Chemical Indicators of Anthropogenic Nitrogen Loading in Four Pacific Estuaries[1]

Brian Fry,[2,3] *Arian Gace*,[2] *and James W. McClelland*[4]

Abstract: Watershed inputs of anthropogenic nitrogen (N) are altering the trophic status of estuaries worldwide. In this study we compared two chemical approaches for assessing watershed N inputs to estuaries: (1) use of conventional nutrient concentration measurements, and (2) use of nitrogen isotope ($\delta^{15}N$) measurements in estuarine sediments and biota. Of special interest was testing whether $\delta^{15}N$ assays were generally robust tracers of watershed N across different estuarine systems. Four Pacific estuaries were chosen for study at widely spaced intervals on the U.S. West Coast: Padilla Bay (northern Washington State), South Slough (southern Oregon), Elkhorn Slough (central California), and Tijuana River (southern California). These estuaries are part of the National Estuarine Research Reserve (NERR) system. They are relatively small and shallow, are well flushed by tides, and can receive substantial natural N-loading from seasonally upwelled offshore waters. Results showed that none of the estuaries was truly pristine, with high watershed DIN (dissolved inorganic nitrogen) concentrations >500 μM especially in Elkhorn and Tijuana estuaries that respectively received high agricultural and sewage inputs. Nitrogen isotope assays failed to detect N-loading under conditions of very high ammonium inputs from sewage, but were otherwise useful indicators of estuarine N status in all four estuaries. Overall, using a combination of nutrient and isotope measurements was the best strategy for detecting watershed N-loading in these estuaries. The combination approach could be used to generate maps of low, medium, and high inputs to each of the four study estuaries. The N isotope measurements appear to be useful especially for tracing historical development of N-based eutrophication and for showing entry of pollutant N into local food webs.

[1] Funding for this study was provided by the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET) (project 99-296 of the University of New Hampshire) through NOAA Grant No. NA870R0512, and also by NSF DEB-9815598. Manuscript accepted 22 April 2002.

[2] Coastal Ecology Institute and Department of Oceanography and Coastal Studies, Louisiana State University, Baton Rouge, Louisiana 70803.

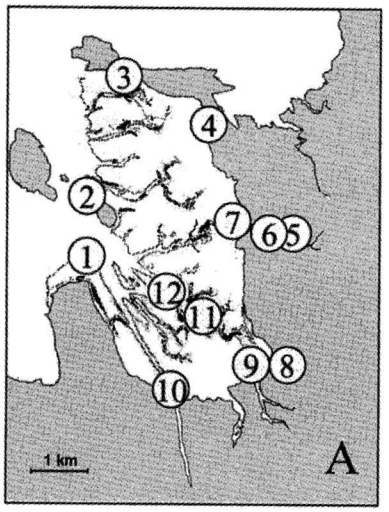
[3] Corresponding author. Current address: Department of Oceanography and Coastal Ecology Institute, Louisiana State University, Baton Rouge, Louisiana 70803 (phone: 225-578-9403; fax: 225-578-6326; E-mail: bfry@lsu.edu).

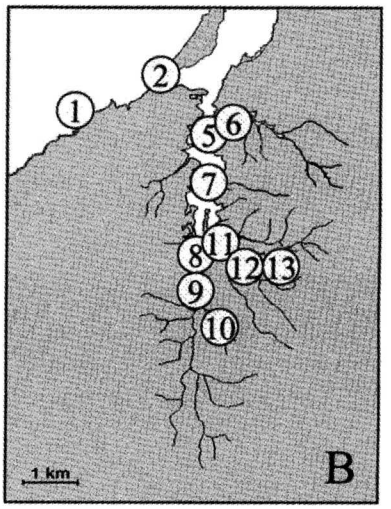
[4] Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 (E-mail: jmclelland@mbl.edu).

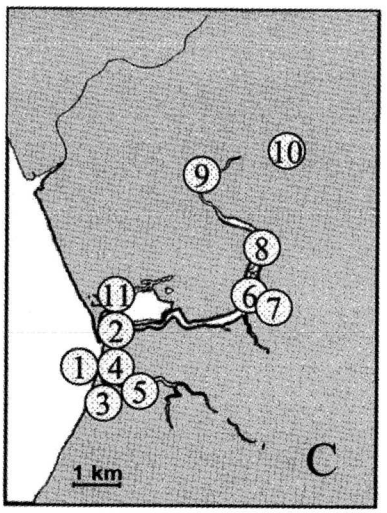
Pacific Science (2003), vol. 57, no. 1:77–101

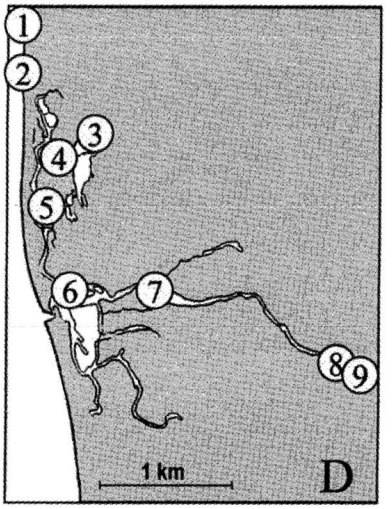
NITROGEN (N) ENRICHMENT of coastal waters is increasing across the globe as a result of anthropogenic activities and is expected to continue with further urbanization of coastal areas. Nitrogen-loading contributes to eutrophication of estuarine communities because primary production is often N-limited (Vitousek et al. 1997, Cloern 2001). A variety of indices has been developed to quantify the extent of eutrophication brought about by N-loading (Schmitt and Osenberg 1995). Most of these indices make use of taxonomic shifts and changes in the abundance of producers and consumers that occur during eutrophication. Chemical measures are also useful in estuarine eutrophication studies. Water quality monitoring of ammonium, nitrate, nitrite, and dissolved organic nitrogen concentrations can help coastal managers detect early changes in N-loading. However,


Padilla Bay (A)
South Slough (B)
Elkhorn Slough (C)
Tijuana Estuary (D)


1 km
A


1 km
B


1 km
C


1 km
D

such monitoring can be costly and sample intensive to obtain broad and representative coverage across whole estuaries because the distribution of nutrients can change strongly in time and space (e.g., Vorosmarty and Loder 1994). A simpler, more integrated chemical approach is needed and may be available via analysis of stable nitrogen isotopes ($\delta^{15}N$) in estuarine sediments and biota. The $\delta^{15}N$ values for these pools integrate many of the water quality fluctuations, making isotope studies complementary to studies based on nutrient concentrations alone (Hobbie et al. 1990).

It is important to understand why the $\delta^{15}N$ approach may be generally useful for estuarine work, and those reasons involve a landscape-level perspective of N cycling. For estuaries receiving anthropogenic N-loading from watersheds, the microbial reactions of nitrification and denitrification are important processes leading to high $\delta^{15}N$ values in the external N load (Mariotti et al. 1981, 1984). Faster processing of the lighter nitrogen isotope (^{14}N) than the heavy nitrogen isotope (^{15}N) results in products enriched in the lighter isotope and leaves residues enriched in the heavy isotope. Thus, nitrification that converts ammonium to nitrite and nitrate leaves residual ammonium enriched in ^{15}N, and denitrification, the conversion of nitrate to nitrogen gas and nitrous oxide, leaves residual nitrate enriched in ^{15}N (Mariotti et al. 1981). Occurrence of these nitrification and denitrification reactions in watershed soils ultimately removes nitrogen as ^{15}N-depleted N_2, leaving residual soil ammonium and nitrate with higher $\delta^{15}N$ values. Watershed processing of N from anthropogenic additions stimulates these soil reactions, with higher system leakiness accompanying the higher N inputs (Hoegberg 1997). Thus, if pristine systems tightly recycle N and have low overall export losses, anthropogenic N-loading increases the amount of N cycling in a system and also increases the fraction of N leaking from the system. According to current understanding, the higher fraction of N lost from watersheds that receive anthropogenic N inputs, and possibly larger fractionations associated with higher N concentrations, is ultimately responsible for increased ^{15}N enrichment versus background conditions (Hoegberg and Johannisson 1993). Following watershed processing of ammonium and nitrate, these ^{15}N-enriched nutrients enter estuaries. Incorporation of ^{15}N-enriched nutrients from watersheds into estuarine algae and food webs leads to general ^{15}N enrichment of organic matter pools in estuaries (Kwak and Zedler 1997, Voss and Struck 1997, McClelland and Valiela 1998, Fry 1999, Costanzo et al. 2001). Similar results are also observed in freshwater systems (Cabana and Rasmussen 1996, Hodell and Schelske 1998, Hebert and Wassenaar 2001, Ogawa et al. 2001). When evaluated with care, the naturally occurring $\delta^{15}N$ distributions can provide valuable information on qualitative and quantitative changes in the nitrogen status of aquatic systems (McClelland et al. 1997).

Complications may arise in this simple model of ^{15}N enrichment. For example, McClelland et al. (1997) showed that ^{15}N enrichment accompanied wastewater inputs from septic fields, but was not strongly associated with lower-level agricultural N inputs from watersheds. Also, Jordan et al. (1997) showed that cold winter temperatures can result in low nitrification rates that limit microbial N processing in wastewater systems and lead to low $\delta^{15}N$ values. Because of these and other complications, and especially that within-estuary nitrification and denitrification can create high $\delta^{15}N$ signals in ammonium and nitrate (Cifuentes et al. 1989, Horrigan et al. 1990), it is not clear that the ^{15}N assay technique is a robust technique for detecting watershed N-loading to estuaries (Waldron et al. 2001). To examine the overall generality of the $\delta^{15}N$ approach, we compared isotope and nutrient-based approaches for estimating N-loading across four U.S. West Coast estuaries that are rich in nitrogen relative to previously studied estuaries.

FIGURE 1. Location of the four National Estuarine Research Reserve (NERR) sites along the U.S. West Coast (*top*) and station locations at Padilla Bay, Washington (A); South Slough, Oregon (B); Elkhorn Slough, California (C); and Tijuana Estuary, California (D). North is toward the top in each panel.

The four study estuaries (Figure 1; see http://inlet.geol.sc.edu/nerrsite.html for general descriptions of the estuaries) are all relatively small and shallow (<10 m maximum depth) and experience large daily tides >1 m amplitude. Consequently, water residence time is short in these estuaries, usually <1–2 days, with longer flushing times during some neap tides. With the strong marine flushing, watershed N-loading is rapidly diluted, and marine N can play a strong role, especially where upwelling brings higher nutrient concentrations to coastal waters. Attached green macroalgae often form a visible green "bathtub ring" in these estuaries, especially in backwater areas where flushing is lower and watershed N-loading high. We expected to see relatively weak N-loading indices in sample types such as dissolved nutrients or particulate organic material that are rapidly flushed and replaced with marine materials, but stronger N-loading indicators in samples of attached filter feeders and macroalgae that are sessile and can accumulate watershed N. For these reasons, we used a variety of sample types in studies of N-loading, including dissolved nutrients and estuarine sediments and biota. We expected strongest N-loading in the two southern estuaries, Elkhorn Slough and Tijuana, where local agricultural and municipal (sewage + urban runoff) inputs are respectively known to be strong. Lesser N-loading was expected for the two northern estuaries (Padilla Bay and South Slough), with Padilla Bay perhaps receiving more nutrients from the Frazer River and local agriculture, and South Slough representing the most pristine site with a generally forested watershed.

MATERIALS AND METHODS

Sampling and Sample Preparation

Water samples and organisms were collected in each estuary at 9–13 stations during October 1998, January 1999, April 1999, and July–August 1999. Estuarine sampling stations were chosen along potential N-loading gradients (Figure 1). Samples were collected for nutrients and $\delta^{15}N$ of sediments, water column particulates, attached macroalgae, and filter-feeding barnacles and clams. Samples were collected by National Estuarine Research Reserve (NERR) personnel, frozen, and then shipped to Louisiana State University (LSU) for analyses. Voucher specimens were also collected and later identified (see Appendix A).

Water samples for nutrient analysis were collected in 4-ml vials and analyzed for NH_4^+, $NO_3^- + NO_2^-$, SiO_3^{2-}, and PO_4^{3-} using a Technicon Autoanalyser at the Coastal Ecology Institute, LSU. Results for representative quality control samples with nutrient concentrations of 13.9, 18.0, and 3.1 μM ammonium, nitrate + nitrite, and phosphate were measured, respectively, at 13.9, 18.6, and 3.4 μM. Precision (C.V.) for the nutrient analyses was typically <5%.

Larger water samples were also collected using 1-liter plastic bottles, transported to NERR laboratories, and frozen. At LSU, samples were thawed and salinity measured with a conductivity meter (YSI 63). Suspended particulate matter was collected by vacuum filtration through precombusted (450°, 4 hr) glass filters (Whatman GF/F). Filtered water was saved for isotope nutrient assays, and $\delta^{15}N$ of ammonium (hereafter $^{15}NH_4^+$) and $\delta^{15}N$ of nitrate + nitrite (hereafter $^{15}NO_3^-$) samples were prepared from filtered samples using an adaptation of the ammonium diffusion method (Sigman at al. 1997, Holmes et al. 1998).

Surface sediments were collected from intertidal and channel locations, with three replicate grabs composited for each sample. Sediments were dried at 60°C in a convection oven. After drying, sediment samples were ground to a fine powder using an amalgamator (Crescent Wig-L-Bug). Powdered sediments were analyzed whole for % N and $\delta^{15}N$.

Common macroalgae attached to shells and pilings were collected at each site, with 3–10 individuals composited for the station average sample. Samples were dried at 60°C in a convection oven. After drying samples were ground to a fine powder using an amalgamator (Crescent Wig-L-Bug).

Common filter feeders, usually barnacles, but occasionally some mussels, were collected

from pilings or channel markers, with 3–10 individuals composited per station. Organic tissues were dissected from specimens, dried at 60°C, and powdered for analysis.

Total organic carbon (TOC), total organic nitrogen (TON), C:N ratio, and $\delta^{15}N$ of the samples were analyzed using an automated analytical system combining an isotope ratio mass spectrometer (ThermoQuest Finnigan Delta Plus) and an elemental analyzer (Carlo Erba NA-1500). All isotopic abundances are given as:

$$\delta^{15}N = ((R_{SAMPLE}/R_{STANDARD}) - 1)^{*}1000 \quad (1)$$

where R is $^{15}N/^{14}N$, and the standard for nitrogen is air N_2 with a $\delta^{15}N$ value of 0.0‰. Analysis of replicate samples usually showed agreement of 0.3‰ or better for particulate $\delta^{15}N$ and 1‰ or better agreement for nutrient $\delta^{15}N$. All reported isotope values have been corrected for blank contributions. Samples that are depleted in the heavy isotope (^{15}N) have lower δ values and are "lighter"; samples enriched in heavy isotope have higher δ values and are the ^{15}N "heavier."

Evaluating Nitrogen Inputs

Values for measured water quality parameters were averaged across the four seasons into single values for each site, with data for the individual analyses in each season listed in a thesis (Gace 2001), a report (Fry et al. 2001), and in electronic spreadsheets available from B.F. The averaging brought the time scale of measurements to the same temporal unit (1 yr) and was done because nutrients integrate N dynamics at short time intervals whereas isotopes integrate N dynamics at much longer intervals.

SALINITY-DIN MIXING DIAGRAMS. To evaluate sources of dissolved inorganic nitrogen (DIN) in estuaries, we used conservative mixing diagrams as conceptual guides (Figure 2*A*) (Day et al. 1989). With no net removal or addition of DIN, conservative mixing between marine and freshwater sources typically results in a straight line with a negative slope when DIN is plotted versus salinity. Three hypothetical cases with low (2 μM), intermediate (6 μM), and high (48 μM) freshwater DIN concentrations are considered in Figure 2. The value of the marine end member was

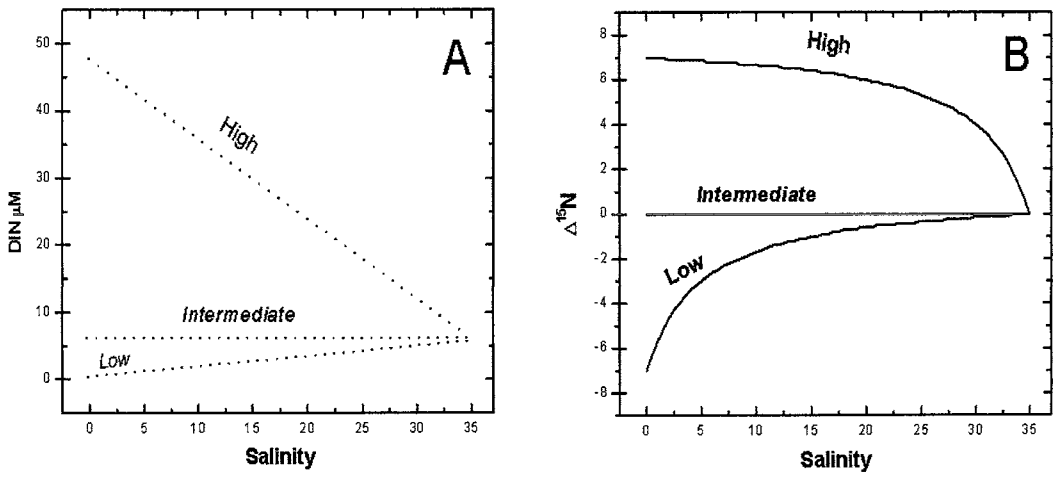


FIGURE 2. Two mixing model approaches for evaluating N-loading in estuaries. *A.* DIN concentrations are linear functions of salinity for conservative mixing of freshwater and seawater end members. The seawater end member has a concentration of 6 μM DIN, and three freshwater concentrations represent low (2 μM), intermediate (6 μM), and high (48 μM) watershed N-loading. *B.* Expected $\Delta^{15}N$ versus salinity in conservative salinity-isotope mixing diagrams. Low, intermediate, and high refer to freshwater DIN-loadings shown in panel *A*, and $\Delta^{15}N$ values are $\delta^{15}N$ values of samples normalized by subtracting the $\delta^{15}N$ values of a reference sample, the marine end member.

set at 6 μM DIN, a value extrapolated from average values measured at the highest salinity, mostly marine stations of this study. These stations included Padilla 1, 2, 11, 12; South Slough 1, 2; Elkhorn Slough 1; and Tijuana 1 and 2. Average salinity and DIN values for these stations were 22 psu and 21.7 μM. We further assumed a DIN ratio of 8:1 for freshwater end member/marine end member values to finally arrive at the extrapolated 6 μM marine DIN value at 35 psu. Higher mixing ratios (e.g., 16:1 instead of 8:1) would result in lower estimates of marine DIN. The extrapolated marine value 6 μM DIN is in the intermediate range of nitrate values (Rau et al. 1998, Pennington and Chavez 2000) measured in Monterey Bay located offshore of one of our sites, Elkhorn Slough.

SALINITY–NITROGEN ISOTOPE MIXING DIAGRAMS. Rather than simple linear mixing, plots of salinity (*x*-variate) versus isotopes (*y*-variate) typically give curvilinear plots for conservative mixing (Figure 2*B*) (Spiker 1980, Fry 2002). Isotopic values of estuarine samples, δ_{MIX}, can be predicted from mixing of riverine and seawater end members by weighting isotopic end member compositions by their respective concentrations:

$$\delta_{MIX}C_{MIX} = (f)\delta_R C_R + (1 - f)\delta_O C_O \quad (2)$$

where subscripts R and O respectively refer to riverine and oceanic end members, and δ and C are isotopic composition and concentration of these end members (Spiker 1980). The fraction f of freshwater in each sample is calculated from salinity,

$$f = (35 - \text{measured salinity})/35 \quad (3)$$

where 35 is taken as the salinity (psu) in the oceanic end member.

We used this salinity-isotope mixing model to investigate possible ^{15}N enrichment that would indicate watershed N-loading. Because ^{15}N enrichment can also occur naturally with increases in trophic level (Fry 1991, Hansson et al. 1997), we normalized data for trophic level before testing for effects of watershed ^{15}N enrichment. We factored out food web effects by normalizing station average $\delta^{15}N$ values through subtraction of marine end member values ($\Delta^{15}N$ normalized = $\delta^{15}N$ measured $- \delta^{15}N$ marine). Filter feeders, green macroalgae, particulate organic nitrogen (PON), and sediments were normalized against respective marine end member $\delta^{15}N$ values of 9.9 ± 0.6‰ (average $\pm$ 95% C.L.), 8.4 ± 0.9‰, 5.8 ± 1.3‰, 8.9 ± 0.8‰, with data from the following high-salinity stations contributing to the marine end member values: Padilla 1, 2, 11, 12; South Slough 1, 2; Elkhorn Slough 1; and Tijuana 1 and 2. When normalizing for sediments, low-N beach sands from stations Padilla 2 and South Slough 1 and 2 were excluded from the averaging as nonrepresentative of marine values (Peters et al. 1978). Using these $\Delta^{15}N$ normalized values, we could plot data for all sample types on the same conservative mixing diagram (Figure 2*B*) to assess overall estuarine ^{15}N enrichment associated with watershed inputs.

Statistics

We used analysis of variance (ANOVA), analysis of covariance (ANCOVA), and Tukey-Kramer *t*-tests (Sokal and Rohlf 1995) in evaluating ^{15}N differences among sites and between bioindicators. To compare nutrient and isotope information, principal component (PC) analysis was performed using eight variables and the correlation matrix approach (Tabachnick and Fidell 2000). Variables were DIN, NH_4^+, NO_3^-, NO_2^- concentrations, and $\delta^{15}N$ of PON ($PO^{15}N$), green macroalgae ($GR^{15}N$), filter feeders ($FF^{15}N$), and sediments ($SED^{15}N$). Because the statistical distribution of the nutrient concentration variables (DIN, NH_4^+, NO_3^-, NO_2^-) was severely skewed, ln-transformations were applied. Isotope values were not ln-transformed. Following ln-transformation (nutrients) or no ln-transformation (isotopes), variables were then standardized by subtraction of the group mean and division of the result by the group standard deviation. Using these inputs, the PCA solution was rotated using VARIMAX (Tabachnick and Fidell 2000) to facilitate the interpretation of the resulting principal components.

To separate more clearly N-loading patterns of DIN versus patterns of $\delta^{15}N$, we also used cluster analysis (Everitt 1993) with DIN variables only or $\delta^{15}N$ variables only. Hierarchical cluster analysis was carried out using the WARD algorithm with DIN variables only (NH_4^+, NO_3^-, NO_2^-) or $\delta^{15}N$ only ($GR^{15}N$, $FF^{15}N$, $PO^{15}N$, $SED^{15}N$), with DIN variables ln-transformed and both ln(DIN) and $\delta^{15}N$ variables standardized as described in the previous paragraph. Analysis was carried out through SAS PRINCOMP and CLUSTER procedures (Tabachnick and Fidell 2000). In the resulting cluster dendrograms, a cutoff distance of 0.2 (semi partial R^2) was used to distinguish "robust" clusters that grouped similar stations into low-impact and high-impact groups (between-estuary clustering results) or low-, intermediate-, and high-impact groups (within-estuary clustering results). (Note: in this study, "impacted" means that high N inputs are occurring and does not necessarily imply that strong biological impacts accompany these high inputs.) In the latter case of clustering stations within single estuaries, cluster groups were assigned to low, intermediate, or high impact based on their average standardized values.

Ranking Systems for N Impact Maps

To generate summary maps of N impacts in each of the four estuaries, we compared our most complete overall evaluation, the PC + cluster results, with a ranking scheme based on a much smaller subset of the data that included only DIN and $\delta^{15}N$ of only one bioindicator, green macroalgae. The smaller data set was of interest because in future studies, measuring DIN and $\delta^{15}N$ of only one bioindicator may be the simplest and most practical approach for evaluating N inputs.

The detailed procedure for the comparison of the two ranking schemes was as follows. To obtain rankings for the PC + cluster results, we assigned scores of 1, 2, and 3 to clusters I, II, and III, respectively indicating low, intermediate, and heavy N-loading impacts. For stations with high ammonium concentrations but low to moderate $\delta^{15}N$ values, we also assigned an overall 3 (highly impacted) ranking.

For rankings based on the smaller data set of just DIN and $\delta^{15}N$ of green macroalgae, we also used an overall 1, 2, 3 scoring system, with 1 representing near-marine conditions, 2 representing enriched versus marine, and 3 representing heavily enriched versus marine. This ranking system was based on average nutrient and isotope values measured at our most marine stations that included Padilla 1, 2, 11, 12; South Slough 1, 2; Elkhorn Slough 1; and Tijuana 1 and 2, with overall average DIN and green macroalgae $\delta^{15}N$ values of 21.7 μM and 8.4‰, respectively. These average values do not reflect strictly marine conditions (average salinity for the stations was 22 psu, not 35 psu), so we refer to these average values as "background" in the following. Stations with DIN values in the ranges 0–1.5:1 background, 1.5–4:1 background, and >4:1 background were assigned respective rankings of 1, 2, and 3. Stations with $\delta^{15}N < +2$‰ versus background, 2–4‰ versus background, and >4‰ versus background were assigned rankings of 1, 2, and 3, respectively. After making the separate DIN and $\delta^{15}N$ rankings, we averaged these values for overall scores, with the caveat that we also gave special consideration to stations where ammonium concentrations averaged >50 μM and assigned those stations overall scores based on DIN alone. This special assignment was made because $\delta^{15}N$ assays of N-loading failed under high ammonium conditions (see Discussion).

RESULTS

All estuaries showed evidence of high N concentrations associated with freshwater or midestuarine inputs, and no estuary was truly pristine (Figure 3). Watershed DIN concentrations were lowest (<5 μM) in streams at the southern end of the forested South Slough watershed and highest in agricultural drainage from Elkhorn Slough and in sewage effluents in Tijuana estuary (Figure 4).

Isotope values of ammonium and nitrate ranged from −0.3 to 19.6‰ (Table 1) and trended toward higher values at high con-

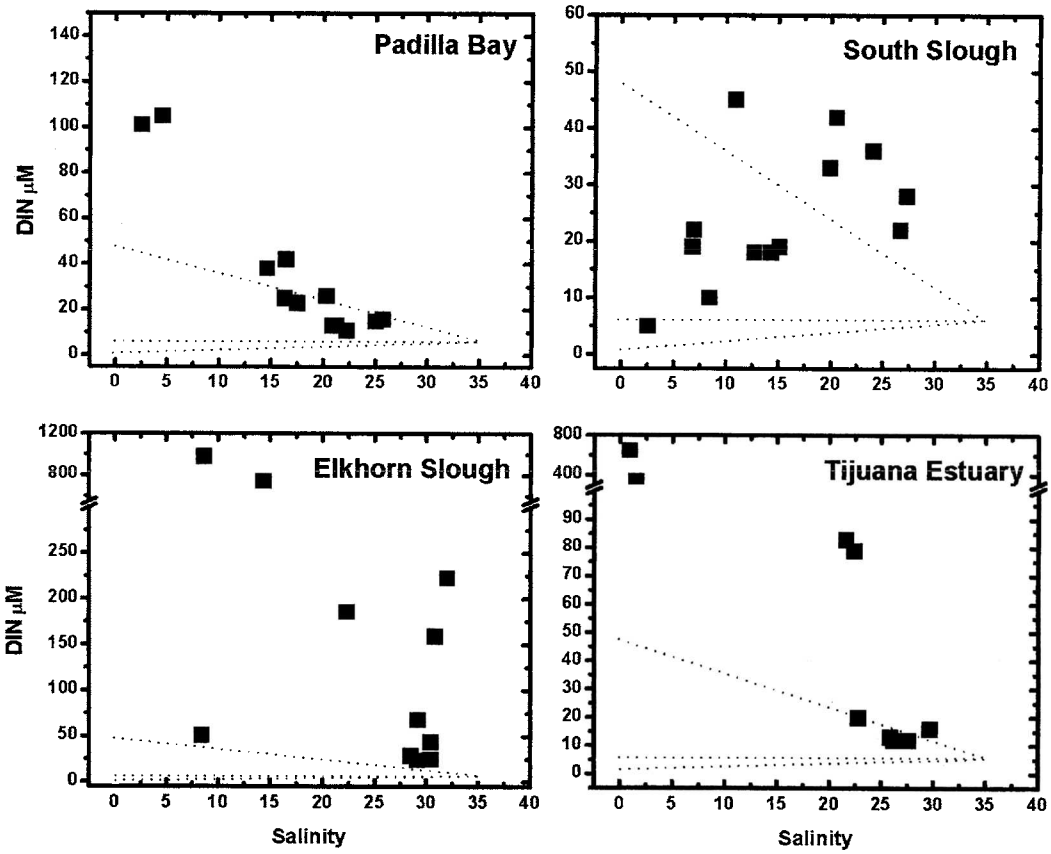


FIGURE 3. Salinity-DIN mixing plots for the four estuaries. Dotted mixing lines from Figure 2 correspond to conservative DIN mixing between marine and three representative freshwater end members.

centrations that would be associated with higher watershed N-loading (Figure 4). On average, ammonium isotope values were 3.4‰ higher than nitrate isotope values, when both ammonium and nitrate were measured from the same station (Figure 5, Table 1). Highest $\delta^{15}N$ values for ammonium and nitrate occurred in the two southern estuaries, Elkhorn Slough and Tijuana (Figure 5).

We studied further uptake of ^{15}N labels in the food web, focusing on four bioindicators. When isotope values in bioindicators were normalized to account for trophic level effects, a one-way ANOVA showed that green macroalgae and filter feeders had very similar normalized values (Table 2). Isotope values for these two bioindicators were enriched in ^{15}N versus PON by 1.3 to 1.4‰ and by 2.6 to 2.7‰ versus sediment N (Table 2), indicating that green macroalgae and filter feeders recorded watershed ^{15}N enrichment patterns more strongly than did PON or sediments. Highest $\Delta^{15}N$ values for bioindicators were found in the two southern estuaries, Elkhorn Slough and Tijuana (Figure 6).

To evaluate differences in N inputs across estuaries in a simple way using the $\delta^{15}N$ approach, we focused on green macroalgae. After accounting for DIN and salinity differences across sites, ANCOVA indicated significant ($P < 0.001$) differences among estuaries for mean $\delta^{15}N$ values for green macroalgae. For this analysis, DIN was logarithmically transformed to improve nor-

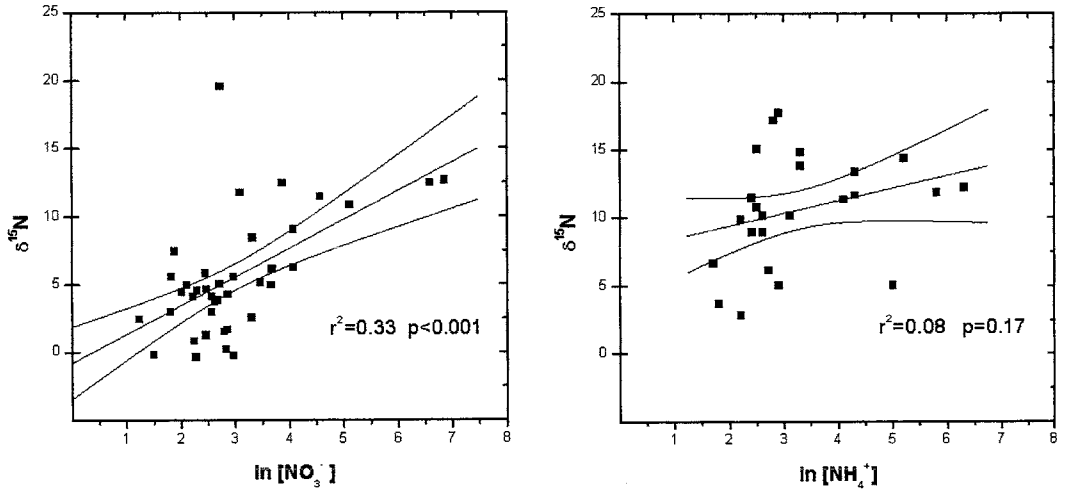


FIGURE 4. $\delta^{15}N$ of NO_3^- (*left panel*) and NH_4^+ (*right panel*) versus their ln-transformed concentrations (data combined across all four estuaries).

mality, whereas salinity was square root transformed before the ANCOVA analyses. Mean $\delta^{15}N$ values for the green macroalgae were 8.3, 8.1, 12.6, and 11.6‰, respectively, for Padilla Bay, South Slough, Elkhorn Slough, and Tijuana Estuary. Tukey-Kramer *t*-tests for differences among mean values showed that values in the two southern estuaries were not significantly different ($P > 0.05$) and similarly that values in the two northern estuaries were not significantly different ($P > 0.05$), but that values in the northern estuaries were significantly ($P < 0.001$) lower than values in the southern estuaries.

We also took more complex approaches in evaluating ^{15}N enrichment patterns across estuaries, considering both isotope and DIN data. We found some parallels between ^{15}N enrichment (Figure 6) and DIN enrichment (Figure 3). Especially, the same overall rankings emerged from the $\delta^{15}N$ data as from the DIN data. South Slough showed the least ^{15}N (DIN) enrichment, Padilla was intermediate, and Elkhorn and Tijuana were highest (compare Figures 3 and 6). However, we also observed low $\delta^{15}N$ values at some high DIN concentrations, especially at Padilla stations 5 and 6 and Tijuana stations 8 and 9 where high ammonium (>50 μM) and low salinities (<6 psu) prevailed (Figure 6, Table 1).

To better understand patterns of DIN and $\delta^{15}N$ across the study sites, we conducted a PC analysis, finding that 77% of the variance in these values could be explained by two principal components (Table 3). PC1 had high loadings for the $\delta^{15}N$ bioindicators (Table 3) and was designated "$\delta^{15}N$"; PC2 was highly correlated with nitrogen concentration indicators (Table 3) and was designated "DIN." A cluster analysis based on these two principal components identified four major station groupings, or four N impact groups (Figure 7). The least impacted stations (group I) had low DIN and $\delta^{15}N$ scores, and most stations from South Slough were in this group. Group II also had low DIN scores, but higher $\delta^{15}N$ values, characteristics expected for stations experiencing the early stages of anthropogenic N-loading (McClelland et al. 1997). Most Padilla Bay stations were in this group, as well as several stations from Tijuana and Elkhorn. Group III stations were high in both $\delta^{15}N$ and DIN, characteristics expected for estuaries with high anthropogenic N-loading, and included stations from Elkhorn and Tijuana estuaries. The final group IV stations had high DIN, but low $\delta^{15}N$ and

TABLE 1

Site Average Values for 45 Stations in the Four Study Estuaries

Station	Salinity	DIN	NO_3^-	NH_4^+	NO_2^-	PO_4^{3-}	SiO_2	C/N_{GR}	$\delta^{15}N$-NO_3	$\delta^{15}N$-NH_4	SED-^{15}N	PON-^{15}N	GR-^{15}N	FF-^{15}N	PC1	PC2	Cluster
P-1	21	13	8	4	0.4	1.1	33	8	4.2	0.0	9.3	6.7	7.9	9.1	0.1	−0.9	2
P-2	16	25	12	12	0.8	1.5	219	9	3.0	10.8	—	4.6	7.2	9.4	−0.4	−0.2	1
P-3	21	13	6	6	0.3	1.2	28	9	4.5	—	9.8	8.0	9.7	11.2	0.7	−1.1	2
P-4	25	15	7	8	0.3	2	44	12	4.6	—	8.8	5.8	9.0	13.6	0.6	−0.9	2
P-5	5	105	31	73	1.2	0.9	252	12	5.2	11.7	3.8	3.6	2.3	—	−2.2	1.2	4
P-6	2	101	38	61	1.6	0.7	311	13	6.2	11.4	6.0	2.7	7.6	10.3	−1.1	1	4
P-7	16	42	18	23	0.9	1	113	12	5.6	10.2	9.8	6.3	8.9	12.6	0.5	0	1
P-8	15	38	26	11	0.6	5.1	47	13	8.5	9.0	6.3	3.9	10.2	—	−0.3	0	1
P-9	17	23	10	12	0.5	1.9	45	11	5.9	—	9.6	5.0	10.0	12.2	0.4	−0.5	2
P-10	20	26	12	14	0.4	1.3	51	9	4.2	9.0	6.6	4.9	7.8	10.7	−0.4	−0.2	1
P-11	26	16	11	5	0.4	1.9	38	9	4.7	—	7.7	4.9	9.8	10.4	0	−0.7	2
P-12	22	11	7	4	0.4	1.3	29	9	5.0	—	8.8	6.8	7.5	10.5	0.2	−1.1	2
S-1	27	22	13	9	0.4	2.1	24	9	3.8	—	3.4	6.7	8.0	10.2	−0.6	−0.3	1
S-2	27	28	13	14	0.5	1.5	23	9	3.9	10.2	4.3	5.4	7.3	8.5	−1	−0.1	1
S-3	11	45	16	28	0.3	0.5	79	11	1.7	—	3.7	0.6	7.6	10.0	−1.6	0.3	1
S-4	15	19	15	4	0.3	1.1	33	9	1.6	—	5.4	4.3	6.9	10.0	−0.7	−0.6	1
S-5	24	36	26	9	0.4	2.3	39	10	2.6	2.9	6.8	4.3	9.5	9.7	−0.4	−0.1	1
S-6	21	42	11	31	0.4	1.6	52	9	1.3	—	6.7	5.2	9.5	10.5	−0.4	0	1
S-7	20	33	8	24	0.3	1.4	50	8	0.9	—	6.0	5.5	9.1	9.7	−0.6	−0.1	1
S-8	7	19	4	14	0.3	0.6	72	14	—	—	7.0	5.1	9.6	11.1	−0.1	−0.6	2
S-9	7	22	16	6	0.3	0.4	61	14	0.3	—	5.2	3.9	10.2	11.2	−0.3	−0.5	1
S-10	3	5	2	3	0.1	0.2	49	17	—	—	3.8	2.4	3.6	—	−1.6	−1.4	1
S-11	13	18	5	12	0.2	0.7	52	9	3.0	—	7.2	3.2	9.1	11.0	−0.4	−0.6	1
S-12	14	18	9	9	0.3	0.9	60	12	−0.3	—	6.0	5.0	7.6	11.3	−0.4	−0.6	1
S-13	8	10	3	6	0.2	0.6	46	15	−0.1	—	4.4	4.2	1.8	—	−1.7	−0.8	1
E-1	30	44	38	6	0.9	1.1	23	9	5.0	6.7	8.4	2.1	9.3	10.9	−0.3	0	1
E-2	29	68	57	9	2.1	1.6	24	10	6.3	—	9.0	5.8	9.3	11.4	0.3	0.3	3
E-3	9	982	947	18	17.4	8.5	149	12	12.7	17.8	9.3	9.7	16.0	14.7	1.8	2.2	4
E-4	14	745	723	12	10.9	5.5	149	10	12.5	15.1	10.0	9.0	13.8	13.6	1.5	1.8	1
E-5	31	159	5	148	5.7	13.7	23	15	7.5	5.1	9.1	9.7	14.0	—	1.1	1	3
E-6	30	25	14	10	0.8	2.3	23	12	5.1	—	8.9	6.5	11.6	12.4	0.7	−0.4	3
E-7	28	29	18	10	0.9	2	29	13	−0.2	—	8.7	6.6	12.7	13.5	1	−0.3	3
E-8	29	24	16	7	1	2.8	22	15	4.3	—	9.8	8.7	12.0	13.3	1.4	−0.6	2
E-9	22	186	162	16	7.6	5.7	67	12	10.9	17.2	11.2	11.5	15.6	15.3	2.4	0.9	2
E-10	8	51	21	26	4	14.4	102	15	11.8	14.9	7.4	9.2	14.2	—	1.1	0.4	2
E-11	32	273	88	174	11.5	15.2	33	12	9.1	14.4	7.9	6.5	10.3	—	−0.1	2.2	3

T-1	28	12	2	9	0.2	0.8	5	17	2.5	9.9	9.3	7.9	9.3	10.7	0.5	−1.1	2
T-2	30	16	1	15	0.2	0.9	13	14	—	6.2	10.0	7.1	10.2	10.1	0.4	−1.1	2
T-3	22	79	47	28	3.8	9.1	119	13	12.5	13.9	9.1	9.3	14.1	13.8	1.4	0.6	3
T-4	26	12	4	6	1.2	4.8	64	14	—	3.7	9.1	7.9	13.3	12.2	1.1	−0.9	2
T-5	23	20	2	18	0.3	3.9	32	14	—	5.1	8.1	6.2	12.9	10.2	0.3	−0.7	2
T-6	26	13	1	12	0.2	1.6	12	15	—	11.5	7.8	5.6	11.9	10.5	0.2	−1	2
T-7	22	83	5	76	1.9	9.8	50	18	5.6	13.4	7.5	4.5	11.8	10.7	−0.3	0.6	1
T-8	2	340	14	323	2.7	38.6	177	—	19.6	11.9	7.6	3.5	—	—	−1.6	1.9	4
T-9	1	649	93	546	10	33.6	182	6	11.5	12.3	9.5	3.4	6.0	—	−1.2	2.8	4

Note: Units: salinity, psu; nutrients, μM; isotopes, ‰; C/N, molar ratio for green macroalgae. FF, filter feeder; GR, green macroalgae; PON, particulate organic nitrogen; SED, sediment. PC1 and PC2 and clusters as in Figure 7.

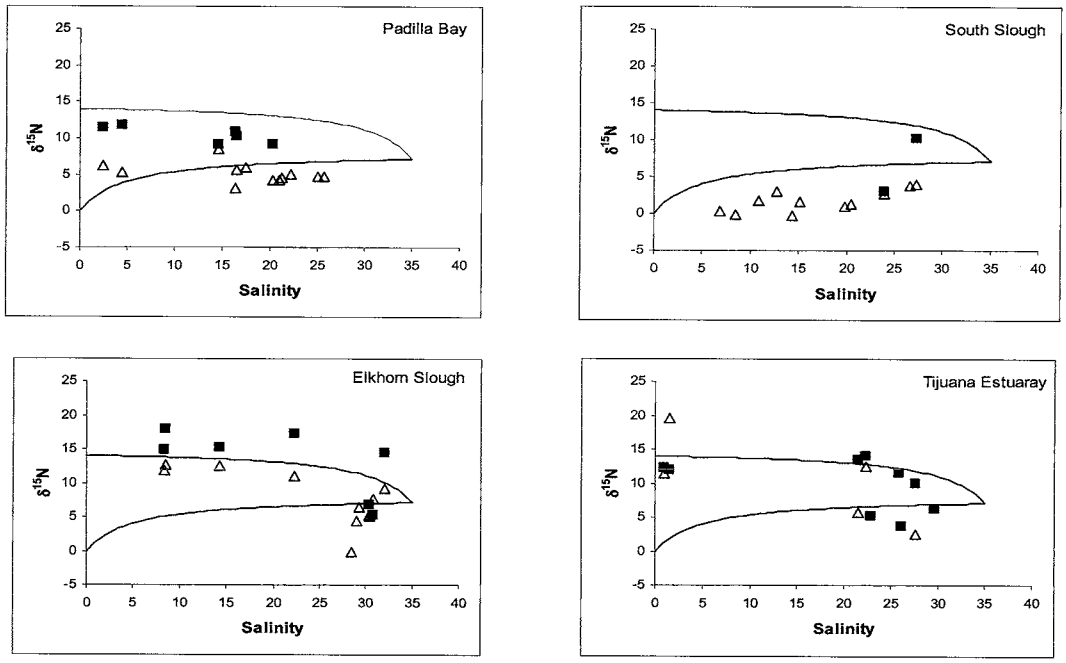


FIGURE 5. Salinity $\delta^{15}N$ mixing plots for ammonium (■) and nitrate (Δ). Mixing lines are based on those of Figure 2B.

TABLE 2

Tukey's Studentized Range (HSD) Test for Normalized $\Delta^{15}N$ between Four Different Bioindicators for All NERR Sites Pooled Together

Indicator Comparison	Difference between Means	Simultaneous 95% Confidence Limits	
FF-GR	0.1	−1.22	1.45
FF-PON	1.4	0.08	2.74***
FF-SED	2.7	1.35	4.03***
GR-PON	1.3	0.04	2.58***
GR-SED	2.6	1.31	3.85***
PON-SED	1.3	0.02	2.54***

Note: ***, significant differences. FF, filter feeder; GR, green macroalgae; PON, particulate organic nitrogen; SED, sediments.

were sites with high ammonium concentrations (>50 μM; Figure 7, Table 1).

In parallel with the overall, combined isotope-DIN analysis shown in Figure 7, we also kept the DIN and isotope variables separate and then performed cluster analyses to directly compare results of DIN versus isotope-based assessments of N-loading. These analyses were performed considering all 45 stations in the four estuaries (Figure 8, left panels) and considering only one estuary at a time (Figure 8, right panels). Results of the cross-estuary comparisons showed 60% agreement between results from DIN clusters versus results from isotope-based clusters (Figure 8, left panels). Results for the individual estuaries showed more prevalent intermediate and high N-loading impacts (higher DIN concentrations and $\delta^{15}N$), especially in South Slough (Figure 8).

Because the statistical results of Figures 7 and 8 indicated that isotopes and nutrients were fairly independent measures of N status, we also considered several schemes for combining nutrient and isotope information to assess overall N status within and across estuaries. Our objective was to produce a summary assessment of N inputs for each of the 45 stations in the four study estuaries. For example, we assigned different weightings to the DIN and $\delta^{15}N$ impact groups of Figure 9, averaged the scores, and plotted the values on

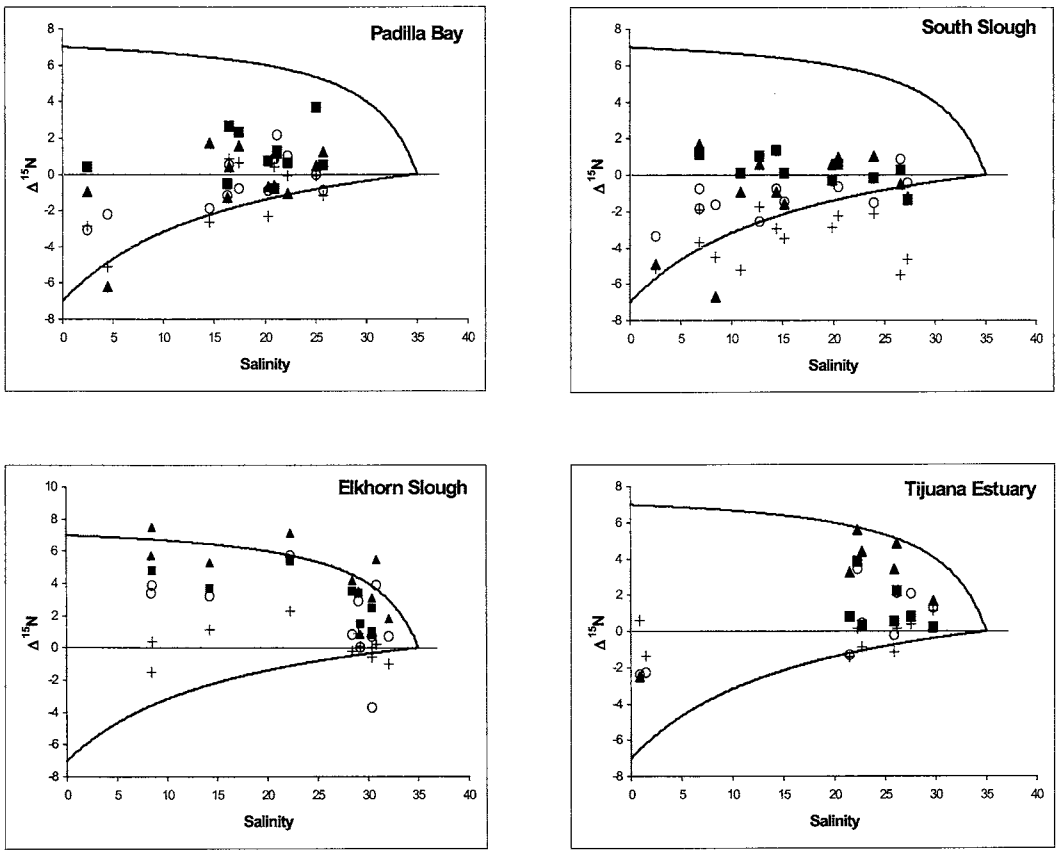


FIGURE 6. Salinity-$\Delta^{15}N$ mixing plots for filter feeders (■), green macroalgae (▲), PON (○), and sediments (+) in the four West Coast estuaries. Mixing lines correspond to conservative mixing for low, intermediate, and high watershed N-loading (see Figure 2).

site maps (Fry et al. 2001, Gace 2001). Different ways of weighting the DIN and isotope information produced similar results for these mapping exercises. Comparisons of ranking schemes based on larger versus smaller data sets (Figure 7 results versus DIN + $\delta^{15}N$ of green macroalgae, respectively, as detailed in Materials and Methods) showed little difference, with an average difference ($\pm$95% C.L.) in pairs of overall scores for the 45 stations of 0.13 $\pm$ 0.17, a result indicating that the two ranking schemes were statistically equivalent. The simpler ranking system, based on averaged rankings for DIN and $\delta^{15}N$ of green macroalgae, produced maps that showed substantial differences in N inputs within and between estuaries (Figure 9). Note that this map actually has four classes of impact stations, the three discussed in Materials and Methods, plus a low-impact "marine" category (see Figure 9 caption for definition of the four categories).

We also examined C/N ratios of bioindicators as possible chemical indicators of N inputs, but generally found that C/N ratios in green macroalgae, PON, and sediments were poorly correlated with DIN concentrations or $\delta^{15}N$ patterns. Only in the least impacted estuary, South Slough, did we observe a consistent relationship, with C/N values of green macroalgae decreasing from values of 15–17 at the low-DIN freshwater end of

TABLE 3

Variance Explained by PC and Loading of Variables on Rotated PC for Eight Variables of Four West Coast Estuaries

PC	Eigenvalue	Difference	Proportion	Cumulative
1	4.13	2.03	0.51	0.51
2	2.09	1.34	0.26	0.77
3	0.74	0.32	0.09	0.87
4	0.41	0.07	0.05	0.92
5	0.34	0.18	0.04	0.96
6	0.15	0.08	0.02	0.98
7	0.07	0.05	0.00	0.99
8	0.02	—	0.00	1.00

Rotated PCA Pattern

Variable	PC 1	PC 2
DIN	0.17	0.97
NO_3^+	0.28	0.76
NH_4^+	−0.18	0.79
NO_2^-	0.40	0.87
FF-^{15}N	0.89	0.10
GR-^{15}N	0.86	0.19
PON-^{15}N	0.86	0.08
SED-^{15}N	0.77	0.11

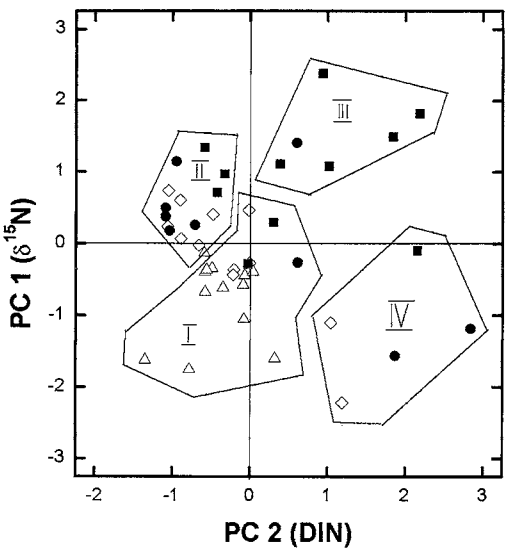


FIGURE 7. PC1 (DIN) versus PC2 (δ^{15}N) scores for 45 individual stations from the four West Coast NERR sites, with the four indicated groups determined from cluster analysis of the PC scores. Padilla Bay, ◇; South Slough, △; Elkhorn Slough, ■; Tijuana Estuary, ●.

the estuary toward C/N values of 8–9 at the marine end of the estuary where DIN levels were higher (Table 1) (Fry et al. 2001).

DISCUSSION

Nutrient Concentrations and Bioindicator δ^{15}N

DIN concentrations in the four study estuaries were almost uniformly high, >10 μM (Table 1), and elevated versus marine end member values. These DIN concentrations indicate substantial N-loading inputs from local watersheds. However, N limitation of estuarine primary production and eutrophication dynamics seemed unlikely because estuarine DIN levels could potentially support substantial levels of phytoplankton biomass. For example, using a Redfield C/N ratio of 6.6 for algal biomass, and a C/chlorophyll-*a* ratio of 42:1 (Valiela 1995), a 10-μM DIN concentration could support algal standing stocks of 23 μg chl-*a*/liter, a value in the usual range for eutrophic estuaries. Very high DIN concentrations (>1000 μM) were observed in some of the study estuaries, especially Elkhorn and Tijuana, and it is probably vigorous tidal flushing with offshore waters that prevents development of persistent algal blooms (not observed except in stagnant portions of these estuaries) and other water quality problems. Valiela et al. (1997*b*) found that strong N-loading in conjunction with strong tidal flushing can result in attached sea grasses and macroalgae dominating primary production in estuaries, because waterborne phytoplankton are flushed by tides from these systems. Occurrence of large visible macroalgal beds and sea grass beds in the study estuaries was observed and is consistent with this type of production regime. N diffusing up from sediments may also contribute to observed abundant macroalgae.

We did not quantify watershed N-loading directly in this study, but high DIN levels were usually associated with higher δ^{15}N values for dissolved nutrients (Figure 4), consistent with watershed sources delivering a high δ^{15}N signal to estuaries. The watershed ^{15}N signal was most evident in two

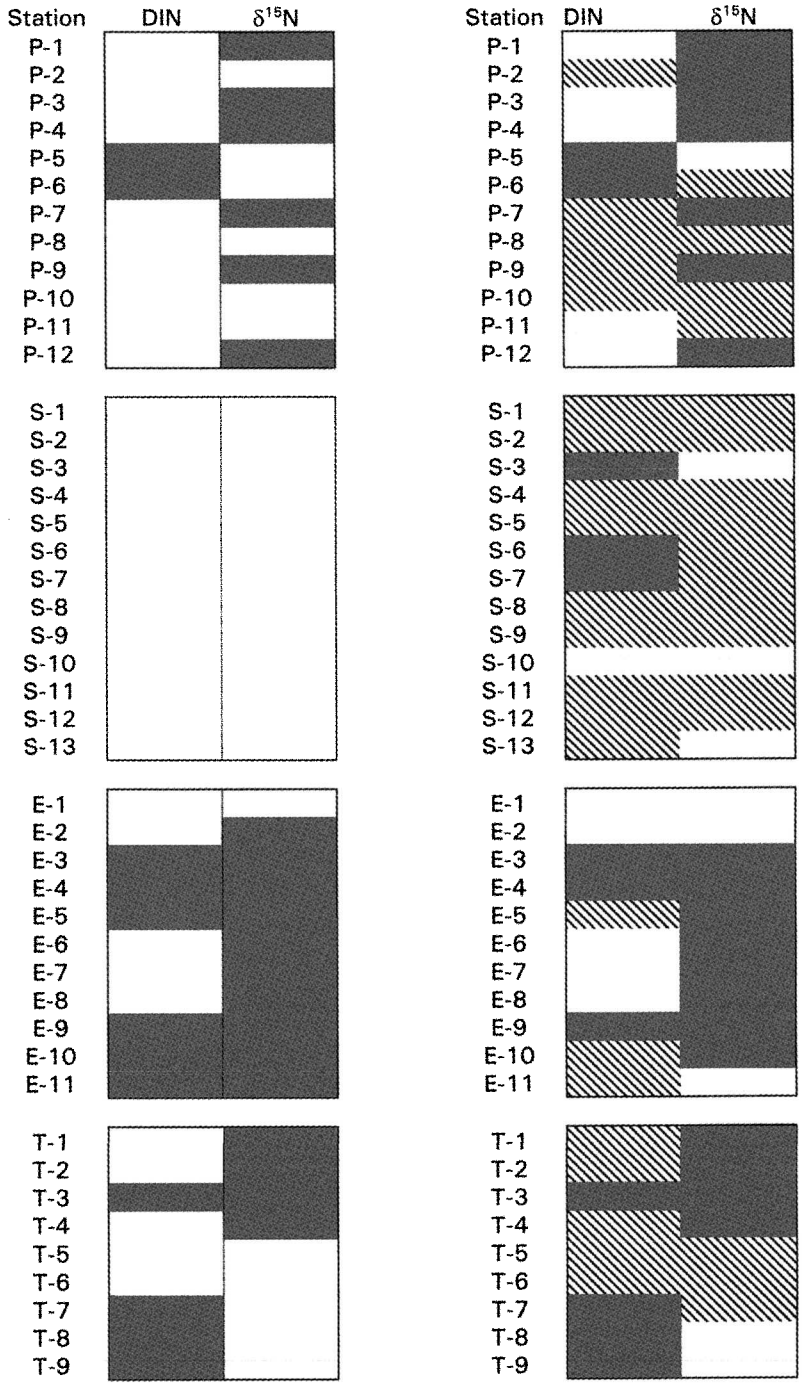


FIGURE 8. N status at NERR sites based on separate DIN and $\delta^{15}N$ cluster analyses. Shading shows overall N status assessment as high impact (heavy shading), intermediate impact (diagonal cross-hatching), and low impact (no shading), with a cutoff distance of 0.2 (semi partial R^2) used in dendrograms (not shown) to separate groups. Left group, cluster analysis for 45 stations pooled together; right group, each estuary was treated separately.

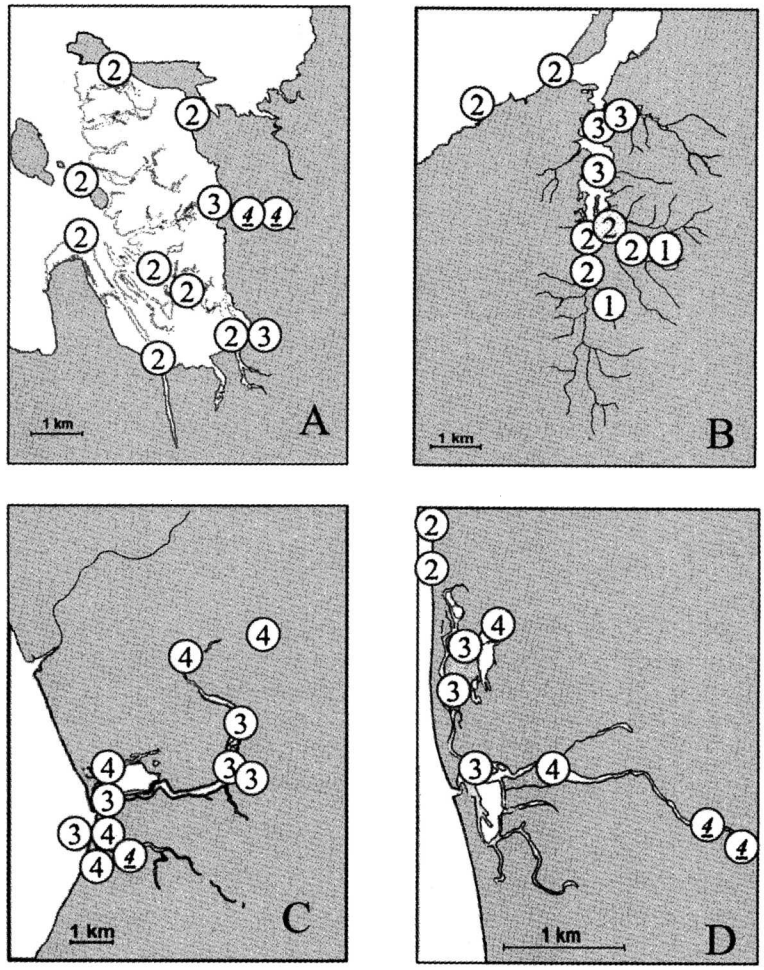


FIGURE 9. Composite index for N-loading at sites in the four study estuaries. Values in site circles range from 1 to 4, with 1 representing low N-loading, 2 representing intermediate loading, and 3 and 4 representing high and very high N-loading, respectively. The index is based on the normalized values versus background values, with background values of 20.7 μM for DIN and 8.4‰ $\delta^{15}N$ for green macroalgae used for normalization. DIN concentrations <0.5:1 background, 0.5–2:1 background, 2–4:1 background, and >4:1 background were given scores of 1, 2, 3, and 4, respectively. Normalized $\delta^{15}N$ values ($\Delta^{15}N$ versus background) that were <−2, −2 to 2, 2 to 4, and >4 were given scores of 1, 2, 3, and 4, respectively. The final score was calculated as the average of the DIN and $\Delta^{15}N$ scores. For stations with >50 μM average ammonium (circles with underlined numbers), only DIN scores were used in averages. We did not use $\delta^{15}N$ data for these stations because the $\delta^{15}N$ assays were not sensitive indicators of N-loading under high ammonium conditions (see Discussion). Note that normal marine sites have values of 2 in this classification scheme.

bioindicators, green macroalgae and filter feeders, but PON and sediments showed relatively little $\delta^{15}N$ enrichment (Table 2, Figure 6) and more strongly recorded marine influences.

Sediments are perhaps the easiest samples to collect for $\delta^{15}N$ analysis. However, the 8.9‰ average value for background marine $\delta^{15}N$ used in this study was relatively high, so that only very strong N-loading in estuaries would be sufficient to produce a noticeable ^{15}N enrichment signal. Thus, although we observed relatively high 9–11‰ $\delta^{15}N$ values for surface sediments, especially in several

Elkhorn Slough stations, these values were only marginally higher than our marine end member value and therefore were not useful for indicating strong N-loading. Investigations of Baltic Sea sediments collected near large rivers showed similar high 8–11‰ values, but those values could be ascribed to riverine N-loading of estuaries, because background, reference sediments from the open Baltic had much lower $\delta^{15}N$ values of 2–4‰ (Voss and Struck 1997, Voss et al. 2000). Other reasons may also contribute to the lack of ^{15}N enrichment in sediments. Smaller river inputs and strong tidal flushing may partially account for the lack of strong $\delta^{15}N$ enrichment in sediments at the NERR study sites. Inputs of terrestrial materials and sewage particulates with low $\delta^{15}N$ values (Peters et al. 1978, Tucker et al. 1999) could also contribute to lack of ^{15}N enrichment in sediments at some of the study sites. Finally, sediments may have a large background of old, low $\delta^{15}N$ nitrogen (Peters et al. 1978) that dilutes new, high $\delta^{15}N$ anthropogenic nitrogen, and sediments may thus represent an organic matter pool that is only slowly labeled in estuaries.

Macroalgae have been shown previously to be good $\delta^{15}N$ bioindicators for tracking entry of watershed N into estuaries (McClelland et al. 1997, McClelland and Valiela 1998, Costanzo et al. 2001). Those studies examined estuarine situations with much lower overall DIN concentrations that are on a par with our cleanest sites (<5 μM, J.W.M. and S. Costanzo, pers. comm.). The higher DIN concentrations at some of our study sampling site locations can bring into play an important confounding factor in relating $\delta^{15}N$ in estuarine biota to watershed N-loading, namely isotope fractionation during DIN uptake by algae at the base of estuarine food webs. Laboratory and field studies have shown that the potential for isotope fractionation during algal DIN uptake increases at higher DIN concentrations (Fogel and Cifuentes 1993, Waser et al. 1998, Altabet 2001), with large fractionations of >20‰ possible, especially when high levels of ammonium (>50 μM) are present (Cifuentes et al. 1989). Large fractionations result in low $\delta^{15}N$ values and thus offset the effect of adding high $\delta^{15}N$ nutrients from watersheds. In such cases, $\delta^{15}N$ assays of estuarine biota are poor indicators of watershed N-loading.

We observed several cases in which high ammonium values were associated with low $\delta^{15}N$ values of bioindicator green macroalgae and filter feeders (stations 5 and 6 in Padilla Bay, station 11 in Elkhorn Slough, and stations 8 and 9 in Tijuana; group IV in Figure 7). Relatively low bioindicator $\delta^{15}N$ values characterized this group of stations, even though the $\delta^{15}N$ values of nutrients were relatively high (Table 1). This situation of high $\delta^{15}N$ values for nutrients, high concentrations of nutrients, and low $\delta^{15}N$ values of estuarine biota is the expected result when isotope fractionation occurs during N uptake by algae. The problem of isotope fractionation by algae partially offsetting watershed $\delta^{15}N$ enrichment signals may be prevalent at our sites that were relatively rich in DIN.

DIN and $\Delta^{15}N$ Mixing Models

Some of our methods for assessing N-loading were based on simple salinity mixing models (Figures 3, 5, and 6). Salinity-based mixing models are sensitive to the selection of end member values at both marine and freshwater ends of estuaries. For example, in DIN salinity mixing models, DIN values lower than 6 μM may be appropriate for marine end members. The choice of 6 μM for marine DIN was an extrapolated value that may need revising in the future using measurements made farther offshore. The isotope mixing models (Figures 5, 6) were also built using several assumptions about end members (for example, that one offshore marine end member is appropriate for all four West Coast sites, and that samples collected at beach sites are representative of open-ocean conditions). Open-coastline beach stations used in this study as marine reference sites may have had some watershed N influence, leading to elevated nutrient and $\delta^{15}N$ values. In that case, estimates of watershed N-loading made in reference to those beach values probably underestimate the true magnitude of the N-loading. Future nutrient and isotope reference samples should be taken at some dis-

tance offshore to ensure no significant terrestrial or watershed influence.

With regard to freshwater end members, there are probably diverse freshwater inputs with differing N contents and isotope values in each of the four study estuaries. For freshwater DIN concentrations, we chose three different end member values for illustrative purposes (Figure 2). For freshwater ^{15}N (Figure 6), we used $\Delta^{15}N$ values of +7 and −7‰ versus a central marine value of 7‰. The range from +7 and −7‰ versus the central marine value was taken from actual low-impact and high-impact sites in the four estuaries (Figure 6, Table 1). Also, Fry (1991) reported $\delta^{15}N$ values consistently close to 0‰ (i.e., −7‰ versus our marine reference point) for macroalgae from relatively pristine freshwater sites sampled over a wide area of the continental United States.

The $\delta^{15}N$ value of watershed N is generally very important for these isotope mixing models. Unfortunately, watersheds do not process nitrogen uniformly to a high and constant $\delta^{15}N$ value. McClelland et al. (1997) found that for subwatersheds of Waquoit Bay, most ^{15}N enrichment was associated with wastewater rather than agricultural inputs, with wastewater N inputs dominating high-load situations. In the study reported here, the highest ^{15}N enrichments were found in Elkhorn Slough, which receives the highest agricultural inputs (Figures 5, 6). Page (1995) also found high $\delta^{15}N$ values in plants and nutrients in a California coastal watershed with extensive agriculture. More recently, Voss et al. (2000) observed ^{15}N enrichment in estuarine systems receiving N inputs from heavily agricultural watersheds, although there was considerable diversity in the degree of ^{15}N enrichment in the different Baltic watersheds they studied. The reasons for these differing results are likely to be found in the details of soil nitrification and denitrification reactions. Temperature, carbon inputs, water residence times, redox status of porewaters, and diffusion rates of nutrients to soil microsites can all affect the rates and extent of these soil microbial processes (Mariotti et al. 1988). Temperature may be the most dominant overall control of microbial activities. Higher temperatures in southern watersheds of this study may have promoted higher microbial activities in soils, and ANCOVA analyses suggested that a factor other than DIN or salinity, a factor such as temperature, was important in explaining higher $\delta^{15}N$ values in southern estuaries.

These studies suggest that watershed N can change from low to high $\delta^{15}N$ with increasing anthropogenic inputs, but the trajectory of this change may vary from site to site, depending on temperature as well as on other controls of microbial nitrification and denitrification in watershed soils. This changing $\delta^{15}N$ trajectory results in some uncertainty in the watershed end member value needed for the mixing models, making it difficult to use these models precisely to estimate watershed N-loading. For this reason, we also used the multivariate analyses of Figures 7 and 8 to assess N-loading because the multivariate approach is free of assumptions about DIN or $\delta^{15}N$ end members. Our simplified evaluation of N-loading (Figure 9) does rely on adopting a marine end member for normalizing purposes, but is free of assumptions about watershed end members. In this simplified evaluation (Figure 9), our use of nearshore, beach stations to estimate the marine end member probably gives a strongly conservative estimate of estuarine N-loading patterns (i.e., a bias toward underestimating watershed N-loading).

Overall Evaluation of N-Loading Patterns

Both nutrient and isotope assays have weak points as indicators of watershed N-loading that depends on both N concentrations and discharge. For example, nutrients are very short-term assays of N status at particular points, and sampling can easily miss nutrient pulses important for primary production. Also, strong nutrient uptake by primary producers at estuarine sites can keep nutrients low when in fact high loading is occurring. For isotopes, artificially low $\delta^{15}N$ values can occur under conditions of high N-loading when ammonium is involved (see previous

paragraphs) or when strong nitrogen fixation is occurring in upper parts of estuaries (A.G. and B.F., unpubl. data). These conditions lead to an underestimate or conservative estimate of watershed N-loading from $\delta^{15}N$ values.

But overestimates of watershed N-loading are also possible if within-estuary processes are creating high $\delta^{15}N$ conditions. Such conditions are well known in some estuaries (Mariotti et al. 1984, Cifuentes et al. 1989, Horrigan et al. 1990, Brandes and Devol 1997) and may have occurred at some of our sites. In particular, strong microbial processing of N in sea grass detritus may have been responsible for elevated $\delta^{15}N$ values at some shoreline sites in Padilla Bay, especially at site 4 in Padilla Bay (Figure 1). There was no evidence of watershed N-loading at that site, which was elevated and near the top of a dike versus adjacent low-lying farmlands. Substantial organic matter cycling probably occurs at that site where sea grass wrack forms heavy seasonal deposits that then decompose. Intense decomposition of sea grasses could represent a natural microbial analogue of sewage treatment processes that generate nutrients with high $\delta^{15}N$. More generally, however, when within-estuary ^{15}N maxima occur in the absence of watershed loading, the maxima tend to occur down-estuary in mesohaline zones, rather than in up-estuary areas expected for watershed N-loading (Cifuentes et al. 1989). In this case, sampling along estuarine gradients should allow identification of within-estuary processing versus watershed sources of elevated $\delta^{15}N$. There are also special cases in which watershed N is strongly processed in the upper (freshwater) end of estuaries (e.g., Brion et al. 2000), and high $\delta^{15}N$ values are sometimes found in these upper estuarine areas (Owens 1985, 1987).

In spite of the various uncertainties, the $\delta^{15}N$ indicators offered a fairly independent way to assess watershed N-loading that complemented assessments based on DIN alone. Multivariate analyses showed that DIN-based estimates of N status were often different than the $\delta^{15}N$ estimates (Figure 8). Comparisons between the clustering results showed that there was more than 50% agreement (better than random agreement) between clustering based on nitrogen nutrient concentrations and clusters based on $\delta^{15}N$ values. South Slough and Elkhorn Slough, representing two opposite cases of low and very high DIN-loading, respectively, showed the highest degree of agreement between different clustering techniques. This may be the result of the homogeneity of these systems in terms of low DIN-loading for South Slough and very high DIN-loading for Elkhorn Slough. More diverse systems such as Padilla Bay had a higher disagreement rate (Figure 8), perhaps due to the fact that DIN concentrations were not high enough throughout the system to be able to "blanket" smaller spatial variability.

Figure 10 shows a possible strategy for evaluating N-loading impacts using a combination of nutrient and isotope information. Samples are collected along estuarine salinity gradients for both nutrients and isotope, then nutrient samples are analyzed first. If DIN is high (>10 μM, or any other limit the investigator may choose), then the site is classified as highly impacted by N-loading. If nutrient concentrations are lower, or if the investigator also generally wishes to rely on isotope assays in addition to nutrients, the isotope samples may then be analyzed for $\delta^{15}N$. In this study, green macroalgae were the samples most widely distributed across salinity gradients, prevalent, easiest to collect, and showed strongest isotope response to watershed N-loading. For these reasons, investigators may wish to analyze and rely on macroalgae $\delta^{15}N$ for further isotope interpretations outlined in Figure 10, although sediments may also provide strong $\delta^{15}N$ signals in some cases (Voss and Struck 1997, Voss et al. 2000). Occurrence of ^{15}N enrichment versus background marine values is used as the next decision point, with low $\delta^{15}N$ values indicative of low impact conditions. If $\delta^{15}N$ values are higher than marine values, then the geographic pattern of the isotope values becomes important, with decreases in $\delta^{15}N$ along salinity gradients pointing toward

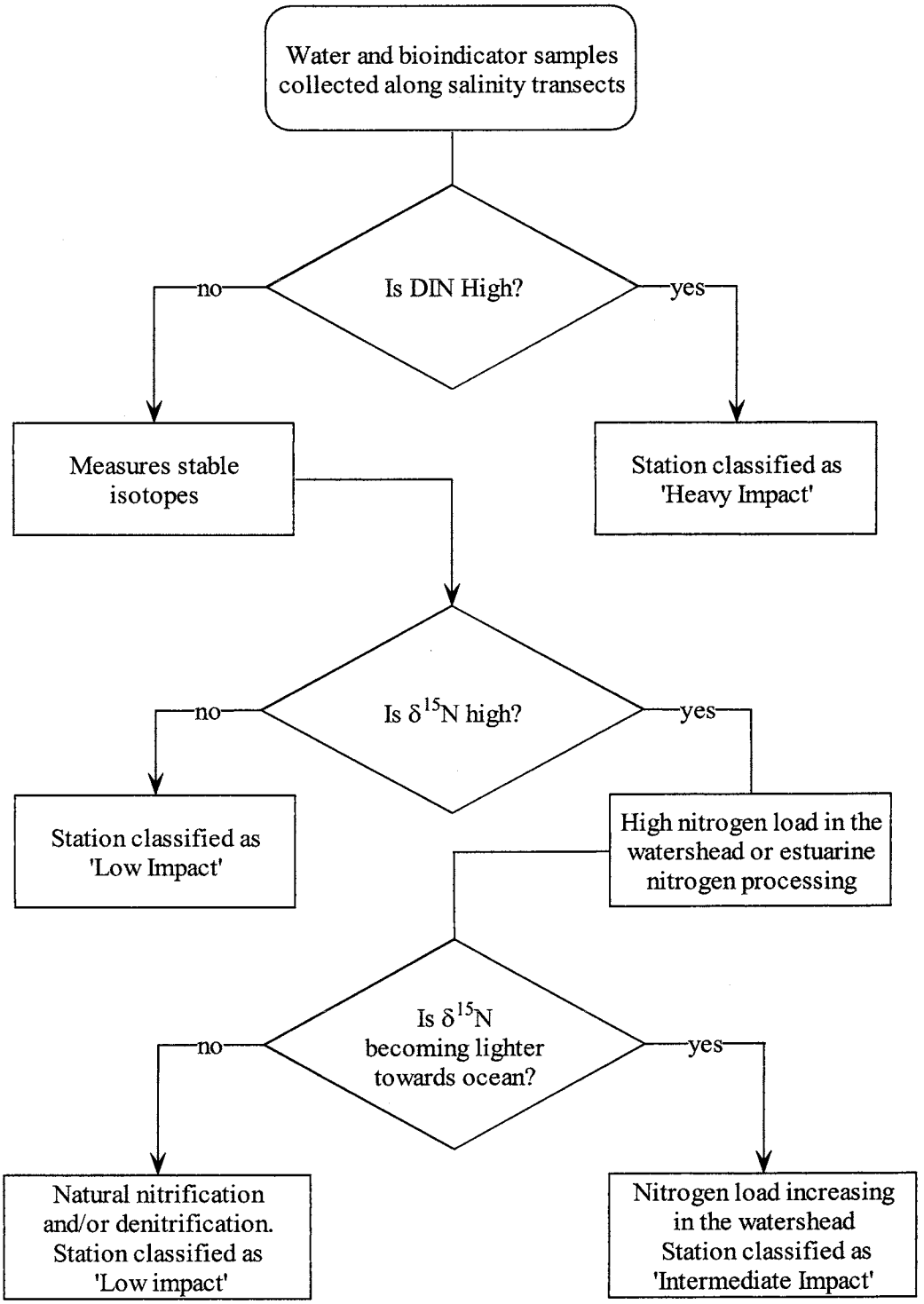


FIGURE 10. Flow chart using nutrient and $\delta^{15}N$ information to classify site N status into one of three categories: low, intermediate, and high watershed N-loading impact.

watershed sources of high $\delta^{15}N$, and mid-estuarine maxima in $\delta^{15}N$ pointing to within-estuary processing of N as a source of ^{15}N-enriched N.

This discussion and flow chart should make clear that there is no "magic bullet" for detecting and assessing strengths of watershed N inputs into estuaries. Nonetheless, a combined approach of measuring DIN concentrations and $\delta^{15}N$ values along estuarine salinity gradients appears useful and relatively cost-effective at this time. A particular strong point of the advocated combined approach is the spatial information obtained, showing which sites and areas within estuaries have high (or low) N-loading impact (e.g., Figure 9). This spatial information may be particularly useful in attempts to localize problem areas within estuaries and to help monitor any cleanup efforts. Finally, these empirical assessments can also provide calibration and validation data for future models of watershed N-loading and estuarine impact, models that combine assessments of watershed land use (Valiela et al. 1997*a*), estuarine hydrodynamics (e.g., Dyer and Taylor 1973, Wood 1979), and estuarine food web dynamics (Holmes et al. 2000, Hughes et al. 2000) to predict the observed spatial pattern of N concentrations, isotopes, and loading impacts.

This study also had some regional lessons for Pacific estuaries of the U.S. West Coast. Strong tidal flushing decreased impacts of watershed N-loading and likely resulted in relatively strong N-loading from oceanic sources where coastal upwelling seasonally increased marine nutrient concentrations. This situation is a departure from usual ideas about N-limited eutrophication dynamics in estuaries. The isotope approach to assessing watershed inputs was also relatively insensitive in this area, because background marine $\delta^{15}N$ values were relatively high (Peters et al. 1978, Liu and Kaplan 1989, Altabet et al. 1999; this study) and near those expected for polluted waters. With high background N concentrations and isotope values, moderate rather than low-level watershed N-loading observed in other studies (McClelland et al. 1997, Costanzo et al. 2001) is probably necessary before isotope metrics begin to show increases in pollutant N levels. Nonetheless, careful measurement showed that watershed N could be detected even above these high backgrounds in green algae and filter feeders, indicating food web incorporation of watershed N. This information about food web uptake cannot be obtained from DIN concentration measurements, but may be important for fisheries concerns (Wainright et al. 1996, Fry 2002). Also, careful isotope measurement done in sediments and cores can show historical development of watershed N-loading (Voss and Struck 1997, Hodell and Schelske 1998, Ogawa et al. 2001), a historical baseline that can be important in restoration efforts.

ACKNOWLEDGMENTS

We acknowledge and hereby thank the many people in the NERR network who participated in this project. Volunteers and staff members from the four West Coast NERR sites worked to make this project a success, collecting and shipping samples to LSU. Robin Cottrell and Doug Bulthuis collected samples from Padilla Bay. Steve Sadro and Steve Rumrill sent samples from South Slough. Volunteers Kupsami Reddy and Janice Jones made the collections at Elkhorn Slough, with coordination help from Jane Caffrey, Becky Christensen, Martha Nitzberg, and Kerstin Wasson. Volunteers Teresa Nystrom, Marya Ahmad, and Guillermo Mendoza collected samples at Tijuana Estuary, with coordination help from Teresa Roper and Phil Jenkins. Chris Kitting of the University of California, Hayward, kindly identified voucher specimens from the field program. Tom Oswald of the Coastal Ecology Institute, LSU, performed the nutrient concentration assays. R. Eugene Turner and K. A. Rose provided helpful editorial comments on an early version of the manuscript. Richard Langan and the CICEET headquarters at the University of New Hampshire helped with financial matters. The Institute of Pacific Islands Forestry, USDA Honolulu, supported the senior author during preparation of this manuscript.

Literature Cited

Altabet, M. A. 2001. Nitrogen isotopic evidence for micronutrient control of fractional NO_3^- utilization in the equatorial Pacific. Limnol. Oceanogr. 46:368–380.

Altabet, M. A., C. Pilskaln, R. Thunell, C. Pride, D. Sigman, F. Chavez, and R. Francois. 1999. The nitrogen isotope biogeochemistry of sinking particles from the margin of the eastern North Pacific. Deep-Sea Res. I 46:655–679.

Brandes, J. A., and A. H. Devol. 1997. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. Geochim. Cosmochim. Acta 61:1793–1801.

Brion, N., G. Billen, L. Guezennec, and A. Ficht. 2000. Distribution of nitrifying activity in the Seine River (France) from Paris to the estuary. Estuaries 23:669–682.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. U.S.A. 93:10844–10847.

Cifuentes, L. A., M. L. Fogel, J. R. Pennock, and J. H. Sharp. 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. Geochim. Cosmochim. Acta 53:2713–2721.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210:223–253.

Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, R. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage inputs. Mar. Pollut. Bull. 42:149–156.

Day, J. W., Jr., C. S. Hall, W. M. Kemp, and A. Yanez-Arancibia. 1989. Estuarine ecology. John Wiley, New York.

Dyer, K. R., and P. A. Taylor. 1973. A simple, segmented prism model of tidal mixing in well-mixed estuaries. Estuarine Coastal Shelf Sci. 1:411–418.

Everitt, B. 1993. Cluster analysis. Halsted Press, New York.

Fogel, M. L., and L. A. Cifuentes. 1993. Isotope fractionation during primary production. Pages 73–100 *in* S. A. Macko and M. H. Engel, eds. Organic geochemistry. Plenum Press, New York.

Fry, B. 1991. Stable isotope diagrams of freshwater food webs. Ecology 72:2293–2297.

———. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Can. J. Fish. Aquat. Sci. 56: 2167–2171.

———. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries 25:264–271.

Fry, B., A. Gace, and J. W. McClelland. 2001. Chemical indicators of anthropogenic nitrogen loading in four NERR estuaries of the U.S. West Coast. Final Report, CICEET project 99-296. Available at NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology, University of New Hampshire, Durham.

Gace, A. 2001. Chemical indicators of anthropogenic nitrogen loading in U.S. estuaries. M.S. thesis, Louisiana State University, Baton Rouge.

Hansson, S., J. E. Hobbie, R. Elmgren, U. Larsson, B. Fry, and S. Johansson. 1997. The stable isotope ratio as a marker of food-web interactions and fish migration. Ecology 78:2249–2257.

Hebert, C. E., and L. I. Wassenaar. 2001. Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. Environ. Sci. Technol. 35:3482–3487.

Hobbie, J. E., U. Larsson, R. Elmgren, and B. Fry. 1990. Sewage derived ^{15}N in the Baltic traced in *Fucus*. EOS 71:190.

Hodell, D. A., and C. L. Schelske. 1998. Production, sedimentation and isotopic composition of organic matter in Lake Ontario. Limnol. Oceanogr. 43:200–214.

Hoegberg, P. 1997. Tansley review no. 95: ^{15}N natural abundance in soil-plant systems. New Phytol. 137:179–203.

Hoegberg, P., and C. Johannisson. 1993. ^{15}N abundance of forests is correlated with

losses of nitrogen. Plant Soil 157:147–150.

Holmes, R. M., J. W. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring $^{15}N^{-}$ NH_4^+ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar. Chem. 60:235–243.

Holmes, R. M., B. J. Peterson, L. A. Deegan, J. E. Hughes, and B. Fry. 2000. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology 81:416–432.

Horrigan, S. G., J. P. Montoya, J. L. Nevins, and J. J. McCarthy. 1990. Natural isotopic composition of dissolved inorganic nitrogen in the Chesapeake Bay. Estuarine Coastal Shelf Sci. 30:393–410.

Hughes, J. E., L. A. Deegan, B. J. Peterson, R. M. Holmes, and B. Fry. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. Ecology 81:433–452.

Jordan, M., K. J. Nadelhoffer, and B. Fry. 1997. Nitrogen cycling in forest and grass ecosystems irrigated with ^{15}N-enriched wastewater. Ecol. Appl. 7:864–881.

Kwak, T. J., and J. B. Zedler. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. Oecologia (Berl.) 110:262–272.

Liu, K.-K., and I. R. Kaplan. 1989. The eastern tropical Pacific as a source of ^{15}N-enriched nitrate in seawater off southern California. Limnol. Oceanogr. 34:820–830.

Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux, and P. Tardieux. 1981. Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. Plant Soil 62:413–430.

Mariotti, A., C. Lancelot, and G. Billen. 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary. Geochim. Cosmochim. Acta 48:549–555.

Mariotti, A., A. Landreau, and B. Simon. 1988. ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. Geochim. Cosmochim. Acta 52:1869–1878.

McClelland, J. W., and I. Valiela. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnol. Oceanogr. 43:577–585.

McClelland, J. W., I. Valiela, and R. H. Michener. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnol. Oceanogr. 42:930–937.

Ogawa, N., T. Koitabashi, H. Oda, T. Nakamura, N. Ohkouchi, and E. Wada. 2001. Fluctuations of nitrogen isotope ratio of gobiid fish (Isaza) specimens and sediments in Lake Biwa, Japan during the 20th century. Limnol. Oceanogr. 46:1228–1236.

Owens, N. J. P. 1985. Variations in the natural abundance of ^{15}N in estuarine suspended particulate matter: A specific indicator of biological processing. Estuarine Coastal Shelf Sci. 20:505–510.

———. 1987. Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol. 24:389–451.

Page, H. M. 1995. Variation in the natural abundance of ^{15}N in the halophyte, *Salicornia virginica*, associated with groundwater subsidies of nitrogen in a southern California salt-marsh. Oecologia (Berl.) 104:181–188.

Pennington, J. T., and F. P. Chavez. 2000. Seasonal fluctuations of temperature, salinity, nitrate, chlorophyll and primary production at station H3/M1 over 1989–1996 in Monterey Bay, California. Deep-Sea Res. II 47:947–973.

Peters, K. E., R. E. Sweeney, and I. R. Kaplan. 1978. Correlation of carbon and nitrogen stable isotope ratios in sedimentary organic matter. Limnol. Oceanogr. 23:598–604.

Rau, G. H., C. Low, J. T. Pennington, K. R. Buck, and F. P. Chavez. 1998. Suspended particulate nitrogen $\delta^{15}N$ versus nitrate

utilization: Observations in Monterey Bay, CA. Deep-Sea Res. II 45:1603–1616.

Schmitt, R. J., and C. W. Osenberg. 1995. Detecting ecological impacts: Concepts and applications in coastal habitats. Academic Press, San Diego, California.

Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method. Mar. Chem. 57:227–242.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: The principles and practice of stratistics in biological research. Freeman & Co., New York.

Spiker, E. C. 1980. The behavior of ^{14}C and ^{13}C in estuarine water: Effects of *in situ* CO_2 production and atmospheric exchange. Radiocarbon 22:647–654.

Tabachnick, B. G., and L. S. Fidell. 2000. Using multivariate statistics. Allyn and Bacon, New York.

Tucker, J., N. Sheats, A. E. Giblin, C. S. Hopkinson, and J. P. Montoya. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Mar. Environ. Res. 48:353–375.

Valiela, I. 1995. Marine ecological processes. 2nd ed. Springer Verlag, New York.

Valiela, I., G. Collins, J. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C. H. Sham. 1997*a*. Nitrogen loading from coastal watersheds to receiving estuaries: New method and application. Ecol. Appl. 7:358–380.

Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh, and K. Foreman. 1997*b*. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42:1105–1118.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. 7:737–750.

Vorosmarty, C. J., and T. C. Loder III. 1994. Spring-neap tidal contrasts and nutrient dynamics in marsh-dominated estuaries. Estuaries 17:537–551.

Voss, M., and U. Struck. 1997. Stable nitrogen and carbon isotopes as indicator of eutrophication of the Oder River (Baltic Sea). Mar. Chem. 59:35–49.

Voss, M., B. Larsen, M. Leivuori, and H. Vallius. 2000. Stable isotopes signals of eutrophication in Baltic Sea sediments. J. Mar. Systems 25:287–298.

Wainright, S. C., C. M. Fuller, R. H. Michener, and R. A. Richards. 1996. Spatial variation of trophic position and growth rate of juvenile striped bass (*Morone saxatilis*) in the Delaware River. Can. J. Fish. Aquat. Sci. 53:685–692.

Waldron, S., P. Tatner, I. Jack, and C. Arnott. 2001. The impact of sewage discharge in a marine embayment: A stable isotope reconnaissance. Estuarine Coastal Shelf Sci. 52:111–115.

Waser, N. A. D., P. J. Harrison, B. Nielsen, S. E. Calvert, and D. H. Turpin. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium and urea by a marine diatom. Limnol. Oceanogr. 43:215–224.

Wood, T. 1979. A modification of existing simple segmented tidal prism models of mixing in estuaries. Estuarine Coastal Mar. Sci. 8:339–347.

Appendix A

Taxonomic Identifications (made by Chris Kitting, University of California, Hayward, Using Frozen Voucher Specimens)

ID	Estuary	Month	Station	Sample Description	Scientific Name
1	Padilla		11	Barnacle	*Balanus*
2	Padilla			Fucus	*Fucus*, young (or Family Dictyotales?)
3	Padilla		7	Green macroalgae	*Enteromorpha*
4	Padilla		8	Filamentous algae	*Cladophora*
5	Padilla			Green macroalgae	*Ulva*
6	Elkhorn	April	1	Barnacle	*Pollicipes polymerus*
7	Elkhorn	July	2	Barnacle	*Tetraclita*
8	Elkhorn	July	2	Fucus (some red algae [Rhodophyta])	Mostly *Rhodoglossum* (some *Polysiphonia*, 1 *Enteromorpha*)
9	Elkhorn	April	4	Filamentous algae	*Cladophora* (fine sp.)
10	Elkhorn	July	7	Green macroalgae	*Ulva*
11	Elkhorn	July	7	Filamentous algae	*Cladophora* (coarse sp.)
12	Elkhorn	July	7	Barnacle	*Balanus glandula*
13	Elkhorn	October	9	Green macroalgae	*Enteromorpha*
14	South Slough	July	1	Barnacle	*Balanus glandula*
15	South Slough	January	3	Green macroalgae	*Enteromorpha*
16	South Slough	October	2	Barnacle	*Pollicipes polymerus*
17	South Slough	July	3	Barnacle	*Balanus (glandula?* smooth, crushed)
18	South Slough	October	2	Green macroalgae	*Ulva*
19	South Slough	October	11	"Filamentous" (tubular) algae	*Enteromorpha*
20	South Slough	July	8	Fucus	*Fucus*
21	Tijuana			Barnacle	*Balanus glandula*
22	Tijuana			Filamentous algae	*Cladophora?* (longer cells)
23	Tijuana			Green macroalgae	*Ulva*