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High congruence of isotope sewage signals in multiple marine taxa

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ABSTRACT

Assessments of sewage pollution routinely employ stable nitrogen isotope analysis ($\delta^{15}N$) in biota, but multiple taxa are rarely used. This single species focus leads to underreporting of whether derived spatial N patterns are consistent. Here we test the question of 'reproducibility', incorporating 'taxonomic replication' in the measurement of $\delta^{15}N$ gradients in algae, seagrasses, crabs and fish with distance from a sewage outfall on the Adelaide coast (southern Australia). Isotopic sewage signals were equally strong in all taxa and declined at the same rate. This congruence amongst taxa has not been reported previously. It implies that sewage-N propagates to fish via a tight spatial coupling between production and consumption processes, resulting from limited animal movement that closely preserves the spatial pollution imprint. In situations such as this where consumers mirror pollution signals of primary producers, analyses of higher trophic levels will capture a broader ambit of ecological effects.

1. Introduction

Nitrogen is pivotal in shaping the structure and function of natural systems at multiple levels of ecological organisation (Conley et al., 2009). Humankind has massively altered the cycling and mobility of nitrogen in the biosphere, adding unprecedented amounts of bioavailable N to ecosystems, most of it since the second half of the last century (Canfield et al., 2010; Vitousek et al., 1997). These anthropogenic inputs of N can have serious and widespread environmental consequences, altering productivity, diversity, trophic connections, species composition, organism health, and habitat quality (Cloern, 2001; Deegan et al., 2012).

Excess nitrogen from human sources is a component of a suite of stressors causing impacts that can be severe and widespread in coastal waters (Lotze et al., 2006; Reopanichkul et al., 2009). Fertilisation of nearshore marine waters with human-derived nutrients is mainly the result of changing land-use, most notably the widespread and escalating urbanisation of coastal areas (Nixon and Buckley, 2002). Ecological effects of nutrient inputs therefore often centre on coastal cities (Diaz and Rosenberg, 2008; Oczkowski et al., 2009).

Nitrogen contained in human metabolic waste products is a major component of nutrient inputs to coastal waters (McClelland et al., 1997; Schlacher et al., 2005), and monitoring the effects of sewage entering marine ecosystems has become an important activity (Costanzo et al., 2001). One of the most widely applied techniques to identify the presence of sewage-N is the analysis of stable nitrogen isotopes in the tissues of biota putatively exposed to inorganic sewage-N or assimilating it from their diet (Cabana and Rasmussen, 1996; Pitt et al., 2009). The technique is based on predictable differences in the abundance of the heavy (^{15}N) and light (^{14}N) isotopes between sewage and other N sources such as fertilizers; nitrogen in treated wastewater is generally enriched in the heavier isotope ^{15}N, and monitoring of sewage inputs exploits this higher concentration of ^{15}N (Cole et al., 2004; Heaton, 1986).

Studies aimed at detecting nitrogen discharged in sewage based on stable nitrogen isotope ratios ($\delta^{15}N$) in organisms have drawn upon a wide variety of taxa, including algae (Dailer et al., 2010; Fernandes et al., 2012), rooted macrophytes (Cole et al., 2004; McClelland et al., 1997), sessile invertebrates (Carmichael et al., 2008, 2012; Fertig et al., 2009, 2010;Risk et al., 2009), motile invertebrates (Bucci et al., 2007), and fish (Hoffman et al., 2012; Schlacher et al., 2005). Most report that the chosen taxon is 'suitable' as an ecological indicator of sewage-N, and that spatial patterns in relation to sewage sources can be mapped, or at least interpreted in a spatial context of known inputs (Costanzo et al., 2001; Northington and Hershey, 2006).

Investigations that include multiple species from different functional groups or from different trophic levels are less common (but see for example Pitt et al., 2009). Arguably, this lack of taxo-

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nomic and functional 'replication' raises two questions in relation to the assessment of isotopic sewage-N distributions: (1) Are spatial patterns of the sewage signal reproducible using different taxa? ('repeatability'), and (2) Are effect sizes (i.e. the magnitude of the isotopic sewage signal) comparable among taxa? ('sensitivity'). Here we examine both these questions using a two-staged approach: (i) we first test for differences in the strength of the δ^{15}N gradient – comparing changes of δ^{15}N in multiple taxa with distance from a defined point source of sewage inputs in a shallow marine gulf of southern Australia, and (ii) we then examine the generality of our findings by comparisons with spatial δ^{15}N gradients in other settings where multiple taxa have been used to measure sewage-N gradients.

2. Methods

Gulf St. Vincent (GSV) is a large, shallow marine embayment in southern Australia, bordering the city of Adelaide (Fig. 1). The general circulation of the gulf is clockwise, the western shores are bordered by mangroves, and the seafloor consists of a mosaic of bare sand and seagrass meadows and reefs (Jones et al., 2008). The GSC is located in an arid climatic zone where the annual evaporation exceeds rainfall, resulting in limited freshwater inputs from small local creeks and rivers (Shepherd and Sprigg, 1976). This makes discharges of sewage effluent on the eastern shores the main source of nutrients to receiving marine waters. Of the 1431 tonne of N entering the gulf waters from the city of Adelaide, 827 tonne (58%) come from waste-water treatment plants (Fernandes et al., 2012). We designed our study to determine spatial gradient of sewage-N in relation to the largest point source, the Bolivar wastewater treatment plant: it is the main contributor of sewage-N to the gulf, discharging 509 tonne of N per annum, or 62% of all treated N discharged in sewage effluents (Fernandes et al., 2012).

We collected multiple taxa to determine the spatial gradients of the sewage-N signal in relation to the defined sewage outfall of the Bolivar treatment plant (Fig. 1). Biota analysed for tissue δ^{15}N included primary producers (the green alga *Ulva lactuca* and two

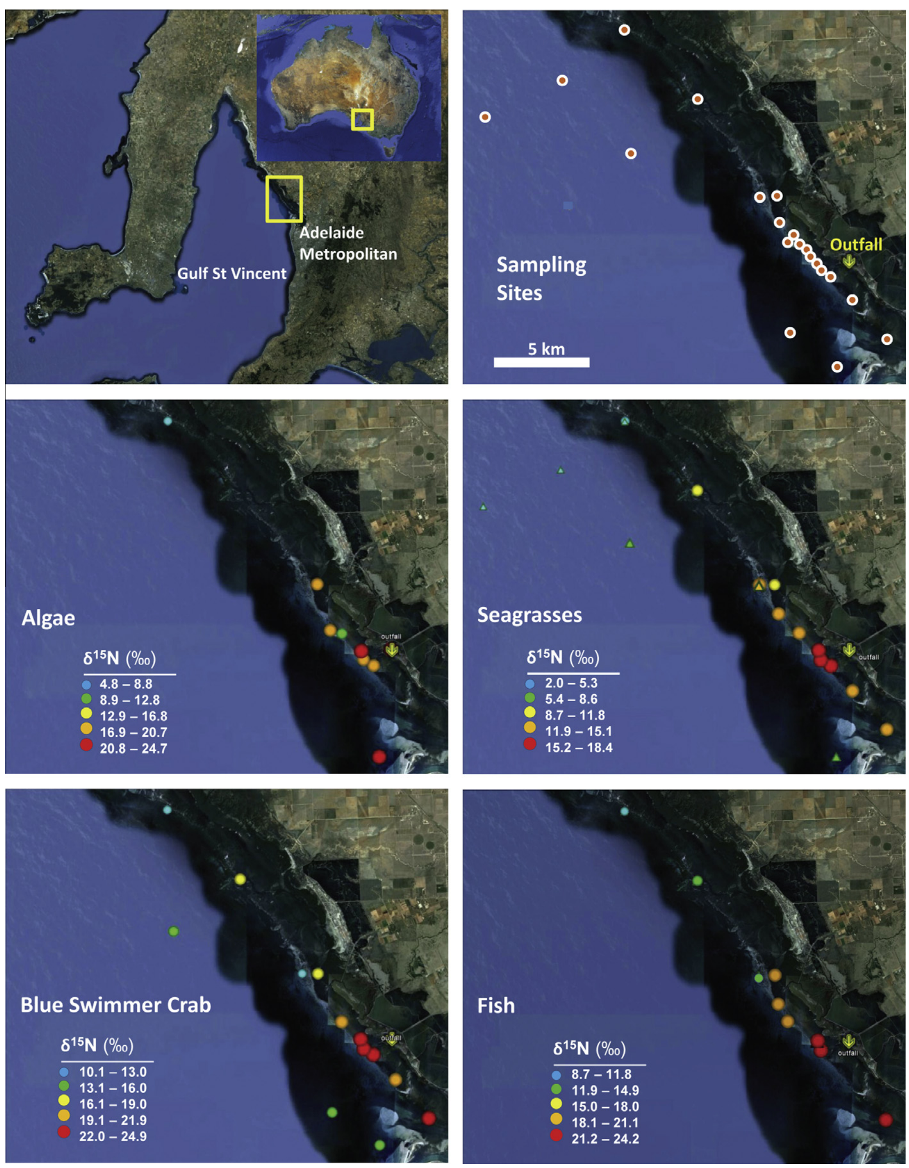

Fig. 1. Location of the study area in Gulf St. Vincent of South Australia near the city of Adelaide and map of the sampling stations in relation to the major sewage outfall point on the eastern shores from the Bolivar treatment plant (top row), and distribution of δ^{15}N values in the tissues of marine biota sampled to determine the spatial gradients of sewage-N signals in the nearshore marine waters of the gulf (middle and bottom row). In the seagrass map circles denote *Zostera* samples and triangles denote *Posidonia*.