analyzer coupled to the mass spectrometer. Results are presented in standard δ notation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [R_{\text{sample}}/R_{\text{standard}}] \times 1000\text{‰}$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standard for carbon is PDB limestone and the standard for nitrogen is atmospheric N_2 . Precision of the stable isotope measurements was better than 0.2‰.

The data were analyzed for spatial trends by linear regression against position (km from the mouth of the bay). For analysis of seasonal trends, the data were grouped by season (winter rainy season, November to April, and summer upwelling season, May to October) and analyzed by 2-way ANOVAs with season and station as factors.

RESULTS

Wide ranges of carbon, nitrogen, and phosphorus content of green leaves of *Zostera marina* were found in the 72 samples collected over the 2 yr of sampling (Table 1). Carbon content varied from 29 to 40.9% of dry weight, with a mean of $36.3 \pm 0.6\%$ (± 1 SE). Nitrogen content averaged $2.33 \pm 0.07\%$, with a range of 1.13 to 3.79%. Phosphorus content averaged $0.38 \pm 0.02\%$, with a range of 0.11 to 0.90%. These wide ranges in C, N and P content resulted in correspondingly wide ranges in elemental ratios (Table 1). C:N, C:P, and N:P ratios all had similar variability as indicated by coefficients of variation (CV = standard deviation/mean) of 0.30, 0.31 and 0.35, respectively. The mean C:N was 19.7 ± 0.7 , with a minimum of 11.5 and a maximum of 38.0. The range of C:P was 106 to 455, with a mean of 273 ± 10 . N:P averaged 14.6 ± 0.6 , with a range of 5.8 to 28.5.

Both the nitrogen and carbon stable isotopic composition of *Zostera marina* leaves from Tomales Bay were quite variable over the course of this study (Table 1). The mean $\delta^{15}\text{N}$ for all samples collected was $9.7 \pm 0.3\text{‰}$. The range of values covered 6.2‰, from a low of 6.3‰ to a high of 12.5‰. Carbon isotopic content had a range of 7.5‰, with a mean $\delta^{13}\text{C}$ of $-9.6 \pm 0.2\text{‰}$. The isotopically heaviest sample encountered had a $\delta^{13}\text{C}$ of -7.5‰ , while the lightest was -15.0‰ .

Table 1. *Zostera marina*. Elemental and stable isotopic composition of eelgrass leaves collected from 23 locations in Tomales Bay, California, USA

	C (% dry wt)	N (% dry wt)	P (% dry wt)	C:N	C:P	N:P	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
August 1992 (n = 23)								
Mean	38.4	2.37	0.34	20.0	304	16.0	9.5	-9.9
SE	0.3	0.12	0.01	1.1	14	1.1	0.3	0.3
Median	38.4	2.29	0.33	19.9	304	14.9	10.0	-9.5
Minimum	35.8	1.46	0.23	13.8	167	8.3	6.9	-15.0
Maximum	40.9	3.35	0.57	32.5	434	25.9	11.7	-7.9
All samples collected between August 1992 and July 1994 (n = 72)								
Mean	36.3	2.33	0.38	19.7	273	14.6	9.7	-9.6
SE	0.6	0.07	0.02	0.7	10	0.6	0.3	0.2
Median	37.1	2.41	0.34	17.5	280	13.1	10.0	-9.3
Minimum	29.0	1.13	0.11	11.5	106	5.8	6.3	-15.0
Maximum	40.9	3.79	0.90	38.0	455	28.5	12.5	-7.5

Spatial variation

Seagrass C, N and P content

The N and P content of leaves of *Zostera marina* from Tomales Bay during August 1992 was more variable than the C content (Table 1). Carbon content ranged from 35.8 to 40.9%, with a mean of $38.4 \pm 0.3\%$. Both N and P content varied by a factor of 2; N content averaged $2.37 \pm 0.12\%$ while P content was $0.34 \pm 0.01\%$. The carbon content of *Z. marina* leaves did not vary as a function of distance from the mouth of the bay (linear regression, $r^2 = 0.00$, $p = 0.94$). There was a strong spatial trend in the N content, but not the P content, of *Z. marina* leaves from Tomales Bay (Fig. 3). The C:N ratio was around 15 near the mouth of the bay and increased linearly with distance into the bay to ~25 near the head of the bay. As indicated by the coefficient of variation, the C:P ratio (CV = 0.22) was as variable as the C:N ratio (CV = 0.26), but showed no relationship to location within the bay (Table 1, Fig. 3).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of seagrass leaves and sediment

The variability of $\delta^{15}\text{N}$ in seagrass leaves in Tomales Bay in August 1992 was very large, with a range of

4.8‰ (Table 1). $\delta^{15}\text{N}$ averaged $9.5 \pm 0.3\%$ for the whole bay. There was a strong spatial pattern in the stable N isotope composition of *Zostera marina* leaves from Tomales Bay (Fig. 4). $\delta^{15}\text{N}$ increased from around 7‰ near the mouth of the bay to near 12‰ at the head of the bay. There was a significant relationship between $\delta^{13}\text{C}$ and location in the bay as well, although less of the variation in $\delta^{13}\text{C}$ could be explained by position than $\delta^{15}\text{N}$. There was a decrease in $\delta^{13}\text{C}$ of 0.1% km⁻¹ from the mouth of the bay towards the head (Fig. 4); seagrass leaves from one bed near the head of the bay had very light $\delta^{13}\text{C}$ (-15.0‰).

Bulk stable carbon isotopic content of sediments from the center of Tomales Bay became lighter from the mouth of the bay ($\delta^{13}\text{C}$ ca -7‰) towards the head of the bay, where $\delta^{13}\text{C}$ approached -24‰ (Fig. 5). Carbon content of the sediments also changed along the transect. From 0 to 8 km from the mouth of the bay the sediment was composed of low organic matter sand, with a bulk carbon content of 0.17 to 0.76% C by weight of dry sediment; from 10 to 20 km from the mouth of the bay the sediment was higher organic matter mud, with bulk C content of 2.01 to 2.25% C. It is quite likely that the stable isotopic C signatures from the low organic matter outer Tomales Bay stations were influenced by inorganic carbon from carbonates; the relatively heavy $\delta^{13}\text{C}$ values of ca -7‰ from Stns 2

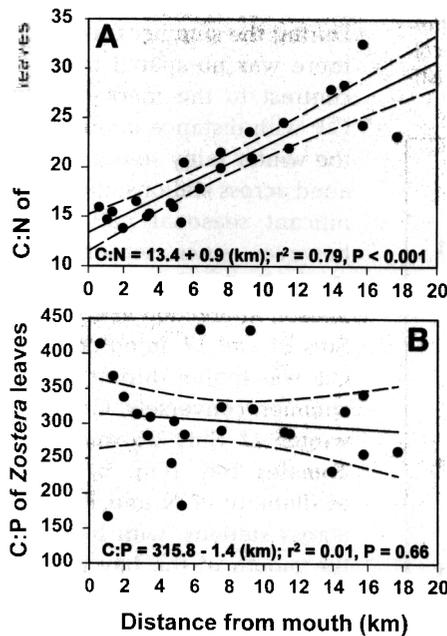


Fig. 3. *Zostera marina*. Spatial pattern in (A) the C:N ratio and (B) the C:P ratio of green leaves in Tomales Bay, August 1992. Position is represented by the distance in km from the mouth of the bay. The least squares linear regression and 95% confidence interval for the regression are shown

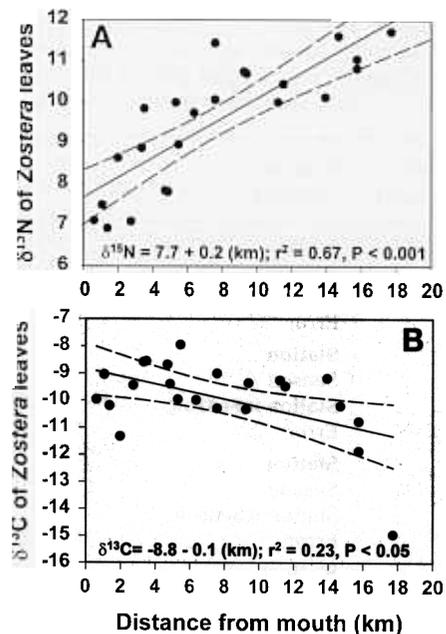


Fig. 4. *Zostera marina*. Spatial pattern in (A) the $\delta^{15}\text{N}$ and (B) the $\delta^{13}\text{C}$ of green leaves in Tomales Bay, August 1992. Position is represented by the distance in km from the mouth of the bay. The least squares linear regression and 95% confidence interval for the regression are shown

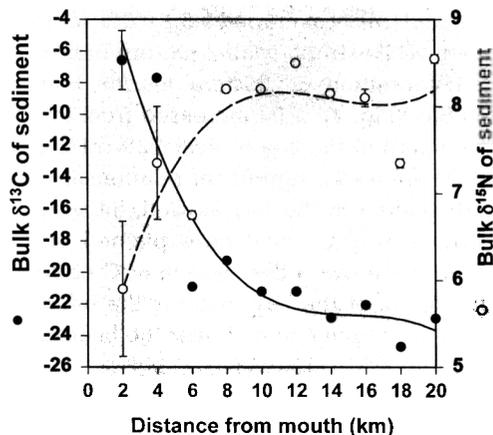


Fig. 5. Bulk stable isotopic composition of sediments from the center of Tomales Bay. Error bars represent the SE from replicate analyses of 1 core per station. The line through each data set is a third-order polynomial least squares regression to indicate the trend in the data

and 4 probably do not reflect the isotopic signature of organic carbon from those sites. The difference in organic matter content of the sediment samples was also evident in the bulk N content of the sediment: from 0 to 8 km, N content was between 0.01 and 0.07%; from 10 to 20 km N content was 0.20 to 0.26% of dry weight. Stable isotopic composition of nitrogen

was relatively low near the mouth of the bay where $\delta^{15}\text{N}$ was ca 6‰ (Fig. 5). Between 8 and 20 km from the mouth of the bay there was little change in the $\delta^{15}\text{N}$ (7.4 to 8.6‰). Pasture grasses and leaves from trees along the streambanks in the watershed had $\delta^{15}\text{N}$ values of -2.4 to 3.8‰. Cow feces collected from pastures adjacent to Tomales Bay had a $\delta^{15}\text{N}$ of 3.0‰.

Temporal variation

Seagrass C, N and P content

Nitrogen content (C:N) of *Zostera marina* leaves varied both among stations and seasons, with a significant Station \times Season interaction (Table 2). During the summer upwelling season (May to October), C:N increased from the mouth of the bay towards the head of the bay, but during the winter rainy season, C:N showed no differences among stations (Fig. 6). The stations from outer Tomales Bay (11 and 17) showed no seasonality in C:N, compared to a large seasonal difference for the stations from the inner bay (26 and 28). Seasonal variation at Stn 28 was especially large, with a low of 17.8 ± 2.2 in the winter rainy season and a high of 31.4 ± 2.9 in the summer upwelling season.

Phosphorus content (C:P) of *Zostera marina* varied significantly between stations, but there was no overall

seasonal effect on C:P averaged across all of the stations (Table 2). During the summer upwelling season, there was no spatial trend in C:P, in contrast to the marked decrease on C:P with distance into the bay during the winter rainy season (Fig. 6). Averaged across stations, there was no significant seasonal pattern (Table 2), however, since season affected the C:P in station-specific ways (Station \times Season interaction in C:P; Table 2). At Stns 11 and 17, in outer Tomales Bay, C:P was higher during winter than in summer; conversely, C:P was lower in winter at Stns 26 and 28 in inner Tomales Bay (Fig. 6). The relative availability of N and P was different across stations, with higher N:P near the mouth of the bay and lower N:P towards the head of the bay in both summer and winter (Fig. 6, Table 2), indicating that during both the summer upwelling season and the winter rainy season, N is more available (relative to P) in outer than in inner Tomales Bay.

Table 2. *Zostera marina*. ANOVA table for analysis of station and season differences in elemental content and stable isotope content of eelgrass leaves collected from Tomales Bay, August 1992 to July 1994. Bold type indicates statistically significant ($p \leq 0.05$) sources of variation

Elemental measurement	Source of variance	df	MS	F	p
C:N	Station	3	170	10.56	<0.001
	Season	1	150	9.28	<0.01
	Station \times Season	3	113	6.99	<0.001
	Error	32	16		
C:P	Station	3	20878	3.27	0.03
	Season	1	563	0.09	0.77
	Station \times Season	3	20960	3.28	0.03
	Error	32	6384		
N:P	Station	3	229	17.91	<0.001
	Season	1	23	1.89	0.18
	Station \times Season	3	16	1.25	0.31
	Error	32	13		
$\delta^{15}\text{N}$	Station	3	26.3	35.43	<0.001
	Season	1	19.2	25.83	<0.001
	Station \times Season	3	1.4	1.90	0.15
	Error	32	0.7		
$\delta^{13}\text{C}$	Station	3	7.2	12.22	<0.001
	Season	1	0.2	0.313	0.58
	Station \times Season	3	0.5	0.920	0.45
	Error	32	0.6		

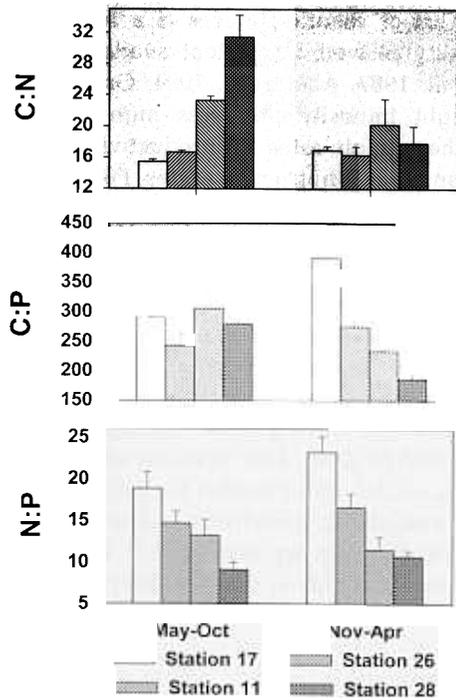


Fig. 6. *Zostera marina*. Seasonal patterns elemental content of green leaves in Tomales Bay. Bars are the mean for all values from a station collected during the indicated season ($n = 5$), error bars are 1 SE. Statistical significance of station and season differences are given in Table 2

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of seagrass leaves

Seagrasses from the outer Tomales Bay stations (11 and 17) had significantly lighter $\delta^{15}\text{N}$ values than seagrasses from the inner bay stations throughout the year (Table 2, Fig. 7). There was a relatively large seasonal pattern in $\delta^{15}\text{N}$ in the outer bay compared to the inner bay. During the summer upwelling season, $\delta^{15}\text{N}$ was between 7 and 8‰ in the outer bay, but during the winter rainy season the seagrass stable N was heavier, between 9 and 10‰. Nitrogen in inner bay seagrasses was isotopically heavier than the N in outer bay seagrasses during both seasons (Fig. 7); summer values were ca 1‰ lighter than winter values. There was no seasonal pattern in $\delta^{13}\text{C}$ in seagrass leaves from Tomales Bay (Table 2, Fig. 7), but there were significant differences in the C stable isotope composition among stations.

DISCUSSION

The nutrient content, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of seagrasses in Tomales Bay vary in both space and time. The variations in these measurements are not random, but follow spatial and seasonal patterns that provide insight

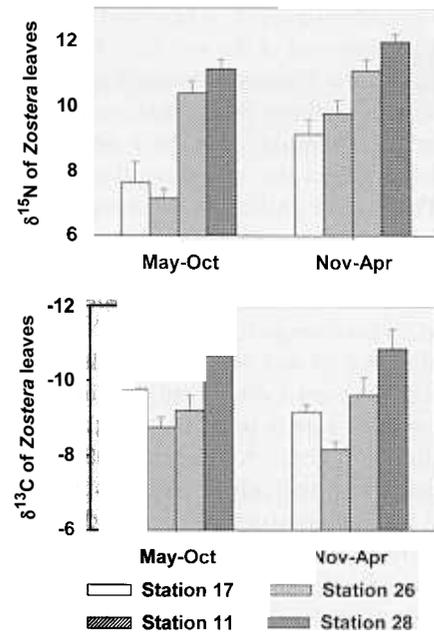


Fig. 7. *Zostera marina*. Seasonal patterns in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of green leaves in Tomales Bay. Bars are the mean for all values from a station collected during the indicated season ($n = 5$), error bars are 1 SE. Statistical significance of station and season differences are given in Table 2

into the sources and processing of nutrients in the Tomales Bay ecosystem. These patterns support the model of elemental cycling that has been developed by S. V. Smith, J. T. Hollibaugh, and coworkers during intensive monitoring and experimentation in Tomales Bay as part of the Land Margin Ecosystem Research project (see Smith et al. 1987, 1989, 1991, 1996, Hollibaugh et al. 1988, 1991). This result suggests the potential for using the distribution of seagrass composition for generating hypotheses about the ecosystem-scale patterns in elemental cycling for other seagrass-dominated ecosystems.

The elemental content of *Zostera marina* leaves in Tomales Bay was quite variable (Table 1), but was within the range of values reported in the literature. Literature values of carbon content of *Z. marina* collected from North America and Europe range from 29 to 46% of dry weight, with a median of 38.5%; nitrogen content ranges from 0.7 to 6.3% with a median of 2.7%; and phosphorus content ranges from 0.15 to 0.63% with a median of 0.20% (Atkinson & Smith 1983, Pedersen & Borum 1992, Abal et al. 1994, Nieuwenhuize et al. 1994, van Lent & Vershuure 1994, Enriquez et al. 1995). The N content of Tomales Bay *Z. marina* (Table 1) was nearly as variable as *Z. marina* collected from the 9 other locations encompassed by these literature values, as a result of the strong seasonality and spatial variability of relative N availability in

Tomales Bay. The strong C:N gradient in Tomales Bay (Fig. 3) is reminiscent of the strong C:P gradient of the tropical seagrass *Thalassia testudinum* from Florida Bay, USA, where there is as much variation in the P content of *T. testudinum* as in the P content of all seagrasses reported in the literature (Fourqurean et al. 1992b). The spatial pattern in P content of seagrasses in Florida Bay was caused by the spatial variability in P availability across that system; similarly the pattern in N content of *Z. marina* from Tomales Bay can be explained by relative availability of N.

The influence of nutrient availability on seagrass growth will be most evident only during times when seagrass growth rate is potentially high enough to be nutrient-limited (Thom & Albright 1990, Pedersen & Borum 1992). In a temperate estuary such as Tomales Bay, light and temperature maxima lead to the greatest potential growth rates in the summer; hence we would expect to see the effects of growth limitation in the summer. During August 1992, near the time of maximum potential seagrass growth rate, there was a strong gradient in the nitrogen content, $\delta^{15}\text{N}$ content and $\delta^{13}\text{C}$ content of seagrasses along the main axis of the bay.

The spatial trend in nitrogen content of seagrass leaves observed during summer 1992 suggested that N availability for *Zostera marina* was high near the mouth of the bay and decreased towards the head of the bay (Fig. 3). In contrast, there was no trend in the phosphorus content of seagrasses (Fig. 3), suggesting that P was in adequate supply across the entire ecosystem. The oceanic source of N and the loss of fixed N from the system via denitrification act to create a gradient in N availability for seagrasses, from relatively available near the oceanic source to relatively scarce far from the source. During summer in Tomales Bay, the loss of fixed N from the system leads to a gradient in the water column concentration of DIN as well as seagrass elemental content, with relatively high DIN near the mouth of the bay and low DIN in the inner bay (Fig. 2). During winter, DIN in the water column is relatively high and constant throughout Tomales Bay; correspondingly the C:N ratio of *Z. marina* leaves shows no significant spatial variation in winter (Fig. 6). Two hypotheses are possible to explain this similarity in the annual patterns of water column DIN and seagrass C:N ratio. Seagrasses and DIN may both be responding to the same factors that control N availability in the ecosystem; or water column DIN may be a significant source of N for *Z. marina* in Tomales Bay. The fact that the $\delta^{15}\text{N}$ of the sediments of inner Tomales Bay (Fig. 5) are not as isotopically heavy as the seagrasses from the inner bay (Fig. 4) may be evidence of the importance of the water column as an N source for seagrasses in this system; unfortunately we have no measurements of the $\delta^{15}\text{N}$ of DIN from Tomales Bay.

The C:N of seagrass leaves is a function of light availability as well as nutrient availability (Zimmerman et al. 1987, Abal et al. 1994, Grice et al. 1996). High light intensity promotes high photosynthetic rates; these high rates of productivity can lead to depletion of local nutrient supplies. For equal nutrient supply rates, a high light environment will have seagrasses with higher C:N than a low light environment (Abal et al. 1994, Grice et al. 1996). We do not believe that differential light availability in Tomales Bay was controlling the spatial pattern of C:N observed in August 1992 (Fig. 3), however. The water near the mouth of Tomales Bay is generally much clearer than at the head of the bay (pers. obs., J. T. Hollibaugh unpubl. Secchi data), and all of our samples were collected in similar water depths (ca 1.5 m). This pattern in light availability would lead to the opposite spatial pattern to the one we observed if light availability were controlling C:N of seagrass leaves from Tomales Bay.

Variability in the content of nutrients in seagrass tissue is potentially a superior indicator of nutrient availability when compared to measures of nutrient standing stocks in the water column and sediments. Concentrations of nutrients in the water column are imperfect indicators of the availability of nutrients to the primary producers because the rate of nutrient supply (i.e. recycling) can be a more important control over availability than standing stock (Howarth 1988). Seagrasses continuously respond to nutrient concentrations in their environment and integrate over short-term variation in nutrient availability; the nutrient content of seagrass tissue is a reflection of the availability of nutrients in the environment over the time scale of the life of the standing biomass (Gerloff & Krombholz 1966, Atkinson & Smith 1983, Fourqurean et al. 1992a, b). While the nutrient content of seagrass leaves decreases as the leaves age due to loss of N from the leaves (Pirc 1985, Pedersen & Borum 1992), the total N content of a whole short shoot is dependent on relative nutrient availability.

The seasonal patterns in elemental and stable isotopic composition of leaves of *Zostera marina* (Figs. 5 & 6) demonstrate the importance of understanding the seasonal variation in relative nutrient supply if spatial variation in nutrient content of seagrasses is to be used as an indicator of nutrient availability across other ecosystems. The striking spatial gradient in N content of *Z. marina* in August 1992 (Fig. 3) was not present during the winter rainy season (Fig. 6). During winter, there was very little variation in N content among the 4 sites that were repeatedly sampled. The seasonal pattern in N content differed between sites in the outer section of Tomales Bay (Stns 11 and 17) versus those from inner Tomales Bay (Stns 26 and 28). The C:N ratio

for the outer bay plants remained relatively constant throughout the year, but there was a summer maximum for the plants from inner bay stations (Fig. 6, and the Station \times Season interaction; Table 2). This arises because of high and relatively constant N availability near the mouth of Tomales Bay, adjacent to the oceanic N source (Smith et al. 1991; Fig. 2). In contrast, inner Tomales Bay experiences limited exchange with the ocean during the summer months, as well as almost no terrestrial runoff (Smith et al. 1991). The combined effect of isolation from an N source, the loss of DIN through denitrification, and rapid uptake of N by primary producers in the summer is a minimum in N availability during summer in the inner parts of Tomales Bay (Fig. 2). This minimum in availability and maximum in nutrient demand by the seagrasses during the summer lead to the low N content of *Z. marina* in inner Tomales Bay in the summer (Fig. 6).

The nitrogen content of seagrasses from Tomales Bay not only indicates nitrogen limitation of primary production in Tomales Bay but it also alludes to the mechanism driving this nitrogen limitation. Net denitrification in the Tomales Bay ecosystem is a major controlling factor of N availability of Tomales Bay and leads to a deficit of DIN for primary production (Smith et al. 1989). This deficit was recorded in the N content of *Zostera marina* in inner Tomales Bay (Fig. 4), as well as by the water column concentrations of DIN during summer (Fig. 2). The gradient in the $\delta^{15}\text{N}$ content of *Z. marina* (Fig. 4) is likely also a result of denitrification. The DIN pool in an ecosystem can become enriched in ^{15}N due to fractionation during denitrification (Cline & Kaplan 1975, Horrigan et al. 1990). Given that the N source for primary production in Tomales Bay is primarily water brought into Tomales Bay by tidal exchange with the Pacific Ocean (Smith et al. 1987, 1989, 1991), progressive denitrification of the DIN from the Pacific Ocean should lead to a gradient of increasing $\delta^{15}\text{N}$ of the DIN in Tomales Bay. Mass balance considerations (Smith et al. 1987, 1989) make it unlikely that plant uptake of nitrate (Horrigan et al. 1990) is the cause of the summer ^{15}N gradient that we hypothesize to exist in the DIN pool. We did not directly measure the isotopic composition of the DIN pool of Tomales Bay; collection, concentration and separation of the components of the DIN pool for stable isotopic measurements is a difficult process (exacerbated by the low concentrations of DIN in Tomales Bay).

Another possible explanation for the gradient in isotopic composition of the seagrass from Tomales Bay is difference in the isotopic composition of N entering the system from the watershed versus the ocean. Unfortunately, we have no data on the isotopic composition of the inorganic N loading from the watershed, but the importance of terrestrially derived DIN in the N pool of

Tomales Bay is not great. A small percentage of the total N loading to Tomales Bay comes from stream flow (Smith et al. 1987, 1991, 1996), and groundwater inputs of N are only 20% as large as stream flow (Oberdorfer et al. 1990). If the pattern in seagrass $\delta^{15}\text{N}$ is a result of a gradient in $\delta^{15}\text{N}$ of the N pool, then extremely heavy N entering Tomales Bay in stream flow from Lagunitas Creek near the head of the bay could generate such a pattern. We know of no reasons to expect isotopically heavy N in Lagunitas Creek flow. The watershed drained by Lagunitas Creek is sparsely populated and rural; there are no obvious potential sources of heavy N in the watershed, save for the small sewage treatment plant in the town of Tomales. Plants from the flood plains of Lagunitas Creek had $\delta^{15}\text{N}$ values of -2.4 to 3.8‰ , much lower than the 12‰ values for seagrasses at the head of the bay. Owing to the isotopic fractionation of N that occurs with each step in a food chain (see Peterson & Fry 1987) and the prevalence of dairy farming in the watershed of Tomales Bay, we measured the $\delta^{15}\text{N}$ of cow feces from the watershed. Feces had a $\delta^{15}\text{N}$ of 3.0‰ ; clearly organic N flowing into Tomales Bay from the watershed did not cause the gradient in $\delta^{15}\text{N}$ of the seagrasses in the bay.

Low organic carbon content of the sediments collected from the outermost stations (2 and 4 km from the mouth of the bay) rendered the bulk $\delta^{13}\text{C}$ for sediments (Fig. 5) uninformative about the isotopic composition of the organic matter in the sediments. Plant (1995) found that sediment organic matter had a $\delta^{13}\text{C}$ of ca -23‰ 2 to 4 km from the mouth of Tomales Bay. Further than 4 km from the mouth there was ample organic carbon in the sediments to yield reliable measurements of organic carbon $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ composition from the sediments from inner Tomales Bay show a trend of decreasing $\delta^{13}\text{C}$ from seaward to landward, with an average $\delta^{13}\text{C}$ of -21.9‰ . This mean value is reflective of the fact that phytoplankton are the most important producers of organic carbon in the system (Smith et al. 1987, 1989). Decreases towards the head of the bay probably arise from the increasing importance of terrestrially derived organic matter (Plant 1995). Carbon isotope ratios of *Zostera marina* leaves also decreased from the mouth of the bay towards the head of the bay (Figs. 4 & 7); this decrease was at the rate of 0.1‰ km^{-1} . This decrease may be a result of the increased dependence of the seagrasses on respiratory CO_2 from the remineralization of organic matter from phytoplankton and terrestrial sources, similar to the finding of Lin et al. (1991) that seagrass $\delta^{13}\text{C}$ in south Florida was in part determined by the availability of CO_2 produced by the respiration of mangrove organic matter.

Seagrasses are good monitors of environmental conditions. The marked spatial and seasonal patterns in elemental and stable isotopic content of *Zostera*

marina in Tomales Bay provide a wealth of information on the environmental conditions of Tomales Bay. Spatial and temporal variation in ecosystem processes of Tomales Bay is well understood as a result of the major, multiyear, multidisciplinary investigation of the system (Smith et al. 1987, 1989, 1991, 1996, Hollibaugh et al. 1988, 1991); consequently, we were able to interpret the major patterns in variation of N content and isotopic composition of *Z. marina* in both space and time for this system as a function of nutrient availability and nutrient source. We have demonstrated the usefulness of analysis of patterns in variation of elemental content and isotopic composition of seagrasses for investigating ecosystem-scale processes in coastal marine systems. Similar analyses of seagrasses from less well studied ecosystems should provide data to generate hypotheses about spatial and temporal variation in processes in these other systems.

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