Estuaries and Coasts (2009) 32:773–786
DOI 10.1007/s12237-009-9148-x

Oyster and Macroalgae Bioindicators Detect Elevated $\delta^{15}N$ in Maryland's Coastal Bays

B. Fertig · T. J. B. Carruthers · W. C. Dennison · A. B. Jones · F. Pantus · B. Longstaff

Received: 20 December 2007 / Revised: 6 February 2009 / Accepted: 6 February 2009 / Published online: 3 April 2009

Abstract Nitrogen loading from anthropogenic sources, including fertilizer, manure, and sewage effluents, has been linked with declining water quality in coastal lagoons worldwide. Freshwater inputs to mid-Atlantic coastal lagoons of the USA are from terrestrially influenced sources: groundwater and overland flow via streams and agricultural ditches, with occasional precipitation events. Stable nitrogen isotopes ratios ($\delta^{15}N$) in bioindicator species combined with conventional water quality monitoring were used to assess nitrogen sources and provide insights into their origins. Water quality data revealed that nutrients derived from terrestrial sources increased after precipitation events. Tissues from two bioindicator species, a macroalgae (*Gracilaria* sp.) and the eastern oyster (*Crassostrea virginica*) were analyzed for $\delta^{15}N$ to determine spatial and temporal patterns of nitrogen sources. A broad-scale survey assessment of deployed macroalgae (June 2004) detected regions of elevated $\delta^{15}N$. Macroalgal $\delta^{15}N$ (7.33±1.15‰ in May 2006 and 6.76±1.15‰ in July 2006) responded quickly to sustained June 2006 nutrient pulse, but did not detect spatial patterns at the fine scale. Oyster $\delta^{15}N$ (8.51±0.89‰) responded slowly over longer time periods and exhibited a slight gradient at the finer spatial scale. Overall, elevated $\delta^{15}N$ values in macroalgae and oysters were used to infer that human and animal wastes were important nitrogen sources in some areas of Maryland's coastal bays. Different nitrogen integration periods across multiple organisms may be used to indicate nitrogen sources at various spatial and temporal scales, which will help focus nutrient management.

Keywords Stable nitrogen isotopes · Coastal lagoons · Human and animal wastes · Biological indicators · Water quality · Spatial analysis

B. Fertig (✉) · T. J. B. Carruthers · W. C. Dennison · A. B. Jones
Integration and Application Network,
University of Maryland Center for Environmental Science,
Cambridge, MD 21613, USA
e-mail: bfertig@hpl.umces.edu

F. Pantus
Division of Marine Research,
Commonwealth Scientific and Industrial Research Organisation,
Cleveland, Queensland 4163, Australia

B. Longstaff
Cooperative Oxford Laboratory,
NOAA-UMCES Partnership (EcoCheck),
Oxford, MD 21654, USA

Introduction

Physical, chemical, and biological indicators are routinely used for monitoring the spatial and temporal extent of eutrophication. However, monitoring eutrophication symptoms does not identify origins of the causative nutrients. In addition, chemical indicators commonly used to measure eutrophication (e.g., total nitrogen or total phosphorus; Nixon 1995; Cloern 2001; Kemp et al. 2005; Bricker et al. 2008) do not detect biologically incorporated nitrogen (Costanzo et al. 2001). Analyzing stable nitrogen isotopes ($\delta^{15}N$) in bioindicator species can be used to address these limitations, as the approach has been shown to identify sources of human and animal wastes (Costanzo et al. 2001; Cohen and Fong 2005). Standard water chemistry measurements of eutrophic symptoms can be complemented with $\delta^{15}N$ in bioindicator species to increase understanding of the location and potentially infer the sources of nitrogen.

Comparison of $\delta^{15}N$ values in bioindicator species has been used to distinguish between chemically synthesized nitrogen fertilizer and human and animal waste sources

(McClelland and Valiela 1998). Fertilizer production fixes atmospheric N_2 (defined as 0‰), and as a result, nitrogen runoff from agricultural areas potentially has lower values of $\delta^{15}N$. Human and animal wastes entering groundwater have $\delta^{15}N$ values that are elevated (e.g., Sweeny and Kaplan 1980; Tucker et al. 1999) due to a combination of volatilization of ammonia and denitrification, which leave the remaining nitrogen pool enriched with ^{15}N (McClelland and Valiela 1998; Fry 2006). Many wastewater treatment plants employ microbial processing to remove nitrogen at rates higher than in natural ecosystems. Microbial nitrogen removal processes, particularly denitrification, favor the isotopically light ^{14}N and enrich the remaining nitrate pool with ^{15}N (Cline and Kaplan 1975; Kendall 1998). Additionally, ammonia from human and animal waste fractionates during volatilization, leaving the non-volatile portion further enriched with ^{15}N (McClelland and Valiela 1998, Fry 2006). Since multiple processes enrich $\delta^{15}N$ values in biological indicator species, interpretations need to be balanced against a set of alternative hypotheses. Measurements of $\delta^{15}N$ in biological indicator species are advantageous over direct measurements that can be made on groundwater (Aravena et al. 1993), the water column, or sediments (Tucker et al. 1999), as biota minimize temporal and spatial variability. In particular, this study focused on nitrogen incorporated into macroalgae and filter feeders.

Integration of nitrogen sources occurs over different timescales in different organisms (Gartner et al. 2002; Dattagupta et al. 2004). While $\delta^{15}N$ integration in diets has been examined across taxonomic groups (including mollusks) and diets (Vanderklift and Ponsard 2003), the temporal integration of $\delta^{15}N$ over various timescales by different organisms, due to species-specific turnover rates, has not been fully explored. Macroalgae uptake nitrogen directly from the water column and have rapid nitrogen turnover rates and so can provide information about available nitrogen over a period of days (Costanzo et al. 2001). Assuming nitrogen limitation, fractionation during assimilation will be minimal (Fry 2006). Oysters are sessile, euryhaline filter feeders that derive nitrogen from a variety of sources, e.g., microorganisms, phytoplankton, detritus, and inorganic particles (Langdon and Newell 1996), and have tissue nitrogen turnover rates in the order of weeks to months depending on the tissue type (Moore 2003). Temporal integration of nitrogen suggested that $\delta^{15}N$ in zebra mussels was appropriate to monitor watershed development and downstream effects despite seasonal variations (Fry and Allen 2003). Feeding over multiple trophic levels in field studies may complicate interpretation of $\delta^{15}N$, which is enriched 3–4‰ over each trophic level (Fry 2006). In certain cases, spatial gradients in $\delta^{15}N$ could reflect variability in available diets. Nevertheless, biological indicators such as macroalgae and oysters allow an assortment of questions to be addressed through manipulative field experiments that provide long-term integration on different timescales, which is missed by water chemistry measurements alone.

Multiple sources of anthropogenic nitrogen affect mid-Atlantic coastal bays. Collectively, agricultural fertilizers as well as human and animal wastes have been directly linked to downstream eutrophication (Kennish 2002; Kiddon et al. 2003; Bricker et al. 2008; Wazniak et al. 2007). Long-term water quality monitoring reported recent degradation and increases in total nitrogen despite historical improvements and decreases in total nitrogen, signaling a need to better understand the driving forces for trend shifts in this region and identify sources of anthropogenic nitrogen (Wazniak et al. 2007). Symptoms of degradation include an approximate doubling of dissolved organic nitrogen, increasing frequency of harmful algal blooms, e.g., brown tide (Glibert et al. 2007), and adverse effects on seagrass distribution and density (Harris et al. 2005; Wazniak et al. 2007). Human population in Maryland's coastal bays watersheds doubled between 1980 and 2000 to ~35,000 people and is expected to double again by 2020 (Hager 1996). Septic and wastewater nitrogen inputs have also increased during this period (MCBP 2005). Identifying and differentiating sources of anthropogenic nitrogen can help target management efforts to reduce inputs.

This paper develops a framework for interpreting $\delta^{15}N$ from macroalgae (*Gracilaria* sp.) and oyster (*Crassostrea virginica*) tissue by addressing three questions: (1) What are the relative capabilities of macroalgae and oysters to detect nitrogen from human and animal wastes? (2) What are the broad-scale spatial patterns of nitrogen from wastes spanning these coastal bays (~600 km^2)? (3) What are the fine-scale spatial patterns of influence by nitrogen from human and animal wastes within regions (ranging from ~10 to 50 km^2) of Maryland's coastal bays?

Methods

Study Location

This study was conducted in a series of coastal lagoons located on the mid-Atlantic coast of the USA (Fig. 1). These coastal lagoons, including Chincoteague Bay (extending from 38°15′14″ N, 75°11′57″ W in the north to 37°54′14″ N, 75°24′38″ W in the south), cover the full length of Maryland's and some of Virginia's Atlantic coastline. The bays comprise a series of shallow (2-m mean depth), well-mixed, lagoonal estuaries behind barrier islands (Fenwick and Assateague Islands).

Due to small watershed areas (totaling 452 km^2) of Maryland's coastal bays, freshwater inputs and activities that result in anthropogenic nitrogen inputs generally occur

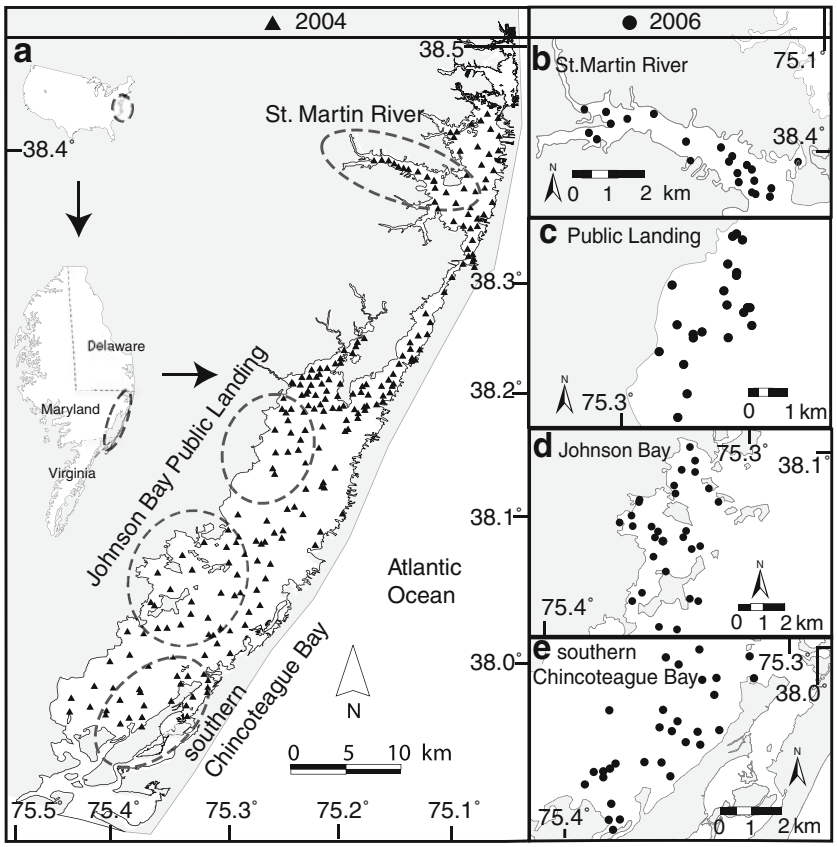

Fig. 1 Geographical reference of Maryland's coastal bays within the Delmarva peninsula. In 2004, macroalgae was deployed at 248 sites (*triangles*) across Maryland's coastal bays (**a**). The 2006 deployment of macroalgae and oyster (*circles*) spanned 100 randomly distributed sites across four regions of interest: **b** St. Martin River, **c** Public Landing, **d** Johnson Bay, and **e** southern Chincoteague Bay

within 6 km of shore as compared to larger ecosystems (e.g., Jordan et al. 1997; Brawley et al. 2000; Turner and Rabalais 2003). Freshwater base flow, transporting nitrate from terrestrial recharge areas, enters Delmarva Peninsula's coastal lagoons via both groundwater (Andres 1992; Bratton et al. 2004; Krantz et al. 2004; Manheim et al. 2004) and overland sources that include riverine (Lung 1994; Schwartz 2003) and agricultural ditches (Schmidt et al. 2007). Seasonal precipitation is variable across these coastal bays (Fig. 2). Salinities range from fresh in some tributaries to polyhaline (30–35‰) in the bays. There is oceanic flushing through two small channels: one near Ocean City (38°19′ 31″ N, 75°05′33″ W) toward the northern end of the bays and the other south of Chincoteague Bay (37°52′36″ N, 75° 25′04″ W; Fig. 1). Flushing rates are around 12 days in St. Martin River and 63 days in Chincoteague Bay (Pritchard 1960; Lung 1994). Land cover in the watersheds of these coastal lagoons is dominated by forests (39.5%) and crop agriculture (31.8%), although industrial poultry feeding operations (1.1%) are also located within the watersheds (Table 1). The region has a high occurrence of septic systems for the residential towns of Berlin, MD and Chincoteague, VA (Souza et al. 1993). Poor water quality has been reported in the northern portion of Chincoteague Bay, which is the receiving waters for the town of Berlin, MD (Boynton 1993; Boynton et al. 1996).

Experimental Design

Macroalgae were used for both broad- and fine-scale surveys. Macroalgae (*Gracilaria* sp.) were deployed at 248 randomly distributed sites throughout all regions of Maryland's coastal bays from 7 to 12 June 2004 (Fig. 1).

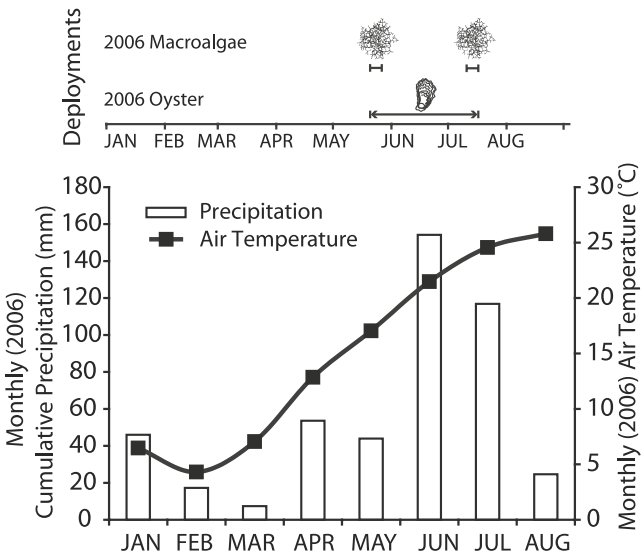

Fig. 2 Precipitation (mm) and air temperature (°C) between macroalgae deployments and during oyster deployment

Table 1 Percent of each sub-watershed of Maryland's coastal bays devoted to various land uses

Land use	Assawoman Bay (%)	Chincoteague Bay (%)	St. Martin River/ Isle of Wight (%)	Newport Bay (%)	Sinepuxent Bay (%)
Residential	18.9	1.5	17.2	6.9	9.4
Urban	6.6	0.2	5.5	2.0	5.9
Crop Agriculture	22.5	32.5	34.1	34.4	11.4
Animal Agriculture	1.4	0.7	1.8	0.8	0.2
Forest	27.9	40.3	37.7	43.5	38.6
Wetlands	21.5	22.9	3.4	12.0	23.1
Bare/Other	1.2	1.9	0.3	0.4	11.4
Total (ha)	2,791	17,340	13,605	11,005	3,080

Data from Maryland Department of Planning, 2002

Finer scale surveys were conducted from 22 to 27 May 2006 and again from 13 to 18 July 2006. Survey dates were not selected a priori for association with precipitation events, yet 12 precipitation events occurred between fine-scale surveys in June 2006 (0.3–53.3 mm; Fig. 2). During the finer scale surveys, macroalgae was deployed at 100 sites randomly distributed across these coastal bays in St. Martin River (21 sites), Chincoteague Bay at Public Landing (22 sites), Johnson Bay (28 sites), and southern Chincoteague Bay (29 sites; see Fig. 1).

Macroalgae surveys followed the deployment methods described by Costanzo et al. (2001). The macroalgae used for deployment were initially collected in Greenbackville, VA near southern Chincoteague Bay 1 day in advance of deployment. Three subsamples (~1.0-g dry weight each) provided an initial $\delta^{15}N$ value (10.0±0.1‰ in June 2004, 5.2±0.2‰ in May 2006, and 9.5±0.6‰ in July 2006). The remaining macroalgae were subsampled (~1.0-g dry weight) for deployment and placed in transparent perforated (35 holes of ~1.0-cm diameter distributed across the side and bottom) containers (130 mL) to allow light, water, and nutrient exchange. For each site, containers (one per site) were attached to anchored surface buoys at a depth of 0.5 of the Secchi depth (rounded to nearest 10 cm).

Oysters (*C. virginica*) were deployed in the fine-scale survey (2006) in a similar manner as macroalgae. Oysters were originally hatchery-reared without shell substrate (cultchless) <1 year old (29.8- to 95.8-mm shell height) and grown in two locations in St. Martin River (8.2± 0.3‰). Oysters were deployed in Johnson Bay and St. Martin River from 21 May to 13 July 2006 and in southern Chincoteague Bay and Public Landing from 22 May to 14 July 2006. Oyster deployments overlapped the June precipitation events. Three oysters from a randomly selected growth location were placed in a mesh (1.9-cm holes) cage, anchored by bricks, and suspended 0.5 m above bottom by surface buoys. The oysters were deployed at the same 100 sites as the macroalgae (Fig. 1).

Data Collection and Analysis

After the deployment period, tissues from both macroalgae and oysters were analyzed for stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$). Upon collection, samples were kept on ice in the field and frozen at the laboratory (−20°C) until processing. Of the surviving oysters from each site, one was selected at random and dissected to recover the adductor muscle for $\delta^{15}N$ analysis. Tissues from both organisms were thawed, rinsed, and oven-dried at 60°C for 48 h or until thoroughly dry. Dried macroalgae tissue was finely ground using a grinding mill (Crescent 3110B Wig-L-Bug), while a mortar and pestle was used for oysters. Subsamples (2.0±0.2-mg dry weight of macroalgae, 1.0±0.2-mg dry weight of oyster) were placed in tin capsules (pressed, standard weight 8×5 mm, elemental microanalysis). Nitrogen and carbon content (μg N and μg C) and natural abundance of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) were analyzed at the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Molecular %N and C/N ratio were calculated. Both $\delta^{15}N$ and $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$, where R was defined as either the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio. The standard reference was atmospheric N_2 (air), with 0.3663 at.% ^{15}N, defined as 0‰ (e.g., Fry 2006), while PDB standard was used for $\delta^{13}C$.

Data on physical parameters and nutrient concentrations were collected and analyzed in conjunction with biological data. Physical (e.g., temperature and salinity) parameters were measured with a WTW Multi 197i water quality probe, and Secchi depth was also recorded. Water samples (20 mL) for nutrient analyses (total nitrogen and total phosphorus) were collected and kept on ice in the field 21 May 2006 (before precipitation events) and 13 July 2006 (after precipitation events) until freezing (−20°C) at the laboratory for analysis. Total nutrients, rather than inorganic species, were analyzed according to standard methods (D'Elia et al. 1977; Kerouel and Aminot 1987). Long-term nitrogen increases and

recycling in these bays have been driven by the dominant dissolved organic fraction (Glibert et al. 2001; Glibert et al. 2007) and locally are at least moderately bioavailable (Seitzinger and Sanders 1999; Seitzinger et al. 2002; Mulholland et al. 2004; Glibert et al. 2006; Wiegner et al. 2006). In culture, *Gracilaria cornea* efficiently grows on organic (urea) or inorganic (NH_4^+, NO_3^-, or NO_3NH_4) nitrogen (Navarro-Angulo and Robledo 1999). Therefore total, rather than dissolved inorganic, nutrients were deemed a better indicator of relative nutrient availability. Water samples (60 mL) for chlorophyll *a* were filtered onto GF/F filter paper (25-mm diameter) in the field and kept on ice until freezing (−20°C) at the laboratory until spectrophotometric analysis, which was conducted according to standard methods (Arar 1997). Data from two statistical outliers (defined as $>\pm 3\sigma$ from mean, verified by Grubb's test) were removed. Precipitation data were collected by National Park Service, Assateague Island National Seashore. Spatial patterns for all parameters were plotted with ArcMap 8.0 geographical information system. The Spatial Analyst functionality of the ArcGIS package was used to Krige raster interpolations for measured variables and their variances. If spatial autocorrelation was not confirmed, the interpolation was removed. Correlations were calculated for physical and nutrient parameters for both months. Assumptions of normality and homogeneity of variances were verified with SAS 9.1.2 (Proc Univariate) and no data transformations were required. Statistical analysis testing for differences between means using two-way analyses of variance (ANOVAs; region, month) was also performed with SAS 9.1.2 (Proc Mixed) for all parameters, except for those involving oysters. Oyster data were analyzed with one-way ANOVAs run on regions since only one deployment was conducted, from May to July.

Physical (including nutrients) and biological (chlorophyll *a*, macroalgae %N, macroalgae $\delta^{15}N$, macroalgae $\delta^{13}C$, and macroalgae C/N) parameters were analyzed with non-metric multidimensional scaling (non-metric MDS) to assess spatial and temporal patterns. Separate analyses were conducted on range standardized physical/nutrient metrics and on biological metrics for each month. A Bray–Curtis similarity matrix produced a distance matrix for each set of variables, which was ordinated by non-metric MDS using PATN (Belbin 1993). Each analysis was conducted in two dimensions with ten random starts. Ordinations had acceptable (0.14 and 0.17, respectively) stress levels (Clarke and Warwick 1994).

Results

Freshwater Inputs Pulsed Nutrients to the Shallow Lagoons

Freshwater inputs were variable across the study period and altered salinity and nutrient concentrations. In 2004, precipitation was consistently low (0.0–15.0 mm) in the spring months (March to May) preceding the broad scale survey. However, there were 12 precipitation events in June 2006 (0.3–53.3 mm; Fig. 2). While total nitrogen was positively correlated with temperature, both total nitrogen and total phosphorus were negatively correlated with salinity (Table 2). Salinity decreased from May 2006 (30.1) to July 2006 (27.7), as precipitation induced a pulse of runoff and diluted the bays. Salinity decreased towards shore at Johnson Bay and upstream at St. Martin River, while salinities at Public Landing and Chincoteague were more homogenous (Fig. 3a–d). Higher concentrations of water column total nitrogen and total phosphorus were found in July 2006 (51.6±15.8 μM N, 4.42±1.04 μM P) than May 2006 (44.6±3.7 μM N, 2.59±0.77 μM P), except for total nitrogen at Johnson Bay. Interpolation of total nitrogen indicated a gradient decreasing offshore (Fig. 3e–h).

Both temporal and regional differences were found in biological parameters in 2006. Chlorophyll *a* in Chincoteague and St. Martin River increased with total nitrogen and total phosphorus temporally (Table 3). Yet all variables had a significant interaction between region and month (Table 4). Nutrients pulsed by precipitation events were also incorporated into macroalgae. Macroalgae %N increased from May (1.5%) to July (2.2%). Non-metric MDS indicated biological parameters grouped temporally, but not regionally (Fig. 4a). Chlorophyll *a* was inversely related to macroalgae $\delta^{15}N$ and $\delta^{13}C$ values and was not related to macroalgae %N or C/N (Fig. 4b). Macroalgae $\delta^{13}C$ was more enriched in July than in May in all regions (Table 3).

Broad Spatial Scale Comparisons (2004)

The broad survey in 2004 showed distinct spatial patterns of total nitrogen concentrations across Maryland's coastal bays. Nutrient concentrations were highest in small creeks and lowest closest to the channels, where bay water exchanges with oceanic water (Fig. 5a). Concentrations of total nitrogen were lowest (0.7 to 38.8 μM) in Isle of Wight Bay, by the channel near Ocean City. Southern Chincoteague Bay, near the other channel, also tended to have low concentrations of total nitrogen (15.8 to 35.0 μM) compared to Public Landing and Johnson Bay (41.2 to 68.8 μM). St. Martin also exhibited moderate concentrations of total nitrogen (42.7 to 72.2 μM). Highest values of total nitrogen were found in Newport Bay (53.7 to 82.1 μM; Fig. 5a). Total nitrogen and total phosphorus concentrations correlated positively with temperature and salinity, but inversely with macroalgal $\delta^{15}N$ values (Table 2).

Macroalgal $\delta^{15}N$ and %N values varied broadly in 2004 across Maryland's coastal bays, and spatial patterns differed from that of total nitrogen concentrations. Highest $\delta^{15}N$ values were found in southern Chincoteague Bay (10.8‰

Table 2 Correlations between nutrients (total nitrogen and total phosphorus) and physical parameters (temperature, salinity) or biological parameters (chlorophyll *a* and macroalgal or oyster $\delta^{15}N$, %N, $\delta^{13}C$, %C, C/N) for broad spatial scale (2004) and fine spatial scale (2006) surveys

Nutrient	Physical parameter	*n*	*r*	*p*
June 2004				
Total nitrogen	Salinity	222	−0.75	**<0.001**
	Temperature	224	0.59	**<0.001**
	Chlorophyll *a*	237	0.72	**<0.001**
	Macroalgae $\delta^{15}N$	231	−0.30	**<0.001**
	Macroalgae %N	231	−0.15	**0.025**
Total phosphorus	Salinity	222	−0.24	**<0.001**
	Temperature	224	0.51	**<0.001**
	Chlorophyll *a*	237	0.84	**<0.001**
	Macroalgae $\delta^{15}N$	231	−0.19	**0.003**
	Macroalgae %N	231	−0.35	**<0.001**
May 2006				
Total nitrogen	Salinity	98	−0.43	**<0.001**
	Temperature	97	0.30	**0.003**
	Chlorophyll *a*	93	0.19	0.067
	Macroalgae $\delta^{15}N$	95	0.14	0.162
	Macroalgae %N	95	−0.05	0.646
	Macroalgae $\delta^{13}C$	94	0.15	0.146
	Macroalgae %C	94	−0.10	0.336
	Macroalgae C/N	94	0.04	0.718
Total phosphorus	Salinity	98	0.03	0.763
	Temperature	97	0.10	0.312
	Chlorophyll *a*	93	0.08	0.433
	Macroalgae $\delta^{15}N$	95	0.17	0.105
	Macroalgae %N	95	−0.26	**0.011**
	Macroalgae $\delta^{13}C$	94	0.24	**0.020**
	Macroalgae %C	94	−0.16	0.116
	Macroalgae C/N	94	0.22	**0.035**
July 2006				
Total nitrogen	Salinity	100	−0.81	**<0.001**
	Temperature	100	0.41	**<0.001**
	Chlorophyll *a*	100	0.55	**<0.001**
	Macroalgae $\delta^{15}N$	99	−0.14	0.158
	Macroalgae %N	99	0.63	**<0.001**
	Macroalgae $\delta^{13}C$	99	−0.44	**<0.001**
	Macroalgae %C	99	0.51	**<0.001**
	Macroalgae C:N	99	−0.57	**<0.001**
	Oyster $\delta^{15}N$	47	0.17	0.265
	Oyster %N	47	0.50	**<0.001**
	Oyster $\delta^{13}C$	47	0.19	0.206
	Oyster %C	47	0.68	**<0.001**
	Oyster C/N	47	0.17	0.249
Total phosphorus	Salinity	100	−0.80	**<0.001**
	Temperature	100	0.06	0.532
	Chlorophyll *a*	100	0.39	**<0.001**
	Macroalgae $\delta^{15}N$	99	0.02	0.853
	Macroalgae %N	99	0.56	**<0.001**
	Macroalgae $\delta^{13}C$	99	−0.21	**0.033**
	Macroalgae %C	99	0.52	**<0.001**
	Macroalgae C/N	99	−0.48	**<0.001**
	Oyster $\delta^{15}N$	47	0.27	0.068
	Oyster %N	47	0.50	**<0.001**
	Oyster $\delta^{13}C$	47	0.27	0.071
	Oyster %C	47	0.71	**<0.001**
	Oyster C/N	47	0.24	0.110

Number of measurements (*n*), correlation value (*r*), and significance (*p*) are reported. Significant relationships are noted in bold

to 26.4‰) and then in St. Martin River (12.1‰ to 22.6‰), but were moderate in Public Landing (12.4‰ to 13.2‰). While macroalgal $\delta^{15}N$ values in Johnson Bay were moderate (9.5‰ to 17.8‰), the higher values tended to lie to the west of islands in Chincoteague Bay (Fig. 5b). Broad spatial patterns of macroalgae %N were similar to that of $\delta^{15}N$. Macroalgae %N was high in Sinepuxent and Newport Bays in addition to St. Martin River and Isle of Wight Bay. Both macroalgae $\delta^{15}N$ and %N were low in Chincoteague Bay, though somewhat elevated around Chincoteague Island (Fig. 5c). Macroalgae %N negatively correlated to total nitrogen (−0.15, $p<0.03$) and total phosphorus (−0.35, $p<0.01$; Table 2). Spatial patterns of total nitrogen concentrations, macroalgae $\delta^{15}N$, and macroalgae %N did not match (Fig. 5a–c). In St. Martin River, total nitrogen concentrations (55.6±3.0 μM), macroalgae $\delta^{15}N$ (15.9±1.2‰), and macroalgae %N (1.5±0.1%) were elevated, but in southern Chincoteague Bay, total nitrogen concentrations (24.1±1.3 μM) and macroalgae %N (1.2±0.1%) were low, while macroalgae $\delta^{15}N$ was elevated (17.3±1.3‰).

Fine Spatial Scale Comparisons (2006)

Regional variations in total nitrogen concentrations were detectable in the finer spatial scale sampling data (Fig. 3e–h). During both fine-scale samplings in 2006, St. Martin River had the highest total nitrogen (54.6±1.2 μM N in May, 71.3±3.6 μM N in July) and Johnson Bay had the highest total phosphorus (3.25±0.10 μM P in May, 5.14±0.17 μM P in July), while southern Chincoteague Bay had the lowest total nitrogen (24.8±1.2 μM N in May, 33.9±0.6 μM N in July) and total phosphorus (1.62±0.05 μM P in May, 3.26±0.05 μM P in July; Table 3). Non-metric MDS showed that physical parameters grouped regionally and that total nitrogen and total phosphorus were inversely correlated with Secchi depth (Fig. 4c, d). Southern Chincoteague Bay

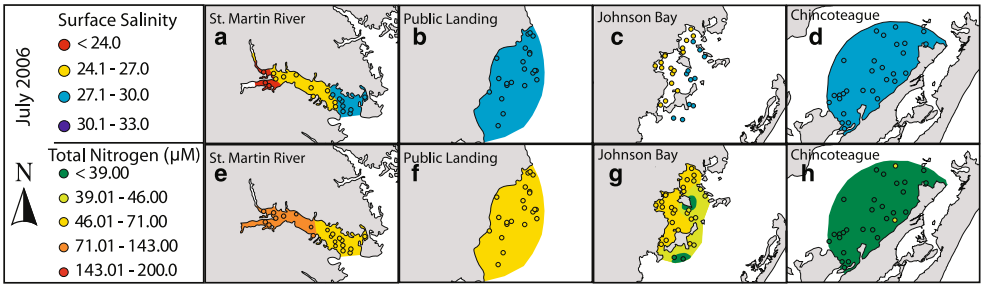

Fig. 3 Spatial patterns of freshwater and total nitrogen moving offshore were observed between May and July 2006. Salinities are reported for St. Martin River (**a**), Public Landing (**b**), Johnson Bay (**c**), and southern Chincoteague Bay (**d**). Total nitrogen is reported for St. Martin River (**e**), Public Landing (**f**), Johnson Bay (**g**), and southern Chincoteague Bay (**h**)

tended to have low total nutrients and increased Secchi depth, while St. Martin River and Public Landing exhibited gradients of nutrients (Fig. 3e–h). While spatial patterns of macroalgal $\delta^{15}N$ were recognizable at the broad scale, they were undetectable at the finer spatial scale within regions in both May and July. The range of macroalgal $\delta^{15}N$ values was bigger at the broad spatial scale in 2004 (8.9‰ to 26.4‰) than at the finer spatial scale in 2006 (5.5‰ to 8.8‰ in May and 2.5‰ to 9.1‰ in July). A slight north–south gradient of oyster $\delta^{15}N$ emerged within these regions (Fig. 6a, b), particularly at Johnson Bay (7.8‰ to 10.3‰) and southern Chincoteague Bay (6.5‰ to 10.0‰).

Bioindicator Species Comparison

Macroalgae and oyster biological indicators both responded, but in different ways, to nutrient concentrations and sources. At the broad scale (2004), macroalgae $\delta^{15}N$ values and total nutrients (total nitrogen and total phosphorus) were inversely related (Table 2). At the finer spatial scale (2006), neither total nitrogen nor total phosphorus significantly related to macroalgae $\delta^{15}N$ (Table 2). Absolute change in macroalgae $\delta^{15}N$ from initial values were greatest in June 2004 and were negative in July 2006, while changes in oyster $\delta^{15}N$ were much smaller than macroalgae, often <± 1.0‰ (Fig. 7a). Meanwhile, macroalgae %N decreased from initial values in June 2004 and changed minimally from initial values in either May or July 2006, while oyster %N values exhibited the greatest absolute change in %N, often > 1.5% (Fig. 7b). Except in Chincoteague, macroalgae %N was higher in July than May, while macroalgae $\delta^{15}N$ decreased from May to July (Table 3). Macroalgae $\delta^{15}N$ and %N were positively correlated in May (0.26, $p<0.01$, $n=95$), but not in July (−0.14, $p=0.17$, $n=99$). Oyster %N

Table 3 Means of physical, nutrient, and biological parameters measured during the fine-scale survey (2006)

Parameter	Units	St. Martin River		Public Landing		Johnson Bay		Chincoteague Bay	
		May n=2	July n=19	May n=21	July n=27	May n=22	July n=28	May n=26	July n=29
Salinity		26.9 (0.3)	25.8 (0.4)	30.4 (0.0)[f]	28.5 (0.0)	31.7 (0.1)[i]	26.6 (0.2)	31.3 (0.0)[h]	29.7 (0.0)
Temperature	°C	22.5 (0.2)	31.8 (0.3)	23.1 (0.1)	29.8 (0.1)	20.3 (0.2)[i]	29.3 (0.2)	21.3 (0.3)[h]	30.6 (0.2)
Dissolved oxygen	mg L^{-1}		6.93 (0.31)	8.10 (0.06)[f]	4.47 (0.08)		5.04 (0.15)	8.17 (0.42)[g]	5.28 (0.07)
Secchi	M	0.6 (0.0)	0.3 (0.0)	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)[i]	0.4 (0.0)	0.9 (0.0)	0.5 (0.0)
Total nitrogen	µM	54.6 (1.2)	71.3 (3.6)	51.0 (0.7)	58.7 (0.8)	51.2 (1.5)	50.7 (1.6)	24.8 (1.2)[g]	33.9 (0.6)
Total phosphorus	µM	2.38 (0.07)	4.79 (0.25)	3.11 (0.04)	4.79 (0.08)	3.25 (0.10)	5.14 (0.17)	1.62 (0.05)[g]	3.26 (0.05)
Total chlorophyll *a*	µg L^{-1}	38.7 (2.9)	66.0 (7.6)	46.9 (4.7)	44.9 (2.0)	41.1 (2.7)	45.4 (3.1)	19.0 (3.0)	37.1 (2.0)
Macroalgae %N	%	1.8 (0.1)	3.1 (0.1)	1.5 (0.0)	1.9 (0.1)	1.5 (0.1)	2.8 (0.1)	1.5 (0.1)	1.4 (0.0)
Macroalgae $\delta^{15}N$	‰	7.4 (0.1)	6.5 (0.2)	7.0 (0.1)	6.8 (0.2)	7.5 (0.1)	6.8 (0.2)	7.0 (0.1)	7.0 (0.3)
Macroalgae $\delta^{13}C$	‰	−20.0 (1.2)[d]	−17.1 (0.2)	−18.8 (0.2)	−16.3 (0.2)	−19.5 (0.3)	−16.1 (0.1)	−19.9 (0.3)	−15.7 (0.2)
Oyster %N	%		13.0 (0.2)[b]		12.1 (0.3)[a]		12.7 (0.3)[c]		11.6 (0.2)[e]
Oyster $\delta^{15}N$	‰		8.8 (0.2)[b]		8.4 (0.2)[a]		9.4 (0.3)[b]		8.1 (0.2)[e]
Oyster $\delta^{13}C$	‰		−21.2 (0.1)[a]		−20.6 (0.1)[a]		−21.0 (0.2)[b]		−21.1 (0.1)[e]

Standard error is reported in parentheses. Sample size (n) is reported for each month in each region, except as noted by superscript
[a] n=8; [b] n=9; [c] n=10; [d] n=20; [e] n=21; [f] n=22; [g] n=25; [h] n=27; [i] n=28

Table 4 ANOVAs run on nutrient and biological parameters (2006 data) identify regional and temporal differences and interactions

Parameter	Variation	*n*	*df*	MSE	*F*	*p*
Total nitrogen	Region	192	3, 184	54.35	184.39	**<0.001**
	Month		3, 184		58.86	**<0.001**
	Region × Month		3, 184		10.84	**<0.001**
Total phosphorus	Region	192	3, 184	0.32	98.61	**<0.001**
	Month		3, 184		527.03	**<0.001**
	Region × Month		3, 184		4.09	**0.008**
Chlorophyll *a*	Region193	193	3, 185	305.76	17.03	**<0.001**
	Month		1, 185		21.93	**<0.001**
	Region × Month		3, 185		6.30	**<0.001**
Macroalgae %N	Region	193	3, 185	0.10	94.02	**<0.001**
	Month		1, 185		245.68	**<0.001**
	Region × Month		3, 185		65.44	**<0.001**
Macroalgae $\delta^{15}N$	Region	193	3, 185	0.72	1.25	0.292
	Month		1, 185		13.01	**<0.001**
	Region × Month		3, 185		3.09	**0.029**
Oyster muscle %N	Region	48	3, 44	0.56	8.86	**<0.001**
Oyster muscle $\delta^{15}N$	Region	48	3, 44	0.61	6.09	**0.002**

Number (*n*), degrees of freedom (*df*), mean square error (MSE), *F* value (*F*), and significance value (*p*) are displayed. Significant relationships are reported in bold

values varied only slightly regionally and exhibited the least increase (1.4%) above initial values at southern Chincoteague Bay. Oyster tissue %N values were spatially consistent with total nitrogen (Table 3). Initial values of macroalgae $\delta^{15}N$ (5.2‰ in May and 9.5‰ in July) and oyster $\delta^{15}N$ (8.2‰) were lower than final measurements after deployment, except at southern Chincoteague Bay (8.1±0.2‰). Spatially, oyster $\delta^{15}N$ values more closely resembled macroalgae $\delta^{15}N$ in May 2006 (Johnson Bay > St. Martin River > Public Landing > southern Chincoteague Bay) than those of July 2006. Overall, both macroalgae and oysters had high isotopic values inshore.

Discussion

Freshwater Inputs Pulsed Nutrients to the Shallow Lagoons

Freshwater inputs in June 2006 pulsed nutrients into the coastal lagoons, resulting in changes to the macroalgae nutrient status and phytoplankton abundance. Salinity and nutrient gradients along St. Martin River (Fig. 3a, e) in conjunction with salinity decreases and spatial patterns of nutrients in Public Landing (Fig. 3b, f) and Johnson Bay (Fig. 3c, g) which emanated from shore implicated transport of total nitrogen from terrestrial sources, either via groundwater or overland flow through streams or agricultural ditches. Similar nutrient pulses (e.g., dissolved nitrate) are common in other comparable coastal ecosys-

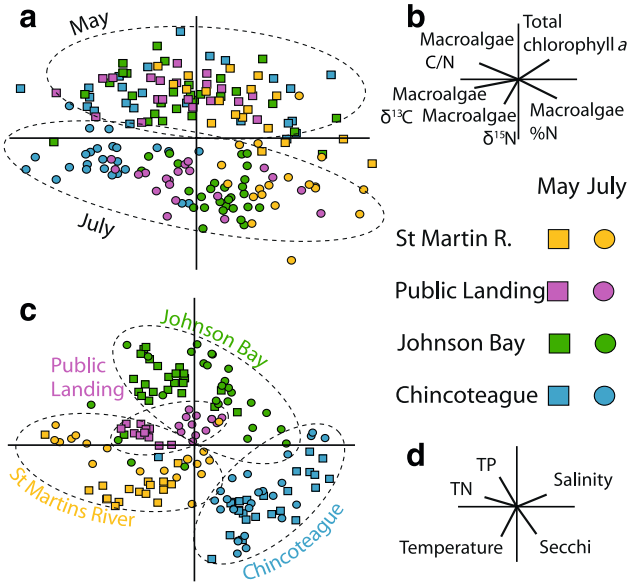

Fig. 4 Non-parametric multidimensional scaling plot for biological parameters (total chlorophyll *a* (the sum of chlorophyll *a* and phaeophytin), macroalgae %N, macroalgae C/N, macroalgae $\delta^{15}N$ values, and macroalgae $\delta^{13}C$ values (**a**). Principal axis correlation plot for biological parameters (**b**). Non-parametric multidimensional scaling plot for physical parameters (Secchi depth, temperature, salinity, total nitrogen, and total phosphorus) (**c**). Principal axis correlation plot for physical parameters (**d**)

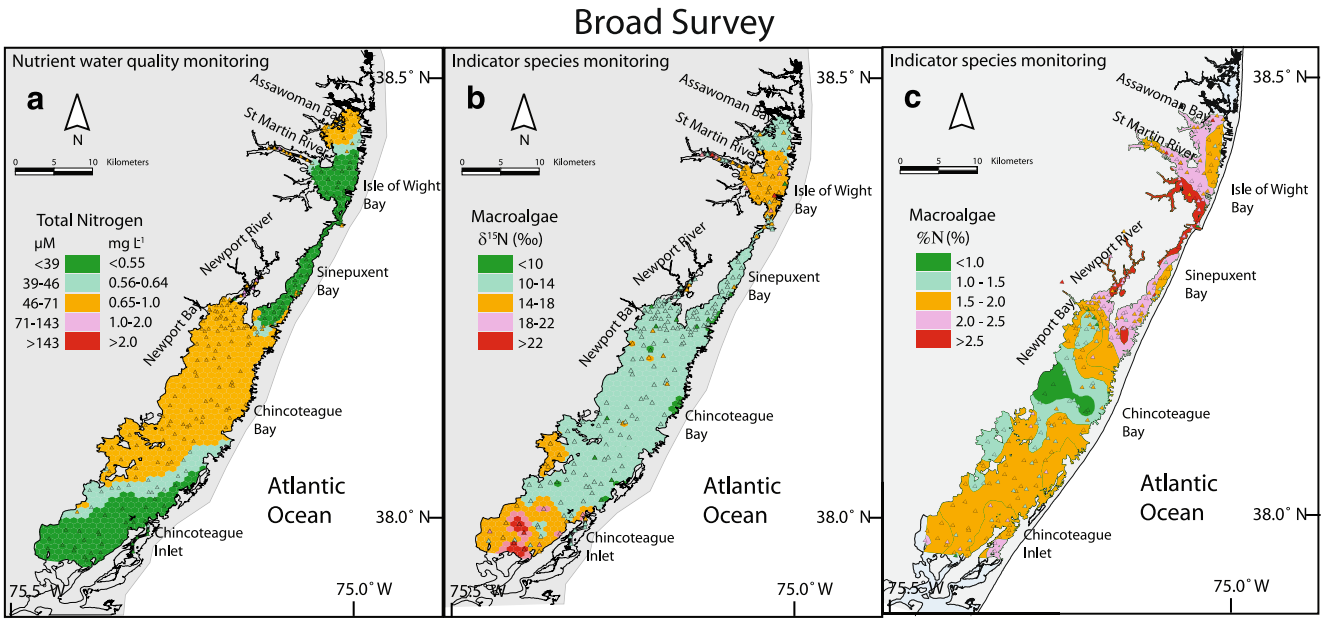

Fig. 5 Measured total nitrogen (**a**), deployed macroalgae $\delta^{15}N$ (**b**), and %N (**c**) values from the broad spatial survey (June 2004)

tems (Valiela et al. 1990; Ullman et al. 2002). Temporal grouping of biological parameters (total chlorophyll *a* and macroalgae %N, C/N, $\delta^{13}C$, and $\delta^{15}N$) in the non-metric multidimensional scaling analysis indicated that the biological response to nutrients was driven by precipitation (Fig. 4a, b). For example, enrichment of macroalgae $\delta^{13}C$ occurred across all regions after June 2006 precipitation events (Table 3). Typical of shallow coastal ecosystems, nutrients increased primary production, which contributed to reduced water clarity via elevated levels of phytoplankton (Fig. 4c, d; Nixon et al. 2001). Macroalgae nitrogen incorporation was inferred from increased %N after freshwater inputs (Table 3).

Patterns at Broad Spatial Scale Identified by Macroalgae

Across these coastal lagoons, total nitrogen concentrations and macroalgae $\delta^{15}N$ values provided different information. Spatial patterns of total nitrogen concentrations (Fig. 5a) reflected physical ecosystem processes such as oceanic exchange, as concentrations were low near the two inlets near both ends of Assateague Island and higher in areas with poor flushing, such as Johnson Bay. Elevated macroalgae $\delta^{15}N$ in St. Martin River and southern Chincoteague Bay potentially indicated nitrogen sources, possibly from human or animal wastes, even though the concentrations of total nitrogen varied (Fig. 5a, b). This highlights the ability to interpret measurements of $\delta^{15}N$ in bioindicators as inputs of human or animal wastes even when total nitrogen concentrations are low. Experimental evidence, such as that with the macroalgae *Enteromorpha*, suggests that $\delta^{15}N$ values are independent of total nitrogen concentration, though the rate of ^{15}N incorporation varies by the form of inorganic nitrogen (Cohen and Fong 2005).

Isotopic values can provide evidence of nitrogen source, particularly in conjunction with land use. Macroalgae were enriched with $\delta^{15}N$ in areas where land uses suggested a possible presence of septic and manure sources of nitrogen. Examples include St. Martin River (17.2% residentially developed watershed largely reliant on septic systems) and the adjacent Assawoman Bay (18.9% residentially developed watershed, Table 1). These results align with quantitative linkages that have been made between urban development and enriched $\delta^{15}N$ values in primary consumers (Vander Zanden et al. 2005). Animal agriculture, with isotopically enriched manure byproducts, was another comparatively prominent land use feature of these regions (1.8% and 1.4%, respectively, Table 1). St. Martin River, the region with the highest total nitrogen, exhibited a gradient decreasing downstream, suggesting terrestrial nitrogen inputs which diluted upon mixing with higher salinity water from ocean exchange. Yet macroalgae $\delta^{15}N$ values in these regions were elevated in the broad (June 2004) survey (Fig. 5b) and in the fine-scale survey prior to rain events (May 2006). While a total nitrogen concentration gradient suggested terrestrially derived nitrogen inputs, septic and/or manure sources were inferred to be important nitrogen sources for St. Martin River and Isle of Wight based upon enriched macroalgae $\delta^{15}N$ values.

The town of Chincoteague, VA (population 4,317; 173.1 people per kilometer, US Census Bureau 2000) is situated atop sandy soils and potentially contributes nitrogen via

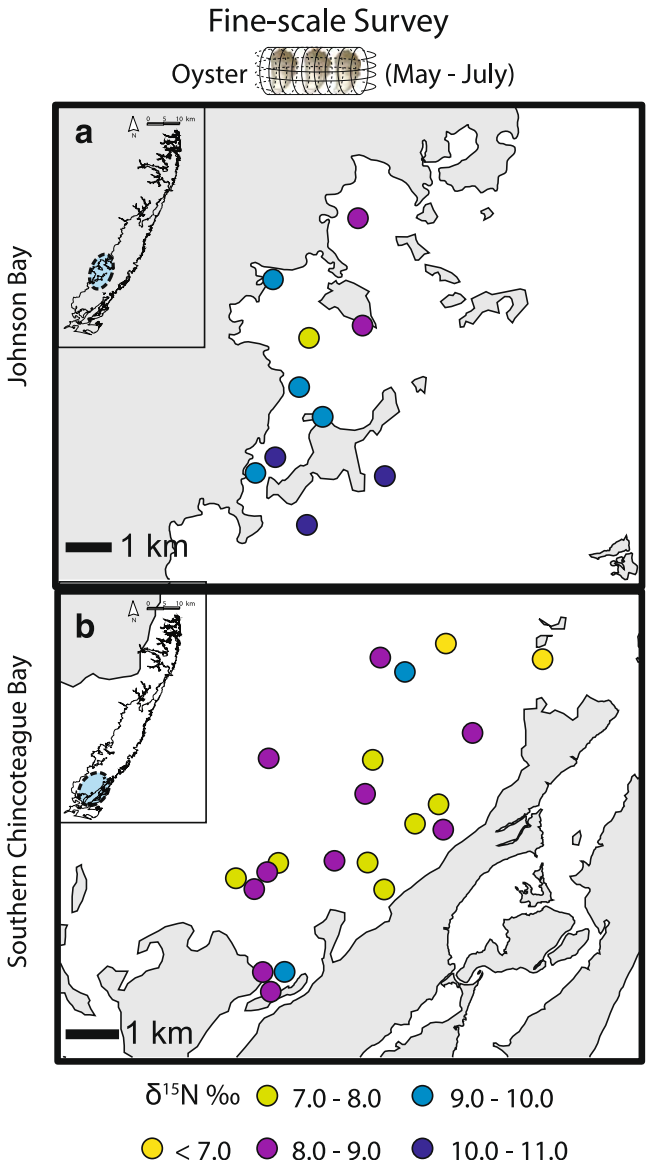

Fig. 6 Fine spatial scale survey (2006) of oyster $\delta^{15}N$ values. Spatial patterns within Johnson Bay (**a**) and southern Chincoteague Bay (**b**) detected with oyster $\delta^{15}N$ values

septic systems, as evidenced by enriched $\delta^{15}N$ values in macroalgae in the surrounding estuarine waters. This town comprises much of the residential development (1.5% of the total watershed area) in the Chincoteague Bay watershed and relies entirely on septic systems. In addition to elevated $\delta^{15}N$, increased concentrations of total nitrogen would be expected from this potential nitrogen source; however, southern Chincoteague Bay had the lowest total nitrogen at both broad (Fig. 5a) and fine spatial scales (Fig. 3h), likely due to small watershed size, expansive and intact wetlands, and physical processes including dilution and ocean flushing (Wazniak et al. 2007).

Fine Spatial Scale Potentially Indicates Sources Despite Lack of Spatial Patterns

At the fine spatial scale, patterns emerged from oyster $\delta^{15}N$ values, but not from macroalgae $\delta^{15}N$ values. North–south gradient patterns in Johnson Bay (Fig. 6a) and southern Chincoteague Bay (Fig. 6b) were detectable in oyster muscle $\delta^{15}N$. These gradients agreed with broad patterns from June 2004 macroalgae $\delta^{15}N$ (Fig. 5b). Though oyster muscle $\delta^{15}N$ gradients were slight, homogeneously elevated values implicated septic sources of nitrogen in southern Chincoteague Bay, likely from the town of Chincoteague, VA. In a similar study, spatial homogeneity of elevated $\delta^{15}N$ among hard clam tissues (*Mercenaria mercenaria*) along a eutrophication gradient has been attributed to anthropogenic sources, suggesting that elevated $\delta^{15}N$ in mollusks can still indicate nitrogen source despite a lack of spatial pattern (Oczkowski et al. 2008). Furthermore, oyster $\delta^{15}N$ values in this study were similar, though somewhat

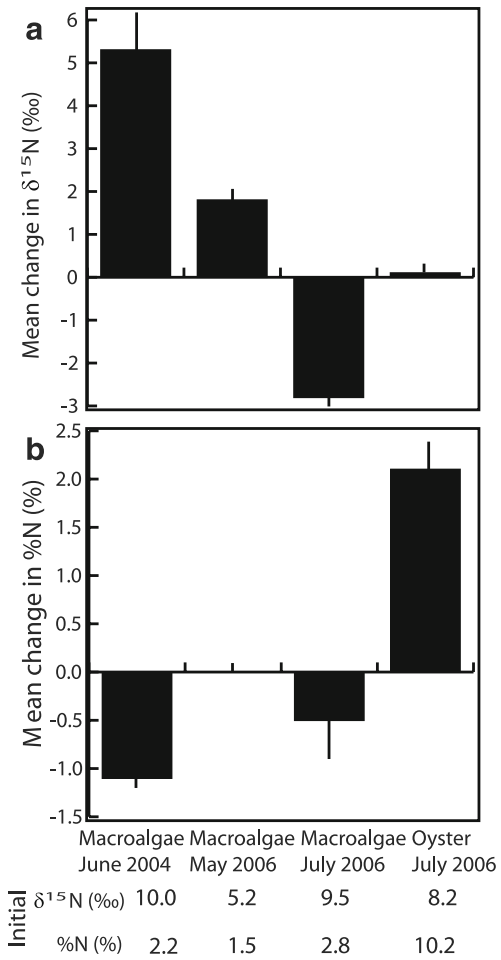

Fig. 7 Change in $\delta^{15}N$ (**a**) and %N (**b**) of macroalgae and oyster from mean initial values in June 2004 and May and July 2006

lower (8.5±0.1‰), to muscle tissue (9.4±0.2‰ and 16.0± 2.3‰) of an Australian oyster species (*Saccostrea glomerata*) influenced by wastewater treatment effluent within 50 m (Piola et al. 2006).

Bioindicator Species Comparison

The growing literature on biological indicators suggests that $\delta^{15}N$ in organisms sampled from natural communities consisting of various taxonomic groups, including macrophytes (e.g., McClelland et al. 1997; Cole et al. 2004; Cohen and Fong 2006), finfish (Lake et al. 2001), and mollusks (Fila et al. 2001; McKinney et al. 2002; Vander Zanden et al. 2005), or some combination (Gartner et al. 2002; Fry et al. 2003) can identify nitrogen sources. Additionally, manipulative deployment of macroalgae has been used to interpolate spatial patterns in anthropogenic sources of nitrogen through experimental field work (e.g., Udy and Dennison 1997; Costanzo et al. 2001). The current study combines the benefits of each technique, providing direct comparison between taxonomic groups of primary producers and consumers along with the ability to interpolate spatial patterns based on a manipulative field design in areas where natural communities may not be currently or readily available.

The presence or absence of spatial patterns in $\delta^{15}N$ in macroalgae and oysters at different spatial scales provides a spatial context in which each can be usefully deployed as a biological indicator. While clear spatial patterns in macroalgae $\delta^{15}N$ and %N emerged at the broad spatial scale in June 2004 (Fig. 5b, c), spatial patterns in δ^{15} N or %N were not obvious for macroalgae deployed at the fine spatial scale (either May or July 2006). Macroalgae $\delta^{15}N$ values were homogenously <10‰ throughout Johnson Bay and southern Chincoteague Bay in both May and July 2006. Therefore, macroalgae may potentially be more usefully deployed as biological indicators of nitrogen source at a broad scale (100 s of km^2) rather than at the fine spatial scale (10 s of km^2). The slight gradients in Johnson Bay (Fig. 6a) and southern Chincoteague Bay (Fig. 6b) suggest that oyster $\delta^{15}N$ may indicate potential nitrogen source at fine spatial scales (10 s of km^2).

In this study, manipulative deployments of macroalgae over multiple years in conjunction with deployment of oysters provided a comparison of isotopic responses to water chemistry factors (i.e., the nutrient pulse) in addition to the comparison between species. Macroalgae $\delta^{15}N$ and %N exhibited smaller changes from initial values after receiving more precipitation during 2006 in the fine-scale survey than the drier 2004 broad-scale survey (Fig. 7a, b). Decreased regional mean macroalgal $\delta^{15}N$ values with increased standard errors from May to July 2006 (Table 3) and the undefined spatial patterns in Johnson Bay can be explained by a relatively short nitrogen turnover rate. Quick turnover rates in macroalgae result in rapid response by $\delta^{15}N$ to environmental conditions as compared to slower tissue turnover rates in tissues of consumers such as oysters (Moore 2003; Cohen and Fong 2005). Similar to other studies (e.g., Gartner et al. 2002 and Fry et al. 2003), responsiveness to nitrogen cycling, as reflected in changes to $\delta^{15}N$ and %N, was greater in macroalgae than in oysters (Fig. 7a, b), likely due to relative physiological turnover times, days for macroalgae and weeks for oysters. Between regions of these coastal lagoons, patterns of oyster tissue $\delta^{15}N$ values (July 2006; Table 3) were more similar to previous macroalgae $\delta^{15}N$ values (May 2006; Table 3) than to concurrent macroalgae $\delta^{15}N$ values (July 2006; Table 3) and did not reflect short-term nutrient pulses from the June 2006 precipitation events.

Interpretations of $\delta^{15}N$ in macroalgae and oysters to infer nitrogen source may be influenced by water chemistry factors as well as the spatial scale of interest. Isotopic signals can be influenced by physical conditions such as salinity, temperature, or depth (Jennings and Warr 2003) and are variable in strength. Water chemistry measurements varied over time in these coastal bays, as evidenced by a wide range of macroalgae $\delta^{15}N$ values in 2004 (Fig. 5b) and a smaller range of macroalgae $\delta^{15}N$ values with no clear finer scale spatial pattern in 2006. Since spatial patterns were detectable in 2006 by oyster $\delta^{15}N$, perhaps these tissues are less susceptible to variability in water chemistry due to oyster physiology (Fig. 7a, b).

A combination of indicator species responsiveness and ecosystem features may affect the success of indicating nitrogen source. For example, oceanic mixing and short residence times in deep waters offshore southwestern Australia may have dispersed $\delta^{15}N$ signals before transmission from organic sources to filter feeders via food sources, though more responsive macroalgae reflected sewage effluent sources (Gartner et al. 2002). In another study in the northeast Atlantic, Jennings and Warr (2003) found that most spatial $\delta^{15}N$ variability in scallops is related to physical conditions (salinity, depth, and temperature). Comparatively, the shallow coastal lagoons of the present study are characterized by residence times on the order of weeks (Pritchard 1960; Lung 1994); potentially enough time for $\delta^{15}N$ signals to persist and be incorporated into oysters, provided a sufficient time period for oyster uptake and assimilation.

Variation in responsiveness based on physiological differences between primary producers and filter feeders may potentially introduce a lag time in oyster $\delta^{15}N$ values compared those of macroalgae. The greater absolute changes found in macroalgae $\delta^{15}N$ compared to oyster $\delta^{15}N$ (Fig. 7a) suggest a more rapid response to nitrogen source, likely due to relative physiological turnover times.

For example, when comparing across regions, oyster $\delta^{15}N$ values (July 2006) more closely resemble macroalgae $\delta^{15}N$ values from May 2006 (Johnson Bay > St. Martin River > Public Landing > southern Chincoteague Bay) than macroalgae $\delta^{15}N$ values from July 2006 (Table 3). This discrepancy between macroalgal and oyster $\delta^{15}N$ values may have been magnified by a time lag due to different rates or modes of nitrogen assimilation. Macroalgae assimilate nitrogen directly from the water column (e.g., Cohen and Fong 2006), while oysters receive their nitrogen indirectly from the water column (via consumption of a variety of nitrogen sources, for example microorganisms, phytoplankton, detritus and inorganic particles) to reflect ambient $\delta^{15}N$ (Newell and Langdon 1996; Cohen and Fong 2005). Due to the rate and timing of nitrogen assimilation, oysters integrate nitrogen in the muscle over longer time periods than macroalgae (Moore 2003). Future studies could investigate the possibility of a lag time in oyster $\delta^{15}N$ response as compared to macroalgae due to variations in length of nitrogen incorporation.

In addition to differences between macroalgae and oysters, different species within a functional group may provide different temporal integrations based on species-specific turnover rates. Muscle tissues in different species of filter feeding bivalves vary, e.g., ~2 months for the eastern oyster (*C. virginica*), >3 months for Sydney rock oyster (*Saccostrea commericalis*; Moore 2003), and >1 year for a methanotrophic hydrocarbon seep mussel (*Bathymodiolus childressi*; Dattagupta et al. 2004). Because the diet of filter feeders includes multiple trophic levels (e.g., primary producers, detritus, etc.), which are separated by 2–3‰ (Fry 2006), mixtures of trophic levels may confound interpretation of $\delta^{15}N$. In certain cases, spatial gradients in $\delta^{15}N$ could reflect variability in available diets. Yet identifying human and animal waste as potential nitrogen source to these bays fits with recent degradation of water quality and increases in total nitrogen identified by long-term monitoring datasets (Wazniak et al. 2007). Multiple temporal integrations among species allow different monitoring questions to be addressed by different biological indicators. Macroalgae and oysters may be suited for different roles as biological indicators, but they both may have the potential to indicate nitrogen sources at various spatial and temporal scales, which will help focus nutrient management.

Acknowledgments Thanks to J. Davis, A. Dungan, M. Hall, J. Schofield, E. Sherry, B. Sturgis, J. Testa, C. Wazniak, and C. Wicks for field and lab assistance, as well as Horn Point Analytical Services and UC Davis Stable Isotope Facility. J. O'Neil provided laboratory space. Thanks also to E. Fertig, T. Fisher, R. Jesien, D. Meritt, R. Newell, B. Sturgis and C. Wazniak for invaluable discussions and insight. Assateague Island National Seashore provided precipitation monitoring data. The authors thank the Maryland Coastal Bays Program and the NOAA OCRM for grant support, and the Maryland Coastal Bays Oyster Gardening Program for oysters. This is UMCES contribution number 4268.

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