Shellfish Nitrogen Content from Coastal Waters of Southeastern Massachusetts

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Abstract

As nitrogen entering coastal waters of Cape Cod continues to be an issue, much attention has been generated to identify potential options which may help alleviate this stressor to estuaries. With Cape Cod's long history of shellfish harvest, the propagation or culture of filter feeding bivalves represents a unique opportunity to potentially help alleviate a growing problem and also generate economic activity. Nitrogen values for shellfish can be found in scientific literature but are most often reported from other distinct regions of the country such as the Chesapeake. Recognizing the importance of locally derived information, oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) from various Cape Cod area sources were recently tested for nitrogen content stored in tissues which would represent a net removal from a water body if harvested. Results showed local oysters have an average of 0.69% nitrogen by total dry weight (about 0.28gN/animal) and quahogs average 0.67% nitrogen by total dry weight (about 0.22gN/animal). However, these values did vary by season and to a lesser extent by location or grow out method (i.e. bottom grown vs. in off-bottom gear). The biggest driver of difference in N content among similarly sized shellfish cohorts was the mass of shell or meat tissue contained as opposed to the percent nitrogen in the tissues. Nitrogen isotope data indicate shellfish from certain water bodies in the region are incorporating significant amounts of nitrogen from anthropogenic sources.

Background

Our 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, though increased human activity has accelerated the rates of N enrichment (Carmichael et al. 2004a). This increase in nitrogen enrichment and eutrophication has a negative impact on coastal waters and can be a root cause of habitat degradation (Bowen et al. 2007, Howarth 2008).

To combat this growing problem in coastal Massachusetts, the approach being taken is reducing nitrogen to thresholds identified as important to maintain 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, and others (Dudley 2003). The use of shellfish production and harvest has also recently garnered interest as an option in plans to reach nitrogen management thresholds and is being initiated in several coastal towns. This holds particular appeal in Massachusetts, as there is a long and continued history of shellfish harvest, a growing shellfish aquaculture industry, and the harvest generates local economic activity.

Bivalve shellfish, including commercially important oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*), live in coastal water bodies and derive their nutrition from filter feeding on available algae or phytoplankton in the water column. Oysters and quahogs are able to pump water into their shell cavity where particles are trapped on their gills and coated in mucus capturing particles extremely efficiently, often close to 100% of the particulates over 5 microns in size (Riisgard 1988). Not all of these particles are ingested as food, and depending on the feed quality and quantity a portion will end up being rejected and released before ingestion; these rejected mucus coated particulates are known as pseudofeces (Beecham 2008, Ward & Shumway 2004). Of the portion of particles ingested, one study has shown about 50% will be incorporated into tissues while the remainder is released as fecal material or metabolites (Newell & Jordan 1983), though this may be more variable depending on food source. This shellfish feeding activity affects the local marine nitrogen cycle through incorporation of nitrogen in shellfish tissues, excretion of inorganic nitrogen metabolites, and the cycling of nitrogen through the particulate waste of feces and pseudofeces (collectively called biodeposits).

There are two general ways in which shellfish may remove nitrogen from their surrounding environment. The first is through direct incorporation of nitrogen acquired through feeding

and use in their body tissues, largely as protein. Shellfish do not absorb nitrogen directly from their environment but act as a first order consumer grazing on phytoplankton. The phytoplankton shellfish feed upon assimilate dissolved nitrogen directly from the surrounding waters, which can be transferred to the shellfish following consumption and digestion. This grazing effect has been measured in both the laboratory (Riisgard 1988) and the field (Grizzle et al. 2008) and can represent substantial amounts of water filtered with every day of feeding activity. As shellfish graze and grow they incorporate more and more nitrogen from the local waters into their tissues (via the plant life they consume) which can be ultimately removed through harvest of the shellfish.

The second general method for potential nitrogen removal is through the interaction of particulates released by shellfish and the surrounding sediment. The particulate waste generated by shellfish, also called biodeposits, falls to the bottom and can become buried or feed an active population of microorganisms there. Under the right conditions these microorganisms can process the particulates resulting in the production of di-nitrogen gas which is released to the atmosphere removing it from the marine environment (for more complete description see Newell et al. 2002, Kellogg et al. 2013).

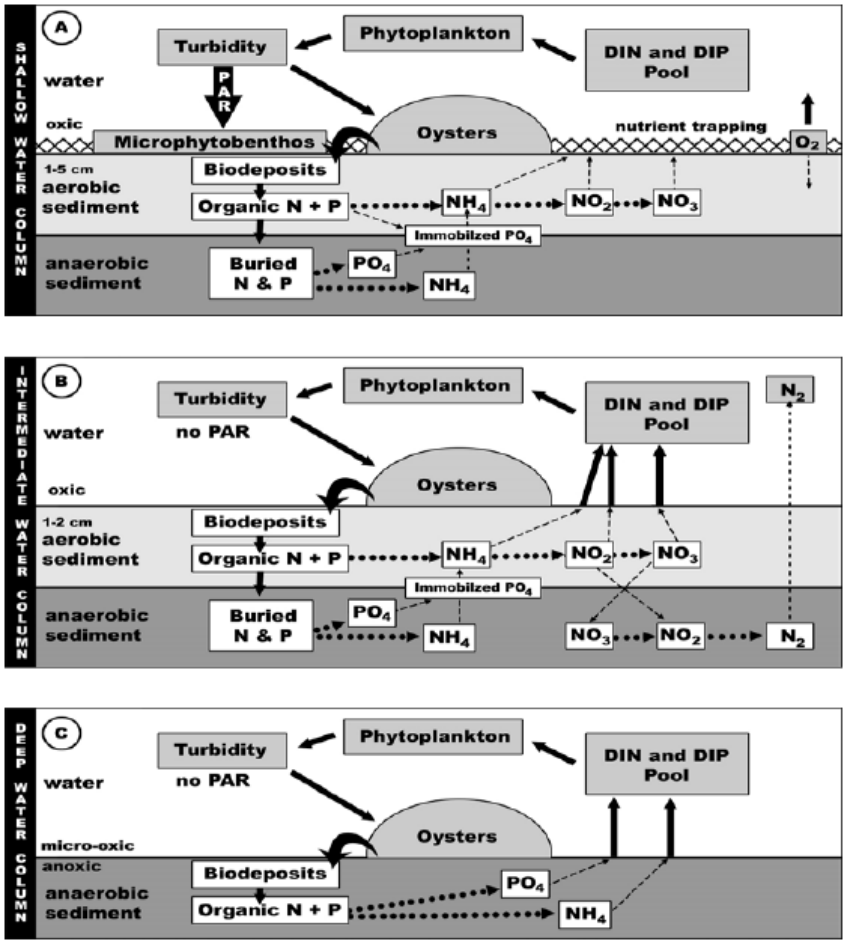

Figure 1 Nitrogen cycling and the denitrification process under different conditions (Newell et al. 2005)

The process of denitrification occurs naturally in coastal water bodies, but may be further stimulated by populations of bivalves. This bivalve stimulated denitrification can become a significant source of nitrogen removal from the water column over the course of a bivalve's life (Newell et al. 2005) but the levels or rates of denitrification can be somewhat variable. For example, oyster reefs in the Chesapeake have been shown to have dramatically higher rates of denitrification compared to control sites without oysters (Kellogg et al. 2013) and could be on the order of about 0.5g of N removed per 3" (1g dry weight) harvest sized oyster (Newell et al. 2005). On the other hand a recent study found no effect on denitrification rates in the sediment where oysters were cultured in floating cages (Higgins et al. 2013), while clam farming has been demonstrated to modestly enhance denitrification rates (Nizzoli et al. 2006). These rates also vary seasonally based on levels of oyster and microbial activity (Kellogg et al. 2013, Newell et al. 2005) and are also based largely on site and sediment conditions (Figure 1) as well as bivalve densities (Burkholder & Shumway 2011).

The natural process of denitrification and potential for further stimulation through bivalve production is intriguing though much is not fully understood, such as the balance of bivalve densities and the sediment conditions. The measurement of potential rates of nitrogen removal through denitrification and/or burial can also be challenging and expensive to determine for practical purposes. Obtaining measures of the nitrogen content within shellfish tissues, or that which could be removed from the water with the harvest of shellfish is more straight forward and may provide the simplest approach to quantification of removal potential through shellfish production.

Oysters		
Meat	**Shell**	**Source**
7%	0.30%	wild Chesapeake oysters, Newell 2004
7.90%	0.20%	float cultured Chesapeake oysters, Higgins et al. 2011
8.60%		juvenile oysters cultured in MA, Carmichael et al. 2012
9.27%	0.21%	wild Virginia oysters, Sisson et al. 2011
Quahogs		
Meat	**Shell**	**Source**
4.20%		calculated based on estimated protein in quahogs, Rice 2001
5.96%	0.15%	variable sized wild Virginia quahogs, Sisson et al. 2011

Table 1 Values reported in the literature of percent nitrogen content in shell and meat tissues of both oysters and quahogs.

Literature values are available for some species of shellfish through estimation using protein content (e.g. Rice 2001) or measurement of the tissue content directly (e.g. Higgins et al. 2011). Values available for the eastern oyster (*Crassostrea virginica*) based on percent nitrogen by dry weight have varied to some degree: in soft tissues or the meat the range is roughly 7-9.3% (Table 1, Newell 2004, Higgins et al. 2011, Carmichael et al. 2012, Sisson et al. 2011) while in the shell the range is 0.2-0.3% (Table 1, Higgins et al. 2011, Sisson et al. 2011, Newell 2004). With quahogs (*Mercenaria mercenaria*) the data is more limited but soft tissue ranges from 4.2-6% (Table 1, Rice 2001, Sisson et al. 2011) and the only shell value available estimated nitrogen at 0.15% in wild quahogs from Virginia (Sisson et al. 2011). While the range of these values are not dramatically different there does appear to be potential for differences in percent nitrogen content between species, location, size, grow out method, as well as by season.

Due to the variability reported elsewhere in the percent nitrogen content data available for the two most commercially important near shore species of shellfish harvested here in Southeastern MA (quahogs and oysters), local values may be necessary before assessing the nitrogen removal capacity available through harvest of shellfish. The goal of this project was to gain a better understanding of the nitrogen content in quahogs and oysters local to

Southeastern MA and Cape Cod at legal harvest size, and what the variation may be considering the above mentioned variables. Overall it is hoped this data will prove useful to those considering shellfish as a potential tool to help alleviate nitrogen enrichment in MA coastal waters.

Methods

With the help of municipal shellfish departments, as well as shellfish growers and harvesters, oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) were collected for nitrogen content analysis from various water bodies in the Cape Cod region to represent what shellfish are typically harvested and a range in local geography (See map in Figure 2). To assess potential differences due to season, a first round of samples was taken in June of 2012, and then a second round 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 basic categories: wild, cultured on-bottom, and cultured off-bottom (a fourth group cultured off-bottom triploid oysters was also included at one site only), while quahogs were separated into 2 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, while wild shellfish should represent native or naturally propagated populations.

Shellfish were selected for inclusion in the field sample at a typical harvest size, which is 3-3.5" (inches) in shell length for oysters, and 1-1.5" in hinge width for quahogs (oysters or quahogs may be harvested at other sizes but to standardize we chose these general size ranges). Four animals were taken for each category or group sampled. Shellfish samples were individually labeled and held refrigerated until measurement for shell length(mm), width(mm), height(mm) as well as whole weight(g) and volume displaced(mL). 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 laboratory methods.

Figure 2 Map showing sample locations

Isotopic signatures of 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. 2004). 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 hours until completely dry and labeled for further analysis at the Stable Isotope Laboratory at Boston University. Isotope analysis was then provided on the adductor muscle tissue using standard methods of the Stable Isotope Laboratory.

Results and Discussion

Oyster and Quahog Comparison

The oysters local to Massachusetts waters examined in this study averaged 83.8mm (3.3”) in shell length, 40.9g of shell, and 2.43g of dried tissue. While quahogs (at the littleneck size sampled) averaged 56.1mm (2.2”) in shell length, 31.2g of shell, and 2.22g of dried tissue (Table

2). Oysters at legal harvest size are significantly larger than just legal (littleneck) quahogs mainly in shell weight and length but not in dried tissue (meat) weight (see Table 2). This difference in shell weight combined with the higher %N in oyster tissue (8.01% oyster, 7.69% quahog) and shell (0.24% oyster and 0.18% quahog) lead to an average weight of N contained in a harvestable animal to be significantly higher for oysters than quahogs (0.28g and 0.22g respectively). The overall percent N per unit dry weight was not different, averaging 0.69% for oysters and 0.67% for quahogs. The size and weight of shell seemed to be the biggest difference between the two species, and interestingly it seems quahogs overall generate more of the N content through meat or soft tissues than oysters (Figures 3 A and B).

Comparison	Oysters	Quahogs	Difference
Shell Length (mm)	83.8	56.1	*Yes
Whole Wt (g)	66.1	51.7	*Yes
Shell DW (g)	40.9	31.2	*Yes
Tissue DW (g)	2.43	2.22	No
Tissue %N	8.01	7.69	*Yes
Shell %N	0.24	0.18	*Yes
Total N (g)	0.282	0.221	*Yes
Total % N (DW)	0.686	0.665	No

Table 2 Comparison of oyster and quahog size and nitrogen content. The difference column indicates if there were statistical differences ($p<0.05$).

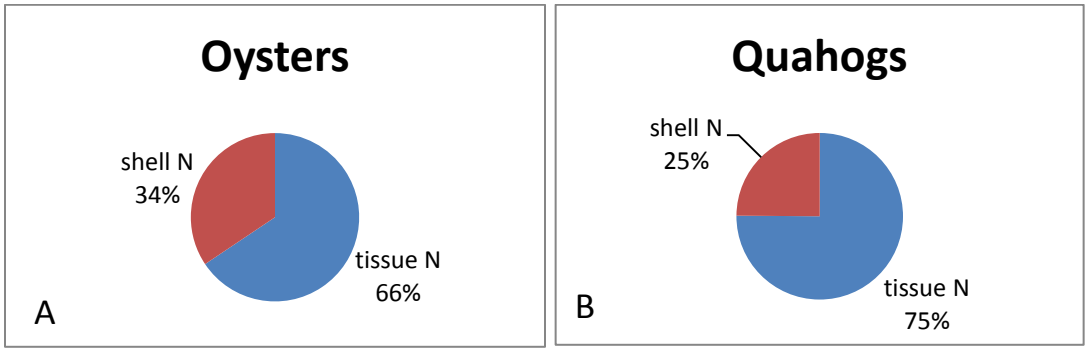

Figure 3 Nitrogen contribution to the total by tissue type in oysters (A) and quahogs (B)

Comparison to literature values

While nitrogen values obtained local to Cape Cod area waters are comparable to the other values available in the literature, some differences are also very apparent (Table 3). The actual

percent nitrogen in the tissue or shell is fairly similar among literature values in comparison to Cape Cod samples, though the samples from Cape Cod do appear to have a higher percent nitrogen in tissues (meat) for oysters and especially quahogs. The wild Chesapeake Bay oysters (Newell 2004) stand out as having much more nitrogen per animal despite a slightly lower shell length (76mm average); this difference is solely related to the dramatically higher shell weight in these animals (Table 3). When breaking the nitrogen content down to a percent of the total dry weight (both tissue and shell) the Cape Cod area shellfish contain a consistently higher level. Since the tissue (meat) contains 66-75% of the oyster or quahog nitrogen, it is worth mentioning the oysters in this study averaged a greater tissue weight (2.43g) which is 143% higher than the wild Chesapeake oysters (1g, Newell 2004) and 54% higher than the cultured Chesapeake oyster average (1.58g, Higgins et al. 2011).

	Shell Length (mm)	**Shell DW (g)**	**Tissue DW (g)**	**Tissue %N**	**Shell %N**	**Total N (g)**	**Total % N (DW)**
Quahogs from Cape Cod (this study)							
Wild	57.1	32.6	2.43	7.50	0.18	0.24	0.67
Cultured	55.0	29.6	1.99	7.90	0.17	0.21	0.66
Quahog avg	56.1	31.2	2.22	7.69	0.18	0.22	0.67
Wild quahogs from Virginia (Sisson et al. 2011)							
	NS	NS	NS	5.96	0.15	NS	NS
Oysters from Cape Cod (this study)							
Wild	82.7	46.0	2.42	8.20	0.26	0.31	0.67
Cultured On	84.9	47.4	2.70	7.89	0.26	0.32	0.65
Cultured Off	83.1	35.7	2.36	7.95	0.21	0.26	0.70
Off Triploid	86.5	22.3	1.36	8.50	0.32	0.19	0.82
Oyster avg	83.8	40.9	2.43	8.01	0.24	0.28	0.69
Wild oysters from reefs in Chesapeake (Newell 2004)							
	76.0	150.0	1.00	7.00	0.30	0.52	0.34
Cultured floating cage oysters - Chesapeake (Higgins et al. 2011)							
	85.5	37.6	1.58	7.28	0.17	0.18	0.45

Adapted from: Newell and Mann 2012

Table 3 Quahog and oyster data summary with literature comparisons. Adapted from Newell and Mann 2012. NS indicates values were not specified.

Wild vs. Cultured, Including Grow Out Method

There was little overall difference between wild or cultured quahogs (Table 3), though there were some significant differences related to the size of quahogs sampled (length 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 (0.67% and 0.66%, for wild and cultured quahogs respectively). However, among the categories of oysters sampled some differences were apparent despite being statistically similar in size or length (Table 4). These differences were largely related to differences in shell weight which equated to overall differences in the amount of N which would be harvested per animal. Differences reflect 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 with 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 have the added growth advantage of conserving reproductive energy loss for growth (Newkirk 1996, Walton & Murphy 2011). 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 while the rest of the fall oysters were at least 1+ in age. Despite these differences in shell weight and mass of N per animal, when considered on percent of the total dry weight per animal ("Total % N" in Table 4) there was no statistical difference between oyster categories.

	Wild	On-bottom	Off-bottom	Off-Triploids	Difference
Shell Length (mm)	82.7	84.9	83.1	86.5	no
Shell DW (g)	46	47.4	35.7	22.3	*yes
Tissue DW (g)	2.42	2.7	2.36	1.36	*yes
Tissue %N	8.2	7.89	7.95	8.5	no
Shell %N	0.263	0.26	0.21	0.316	no
Total N (g)	0.306	0.317	0.255	0.189	*yes
Total % N (DW)	0.666	0.653	0.704	0.82	no

Table 4 Oyster category summary data. Colors indicate statistically different groups.

Seasonal Differences

One of the larger factors shaping differences seen in both quahogs and oysters was related to seasonal differences between the spring and fall sampling periods (Table 5). There was no difference in size (measured as length) of the animals sampled between the seasons, but the amount of tissue (meat) went up 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 in the Total N and Total % N as well, which shouldn't be understated as a harvested quahog or oyster in fall would have 44% or 28% more N per animal than if harvested in the spring at the same size. The tissue of 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 glycogen which builds going into the fall season, leaving a lower relative percent protein composition (Thompson et al. 1996), which also translates to reduced tissue %N seen in the fall with the Cape Cod oysters and quahogs. The differences in %N of the shell by season, albeit in different directions is also notable. The June and October samples taken in this study represent seasonal tissue difference extremes, so an average may be helpful as an estimate for full year harvest information.

Seasonal	Quahogs			Oysters		
Comparison	Spring	Fall	Difference	Spring	Fall	Difference
Shell Length (mm)	56.5	55.6	No	82.5	85.1	No
Shell DW (g)	32.3	29.9	No	38.3	43.4	No
Tissue DW (g)	1.71	2.78	*Yes	1.63	3.23	*Yes
Tissue %N	8.07	7.29	*Yes	8.89	7.14	*Yes
Shell %N	0.15	0.20	*Yes	0.27	0.22	*Yes
Total N (g)	0.18	0.26	*Yes	0.25	0.32	*Yes
Total % N (DW)	0.53	0.82	*Yes	0.63	0.73	*Yes
Condition Index	7.91	14.32	*Yes	6.99	12.44	*Yes

Table 5 Differences in quahogs and oysters between season sampled.

Site or Water Body Differences

There were some differences apparent due to the site or water body the shellfish were grown in (Table 6, summarized groups with differences). When using total percent nitrogen as the point of comparison (which should normalize for any difference in size) and comparing like groups within a single season (to eliminate the potential for species, grow out and seasonal

variation) across water bodies differences were seen but not in every comparison. Oysters cultured off-bottom showed differences between water bodies in both spring and fall (Figure 4A), oysters cultured on-bottom only showed differences in fall, while wild oysters did not show any difference across sites. With quahogs total percent nitrogen content varied among cultured cohorts from different water bodies in spring and fall (Figure 4B), while there was no apparent variability with wild quahogs. Differences were also seen for percent nitrogen in the soft or meat tissues by water body and to a much smaller extent for percent nitrogen in the shell (See Appendix Table A, B, and C for a summary of spring, fall, and seasonal average values by location).

Differences:	**Quahog**				**Oyster**					
by water body	**Spring**		**Fall**		**Spring**			**Fall**		
Parameter	**Cultured**	**Wild**	**Cultured**	**Wild**	**Off-bottom**	**On-bottom**	**Wild**	**Off-bottom**	**On-bottom**	**Wild**
Total %N	yes	no	yes	no	yes	no	no	yes	yes	no
N/animal	yes	yes	no	yes	yes	yes	no	yes	yes	no
Meat %N	yes	yes	yes	yes	yes	no	no	no	yes	no
Shell %N	yes	no	no	no	no	no	no	yes	yes	no
Length	yes	yes	no	no	yes	no	no	yes	yes	no
Whole Wt	yes	yes	no	no	yes	yes	no	yes	yes	no
Condition Index	yes	yes	yes	yes	yes	no	no	yes	yes	no

Table 6 Differences (indicated as yes) when comparing across water bodies within cohorts

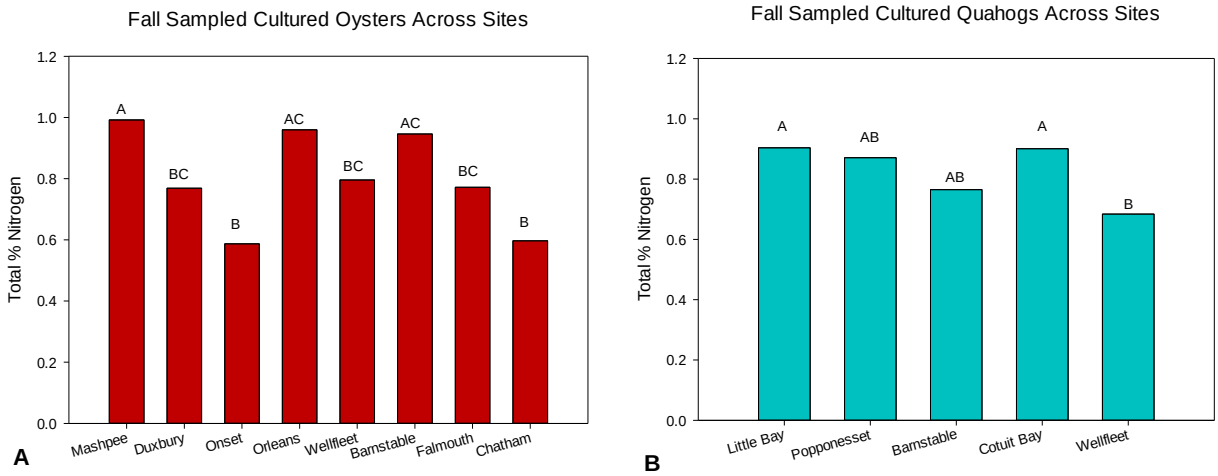

Figure 4 Differences across sites or water bodies in fall samples among (A) oysters cultured off-bottom and (B) cultured quahogs. Letters indicate statistical differences.

Variables not accounted for when sampling could easily affect nutritional status of the sampled animals. 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 which would include the gut. In addition, animals from any one particular water body may be affected at the time of sampling by a disease, environmental stressor, or available food leading to a different nutritional status which can lead to 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 & Scott 1982, Rainer & Mann 1992). When we look at condition of the shellfish sampled there is a positive correlation between condition index and percent nitrogen content, with oysters (r=0.38) and especially with quahogs (r=0.83) indicating this may explain some of the variability seen between water bodies as differences were also seen for condition index or nutritive status of the shellfish between water bodies (Table 6).

Size and Weight Considerations

Size is a critical factor when looking at shellfish and the potential for nitrogen removal through harvest of their tissues. Higgins et al. (2011) previously reported that between harvest classes of oysters from the Chesapeake Bay (cocktail- at least 50mm or 2", regular at least 76mm or 3", jumbo at least 102mm or 4") there was at least a 1.5 fold difference in total weight of nitrogen contained, and in fact jumbos (117mm length with 0.4g N) actually contained 4 fold more N than the cocktails (65mm length with 0.1g N). In the MA shellfish sampled in this study, an attempt was made to keep sizes similar but even so a correlation was seen between length and the quantity of nitrogen contained (Figures 5A and 5B). Pearson correlation values between length and N content were lower for oysters (0.50 in spring, 0.44 in fall, 0.49 overall) than for quahogs (0.80 in spring, 0.75 fall, 0.61 overall). While with the total %N content the correlation values were much lower and variable -0.03 in spring, -0.25 in fall, and -0.08 overall for oysters, and 0.33 in spring, -0.27 in fall, and -0.04 overall for quahogs, indicating little relationship between size of the animal and the total percent nitrogen contained.

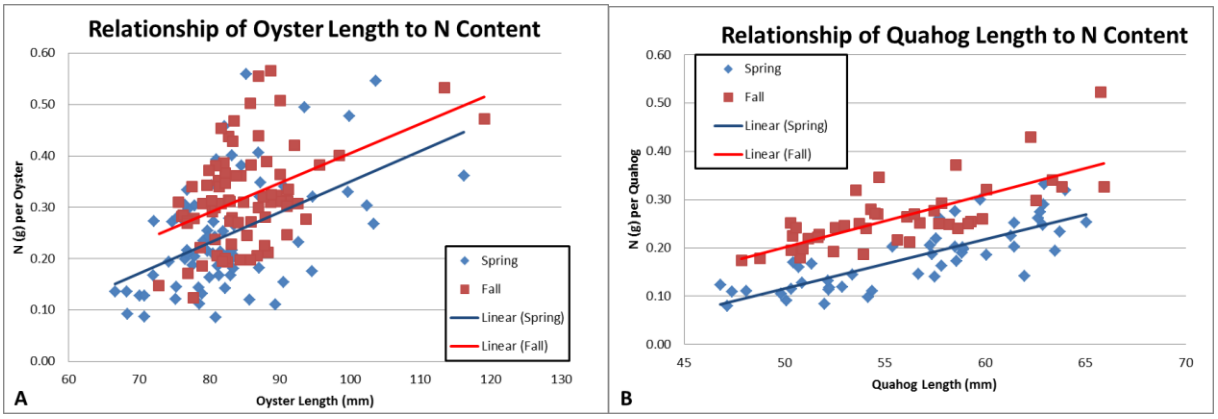

Figure 5 Relationship of size and N content by weight in (A) oysters and (B) quahogs

With size being so strongly tied to the weight or mass of N content, it would be recommended that for the greatest accuracy the measure used to estimate nitrogen removal potential be based on the less size dependent total % N content and the total dry weight of animals harvested so that size and weight is fully considered. Dry weights could be obtained from typical sizes of shellfish being harvested fairly easily, or may be extrapolated to average weights of bushels or bags being harvested. Estimating removal potential based on the weight of N seen in the data reported here (shellfish at just over legal size) may significantly underestimate removal potential if shellfish are typically harvested at larger sizes, this is especially important with quahogs which may be harvested at various sizes from the wild fishery. Using weight estimates and the total % N values obtained here should provide a reasonable estimate, or if a potential conservative or underestimate is acceptable measuring by the piece may suffice.

Estimating dry weight based on whole weight is an approach worth considering to streamline nitrogen removal estimates. The tissue or meat of oysters contains about 80% water before being dried (Galstoff 1964) so dry weights would be much more reliable. The dry meat tissue of oysters averaged 3.76% (SD 1.5%) of the whole (live) weight, and the shell was 61% (SD 6.4%) of whole weight. It should be mentioned whole weights in the shellfish used in this study were done on live animals after removing any fouling organisms and patting dry. These values should be taken conservatively though as there are significant differences in dry meat content between spring and fall, and with shell content between groups of oysters, those on bottom or wild with a greater percent of total weight in shell. 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. While there are differences between fall and spring in meat content of quahogs, the shell was consistent and there is little difference between wild and cultured in the proportion of tissues.

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 isotopic signature, expressed as ^{15}N:^{14}N ratio in permill (‰) then allows for an estimation of whether shellfish from our area are indeed incorporating wastewater derived nitrogen, with heavier signature values indicating a larger portion of the nitrogen is likely from waste water derived nutrient sources. The average signature of quahogs (9.43‰) was significantly heavier than the average for oysters (8.75‰), and when looking at differences between the species within the same water body (Barnstable and Wellfleet Harbors) quahogs remained heavier in isotopic signature than oysters off-bottom but not necessarily from oysters on-bottom, which had a heavier signature than the off-bottom oysters

(data summarized in Appendix Table D). This difference between species may indicate a different preference or availability in food source between the species with quahogs tending towards a greater anthropogenic source of nitrogen, or the results may be skewed due to the different water bodies, or areas of water bodies sampled for oysters and quahogs. There were no differences between cultured (9.41‰) or wild (9.44‰) quahogs, or among the categories of oysters (8.71‰ off-bottom, 9.02‰ on-bottom, 8.74‰ wild). The only exception being if the off-bottom triploid oysters (7.37‰) were to be included, which represented much younger animals from only one site but were significantly lighter in isotopic signature than oysters cultured on-bottom.

Aside from the difference seen between the two species, the main differences in isotopic signatures were between 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 (Figure 6A and 6B, Appendix D), and to further simplify the analysis and demonstrate the differences in isotopic signatures, sites were further lumped into regions. The regions are those sites bordering Buzzards Bay, the South Cape area involving water bodies bordering Nantucket Sound, the Cape Cod Bay region, and the Outer Cape region (Town Cove for quahogs, and Pleasant Bay for oysters). Buzzards Bay and South Cape sites showed significantly heavier isotopic signatures than the Cape Cod Bay or Outer Cape regions for both quahogs and oysters (Figures 7A and 7B) indicating shellfish from these waters are incorporating a greater amount of the available nitrogen from anthropogenic sources.

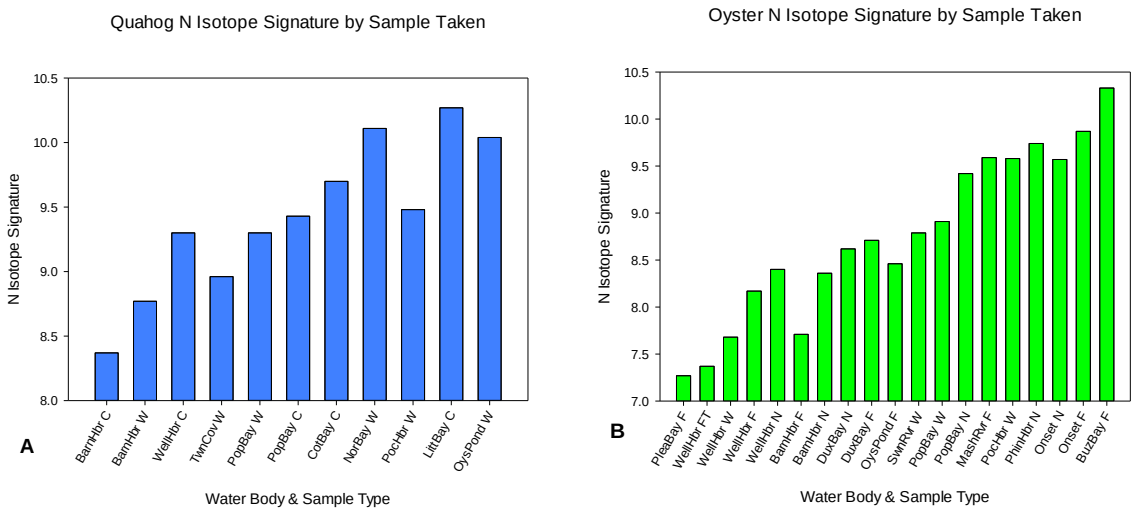

Figure 6 Nitrogen isotope signature by sample taken in (A) quahogs and (B) oysters

For reference, 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 South Cape estuaries are likely receiving and incorporating the majority of the nitrogen in their tissues from anthropogenic wastewater sources, while in the more open sites of Cape Cod Bay and the Outer Cape the wastewater contribution is much less or more dilute with a bigger influence of offshore nitrogen (sources which tend to have a lighter isotope signature). The same trend is apparent with oysters by region though signatures are slightly lower overall, and Buzzards Bay showed an even heavier signature than the South Cape water bodies.

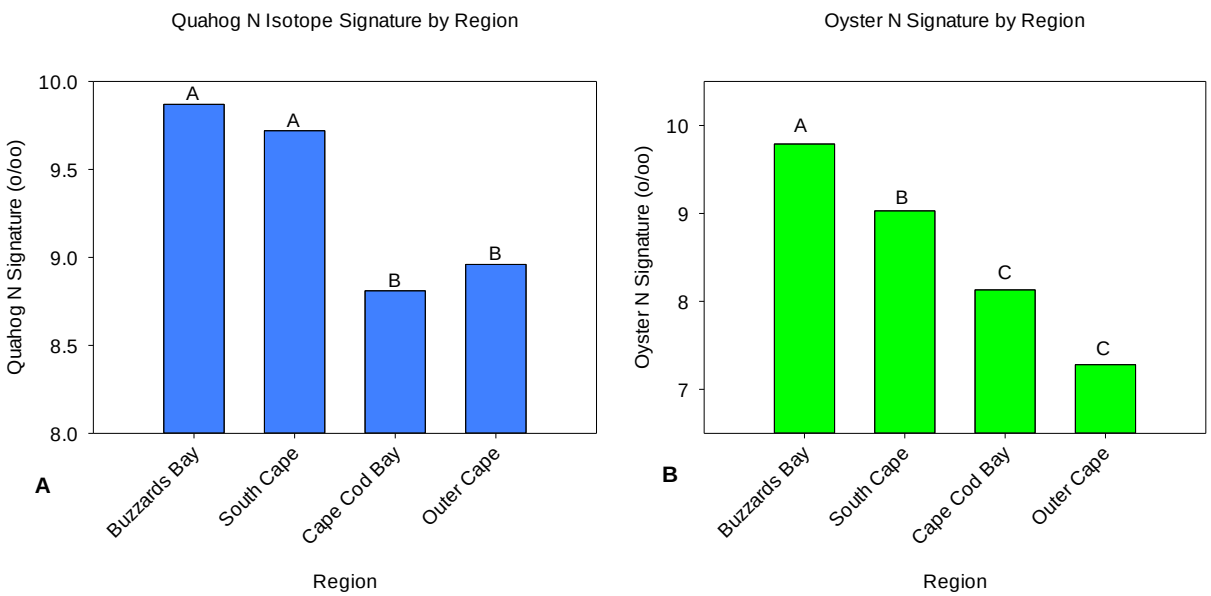

Figure 7 Nitrogen isotope signature by region in (A) quahogs and (B) oysters

Previous studies have indicated shellfish may grow more rapidly and benefit from increased inputs of nitrogen (and increased waste water 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 isotopic signature of the nitrogen (or nitrogen source) correlations were done comparing isotopic signature with %N in the tissue, %N in the shell, and total %N by dry weight in the soft tissues. Correlation values were weak overall, slightly negative with oysters, and slightly positive with quahogs. Though growth may increase with increased nitrogen inputs, no trend was seen to indicate there is an increased proportion of nitrogen in tissues of shellfish with heavier nitrogen isotopic signatures.

Carbon Content

Analyzing the carbon content of shellfish was not a goal of this study, but was available due to the way in which the laboratory obtained nitrogen data and is provided here for reference. Oysters averaged 42.2% carbon in their meat or soft tissues (42.7% spring, 41.7% fall) for an average of about 1g of carbon per oyster (0.70g spring, 1.36g fall). Quahogs averaged 38.5% carbon in the soft tissues (39% spring, 38% fall) for a content of about 0.86g per quahog (0.67g spring, 1.06g fall).

The shell tissue was run differently for the spring and fall samples, because the high carbonate content can sometimes cause problems in analysis the spring shell samples were acidified before assay of nitrogen and carbon content leaving just the organic carbon. The fall shell samples were assayed without the acidification step (and without any problems) so that the carbonate content as well as the organic carbon was included. Spring samples showed oyster shells to contain about 0.77% (0.3g) organic carbon, while quahogs had 0.48% (0.16g) organic carbon in the shell. With the fall samples, including the carbonate portion, oyster shells were 12% carbon with about 5.2g by weight, and quahog shells were 12.2% carbon with about 3.67g by weight.

Summary

The shellfish sampled in this study showed tissue nitrogen contents to be fairly similar in range to literature values but not exactly the same. Differences in tissue content at legal size, either in shell weight or dried meat tissues, 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 these previous reports. Among the animals sampled oysters were heavier and contained more nitrogen than littleneck sized quahogs, with oysters containing 66% of the total nitrogen content from soft tissues and quahogs 75% from the soft tissue. By season, a large difference was seen in the potential for nitrogen removal as both quahogs and oysters contained significantly more meat 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, while 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. Small differences between water bodies were 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.

Isotopic signatures indicate shellfish being grown in waters on the south side of Cape Cod and Buzzards Bay are obtaining a significant portion of their nitrogen from anthropogenic sources. This along with the available nitrogen data indicate shellfish are indeed an important part of nutrient cycling in near shore waters such that propagation and harvest of their tissues may represent a method to help alleviate increasing nitrogen levels in local embayments. It is recommended if nitrogen removal is to be credited to shellfish harvest, it be done on a unit weight basis due to the fact that size (i.e. amount of tissue) contained in an individual shellfish is directly correlated to the amount of nitrogen contained. Estimates based on the number of animals harvested may significantly under or over estimate nitrogen based on the value being used. For example, a chowder sized quahog may weigh about a pound (450g) in whole live weight, and if tissue proportions stay similar throughout growth this large quahog (19.5g dry tissue, 272g shell) would contain almost 2g of nitrogen, the equivalent of almost 8 littlenecks.

Example of data use

To quickly demonstrate how this data could be utilized: a town shellfish department estimates on average 5,000 pecks of oysters (~50 oysters per peck basket) or about 250,000 oysters are harvested annually from a shellfishing area supported by an oyster propagation program. Using a per oyster estimate from the values obtained here for the off-bottom technique used (0.255g of N per oyster) would equate to 63.75 Kg of nitrogen removed per year. After considering the size of oysters harvested, the town finds a lot of the oysters being harvested seem closer to 4" and is interested in more accurately quantifying the nitrogen contained in these oysters. Further analysis shows the oysters recreational harvesters are taking to be on average 55g in total dry weight (49.1g shell, 2.9g tissue), equating to about 2,455g of shell and 145g of meat tissue per 50 oyster peck basket. This ultimately computes to 17.5g of nitrogen per basket using 0.24% nitrogen in the shell and 8% nitrogen in tissue, and a total of 87.5kg of nitrogen removed with the 5000 basket total. Alternatively, one could also use the total dry weight (2,600g per basket) and the general oyster percent nitrogen by dry weight estimate of 0.686% to obtain a value of 17.8g per basket. Fine tuning the numbers for greater accuracy showed the nitrogen removal estimate to be about 37% higher than the conservative estimate standardized for an oyster just greater than the legal limit.

Acknowledgements

We would like to thank all of the municipal shellfish departments and the individual shellfish growers or harvesters for their help in obtaining samples. We are also very grateful to Bob Michener and the efforts of the Stable Isotope Laboratory at Boston University for all the hard work in processing samples, and for comments on the use and analysis of the collected data.

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Appendix A: Spring shellfish nitrogen content sample group averages

Shellfish Nitrogen Content - Average Values Per Sample Group																	
						Volume	Length	Whole	Dry Shl	Dry Tiss	Cond	Soft Tissue Values		Shell Values		Total (g)	Total (%)
Season	Town	Water Body	Species	Cult/Wild	Bottom	(ml)	(mm)	Wt (g)	Mass(g)	Mass(g)	Index	Meat%N	MeatN(g)	Shl %N	Shl N(g)	N/animal	N/animal
Spring	Wellfleet	Wellfleet Hbr	Cv	wild	on	50.0	76.43	79.26	53.76	1.68	6.7	9.40	0.16	0.23	0.12	0.27	0.51
Spring	Wellfleet	Wellfleet Hbr	Mm	cultured		30.0	54.05	45.73	28.90	0.88	5.1	8.54	0.07	0.14	0.04	0.11	0.38
Spring	Wellfleet	Wellfleet Hbr	Cv	cultured	on	42.5	78.70	62.95	38.31	1.47	6.1	8.45	0.12	0.45	0.17	0.30	0.75
Spring	Wellfleet	Wellfleet Hbr	Cv	cultured	off	33.8	82.06	44.40	22.70	1.05	4.8	8.47	0.09	0.17	0.04	0.13	0.54
Spring	Wellfleet	Wellfleet Hbr	Cv	cultured	off-trip	35.0	88.59	48.37	25.93	0.89	4.0	8.43	0.07	0.34	0.09	0.16	0.61
Spring	Barnstable	Cotuit Bay	Mm	cultured		40.0	61.33	63.51	37.23	2.11	8.0	7.24	0.15	0.11	0.04	0.20	0.49
Spring	Barnstable	North Bay	Mm	wild		42.5	61.57	64.50	37.18	2.01	7.4	7.99	0.16	0.15	0.06	0.22	0.56
Spring	Bourne	Pocasset Rvr	Cv	wild	on	42.5	85.60	67.82	39.20	2.48	8.7	8.99	0.22	0.37	0.15	0.37	0.88
Spring	Bourne	Little Bay	Cv	cultured	on	36.3	83.58	57.59	35.20	1.57	7.0	8.68	0.14	0.24	0.09	0.22	0.61
Spring	Bourne	Pocasset Rvr	Mm	wild		41.3	61.01	62.03	36.82	1.61	6.4	8.42	0.14	0.18	0.07	0.20	0.52
Spring	Bourne	Little Bay	Mm	cultured		30.0	55.41	47.85	28.90	1.16	6.0	8.66	0.10	0.17	0.05	0.15	0.48
Spring	Barnstable	West Bay	Mm	cultured		21.3	50.39	39.60	25.20	1.12	7.7	8.24	0.09	0.10	0.03	0.12	0.45
Spring	Barnstable	Barnstable Hbr	Mm	cultured		36.3	56.21	51.84	30.42	2.31	10.8	7.33	0.17	0.19	0.06	0.23	0.69
Spring	Orleans	Town Cove	Mm	wild		42.5	60.76	68.17	41.67	3.40	12.8	6.22	0.21	0.12	0.05	0.26	0.58
Spring	Orleans	Pleasant Bay	Cv	cultured	off	41.3	77.73	64.86	37.81	2.31	8.5	9.35	0.22	0.20	0.07	0.29	0.72
Spring	Barnstable	Barnstable Hbr	Cv	cultured	off	41.3	82.94	64.77	42.26	2.38	10.7	7.53	0.18	0.33	0.15	0.32	0.73
Spring	Chatham	Oyster Pond	Cv	cultured	off	52.5	81.41	82.30	50.39	1.45	4.7	8.26	0.12	0.12	0.06	0.18	0.35
Spring	Chatham	Oyster Pond	Mm	wild		25.0	51.24	40.76	25.17	1.17	7.4	7.84	0.09	0.08	0.02	0.11	0.42
Spring	Barnstable	Barnstable Hbr	Cv	cultured	on	48.8	84.01	79.66	52.12	2.40	8.7	8.78	0.21	0.28	0.15	0.36	0.65
Spring	Barnstable	Barnstable Hbr	Mm	wild		25.0	51.23	40.67	24.65	1.21	7.5	8.92	0.11	0.14	0.03	0.14	0.55
Spring	Wareham	Buzzards Bay	Cv	cultured	off	22.5	72.85	37.47	23.30	1.09	7.7	9.18	0.10	0.18	0.04	0.14	0.60
Spring	Wareham	Buzzards Bay	Cv	cultured	on	45.0	83.91	75.65	48.96	1.84	7.0	8.79	0.16	0.15	0.08	0.24	0.49
Spring	Mashpee	Popp Bay	Mm	wild		36.3	57.54	57.93	34.88	1.75	7.9	8.57	0.15	0.19	0.07	0.22	0.60
Spring	Mashpee	Popp Bay	Mm	cultured		33.8	57.37	58.74	36.79	1.75	8.0	8.80	0.15	0.20	0.08	0.23	0.59
Spring	Mashpee	Popp Bay	Cv	wild	on	35.0	84.34	57.85	35.69	1.56	7.3	9.17	0.14	0.40	0.12	0.26	0.79
Spring	Mashpee	Popp Bay	Cv	cultured	on	36.3	86.15	57.23	32.99	1.42	5.8	9.37	0.13	0.38	0.13	0.27	0.76
Spring	Mashpee	Mashpee Rvr	Cv	cultured	off	80.0	105.79	117.24	64.89	2.64	5.1	8.66	0.23	0.32	0.18	0.41	0.67
Spring	Falmouth	Buzzards Bay	Cv	cultured	off	41.3	85.36	65.44	44.79	1.27	6.1	8.71	0.11	0.19	0.09	0.20	0.43
Spring	Dennis	Swan River	Cv	wild	on	41.3	83.15	69.02	44.61	1.63	6.9	9.00	0.15	0.24	0.11	0.26	0.56
Spring	Duxbury	Duxbury Bay	Cv	cultured	on	21.3	73.55	34.00	21.51	1.01	8.2	9.62	0.10	0.26	0.06	0.15	0.68
Spring	Duxbury	Duxbury Bay	Cv	cultured	off	15.0	70.51	22.93	13.76	0.81	8.8	10.06	0.08	0.27	0.04	0.12	0.82

Appendix B: Fall shellfish nitrogen content sample group averages

Shellfish Nitrogen Content - Average Values Per Sample Group																	
						Volume	Length	Whole	Dry Shl	Dry Tiss	Cond	Soft Tissue Values		Shell Values		Total (g)	Total (%)
Season	Town	Water Body	Species	Cult/Wild	Bottom	(ml)	(mm)	Wt (g)	Mass(g)	Mass(g)	Index	Meat%N	MeatN(g)	Shl %N	Shl N(g)	N/animal	N/animal
Fall	Dennis	Swan River	Cv	wild	on	48.8	84.68	80.50	51.11	3.55	12.1	7.08	0.25	0.20	0.10	0.35	0.64
Fall	Wareham	Buzzards Bay	Cv	cultured	off	37.5	83.50	63.62	41.11	2.66	11.8	7.44	0.20	0.14	0.06	0.25	0.59
Fall	Wareham	Buzzards Bay	Cv	cultured	on	52.5	82.96	81.88	53.49	2.72	9.6	7.44	0.20	0.21	0.11	0.31	0.56
Fall	Mashpee	Popp Bay	Mm	wild		36.3	58.89	58.00	35.86	3.87	17.4	7.58	0.29	0.22	0.08	0.37	0.93
Fall	Mashpee	Popp Bay	Mm	cultured		26.3	53.90	42.61	26.77	2.62	16.8	7.92	0.21	0.17	0.05	0.25	0.87
Fall	Mashpee	Mashpee Rvr	Cv	cultured	off	33.8	85.69	51.23	26.37	3.61	14.5	6.73	0.24	0.20	0.05	0.29	0.99
Fall	Mashpee	Popp Bay	Cv	cultured	on	55.0	87.67	88.14	53.57	4.84	14.0	6.42	0.31	0.15	0.08	0.39	0.67
Fall	Mashpee	Popp Bay	Cv	wild	on	45.0	83.31	72.18	45.58	3.63	13.7	6.90	0.25	0.15	0.07	0.32	0.68
Fall	Barnstable	Barnstable Hbr	Mm	wild		28.8	54.73	47.59	28.82	2.85	15.5	7.04	0.20	0.21	0.06	0.26	0.84
Fall	Barnstable	Barnstable Hbr	Cv	cultured	on	53.8	86.22	86.33	49.94	5.21	14.5	6.16	0.32	0.33	0.16	0.48	0.88
Fall	Barnstable	Barnstable Hbr	Cv	cultured	off	47.5	86.37	66.14	36.10	4.40	14.8	7.34	0.33	0.16	0.06	0.38	0.95
Fall	Barnstable	Cotuit Bay	Mm	cultured		25.0	50.84	37.80	21.34	2.08	12.6	8.36	0.17	0.17	0.04	0.21	0.90
Fall	Barnstable	North Bay	Mm	wild		32.5	57.96	53.68	30.23	3.09	13.3	6.62	0.21	0.18	0.06	0.26	0.78
Fall	Duxbury	Duxbury Bay	Cv	cultured	off	31.3	85.51	43.40	24.98	2.19	11.9	7.17	0.16	0.20	0.05	0.21	0.77
Fall	Duxbury	Duxbury Bay	Cv	cultured	on	45.0	85.03	77.98	52.73	3.02	12.1	6.65	0.20	0.18	0.08	0.28	0.56
Fall	Chatham	Oyster Pond	Cv	cultured	off	51.3	88.91	81.11	50.23	4.11	13.3	6.03	0.25	0.14	0.07	0.32	0.60
Fall	Chatham	Oyster Pond	Mm	wild		43.8	61.80	70.60	42.86	3.33	12.4	6.80	0.23	0.27	0.12	0.35	0.76
Fall	Wellfleet	Wellfleet Hbr	Cv	wild	on	41.3	85.02	64.57	42.61	2.50	12.0	7.80	0.20	0.24	0.10	0.30	0.69
Fall	Wellfleet	Wellfleet Hbr	Mm	cultured		30.0	54.60	51.24	31.90	2.28	12.0	7.41	0.17	0.20	0.06	0.23	0.68
Fall	Wellfleet	Wellfleet Hbr	Cv	cultured	on	43.8	80.49	76.94	52.29	2.81	11.6	7.61	0.21	0.26	0.14	0.35	0.65
Fall	Wellfleet	Wellfleet Hbr	Cv	cultured	off	42.5	82.46	69.67	44.53	3.08	12.3	6.86	0.21	0.37	0.17	0.38	0.80
Fall	Wellfleet	Wellfleet Hbr	Cv	cultured	off-trip	27.5	84.50	36.90	18.73	1.83	10.1	8.57	0.16	0.29	0.05	0.21	1.03
Fall	Bourne	Phinneys Hbr	Cv	cultured	on	82.5	106.68	122.07	77.79	4.14	10.0	6.69	0.28	0.22	0.17	0.45	0.57
Fall	Bourne	Pocasset Hbr	Cv	wild	on	45.0	78.84	76.59	55.77	2.29	11.1	7.26	0.17	0.29	0.15	0.32	0.57
Fall	Bourne	Pocasset Hbr	Mm	wild		23.8	52.97	42.92	26.74	2.12	13.4	7.60	0.16	0.17	0.05	0.21	0.74
Fall	Bourne	Little Bay	Mm	cultured		27.5	54.36	44.73	26.72	2.69	15.2	7.10	0.19	0.27	0.07	0.26	0.91
Fall	Orleans	Pleasant Bay	Cv	cultured	off	35.0	80.92	54.26	27.90	2.99	11.3	7.78	0.23	0.23	0.06	0.30	0.96
Fall	Orleans	Town Cove	Mm	wild		26.3	55.94	46.47	26.56	2.79	14.1	6.43	0.18	0.20	0.05	0.23	0.79
Fall	Barnstable	Barnstable Hbr	Mm	cultured		28.8	56.07	51.31	31.59	2.90	14.8	7.28	0.21	0.16	0.05	0.26	0.77
Fall	Falmouth	Buzzards Bay	Cv	cultured	off	17.5	77.37	31.13	19.55	1.72	15.6	7.65	0.13	0.15	0.03	0.16	0.77

Appendix C: Overall (annual) shellfish nitrogen content average by sample location

Shellfish Nitrogen Content - Average Values Per Sample Group																	
						Volume	Length	Whole	Dry Shl	Dry Tiss	Cond	Soft Tissue Values		Shell Values		Total (g)	Total (%)
Season	Town	Water Body	Species	Cult/Wild	Bottom	(ml)	(mm)	Wt (g)	Mass(g)	Mass(g)	Index	Meat%N	MeatN(g)	Shl %N	Shl N(g)	N/animal	N/animal
Average	Barnstable	Barnstable Hbr	Cv	cultured	off	44.4	84.65	65.45	39.18	3.39	12.8	7.43	0.25	0.25	0.10	0.35	0.84
Average	Barnstable	Barnstable Hbr	Cv	cultured	on	51.3	85.11	82.99	51.03	3.80	11.6	7.47	0.27	0.31	0.16	0.42	0.77
Average	Barnstable	Barnstable Hbr	Mm	cultured		32.5	56.14	51.57	31.00	2.60	12.8	7.31	0.19	0.18	0.05	0.25	0.73
Average	Barnstable	Cotuit Bay	Mm	cultured		32.5	56.08	50.65	29.28	2.09	10.3	7.80	0.16	0.14	0.04	0.20	0.70
Average	Barnstable	Barnstable Hbr	Mm	wild		26.9	52.98	44.13	26.73	2.03	11.5	7.98	0.15	0.18	0.05	0.20	0.69
Average	Barnstable	North Bay	Mm	wild		37.5	59.76	59.09	33.71	2.55	10.4	7.31	0.18	0.17	0.06	0.24	0.67
Average	Bourne	Little/Phinneys	Cv	cultured	on	59.4	95.13	89.83	56.49	2.85	8.5	7.68	0.21	0.23	0.13	0.34	0.59
Average	Bourne	Pocasset Hbr	Cv	wild	on	43.8	82.22	72.20	47.48	2.38	9.9	8.13	0.19	0.33	0.15	0.34	0.72
Average	Bourne	Little Bay	Mm	cultured		28.8	54.88	46.29	27.81	1.93	10.6	7.88	0.15	0.22	0.06	0.21	0.69
Average	Bourne	Pocasset Hbr	Mm	wild		32.5	56.99	52.47	31.78	1.86	9.9	8.01	0.15	0.18	0.06	0.20	0.63
Average	Chatham	Oyster Pond	Cv	cultured	off	51.9	85.16	81.71	50.31	2.78	9.0	7.14	0.18	0.13	0.06	0.25	0.47
Average	Chatham	Oyster Pond	Mm	wild		34.4	56.52	55.68	34.01	2.25	9.9	7.32	0.16	0.18	0.07	0.23	0.59
Average	Dennis	Swan River	Cv	wild	on	45.0	83.91	74.76	47.86	2.59	9.5	8.04	0.20	0.22	0.11	0.31	0.60
Average	Duxbury	Duxbury Bay	Cv	cultured	off	23.1	78.01	33.16	19.37	1.50	10.4	8.62	0.12	0.24	0.04	0.16	0.79
Average	Duxbury	Duxbury Bay	Cv	cultured	on	33.1	79.29	55.99	37.12	2.02	10.1	8.14	0.15	0.22	0.07	0.22	0.62
Average	Falmouth	Buzzards Bay	Cv	cultured	off	29.4	81.36	48.29	32.17	1.50	10.9	8.18	0.12	0.17	0.06	0.18	0.60
Average	Mashpee	Mashpee Rvr	Cv	cultured	off	56.9	95.74	84.23	45.63	3.13	9.8	7.69	0.24	0.26	0.12	0.35	0.83
Average	Mashpee	Popp Bay	Cv	cultured	on	45.6	86.91	72.68	43.28	3.13	9.9	7.90	0.22	0.27	0.11	0.33	0.71
Average	Mashpee	Popp Bay	Cv	wild	on	40.0	83.82	65.01	40.63	2.60	10.5	8.04	0.20	0.27	0.09	0.29	0.74
Average	Mashpee	Popp Bay	Mm	cultured		30.0	55.63	50.67	31.78	2.18	12.4	8.36	0.18	0.19	0.06	0.24	0.73
Average	Mashpee	Popp Bay	Mm	wild		36.3	58.21	57.96	35.37	2.81	12.6	8.08	0.22	0.20	0.07	0.29	0.77
Average	Orleans	Pleasant Bay	Cv	cultured	off	38.1	79.32	59.56	32.85	2.65	9.9	8.56	0.22	0.21	0.07	0.29	0.84
Average	Orleans	Town Cove	Mm	wild		34.4	58.35	57.32	34.12	3.10	13.4	6.32	0.20	0.16	0.05	0.25	0.69
Average	Wareham	Buzzards Bay	Cv	cultured	off	30.0	78.17	50.54	32.21	1.88	9.7	8.31	0.15	0.16	0.05	0.20	0.59
Average	Wareham	Buzzards Bay	Cv	cultured	on	48.8	83.44	78.76	51.22	2.28	8.3	8.12	0.18	0.18	0.09	0.27	0.52
Average	Wellfleet	Wellfleet Hbr	Cv	cultured	off	38.1	82.26	57.03	33.62	2.07	8.6	7.66	0.15	0.27	0.10	0.25	0.67
Average	Wellfleet	Wellfleet Hbr	Cv	cultured	off-trip	31.3	86.54	42.63	22.33	1.36	7.0	8.50	0.12	0.32	0.07	0.19	0.82
Average	Wellfleet	Wellfleet Hbr	Cv	cultured	on	43.1	79.60	69.94	45.30	2.14	8.8	8.03	0.17	0.36	0.16	0.32	0.70
Average	Wellfleet	Wellfleet Hbr	Cv	wild	on	45.6	80.73	71.91	48.18	2.09	9.4	8.60	0.18	0.24	0.11	0.29	0.60
Average	Wellfleet	Wellfleet Hbr	Mm	cultured		30.0	54.32	48.49	30.40	1.58	8.6	7.97	0.12	0.17	0.05	0.17	0.53

Appendix D: Average nitrogen isotope signature values by sample group. Shading differentiates regions of interest.

Region	Town	Water Body	Species	Cult/wild	Bottom	Vol (ml)	Length (mm)	Whole Wt(g)	N15 Ratio
Buzzards Bay	Bourne	Phinneys Harbor	oyster	cultured	on	115.0	122.46	147.53	9.75
Buzzards Bay	Bourne	Pocasset Harbor	oyster	wild	on	38.8	77.88	69.65	9.58
Buzzards Bay	Falmouth	Buzzards Bay	oyster	cultured	off	13.3	63.10	20.87	10.33
Buzzards Bay	Wareham	Buzzards Bay	oyster	cultured	off	38.8	81.08	61.77	9.87
Buzzards Bay	Wareham	Buzzards Bay	oyster	cultured	on	48.8	89.76	72.15	9.59
South Cape	Chatham	Oyster Pond	oyster	cultured	off	52.5	97.16	91.64	8.46
South Cape	Dennis	Swan River	oyster	wild	on	48.8	88.25	78.89	8.79
South Cape	Mashpee	Mashpee River	oyster	cultured	off	40.0	84.09	57.94	9.59
South Cape	Mashpee	Popponesset Bay	oyster	cultured	on	55.0	92.08	98.59	9.42
South Cape	Mashpee	Popponesset Bay	oyster	wild	on	45.0	86.83	64.88	8.91
Cape Cod Bay	Barnstable	Barnstable Harbor	oyster	cultured	off	38.8	83.42	55.48	7.71
Cape Cod Bay	Barnstable	Barnstable Harbor	oyster	cultured	on	50.0	82.60	80.12	8.36
Cape Cod Bay	Duxbury	Duxbury Bay	oyster	cultured	off	31.3	83.93	44.19	8.71
Cape Cod Bay	Duxbury	Duxbury Bay	oyster	cultured	on	43.8	86.28	73.44	8.62
Cape Cod Bay	Wellfleet	Wellfleet Harbor	oyster	cultured	off	41.3	81.84	63.77	8.17
Cape Cod Bay	Wellfleet	Wellfleet Harbor	oyster	cultured	off-triploid	21.3	81.26	28.76	7.37
Cape Cod Bay	Wellfleet	Wellfleet Harbor	oyster	cultured	on	40.0	78.37	68.50	8.40
Cape Cod Bay	Wellfleet	Wellfleet Harbor	oyster	wild	on	40.0	93.83	57.42	7.68
Outer Cape	Orleans	Pleasant Bay	oyster	cultured	off	31.3	79.83	49.51	7.28
Buzzards Bay	Bourne	Little Bay	quahog	cultured		28.8	54.64	46.18	10.27
Buzzards Bay	Bourne	Pocasset Harbor	quahog	wild		21.3	51.00	39.10	9.48
South Cape	Barnstable	Cotuit Bay	quahog	cultured		23.8	51.69	35.40	9.71
South Cape	Barnstable	North Bay	quahog	wild		32.5	57.09	54.03	10.11
South Cape	Chatham	Oyster Pond	quahog	wild		71.3	73.03	116.31	10.04
South Cape	Mashpee	Popponesset Bay	quahog	cultured		30.0	54.13	46.59	9.43
South Cape	Mashpee	Popponesset Bay	quahog	wild		40.0	60.75	71.33	9.30
Cape Cod Bay	Barnstable	Barnstable Harbor	quahog	cultured		28.8	56.02	49.18	8.37
Cape Cod Bay	Barnstable	Barnstable Harbor	quahog	wild		31.3	54.05	50.21	8.77
Cape Cod Bay	Wellfleet	Wellfleet Harbor	quahog	cultured		25.0	53.36	44.92	9.31
Outer Cape	Orleans	Town Cove	quahog	wild		26.3	54.32	47.80	8.96