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Inputs of anthropogenic nitrogen influence isotopic composition and trophic structure in SE Australian estuaries

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ABSTRACT

Urban development in coastal settings has increased the input of nitrogen into estuaries globally, in many cases changing the composition of estuarine ecosystems. By focussing on three adjacent estuaries with a gradient of anthropogenic N loadings, we used stable isotopes of N and C to test for changes due to increased anthropogenic N input on the structure of some key trophic linkages in estuaries. We found a consistent enrichment in $\delta^{15}N$ corresponding to increased anthropogenic N at the three ecosystem levels studied: fine benthic organic matter, grazing invertebrate, and planktivorous fish. The degree of enrichment in $\delta^{15}N$ between fine benthic organic matter and the grapsid crab *Parasesarma erythrodactyla* was identical across the three sites. The glassfish *Ambassis jacksoniensis* showed lower levels of enrichment compared to basal food sources at the higher N-loaded sites, suggesting a possible effect of anthropogenic N in decreasing food-chain length in these estuaries.

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1. Introduction

Anthropogenic influences on nutrient inputs into estuaries, in particular the elevation of nitrogen levels, have the potential to alter the structure and function of estuarine ecosystems. The accumulation of biomass in most coastal systems is nitrogen (N) limited (Rabalais, 2002). The nitrogen enrichment in the coastal water is increasing as a result of increasing anthropogenic activities (Fry et al., 2003) and globally several large estuarine and nearshore systems have been subject to eutrophication and hypoxia (Rabalais, 2002). In a global review, Leavitt et al. (2006) report a continued trend in the period following 1950 of increases in N and P leading to oxygen depletion and alterations to primary productivity, as a direct result of point and nonpoint pollution.

One effect of elevated N in estuarine waters is a shifting of the production base from macrophytes to microphytic algae. Macreadie et al. (2012) demonstrated a shift in C to N ratios of organic carbon, consistent with a transition from macrophyte to algal carbon sources in estuarine cores from Botany Bay, Australia. The period of transition corresponded to catchment development and alterations to nutrient concentrations following European colonisation and catchment development. Such changes have the potential to alter the diet of benthic and intertidal herbivores, and Macreadie et al. (2012) called for further work to determine how increases in microalgae in Botany Bay have affected food web dynamics and ecosystem function within the Bay.

The trophic structure of benthic communities in Botany Bay has been extensively studied using stable isotopes of carbon and nitrogen (Mazumder et al., 2011; Saintilan and Mazumder, 2010). Generally crabs graze within narrowly defined feeding ranges (Guest et al., 2004), which allows the contrasting carbon isotope signatures of C_3 and C_4 saltmarsh plants, growing in mosaics, to be used to identify the relative contribution of macrophyte carbon in crab diets (Guest and Connolly, 2005). These studies have demonstrated a mixture of microphytobenthos and macrophyte detritus contributing to the diet of grapsid crabs, both at Botany Bay (Saintilan and Mazumder, 2010), and at nearby Brisbane Water (Alderson et al., 2013). Grapsid crabs play a keystone role in estuarine ecosystems in SE Australia, releasing larvae into the ebbing spring tide waters which provides an important source of nutrition for small fish (Mazumder et al., 2006) with a trophic relay traceable to commercially important predatory species (Mazumder et al., 2011). Shifts in the productivity base are therefore likely to show flow-on effects through the entire ecosystem.

Stable nitrogen isotope analysis has emerged as an effective way of exploring the uptake of anthropogenic N in estuarine ecosystems. Inputs of nitrogen in catchments increase the amount of N cycling in soils, a process that results in enrichment of the heavier ^{15}N isotope (Fry et al., 2003). Nitrate from human wastewater is also enriched in ^{15}N as a result of de-nitrification and volatilization of ammonia (Kendall et al., 2007), with $\delta^{15}N$ values typically in the range of +10‰ to +20‰ (Hoffman et al., 2012). Surface and groundwater bearing atmospherically derived N typically support background $\delta^{15}N$ signatures of +2‰ to +8‰ (McClelland et al., 1997).

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This alteration to the $\delta^{15}N$ signatures resulting from anthropogenic nitrogen inputs has been detected in an extensive range of ecosystem components. The nitrogen isotope ratios of primary producers reflect that of their nitrogen source, though fractionated (differential use of ^{15}N and ^{14}N) during uptake. Consumers typically show a +3‰ to +5‰ enrichment in $\delta^{15}N$ with respect to their diet (De Niro and Epstein, 1981; Minagawa and Wada, 1984). Elevated levels of $\delta^{15}N$ have been identified in sediment and suspended particulate organic matter (Kohzu et al., 2009); macroalgae and macrophytes (Cole et al., 2004; Kaldy, 2011); bivalves (Oczkowski et al., 2008); macroinvertebrates and zooplankton (Peterson et al., 2007), and fish (Bannon and Roman, 2008; Hoffman et al., 2012). The $\delta^{15}N$ values of nitrogen from animal waste, agricultural fertilisers and urban effluent sources are isotopically distinct from each other (Peterson, 1999; Costanzo et al., 2001, 2003), therefore the use of $\delta^{15}N$ provides the advantage of integrating, through the relatively long absorption time (3–5 weeks) water quality fluctuations occurring over the short term. However, the approach may not detect very high ammonium inputs from sewage (Fry et al., 2003).

Multi-estuary comparisons using gradients of N input have demonstrated the utility of $\delta^{15}N$ as a potential indicator of N loading. McClelland et al. (1997) sampled a range of trophic levels in a series of estuaries in Cape Cod (USA) in which nitrogen loads varied from 2 to 467 kg N $ha^{-1}yr^{-1}$, and found that anthropogenic N was detectable even at relatively low loadings. Hoffman et al. (2012) compared larval fish $\delta^{15}N$ across three watersheds spanning a large population density gradient in Lake Superior (USA), finding that $\delta^{15}N$ increased with N concentration, reflecting higher anthropogenic inputs. Fry et al. (2003), compared N loading with $\delta^{15}N$ in components of the estuarine biota in four West-Coast American systems, none low impact in respect to nitrogen loading, finding that macroalgae provided a good indicator of anthropogenic N.

Several issues remain unresolved in relation to the impacts of nitrogen loading in the estuarine environment. Although numerous studies have demonstrated significant uptake of anthropogenic N in tissues of estuarine organisms, it is unclear in many cases whether this amounts to an environmental problem such that ecosystem structure and function are disrupted, and if so, at what thresholds of N loading these changes occur (Rabalais, 2002). The exploration of ecosystem-level effects of elevated anthropogenic N requires consideration of trophic linkages using multiple stable isotope tracers across impact gradients.

In this study, we use stable isotopes of C and N in three estuarine settings that span a range of N loadings to explore the effect of elevated anthropogenic N on ecosystem trophic structure. Because $\delta^{15}N$ is fractionated between consumer and prey by predictable amounts the measure can be used to determine trophic position, where the signature of basal $\delta^{15}N$ is known. The dietary source is identified using $\delta^{13}C$, which has a lower degree of fractionation between diet and consumer (De Niro and Epstein, 1978). We utilise a well-studied trophic link between benthic detrital sources of carbon and nitrogen, and the dietary carbon source and trophic position of grazing crabs of the species *Parasesarma erythrodactyla*, and the glassfish *Ambassis jacksoniensis*, known to predate on the larvae of *P. erythrodactyla*, amongst other zooplankton sources. Three adjacent estuaries in the Sydney region, SE Australia, provide contrasting levels of anthropogenic N input while supporting common species and habitats. Our hypothesis is that the gradient of anthropogenic nitrogen input will correspond with a gradient of $\delta^{15}N$ values at all trophic levels, but that the trophic position and dependencies of organisms will be otherwise unchanged.

2. Methods

2.1. Site selection

Samples were collected from the three SE Australian estuaries with variable anthropogenic nitrogen inputs (Fig. 1). Brisbane Water is situated on the northern entrance of Broken Bay, and the intertidal wetlands form on flood-tidal deltaic sands. The primary hydrological input is oceanic tidal water with no major rivers contributing sediment or water. This site is the least disturbed of the three chosen, with the lowest level of catchment clearance, lowest population density, and lowest percentage increase in anthropogenic N against estimated pre-development levels (Table 1). The mangrove community at the site is dominated by *Avicennia marina*, fronted at the seaward edge by Zostera seagrass meadows.

Botany Bay represents an intermediate level of anthropogenic modification amongst the three sites, in percentage clearance, and population density. Though current N inputs are lower than Brisbane Water, the Bay receives inputs from the Georges River, the major tributary, which contains a relatively high level of anthropogenic N (Table 1). Water clarity at the sampling site of Towra Point, Botany Bay is sufficient to support the seagrasses *Posidonia australis* and other species in the families Zosteraceae and Hydrocharitaceae. The mangrove community is dominated by *A. marina*, and the wetland consists of a diverse saltmarsh assemblage in the upper intertidal zone. In the late 1970s Towra Point was declared a nature reserve and in the mid-1980s its adjacent waters were declared an aquatic reserve.

Homebush Bay, within the Parramatta River estuary, is the site of highest anthropogenic disturbance with the highest population density, highest level of clearance and highest levels of local anthropogenic nitrogen input of the three sites. The wetlands are situated on estuarine silts approximately 20 km from the ocean. From the late 1920s until the mid-1980s, the eastern shore of Homebush Bay was the site of various industrial facilities at which timber preservatives, tar-based products, herbicides, pesticides, chlorine gas and plastics were produced (Alexander, 2002). Some of the by-product material was disposed of on site, and contaminated spoil was used to reclaim land for the expansion of adjacent industrial facilities (Birch et al., 2007). A National Dioxin Study identified the highest dioxin and furan concentrations in Australia to be from Homebush Bay (Mueller et al., 2004). The industrial sites on the east side of the bay have been progressively replaced with residential apartments, with conditions of approval requiring the dredging, removal and treatment of contaminated soil. In the upper portion of the bay, brickworks and an abattoir operated for many decades but were replaced in the late 1990s to provide the site for the Sydney 2000 Olympics. Two small creeks, channelized and hence vastly modified from their natural condition, enter Homebush Bay from the south. Mangrove (*A. marina*) and saltmarsh are present, but seagrass is not, presumably due to reduced water clarity at this location. There were no sewage treatment facilities in the vicinity of any of the three sampling sites.

2.2. Sampling and analytical methods

Three components of the food chain: sediment organic matter (SOM), burrowing crabs (*P. erythrodactyla*) and estuarine glassfish (*A. jacksoniensis*) were collected from saltmarsh and mangrove habitats of the three estuaries. Sample replication was determined using the prior power analysis of Mazumder et al. (2008).

Sediment organic matter is the likely base of the food chain, and was collected from saltmarsh/mangrove habitats of the three estuarine ecosystems ($n = 9–17$). Sediment organic matter includes variety of living and non-living sources including microalgae (Nils, 2003). Sediment scrapings were collected from the top 1 cm of surface sediment (Melville and Connolly, 2003) and the samples were washed through 4-mm, 1-mm, 0.5-mm, and 0.25-mm sieves following the method of (Loneragan et al., 1997). Material elutriated from the finest size-fractions (<0.25 mm) were analysed for isotopic values. As stable isotopes used in trophic ecology rely on C fixed in the organic form (Mateo et al., 2008), acid-washing of sediment samples was performed prior to analysis. To avoid bias introduced by inorganic C, a small volume of 1 M hydrochloric acid was added to sediment sub-samples and left

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