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Nitrogen extraction potential of wild and cultured bivalves harvested from nearshore waters of Cape Cod, USA

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ABSTRACT

As nitrogen entering coastal waters continues to be an issue, much attention has been generated to identify potential options that may help alleviate this stressor to estuaries, including the propagation of bivalves to remove excess nitrogen. Oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) from numerous Cape Cod, MA, (USA) sources were analyzed for nitrogen content stored in tissues that would represent a net removal of nitrogen from a water body if harvested. Results showed local oysters average 0.69% nitrogen by total dry weight (mean 0.28 g N/animal) and quahogs average 0.67% nitrogen by total dry weight (mean 0.22 g N/animal); however, these values did vary by season and to a lesser extent by location or grow-out method. The differences in nitrogen content were largely related to the mass of shell or soft tissue. Nitrogen isotope data indicate shellfish from certain water bodies in the region are incorporating significant amounts of nitrogen from anthropogenic sources.

1. Introduction

Coastal water bodies and estuaries are essential habitat for many species and are also important to the economic health of coastal communities. While nitrogen (N) is a vital nutrient to the marine environment, in excess it causes eutrophication or an increase in the rate of supply of organic matter to a system (Nixon, 1995). Nitrogen can enter coastal waters from various point and non-point sources, although increased human activity has accelerated the rates of N enrichment (Carmichael et al., 2004a). This increase in nitrogen enrichment and subsequent eutrophication has a negative impact on coastal waters and can be a root cause of habitat degradation (Bowen et al., 2007; Howarth, 2008).

The approach to combat this growing problem in coastal Massachusetts is to reduce nitrogen to threshold levels identified as important in maintaining ecosystem health in coastal waters. Strategies being considered for reduction of nitrogen include centralized or improved wastewater treatment, stormwater treatment, increased tidal flushing, enhanced attenuation via wetlands, in addition to other techniques (Dudley, 2003). The use of shellfish production and harvest has also recently garnered interest as an option in plans to reach nitrogen management thresholds (Bricker et al., 2014; Carmichael et al., 2012; Grizzle et al., 2016; Higgins et al., 2011; Rose et al., 2014).

The removal of nitrogen via bivalves can occur through harvest of tissue and shell, long-term burial in the sediment, or conversion of N in biodeposits to di-nitrogen gas through stimulated microbial activity (reviewed in Kellogg et al., 2014 and references therein). While the latter pathways of burial and denitrification have been demonstrated in relation to oyster reefs (Kellogg et al., 2013), significant variability exists as to rates and quantifiable numbers (Kellogg et al., 2014). Current data have shown that oyster aquaculture has some ability to stimulate denitrification (Higgins et al., 2013; Testa et al., 2015; Humphries et al., 2016), though results are limited regarding the impact of clam farming (Nizzoli et al., 2006; Murphy et al., 2015). The variability of site and bivalve density may also have significant impacts (Burkholder and Shumway, 2011).

The "nutrient bioextraction" potential of filter feeding bivalves may be most directly quantifiable through the quantity of N contained in harvested shellfish. Reported values have indicated that percent N in the soft tissue of Eastern oysters (*Crassostrea virginica*) may range from 7–9.3% (Newell, 2004; Higgins et al., 2011; Carmichael et al., 2012; Sisson et al., 2011; Grizzle et al., 2016), whereas the shell range is 0.2–0.3% (Higgins et al., 2011; Sisson et al., 2011; Newell, 2004; Grizzle et al., 2016). It has been suggested that oysters vary significantly in morphology, and may also vary in nitrogen content by space and season such that values for N removal through oysters will likely be location specific (Kellogg et al., 2014; Grizzle et al., 2016). The data for quahogs (*Mercenaria mercenaria*) are more limited, but nitrogen content in soft tissue ranges from 4.2–6% (Table 1, Rice, 2001, Sisson et al., 2011) and shell nitrogen was reported at 0.15% in wild quahogs from

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Table 1
Samples collected by site, and the larger region of influence.

Site	Waterbody	Region	Quahogs		Oysters			
			Wild	Cultured	Wild	On-bottom	Off-bottom	Off-triploid
DB	Duxbury Bay	Cape Cod Bay				X	X	
BH	Barnstable Harbor	Cape Cod Bay	X	X		X	X	
WH	Wellfleet Harbor	Cape Cod Bay		X	X	X	X	X
TC	Town Cove	Atlantic Ocean		X				
PB	Pleasant Bay	Atlantic Ocean					X	
OP	Oyster Pond	Nantucket Sound	X				X	
SR	Swan River	Nantucket Sound			X			
CB	Cotuit Bay	Nantucket Sound	X	X				
PP	Popponesset Bay	Nantucket Sound	X	X	X	X	X	
SB	South Buzzards Bay	Buzzards Bay					X	
BO	Bourne Harbors	Buzzards Bay	X	X	X	X	X	
NB	North Buzzards Bay	Buzzards Bay				X	X	

Virginia (Sisson et al., 2011). The goal of this study was to examine oysters and quahogs from Cape Cod, MA as a nitrogen bioextraction tool through harvest of both species from a variety of sources and during differing seasons.

2. Methods

Oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) were collected for nitrogen content analysis from various water bodies in the Cape Cod, MA, (USA) region to represent the predominant shellfish commercially harvested and a range in local geography (Table 1 and Fig. 1). To assess potential differences related to season, a first set of samples were collected in June 2012, and then a second collection later in October 2012. Wherever possible, both oysters and quahogs were taken from the same water body for comparison. Considering potential differences in the life history or type of grow-out used, oysters were separated into 3 main categories: wild, cultured on-bottom, and cultured off-bottom. A smaller fourth group of cultured off-bottom triploid oysters was included at one site only. Quahogs were separated into two categories: wild and cultured. For the purposes of this study, an animal was considered cultured if held in shellfish culture gear at any portion of the life cycle, whereas wild shellfish represented native or naturally propagated populations.

Shellfish were selected for inclusion in the field samples at typical local harvest sizes, which is 3–3.5 in. (76–89 mm) in shell height (measured as the longest axis) for oysters, and 1–1.5 in. in shell hinge width (as measured between the convex apex of the right shell and the convex apex of the left shell) for quahogs. Four animals were collected for each category or group sampled. Shellfish samples were individually labeled and held refrigerated until measurements for shell height, length, and width to the nearest 0.01 mm using digital calipers, and whole wet weight to the nearest 0.01 g were recorded. After initial processing, samples were frozen and delivered to the Boston University Stable Isotope Laboratory for separation of the shell and soft tissues, drying, and measurement of dry tissue weights. Percent nitrogen and carbon analysis was provided on dried ground shell and soft tissues (gut intact) using standard Eurovector CN analyzer methods for the laboratory.

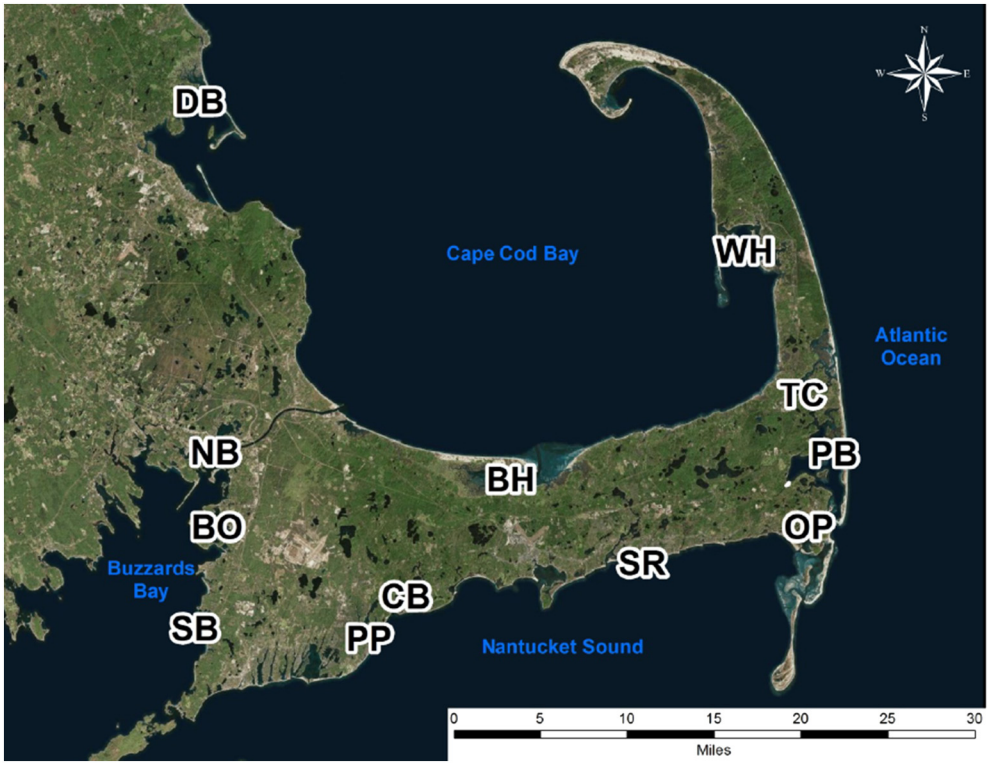

Fig. 1. Map showing sample locations (abbreviated), and proximity to regional water bodies.

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Isotopic signatures of nitrogen, expressed as ^{15}N:^{14}N ratio in per mill (‰), contained in shellfish have been shown to vary in relation to the amount of wastewater contribution to the nitrogen loading of a water body (Carmichael et al., 2004a). To assess for potential differences in the ultimate source of nitrogen in shellfish tissues, four additional samples were taken from each group in fall 2012 for stable isotope analysis. These samples were measured and shucked immediately to remove the adductor muscle, a tissue selected as a representative for incorporation of nitrogen. The adductor muscle was then dried for at least 24 h at 70 °C until completely dry and marked for further analysis using standard methods at the Stable Isotope Laboratory at Boston University.

Statistical analysis was performed using SYSTAT 13, with statistical significance set at $p < 0.05$. The categories of oysters were compared using a one-way ANOVA with post hoc pairwise comparisons where appropriate using the Games-Howell for unequal variance, as the homogeneity of variance could not always be assumed. Wild and cultured quahogs were compared using a separate variance *t*-test. The spring (June) samples of each species were compared to their fall (October) cohorts using a separate variance *t*-test. To examine for differences among sites (water bodies), ANOVA comparisons were made by grow-out type within a particular season to eliminate the potential effect of these variables (i.e. cultured quahogs in spring or off-bottom cultured oysters in fall, etc.). Condition index was calculated per the method of Lawrence and Scott (1982), and correlated to total %N of individual quahogs and oysters using Pearson's correlation. Comparisons of isotopic signatures were performed using *t*-tests or ANOVA with post hoc Tukey's HSD where appropriate. Pearson's correlations between isotopic signature and %N levels were made using sampling group means ($n = 4$ per group).

3. Results and discussion

3.1. Oysters

The oysters local to Massachusetts' waters examined in this study averaged 83.8 mm (3.3 in.) in shell height; dry weights for shell and soft tissue were 40.9 g and 2.43 g, respectively. The dried soft tissue of oysters averaged 3.76% (SD 1.5%) of the whole (live) weight, and the shell was 61% (SD 6.4%) of whole (live) weight. Of the total, 66% of the nitrogen in oysters was found in the soft tissues despite shell weight comprising most of the total weight (Table 2).

Among the categories of oysters sampled, some differences were apparent despite being statistically similar in size by shell height (Table 2). These differences were largely related to differences in shell weight that equated to overall differences in the amount of N that would be harvested per animal. Differences reflect culture gear and origin of oysters; wild oysters and oysters grown on bottom were higher in N content than cultured oysters grown off the bottom.

Growing oysters off the bottom or above the sediment surface tends to reduce predation pressure and provide better access to food, and as such tends to promote rapid growth resulting in thinner, lighter shells (Higgins et al., 2011; Newell and Mann, 2012). The cultured off-bottom triploids were only sampled at one site and may represent an extreme in growth as reproductively sterile triploids can have the added growth advantage of conserving reproductive energy loss for growth (Shpigel et al., 1992; Newkirk, 1996). The triploid data should be taken conservatively as it only represents one site and the age of these animals was likely much younger; for example the fall triploid samples were only ~6 months old, whereas the rest of the fall oysters were at least 1+ years in age. Despite these differences in shell weight and mass of N per animal, when considering percent N of the total dry weight per animal ("Total % N" in Table 2), there was no statistical difference among oyster categories.

While nitrogen values obtained from local Cape Cod area waters are comparable to other values available in the literature, some differences are also apparent (Table 2). The actual percent nitrogen in the tissue or shell is fairly similar among literature values in comparison to Cape Cod samples. The wild Chesapeake Bay oysters (Newell, 2004) stand out as having much more nitrogen per animal despite a slightly lower shell height (76 mm average); this difference is solely related to the higher shell weight in these animals (Table 2). Since the tissue (meat) contains 66% of the total nitrogen in Cape Cod oysters, it is worth mentioning that the oysters in this study averaged a greater tissue weight (2.43 g), 143% higher than the wild Chesapeake oysters (1 g, Newell, 2004) and 54% higher than the cultured Chesapeake oyster average (1.58 g, Higgins et al., 2011). When breaking the nitrogen content down to a percent of the total dry weight (both tissue and shell) the Cape Cod area shellfish contained a consistently higher level, but this difference is largely related to quantity of tissue as opposed to %N in the tissues.

3.2. Quahogs

Quahogs (at the littleneck size sampled) averaged 56.1 mm (2.2 in.) in shell height, 31.2 g of dried shell, and 2.22 g of dried tissue (Table 3). Quahog meats when dried were 4.34% (SD 1.57%) of whole weight, and the shell averaged 60.4% (SD 2.7%) of whole weight. Of the total, 75% of the N in quahogs was contained in the soft tissue, despite shell being a much greater percentage of total weight.

There was little overall difference between wild or cultured quahogs (Table 3), although there were some significant differences related to

Table 2
Oyster data with comparison to literature values of oysters of similar size. Total %N for literature values was calculated based on reported mean weights and %N content as (((Shell %N * Shell DW) + (Tissue %N * Tissue DW)) / (Shell DW + Tissue DW)) * 100, while data shown from the present study are group mean values calculated by each category of oysters. Bold values are statistically significant ANOVA results.

Oysters	N	Height (mm)	Whole wt (g)	Shell		Tissue		Total		Source
				DW (g)	%N	DW (g)	%N	N (g)	% N (DW)	
Wild	32	82.7	71.0	46	0.26	2.42	8.2	0.31	0.67	MA, present study
Cultured on	48	84.9	75.0	47.4	0.26	2.70	7.89	0.32	0.65	MA, present study
Cultured off	64	83.1	60.0*	35.7*	0.21	2.36	7.95	0.26*	0.70	MA, present study
Off triploid	8	86.5	42.6**	22.3**	0.32	1.36*	8.5	0.19**	0.82	MA, present study
p-Value		0.410	<**0.001**	<**0.001**	0.064	**0.025**	0.475	**0.001**	0.137	
Mean value	152	83.8	66.1	40.9	0.24	2.43	8.01	0.28	0.69	MA, present study
Literature values										
Wild		76.0	NS	150	0.30	1.00	7	0.52	0.34	VA, Newell, 2004
Cultured off[a]		85.5	NS	37.6	0.17	1.58	7.28	0.18	0.45	VA, Higgins et al., 2011
Cultured off[a]		86.0	NS	38.5	0.13	1.20	7.3	0.14	0.35	NH, Grizzle et al., 2016
Cultured off[b]		82.0	NS	29.28	0.20	2.56	7.65	0.26	0.80	NY, Sebastiano et al., 2015

NS indicates values were not specified.
* or ** indicate statistical difference among oysters from wild or cultured categories.
[a] Values reported for "regular" size oysters (85–86 mm mean shell height).
[b] Values reported for shell height most similar to the present study (82 mm).

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Table 3
Quahog data and literature value comparison. Bold values are statistically significant *t*-test results.

Quahogs	N	Height (mm)	Whole wt (g)	Shell		Tissue		Total		Source
				DW (g)	%N	DW (g)	%N	N (g)	% N (DW)	
Wild	48	57.1	54.4	32.6	0.18	2.43	7.5	0.24	0.67	MA, present study
Cultured	44	55.0	48.6	29.6	0.17	1.99	7.9	0.21	0.66	MA, present study
p-Value		**0.032**	**0.030**	0.064	0.806	**0.016**	**0.035**	0.056	0.691	
Mean value	92	56.1	51.7	31.2	0.18	2.22	7.69	0.22	0.67	MA, present study
Literature value										
Wild		NS		NS	0.15	NS	5.96	NS	NS	VA, Sisson et al., 2011

NS indicates values were not specified.

the size of quahogs sampled (height and tissue weight) as well as %N in tissue and total N per animal. There was no difference in total %N based on complete dry weight between cultured or wild (0.67% and 0.66%, for wild and cultured quahogs, respectively). This result is not unexpected in that quahog culture practices on Cape Cod, MA, usually involve bottom planting and covering with protective netting that may not affect growth characteristics significantly from wild quahog growth.

Values for comparison of nitrogen in quahog tissues are limited, although the samples from the present study do appear to have a higher percent nitrogen in at least the shell (Table 3). Mean quahog soft tissue %N values of 7.7% appear within range (~7–10%) of MA quahogs of various ages and sizes (Carmichael et al., 2004b). Further study will be required to determine how size and age may relate to a quahog's nitrogen assimilation status. Collectively, these results indicate quahogs contain an appreciable amount of N, with total content at popular harvest size approaching that reported for oysters.

3.3. Seasonal differences

One of the major factors shaping differences seen in both quahogs and oysters was related to seasonal differences between the spring and fall sampling periods (Table 4). There was no difference in size (height or whole live weight) of quahogs sampled between the seasons, although oysters were marginally different in height (<3 mm difference, $p = 0.049$) but not in whole weight ($p = 0.064$). Although size may have had an influence on oysters, it is interesting to note the amount of soft tissue increased by 98% for oysters and 63% for quahogs from spring to fall. Since the tissue contains a greater %N than the shell, this had an impact on the total N content and total % N, such that shellfish harvested in fall would have 44% (quahog) or 28% (oyster) more N per animal than if harvested in the spring at the same size. There were also differences in %N of the shell between seasons, although it is difficult to make any conclusions due to the high degree of variability (coefficient of variation, CV = 57% with oysters, CV = 46.3% with quahogs) and the very low nitrogen content of the shell.

The soft tissue in oysters is known to change over the course of a season, in spring as they prepare to spawn, during the spawn, and during fattening after the spawn in the fall (Newell and Mann, 2012). This change in composition is largely related to increasing glycogen reserves going into the fall season, leaving a lower relative percent protein composition (Thompson et al., 1996), which translates to the reduced tissue %N seen in the fall with the Cape Cod oysters and quahogs. The June and October samples collected in this study may represent seasonal tissue difference extremes, though further sampling would be required to examine changes over an entire year. Results for both oysters and quahogs indicate that the maximum potential for nitrogen assimilation would occur in the fall when tissue content is much higher.

Table 4
Seasonal mean values for quahogs and oysters. Bold *p*-values indicate a statistically significant *t*-test result when comparing between the two seasons.

Seasonal	Quahogs			Oysters		
Comparison	Spring	Fall	p-Value	Spring	Fall	p-Value
Shell height (mm)	56.5	55.6	0.401	82.5	85.1	**0.049**
Whole wt (g)	53.4	49.7	0.171	62.6	69.7	0.064
Shell DW (g)	32.3	29.9	0.15	38.3	43.4	0.060
Shell %N	0.15	0.2	**0.001**	0.27	0.22	**0.016**
Tissue DW (g)	1.71	2.78	<**0.001**	1.63	3.23	<**0.001**
Tissue %N	8.07	7.29	<**0.001**	8.89	7.14	<**0.001**
Total N (g)	0.18	0.26	<**0.001**	0.25	0.32	<**0.001**
Total % N (DW)	0.53	0.82	<**0.001**	0.63	0.73	**0.005**
Condition index	7.91	14.32	<**0.001**	6.99	12.44	<**0.001**

3.4. Site or water body differences

There were differences in the size of the shellfish sampled in some of the comparisons between water bodies despite attempts to standardize size. Size of oysters has previously been demonstrated to have a proportional relationship to quantity of nitrogen contained (Higgins et al., 2011). For this reason, comparisons among sites focus on unit-less measures such as percent nitrogen content and condition index, although whole weight and total nitrogen content are also shown for reference (Table 5).

There were some differences apparent due to the site or water body where the shellfish were taken, even when using unit-less measures and eliminating the effect of season and grow-out method, although this did not occur in every comparison. When using total percent nitrogen as the point of comparison, there were significant differences in 2 out of 4 quahog comparisons and 3 of 6 oyster related comparisons. In one comparison, oysters sampled in the spring from off-bottom culture scenarios, the total %N more than doubled from the lowest water body mean to the highest (0.35–0.82). These oysters also had the largest degree of variability in total weight.

The percent of nitrogen in the shell as well as in the tissue also varied significantly across water bodies with both species, but not in all comparisons. As mentioned previously, shell %N varies to a degree within a group, but also varied between sites in 1 out of 4 quahog comparisons, and 2 out of 6 oyster comparisons. The tissue % N, however, was significantly different among water bodies in all 4 quahog comparisons, although only in 2 out of 6 oyster comparisons. The largest difference in means was from wild quahogs sampled in spring, 6.2–8.9%.

Variables not addressed when sampling could easily affect nutritional status of the sampled animals and possibly the nitrogen contained. Age class was not considered between sites and has been shown to have some impact on N sequestration of oysters (Dalrymple and Carmichael, 2015). Also, leaving the gut and its contents intact as was done in this study may increase variability, but our goal was to simulate a typical harvest of shellfish that would include the gut. In addition, animals from any one particular water body may be affected at the time of sampling by disease(s), environmental stressors, or available food leading to a different nutritional status. Such variables may all influence overall differences in tissue content and thus nitrogen removal capacity.

Condition index is often used as a measure of nutritive status in shellfish, as it gives a relative measure of the amount of tissue occupying the available shell cavity (Lawrence and Scott, 1982). Condition index of the shellfish sampled in the current study show a positive correlation

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Table 5
Sample category, number of sites, and range of means between sites, low to high, within each sample group. An * indicates there was a statistically significant ANOVA when comparing sites.

Species	Season	Grow Out	Sites (n)	Whole wt (g)	Shell %N	Tissue %N	Total N (g)	Total %N	Condition index
Quahog	Spring	Cultured	6	39.6–63.5*	0.10–0.21*	7.2–8.8*	0.11–0.23*	0.38–0.70*	5.1–10.8*
		Wild	6	40.7–68.2*	0.09–0.19	6.2–8.9*	0.11–0.26*	0.42–0.60	7.4–12.8*
	Fall	Cultured	5	37.8–51.3	0.17–0.27	7.1–8.4*	0.21–0.26	0.68–0.90*	12.0–16.8*
		Wild	6	42.9–70.6	0.18–0.27	6.4–7.6*	0.21–0.37*	0.74–0.93	12.4–17.4*
Oyster	Spring	Off-bottom	8	22.9–117.2*	0.12–0.33	7.5–10.1*	0.12–0.42*	0.35–0.82*	4.7–10.7*
		On-bottom	6	34.0–79.7*	0.15–0.45	8.5–9.6	0.15–0.36*	0.49–0.76	5.8–8.7
		Wild	4	57.9–79.3	0.23–0.40	9.0–9.4	0.26–0.37	0.51–0.88	6.7–8.7
	Fall	Off-bottom	8	31.1–81.1*	0.14–0.37*	6.0–7.8	0.16–0.38*	0.60–0.96*	11.3–15.6*
		On-bottom	6	76.9–122.1*	0.15–0.33*	6.2–7.6*	0.28–0.49*	0.56–0.88*	9.6–14.5*
		Wild	4	64.6–80.5	0.15–0.29	6.9–7.8	0.30–0.35	0.57–0.69	11.1–13.7

between condition and total percent nitrogen content, with a stronger relationship among quahogs ($r = 0.83$) than with oysters ($r = 0.38$). Condition of animals by site may explain some of the variability seen among water bodies, as differences were also seen for condition index of the shellfish among water bodies, which can ultimately affect total %N (Table 5) by quantity of tissue per unit size.

3.5. Nitrogen isotope signatures

Carmichael et al. (2004a) demonstrated locally that the signature of nitrogen isotopes in shellfish tissues tends to be heavier with an increasing proportion of wastewater to the nitrogen load of an estuary. The average isotopic signature of quahogs was significantly heavier than the average for oysters (9.43‰ and 8.75‰, respectively, $p < 0.001$). Within the same water body, quahogs remained heavier in isotopic signature than oysters in Barnstable Harbor ($p = 0.047$), Oyster Pond ($p = 0.015$), and Wellfleet Harbor ($p < 0.001$), but not in Popponesset Bay ($p = 0.422$) or Bourne Harbors ($p = 0.759$). It is possible the isotopic signature difference seen between the two species may indicate a difference in food selection and assimilation (Dalrymple and Carmichael, 2015; Ward and Shumway, 2004), but the results may also be skewed due to location of sites sampled for oysters and quahogs.

There were no differences ($p = 0.893$) between cultured (9.41‰) or wild (9.44‰) quahogs, or among the different categories of oysters (8.71‰ off-bottom, 9.02‰ on-bottom, 8.74‰ wild, $p = 0.474$). The only exception being if the off-bottom triploid oysters (7.37‰) were to be included, these animals showed a lighter signature than oysters cultured on-bottom (8.40‰) at the same site ($p = 0.023$). This may be attributable to the young age of the triploid oysters, as they have only fed through approximately 6 months at the site and this would not allow them to fully represent the signature of oysters having grown at the site for greater than a year. In support of this possibility, there were no differences from wild (7.68‰) or diploid off-bottom oysters (8.17‰) from the same water body. This may also be the result of differences in food selection and assimilation (Dalrymple and Carmichael, 2015).

The other main differences in isotopic signatures were among the sites or water bodies from which the shellfish were harvested, as has been demonstrated previously (Carmichael et al., 2004a; Oczkowski et al., 2008). These differences were evident through all categories of oysters and quahogs, wild or cultured. Since there was no difference between wild or cultured animals all were included together for analysis, although species were kept separate. After initial plotting of the mean nitrogen isotope signature, a pattern emerged based on the region where the sites were located, so comparison by site proceeded after pooling samples by region. The regions are those sites bordering Buzzards Bay, sites bordering Nantucket Sound, the Cape Cod Bay region, and the region bordering the Atlantic Ocean directly (Fig. 1). Buzzards Bay and Nantucket Sound sites showed significantly heavier isotopic signatures than the Cape Cod Bay or Atlantic Ocean regions for both quahogs and oysters (Fig. 2a and b), indicating shellfish from the first two regions contain a proportionally greater amount of nitrogen from anthropogenic sources.

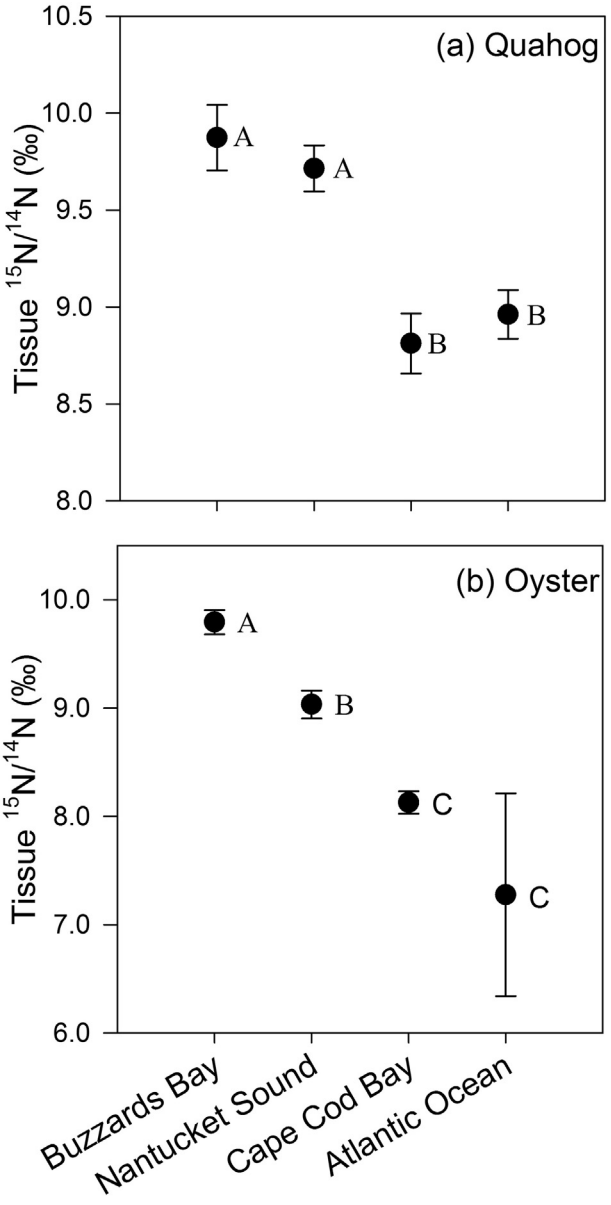

Fig. 2. Mean nitrogen isotope signature by general region in (a) quahogs and (b) oysters. Error bars indicate SE. Letters beside dots indicate statistical differences between regions, those which share a letter are not statistically different.

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Carmichael et al. (2004a) showed that in Cape Cod water bodies receiving 50–100% of the nitrogen available from waste water, isotopic signatures in quahog tissue were roughly 9–10‰. This would indicate that quahogs from Buzzards Bay and the Nantucket Sound estuaries are likely receiving and incorporating the majority of the nitrogen in their tissues from anthropogenic wastewater sources. It's not surprising that the other regions, Cape Cod Bay with a larger tidal range of 3–4 m (as opposed to 1–2 m elsewhere) and sites feeding directly to the Atlantic Ocean, had a more dilute signal of anthropogenic wastewater related N (lighter isotope signature). The same trend is apparent with oysters by region, although signatures are slightly lower overall, and Buzzards Bay oysters showed a heavier signature than the Nantucket Sound oysters.

Previous studies have indicated that shellfish may grow more rapidly and benefit from increased inputs of nitrogen (and increased wastewater inputs) due to increased quantities of food available (Carmichael et al., 2004b; Weiss et al., 2002). To investigate if there is a relationship between the proportion of nitrogen content in tissues and the N isotopic signature, correlations were obtained using mean isotopic signature by group and %N in the tissue, %N in the shell, and total %N by dry weight. Pearson correlation values were weakly negative with tissue %N ($r = -0.025$ quahogs, $r = -0.288$ oysters), slightly positive with quahog ($r = 0.491$) and negative with oyster ($r = -0.392$) shell %N, and weakly positive with quahog ($r = 0.179$) and negative with oyster ($r = -0.511$) total %N. Although growth may increase with increased nitrogen inputs, no strong correlation was evident to indicate there is an increased proportion of nitrogen in tissues of shellfish with heavier nitrogen isotopic signatures.

4. Implications for use in management

Isotopic signatures indicate shellfish being grown in waters feeding the regions of Nantucket Sound and Buzzards Bay are obtaining a significant portion of their nitrogen from anthropogenic sources. This, along with the available nitrogen data, indicate shellfish contain a small but appreciable amount of nitrogen, such that propagation and harvest of their tissues in sufficient quantities may represent a method to help alleviate increasing nitrogen levels in local embayments. The shellfish sampled in this study showed tissue nitrogen content to be fairly similar in range to literature values, but not exactly the same. Differences in dry weights at legal size, either in shell or tissue, lead to most of the differences from the literature values in the mass of nitrogen per harvestable oyster and the overall total percent nitrogen. Differing from limited literature values, the percent nitrogen content in both quahog shell and meat tissue reported here is higher than previous reports.

Between the seasons sampled in the current study, a large difference was seen in the potential for nitrogen removal as both quahogs and oysters contained significantly more tissue, and thus nitrogen, in the fall as opposed to the spring. Aside from the seasonal difference, quahogs were not much different whether of cultured or wild origin, whereas oysters showed differences in the form of grow-out largely related to thicker heavier shells apparent in oysters of wild origin or cultured on the bottom. Differences among sites were also seen, but these differences were not as dramatic as the seasonal differences and it may be at least somewhat related to nutritional status of the shellfish at the time of sampling.

Previous reports demonstrated that between harvest size classes of oysters there are up to 1.5 fold differences in total weight of nitrogen contained (Higgins et al., 2011, Grizzle et al., 2016). It is recommended that for greatest accuracy that the measure used to estimate nitrogen removal be based on weight of animals harvested, rather than number of shellfish harvested. Given the variability in N levels seen in the current study, it is further recommended that for harvested shellfish to be credited for N removal from a water body, N content be measured at each point of harvest, as size, season, site, and grow out methodology can impact N bioextraction potential.

As an example of how this information may be used in practice, the town of Mashpee, MA, USA has developed a Comprehensive Watershed Nitrogen Management Plan (CWNMP) addressing Total Maximum Daily Loads (TMDL) of nitrogen entering coastal embayments of the town (Town of Mashpee Sewer Commission, 2015). While the plan incorporates several approaches to nitrogen reduction, harvest of shellfish through aquaculture and propagation for fishery enhancement has been recommended for a number of embayments. The Mashpee plan used data provided in this study as a starting point and further analysis allowed for better estimation of N reduction through harvest of shellfish based on larger harvest size than the current study, and based on season of harvest. In one area, the plan targets culturing oysters for harvest in the Mashpee River system to remove 50% of the TMDL, or 2500 kg N per year to be removed, through harvest of 500,000 kg of oysters or 5,000,000 oysters at the intended harvest size of 100 g (0.5 g N per oyster). While quahog production and harvest of 292,000 kg, or 4,870,000 quahogs at an average size of 60 g (0.3 g N per quahog), is recommended for the complete TMDL of 1460 kg N per year in the Popponesset Bay system. The challenges to maintaining annual shellfish harvest at these levels are not insignificant, but a plan is in place to pursue shellfish aquaculture and subsequent harvest as an option while other portions of the plan such as more traditional source control strategies like wastewater treatment facilities are much slower to come online.

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