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Food resource partitioning among fishes in an estuarine nursery as revealed by stable isotope analysis

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Abstract: Various coastal fishes use shallow estuarine habitats as nurseries. To investigate the extent of food resource partitioning among juvenile fishes occurring sympatrically in such nurseries, we analyzed carbon and nitrogen stable isotope ratios from three dominant coastal fishes (Japanese seaperch *Lateolabrax japonicus*, yellowfin goby *Acanthogobius flavimanus*, and flathead mullet *Mugil cephalus cephalus*) and their food source on a tidal mudflat in the Tama River estuary, central Japan. Our isotopic data indicated that Japanese seaperch mainly feed on fish or benthic crustaceans, and yellowfin goby feed on polychaetes or benthic crustaceans. Plots of stable isotope ratios of flathead mullet showed to be far from those of sediments or deposit organic material values, signifying their food source were not of such materials. However, comparing with published literature showed that benthic microalgae maybe their plausible food source because of similarity in δ^{13}C values and δ^{15}N enrichment. Thus, isotopic compositions of those juvenile fishes differed greatly among species, indicating the evident food resource partitioning. Such resource partitioning may play an important role in reducing inter–specific competition on the estuarine mudflat.

Keywords: *Juvenile fishes, Stable isotope, Food resource partitioning, Tidal mudflat*

1. Introduction

Tidal mudflats in temperate estuaries function as nursery grounds for many coastal and euryhaline fishes, including several of commercial importance (KANOU *et al*., 2000; MORRISON *et al*., 2002). On a tidal mudflat in the Tama River estuary, central Japan, there exist the diverse communities of juvenile fishes, which may attain densities of up to 30 species and > 7000 individuals in a 100 − m^2 area during spring and early summer (April to June) (KANOU, 2003; KANOU *et al*., 2007). Many of these fish species are very similar in patterns of microhabitat use (e.g., vertical distributions and intertidal movements) (KANOU *et al*., 2004a, 2005a), implying that dietary niche segregation may play an important role in reducing inter–specific competition and maintaining high species diversity. Most recent study (KANOU *et al*., 2004b) has found significant variation of stomach contents among these fishes, even in highly productive estuarine mudflats with plentiful food supply. However, the results of stomach contents analysis represent food consumed over a small time period and within a small area (KANOU *et al*., 2005b). In addition, other disadvantages of stomach content analyses include difficulty of identification and uncertainty over whether all observed stomach contents, such as microalgae and cyanobacteria, are indigestible. One method that allows measurement of assimilated, and therefore nutritionally important,

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materials is stable isotope analysis. The stable isotope ratios of carbon and nitrogen differ among autotrophs (FRY, 1984; BOON *et al.*, 1997; BOUILLON *et al.*, 2002). This ratio is taken on by consumers and reflected in their tissues at whatever trophic level they occur (PETERSON, 1999). Generally, carbon isotope ratio is useful in estimating the source of consumers' diets where enrichment caused by fractionation or metabolic effects is small or negligible, with reported enrichment of only 0 – 1 ‰ (e.g. DENIRO and EPSTEIN, 1978). For nitrogen isotope value, a higher enrichment by fractionation ranging from 2.6 ‰ (OWENS, 1987) to 3.4 ‰ (MINAGAWA and WADA, 1984) is usually assumed, and is used to estimate consumer trophic levels. These analyses also have the advantage of representing food consumed over a relatively long period of time. In this study, therefore, we use stable isotope analysis to attempt to determine which autotrophs provide nutrition to juvenile fishes occurring sympatrically on an estuarine mudflat.

2. Materials and Methods

Sampling was carried out on a tidal mudflat in the Tama River estuary (35° 32' N, 139° 46' E), located on the western shore of Tokyo Bay, central Japan. The estuary is subjected to semidiurnal tides with a tidal range of up to about 2 m. The tidal current from the bay flows along the estuary at flood tide, whereas the effect of freshwater inflows is significant at low tide. The sampling site was located on a tidal mudflat approximately 3 km from the river mouth. A map of the study site was given by KANOU *et al.* (2005a). Sediment of the surface layer in the subtidal zone consisted of about 20% silty clay and 80% sand. There was no rooted macrophyte vegetation in the site during the study period. Samples of Japanese seaperch *Lateolabrax japonicus* [n=5, 45–50 mm in standard length (SL)], yellowfin goby *Acanthogobius flavimanus* (n=5, 40–57 mm SL), and flathead mullet *Mugil cephalus cephalus* (n=19, 34–60 mm SL) were caught using cast nets on 14 May 2005. These fish species appear abundantly in shallow estuaries and mudflats at the end of pelagic stage (< 15 mm SL) in early spring (March), subsequently growing within the habitat by late summer (September). Recent examinations of the stomach contents of these sympatric juveniles on the mudflat of Tama River estuary (KANOU *et al.*, 2004b) indicated that Japanese seaperch, yellowfin goby, and flathead mullet belong to crustacean/fish feeders [the main food item being small crustaceans (i.e., mysids, gammaridean amphipods, and cumaceans) and juvenile fishes], polychaete feeders (polychaetes with small crustaceans), and detritus feeders, respectively. All prey items, observed as stomach contents of these fishes by previous studies (KANOU *et al.*, 2004b, 2005b), were collected on the mudflat immediately after fish sampling. Planktonic prey animals, mainly including cladocerans, were collected using a 0.3 mm–mesh conical net (45 cm mouth diameter and 180 cm long). Benthic prey animals, such as cumaceans, gammaridean amphipods, mysids, shrimps and polychaetes, were collected with a cylindrical core sampler (11 cm diameter) that was used to extract a 300 cm^3 volume (3.2 cm depth) of sediment in the subtidal zone. Epilithic macroalgae, deposit organic mateirials [detritus in KANOU *et al.* (2004b)], and sediments were collected by hand. During the sampling periods, the water was turbid, salinity ranged from 9.6 to 15.2 ‰ and surface water temperature from 19 to 24℃.

All samples were kept in a cool bag during transport to the field laboratory, and then washed with distilled water. The samples except fishes were immediately dried at 60 ℃ for at least 24 h. Muscle tissues of fishes were taken directly from the dorsal area, and they were also dried at 60 ℃ for at least 24 h. After drying, all samples (fish tissues and potential prey items) were ground to a fine powder and they were treated with chloroform:methanol (2:1) for 3 h and 0.1N HCl for 24 h to remove lipid and carbonates, and then re–dried. Such removal process was conducted to eliminate lipid and carbonate effects on muscle $\delta^{13}C$ measurements. Isotopic analyses were carried out on an isotope–ratio mass spectrometer (Thermo/Finnigan Delta plus XP), and expressed relative to conventional standards, i.e. PeeDee Belemnite for carbon, and atmospheric air for nitrogen, as ‰ values, defined as: δ X

Table 1. Carbon and nitrogen stable isotope ratios (mean ± SD) of the fishes and their potential food sources collected on 14 May 2005 from a tidal mudflat in the Tama River estuary. Cumaceans, shrimps and polychaetes samples were pooled for isotope analysis due to small amounts collected.

Samples	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	*n*
Fishes			
Lateolabrax japonicus	−16.6±0.4	11.7±1.6	5
Acanthogobius flavimanus	−18.8±0.9	11.4±0.7	5
Mugil cephalus cephalus	−15.6±2.5	9.0±1.1	19
Food sources			
Cumaceans	−19.0	5.7	3 (pooled)
Gammaridean amphipods	−20.5±1.7	5.5±0.5	3
Mysids (5−9 mm in Body Length, BL)	−17.8±0.6	7.5±0.6	3
Mysids (10−15 mm BL)	−18.6±0.3	7.5±0.6	3
Shrimps	−18.3	10.6	3 (pooled)
Polychaetes (5−9 mm BL)	−20.3	10.2	3 (pooled)
Polychaetes (10−25 mm BL)	−21.5	8.2	3 (pooled)
Zooplankton	−22.6±0.1	6.3±0.1	3
Epilithic macroalgae	−19.7±1.2	0.7±1.8	3
Deposit organic materials	−27.2±0.4	2.0±0.5	3
Sediments	−25.0±1.2	5.1±0.8	3

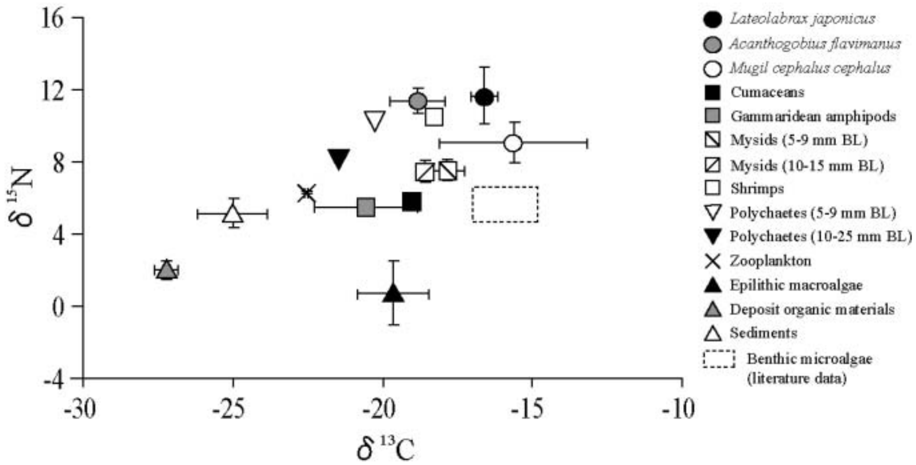

Fig. 1. $\delta^{13}C$ and $\delta^{15}N$ plot of fishes and their food sources from a tidal mudflat in the Tama River estuary, central Japan. Broken line shows typical $\delta^{13}C$ and $\delta^{15}N$ range for benthic microalgae collected from similar intertidal mudflat (Riera *et al.*, 1996; Yokoyama and Ishii, 2003; Al-Zaidan *et al.*, 2006). Error bars indicate standard deviations. See Table 1 for detailed values for samples.

= (R sample − R standard) / R standard × 10^3 (‰), where X = ^{13}C or ^{15}N, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Experimental precision (based on standard deviation of replicates of an alanine standard) was better than 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

3. Results

The carbon and nitrogen stable isotope ratios of samples are summarized in Table 1. Isotopic compositions among three fish species showed different values (Fig. 1). The mean $\delta^{13}C$ value from *L. japonicus* was similar to those of *M. cephalus cephalus* or benthic crustaceans (mysids or cumaceans), and its $\delta^{15}N$ value was enriched by about 3 ‰ by these two food items. *A. flavimanus* showed similar $\delta^{13}C$ value with polychaetes or benthic crustaceans (mysids, cumaceans or gammaridean amphipods), and with an enriched $\delta^{15}N$ value comparable with to these food resources. Isotopic compositions of flathead mullet were greatly different from those of sediments or deposit organic materials.

4. Discussion

In our study, isotopic compositions of three fishes differed greatly among species (Fig.1), indicating their food preferences was different. Such dietary niche segregation may play an important role in reducing interspecific competition and maintaining high species diversity on tidal mudflat as nursery grounds.

Our isotopic data indicated that Japanese seaperch and yellowfin goby mainly fed on fish or benthic crustaceans and polychaetes or benthic crustaceans, respectively (Fig. 1). The results were consistent with previous reports (KANOU *et al.*, 2004b; KANOU *et al.*, 2005b) using stomach content analysis. However, isotopic result from flathead mullet was different. Stable isotope ratios of mullet were plotted far from sediments or deposit organic material values (Fig. 1), indicating their food source were not such materials. Their plausible food source would be benthic microalgae because they had similar $\delta^{13}C$ value to typical benthic microalgae which collected at similar intertidal mudflat ($\delta^{13}C = -14.7$ to -16.9 ‰; RIERA *et al.*, 1996; YOKOYAMA and ISHII, 2003; AL–ZAIDAN *et al.*, 2006), and had 2.4 – 4.4 ‰ enriched $\delta^{15}N$ value compared to microalgae ($\delta^{15}N = 4.6$ to 6.6 ‰; RIERA *et al.*, 1996; YOKOYAMA and ISHII, 2003). Indeed, LIN *et al.*, (2007) reported detritivorous fish including mugilidae (*Liza macrolepis*) assimilated benthic microalgae in tropical or subtropical region.

Detritus, such as deposit organic materials, is generally considered to be one of the most abundant food resources in tidal flat sediments, being utilized by most small invertebrates (e.g. copepods, ostracods, amphipods, annelids, and snails) that are in turn commonly consumed by tidal flat fishes (REISE, 1985). On the mudflat in Tokyo Bay, mugilids, blennids, clupeids, and cyprinids fed largely on detritus (KANOU *et al.*, 2004b). Despite the abundance of detritus, it is well known that the direct nutritional values of them are very low for fishes (e.g. PRINSLOW *et al.*, 1974; D' AVANZO and VALIELA, 1990; LARSON and SHANKS, 1996). Therefore, the specialist feeders, such as flathead mullet in this study, might select higher quality detritus including microalgae by the taste buds on the gill arches (HOSSLER and MERCHANT, 1983; LARSON and SHANKS, 1996), while the generalist feeders possessing a bit of detritus might swallow it without selection when they fed on other foods. In any case, such feeding at a lower trophic level may play a role at rapidly transferring energy and materials up the food web.

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