Marine Pollution Bulletin 90 (2015) 41–47

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Tracking spatial distribution of human-derived wastewater from Davis Station, East Antarctica, using δ15N and δ13C stable isotopes

Patricia A. Corbett [a,*], Catherine K. King [b], Julie A. Mondon [a]

[a] Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Princes Highway, Warrnambool, Victoria 3280, Australia
[b] Terrestrial and Nearshore Ecosystems, Australian Antarctic Division, 203 Channel Highway, Kingston, Tasmania 7050, Australia

ARTICLE INFO

Article history:
Available online 5 December 2014

Keywords:
Trematomus bernacchii
Sewage
Fish
Antarctic rock-cod
Stable isotope

ABSTRACT

Stable isotope ratios, δ15N and δ13C were effectively used to determine the geographical dispersion of human derived sewage from Davis Station, East Antarctica, using Antarctic rock cod (*Trematomus bernacchii*). Fish within 0–4 km downstream of the outfall exhibited higher δ15N and δ13C values relative to reference sites. Nitrogen in particular showed a stepped decrease in δ15N with increasing distance from the discharge point by 1–2‰. Stable isotopes were better able to detect the extent of wastewater contamination than other techniques including faecal coliform and sterol measures. Uptake and assimilation of δ15N and δ13C up to 4 km from the outfall adds to growing evidence indicating the current level of wastewater treatment at Davis Station is not sufficient to avoid impact to the surrounding environment. Isotopic assimilation in *T. bernacchii* is a viable biomarker for investigation of initial sewage exposure and longer term monitoring in the future.

1. Introduction

Antarctic waters are under increasing pressure from human activity. Research station waste has traditionally been disposed of by incineration, placement onto sea ice, or via direct discharge into the sea (Tin et al., 2009). Over 35% of permanently-staffed Antarctic stations lack any form of wastewater treatment prior to discharge (Hughes, 2004; Gröndahl et al., 2009). Several factors affect operational efficiency of wastewater treatment in Antarctica: logistical constraints on supply of equipment; limited potable water; fluctuating station populations and corresponding variation in wastewater volume; significant heating and maintenance costs associated with wastewater containment and discharge infrastructure (Stark et al., Unpublished-a).

Faecal material represents a significant portion of the discharge wastewater (Stark et al., Unpublished-a). Breakdown and degradation rates of organic matter in ambient −1.8 °C polar seawaters are three times slower than in temperate seawaters of 20 °C (Howington et al., 1994). Consequently, if wastewater treatment is inadequate for polar regions, receiving environments will accumulate anthropogenic contaminants. Wastewater-derived non-native microbial species for example are present in near-shore environments at Davis Station, with microbes remaining viable for at least 54 days (Smith et al., 1994; Hughes and Nobbs, 2003).

Sewage and other wastewater generated at Antarctic Stations, to the maximum extent practical, must be minimised or removed to reduce potential human impact (Antarctic Treaty, 1991, Annex III, Waste Disposal and Waste Management – Article 2). Whilst direct disposal of wastewaters onto sea ice, ice shelves or grounded ice-sheets is prohibited (Article 4), direct discharge into the sea is permitted at stations supporting 30 people or more, provided maceration occurs prior to discharge, and conditions exist for initial dilution and rapid dispersion resulting in nil adverse impact to the receiving environment (Article 5).

Prior to 1991 Davis Station wastewater disposal was via combustion. A secondary wastewater treatment system began operation during the summer field season of 1990/91 (Green and Nichols, 1995), and later decommissioned with removal of infrastructure in 2005 (Stark et al., 2011). Since this time, wastewater has undergone simple maceration prior to shoreline discharge, either into the sea at high tide, or onto the beach at low tide. (Stark et al., Unpublished-a). Davis typically supports a population between 80–100 over summer, and 20–25 over winter, generating an overall average daily wastewater load of 1600–4300 L (Smith and Riddle, 2009). Relative to standard municipal wastewaters, Davis wastewater is more concentrated and highly variable in composition and volume, with significant inconsistency in

* Corresponding author at: 4 Paratea Crt, Greensborough, Victoria 3088, Australia. Tel.: +61 04 1114 3234.
E-mail addresses: pacor@deakin.edu.au (P.A. Corbett), Cath.King@aad.gov.au (C.K. King), julie.mondon@deakin.edu.au (J.A. Mondon).

http://dx.doi.org/10.1016/j.marpolbul.2014.11.034
0025-326X/

temporal discharge to the environment ranging from hours to years (*Ibid.*). Consequently, faecal load and biological oxygen demand in wastewater can be very high. Human faecal indicator bacteria in the discharge plume disperse up to 1.5 km south, 1.25 km north and 100 m west of the outfall (Stark et al., 2011). Human faecal sterols in sediments are highest within 1 km of the outfall and detectable up to 2 km south, downstream of the outfall (Leeming et al., 2014).

Faecal sterols indicate an intensification of nutrient enrichment (manuring) closest to the outfall, but a paucity of data related to the persistence of sterols to *in situ* aerobic microbial degradation, and exposure to native biota inhibits interpretation of environmental impact. Numerous studies elsewhere have used stable isotope δ15N: δ14N ratios to track sewage derived nitrogen incorporation into food webs across different habitats, species and trophic levels (Costanzo et al., 2001; Gaston et al., 2004; Savage, 2005; Schlacher et al., 2005; Connolly et al., 2013). Sewage yields higher δ15N values relative to δ14N. Sediment and biota exhibiting elevated δ15N signatures effectively 'map' the spatial range of sewage derived nitrogen transported physically and biologically through ecosystems (Costanzo et al., 2001; Savage, 2005). δ15N assimilation assumes transfer of nitrogen via successive consumption and incorporation in organisms across trophic levels (Schlacher et al., 2005; Piola et al., 2006).

Similarly, integration of δ13C in sediment and biota has been used to identify sewage exposure, albeit with variable results (Rogers, 1999, 2003; Gaston et al., 2004; Conlan et al., 2006). δ13C assimilation denotes both dietary carbon source and the organism's trophic level in a food web (Chen et al., 2012; Gillies et al., 2012a). Correlation between isotopic signatures and food-web-structure is weaker for δ13C than δ15N, where variance in trophic separation (i.e. the difference in δ13C between a consumer and its food source) is often considerably smaller (potentially up to 50%), and less consistent (Fry, 1988; Hansson et al., 1997; Gaston and Suthers, 2004). Consequently, the δ15N signature is stronger (Moore and Suthers, 2005; Vizzini and Mazzola, 2006), particularly when used as a standalone measure for tracking human derived sewage (Connolly et al., 2013). However, the use of δ13C in this context is not invalid, particularly when applied to support additional stable isotope analyses including nitrogen and sulphur (Gaston et al., 2004). δ13C itself is not necessarily indicative of sewage exposure, but provides an additional line of evidence supporting the δ15N sewage signal. Theoretically, application of both independent lines of evidence should strengthen interpretation of sewage exposure and dispersion.

Mapping the geographical extent of sewage dispersal via δ15N signatures is expedient over the more traditional techniques, including standard physico-chemical analyses of sediments and waters (e.g. DO and BOD), faecal indicator bacteria and human faecal sterols. Each is expected to be highly variable through time due to large fluctuations in volume, composition and timing of wastewater release. Indicator bacteria and sterols effectively identify presence of human faecal matter in sediment across varying temporal scales (Bull et al., 1999; Hughes and Nobbs, 2003), although a number of factors exist which confound interpretation of results. None of the traditional analyses currently measure exposure and bioavailability. Additional support for advocating the stable isotope approach in Antarctic environments relates to cost effectiveness of the technique, relative ease of interpretation, and scientific recognition that the methodology is robust for mapping the spatial extent of sewage contamination in sediments, and as a biomarker of sewage exposure by uptake and assimilation in organisms. Collectively, a 'sewage hierarchy' approach could be adopted where faecal indicator bacterial, faecal sterol and sewage isotopic signatures are applied; from an initial short-term contamination measure (indicator bacteria), to longer term environmental accumulation (faecal sterols), to biological uptake and assimilation by biota (stable isotopes).

Fish are considered ideal 'assimilation' organisms for detecting sewage exposure. Slow tissue turnover rate and longer life span relative to macroalgae and invertebrates (Hesslein et al., 1993; Gartner et al., 2002), enables detection of very low effluent volume impact evidenced by δ15N in muscle tissue (Schlacher et al., 2005). In the Antarctic context, *Trematomus bernacchii* (Antarctic rock cod) represents an ideal assimilation species. Rock cod are relatively abundant, with a wide geographical coastal distribution (Romano et al., 1997), yet the home range of individuals is narrow, within an estimated radius of 500 m (Kawaguchi et al., 1989; Evans et al., 2000; Herbert et al., 2003). Additionally, rock cod are opportunistic predators of benthic and epibenthic invertebrates (Kiest, 1993), potentially exposed to contaminants via diet and direct sediment contact.

Previous research at McMurdo Station suggests isotopic nitrogen signatures in rock cod are not effective at tracing wastewater dispersion across a narrow spatial gradient of 600 m (Conlan et al., 2006). At Davis Station indicator bacteria and faecal sterols are detected at 1.5 km and 2 km from the discharge, respectively. If a 'sewage hierarchy' approach were to be applied at Davis, the addition of sewage nitrogen signatures would confirm the spatial range of sewage detection within, and potentially beyond a 2 km radius above and below the outfall. To evaluate the hierarchical measurement approach and ascertain the spatial extent of biological exposure from wastewater disposal, this study investigates δ15N and δ13C in *T. bernacchii* tissue to elucidate the effectiveness of isotopic signatures in detecting and tracking human derived sewage dispersion and bioavailability in Antarctic coastal waters.

2. Materials and methods

2.1. Sampling sites and fish collection

A total of 60 fish were caught by line and in box traps from four sites along a spatial gradient from the Davis Station wastewater outfall: 0 km (within 250 m of the point of discharge), 1 km, 4 km and 9 km south of the discharge point in the direction of the predominant current. Additionally, two reference sites were sampled 9 km and 16 km north of the discharge point (Fig. 1). Once collected, fish were immediately returned to the Davis Station laboratories and sacrificed individually by immersion in an Aqui-S solution (~15 ml/L). At the onset of death (approximately 5 min), length and weight were measured and sex of fish was determined. Dorsolateral muscle tissue sections (approximately 1×1 cm^3) from the left side of each individual was removed using a scalpel, and was placed in aluminium foil and frozen at −20 °C for later analysis.

2.2. Tissue processing

Frozen muscle tissue sections were placed into a clean, acid washed glass crucible and cut into to smaller pieces. Tissue was dried for 48 h at 60 °C, carefully placed into separate 2 ml Eppendorf tubes, each containing a washed, dried stainless steel ball bearing. Lids were closed tightly to prevent any moisture entry, tubes were shaken for 6 min in a Tissue II Lyser to generate a fine powder. Ball bearings were removed from the tubes and powdered tissue samples sent to Cornell University Stable Isotope laboratory for δ15N and δ13C analysis. Stable isotope ratios are expressed in ‰ units using the standard delta (δ) notation, δ15N and δ13C.

2.3. Statistical analysis

ANOVA and post hoc Tukey Kramer tests were performed on the following data sets: δ13C and site; δ13C and length; δ13C and sex;