Spatial dietary shift of the intertidal snail, *Batillaria multiformis*: stable isotope and gut content analyses

HISASHI YOKOYAMA¹,*, JING FU²,†, YUJI TAMURA³,‡ & YOH YAMASHITA¹

¹Field Science Education and Research Center, Kyoto University, Oiwake, Sakyo, Kyoto 606–8502, Japan
²Graduate School of Global Environmental Studies, Kyoto University, Yoshida-Honnachi, Sakyo, Kyoto 606–8501, Japan
³Shallow/Fresh Water Group, Fisheries Research Division, Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Kuresaki, Bungo-Takada, Oita 879–0608, Japan

¹Present address: East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 300 Jungkin Road, Shanghai 200090, China
²Present address: Research and Guidance Coordinator, Fisheries Research Division, Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Tsuiura, Kamiura, Saiki, Oita 879–2602, Japan

Received 4 September 2018; Accepted 6 February 2019  Responsible Editor: Gen Kanaya
doi: 10.3800/pbr.14.86

Abstract: In order to identify the food sources of the intertidal snail, *Batillaria multiformis*, stable isotope (δ¹³C, δ¹⁵N) and gut content analyses were conducted. Snails and their possible food sources were collected from the river-sea transects at the mouths of the Iroha and Katsura rivers in northeastern Kyushu, Japan, which differ in land use parameters (e.g., the ratio of agricultural area in the catchment area). There was a shift in the δ¹³C of the snail along the transects, showing higher values at the upstream stations (−15.8 and −14.8‰) and lower values (−17.5 and −17.1‰) at the seaward stations. The δ¹⁵N showed no significant spatial gradient along the transects, but a significant difference (t-test, p<0.001) was observed between the Iroha (mean, 10.0‰) and Katsura (11.1‰) rivers. A Bayesian mixing model and the biomass of possible food sources on the tidal flats showed that (1) the major food sources were marine phytoplankton, seaweeds, and benthic microalgae, and that (2) the dependency on marine phytoplankton increased in the seaward stations. However, gut content analysis revealed that most of the identifiable dietary items across all stations were benthic diatoms, which is considered to be due to the short-term result of ingestion on the sediment surface at low tide. Increasing dependency on phytoplankton at seaward stations was considered to be due to consumption of suspended particles in the water column at high tide by filter feeding. Differences in the δ¹⁵N between the two rivers suggest the possibility of using the δ¹⁵N of snails as an indicator of watershed characteristics.

Key words: Bayesian mixing model, benthic diatom, gastropod, intertidal flat

Introduction

Estuaries have been studied intensively to clarify a mechanism of how to maintain high biodiversities and productivities (e.g. Daborn & Redden 2016). Such ecosystems are considered to be supported by materials including nutrients and organic and inorganic particles that are discharged from terrestrial areas and are carried by rivers (Yamashita 2014).

Carbon and nitrogen stable isotope analyses have contributed to elucidate the sources and trophic transfer of organic matter in estuaries. The stable carbon isotope ratio (δ¹³C) has been widely used as a natural tracer to follow the flow of food sources (e.g., Fry & Sherr 1984), whereas the stable nitrogen isotope ratio (δ¹⁵N) has been used to show the trophic levels of consumers in food webs (e.g., Owens 1987). Carbon isotope signatures of organic matter sources are determined by isotope fractionation of primary producers according to their photosynthetic pathways. The ¹³C fractionation of aquatic plants and algae varies depending on their internal and external environments, resulting in taking a wide range of δ¹³C values even within each producer group (Fogel et al. 1992, Simenstad et al. 1993,
Fry 1996, Gervais & Riebesell 2001, Cloern et al. 2002, Trudeau & Rasmussen 2003). Biotic and abiotic nitrogen are subject to various kinds of trophic transfer and/or biogeochemical processes, resulting in a large spatiotemporal variability in their δ¹⁵N signatures in aquatic ecosystems (Cifuentes et al. 1988, McCutchan et al. 2003, Middelburg & Herman 2007). The large variability in the isotopic composition of possible food sources may involve drawbacks; however, simultaneous use of dual isotopes and frequent sampling of food sources may compensate for such drawbacks and provide signatures that are integrated over a period corresponding to the turnover time of the analyzed tissues. On the other hand, traditional gut content analysis provides evidence of the dietary items that individuals actually consumed, though this analysis is biased towards recent dietary items that do not digest readily (e.g., Polito et al. 2011). Complementary stable isotope and gut content analyses thus provide valuable ecological insights.

Extensive data on the diets for estuarine animals are available. Of a number of these case studies, most have noted the importance of benthic or pelagic microalgae (e.g., Persic et al. 2004, Kang et al. 2015). On the other hand, several studies have indicated that riverine terrestrial organic matter (Kasai & Nakata 2005, Hoffman et al. 2008, Cole & Solomon 2012) and anthropogenic effluents (e.g. Hadwen & Arthington 2007) play an important role in estuarine food webs. Several studies have also suggested that significant incorporation of terrestrial organic matter by estuarine animals occurs in the upper estuarine reaches (Kikuchi & Wada 1996, Fry 1999, Antonio et al. 2012) or during freshet periods (Riera & Richard 1997, Chanton & Lewis 2002). Thus, there are large variations in food utilization by estuarine consumers among estuaries depending on the differences in food supply which are usually influenced by the mixing ratio between seawater and freshwater. It is, therefore, important to understand the flow of organic matter and energy in estuaries in order to clarify the spatial variability in the food sources of consumers.

The bivalve Batillaria multiformis (Lischke, 1869) (Gastropoda, Batillariidae) is distributed in estuaries in Japan (southern Hokkaido to Kyushu) (Japanese Association of Benthology 2012) and Korea (Lee et al. 2016). This snail prefers sand and mud substrata in the middle and low intertidal zones, and feeds on benthic diatoms and marine phytoplankton rather than terrestrial plants (Liu et al. 2014). It produces egg capsules that hatch into planktonic larvae (Furota et al. 2002). This species has been commonly found in Japan; however, during the latter half of the 20th century, populations in many areas of Japan such as at the mouth of the Nanakita River (Sendai City) and in Tokyo Bay (Wada 2001, Furota et al. 2002) have decreased drastically due to land reclamation and water pollution. As a result, B. multiformis has been designated as a near threatened species in 2007 (Ministry of the Environment, Japan 2014).

In the area of the Kunisaki Peninsula and in the nearby Usa region, northeastern Kyushu, the amount of precipitation is relatively small compared with that in other parts of Japan (<1.600 mm yr⁻¹, Oita Meteorological Office, http://www.jma-net.go.jp/oita/oita-kikou.htm: June 19, 2018). In order to develop agriculture in this region, which has low rainfall and steep slopes, residents have constructed reservoirs called ‘Tameike’ and terraced paddy fields in a staircase pattern on very steep slopes called ‘Tanada,’ resulting in a unique landscape. Residents have also been utilizing deciduous fagaceous vegetation dominated by sawtooth oak (Quercus acutissima) as materials for culturing Shiitake mushrooms (Lentinus edodes) and producing charcoal. Such a traditional farming system has been recently certified as a site for Globally Important Agricultural Heritage Systems (GIAHS) by the Food and Agriculture Organization of the United Nations under the name of ‘Kunisaki Peninsula Usa integrated forestry, agriculture and fisheries system’ (http://www.kunisaki-usa-giahs.com/en/: June 19, 2018).

During field surveys around the Kunisaki Peninsula that aimed to verify the effectiveness of the GIAHS against production in coastal waters, we often found dense populations (~2,000 individuals m⁻²) of B. multiformis on the tidal flats of river mouths. Comparison of the snail populations around the Kunisaki Peninsula with those in industrialized areas of Japan where the populations have been disappearing encouraged us consider that the dense populations were supported by fertile GIAHS environments which were characterized by the harmonious coexistence of nature and humans. We therefore tried to clarify the mechanism responsible for the establishment of these dense populations. In the present study, we tried to clarify what this snail feeds on by examining the δ¹³C and δ¹⁵N and gut contents of B. multiformis collected from intertidal stations along river-sea transects at the mouths of the two rivers, which differ in topographic, geologic, and land use parameters.

**Materials and Methods**

**Study area and sample collection**

The Kunisaki Peninsula is formed from a round volcano (diameter, ∼30 km) with a peak (Mt. Futago, 721 m above sea level) at the center (Fig. 1). Much of the Kunisaki Peninsula is covered by volcanic rock that was produced by the Futago Volcanic Group at a geological age of 1.19 Ma (Horikawa et al. 2016). A number of short and steep rivers occur radially and flow into Suo-nada and Iyo-nada in Seto Inland Sea. In this region, plain areas are scarce, whereas the Nakatsu and Usa districts located to the west of the Kunisaki Peninsula have an alluvial plain created by several rivers such as the Yamakuni and Yakkan rivers.

The present study aimed to reveal the diet of B. multiformis inhabiting the tidal flats at the mouths of the Iroha and Katsura rivers [Fig. 1a, b: Stations (Stns) 1–13]. This spe-
cies occurs as an exclusive dominant species on the tidal flats in these two rivers as well as in other rivers in the Nakatsu and Usa districts and the Kunisaki Peninsula area. We considered that this snail is a model species that might demonstrate the watershed and estuarine linkage.

The Iroha River (hereafter, IR) flows in the Nakatsu and Usa districts where the ratio of agricultural area in the catchment area is relatively high (25.8% vs. 17.0% in the Katsura River area), whereas the water catchment area of the Katsura River (hereafter, KR) is located mainly in Kunisaki Peninsula where forested land covers a large proportion of the area (79.1% in the catchment area vs. 67.1% in IR). *Batillaria multiformis* were collected from six stations in IR (Fig. 1a, Stns 1–6) and seven stations in KR (Fig. 1b, Stns 7–13) on April 20 and 21, 2015. These stations were arranged along the river-sea transects in the mediolittoral zone to evaluate the spatial dietary shift in *B. multiformis*. The surface sediments at the stations were composed of sand or muddy sand.

The tidal flats in the study area are covered by a variety of potential food sources including autochthonous (benthic microalgae, various seaweed species, and salt marsh plants) and allochthonous (terrestrial plants, reeds, marine phytoplankton, and seagrasses) primary producers. In order to determine the diet of *B. multiformis*, we collected seven primary producers from the tidal flats monthly, over a 2-month period from February to April in 2015, preceding the animal collection, assuming that the majority of the snail’s tissues are formed during this period.

In order to collect a sufficient amount of benthic microalgae, we looked for surface sediments that had turned yellowish brown as a sign of high growth of benthic microalgae on the tidal flat in IR and KR on four occasions: February 19, March 20, April 19, and April 21 in 2015. We collected approximately 500 mL of surface sediments from the whole area of the tidal flats, and brought the sediments to the laboratory and extracted the microalgae following the procedure described by Couch (1989) as modified by Yokoyama & Ishihi (2003) (total number of samples = 8).

Living seaweeds growing on the pebbles and rocks on the tidal flats or on seawalls mainly around Stns 1–4 (IR) and Stns 7–10 (KR) and the debris of terrestrial plant material composed mostly of broad-leaved trees and dead leaves and stems of the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. were collected from the two tidal flats on three occasions, i.e., February 19, March 20, and April 21 in 2015. A living salt marsh plant, *Zoysia sinica* Hance, 1869 was collected on the same three occasions, whereas such vegetation was not found in IR. This plant covered a small part (∼580 m²) of the high intertidal zone at the KR mouth (Fig. 1b). On the tidal flats, we could not find any debris that originated from this plant during the survey. A small amount of drifted seagrass fragments was occasionally found on the tidal flats. The seagrass *Zostera marina* Linnaeus, 1753 was collected from the IR tidal flat in February and from the KR tidal flat in April, whereas a fragment of *Z. japonica* Ascherson & Graebner, 1907 was collected from the KR tidal flat in April alone (a total...
of four fragments of Zostera species collected from two tidal flats during a total of 6 hours in the sampling period). These materials were stored in a freezer, defrosted, rinsed with distilled water, dried at 60°C, and ground to a fine powder.

We collected seawater including particulate organic matter (POM) in Suo-nada from a depth of 5 m at Stn 14 (approximately 11 m deep, Fig. 1c) that is located 3 km from the mouth of KR on three occasions: February 4, March 2, and April 13 in 2015. This sampling location and layer was determined from the high salinity (>32) and at an intermediate layer of 6 m above the seabed, which suggest minimal influence of freshwater, high growth rate of phytoplankton (mean±SD of chlorophyll a in collected seawater=2.7±0.6 μg L⁻¹, http://www.pref.oita.jp/soshiki/15090/jigyouhoukoku.html: November 9, 2018), a low level of resuspension of sediments, and lack of any estuarine turbidity maximum (ETM). Therefore, we regarded the collected POM as a representative of marine end-members (marine phytoplankton). The collected seawater was passed through a 0.125 mm mesh net to remove large particles and was filtered on a pre-combusted (450°C, 2 hours) Whatman GF/F glass fiber filter, which was then acidified samples.

The stomach and intestine of B. multiformis were extracted from 1–5 individuals collected from each of the Stns 1–13 (excluding Stn 3) and mixed with distilled water. The gut contents were observed, diatom cells were identiﬁed to the genus level, and their number of cells was counted under an optical microscope. We distinguished benthic and pelagic diatoms as described by Round et al. (1990). We did not include formless detrital particles as an analysis target.

Adoption of a mixing model

The Bayesian stable isotope mixing model, SIAR (Stable Isotope Analysis in R: Parnell et al. 2010) was used to estimate the contributions of seven different primary producers to the B. multiformis diet. This model enables calculating the contribution ratios of food sources to the consumer’s diet as credible intervals, even if the number of sources is more than (n+1) relative to the number of isotopes used (n). We used the means and standard deviations (SD) of the seven primary producers and the widely applied fractionation values, 1.0% for 13C (DeNiro & Epstein 1978, Fry & Sherr 1984) and 3.4% for 15N (DeNiro & Epstein 1981, Minagawa & Wada 1984), which were determined by analyzing the non-defatted whole-body samples of consumers. Contributions of dietary sources (proportion, %) were represented as 95%, 75%, and 25% credible intervals (CI) and as the mean and mode values for proportional source contributions.

Gut content analysis

The stomach and intestine of B. multiformis were extracted from 1–5 individuals collected from each of the Stns 1–13 (excluding Stn 3) and mixed with distilled water. The gut contents were observed, diatom cells were identified to the genus level, and their number of cells was counted under an optical microscope. We distinguished benthic and pelagic diatoms as described by Round et al. (1990). We did not include formless detrital particles as an analysis target.

Results

Gut content of B. multiformis

Gut content analysis of B. multiformis collected from the two river mouths showed no traces of terrestrial plants, reeds, seaweeds, seagrasses, salt marsh plants, or animal tissues and indicated that the only identifiable primary producer was diatoms. We confirmed the occurrence of 32 species/species groups, which were classified as 27 species/species groups of benthic diatoms, four species/species groups of pelagic diatoms, and species belonging to the genus Odontella, for which we could not determine the benthic or pelagic type (Table 1). The species compositions in IR and KR were similar; in both river mouths, (1) benthic diatoms accounted for the majority of cells, i.e., 95.6% in IR and 98.0% in KR, whereas the composition of pelagic diatoms was quite small, 4.2% (range=1.0% to 7.0%) in IR and 1.2% (0% to 4.4%) in KR, and there was no gradient in the composition of benthic and pelagic diatoms along the river-sea transects, (2) Navicula spp., unidentified Diatomaceae, and Nitzschia spp., all benthic diatoms, were the dominant species groups, and (3) there was no gradient in the species composition except for the composition of Diatomaceae in IR, which showed lower values at
more seaward stations along the river-sea transects (Fig. 2).

Isotopic compositions of potential food sources

Among the possible food sources, terrestrial plants showed the lowest $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 3a, b: mean $\pm$ SD = $-30.6 \pm 1.1%$ and $2.8 \pm 1.0%$ in IR, $-29.8 \pm 0.7%$ and $0.6\pm 0.6%$ in KR, respectively). The reeds had higher $\delta^{13}C$ and $\delta^{15}N$ values ($-25.8 \pm 2.4%$ and $4.1\pm 1.3%$ in IR, $-25.7 \pm 1.6%$ and $5.5\pm 0.7%$ in KR, respectively) than those of terrestrial plants. The highest levels of $\delta^{13}C$ and $\delta^{15}N$ were found in seagrasses ($-14.4%$ and $6.4%$ in IR, $-11.0\pm 0.6%$ and $5.6\pm 1.3%$ in KR, respectively) and salt marsh plants ($-14.5\pm 0.5%$ and $6.6\pm 3.0%$ in KR alone). The $\delta^{15}N$ of marine phytoplankton ($6.9\pm 0.7%$), seaweeds ($5.7\pm 1.4%$ in IR and $6.8\pm 1.4%$ in KR) and benthic microalgae ($7.1\pm 2.3%$ in IR and $4.5\pm 3.0%$ in KR) showed similar values to the seagrasses and salt marsh plants. Among these three kinds of algae, marine phytoplankton showed the lowest $\delta^{13}C$ ($-22.7\pm 0.7%$), followed by seaweeds ($-19.5\pm 5.2%$ in IR and $-19.3\pm 5.4%$ in KR), whereas benthic microalgae showed the highest value ($-14.6\pm 2.3%$ in IR and $-17.0\pm 0.2%$ in KR).

The mean $\delta^{13}C$ values of seaweeds were determined based on the 26 species collected from IR and 12 species collected from KR, which showed wide ranges of the mean $\delta^{13}C$ of each species i.e., from $-35.1%$ of *Plocamium leptophyllum* Kützing, 1849 (No. 21, an open circle in Fig. 3c) to $-13.4%$ of *Ulva* sp. (No. 3, an open circle) in IR and from $-36.3%$ of *P. leptophyllum* (No. 21, a filled circle) to $-13.9%$ of *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 (No. 23, a filled circle) in KR, resulting in large SD
values.

The $\delta^{15}$N of benthic microalgae showed large temporal variations, as shown by the wide ranges from 5.2 to 10.3% in IR and from 0.7 to 10.9% in KR (Fig. 3c). In April, when samples were collected on two occasions at a two-day interval, the difference in the $\delta^{15}$N between these two occasions reached 3.3% in IR and 6.0% in KR. The $\delta^{13}$C of benthic microalgae in IR also showed a wide fluctuation range (4.3%).

**Isotopic compositions of *Batillaria multiformis* along river-sea transects**

One-way ANOVA showed significant differences in the $\delta^{13}$C of *Batillaria multiformis* among the station groups in IR ($F=10.6$, $p<0.001$) and in KR ($F=9.6$, $p<0.001$). The Tukey–Kramer test shows that (1) the mean $\delta^{13}$C values at the upstream stations in IR (Stns 1–3) ranged from −16.1 to −15.8%, which were significantly different from the
values obtained from the seaward stations (−17.5‰ and −17.4‰ at Stns 5 and 6) (Figs. 3a and 4a); (2) at the more seaward stations, the δ13C in KR also showed a gradual decreasing tendency from −15.2‰ (Stn 7) or −14.8‰ (Stn 8) to −17.1‰ at the seaward station (Stn 13) (Figs. 3b and 4b).

Regarding the δ15N of B. multiformis, there was a significant difference among the stations in IR (one-way ANOVA, F = 4.6, p = 0.01); however, the significant difference was found only between Stn 1 and Stns 3 and 4 (Tukey–Kramer test, Fig. 4c). One-way ANOVA showed no significant difference in the δ15N of B. multiformis among the stations in KR (F = 0.21, p = 0.97). Since a gradient of δ15N along the river-sea direction was not found for the two rivers, values obtained from each river were compiled, and the two-sample t-test was used to evaluate the difference between the two rivers, resulting in a significant difference (p < 0.001) between IR (mean ± SD = 10.0 ± 3.‰) and KR (11.1 ± 0.5‰).

**Contribution of primary producers to the B. multiformis diet**

In accordance with the presumptive 13C and 15N fractionation of consumers (1.0‰ and 3.4‰, respectively), the estimated mean δ13C and δ15N values of the diet for B. multiformis at each station should be −18.5‰ to −16.8‰ and 6.1‰ to 6.8‰ in IR, and −18.1‰ to −15.8‰ and 7.5‰ to 8.0‰ in KR, respectively (Fig. 3a, b). There was a ≥7.2‰ difference in the δ13C between the estimated diet of B. multiformis and the reed and terrestrial plant debris. On the other hand, differences in δ13C between the estimated diet and other primary producers were generally within 7.0‰ in general and the dietary δ13C values were between the relatively low value of marine phytoplankton (mean = −22.7‰) or seaweeds (−19.5‰ in IR, −19.3‰ in KR) and the high values of primary producers such as benthic microalgae (−14.6‰ in IR, −17.0‰ in KR), a salt marsh plant (−14.5‰ in KR) and seagrasses (−14.4‰ in IR, −11.0‰ in KR). At more seaward stations, the dietary δ13C shifted to values nearer to marine phytoplankton in both the localities.

The SIAR mixing model showed the contribution (proportion, %) of the six producers in IR and the seven producers in KR to the diet of B. multiformis (Figs. 5, 6). The contribution of terrestrial plants was very low in both IR and KR, as shown by the low mean (4–7% in IR, 5–7% in KR) and mode (1% in IR and KR) values, although the 95% credible interval (95% CI) showed a relatively large variation (0–17% in IR, 0–18% in KR) (Figs. 5a, 6a). The reed also showed a minor contribution (95% CI = 0–23% in IR, 0–25% in KR; mean = 6–10% in IR, 7–12% in KR; mode = 1–2% in IR, 1–4% in KR) (Figs. 5b, 6b). The contribution of marine phytoplankton was relatively low at upstream stations in IR (Stns 1–3: 95% CI = 0–34%, mean = 14–16%, mode = 6–14%) and in KR (Stns 7–9: 95% CI = 0–26%, mean = 10–12%, mode = 2–5%), whereas at more seaward stations, their contribution increased (Stns 5–6 in IR: 95% CI = 1–42%, mean = 21–23%, mode = 23–26%; Stns 13 in KR: 95% CI = 0–29%, mean = 15%, mode = 18%) (Figs. 5c, 6c). The 95% CI, mean, and mode for the contribution of seaweeds in IR and KR were within the range of 0–33% and 0–28%, 11–15% and 10–14%, and 3–8% and 2–14%, respectively (Figs. 5d, 6d). In IR, there was no clear trend in the spatial pattern of seaweed contribution, whereas at the seaward station in KR (Stn 13), the mean and mode (both 14%) showed higher values than those at other stations. The highest values of the contribution were found in seagrasses (IR: 95% CI = 5–65%, mean = 29–35%, mode = 27–32%); KR: 95% CI = 2–50%, mean = 19–28%, mode = 20–28%) (Figs. 5f, 6f). The second highest values were found in benthic microalgae (IR: 95% CI = 0–46%, mean = 18–26%, mode = 19–26%; KR: 95% CI = 0–37%, mean = 15–17%, mode = 8–18%) (Figs. 5e, 6e). In KR, the salt marsh plant showed high contributions (IR: 95% CI = 0–46%, mean = 18–24%, mode = 20–24%) (Fig. 6g). There was no clear trend in the spatial pattern of the contribution by these three sources (benthic microalgae, seagrasses, and salt marsh plant).

**Discussion**

Large differences in the δ13C between B. multiformis and terrestrial plants or reeds and the SIAR mixing model showed minimal contributions of terrestrial plants and reeds to the diet of this snail. Vascular plants including detrital terrestrial plants and reeds have previously been considered to have little nutritional importance for intertidal and/or estuarine animals due to the presence of a large amount of refractory substances such as cellulose and lignin (e.g., Fontugne & Jouanneau 1987, Cividanes et al. 2002). The model also showed that the mean and mode values of seagrasses and salt marsh plants were very high. However, the credible intervals (CI) found in these produc-
Fig. 5. Proportional contribution of six primary producers to the diet of *Batillaria multiformis* at six stations (1–6) in the Iroha River. The SIAR mixing model was used for the calculation. Dark gray in the center, light gray in the middle, and white areas in the outer area indicate 25%, 75%, and 95% credible intervals, respectively. Means and modes of contributions are indicated by lines and filled circles in the boxes and numerals in roman (mean) and italics (mode) beside the boxes, respectively.

Fig. 6. Proportional contribution of seven primary producers to the diet of *Batillaria multiformis* at the seven stations (7–13) in the Katsura River. The SIAR mixing model was used for the calculation. Dark gray in the center, light gray in the middle, and white areas in the outer area indicate 25%, 75%, and 95% credible intervals, respectively. Means and modes of contributions are indicated by lines and filled circles in the boxes and numerals in roman (mean) and italics (mode) beside the boxes, respectively.
ers showed large variabilities (2–65% and 0–46%, respectively), indicating the possibility of scarce contribution. In addition to this, the biomasses of these producers were small, and the biochemical composition of these plants is similar to that of terrestrial vascular plants. It is thus difficult to conclude that seagrasses and salt marsh plant made a notable contribution to the diet.

The SIAR mixing model (Figs. 5, 6) and observations on the biomass of living and fragmentary primary producers on the tidal flats showed that (1) the major food sources were marine benthic microalgae, phytoplankton, and seaweeds, and (2) the dependency on marine phytoplankton increased at the seaward stations. On the other hand, gut content analysis revealed that the only identifiable dietary items were diatoms and that most of them were benthic diatoms across all stations (Fig. 2). Visual observations in the daytime on the KR tidal flat showed that *B. multiformis* left gray trails on the sediment surface that looked yellowish brown (Fig. 7). Kikuchi (1993) described that intertidal benthic diatoms move to the sediment surface or near-surface during the daytime, resulting in yellowing of the sediment surface. These observations suggest that the snails grazed on the yellowish sediments including benthic microalgae while crawling on the sediment surface. Thus, it is a non-controversial fact that microalgae on tidal flats are an important food source for *B. multiformis*. However, it should be noted that the gut content analysis is biased towards recent dietary items that do not digest readily (Polito et al. 2011). Accordingly, the role of phytoplankton and seaweeds as a diet for this species needs further discussion.

*Batillaria* species are reported to be deposit feeders and grazers of benthic diatoms (Morton and Morton 1983, Koike et al. 1989, Ozawa 1996). However, Kamimura and Tsuchiya (2004) observed ctenidial filter feeding behavior of *Batillaria zonalis* occurring in subtropical tidal flat habitats. *Batillaria zonalis* in seawater sucks suspended particles through the siphonal canal, forms a mucus cord to entangle particles, and then ingests it. Itoh et al. (2018) found a similar feeding behavior in *B. multiformis*. These findings indicate that *B. multiformis* has an ability to consume living phytoplankton in the eutrophic water column at high tide. It is also possible that snails consume the detrital phytoplankton accumulated in sediments as well as living phytoplankton by their filter-feeding behavior. The proportion of phytoplankton-derived organic matter in estuarine sediments has been found to increase at more seaward areas (e.g., Yokoyama et al. 2006). The gradient in the concentration of detrital phytoplankton may also be related to the observed spatial shift in the snail’s δ¹³C. Regarding the utilization of seaweeds as the diet of *B. multiformis*, we did not observe any behavior indicating that the snails grazed on living or fragmentary seaweeds during the survey; however, the large biomasses of seaweeds in IR and KR and the SIAR analysis indicate the possibility of consumption of detritus originating from seaweeds.

Fig. 7. Photograph showing trails of *Batillaria multiformis* on the sediment surface at the mouth of the Katsura River, taken at Stn 11 on April 21, 2015. Note the lower left of the photograph where the change in color from yellowish brown to gray after scraping the surface sediments with a spatula is shown.

It has been reported that isotopic compositions of consumers shift along the estuarine gradient, accompanied by shifts in isotopic values of food sources (Chanton & Lewis 1999, Fry 1999) or in the relative contribution of different sources (Kikuchi & Wada 1996, Kasai & Nakata 2005, Dias et al., 2014). These studies show that the estuarine gradients extend over several kilometers or several tens of kilometers. In contrast, the present study showed a shift of the δ¹³C in the snail tissues over relatively short distances (<400 m). These findings suggest that the relative contribution of benthic microalgae and marine phytoplankton to the diet of snails shifts even at such short distances and that the behavior range of snails is relatively small throughout their whole benthic life.

Estimated δ¹⁵N values of the snail’s diet (6.1–6.8‰) in IR calculated from the currently accepted ¹⁵N fractionation (3.4‰) are close to the mean values of marine phytoplankton (6.9‰) and benthic microalgae (7.1‰), suggesting the direct consumption of these primary producers by the snail. On the other hand, estimated δ¹⁵N values of the snail’s dietary items (7.5–8.0‰) in KR are higher than the values of the three producers (4.5–6.9‰). One possible explanation for the difference in the estimated δ¹⁵N values may be that the snails consume these producers through intermediate animals such as foraminifers, copepods, and the eggs of turbellarians, which has been previously demonstrated in the intertidal snail *Cerithium atratum* (Marcus and Marcus 1964). It appears likely that these animals were not recognized during the gut content analysis because the animal tissues had been broken by the radulae of the snails and/or the tissues had been digested. Another explanation is that the δ¹⁵N values of detrital organic matter may increase in comparison with those of the original primary producers under deoxygenated and enriched environments (e.g. Koba et al. 1997).
In recent years, the $\delta^{13}$C and $\delta^{15}$N values have been commonly used in determining the food sources of consumers; however, when applying the analysis and mixing models, attention should be paid to the positive contributions of possible food sources to the consumers’ diets. The spatial and temporal variability in isotopic compositions of possible food sources needs to be considered. In the present survey, we found a large variability in the isotopic composition among seaweed species and also among the sampling occasions of benthic microalgae. Averages of such values do not always represent the true isotopic composition of each organic component in the diet. For example, if detritus originating from seaweeds contributes significantly to the animals’ diet, the quality and quantity of such organic matter will be determined by many factors such as the biomass, productivity, degradability, and origin (allochthonous or autochthonous) of each seaweed species. Thus, the detrital seaweed values cannot be determined from a simple arithmetic mean of the seaweeds collected from the survey. Frequent samplings to determine the representative values for possible food sources are also required. We used the currently accepted fractionation, because we have no information about the fractionation regarding B. multiformis. Laboratory-determined species-specific and diet-specific fractionations are necessary to more comprehensively interpret the stable isotope data from this study area.

In conclusion, stable isotope analysis of the intertidal snail, Batillaria multiformis and its possible food sources showed that the snail’s diet was a mixture of marine phytoplankton, seaweeds, and benthic microalgae, and that the dependency on marine phytoplankton increased at the seaward stations. This tendency may be explained by the filter feeding on phytoplankton at high tide. However, the gut content analysis revealed the predominant occurrence of benthic diatoms throughout all stations, probably due to the short-term result of ingestion on the sediment surface at low tide. The snails’ $\delta^{15}$N showed no significant gradient along the river-sea transects but a significant difference between the two rivers, suggesting the possibility of the snail’s $\delta^{15}$N as an indicator of watershed characteristics.

Acknowledgements

We are grateful to the two anonymous reviewers and the editor of Plankton and Benthos Research, Dr. Gen Kanaya for valuable comments and suggestions. The stable isotope analysis was conducted using the Cooperative Research Facilities (Isotope Ratio Mass Spectrometer) of the Center for Ecological Research, Kyoto University. This study was partly supported by the Link Again Program of the Nippon Foundation-Kyoto University Joint Project.

References


Furota T, Sunobe T, Arita S (2002) Contrasting population status between the planktonic and direct developing batillariid snails Batillaria multiformis (Lischke) and B. cumingi (Crosse) on an...


