δ^{15}N and δ^{13}C reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass

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ABSTRACT: Eutrophication forces shifts in estuarine producer assemblages from seagrass meadows to communities dominated by macroalgae. This restructuring of the benthic producer assemblage presents challenges and alters food resources available to consumers. We examined food web relationships in 3 sub-estuaries of the Waquoit Bay system, Massachusetts, USA, that span a gradient of relatively low nitrogen loads of 5, 10, and 34 kg N ha^{-1} yr^{-1}. We detected effects on benthic macrophytes across this range of N loads with reduced seagrass cover and higher macroalgal biomass as N loading increased. The difference in N loading to these estuaries could also be detected in δ^{15}N signatures of the benthic community. Producers and consumers were enriched in δ^{15}N in response to increasing N load and incorporation of isotopically heavy nitrogen from wastewater sources. The differences in the relative availability of seagrass and macroalgae associated with the N load gradient were reflected in consumer diets. δ^{13}C of producers were similar among the estuaries, but for consumers the isotopic signatures differed, suggesting they did not feed on the same primary producer sources. Using IsoSource, we modeled the diets of 2 herbivores, the amphipod *Cymadusa compta* and the isopod *Erichsonella filiformis*. At the lower N loads, more seagrass and detritus were consumed, whereas at the high N load where macroalgae was the dominant vegetation type, algae dominated the diet. The resulting C isotopic signatures appear to have permeated to higher trophic levels, suggesting C flow through the system differs across the range of N loads sampled. In the most pristine estuary, the overall food web was supported mainly by seagrass, its epiphytes and detritus, but macroalgal C was more important where this was the dominant type of macrophyte. These results demonstrate that C pathways through coastal food webs may be altered at earlier stages of eutrophication than previous studies have suggested.

KEY WORDS: Eutrophication · Nitrogen load · *Zostera marina* · Herbivore · Isotope tracers · Wastewater · Macrophyte

INTRODUCTION

Nutrient inputs from anthropogenic activities have led to widespread eutrophication of estuarine and coastal environments (Duarte 1995, Bricker et al. 2007). The effects of excessive nutrient inputs include altered structure of benthic primary producer commu-nities through proliferation of rapidly growing macro-algae and phytoplankton, which shade seagrasses and cause a shift from seagrass meadows to algal-dominated communities in estuaries (Valiela et al. 1992, 1997, Hauxwell et al. 2001, 2003, McGlathery 2001). The increase of free-floating macroalgae at the expense of rooted vegetation brings about changes to

the abiotic environment that present challenges to benthic fauna including loss of habitat structure and changes to the physical and chemical conditions of the sediment and water column (Orth et al. 2006, McGlathery et al. 2007). Shifts in benthic producer communities also alter the quality of live and detrital food resources available to consumers because macroalgae and seagrasses differ considerably in nutritional value, chemical defenses, and structural components (Harrison & Mann 1975, Zapata & McMillan 1979, Thayer et al. 1984, Hay & Fenical 1988). The composition of benthic consumer diets may change in relation to the availability, supply and quality of different food sources. Fish and invertebrates have been shown to change diets along a gradient from upper to lower estuary, reflecting changes in the relative contribution of terrestrial and estuarine or marine sources of organic matter (Peterson et al. 1985, Deegan & Garritt 1997, Doi et al. 2005, Yokoyama & Ishihi 2007). Shifts in the estuarine benthic producer community composition also affect consumer diets, e.g. as seagrass is lost (McClelland & Valiela 1998), or are due to changes in the composition of detritus (Keats et al. 2004). Because of the apparent plasticity in benthic consumer diets and their ability to opportunistically make use of food available in their surroundings, the relative density and distribution of different food sources are also likely to be important in determining dietary composition. Eutrophication-induced shifts in benthic producer community composition may therefore have implications for carbon pathways through the benthic food web.

To study changes in consumer diets we can use stable isotopic techniques. Producer $\delta^{13}C$ depends largely on the degree of fractionation during C assimilation (Smith & Epstein 1971, Lajtha & Marshall 1994) and the signatures of the available C sources (Beer et al. 2002, Raven et al. 2002), resulting in different ranges of $\delta^{13}C$ signatures for different producer types (Fry & Sherr 1984, Hemminga & Mateo 1996). $\delta^{13}C$ is most useful for differentiating among producer sources at the base of food webs, because consumers tend to have $\delta^{13}C$ signatures similar to those of their food (DeNiro & Epstein 1978, Fry & Sherr 1984). For estuarine food webs, the average ^{13}C enrichment is +0.5‰ per trophic level (Peterson & Fry 1987, France & Peters 1997).

N isotopes have also been used to define food web relationships (Peterson & Fry 1987). Typically, consumers become enriched in $\delta^{15}N$ compared to their food source by 3 to 4‰ (Minagawa & Wada 1984, Michener & Schell 1994, Post 2002, Vanderklift & Ponsard 2003), so that the $\delta^{15}N$ of an organism can predict trophic position in the food web (Wada et al. 1991). There is, however, considerable variation in fractionation both among species (Parker et al. 2008)

and depending on the food source (Crawley et al. 2007).

N stable isotopes can be used as sensitive tools to detect incipient nutrient enrichment. Nutrients transported from the watershed to the estuary carry isotopic signatures that are characteristic of land uses on the watershed (McClelland et al. 1997, Cole et al. 2004). Nitrate from atmospheric sources typically have $\delta^{15}N$ signatures between 0.5 and 8‰ (Kreitler & Jones 1975, Gormly & Spaulding 1979, Kellman & Hillaire-Marcel 2003), $\delta^{15}N$ signatures of nitrate from synthetic fertilizers range from −3 to 3‰ (Freyer & Aly 1974, Macko & Ostrom 1994, Kellman & Hillaire-Marcel 2003), and nitrate from human and animal wastes typically have a $\delta^{15}N$ signature that is enriched relative to other sources of N ranging from 9 to 25‰ (Kreitler et al. 1978, Kreitler & Browning 1983, Rolston et al. 1996). Several studies have shown an overall shift in producers and consumers towards heavier $\delta^{15}N$ signatures as a result of increasing urbanization of watersheds leading to increases in the N loads and relative contribution by wastewater (McClelland et al. 1997, Yamamuro et al. 2003, Tewfik et al. 2005, Martinetto et al. 2006).

Despite the large amount of published literature describing the effects of seagrass loss on both producer and consumer communities, there is little information on what changes take place in seagrass communities during the early stages of eutrophication, before seagrass is completely lost from an estuary. In this study, we take advantage of the sub-estuaries of Waquoit Bay, Massachusetts, USA, that receive different N loads imparted from different land uses on their watersheds (Valiela et al. 1992, 2004, Kroeger et al. 1999, Valiela & Bowen 2002, Bowen et al. 2007). We examined 3 of the sub-estuaries that receive relatively low N loads (<35 kg N ha^{-1} yr^{-1}) and still support eelgrass *Zostera marina* meadows. The overall goal of this study was to examine the effects of small differences in N loading on benthic producers and consumers and C flow through the food web. Our aims were 3-fold. First, we examined whether the community structure of benthic macrophytes showed differences associated with N loading. Second, we assessed whether $\delta^{15}N$ signatures of estuarine producers and consumers are useful tracers of increases in anthropogenically derived N at low N loads. Third, we examined whether changes in producer community structure, specifically the relative biomass of seagrass and macroalgae, affected C flows through estuarine benthic food webs.

MATERIALS AND METHODS

Site description. Waquoit Bay and its sub-estuaries, located on the south coast of Cape Cod, Massachu-

setts, are representative of shallow groundwater-fed estuaries in the northeastern USA (Fig. 1). The estuaries in the system are similar in residence time (1 to 2 d), depth (1 to 3 m), and experience similar tidal and climatic patterns, but receive different N loads due to differences in land uses and degrees of watershed urbanization (Valiela et al. 2000, 2004, Bowen & Valiela 2001; see Table 1). Over the past decades, benthic macrophyte communities have been restructured in these estuaries as a result of increasing N loads (Valiela et al. 1997, McClelland & Valiela 1998, Fox et

al. 2008). The estuaries with the highest N loads have high macroalgal biomass (Valiela et al. 1992, 1997, Fox et al. 2008) and have seen dramatic declines in eelgrass owing to macroalgal shading (Valiela et al. 2000, Hauxwell et al. 2003).

The sub-estuaries of the Waquoit Bay system fulfill the assumptions for space-for-time substitution (Picket 1991), which makes this system an ideal location to study alterations to estuarine communities that occur over time in response to long-term watershed-scale changes in N inputs. The major mechanism of change
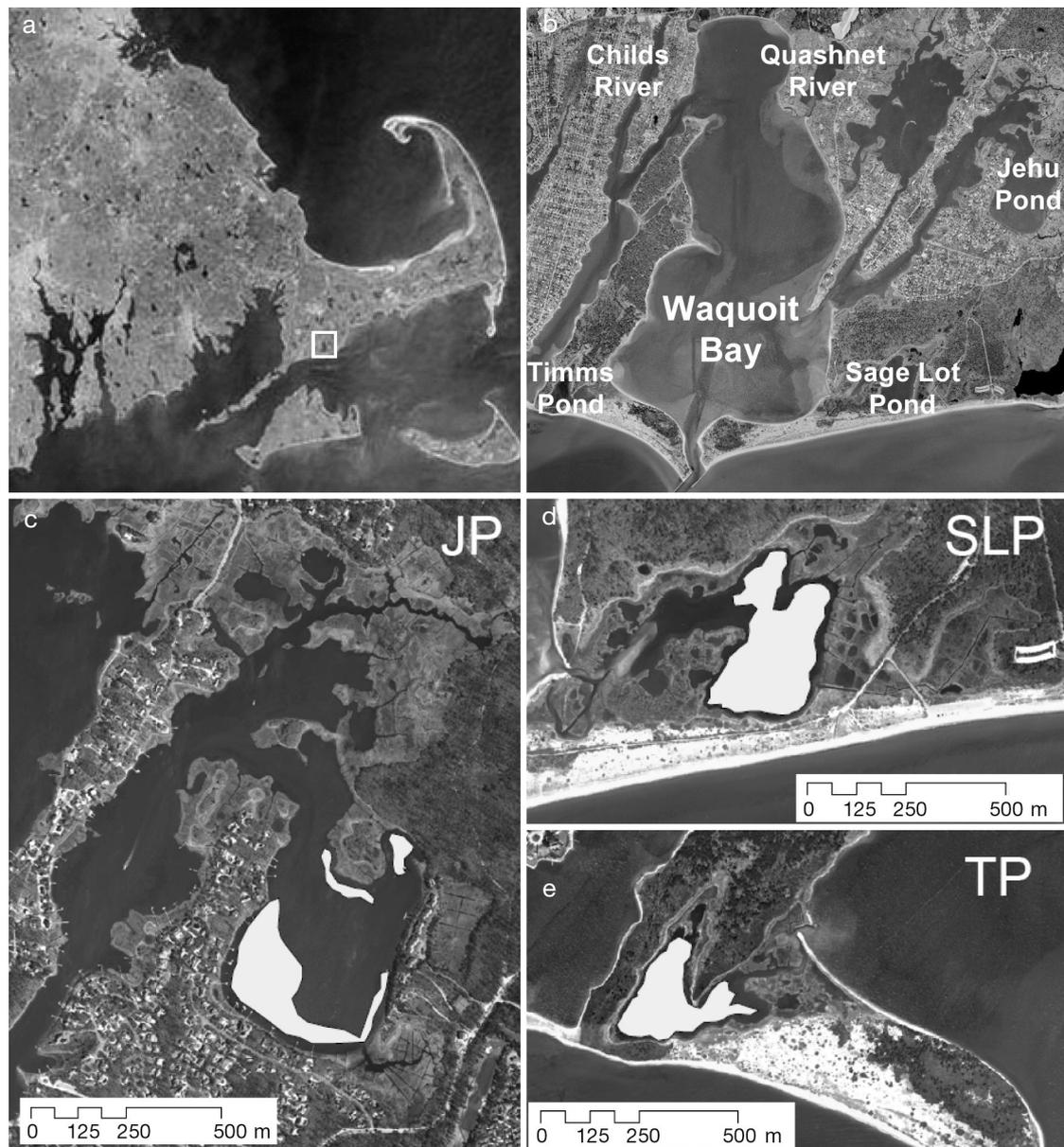

Fig. 1. (a) Waquoit Bay in Cape Cod, Massachusetts, USA, and (b) its sub-estuaries. White areas: seagrass areas in (c) Jehu Pond (JP), (d) Sage Lot Pond (SLP), and (e) Timms Pond (TP). Satellite images provided by Mass GIS

forced on the Waquoit Bay environment is develop-
ment of the watershed, specifically the increase in the
numbers of houses and decrease in the area of fringing
salt marsh, that have increased delivery of N to the
estuarine waters (Valiela et al. 1992, 1997). Previous
studies in Waquoit Bay have argued that although
there may be differences among the estuaries, the dif-
ferences in N loading received from land overwhelm
the influence of other potential controls on nutrient
and oxygen concentrations, phytoplankton, microphy-
tobenthos, macrophyte biomass, and higher trophic
levels and food webs. Comparisons among the sub-
estuaries of Waquoit Bay have previously been used to
examine the effects of different N loads at the water-
shed–estuary scale, both on abundances and diversity
of estuarine flora and fauna (Valiela et al. 1992, Dee-
gan et al. 2002, Fox et al. 2008, 2009) and on their sta-
ble isotopic signatures and food web relationships
(McClelland et al. 1997, McClelland & Valiela 1998,
Martinetto et al. 2006, Fox et al. 2009).

Today, eelgrass *Zostera marina* is present in only 3
Waquoit Bay sub-estuaries (see Table 1, Fig. 1). N
loads in these estuaries are the lowest found in the
Waquoit Bay system (see Table 1), with a very low load
in Timms Pond (5 kg N ha^{-1} yr^{-1}, 0% wastewater), low
in Sage Lot Pond (11 kg N ha^{-1} yr^{-1}, 23% wastewater),
and moderate in Jehu Pond (34 kg N ha^{-1} yr^{-1}, 58%
wastewater). To examine differences in the benthic
producer community structure and benthic food webs
across a range of relatively low N loads we sampled
the benthic communities in these 3 estuaries.

**Mapping eelgrass cover and estimating macrophyte
biomass.** Eelgrass in the Waquoit Bay estuaries can be
found growing in patches and larger continuous beds
(Fig. 1). We examined differences in the producer com-
munities among the 3 estuaries in 2 ways. First, we
mapped the locations and extent of eelgrass cover with
visual surveys by snorkeling and boat observations
and entered the data into ArcView (ESRI) (Fig. 1). Sec-
ond, we estimated shoot density, and eelgrass and
macroalgal biomass by sampling inside the continuous
eelgrass beds. This was done by collecting all above-
ground vegetation within a 0.073 m^2 circle at 8 random
locations within the seagrass bed in each estuary. We
counted the number of shoots and cleaned and dried
the seagrass blades and macroalgal fronds at 60°C to
determine dry wt.

**Determination of producer and consumer stable
isotopes.** To investigate the effects of N loads on pro-
ducer stable isotope ratios we sampled macroalgae
and seagrass from within the eelgrass beds in Timms
Pond, Sage Lot Pond, and Jehu Pond in August 2006.
Seagrass was collected from 3 randomly selected sites
in each estuary. Macroalgae were collected by hand or
using a net and pooled into 1 sample per species per

estuary. Samples were cleaned with deionized water.
It was not possible to distinguish between eelgrass and
microalgal seagrass epiphytes using δ^{15}N and δ^{13}C sig-
natures, so we measured the average isotopic signa-
ture of this 'seagrass–epiphyte complex', which will be
referred to as 'seagrass' from this point forward.

Benthic microalgal samples were collected from 3
randomly selected locations in Timms Pond, 3 in Sage
Lot Pong, and 2 in Jehu Pond and were isolated using
methods described by Couch (1989). Sediment was
collected from the top 0.5 cm and placed in a 1 cm thin
layer in 10 × 20 cm^2 dishes. A 63 µm mesh was placed
on the sediment and covered with a 2 to 3 mm thick
layer of combusted dune sand. The sand was moist-
ened with filtered seawater and a second mesh placed
on top. The dishes were placed under fluorescent light
for 12 h to allow upward migration of the microalgae.
The microalgae were then collected from the upper-
most mesh, filtered onto glass fiber filters and dried at
60°C in preparation for stable isotope analyses.

Benthic particulate organic matter (POM) was ex-
tracted from replicate samples of the top 2 cm of sedi-
ment from 3 randomly selected locations within each
estuary. Each sediment sample was decarbonated
using 0.1N HCl to remove carbonates for δ^{13}C analysis
(Jacob et al. 2005). δ^{15}N analysis was analyzed on sed-
iments that were not acidified.

To determine how linkages among species in the
benthic food webs differed among the 3 estuaries we
pooled fauna collected from 3 randomly selected sites
within the eelgrass beds in each estuary. Samples
were collected by hand or with a net, put on ice, and
taken to the laboratory for sorting and processing.
Fish were dissected to remove their digestive tracts,
bones, and scales. Large specimens with calcified
shells were dissected, and guts and shell material
were discarded. Smaller specimens were processed
whole after placing individuals in containers with fil-
tered seawater for 24 h to allow for evacuation of gut
contents. At least 7 to 10 specimens of the same spe-
cies from each estuary were pooled. Consumer sam-
ples were rinsed with deionized water and dried at
60°C. All sediment, algal, seagrass, and consumer
samples were then ground to fine powder, weighed,
and loaded into tin capsules.

C and N stable isotope analyses were performed by
mass spectrometry at the stable isotope facilities at
University of California (UC) Davis, CA, or at The
Ecosystems Center, Marine Biological Laboratory,
Woods Hole, MA. Results were calculated based on
comparisons with known laboratory standards (citrus
leaves at The Ecosystems Center and a mixture of
ammonium sulfate and sucrose at UC Davis) that had
been calibrated against NIST Standard Reference
Materials. Stable isotope ratios were reported as per

mille (‰) using delta notation determined from the equation:

$$\delta^{13}C \text{ or } \delta^{15}N \, (‰) \; = \; [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3 \tag{1}$$

where R is ($^{15}N/^{14}N$) or ($^{13}C/^{12}C$). Duplicate determinations on the same sample differed by <0.2‰. Tissue %C and N were also obtained from this analysis.

Using $\delta^{15}N$ to trace N sources. To determine if $\delta^{15}N$ of producers and consumers were correlated to N load of the estuary where they were collected we first assigned the taxa to trophic levels based on the published literature. We then pooled species into groups of herbivores, detritivores, omnivores, and predators based on their feeding preferences. The isotopic signature for each group was plotted as a function of N load. To give a more comprehensive view of $\delta^{15}N$ signatures of producers and consumers over the full range of N loads to Waquoit Bay sub-estuaries we included isotopic data from 2 estuaries at the higher end of the N load spectrum (Quashnet and Childs Rivers) that were collected during a previous study done in these estuaries (Martinetto et al. 2006).

Establishing food web links. We evaluated differences in consumer diets and food webs among the 3 estuaries sampled in 2 ways. First, we examined biplots of $\delta^{15}N$ and $\delta^{13}C$ signatures of producers and consumers from each of the 3 estuaries. Second, to identify the relative proportions of different producers in herbivore diets we modeled dietary composition for the 2 most abundant herbivores, the amphipod *Cymadusa compta* and the isopod *Erichsonella filiformis* using the stable isotope mixing model IsoSource version 1.3 (Phillips & Gregg 2003, Phillips et al. 2005). Consumer $\delta^{15}N$ signatures were low compared to those of primary producers, and using typical rates for trophic enrichment did not yield any model results. We therefore modeled $\delta^{13}C$ mixing in the estuarine consumers in IsoSource, since $\delta^{13}C$ signatures are the most useful for identifying C flow from producers to consumers in food webs (DeNiro & Epstein 1978, Fry & Sherr 1984). This approach has been successfully used in previous studies of benthic consumer diets where consumer $\delta^{15}N$ signatures did not reveal clear enrichment to any predictable food sources (Jephson et al. 2008). The model calculates all possible diet source combinations using diet composition increments of 5% where the predicted and observed mixture $\delta^{13}C$ signatures matched within ±0.1‰. We used a trophic fractionation of 0.5‰ based on literature values for estuarine herbivores (France & Peters 1997, Post 2002). To evaluate the importance of different functionally related producer groups in herbivore diets, we combined the results for macroalgae *a posteriori* according to Phillips et al. (2005).

Statistical analysis. To assess differences in the macrophyte communities among the 3 estuaries mean biomasses of seagrass and macroalgae and seagrass shoot density were compared using 1-way ANOVAs with Tukey's post hoc tests. The relationship of $\delta^{15}N$ signatures of primary producers and consumers versus N load was described using linear regressions. $\delta^{15}N$ data were $\log_{10}$-transformed to fulfill the assumption of normality, and a regression analysis was carried out for each group. Although the statistical comparison was conducted on transformed data, non-transformed values (means ± SE) are shown in figures and tables.

Percent tissue C and N of potential food sources classified as macroalgae, seagrass, detritus, and benthic POM were compared among estuaries using 1-way ANOVAs and pairwise comparisons with Tukey's post hoc tests. To evaluate differences in C flow among estuaries differences in $\delta^{13}C$ signatures were tested for producers and consumers. Producers were split into functional groups (seagrass, macroalgae, microalgae, and POM) and fauna were split into 3 trophic levels (herbivores + detritivores, omnivores + deposit feeders, and predators). The $\delta^{13}C$ signatures of each group were then examined for inter-estuary differences using 1-way ANOVAs and pairwise comparisons with Tukey's post hoc tests. Descriptive statistics for the diets of selected herbivores were obtained from the IsoSource program.

RESULTS

Seagrass bed extent and macrophyte biomass

Benthic macrophyte assemblages displayed changes associated with the relative degree of N loading in the estuaries. The percent of estuary covered by eelgrass decreased along the gradient of increasing N load (Table 1, Fig. 1), but there were no significant differences among the 3 estuaries in eelgrass biomass and shoot density in the interior of the seagrass meadows (Table 1, Fig. 2). Macroalgal biomass sampled inside the seagrass beds was significantly different among the estuaries and was related to N load. Macroalgal biomass was lowest in the least impacted estuary and increased with increasing N load (Table 1, Fig. 2). As a result, the ratio of macroalgal to seagrass biomass differed among the estuaries (Fig. 2). At the very low N load, seagrass biomass dominated, and macroalgal biomass was 0.16 times that of seagrass. At the intermediate N load, the ratio of macroalgal to seagrass biomass was 0.67, and at the moderate N load, the biomass of macroalgae was larger than that of seagrass (ratio = 1.30).

Table 1. Nitrogen load, sources, water column nutrient concentrations, and seagrass shoot density and biomass in sub-estuaries of Waquoit Bay. TP: Timms Pond; SLP: Sage Lot Pond; JP: Jehu Pond; QR: Quashnet River; CR: Childs River. Mean ± SE shown where available. Data for N load and relative contributions of the different sources were calculated using the NLM model within NLOAD (http://nload.mbl.edu). One-way ANOVAs were performed on seagrass biomass and shoot density, and on macroalgal biomass. No significant differences found between estuaries for seagrass biomass and shoot density (1-way ANOVA, biomass, df = 2, $F = 0.027$, p > 0.05; shoot density, df = 2, $F = 0.996$, p > 0.05). TP < SLP < JP in terms of macroalgal biomass (1-way ANOVA, df = 2, $F = 19.15$, p < 0.001). Sources: A, unpubl. data; B, present study; C, annual QR and CR means from Holmes (2008)

| | Estuary | | | | | Sources |
	TP	SLP	JP	QR	CR	
N load (kg N ha^{-1} yr^{-1})	5	11	34	404	616	A
Nitrogen sources (%)^a						
Wastewater	0	23	58	29	67	A
Fertilizer	0	45	21	22	12	A
Atmosphere	100	32	20	32	17	A
Nutrient concen. (µM)						
NO$_3$	1.4 ± 0.2	0.7 ± 0.01	2.1 ± 0.7	8.0 ± 0.8	20.6 ± 2.5	B, C
NH$_4$	0.3 ± 0.03	1.5 ± 0.1	0.3 ± 0.01	2.3 ± 0.3	1.6 ± 0.2	B, C
PO$_4$	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.04	0.23 ± 0.02	0.22 ± 0.04	B, C
Macrophytes						
Within seagrass bed						
Seagrass biomass (dry wt g m^{-2})	95 ± 31	104 ± 26	99 ± 29	0	0	B
Seagrass shoot density (no. m^{-2})	387 ± 76	345 ± 48	279 ± 30	0	0	B
Macroalgal biomass (dry wt g m^{-2})	14 ± 2	70 ± 7	128 ± 22	0	0	B
Within estuary						
Seagrass cover (% of estuary area)	70	54	12	0	0	B

^{a}Where individual sources do not add up to 100%, there was an additional source of N from up-gradient intercepting ponds
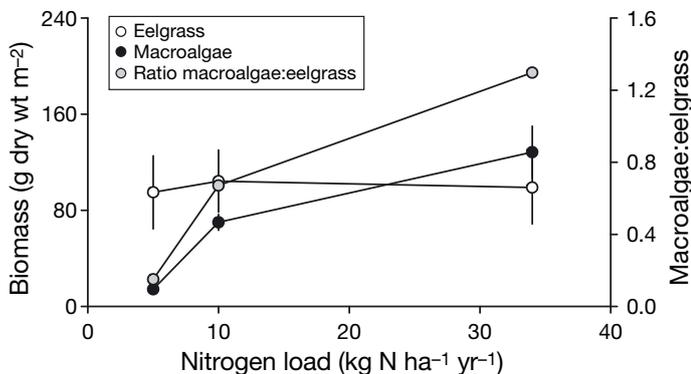

Fig. 2. Biomass (means ± SE) of eelgrass *Zostera marina* and macroalgae measured within seagrass beds in Waquoit Bay sub-estuaries receiving different N loads. Grey circles indicate the ratio of macroalgae to seagrass biomass by dry weight

δ^{15}N signatures of consumers and producers in relation to N loading

δ^{15}N signatures differed considerably among estuaries across the gradient of N loading and reflected inputs of nitrate from wastewater with a heavy δ^{15}N signature. Seagrass, microalgae and macroalgae increased in δ^{15}N by ~0.7‰ from the lowest to the intermediate load, and 1.7 to 2‰ in the highest N load.

The increase in detritus δ^{15}N was somewhat steeper, which was a reflection of differences in the composition of the material in the detrital pool as well as an increase in the δ^{15}N signature of its individual components. Consumer δ^{15}N signatures increased from the lowest N load to the intermediate by 1 to 2‰, and to the highest N load by 3.7 to 4.6‰. Regression analysis showed significant positive increases in δ^{15}N with increasing N load for all groups with the exception of microalgae and strict deposit feeders and detritivores, most likely due to small number of replicates in these 3 categories (Table 2, Fig. 3).

δ^{15}N and δ^{13}C signatures of producers and consumers

Within each estuary, δ^{15}N signatures reflected the different trophic levels of producers and consumers. Seagrass had the lowest δ^{15}N signatures whereas macro- and microalgae were heavier by ~3 and 2‰ respectively. The δ^{15}N signatures of detritus were similar to those of seagrass, reflecting a large contribution of seagrass to this pool of material (Table 2, Fig. 4). POM had δ^{15}N signatures slightly heavier (0.2 to 1‰) than those of seagrass and ~2.5‰ lighter than macroalgae in all 3 estuaries.

Herbivores had very low δ^{15}N signatures compared to primary producers in the system. In the estuary with the

lowest N load, herbivore δ^{15}N signatures were <0.6‰ heavier than potential food sources, in the intermediate estuary they were <1.8‰, and in the estuary with the moderate N load <3.1 ‰ (Fig. 4). Omnivores were enriched compared to herbivores by 1.8 to 2.6‰ (Table 2). The deposit feeder *Sclerodactyla briareus* had a signature intermediate to those of omnivores and predators. Differences in δ^{15}N signatures among consumers indicated typical fractionation with an average of 3.7 to 4.5‰ between herbivores and predators.

Table 2. δ^{13}C and δ^{15}N signatures (mean ± SE) of benthic producers and consumers for Timms Pond, Sage Lot Pond, and Jehu Pond. Each replicate sample (n) was pooled from a minimum of 7 specimens. Organisms are divided into trophic groups determined from published literature (Hsueh et al. 1992, Pollock 1998, Stachowicz & Hay 1999, Rouse & Pleijel 2001). POM: particulate organic matter; unid.: unidentified

| Taxa	Timms Pond			Sage Lot Pond			Jehu Pond		
	δ^{13}C (‰)	δ^{15}N (‰)	n	δ^{13}C (‰)	δ^{15}N (‰)	n	δ^{13}C (‰)	δ^{15}N (‰)	n
Producers									
Seagrass									
Zostera marina	−7.7 ± 0.8	1.2 ± 0.3	3	−10.9 ± 0.7	1.9 ± 0.4	3	−11.2 ± 0.3	3.5 ± 0.1	3
Macroalgae									
Agardhiella sp.	−21.3	4.8	1	−18.6	3.8	1	−18.7	6.9	1
Chaetomorpha sp.				−19.1	5.8	1			
Codium fragile	−11.3	5.9	1	−12.3	5.7	1	−14.0	5.9	1
Dasya baillouviana	−15.6	4.6	1				−18.6	7.5	1
Enteromorpha sp.	−17.3	4.7	1				−20.8	7.4	1
Fucus vesiculosus	−12.7	4.7	1	−14.2	5.8	1			
Gracilaria tikvahiae				−19.1	5.8	1	−17.6	6.6	1
Polysiphonia sp.	−17.7	5.6	1	−21.9	5.7	1	−21.7	5.5	1
Red epiphyte unid.	−16.0	2.4	1				−20.7	4.7	1
Ulva lactuca	−12.5	4.3	1	−14.6	5.1	1	−12.7	5.7	1
Benthic microalgae	−13.7 ± 0.5	3.0 ± 0.5	3	−12.4 ± 0.5	3.6 ± 0.7	3	−17.7 ± 1.6	5.1 ± 1.0	2
Detritus	−9.0	1.4	1	−11.4	1.7	1	−14.1	4.3	1
POM (sediment)	−16.1± 0.1	2.1 ± 0.7	3	−17.6 ± 0.4	2.5 ± 0.4	3	−19.2± 0.2	3.4 ± 0.2	3
Detritivores									
Orbinia ornata				−13.7	4.1	1	−18.3	6.6	1
Herbivores									
Edotea triloba				−10.9	3.3	1			
Erichsonella filiformis	−10.0 ± 0.3	1.9 ± 0.2	3	−11.3 ± 0.5	3.9 ± 0.0	3	−15.3 ± 0.4	6.0 ± 0.3	3
Cymadusa compta	−9.7 ± 0.2	1.5 ± 0.5	2	−12.7 ± 0.2	3.6 ± 0.2	4	−18.8 ± 0.3	6.7 ± 0.2	2
Haminoea sp.				−11.0	1.3	1			
Microdeutopus gryllotalpa				−12.7	3.3	1			
Omnivores									
Pagurus longicarpus				−13.6	5.7	1			
Hippolyte zostericola	−10.1	4.1	1	−12.8	7.2	1	−16.9	8.4	1
Libinia dubia	−8.9	2.9	1	−14.5	6.0	1	−19.0	8.1	1
Nassarius obsoletus	−10.9	5.3	1	−14.0	5.1	1	−15.7	8.1	1
Nereis succinea	−9.3	3.0	1	−11.2	4.6	1	−19.7	6.9	1
Palaemonetes pugio	−10.3	4.7	1	−13.5	6.6	1	−17.4	8.1	1
Palaemonetes vulgaris	−9.8	4.6	1	−12.8 ± 0.4	5.7 ± 0.3	3	−17.5	7.9	1
Panopeus herbstii				−11.5	5.1	1	−16.6	7.9	1
Rhithropanopeus harrisii							−16.4	8.2	1
Deposit feeders									
Sclerodactyla briareus	−12.1	5.1	1	−13.4	7.1	1	−15.6	8.9	1
Predators									
Anguilla rostrata				−22.1	8.8	1			
Callinectes sapidus	−10.2	5.2	1	−13.2 ± 1.1	5.9 ± 0.7	2			
Gobiosoma sp.	−9.6	6.8	1				−18.8	11.3	1
Lysianopsis alba				−12.23	5.5	1			
Nemerteans	−11.2	6.5	1	−13.7 ± 0.1	6.9 ± 0.2	2	−19.5	9.1	1
Podarke obscura	−11.9	5.5	1	−13.6	7.6	1	−17.7	9.4	1
Apeltes quadracus	−11.0	8.2	1	−16.3	10.1	1	−18.5	11.1	1
Tautoga onitis	−11.3	6.9	1						
Syngnathus fuscus	−10.6	7.3	1	−14.0	8.2	1	−18.9	9.9	1
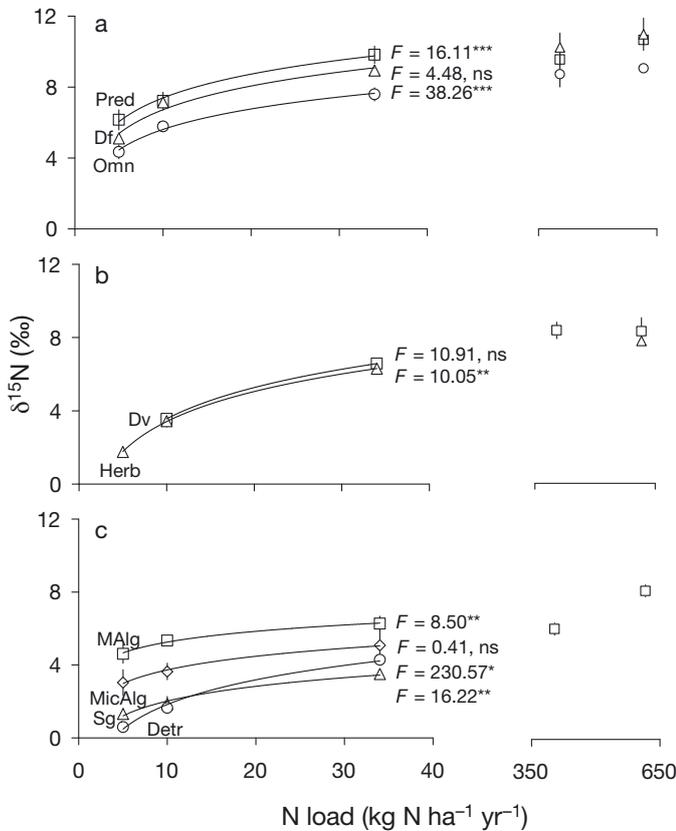
Fig. 3. δ¹⁵N (mean ± SE) for (a,b) consumers (Df: deposit feeders, Omn: omnivores, Pred: predators; Herb: herbivores, Dv: detritivores) and (c) producers (Detr: detritus, Sg: seagrass, MAlg: macroalgae, MicAlg: benthic microalgae) as a function of estuarine N load. Consumers are averaged by trophic level and split into 2 panels (a,b) to show the data more clearly. Data for Timms, Sage Lot, and Jehu Ponds (N loads: 5, 10, and 34 kg N yr⁻¹ ha⁻¹, respectively, left side) collected in August 2005. Data for Quashnet and Childs Rivers (404 and 616 kg N yr⁻¹ ha⁻¹, respectively, right side) from Martinetto et al. (2006) are included to allow comparison across a wider range of N loads. F-values show result of regression analyses carried out on log-transformed data (data for N loads <34 kg yr⁻¹ ha⁻¹ only). *p < 0.05, **p < 0.01, ***p < 0.001. ns: not significant

Isotopic δ¹³C signatures clearly differed among primary producer taxa. Seagrasses were lighter in δ¹³C (−7.2 to −11.2‰) compared to macroalgae (−11.3 to −21.9‰), reflecting differences in their modes of C fixation and use of different C sources (Smith & Epstein 1971, Lajtha & Marshall 1994). Benthic microalgae (−17.7 to −12.4‰) were lighter in δ¹³C than seagrass and exhibited similar signatures to macroalgae in 2 out of the 3 estuaries. The δ¹³C signatures of detritus differed among the sites (Table 2, Fig. 4). In the 2 estuaries with the lowest N loads, δ¹³C of detritus was relatively heavy (very low N load: −9‰, low N load: −11.4‰), whereas at the moderate N load, the signature was lighter (−14.1‰). Benthic POM showed slight

depletion in ¹³C with increasing N load, which may be due to seagrass being a more important contributor at the lower N loads. Overall, POM δ¹³C signatures were similar to macroalgal isotope signatures in all 3 estuaries (Table 2, Fig. 4).

Seagrass δ¹³C differed among estuaries with lower signatures in the estuaries with the moderate and intermediate N loads (−11.2 ± 0.3‰ and −10.9 ± 0.7‰) compared to signatures at the low N load (−7.7 ± 0.8‰) (Table 3). The mean δ¹³C signatures for macroalgae and microalgae did not differ significantly among estuaries (Table 3). In addition, macroalgal δ¹³C signatures spanned a similar range in all 3 estuaries (Table 2). Benthic POM was most enriched in ¹³C in the estuary with the lowest N load (−16.1 ± 0.1‰) and became depleted in ¹³C with increasing N load (−17.6 ± 0.4‰ and −19.2 ± 0.2‰) (Table 3).

δ¹³C signatures of fauna grouped by trophic level into primary consumers, omnivores (and deposit feeders) and predators revealed significant differences among the 3 estuaries. Consumers of all 3 trophic
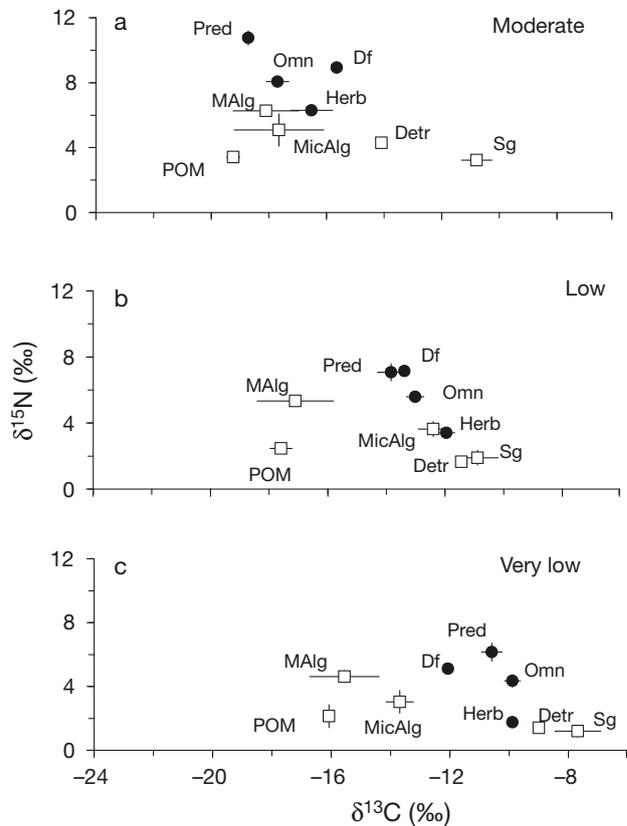

Fig. 4. δ¹³C and δ¹⁵N (mean ± SE) of consumers and producers in the estuary with (a) moderate, (b) low and (c) very low N loads. ●: consumers (Pred: predators, Omn: omnivores, Herb: herbivores, Df: deposit feeders). □: potential food sources (Sg: seagrass, MAlg: macroalgae, MicAlg: benthic microalgae, Detr: detritus, POM: benthic particulate organic matter)

groups were most enriched in the estuary with the lowest N load. In comparison to very low, consumers in the estuaries with low and moderate N loads were depleted in ^{13}C by 2.2 to 3.2‰ and 6.9 to 7.8‰, respectively (Table 3). The same pattern of depleted δ^{13}C signatures with increasing N load was evident for individual consumer taxa (Fig. 5).

Consumer diets

An initial examination of the stable isotope biplot showed differences in the overall food webs among estuaries (Fig. 4). δ^{13}C signatures of producers were relatively similar among estuaries, but there was large inter-estuary variation in the δ^{13}C signatures of consumers. For all consumer trophic levels, δ^{13}C signatures became lighter as N load increased (Fig. 4, Table 3). Consumers have δ^{13}C signatures similar to their food source since there is little C fractionation with each trophic step (typically 0.5‰; France & Peters 1997, Post 2002). In the estuaries with the lowest N loads, seagrass and detritus appeared to form the base of the food web, whereas macro- and microalgae were more important food sources in the estuary with the moderate load (Fig. 4). This pattern is even more apparent when the δ^{13}C of each species is examined in relation to the producer sources (Fig. 5).

These among-estuary patterns were also evident in the IsoSource modeling

of diets for 2 dominant herbivores: the amphipod *Cymadusa compta* and the isopod *Erichsonella filiformis*. The model results showed a large contribution of seagrass (28 to 52%) and detritus (25%) to diets of
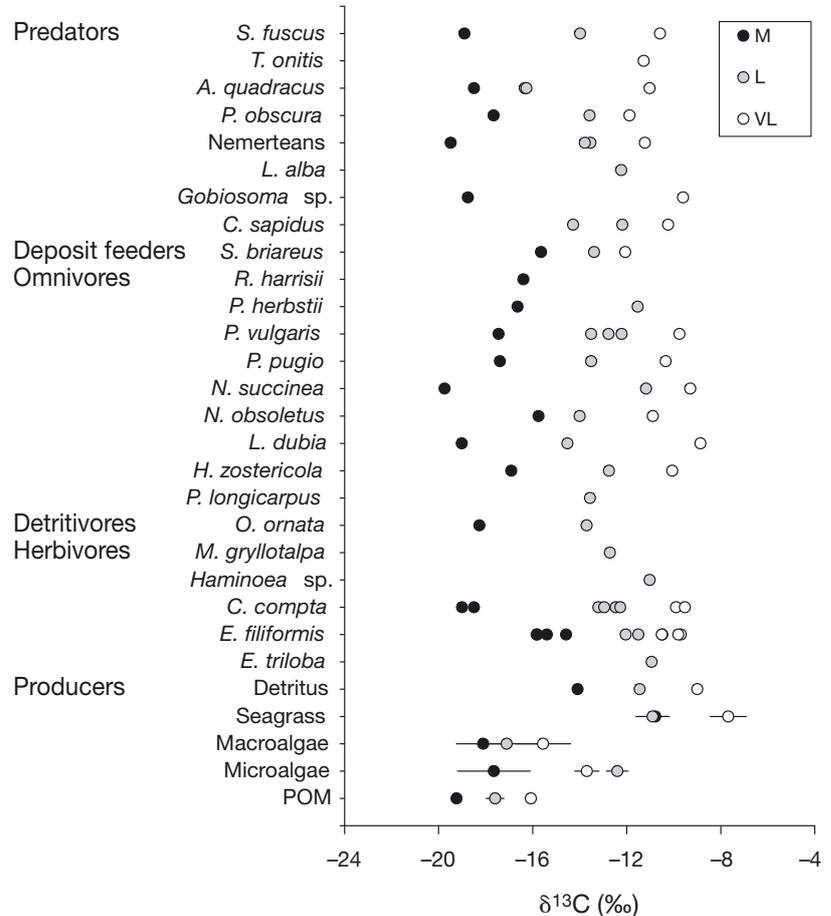

Fig. 5. δ^{13}C of producers and consumers collected in Waquoit Bay estuaries with very low (VL, ○), low (L, ◉), and moderate (M, ●) N loads. Values are: means ± SD. Sample sizes and full species names are given in Table 2. POM: particulate organic matter

Table 3. δ^{13}C (means ± SE) of producer and consumer groups from the 3 estuaries (very low, low and moderate N load). Results from 1-way ANOVAs testing inter-estuary differences are shown (ns = not significant, *p < 0.05, ***p < 0.001), df for site = 2 for all comparisons. Values within a row with the same superscript letter are not significantly different. POM: particulate organic matter

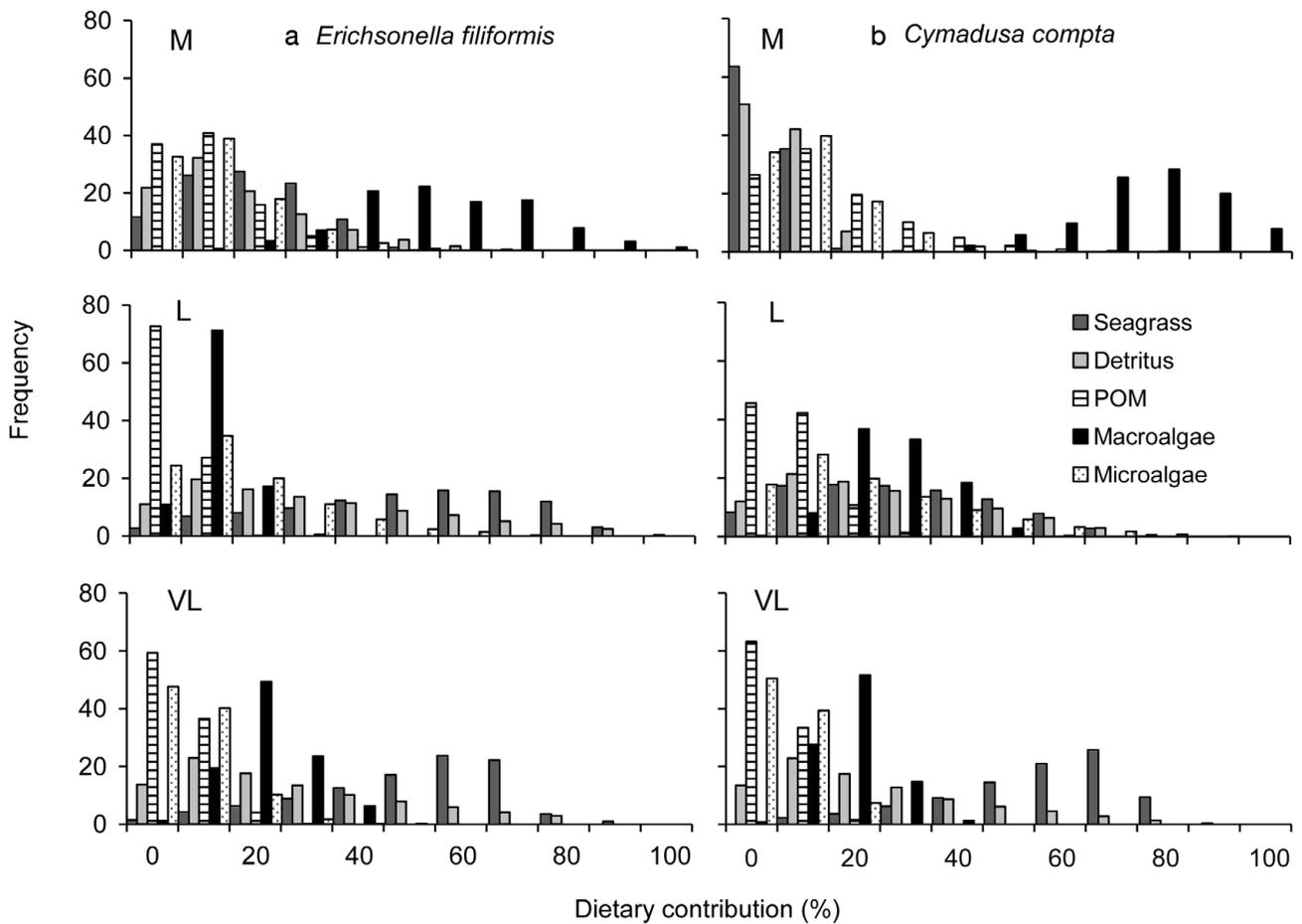|	δ^{13}C (‰)			MS	F	p
	Very low	Low	Moderate			
Producers						
Macroalgae	−15.6[a] ± 1.2	−17.1[a] ± 1.3	−18.1[a] ± 1.1	13.1	1.2	ns
Microalgae	−13.7[a] ± 0.5	−12.4[a] ± 0.5	−17.7[a] ± 1.6	17.4	2.9	ns
Seagrass	−7.7[a] ± 0.8	−10.9[b] ± 0.7	−11.2[b] ± 0.5	10.1	7.3	*
POM	−16.1[a] ± 0.1	−17.6[b] ± 0.4	−19.2[c] ± 0.2	34.9	34.9	***
Consumers						
Herbivores + detritivores	−10.0[a] ± 0.2	−12.1[b] ± 0.3	−16.8[c] ± 0.7	78.2	52.7	***
Omnivores + deposit feeders	−10.2[a] ± 0.4	−13.0[b] ± 0.3	−17.2[c] ± 0.5	102	74.5	***
Predators	−10.8[a] ± 0.3	−14.0[b] ± 0.5	−18.7[c] ± 0.3	84.4	70.8	***

Fig. 6. Relative contributions of seagrass, detritus, particulate organic matter (POM), macroalgae, and microalgae to diets of (a) *Erichsonella filiformis* and (b) *Cymadusa compta* in Waquoit Bay estuaries with moderate (M), low (L), and very low (VL) N loads. Dietary contributions have been calculated using δ^{13}C signatures modeled in IsoSource for all iterations (in 5 % increments) and are expressed as % frequency of all possible solutions

these consumers at the lowest N loads (Fig. 6, Table 4). Benthic micro- and macroalgae made up an average of 13 to 19 % and 4 to 24 %, respectively. The proportion of POM in diets was relatively low at ~2 to 5 %. At the moderate N load, macroalgae was the most important food source (51 to 73 %) especially for *C. compta* (Fig. 6, Table 4). Seagrass (2 to 18 %), detritus (4 to 15 %), POM (7 to 12 %) and benthic microalgae (8 to 9 %) contributed less to consumer diets.

Producer tissue nutrient content did not differ among the 3 estuaries (Table 5). The %N was similar for macroalgae and seagrass across all 3 estuaries (1.9 to 2.2 %). Detritus ranged from 2.2 to 2.9 % N and benthic POM was much lower at 0.07 to 0.16 %. Percent tissue C did not differ among estuaries, but was lower for macroalgae (29.6 to 33.2 %) compared to seagrass (36.9 to 38.0 %) and detritus (37.4 to 40.6 %). POM had

Table 4. Mean relative contribution (%, ± SD) of potential food sources to diets of 2 herbivores, the amphipod *Cymadusa compta* and the isopod *Erichsonella filiformis*, calculated using the IsoSource model. POM: particulate organic matter

| | N load | | |
	Moderate	Low	Very low
Cymadusa compta			
Seagrass[a]	2.3 ± 3.5	27.5 ± 18.3	52.4 ± 18.6
Detritus	4.0 ± 5.0	24.4 ± 18.9	24.6 ± 22.0
POM	12.3 ± 13.0	5.0 ± 6.0	2.6 ± 3.9
Benthic microalgae	8.4 ± 9.3	19.0 ± 17.7	16.2 ± 7.1
Macroalgae	73.0 ± 14.1	24.0 ± 9.0	4.3 ± 5.6
Erichsonella filiformis			
Seagrass[a]	17.5 ± 11.6	47.3 ± 22.9	48.1 ± 18.6
Detritus	14.8 ± 14.2	29.7 ± 24.1	25.3 ± 21.9
POM	7.4 ± 8.3	1.6 ± 2.8	3.0 ± 4.4
Benthic microalgae	9.1 ± 10.0	13.2 ± 13.4	18.7 ± 7.6
Macroalgae	51.3 ± 17.1	8.2 ± 5.0	4.9 ± 6.2
[a]Includes microalgal epiphytes growing on seagrass blades			

Table 5. Tissue %N and %C (mean ± SE) originating from potential food sources. Results from 1-way ANOVAs testing differences in %N and %C among sites (ns: no significant difference, p > 0.05). df for site = 2 for all comparisons. POM: benthic particulate organic matter

	N load			MS	F	p
	Moderate	Low	Very low			
%N						
Macroalgae	2.3 ± 0.3	1.9 ± 0.5	2.3 ± 0.5	0.4	0.34	ns
Seagrass	2.0 ± 0.3	2.1 ± 0.3	2.2 ± 0.2	0.01	0.07	ns
Detritus	2.6	2.9	2.2			
POM	0.2 ± 0.05	0.1 ± 0.01	0.1 ± 0.01	0.01	3.09	ns
%C						
Macroalgae	29.6 ± 2.3	31.1 ± 2.5	33.2 ± 2.5	25.6	0.56	ns
Seagrass	36.9 ± 0.5	38.0 ± 0.02	37.6 ± 0.2	1.0	2.72	ns
Detritus	40.1	40.6	37.4			
POM	1.2 ± 0.5	0.2 ± 0.07	0.3 ± 0.07	0.9	3.01	ns

the lowest C content at 0.22 to 1.2%, probably due to a relatively large proportion of inorganic sedimentary material in the samples.

DISCUSSION

Producer community structure

In this study, we measured the changes taking place in macrophyte communities at the early stages of the transition from a benthic community dominated by seagrasses to a macroalgal-dominated system. The producer community structure differed among estuaries with seagrass being dominant at the lowest N load and macroalgae being equal in biomass in the estuary with the moderate N load. In a compilation of world-wide seagrass loss over a range of reported N loads, Valiela & Cole (2002) showed that substantial seagrass losses (>50%) occurred between 50 to 100 kg N ha^{-1} yr^{-1}, and seagrasses were completely replaced by macroalgae at loads exceeding 100 kg N ha^{-1} yr^{-1}. In the present study, all 3 estuaries had N loads below these thresholds (<34 kg N ha^{-1} yr^{-1}). The estuary with the moderate N load may have a reduced extent of sea-grass cover, but the biomass and density within the beds were not significantly affected at these low loads. If N inputs increase or remain high and macroalgal proliferation continues in this estuary, we would expect considerable losses of eelgrass.

This transition from a seagrass-dominated benthic community to roughly equal proportions of seagrass and macroalgae as a result of N loading therefore alters the composition of the carbon pool available to consumers both as live biomass and as detritus and POM. Indirectly, N loading thus influences consumer diets and C pathways in the benthic food web.

δ^{15}N and δ^{13}C signatures of producers and consumers

The isotopic signatures of producers and consumers differed among estuaries and taxa. The increase in δ^{15}N signatures of primary producers with increasing N load demonstrates that the isotopic signatures characteristic of the land uses contributing N to the different estuaries were incorporated into the chemical composition of the primary producers. Differences in the δ^{15}N signatures of seagrasses (1.2 to 3.5‰) and macroalgae (4.6 to 6.3‰) may reflect differences in uptake rates and internal nutrient cycling, preference for ammonium or nitrate, or be due to the fact that seagrasses have access to both water column and sediment pools of N (Cole et al. 2004). Despite these differences, δ^{15}N signatures of both types of macrophytes increased significantly in response to wastewater N inputs and could be useful predictors of wastewater N inputs to estuaries.

Consumers displayed a similar pattern to those of macrophytes for different feeding guilds, indicating that the δ^{15}N signatures incorporated into the primary producers were passed onto herbivores and consumers at higher trophic levels. All components of the benthic food web therefore appeared to be coupled to their watersheds. The response of δ^{15}N was most sensitive to N source detection at the low end of the range of N loads, which is supported by earlier work in Waquoit Bay (McClelland et al. 1997, Cole et al. 2004). This study corroborates the notion that δ^{15}N signatures of biota can be used to detect early stages of eutrophication, and that there are a suite of taxa that may be suitable as indicators for monitoring incipient N loading to coastal estuaries.

Since there were significant differences in the relative biomasses of seagrasses and macroalgae among the 3 estuaries, we expected and observed inter-estuary differences in food web structure. Carbon stable isotopes were used to determine the major food groups available to consumers, since δ^{13}C signatures of primary producers were isotopically distinct. Our study shows evidence of significant differences in the composition of detritus across the N loads sampled. The δ^{13}C signatures for detritus were heavier in the estuaries with the lowest N loads, suggesting eelgrass was an important component of the detrital pool. Detritus in the estuary with moderate N load had a lighter δ^{13}C signature, which is consistent with it containing a larger proportion of algal material. This is not surprising given the large amount of macroalgae found here. Based on δ^{15}N and δ^{13}C of primary consumers, in the very low N

loaded estuary, herbivores appear to feed on poor quality food sources, such as seagrass and detritus. As N load increased, they switched to more nutritious food sources, such as algae, as these became more abundant. This corresponds well to the difference in the ratio of seagrass to macrophytes among the 3 estuaries.

Omnivores and predators displayed trophic N isotope fractionation typical for estuarine consumers, but herbivores had very low δ^{15}N signatures compared to any of the predictable food sources. This is similar to what has been observed for small grazers in other systems. For example, Jephson et al. (2008) reported a similar apparent lack of fractionation for crustacean (*Gammarus locusta*, *Idotea baltica*, and *Microdeutopus gryllotalpa*) and gastropod (*Rissoa* spp., *Littorina* spp., *Radix baltica*, and *Theodoxus fluviatilis*) grazers in the Baltic Sea. It seems that N fractionation in the 2 species chosen is smaller than the 3 to 4‰ per trophic level that is commonly accepted for most organisms (Minagawa & Wada 1984, Michener & Schell 1994, Post 2002). Considerable variation in trophic enrichment of ^{15}N in invertebrates has been found (McCutchan et al. 2003). Vanderklift & Ponsard (2003) suggested an average δ^{15}N enrichment of only 0.53‰ per trophic level for organisms feeding on detritus. Vander Zanden & Rasmussen (2001) also suggested differences in fractionation depending on diet with herbivores having less and more variable trophic enrichment compared to carnivores. Another possibility is that we missed a major food source. The consumers we sampled are known to feed on macroalgae, seagrass and its detritus, and epiphytes (Nelson 1979, Zimmerman et al. 1979, Luczkovich et al. 2002), which were all analyzed, but it may also be possible that these herbivores feed selectively on isotopically lighter material within the pools of producers measured.

Implications of increased N load for C pathways through benthic food webs

Our results showed differences in the major components of benthic consumer diets among the 3 estuaries sampled. Diets in these estuaries reflect both the relative availability of potential food items and herbivore preference for more palatable and nutritious primary producers. Herbivores are able to choose their food based on palatability using cues like tissue %N (Mattson 1980, Boyer et al. 2004), plant morphology (Hay 1997), the presence of structural compounds like cellulose and lignin (Cebrián & Duarte, 1998), or grazing deterrents (Hay & Fenical 1988). Augmented nutrient supply has been shown to increase tissue %N of macrophytes in Waquoit Bay (Teichberg et al. 2007, 2008), and we might expect to see differences in the nutrient content of primary producers across the gradient of N loads. Uptake and assimilation of the available nutrients reduces the C to nutrient ratio of seagrass or algal tissues, making producers a better quality food source for grazers (McGlathery 1995, Hemmi & Jormalainen 2002, Boyer et al. 2004, Olsen & Valiela 2010). This could lead to increased grazing rates where N supply is enhanced. In the present study, however, increased reliance on macroalgae in the estuary with the moderate N loading did not appear to be a result of better nutritional quality of algal material with increased nutrient availability because %N and %C did not differ significantly among estuaries. The increased amounts of macroalgae in herbivore diets with increasing N load, therefore, appears to be determined by encounter rate rather than being mediated by inter-estuary differences in macroalgal palatability. The preference for macroalgae over seagrass and detritus may be determined by palatability, but in the lowest N loaded estuaries, there is not sufficient supply of macroalgae to allow for herbivore choice. The differences in herbivore diets among estuaries resulted in differences in δ^{13}C signatures of primary consumers that were transferred to higher trophic levels.

McClelland & Valiela (1998) suggested that losses of eelgrass due to eutrophication may eliminate an important pathway for N cycling through the food web. When eelgrass is replaced by algae, resources locked up in detritus become available to consumers at a faster rate because macroalgal detritus is readily available to consumers while refractory eelgrass detritus must be microbially altered first. Macroalgae are more easily digested and can, to a larger extent, be used by higher trophic levels. This may result in faster rates of turnover through the detrital pathway and a food web that is more tightly coupled to annual production cycles. The C flow through the benthic food web can thereby become driven by more rapid cycles of shorter-lived food resources, which may lead to an overall loss of ecosystem stability (McClelland & Valiela 1998, Keats et al. 2004).

The results of the present study indicate that relatively small increases in N inputs to estuaries could result in shifts in the patterns of C flow through the benthic food web. The differences observed here may or may not be considered ecologically detrimental, but they highlight the potential of incipient levels of eutrophication to alter carbon pathways and foster measurable changes in coastal food webs.

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