Food web and transports of methane-derived carbon in eutrophic shallow lake using carbon and nitrogen stable isotope analyses

In this study, I used carbon and nitrogen stable isotope analyses to elucidate food web structure of eutrophic shallow lake, Izunuma, and transports of methane-derived carbon to whole-lake food web via benthic invertebrates.

1. I measured carbon and nitrogen stable isotope ratios of 3 sympatric species of larval chironomids (Chapter 2). Markedly lower $\delta^{13}C$ values were reported in *Chironomus plumosus* (–51.2‰) and *Tanypus* sp. (–43.5‰) than those of photoautotrophic carbon sources (particulate organic matter [POM] and sediment). There were positive correlations between $\delta^{13}C$ and $\delta^{15}N$ in the 2 chironomid species. These results indicated that they assimilated carbon derived from biogenic methane by exploiting methane-oxidizing bacteria (MOB). In contrast, *Propsilocerus akamusi* exhibited similar $\delta^{13}C$ values to those of POM or sediment, indicating that they assimilated carbon derived from photoautotrophs. A $\delta^{13}C$-based isotope mixing model was used to estimate the dietary contributions of MOB to each chironomid species. The mean contributions ranged from 11 to 15% in *C. plumosus*, 13 to 19% in *Tanypus* sp., but only up to 5% in *P. akamusi*. In an aquarium, I observed that *C. plumosus* and *Tanypus* sp., which exhibited low $\delta^{13}C$ values, built U-shaped tubes in the sediment with an oxidized layer developed around their tubes, whereas *P. akamusi* did not exhibit this behavior. These results suggest that tube building may provide larval chironomids with greater access to methane-derived carbon through increased opportunities to feed on MOB.

2. I investigated the seasonal patterns of $\delta^{13}C$ of *C. plumosus* and methane concentrations in the sediment in Lake Izunuma (Chapter 3). Larval $\delta^{13}C$ was depleted in late summer and autumn. Methane concentrations above a 6 cm depth peaked in late summer or autumn, while those in the 10–11 cm and 20–21 cm layers peaked in October. Negative correlations between methane concentrations in the 5–6 cm/10–11 cm layers and larval $\delta^{13}C$ were found. This suggests that an increase in the supply of methane stimulated the activity of MOB, thus increasing the dietary contribution of MOB for larval chironomids.

3. Freshwater mussels have often been used as indicators of a trophic baseline (i.e., primary

consumers) in the food web. To assess the utility of a large filter-feeding mussel, *Cristaria plicata*, as an isotopic indicator, I compared $\delta^{13}C$ and $\delta^{15}N$ of the mussel with those of its potential food sources, POM and sediment organic matter [SOM] (Chapter 4). The $\delta^{13}C$ values of large mussels (shell length > 140 mm, –31.0‰ to –29.2‰) were similar to those of POM (–30.2‰) indicating that mussel carbon was derived from POM (mainly phytoplankton). In contrast, the mussels exhibited 6.3‰ to 9.0‰ higher $\delta^{15}N$ values than did POM. The trophic level estimate of the mussel ranged from 2.9 to 3.6, indicating that they functioned as secondary rather than primary consumers. These results also revealed positive correlations between shell lengths and $\delta^{15}N$ values, suggesting that the mussels changed their trophic position from primary consumer (shell length < 140 mm) to secondary consumer (shell length >140 mm) with growth.

4. I measured $\delta^{13}C$ and $\delta^{15}N$ of zooplanktivorous *Hypomesus nipponensis*, and five dominant species of small-sized omnivorous fishes. Stable isotope analyses confirmed that zooplanktivorous *H. nipponensis* fed on zooplankton. For the omnivorous fishes, I estimated dietary contributions of 3 potential food sources, zooplankton, benthic chironomids and epiphytic algae to their biomass using isotope mixing model. The results showed 4 omnivorous fishes, *G. elongates elongates*, *P. parva*, *B. zezera*, and *T. obscures* relied largely on epiphytic algae (30-56%), zooplankton (24-68%), and rarely on benthic invertebrates (0-20%), though *A. rhombeus* depended mostly on epiphytic algae (82%). Furthermore, there was negative relationship between $\delta^{13}C$ and $\delta^{15}N$ in small size fishes, including five omnivorous fishes and one zooplanktivorous *H. nipponensis*, indicating that the small fishes exploited on two isotopic distinct food sources; lower $\delta^{13}C$ and higher $\delta^{15}N$ sources (i.e. zooplankton) and higher $\delta^{13}C$ and lower $\delta^{15}N$ sources (i.e. epiphytic algae). Thus in Lake Izunuma, the omnivorous fishes incorporated littoral and pelagic production into the food web, whereas they were rarely supported by benthic production.

5. In General discussion, I discussed the food web structure of Lake Izunuma as a small shallow eutrophic lake, and the methane-derived carbon transport via benthic invertebrates.