



Temporal changes in carbon and nitrogen stable isotope ratios of macrozoobenthos on an artificial tidal flat facing a hypertrophic canal, inner Tokyo Bay

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ABSTRACT

Temporal changes in benthic food web structure were analyzed in an artificial tidal flat in inner Tokyo Bay, Japan, using carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Microphytobenthos were the most important food sources of macrozoobenthos, due to high microphytobenthic biomass on the tidal flat, while phytoplankton in canal water (canal POM_{pp}), terrestrial materials from urban surface runoff (canal POM_{tm}), and marsh plants were less important. Dietary contribution of microphytobenthos was highest in April to June, while decreased towards December owing to the supply of canal POM_{pp} and canal POM_{tm} following red tides and heavy rainfall events in summer to fall. Temporal changes in $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) of consumer corresponded well to the ^{15}N -enrichment in canal POM_{pp} in summer. A meta-analysis showed that the consumer- $\Delta\delta^{15}\text{N}$ was considerably larger in inner Tokyo Bay than those in other estuaries, which may be a specific characteristic of benthic food web in highly urbanized estuaries.

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1. Introduction

Estuaries and coastal wetlands function as sinks for nutrients and organic materials from a variety of sources, and provide habitats for associated organisms including macrozoobenthos, fishes, and birds (Levin et al., 2001). Sufficient supply of nutrients assures high primary production in estuaries, which supports the high secondary production of benthic and pelagic consumers (Heip et al., 1995). In estuarine soft-bottom habitats, organic materials in the water column and sediment are assimilated by benthic consumers such as bivalves and polychaetes (Riera and Richard, 1996; Chanton and Lewis, 2002; Page and Lastra, 2003; Antonio et al., 2010), and transferred to the biomass of consumers at higher trophic levels. Macrozoobenthic organisms can modify water quality, sediment properties, and associated biota through activities including feeding, burrowing, and sediment reworking (i.e., bioturbation; Kinoshita et al., 2003; Kanaya et al., 2005; Wall et al., 2011). Therefore, macrozoobenthos play an important role in material flow and biogeochemical cycling in estuaries, and thus strongly shape the ecological functions (Levin et al., 2001).

Stable isotope signatures have been widely applied to estuarine ecosystems to trace organic matter flow through food webs based on the dietary components of macrozoobenthos (Riera and

Richard, 1996; Chanton and Lewis, 2002; Page and Lastra, 2003). This method can provide historical information about a consumer's diet on a scale of several months (e.g., Post, 2002; Fukumori et al., 2008). Because $\delta^{13}\text{C}$ is nearly constant throughout a trophic chain (0–1.0‰ increase per a trophic level) (Fry and Sherr, 1984), but $\delta^{15}\text{N}$ increases by 3–4‰ per a trophic level (Post, 2002), we can estimate the major food resources and trophic level of an animal from its stable isotope signatures. In aquatic food webs, $\delta^{15}\text{N}$ values of long-lived herbivores (e.g., filter-feeding bivalves and grazing gastropods) have been used as a trophic baseline because they reflect those of primary producers over certain time periods and are temporally more stable than those of short-lived species with rapid tissue turnover (Post, 2002). However, a recent review pointed out that the isotopic turnover of benthic consumers occurs over much shorter time frames than was expected (Woodland et al., 2012). In fact, in estuaries, macrozoobenthos sometimes show temporal changes in food components in relation to seasonal environmental changes, such as river flow regime (e.g., Chanton and Lewis, 2002; Kasai et al., 2004) and phytoplanktonic blooms (e.g., Page and Lastra, 2003). Accordingly, it is necessary to know the extent of temporal variability in the dietary components of macrozoobenthos to ascertain the pathways of material flow through an estuarine food web.

Estuarine and coastal waters have been disturbed by anthropogenic activities due to increasing human coastal populations, resulting in losses of ecological function (Lotze et al., 2006).

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Recently, several studies have highlighted the anthropogenic modification of benthic food web structures (e.g., Hadwen and Arthington, 2007; Yokoyama and Ishihi, 2007; Schaal et al., 2010; Olsen et al., 2011). For example, the inflow of ^{15}N -enriched sewage increases the $\delta^{15}\text{N}$ of primary producers, resulting in an overall increase in $\delta^{15}\text{N}$ values of consumers in the estuary (Hadwen and Arthington, 2007; Olsen et al., 2011). The deposition of fish food from aquaculture cages can induce changes in the dietary components of macrozoobenthic organisms in fish-farming areas (Yokoyama and Ishihi, 2007). Changes in the biomass of primary producers along a eutrophication gradient can also change the food composition of benthic invertebrates (Kanaya et al., 2007, 2008; Olsen et al., 2011). Such findings suggest that anthropogenic-induced changes in food composition and nutrient supply could modify the benthic food web structure on different scales.

Woodland et al. (2012) conducted a meta-analysis and found that temporal changes in the stable isotope signatures (especially in $\delta^{15}\text{N}$) of aquatic invertebrates are, in general, more conspicuous in eutrophic waters than in natural and more oligotrophic waters. This suggests that benthic food web structure is temporally more unstable in eutrophic systems, especially in the trophic baseline (i.e., $\delta^{15}\text{N}$ of primary consumers). Accordingly, we need to know how benthic food web structure is modified by periodic or long-term anthropogenic disturbances; such information could then be used to interpolate the ecological functions of estuarine ecosystems.

In this ecological context, we assessed temporal changes in the structural and functional characteristics of a benthic food web on an artificial tidal flat, the Oi tidal flat, which faces a hypertrophic canal system (inner Tokyo Bay, Japan). Canals and adjacent waters in inner Tokyo Bay are highly eutrophic, and red tides occur frequently in warmer seasons (Tokyo Metropolitan Government). The catchment is heavily urbanized, and shortly after rainfall, a huge amount of urban surface runoff, which is a significant source of land-derived nutrients and organic particles (Kojima et al., 2011), is discharged into the canal through rivers and sewage overflow (Maki et al., 2007). In the present study, we focused on whether such seasonal pulses in human-induced environmental changes affect the local food web structure. More specifically, we investigated how seasonal red tides and urban surface runoff in the hypertrophic canals affect the availability of food components of macrozoobenthos, and whether the trophic baseline in the tidal flat tends to fluctuate more than those in other less urbanized and natural estuaries.

2. Materials and methods

2.1. Study site

Field surveys were conducted in 2009 and 2010 on the Oi artificial tidal flat ($35^{\circ}35'30\text{N}$, $139^{\circ}44'58\text{E}$, 0.01 km^2 , Fig. 1), created in 1978 on the eastern side of the Keihin Canal in the innermost part of Tokyo Bay, where extensive reclamation has proceeded during the 20th century. There are two sewage treatment plants located along the Keihin canal (Bureau of Sewerage, Tokyo Metropolitan Government; <http://www.gesui.metro.tokyo.jp/english/english.htm>). After heavy rainfall, urban surface runoff is discharged through rivers and the drainage system as sewage overflow, which is a significant source of land-derived nutrients and organic particles in the canal system (Maki et al., 2007). The canal is hypertrophic, with an annual average $[\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+]$ of $226 \pm 82\ \mu\text{M}$ (2010–2011, $n = 17$, monthly monitoring by the authors). Salinity fluctuates between 10 and 30, and hypoxic water often develops in summer, especially in the bottom layer (Nakamura et al., 2009). From spring to fall, red tides frequently occur in inner Tokyo Bay (Tokyo Metropolitan Government).

At the tidal flat, the intertidal zone measured about 20–60 m in width, and was characterized by sandy sediment in most parts (silt-clay < 10%). Tides were semi-diurnal, with a range of 1.5–2.0 m in spring tides. There was a boulder area (50 m \times 20 m) densely covered by the invasive mussel species *Xenostrobus securis* in the middle part of the flats. Benthic invertebrates, including crabs, bivalves, and polychaetes, were found in the tidal flats. The marsh plants *Phragmites australis* and *Carex scabrifolia* grew densely in the upper-tidal zone, while macroalgae was scarce and seagrass was absent.

2.2. Collection of macrozoobenthos for stable isotope analysis

One to eight individuals of invertebrate species (see Table 2) were collected around St. T1 during eight sampling events from April 2009 to May 2010. Sessile species including the anemone *Haliplanella lineata* and the bivalves *Perna viridis*, *X. securis*, *Musculus senhousia*, and *Crassostrea gigas* were sampled at the dike and in the boulder zone (Fig. 1). Other species were picked up from the sediment around St. T1, and species including the crabs *Scopimera globosa* and *Macrophthalmus japonicus* and the bivalve *Corbicula japonica* were collected from the upper tidal zone near the marsh edge. In the laboratory, the body sizes were measured using calipers and gut contents were removed under a dissecting microscope. For stable isotope analysis, muscle tissue was washed with deionized water, freeze-dried (24 h), and powdered using a mortar. Lipids were removed using a chloroform-methanol solution (2:1 by volume) following the method of Folch et al. (1957).

2.3. Collection of potential food sources

To collect particulate organic matter in the canal (canal POM), three bottles of surface water were collected at St. T2 in May, June, July, September, October, and December 2009 and March 2010 (i.e., three replicates for each sampling occasion). Surface water for marine POM was collected with 1–4 replicates from inner Tokyo Bay (Sts. M1 to M3) in May, July, and August 2009 using a motor boat. Red tides occurred in June 2009 in the canal (Kanaya, pers. obs.), and in April to September in inner Tokyo Bay (Tokyo Metropolitan Government). Sampling in September 2009 was conducted shortly after a heavy rainfall (80 mm d^{-1} ; Tokyo, Japan Meteorological Agency), when the canal water became highly turbid with fine detrital particles from urban surface runoff. In the laboratory, POM in the water was concentrated onto a pre-combusted GF/F (500 $^{\circ}\text{C}$, 2 h) after being pre-filtered through a 0.125-mm mesh. Subsamples ($n = 3$) were taken from the bottles for chlorophyll measurements (see below).

Sediment organic matter (SOM; 0–1 cm deep) was collected from St. T1 on the same days as the canal POM sampling (also in April 2010 for SOM). SOM was sampled in the field using a spatula, and placed in three plastic tubes (i.e., $n = 3$). Microphyto-benthos were collected from the tidal flat on six occasions (see Table 1) when visible diatom mats developed. Collection was made from various sites (e.g., near the marsh, lower tidal zone, or just around St. T1) due to their high spatiotemporal heterogeneity in their biomass. To collect them, surface sediments (0–1 cm deep) were placed on three polyethylene trays and microphyto-benthos were extracted by exploiting their phototaxis in the laboratory (Riera and Richard, 1996). Then they were suspended in deionized water, concentrated onto a pre-combusted GF/F, acidified with 1 M HCl to remove carbonates, and freeze-dried (24 h). The C_3 -marsh plants *P. australis* and *C. scabrifolia* were sampled from the marsh, brushed with deionized water, freeze-dried (24 h), and powdered.

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