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A stable isotopic approach to investigate nitrogen pathways in a coastal aquaculture area

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Abstract: Coastal eutrophication and consequent environmental deterioration have been problematic in many parts of the world. However, oligotrophication and resulting reduction in coastal fisheries and aquaculture productivity have also been a recognized problem in some parts of Japan. Some studies argue that declining fisheries production of coastal resources and unfed aquaculture production is partially attributable to the reduced nutrient concentrations in the coastal waters in recent years. Eutrophication mitigation efforts have reduced the terrestrial nutrient load to the coastal environment over the past forty years in Japan. Excessive oligotrophication has allegedly reduced primary productivity and hence carrying capacity of coastal ecosystems supporting coastal fisheries and aquaculture. Fisheries production of the Manila clam, *Ruditapes philippinarum*, has decreased by 95% over the past three decades. Insufficient food supply is one amongst many speculated factors causing the reduction. However, complex nutrient flow in coastal environments has not necessarily been elucidated. Our stable isotopic (δ^{13}C and δ^{15}N) studies have suggested that there are large and small scale variations in the nutrient pathways from inorganic nutrients to the clam. Clams inhabiting the same tidal flat only 10 m apart from each other can have a different food environment as indicated by δ^{13}C. Pervasive effects of terrestrial nutrient load on the food availability of the clam may be ascertained by δ^{15}N. Stable isotopic methods may also be used to determine nutrient flow within an integrated multi-trophic aquaculture (IMTA) system, which has potential to enhance unfed aquaculture production in an oligotrophic environment.

Key words: eutrophication, oligotrophication, stable isotope ratio, nitrogen, carbon, IMTA

Introduction

Coastal eutrophication due to increasing anthropogenic activities and consequent environmental deterioration, such as anoxia, harmful algal blooms and hydrogen sulfide emission have been problematic in many parts of the world, sporadically causing mass mortality of aquatic organisms. While this holds true for Japan, oligotrophication and resulting reduction in coastal fisheries and unfed-aquaculture productivity have also been a recognized problem in some parts of Japan. Some studies argue that ever-dwindling fisheries production of coastal resources, as well as unfed aquaculture production of inorganic and organic extractive species is partially attributable to the reduced nutrient levels in the coastal waters in recent years (Tanda and Harada, 2012; Yamamoto, 2003).

Eutrophication mitigation efforts have reduced the load of nitrogen and phosphorus of terrestrial and aquaculture origins to the coastal environment over the past forty years in Japan. Allegedly excessive oligotrophication has reduced primary productivity and hence carrying capacity or coastal ecosystems supporting coastal fisheries and aquaculture. However, complex nutrient flows within coastal ecosystems are not necessarily well understood.

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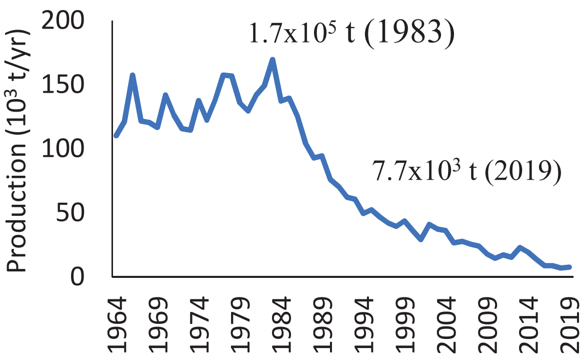

Fig. 1. Manila clam fishery production in Japan.

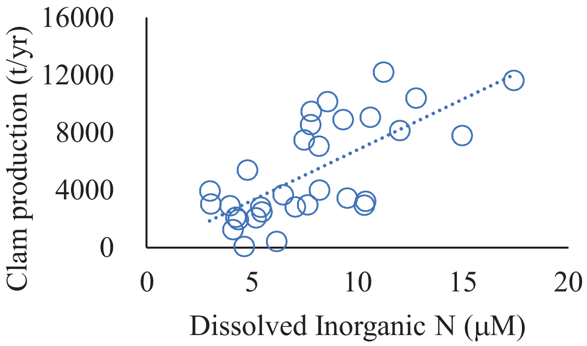

Fig. 2. Relationship between the annual mean dissolved inorganic nitrogen level and Manila clam fisheries production in Ise, Bay, Mie Prefecture from 1985 to 2015.

This paper describes a stable isotopic approach to investigate nutrient pathways in coastal aquaculture areas, with Manila clam, *Ruditapes philippinarum*, and Integrated Multi-Trophic Aquaculture (IMTA) as examples.

Manila Clam Fisheries Production in Japan

Manila clam is one of the iconic coastal species facing serious stock depletion in Japan. National fisheries production, inclusive of bottom culture, of the Manila clam has decreased by 95% over the past three decades (**Fig. 1**) (e-Stat). The production reached the peak at 169,621 t in 1983, and it gradually and continuously decreased to 7,736 t in 2018. Japan used to be the largest producer of the clam in the world, but it imported 42,482 t of the clam, amounting to JPY 8.6 billion from countries such as China and South Korea in 2017 (Ministry of Finance, Trade statistics of Japan).

Although the mechanism of the clam production decline is not necessarily elucidated, insufficient food supply associated with recent coastal oligotrophication is one amongst many speculated factors causing the reduction. The relationship between the annual mean dissolved inorganic nitrogen (DIN) level (Mie Prefecture) and the Manila clam fisheries production in Ise Bay from 1985 to 2015 (e-Stat) showed a significant negative correlation (**Fig. 2**) (r^2=0.49, $p < 0.001$, N=30). Like many other bivalves, the Manila clam is thought to filter-feed microalgae. Reduced nutrient level in coastal waters is thought to diminish primary productivity, thereby reducing the carrying capacity of the area. However, this hypothesis has not been supported yet because of the complexity of nutrient cycles in coastal areas.

Small Scale Variation in Food Items of the Clam

Food availability to the clam is often represented by chlorophyll *a* levels in a water column. In many studies, however, researchers take water samples from water columns far above the bottom water that the clams inhale and filter-feed. Collection of bottom water within a few centimeters from the bottom is laborious, and water parameters measured for other general purposes are often used to ascertain the food availability. Also, information on the availability and nutritional quality of different microalgal species is limited. These issues make accurate determination of food availability and nutrient flow within the food chain difficult.

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are widely used in the studies of interactions of food webs in aquatic ecosystems. Our stable isotopic study (Watanabe *et al.*, 2009a) has suggested that the food source of the clam may differ within a small area depending on the bottom topography of the habitat.

Many filter feeding bivalves are generally held to be phytoplanktivorous; however, they also feed on benthic microalgae. Provisional ratio of the planktonic (*i.e.* pelagic) to benthic microalgae seems to be affected by the extent of resuspension of the latter from the sediment to the bottom water to make them available to the clam. In our study

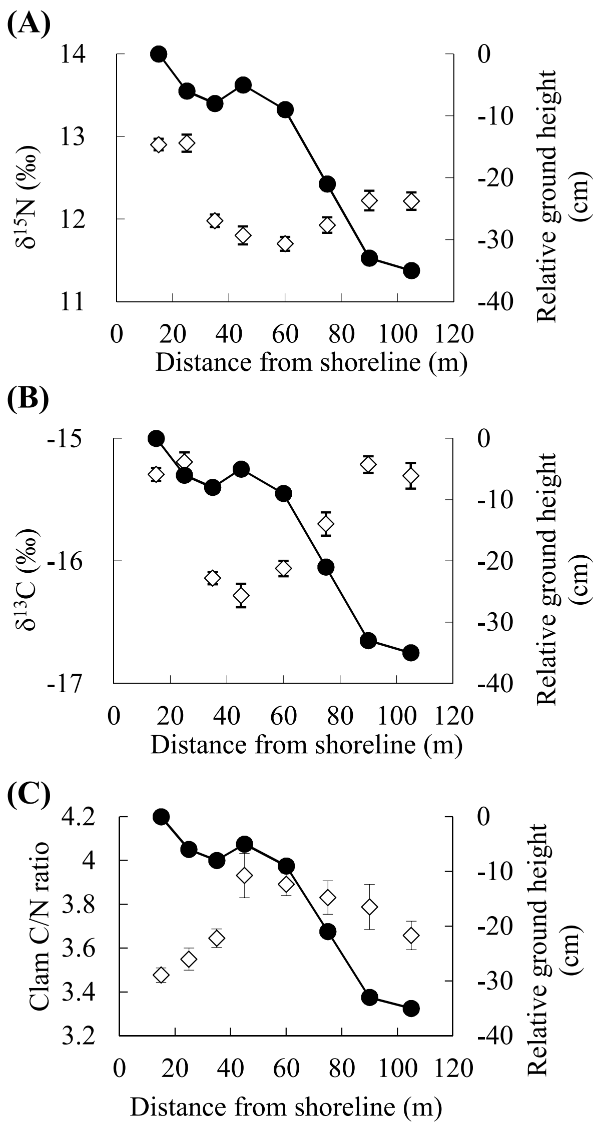

Fig. 3. $\delta^{15}N$ (A), $\delta^{13}C$ (B) and C/N ratio (C) of whole soft tissue of Manila clam at eight stations on an inshore – offshore transect in tidal flat in Yokohama Marine Park (after Watanabe *et al.*, 2009a).

of $\delta^{13}C$ and $\delta^{15}N$ of the clams collected along a inshore-offshore transect in an artificial tidal flat in Yokohama Marine Park, clams inhabiting the same tidal flat only 10 m apart from each other had a different $\delta^{13}C$ and $\delta^{15}N$ signature, as well as carbon to nitrogen ratio (C/N) depending on the position on the sandwave (**Fig. 3**). This indicates that the clam assimilates different food items depending on the position on the sandwave. In general, benthic microalgae tend to have higher $\delta^{13}C$ than do phytoplankton, and higher C/N indicates higher glycogen (*i.e.* energy reserve for bivalves) content of the clam. Resuspension of benthic microalgae may be more active near a dune presumably because of higher wave action, increasing the availability of benthic microalgae to the clam. The clams utilizing both planktonic and benthic microalgae near the dune had higher $\delta^{13}C$, and they seemed to have better nutritional condition as indicated by higher C/N.

Benthic microalgae are important food source for the Manila clam, and chlorophyll *a* concentration in the bottom water should be analyzed to determine food availability to the clam.

Large Scale Variation in Nutrient Pathway to the Clam

Anthropogenic nitrogen is known to have a higher nitrogen stable isotopic ($\delta^{15}N$) signature than nitrogen from atmospheric deposition (2‰ – 8‰) and nitrogen fixed by cyanobacteria (−2‰ – 0‰) (McClelland *et al.*, 1997; Oowada *et al.*, 2003). Treated water of sewage, for example, contains DIN with higher $\delta^{15}N$ elevated by denitrification during the treatments (Macko and Ostrom, 1994). Agricultural fertilizer also enhances soil denitrification and increases $\delta^{15}N$ in groundwater (Ogawa *et al.*, 2001). Thus, $\delta^{15}N$ in DIN acts as an indicator of the level of anthropogenic nitrogen loads to coastal waters.

The $\delta^{15}N$ in the soft tissues of the Manila clam was found to be positively correlated to the total DIN (*i.e.* sum of nitrate, nitrite and ammonium nitrogen) concentration in the bottom water in tidal flats in Kanagawa, Shizuoka and Fukuoka Prefectures with different eutrophication levels (Watanabe *et al.*, 2009b). This indicates that the clam $\delta^{15}N$ can be a proxy for the anthropogenic nitrogen load to the coastal environment.

The concentration and $\delta^{15}N$ of the total DIN were positively correlated with each other in the studied areas (**Fig. 4**), indicating that input of terrestrial nitrogen with higher $\delta^{15}N$ elevates the DIN level in coastal waters. The $\delta^{15}N$ of the clam soft tissue, particulate organic matter (POM) in seawater and sediment organic matter (SOM) in tidal flat surface were higher in areas with higher total DIN concentration of bottom water (**Fig. 5**). The higher $\delta^{15}N$ in the clam was considered to be attributable to higher $\delta^{15}N$ in the food particles (POM and SOM),

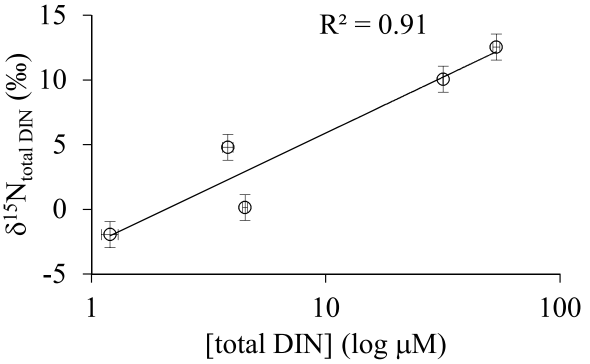

Fig. 4. Relationship between the mean concentration and mean δ^{15}N in total DIN in bottom water collected from different tidal flats in Japan (after Watanabe *et al.*, 2009b).

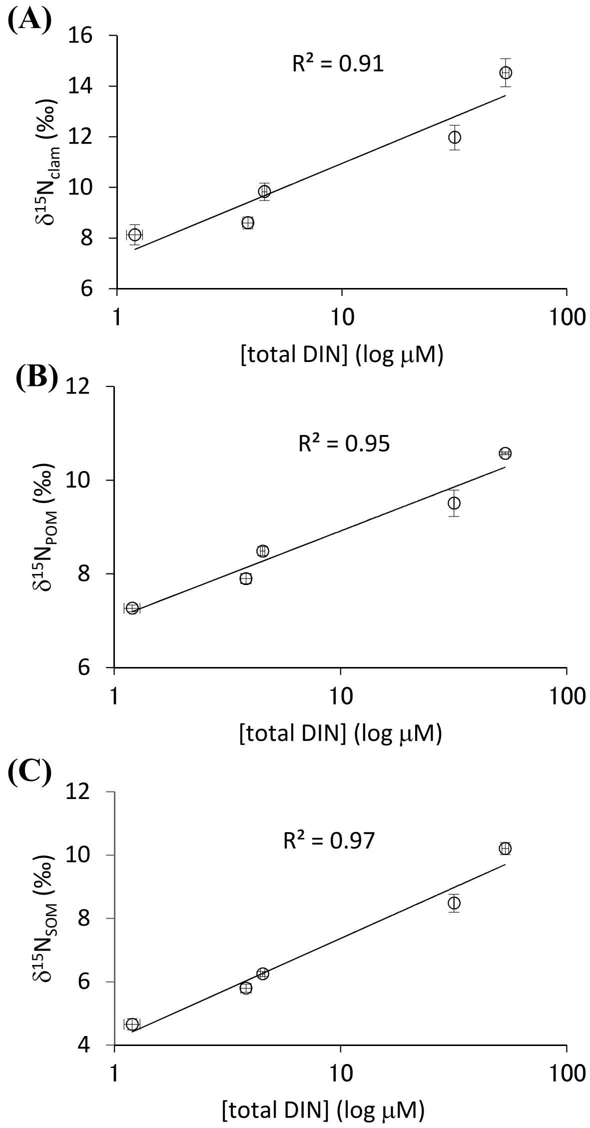

Fig. 5. Relationship between the mean concentration (±SE) of the total DIN and δ^{15}N in Manila clam (A), POM in the bottom water (B) and SOM in the tidal flat surface (C) (after Watanabe *et al.*, 2009b).

especially those in SOM.

This study demonstrates that elevated δ^{15}N in DIN in coastal waters due to anthropogenic nitrogen loads is reflected in the δ^{15}N in the clam. Thus, pervasive effects of terrestrial nutrient load to the food availability to the clam may be ascertained by the clam δ^{15}N. This can be a powerful tool to understand the effects of eutrophication and oligotrophication of coastal waters to the clam fisheries productivity.

Nutrient Flow within Integrated Multi-Trophic Aquaculture

Integrated multi-trophic aquaculture (IMTA) is a technique to use unfed aquaculture of inorganic and organic extractive species (*e.g.* algae and macrobenthos) to consume effluent from fed aquaculture of finfish (Chopin, 2006). Although IMTA is usually proposed as a mitigation measure for eutrophication by intensive fed aquaculture, it has a potential to alternatively enhance unfed aquaculture production in an oligotrophic environment. In either case, nutrient tracing is important to design an efficient IMTA system.

In order to trace nutrient flow within an IMTA system using stable isotopic method, not only is it necessary to determine isotopic fractionation between organic extractive species (*e.g.* sea cucumber, Watanabe *et al.*, 2013) and food, but to have information on how stable isotopic signatures differ between the aquaculture feed and fish feces, both of which can be a nutrient source for the organic extractive species.

We compared the δ^{13}C and δ^{15}N of feces collected from five different finfish species (Asian seabass, mangrove red snapper, milkfish, snubnose pompano and orange-spotted spinefoot) commonly produced in aquaculture in Southeast Asia (Watanabe unpubl. data). We fed the same formulated feed to these fish to determine difference in δ^{15}N, δ^{13}C, and contents of nitrogen and carbon between the feed and feces. We found that while the δ^{15}N of feces showed no significant difference from that of the feed among all the species, δ^{13}C and C/N ratio were different among the species. Therefore, δ^{15}N can be used to determine the contribution of the nitrogen from fed aquaculture to growth of organic extractive species, but it cannot determine whether the nitrogen was used by the organic extractive species as leftover feed or fish feces.

In order to using δ^{13}C to trace carbon flow within an IMTA system, the relationship of δ^{13}C between feed and fish feces must be determined beforehand for each fish species and feed. The difference we observed in δ^{13}C and C/N ratio may be attributable to different digestibility of each fish species. Formulated feed usually contains plant ingredients such as wheat flour and rice bran as a binder. Herbivorous species (orange-spotted spinefoot) may be able to digest and assimilate more plant ingredients (*i.e.* rich in carbon) than do carnivorous species (Asian seabass).

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Annotated Bibliography of Key Works

(1) Watanabe S., Kodama M., Sumbing J. G., and Lebata-Ramos M. J. H., 2013: Diet-tissue stable isotopic fractionation of tropical sea cucumber, *Holothuria scabra*. *JARQ*, **47**, 127–134.

In order to provide a basis for stable carbon and nitrogen isotope ratio (δ^{13}C / δ^{15}N) analysis that will allow determination of assimilated organic matter in the sea cucumber, *Holothuria scabra*, diet-tissue fractionations were experimentally determined by mono-feeding rearing with diatoms. While δ^{15}N fractionation of the whole body wall (2.4‰) was similar to the commonly accepted value (2.6–4‰),

$\delta^{13}C$ fractionation of the body wall (4.2‰) showed considerable discrepancy with the commonly accepted value (0–1‰) due to the high content (35% dry wt/wt) of calcareous spicules ($CaCO_3$) in the body wall, which had significantly higher $\delta^{13}C$ (−8.6‰) than the organic fractions. Computational elimination of spicules based upon spicule content and spicule $\delta^{13}C$ reduced the $\delta^{13}C$ fractionation of the body wall to 1.5‰, close to the common value. $\delta^{13}C$ fractionation after spicule removal by acid decarbonation and subsequent rinsing (3.2‰) did not agree with the common value, and $\delta^{15}N$ fractionation was significantly elevated by decarbonation. $\delta^{15}N$ and $\delta^{13}C$ fractionations of the intestine (1.5 and 2.2‰, respectively) did not agree with the common values. Since $\delta^{13}C$ and $\delta^{15}N$ of the feces did not differ significantly from those of the diet, feces may be used to determine ingested organic matter in the wild.

(2) Watanabe S., Kodama M., Zarate J. M., Lebata-Ramos M. J. H., and Nievales M. F. J., 2012: Ability of sandfish (*Holothuria scabra*) to utilise organic matter in black tiger shrimp ponds. *ACIAR Proceedings*, **136**, 113–120.

Due to frequent viral disease outbreaks, a large proportion of shrimp aquaculture in South-East Asian countries has switched from black tiger shrimp (*Penaeus monodon*) to *P. vannamei*, an exotic species originally imported from Latin America. One of the causes of disease outbreaks is thought to be poor water and sediment conditions in the shrimp ponds, which may aggravate disease symptoms. To obtain basic information for co-culture methods of black tiger shrimp and sandfish (*Holothuria scabra*) for possible mitigation of shrimp-pond eutrophication and prevention of disease outbreaks, basic laboratory experiments were conducted. A feeding trial of juvenile sandfish showed that they do not grow well with fresh shrimp feed on hard substrate. Another trial indicated that sand substrate enhances the growth of juvenile sandfish fed with shrimp feed. A feeding trial using shrimp tank detritus, shrimp feces and *Navicula ramosissima* (a benthic diatom) as food sources showed that sandfish grew fastest with the feces, followed by detritus and *N. ramosissima*. Dissolved oxygen consumption and acid-volatile sulfur levels in the shrimp tank detritus were reduced by sandfish feeding. This suggests that sandfish are capable of growing with organic matter in shrimp ponds and can bioremediate shrimp-pond sediment.