INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume 1, No 5, 2011

Research article **ISSN 0976 – 4402**

Evidence for Tracing Incorporation of Sewage-Derived Organic Matter into an Urbanized Aquatic Food Web Though Stable Nitrogen Isotope Analysis

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ABSTRACT

Previous studies have demonstrated variable success in using stable nitrogen isotope analysis (ratio of ^{15}N to ^{14}N) as a means to gauge the incorporation of sewage-derived organic material (SDOM) into coastal food webs. In this study, 7 species of macroinvertebrates and 7 species of fish, selected to span a wide range in both trophic position as well as food source provenance, were sampled from the food web in Boston Harbour. Results showed that the more that organisms were dependent on benthic food (as measured by stable carbon isotope analysis), the greater their likelihood of ^{15}N-depletion, and presumably, therefore, SDOM incorporation.

Key words: Sewage incorporation, benthic foodweb, ^{15}N isotope analysis

1. Introduction

The collection, treatment and disposal of human waste has always been a defining characteristic of city planning, and even today remains an important concern for environmental and human health. For example, for more than a century, until 1991, Boston Harbour received considerable quantities of untreated sewage sludge in addition to annual discharges of effluent in the range of 4 to 13 billion L during the late eighties and early nineties (Lavery *et al,* 1994, Leo *et al,* 1995). The result was that Boston Harbour had among the highest recorded loadings of nutrients for any American estuary (Kelly, 1997). Water quality problems caused by antiquated and inadequately functioning waste water treatment led Boston Harbour to receive considerable attention in the United States media, first as the bestselling hit song "Dirty Water" in 1966, and then through playing a significant role in the Presidential election in 1988. Since that time, sludge dumping has ceased, the wastewater plant has been upgraded for secondary treatment, and a new outfall pipe has been built to divert treated effluent far out into Massachusetts Bay. However, throughout the 1990s, deposits of sewage sludge lingered and continued to be a source of contamination (Moore *et al*, 1996, Tucker *et al*, 1999).

In the last several decades numerous studies have posited the utility of using stable nitrogen isotope ratios of ^{15}N to ^{14}N (ó^{15}N expressed as parts per thousand in relation to the isotopic standard) to trace the incorporation of sewage-derived nitrogen into food webs. Due to fractionation processes during microbial decomposition, treated effluent often exhibits elevated ó^{15}N values of its dissolved inorganic nitrogen (DIN) compared to marine values. As a result, uptake of sewage-derived DIN in plants and the animals that assimilate them can be

Received on February, 2011 Published on February 2011

traced through this technique (e.g. Savage, 2005). Because Boston Harbour is rapidly flushed (Signell and Butman, 1992) nutrient levels are low, and as such, it is doubtful that aqueous DIN from sewage discharges would linger long enough to be taken up by biota (Tucker *et al*, 1999). The possibility still exists, however, that particulates originating from sewage sludge dumping and non point-source combined sewage overflows (CSOs) after storm events could become incorporated into benthic or deposit-feeding food webs.

The progressive ^{15}N-enrichment of particulate organic matter from terrestrial to freshwater to marine environments (France, 1995a), has been used to assess the transport of terrigeneous-derived material into coastal areas (e.g. Peters *et al,* 1978, Sweeney and Kaplan, 1980a, Owens, 1985). For the same reason, ó^{15}N analysis can provide a convenient measure of the transport and biological incorporation of particulate organic matter across ecotones, as for example, from terrestrial to estuarine (France, 1997), estuarine to marine (France, 1994, 1995b), marine to freshwater (Kline *et al,* 1990, Bilby *et al,* 1996), and terrestrial to freshwater (France, 1996a) environments. Of interest to environmental scientists is the ability to exploit these source differences in ó^{15}N in order to trace the transport and possible biological incorporation of domestic and industrial sewage (Macko and Ostrom, 1994).

Because sewage-derived organic material (SDOM), composed of human waste products originating predominantly from terrestrial diets (Schoenenger *et al,* 1983), is depleted in ^{15}N compared to marine organic matter (Sweeney and Kaplan, 1980b, Sweeney *et al*, 1980), it may be possible to use ó^{15}N analysis to trace the movement of SDOM into marine food webs. In other words, organisms which incorporate (either directly or facultatively) SDOM for a portion of their diets would be expected to be depleted in ^{15}N compared to individuals of the same species relying more substantially on marine-derived food sources (Rau *et al,* 1981, Spies *et al,* 1989, Van Dover *et al,* 1992).

Sludge and sediments near the major sewage outfall in Boston Harbour have been found to be depleted in ^{15}N (Giblin *et al*, 1992, Tucker *et al*, 1996). And results for one species of fish (Hillman *et al,* 1994, Michener, 1995, Moore *et al*, 1996) and several species of macroinvertebrates (Tucker *et al*, 1999), have suggested the utility of ó^{15}N analysis for tracing incorporation of SDOM for this system. In this study I wish to extend these results for a larger portion of the food web composed of 14 different species of fishes and macroinvertebrates.

As a result of increased boundary layer diffusion resistance, algae in protected waters become enriched in ^{13}C such that their ó^{13}C isotope ratios (^{13}C to ^{12}C expressed as parts per thousand in relation to the isotopic standard) will be elevated (France and Holmquist, 1997). In consequence, benthic algae are frequently enriched in ^{13}C compared to planktonic algae (France 1995b, Doi *et al*, 2010) to such an extent as to enable separation of food webs (Hecky and Hesslein 1995, France, 1995c,b, France *et al*, 1998a). Ingestion of aqueous DIN derived from human waste may elevate ó^{15}N values of planktonic consumers and plants or filter feeders assimilating DIN (Hansson et al. 1997, Savage, 2005, Wigand *et al*, 2007, Bannon and Roman, 2008, Carmichael *et al*, 2008). Benthic food webs may be functionally isolated from such processes, however, (France, 1996b) instead ultimately relying upon ingestion of attached algae, detritus, and organic deposits, a portion of the latter of which may contain SDOM. This should lead, as outlined above, to a depletion, not an enrichment, in ^{15}N (Rau *et al,* 1981, Spies *et al,* 1989, Van Dover *et al,* 1992, Tucker *et al*, 1999). Such a scenario might explain why Van Dover *et al.* (1992) found planktonic-feeding organisms such as shrimp and coral to be essentially isotopically unchanged following exposure to

SDOM, whereas deposit-feeding organisms such as sea urchins and cucumbers were depleted in ^{15}N by about 3 o/oo.

I was interested in testing the hypothesis of benthic-pelagic differences in sewage incorporation by formulating the prediction that the more closely animals are dependent on a benthic diet, as reflected by their ó^{13}C values, the more likely they will be to demonstrate lower ó^{15}N values through presumed incorporation of SDOM. Reciprocally, the more that species rely on planktonic-derived organic matter, the greater the likelihood will be that their ó^{15}N values will remain unchanged or perhaps even be slightly elevated in association with sewage waste.

2. Methods

As part of a multi-faceted study on the effects of the deterioration of seagrass beds in Boston Harbor (Chandler *et al* 1996), fishes and macroinvertebrates were sampled for stable isotope analyses during June-July 1995 with use of gillnets, minnow traps and quadrats. Samples were also obtained of benthic algae, seagrass-epiphyton detritus, and organic deposits overlying sediments located 10 m away from seagrass beds. As described in France *et al.* (1998b,c) samples collected from Boston Harbour for isotope analysis were acid washed and hand-cleaned to remove inorganic contaminants. Dissected muscles or whole organisms were ground and samples stored frozen until they could be analyzed for stable isotopes on a Europa Tracermass mass spectrometer interfaced with a Roboprep-CN analyzer or on a VG Micromass 903E triple-collector mass spectrometer Duplicate determinations on either machine did not differ. Analytical variability averaged ± 0.3 SD for standards following weight-related bias correction. Two to three analytical replicates were run for each biological sample. Sample sizes ranged from 5 to 13 individuals for each species.

Animals were collected from three sites within greater Boston Harbour: a small (0.25 ha) and rapidly disappearing seagrass bed in the inner protected harbour at Hingham which is located 4 to 6 km from the former sewage sludge dumping site and one of the two major sewage outfalls for Boston and which is also exposed to CSO pollution during storm events; and two large (2-4 ha and 30-40 ha), proximally located and healthy seagrass beds in the outer harbour off the Nahant Peninsula which, although located 8 km from the other major sewage outfall, are exposed to rapidly flushing tidal currents and therefore not as seriously affected by sewage sludge dumping and effluent or CSO pollution (Chandler *et al,* 1996). The following 14 species, ordered below in relation to their increasing trophic position as determined by ó^{15}N analysis for individuals from the outer harbor (France *et al,* 1998b), were collected to span the complete food web: hermit crabs (*Parurus longicarpus*), blue mussels (*Mytilus edulis*), chink shells (*Lacuna vincta*), epiphytic sponges, periwinkle snails (*Littorina littorea*), rock crabs (*Cancer irrotatus*), Jonah crabs (*Cancer borealis*), shorthorn sculpin (*Myoxcephalus scorpius*), ollock (*Pollachius virens*), cunner (*Tautogolabrus adspersus*), menhaden (*Brevoortia tyrannus*), little skates (*Raja erinacea*), winter skates (*Raja ocellatus*) and striped bass (*Morone saxatilis*). These species were also selected to span the range found in food provenance (as measured by ó^{13}C analysis – France, unpubl. Data) from benthic sources (chink shells ó^{13}C = – 6 o/oo) to planktonic sources (menhaden ó^{13}C = – 19 o/oo). It is important to note that there were no differences in species-specific ó^{13}C values among the study sites.

Measurement of the presumed incorporation of SDOM into organisms from the inner (polluted) compared to the outer harbour sites, was examined by tabulating all possible differences in ó^{15}N values on an individual-to-individual, species-by-species basis (in order to remove trophic position effects as in France and Peters, 1997). Species were then ranked along a spectrum of benthic to planktonic food provenance based on their ó^{13}C values (France, unpubl. Data).

Table 1: Stable nitrogen isotope ratios (± SD) of species sampled from outer (unpolluted) and inner (polluted) sites in Boston Harbour. Species are arranged in order from top to bottom based on their increasing dependence on pelagic over benthic food sources as measured by stable carbon isotopes and described in the text. Brackets designate food categories shown in Table 2.

	Outer Harbour	Inner Harbour	Difference
Lacuna snail (1)	6.5 ± 0.6	5.1 ± 0.8	- 1.4
Rock crabs (1)	9.1 ± 1.6	7.7 ± 1.0	- 1.4
Little skate (2)	13.0 ± 0.5	11.8 ± 0.7	- 1.2
Periwinkle snail (2)	8.6 ± 0.7	7.7 ± 0.5	- 0.9
Winter skate (3)	12.4 ± 0.5	11.9 ± 0.5	- 0.5
Sculpin (3)	11.0 ± 0.7	10.2 ± 0.4	- 0.8
Jonah crab (4)	10.8 ± 1.2	9.6 ± 0.5	- 1.2
Hermit crab (4)	5.8 ± 0.6	6.0 ± 0.4	+ 0.2
Cunner (5)	12.4 ± 0.8	11.6 ± 0.6	- 0.8
Pollack (5)	11.8 ± 0.5	11.4 ± 0.5	- 0.4
Striped bass (6)	15.8 ± 1.4	15.8 ± 1.2	0.0
Sponge (6)	6.4 ± 0.5	6.5 ± 0.4	+ 0.1
Blue mussel (7)	6.2 ± 0.6	6.6 ± 0.3	+ 0.4
Menhaden (7)	12.6 ± 0.6	12.6 ± 0.9	0.0

Table 2: Differences in stable nitrogen isotope ratios (± SD) between animals sampled from outer (unpolluted) and inner (polluted) sites in Boston Harbour. Species are paired (see Table 1) in food categories based on their increasing dependence on planktonic over benthic food sources as measured by their stable carbon isotopes (ó^{13}C± SD) and described in the text.

Food category	Mean ó^{13}C	ó^{15}N difference
1 (benthic)	- 7.2 ± 3.2	-1.3 ± 0.3
2	-14.3 ± 1.2	-0.9 ± 0.2
3	-15.8 ± 0.9	-1.2 ± 0.2
4	-17.0 ± 0.6	-0.8 ± 0.1
5	-17.7 ± 0.3	-0.2 ± 0.3
6	-18.3 ± 0.3	+0.3 ± 0.2
7 (planktonic)	-19.0 ± 0.1	+0.4 ± 0.2

Seven categories were obtained from paired species rankings (there are no substantive trophic position effects on ó^{13}C – France and Peters, 1997): (1) chink shells and rock crabs being the most dependent on benthic material, and moving through the species pairings of (2) little skates and periwinkles, (3) Jonah crabs and winter skates, (4) hermit crabs and shorthorn sculpins, (5) cunner and ollock, (6) sponges and striped bass, to (7) blue mussels and menhaden being the most dependent on pelagic material. For each category of paired species, the average ó^{15}N difference between outer and inner harbor sites was calculated.

3. Results and Discussion

Potential benthic food sources displayed similar ó^{15}N values between the outer and inner harbor sites (epiphyton = 3.8 ± 1.7 SD o/oo outer harbor (France *et al*, 1998b) and 4.2 ± 0.9 o/oo inner harbour; macroalgae = 6.3 ± 0.3 o/oo outer harbor and 6.6 ± 0.5 o/oo inner harbor for a mean of 6.5 ± 0.7 o/oo (France *et al*, 1998c); and detritus composed of eelgrass (*Zostera marina*) and epiphyton = 4.5 ± 1.0 o/oo outer harbor and 4.8 ± 1.0 o/oo inner harbor for a mean of 4.6 ± 1.2 o/oo (France *et al*, 1998c). However, differences were found to be more substantial in the ó^{15}N values of organic deposits overlying sediment between the two sites (5.5 ± 0.5 o/oo outer harbor and 4.3 ± 0.6 o/oo inner harbour). Is this 1.2 o/oo depletion in ^{15}N observed for OM at the inner habour site of a sufficient magnitude to be detected in animals feeding there?

Nine of the 14 sampled species were found to display lower ó^{15}N values when collected from the inner compared to the outer harbor (Table 1). There was a relationship observed between food source provenance (as indicated by ó^{13}C analysis) and the ó^{15}N differences found between animals collected from the outer and inner harbour sites (Table 2); i.e. the more dependent organisms were on benthic food (categories 1, 2, 3 and 4) compared to planktonic food (categories 5, 6 and 7), the greater their ^{15}N-depletion, possibly due to incorporation of SDOM.

Sewage sludge in Boston Harbour has a ó^{15}N value of 3.3 o/oo (Giblin *et al*, 1992). Tucker *et al*. (1999) found the ó^{15}N values of OM in surface sediments to be lower in areas closest to sites were sewage sludge was dumped or discharged after minor treatment (about 4.1 o/oo) compared to sites in the southern harbor (5.3 o/oo), outer harbor (5.6 o/oo), and Massachusetts Bay (6.3 o/oo). Further, sediment profiles collected from a polluted site revealed that OM became progressively enriched in ^{15}N following cessation of sludge dumping in 1991 and the presumed dissolution of the contaminated material (4.8 o/oo in 1991, 5.2 o/oo in 1993, and 5.5 o/oo in 1994).

In previous research in Boston Harbour, Hillman *et al*. (1994) and Moore *et al*. (1996) found scales of winter flounder (*Pleuronectes americanus*) collected from inner polluted regions to be depleted in ^{15}N by 1 to 4 o/oo compared to scales of fish from outer less polluted regions. Likewise, unidentified gut contents from these same fish also demonstrated a 3 o/oo ^{15}N-depletion in regions near the sewage outfall. Michener (1994) found body tissues of winter flounder from sites near the two sewage outfalls to be ^{15}N-depleted by 1 o/oo compared to other sites from within the inner harbour, but located more distant from the actual outfall pipes. Tucker *et al*. (1999) found blue mussels to be depleted in ^{15}N by 1-2 o/oo at more polluted harbor sites and polychaetes to be 1-2 o/oo lower in ^{15}N compared to worms from the outer Massachusetts Bay. Intriguingly, and in support of the present findings, Tucker *et al*. (1999) also present some preliminary data for two species of amphipods suggesting that the species which is a deposit-feeder has a ó^{15}N value more closely identifiable with sewage sludge compared to the other species which is a suspension-feeder. Clearly, as the present study shows, where, and more importantly, what a species eats will determine whether stable nitrogen isotope analysis can be used to race the incorporation of SDOM into food webs.

Inter-site differences found in our study were less than those of Spies *et al.* (1989) and Rau *et al.* (1981) and Van Dover *et al.* (1992). This might result from either the outer harbour site being exposed to some influences of sewage effluent and/or a lack of complete habitat-site fidelity among some of the study organisms. Had animals instead been sampled from a truly pristine site far removed from the influences of greater Boston Harbour, or inner harbour animals been sampled from directly at the mouths of the sewage outfall pipes or where sludge had been dumped, there might have been a greater separation in ó^{15}N values as was observed by Tucker *et al.* (1999) who undertook just such a sampling strategy. That the present study found benthic OM and several benthic-feeding organisms to be depleted in ^{15}N at a inner harbor site located almost 5 km distant from the major sewage outfall and 4 years after sludge dumping was abated is somewhat surprising given that sediment data from Tucker *et al.* (1999) imply that the system can recover relatively quickly following improvements in sewage treatment management. It is possible that the inner harbor site in this study was much more protected than the sites sampled in the previous study and thus the harbor-wide legacy of sludge dumping is more evident at this location despite it being more distant from the actual site of the dumping that was sampled by Tucker *et al*. (1999). SDOM in Boston Harbour also originates from non point-source CSO pollution which was considerable in the early-to-mid 1990s (Lavery *et al*, 1994, Chandler *et al*, 1996). And it is entirely feasible that the inner harbor site sampled in this study could be a sink for the deposition of such material. Regardless of the precise pollution source, the present results support earlier work from southern California (Rau *et al,* 1981; Spies *et al,* 1989), New Jersey (Van Dover *et al*, 1992) and Boston Harbour (Tucker *et al*, 1999) in suggesting that ó^{15}N analysis can be used to successfully trace the incorporation of urban SDOM into certain components of benthic food webs.

Acknowledgements

M. Chandler, C. Davidson, P. Sackley, L. McCabe, C. Rigg, S. Mazumder, M. France and R. Drimmie are thanked for logistical assistance

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