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Monitoring nitrogen pollution in seasonally-pulsed coastal waters requires judicious choice of indicator species

Daniel Gorman[a,*], Alexander Turra[a], Rod M. Connolly[b], Andrew D. Olds[c], Thomas A. Schlacher[c]

[a] Departamento de Oceanografia Biológica, Instituto Oceanográfico, University of São Paulo, Praça do Oceanográfico, 191, CEP 05508-120 São Paulo, SP, Brazil
[b] Australian Rivers Institute - Coast & Estuaries, and School of Environment, Gold Coast campus, Griffith University, Queensland, Australia
[c] School of Science & Engineering, University of the Sunshine Coast, Queensland, Australia

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ABSTRACT

We compared the sensitivity of algae and hermit crabs to seasonal shifts in the dominance of continuous sewage discharge vs. pulsed inputs of terrestrial material to a subtropical bay. During periods of low rainfall, when sewage was proportionately more important than diffuse loads from adjacent catchments, algae and crabs provided comparable information on the spatial distribution of N pollution. Conversely, during the wet season, when diffuse nitrogen loads from the catchment were of greater importance, the isotope signal of algae decoupled from that of crabs, indexing a greater magnitude of change and a more pronounced spatial gradient. Overall, algae better indexed the short-term impacts of anthropogenic nitrogen pollution whereas the signals provided by crabs provided a longer-term integrated measure of N inputs. Our results demonstrate the value of including multiple taxa with variable traits when monitoring the spatial and temporal extent of nitrogen inputs to coastal waters.

1. Introduction

Nitrogen contained in human sewage can be a major source of pollution in many coastal waters (McClelland et al., 1997; Schlacher et al., 2005). Elevated levels of the bioavailable forms of N discharged to near-shore waters can significantly impact productivity, biodiversity, organism health, habitat quality, food safety, and the delivery of ecosystems services (Cloern, 2001; Deegan et al., 2012; Diaz and Rosenberg, 2008; Schlacher et al., 2007). This makes reliable monitoring of the spatial and temporal signals of sewage pollution an important task in many environmental management settings (Costanzo et al., 2001).

Measuring stable isotope ratios (δ^{15}N and δ^{13}C) in the tissues of organisms in receiving waters has become a widely-used technique to trace the origin of inputs and map their distribution (Corbett et al., 2015; Costanzo et al., 2003; Gorman, 2009). While spatial signals in tissue δ^{15}N of organisms exposed to sewage reflect proximity to known point sources (Connolly et al., 2013; Oakes and Eyre, 2015), it is not widely known how temporal variation in upland nitrogen and carbon sources (e.g. with variation in rainfall or river discharge) affect isotope ratios in the tissues of indicator species (but see; Fertig et al., 2009; Schlacher and Connolly, 2009). For example, variation in δ^{15}N signals of algae have been reported over spatial (Orlandi et al., 2014), seasonal (Lemesle et al., 2016) and human -stress gradients (Calizza et al., 2015). Such variation is likely to be particularly important in systems where inputs from adjacent catchments are strongly pulsed, notably during quasi-predictable seasonal variation in peak rainfall that drives diffuse run-off and river discharge (Caffrey et al., 2007; Gorman et al., 2009; Schlacher et al., 2008; van de Merwe et al., 2016).

Choice of indicator taxa is a critical decision step in any environmental monitoring program (Schlacher et al., 2014). There are three important considerations for studies that use tissue- δ^{15}N of exposed organisms to monitor nitrogen pollution: a) source of uptake (inorganic N from water in plants or organic N via food in animals, b) tissue turnover rates which determine the period prior to sampling over which a taxon is likely to reflect ('integrate') a pollution signal; and c) organism mobility, which determines the spatial scale over which a pollution signal is integrated; not surprisingly then, a diverse range of taxa has been used to map sewage (e.g. seagrass, algae, mussels, corals, clams, fishes; Gappa et al., 1990; Gartner et al., 2002; Jones et al., 2001; Reopanichkul et al., 2009; Schlacher et al., 2005; Watanabe et al., 2009).

Testing how the specific biological attributes or traits of indicators vary in their capacity to provide spatial and temporal information is vital for pollution monitoring studies. Here we compare two organisms; a sessile autotrophic green alga, and a mobile heterotrophic hermit

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[*] Corresponding author.
E-mail addresses: dgorman@usp.br (D. Gorman), tschlach@usc.edu.au (T.A. Schlacher).

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crab, to assess their capacity to provide both singular and cumulative information about the nitrogen inputs to a coastal bay. Because sources of nitrogen to the study system are variable and comprise of a combination of continuous sewage release and a variety of riverine and terrestrial inputs driven by seasonal rainfall we expected considerable spatio-temporal variation in isotope signals. Our hypothesis was that spatial (across sites) and temporal (seasonal) change in the response of indicators (change in tissue δ^{15}N and δ^{13}C) would be greater for marine algae than it would be for mobile benthic invertebrates. This type of information is particularly important for monitoring programs that require reliable and integrated measures of pollution sources that can change in response to a range of environmental factors. Our model system is a subtropical bay subjected to multiple forms of nitrogen input (sewage as a distinct point source vs. diffuse urban run-off and catchment inputs) and where regular seasonal cycles in rainfall lead to predictable seasonal peaks in the contribution of catchment-derived nitrogen inputs. We compare the alga *Ulva lactuca* (an organism that assimilates N from the water column) and the hermit crab *Clibanarius vittatus* (indirect assimilation from ingested food) with respect to; (a) the magnitude of change in isotope signals between seasons; and (b) the degree of spatial concordance of isotope signals between algae and crabs and whether this changes over time.

2. Methods

We tested the sensitivity of indicator species to seasonal variation in sewage-signals in Araçá Bay, São Paulo state (Brazil; Fig. 1). This is a relatively shallow bay (max. depth ~ 11 m at the eastern opening), located on the mainland-side of the São Sebastião Channel, the location of Brazil's largest oil shipping port. The area has a meso-tidal range of < 2 m, and low to moderate wave exposure (Amaral et al., 2010). Water temperature in the channel typically ranges between 21 °C in winter and 26 °C in summer (Cerda and Castro, 2014). The main current flow within the channel is from the south (Castro-Filho, 1990), but tidal oscillations give the site a characteristic counter-clockwise circulation pattern (Siegle et al., 2014). During the winter 'dry season' (April to September) storm mixing of the water column occurs, resulting in low but relatively constant inputs of nutrients. This contrasts summer 'wet season' (November to March) were rainfall-driven pulses of riverine inputs (from the Mae Isabel river) and restricted local upwelling events lead to a strong spatial gradient in nutrient concentrations within the bay (Dottori et al., 2015). The ratio of Dissolved Inorganic Nitrogen (DIN) inputs from the Mae Isabel river vs. outfall varied from ca. 1:1 during the dry season, to ca. 6:1 during the wet season (Carrilho, 2015; SABESP, 2015). Input of these materials results in a persistent enrichment of bay waters when compared to the São Sebastião Channel; observations validated by measures of mean nitrate plus nitrite concentrations, which are consistently greater within the bay when compared to channel (i.e., means: 31.6 vs. 18.6 mg/L over the same study period; Giannini and Ciotti, 2016).

Araçá Bay has a long history of human modification, with the area incrementally reduced in size through consecutive expansions of an adjacent port since 1936 (Mani-Peres et al., 2016) and a population that has quadrupled over a similar period (SEADE, 2015). Primary-treated sewage (ca. 4.4×10^{6} m^{3}·per year) is discharged via a 1 km long pipe at the bay's southern-most edge (Fig.1; CETESB, 2005). The bay also receives large volumes of diffuse surface runoff from urban and industrial areas (notably from the port of São Sebastião), and illegal dumping of commercial and domestic waste is common. While the flow of sewage into the bay is fairly constant (ca. 140 L s^{-1}), seasonal rainfall increases river flow from ca. 84 L s^{-1} (dry season) to > 250 l·s^{-1} (wet season)(Carrilho, 2015).

The biological indicators assessed in this study were the green alga *Ulva lactuca* (Linnaeus, 1753) and the hermit crab *Clibanarius vittatus* (Bosc, 1802). Collections were made at 45 sites (24 during the dry season and 21 during the wet season) spread throughout the bay where both algae and crabs were found. While it was not possible to replicate the exact sampling locations (i.e., collection was done opportunistically) the spatial extent and depth range of sampling sites was concurrent for both periods. To capture seasonal signals related to changes in the amount of diffuse surface run-off driven by variations in rainfall, we sampled during two periods; (1) October 2013 at the end of the dry season (April to September) with mean daily rainfall of 1.67 ± 0.35 (se) mm·d^{-1}; and (2) March 2014 at the end of the wet season (November to March: 3.18 ± 0.80 (se) mm·d^{-1}; source CEBIMar weather station). The rational for sampling at the end of each distinct rainfall period was to allow organisms to reach isotopic equilibrium in their tissues if the input signals were to change.

For stable isotope analysis, we excised muscle tissue from the chelae of crabs (samples comprising material from 3 individuals pooled for each site × time) and rinsed the entire thalli of algae (~5 g of wet material) that had been cleaned of epiphytes by scraping with a scalpel. Given the small amount of material obtained from each crab specimen, we were unable to derive measures of variability in δ^{13}C and δ^{15}N signals among individuals for any particular site. Stable isotope mass spectrometry was done at the University of California, Davis. Precision (standard deviation) was 0.09‰ for δ^{13}C and 0.05‰ for δ^{15}N.

Data for each site and season were integrated into a Geographic Information System (ArcGIS version 10.1), where Kriging methods were used to produce continuous raster maps for each indicator, signal and season. The magnitude of seasonal change (‰) was calculated using the map algebra geoprocessing tool that calculated each pixel value using the equation $T2i - T1i$, (where i is the Kriging value for each point on the map). The function permitted the creation of spatially explicit maps describing the degree of seasonal change in the isotope signals of each indicator species.

We tested for seasonal differences in the mean value of δ^{15}N and δ^{13}C with PERMANOVA and for differences in the dispersion of these signatures with PERMDISP (Anderson, 2001); both analyses were based on Euclidean distances. In this way, the experimental design compared both the spatial (i.e., replicate sites across the bay) and temporal (seasonal variation in relative importance of various inputs) variability in indicator species. We tested the general hypothesis that the spatial and temporal variation in indicator response (i.e., magnitude of difference % in δ^{15}N and δ^{13}C between the dry- and wet-seasons) would be greater for algae than it would be for hermit crabs. We also tested the consistency in spatial patterns by assessing variation in isotope signals with distance across the bay (i.e., using ANCOVA based on the distance from river and sewage inputs) and by testing the degree of spatio-temporal concordance between algae and crabs (i.e., do these indicators tell the same story across seasonal cycles).

3. Results

The δ^{15}N values of crab tissues were typically enriched by 1.92‰ (wet season) and 2.87‰ (dry season; Fig. 2) in relation to algae. Both algae and hermit crabs showed a significant shift towards heavier nitrogen isotope ratios after the wet season (Table 1); this seasonal change was larger in algae (1.3 ×) than in crabs (1.1 ×; Table 1; Fig. 2). Spatial variability in δ^{15}N did not change significantly ($P = 0.426$) in hermit crabs between the dry and wet season (Table 1; Fig. 2). By contrast, δ^{15}N values of algae became significantly ($P = 0.019$) less variable after the wet season (Table 1; Fig. 2). Tissue δ^{13}C of algae did not change between seasons ($P = 0.871$) whilst hermit crabs became slightly (delta $\delta^{13}C = -0.96$) but significantly ($P = 0.028$) more depleted after rain (Table 1). Similar to δ^{15}N, spatial variability in δ^{13}C of hermit crabs did not vary between season (Table 1; Fig. 2); but was significantly ($P = 0.002$) greater for algae after the wet season (Table 1; Fig. 2).

Nitrogen and carbon isotope signals showed spatial variation across Araçá Bay that differed between season and indicator (supplementary data). In general, signals demonstrated a north-to-south gradient across