

Seasonal dynamics of a coastal food web: Stable isotope analysis of a higher consumer

Hideki Hamaoka^{1,*}, Noboru Okuda², Toru Fukumoto³,
Hitoshi Miyasaka¹, and Koji Omori¹

Abstract

We examined the structure and dynamics of the demersal food web in the Uwa Sea, focusing on seasonal changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the glowbelly fish *Acropoma japonicum*, a higher consumer dominating the demersal community. Contrary to the conventional “pelagic-benthic coupling” theory, the demersal food web was subsidized by benthic production, although it was under an aphotic environment where benthic microalgae could not grow. For *A. japonicum*, which fed on a variety of prey organisms ranging from small zooplankton to benthic gobies and shrimps, its reliance on pelagic production varied seasonally and corresponded to its feeding habits; it shifted its diet from benthic to pelagic prey in the spring, subsequently depleting its $\delta^{13}\text{C}$. Although this fish became enriched with $\delta^{15}\text{N}$ with ontogenetic growth, large adults showed substantial $\delta^{15}\text{N}$ depletion early in the summer, suggesting seasonal lowering of their trophic level. One possible reason for this $\delta^{15}\text{N}$ depletion is overfishing of the larval anchovy *Engraulis japonicus*, which was previously a valuable food resource for the large adults, resulting in low availability of prey with a high trophic level. Our approach to examining temporal changes in a higher consumer’s stable isotopic signature was demonstrated to efficiently monitor food web dynamics and will be useful for ecosystem management.

Key Words: *Acropoma japonicum*, overfishing, pelagic-benthic coupling, trophic level, trophodynamics

¹ Center for Marine Environmental Studies, Ehime University ² Center for Ecological Research, Kyoto University ³ Saga Prefectural Genkai Fisheries Research and Development Center

*Corresponding author: E-mail: jako-ten@mail.goo.ne.jp

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INTRODUCTION

It is a matter of recent concern that anthropogenic disturbances have resulted in deterioration of aquatic ecosystems all over the world. Many aquatic ecologists have pointed out that a variety of human activities, such as nutrient loading, overfishing, and introduction of exotic species, have caused eutrophication and biodiversity loss, leading to simplification and destabilization of aquatic ecosystems and a resultant reduction in ecosystem services (McClelland and Valiela 1998; Jackson 2001; Jackson et al. 2001; Tewfik et al. 2005). To depict such ecosystem alterations, we must be able to monitor the dynamics of energy flow within a biological community through trophic relationships. One conventional way of identifying energy pathways among organisms embedded in the food web is direct observation of their stomach contents. Dietary analysis provides intact information on the feeding habits of focal species; however, it reflects only transient diets (Vander Zanden et al. 1997). Moreover, as there are great individual variations in diet, substantial effort must be expended to quantitatively evaluate feeding habits at the population or species level.

As an alternative method, aquatic ecologists have recognized that stable isotope analysis is a useful tool for quantification of energy or material flow within food webs (Peterson and Fry 1987; Fry 1988; Gu et al. 1996; Vander Zanden and Vadeboncoeur 2002). General application of this technique relies on the empirical rule that carbon and nitrogen stable isotope ratios are enriched by a given fractionation factor when organic matter is trophically transferred from prey to a consumer (Gannes et al. 1997). The carbon isotope ratio ($\delta^{13}\text{C}$) changes by only 0.8‰ as a result of a trophic interaction and thus, it can be used to identify basal carbon sources for consumers and trophic transfer pathways leading to them within the food web (DeNiro and Epstein 1978; Fry and Sherr 1984). On the other hand, the nitrogen isotope ratio ($\delta^{15}\text{N}$) of consumers is enriched by an average of 3.4‰ relative to their diets (Minagawa and Wada 1984; Post 2002). Thus, $\delta^{15}\text{N}$ is a good indicator of the trophic level of a focal consumer. Based on these principles of isotopic trophic enrichment, we can estimate the trophic positions of consumers embedded in the food web by plotting their isotopic signatures and those of their prey on a $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map. Stable isotope analysis, which is based on metabolic physiology, can quantitatively estimate trophically assimilated materials, but is not suitable for identifying specific trophic links between prey and consumer species embedded in the food web, unless detailed dietary information is available. The effectiveness of stable isotope analysis is thus improved when it is used in combination with conventional dietary analysis (Vander Zanden et al. 1997).

Although it is without doubt that food web research has progressed with the adoption of stable isotope analysis, obstacles have been encountered in accurate characterization of the configuration of complex and varying food webs that are composed of innumerable trophic links. One of the obstacles in aquatic food web studies is spatio-temporal heterogeneity in the quality and quantity of primary production. Aquatic ecosystems are supported by a variety of autochthonous primary producers, such as phytoplankton, benthic microalgae,

benthic macroalgae, and macrophytes, and by allochthonous organic input from terrestrial ecosystems, and they are also characterized by spatio-temporal variations in the extent to which each consumer relies on these carbon sources. For example, it is well known that $\delta^{13}\text{C}$ differs among consumers in littoral and pelagic habitats in lake and marine ecosystems, reflecting differences in $\delta^{13}\text{C}$ between their basal foods, phytoplankton and benthic microalgae (France 1995a, b). In coastal ecosystems, there are remarkable spatial variations in the stable isotopic signatures of consumers even within a small area, depending on the relative abundance of each primary producer (Vizzini and Mazzola 2006; Richoux and Froneman 2007). In addition, the stable isotopic signatures of primary producers show marked seasonal changes that may account for the dynamic patterns of consumers' stable isotopic signatures (Leggett et al. 2000; Lehmann et al. 2004).

To overcome such difficulties, recent food web studies have frequently focused on the isotopic signatures of consumers rather than those of primary producers. Because the consumers have larger body sizes and longer turnover times than primary producers, they integrate spatio-temporal changes in the stable isotope ratios of primary producers; i.e., they have an averaging effect (Cabana and Rasmussen 1996). For instance, Fukumori et al. (2008a, b) conducted stable isotope analysis of the suspension-feeding bivalve *Pinctada fucata martensii* and demonstrated that the relative importance of phytoplankton and associated microalgal production varies on a local and seasonal scale. The isotopic signatures of higher consumers can also provide more time-integrated information on the trophic structure of the food web (Vander Zanden and Rasmussen 1999). In aquatic ecosystems, higher consumers, such as piscivorous fishes and marine mammals, have a wider home range and feed on prey across various habitats (McCann et al. 2005). Because of these feeding habits, they integrate energy and materials from different food chains constituting the aquatic ecosystem, recording this information in their body tissues (Hobson 1999; Vander Zanden and Vadeboncoeur 2002; Fukumori et al. 2008b). Recently, empirical work has stressed that stable isotopic signatures of higher consumers can be a convenient indicator of overall changes in the food web structure due to human disturbance; e.g., it has been reported that habitat fragmentation collapsed the niche width of carnivorous fish (Layman et al. 2007), eutrophication caused seasonal or interannual feeding shifts of fish from benthic to pelagic prey (Yoshioka et al. 1994; Vander Zanden et al. 2003), and overfishing and human introduction of exotic predatory fish distorted the trophic level of a native top predator (Pauly et al. 1998; Vander Zanden et al. 1999).

In this study, we aimed to characterize the food web structure of the coastal ecosystem in the Uwa Sea that is vulnerable to modern human activities by conducting stable isotope analysis of organisms embedded in the food web. We also monitored the seasonal dynamics of the coastal food web, focusing on the stable isotope ratios of a higher consumer, and associated its trophodynamics with human disturbances in the Uwa Sea.

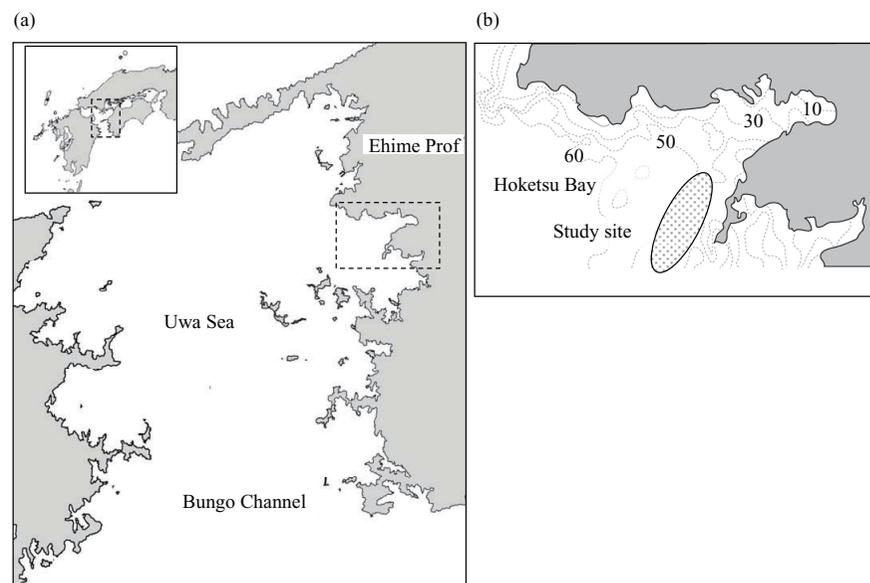


Fig. 1. Locations of (a) Uwa Sea, Japan and (b) our study site (dotted area) in Hoketsu Bay.

Materials and Methods

Study site and subjects

The Uwa Sea fronting the Bungo Channel is located southwest of Shikoku Island, Japan (Fig. 1a). Because this sea is famous for its good fishing grounds, coastal fisheries have flourished. In the summer, nutrient-enriched deep-sea waters intrude into this area from the slope of the offshore continental shelf under the influence of the Kuroshio Current, the so-called “bottom intrusion” (Kaneda et al. 2002). It is believed that the plentiful nutrient supply provided by the bottom intrusion enhances productivity in the coastal ecosystem of the Uwa Sea, although the detailed mechanism is unexplored (Koizumi et al. 1997; Nakano et al. 2004). However, despite the potential for high fisheries productivity, total annual fish catches in the Uwa Sea have decreased from 12.8 to 4.2 t in the past two decades (Sasaki 2005). Some possible anthropogenic factors contributing to this drastic decrease in fisheries catch include overfishing, eutrophication due to aquaculture, and global warming, which may influence the meandering of the Kuroshio Current.

The present study was carried out in Hoketsu Bay, located in the northern part of the Uwa Sea (Fig. 1b). We focused on the glowbelly *Acropoma japonicum* as an indicator species for monitoring coastal food web dynamics. This fish is a demersal species that lives near the bottom of the Uwa Sea (mean depth 71.8 m). The common name “glowbelly” was derived from the glowfly because it has a luminous organ in its abdomen (Haneda 1950).

Although its ecological function is unknown, it may be used for communication in deep, dark environments. This fish is a higher consumer dominating the demersal food web of the Uwa Sea (Okuda et al. 2005). Prior to the breeding season (June–September), it migrates from deep areas offshore to coastal waters (Okuda et al. 2005). In the Uwa Sea, it is caught primarily by trawlers as a commercially important fishery resource, used for the local food “jakoten.”

Sample collection

Specimens of *A. japonicum* were caught using traditional trawls (mesh size: 43.3–50.5 mm) towed along the bottom ~60 m in depth from ~1 km offshore toward the shore (Okuda et al. 2005). Fish specimens were collected monthly from October 2002 to December 2003, except in December 2002 when the trawls could not be operated due to bad weather. In each month, 40 fish were examined for dietary analysis and 20 fish (10 fish in October 2002) underwent stable isotope analysis, after measuring total length to the nearest 0.1 mm. To fix the stomach contents, we injected 100% formalin into the stomach immediately after landing the fish. For stable isotope analysis, we excised muscle tissues from the dorsal part of the lateral body. The muscle tissues were frozen at -20°C until analysis.

To delineate the food web configuration using stable isotope analysis, primary producers and potential prey organisms of *A. japonicum* were collected in Hoketsu Bay in June (early breeding season) and October (immediately after the breeding season) 2003. In this study area, the main primary producers were phytoplankton, benthic microalgae, and macroalgae. We collected particulate organic matter (POM) in six replicate samples from surface waters offshore (mean depth ~60 m) with a 100- μm mesh plankton net. POM was screened with a 200- μm mesh to sort it from zooplankton. POM of this size fraction (100–200 μm) was trapped on GF/A glass filters that were precombusted at 550°C for 3 h, and was regarded as a proxy for phytoplankton. Microscopic observations confirmed that the POM consisted of almost entirely phytoplankton. As a proxy for benthic microalgae, epilithic organic matter (EOM) was collected in shallow waters <5 m deep by SCUBA divers. We collected six submerged pebbles from the bed along the shore and exfoliated EOM from the stones with a brush after rinsing sediments from the stones with seawater. The EOM was screened with a 200- μm mesh net to sort it from meiobenthos and was trapped on precombusted GF/A glass filters. Benthic macroalgae were also collected in shallow waters <5 m deep. Dominant species were the green macroalgae *Codium fragile* and *C. cylindricum* and the brown macroalgae *Dictyopteris prolifera* and *Colpomenia sinuosa*. For each species, six fronds from different stocks were softly rinsed with seawater to remove attached microalgae. We could not collect macroalgae in October 2003 because they had already disappeared.

Collection of zooplankton and zoobenthos was conducted in offshore waters. We collected zooplankton by towing a 200- μm mesh plankton net vertically from the bottom to the surface. Small zoobenthos were collected from the sandy-mud bottom (approximately 60, 30, and 7 m in depth) using an Ekman-Berge grab sampler and then sorted with a 1-mm mesh screen. Zooplankton and small zoobenthos were identified to at least the order

level, if possible to the species level, under a binocular microscope. Large zoobenthos and small demersal fishes were collected by fishery trawls. The cephalopods and goby fishes collected by the trawls were much larger than those found in the stomach contents of *A. japonicum*. For cephalopods inhabiting the Seto Inland Sea adjacent to the Uwa Sea, it has been reported that their stable isotopic signatures vary with increasing body size (Takai et al. 2002a); therefore, we use these data with caution to delineate food web configuration. Juveniles of the anchovy *Engraulis japonicus*, one of the primary prey items, were collected by boat seines. The anchovy specimens were also slightly larger than the prey items of *A. japonicum*, which feed on anchovy at the larval stage rather than the juvenile stage.

For stable isotope analysis, muscle tissues were excised from the bodies of the fish and large zoobenthos. For zooplankton and small zoobenthos, whole bodies or bulk samples were used. The samples were frozen at -20°C until analysis.

Dietary analysis

We conducted dietary analysis for *A. japonicum* from November 2002 to December 2003, except in December 2002. Soon after collecting specimens of *A. japonicum*, we injected 100% formalin into their abdominal cavities to prevent their stomach contents from being digested. We dissected specimens in the laboratory to collect the stomach contents. Prey items were sorted and identified to at least the order level under a binocular microscope. The number of prey items was also counted for each taxonomic group. We measured the prey items' body lengths and dry weights if they were intact. We evaluated the dietary composition of *A. japonicum* using two indices: 1) %N, the percentage of each prey taxon with respect to the total number of prey items and 2) %W, the percentage weight of each prey taxon with respect to the total weight of the prey items. For the latter index, the weight of each prey taxon was determined by multiplying the number of individuals by the average individual dry weight. %W rather than %N serves as a better proxy for feeding habits based on quantitative evaluation, and thus is more compatible with stable isotope analysis, which reflects the mass balance of assimilated foods. These two indices were calculated in bulk by pooling all specimens, rather than for each specimen. This method efficiently quantifies feeding habits as population averages for *A. japonicum*. To evaluate seasonal changes in feeding habit, we also calculated %W for specimens collected each month.

Stable isotope analysis

Prior to stable isotope analysis, animal samples were dried at 60°C for 24 h, pulverized, and immersed in chloroform: methanol (2: 1) solution for 24 h to remove lipids for lipid correction, according to Bligh and Dyer (1959). POM and EOM were exfoliated from the glass filters and pulverized. For POM, EOM, and macroalgae, the powder samples were exposed to 12 N HCl vapor for 24 h to remove carbonate carbon. The animal and plant samples were dried again at 60°C for 24 h. The dried samples were wrapped with tinfoil and their carbon and nitrogen stable isotope ratios were measured using a mass spectrometer (ANCA-SL; PDZ Europa, Elworth, UK).

The carbon and nitrogen isotope ratios are expressed in δ notation and defined as the per mil deviation from the standard, as follows:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$$

where $\text{R} = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standards used were Pee Dee belemnite (PDB) limestone carbonate for $\delta^{13}\text{C}$ and atmospheric nitrogen (N_2) for $\delta^{15}\text{N}$.

Estimation of trophic level and reliance on primary productivity

From the stable isotope ratios of *A. japonicum* and its basal prey, we estimated its trophic level and relative reliance on each of the two primary producers, phytoplankton and benthic microalgae, in June and October 2003, using a two-end-member mixing model according to Post (2002). Assuming that the trophic enrichment factor is 0.8‰ for $\delta^{13}\text{C}$ (DeNiro and Epstein 1978; Fry and Sherr 1984) and 3.4‰ for $\delta^{15}\text{N}$ (Minagawa and Wada 1984), trophic level and relative reliance were calculated using the following equations:

$$r = [\delta^{13}\text{C}_A - \delta^{13}\text{C}_B - 0.8(TL_A - 1)] / (\delta^{13}\text{C}_P - \delta^{13}\text{C}_B)$$

$$TL_A = 1 + (\delta^{15}\text{N}_A - [r \times \delta^{15}\text{N}_P + (1 - r) \times \delta^{15}\text{N}_B]) / 3.4$$

where r is the relative reliance on phytoplankton production for *A. japonicum*, TL is the trophic level, and subscripts A, P, and B are *A. japonicum*, phytoplankton, and benthic microalgae, respectively. We did not consider reliance on benthic macroalgal production for the following reasons: 1) the seasonal disappearance of benthic macroalgae did not account for the seasonal pattern of $\delta^{13}\text{C}$ for *A. japonicum* (Okuda et al. 2004; also see Results) and 2) it is unlikely that prey organisms of *A. japonicum* were supported by the production of shallow-water-dwelling benthic macroalgae, as shown by their stable isotopic signatures characteristic of deep water habitats (see Results), which are considerably distant from shallow water habitats. Although many stable isotopic studies have reported that terrestrial organic matter can influence coastal food webs through riverine influx (Darnaude et al. 2004a, b; Darnaude 2005), terrestrial input is negligible in this study area because there are no large rivers around Hoketsu Bay. In fact, no prey organisms reflected the stable isotopic signal of terrestrial POM ($\delta^{13}\text{C} = -26.9$ to -25.2‰ ; N. Okuda, unpublished data) in the demersal food web of our study area (see Results).

We also compared the trophic level and primary productivity reliance of *A. japonicum* between June and October 2003. To evaluate the seasonal dynamics of the demersal food web from the perspective of a higher consumer, we statistically analyzed monthly changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *A. japonicum*, separating the fish into two age classes, 0+ and 1+ or older fish (hereafter, 1+ \leq), based on their size, according to Okuda et al. (2005). Because data were available only in June and October for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the primary producers needed for estimation of trophic level and primary production reliance, intact isotopic values for *A. japonicum* were compared using a two-way ANOVA, incorporating seasonal (month) and life history (age) effects on isotopic signature into the model. We used the contrast method for post-hoc comparison after the two-way ANOVA.

Results

Feeding habits of *A. japonicum*

The diet of *A. japonicum* is shown in Table 1. A variety of prey items, including pelagic prey such as copepod zooplankton, larvae of the anchovy *E. japonicus*, and juvenile cephalopods, and benthic prey such as the decapod shrimp *Metapenaeopsis barbata* and juvenile gobies, were found in stomach contents throughout the year, for a total of 21 taxonomic groups. Small zooplankton, particularly calanoid copepods, were dominant in number (86.0%) in the diet (Table 1). In terms of %W, in contrast, *A. japonicum* primarily fed on large prey such as gobiid juveniles (69.9%), the decapod shrimp *M. barbata* (10.3%), and cephalopod juveniles (7.3%) (Table 1). Its feeding habits also varied seasonally (Fig. 2). Although in most months a large percentage of its food items consisted of large benthic prey such as shrimp and juvenile gobies, it shifted its food items toward pelagic prey such as copepods, larval anchovy, and cephalopod juveniles in the spring.

Small *A. japonicum* fed primarily on small zooplankton and thereafter shifted toward larger prey, such as decapod shrimp, cephalopod juveniles, larval anchovies, and juvenile gobies, as its body size increased. Despite this size-dependent diet, small prey organisms were found in the stomach contents of large ($1 + \leq$) as well as small ($0 +$) fish (Fig. 3). This was attributed to the seasonal feeding shift toward small zooplankton in spring (Fig. 2).

Demersal foodweb

The trophic positions of *A. japonicum* and its prey organisms were plotted on a $\delta^{13}\text{C} - \delta^{15}\text{N}$ map (Fig. 4). The $\delta^{13}\text{C}$ s and $\delta^{15}\text{N}$ s of POM and EOM (proxies for phytoplankton and benthic microalgae, respectively) did not significantly differ between June and October (t-test, POM $\delta^{13}\text{C}$: $t_{6,3} = 1.07$, $p = 0.32$; POM $\delta^{15}\text{N}$: $t_{6,2} = 1.85$, $p = 0.11$; EOM $\delta^{13}\text{C}$: $t_{3,6} = 1.40$, $p = 0.21$; EOM $\delta^{15}\text{N}$: $t_{3,6} = 2.24$, $p = 0.06$; see also Okuda et al. 2004); thus, their seasonal data were pooled for plotting. The trophic positions of *A. japonicum* and all of the prey species other than cephalopod juveniles were positioned in the food chain between POM and EOM (Fig. 4). The $\delta^{13}\text{C}$ of the copepods was among the lowest of the prey organisms, indicating that they relied most on pelagic production. By contrast, anchovies were located directly on the food chain originating with benthic microalgae, although they are regarded as pelagic species, suggesting that they had no reliance on phytoplankton production. The body sizes of the anchovy specimens were 26.2–97.0 mm in total length and were larger than the prey size (14.0–36.0 mm) of *A. japonicum*. Our result is consistent with a previous study reporting that anchovies exhibited a substantial increase in $\delta^{13}\text{C}$ after reaching a length of approximately 30 mm, at which point they metamorphosed from the larval stage to the juvenile stage (Lindsay et al. 1998). Decapod shrimp were also located on the benthic food chain. Assuming that they are entirely reliant on benthic production, trophic levels were estimated as 2.7 ± 0.2 for anchovy juveniles and 2.6 ± 0.1 for decapod shrimp. Although gobiid and cephalopod juveniles were significant food items for *A. japonicum*,

Table 1 Feeding habits of *A. japonicum* (n = 500) in the Uwa Sea. Dietary data are expressed as percentage number (%N) and percentage weight (%W) of each prey item.

Prey item	%N	Mean individual dry weight (mg)	%W
Cephalopoda			
Teuthoidea	0.2	75.1	7.3
Octopoda	0.0	2.0	0.0
Ostracoda			
Myodocopida			
Halocyprididae			
<i>Conchecia</i> sp	3.1	0.1	0.2
Copepoda			
Calanoida			
Calanidae			
<i>Calanus sinicus</i>	78.5	0.1	3.9
Euchaetidae			
<i>Paraeuchaeta plana</i>	6.3	0.1	0.3
Candacidae			
<i>Candacia</i> sp	0.2	0.1	0.0
Paracalanidae			
<i>Paracalanus</i> sp	1.0	0.1	0.0
Pocilostmatoidea			
Oncaeiidae			
<i>Oncaea</i> sp	0.3	0.1	0.0
Haplocarida			
Stomatopoda	0.0	5.3	0.1
Eumalacostraca			
Mysida	2.8	0.7	1.0
Amphipoda			
Gammaridea	0.2	0.1	0.0
Hyperiidea	0.5	0.0	0.0
Caprelliidea	0.3	0.5	0.1
Euphausiacea	0.6	6.5	2.1
Decapoda			
Luciferiidea	0.1	1.8	0.1
Penaeiidea			
<i>Metapenaeopsis barbata</i>	0.9	23.9	10.3
Alpheidae	0.0	118.3	1.6
Diogenidae	0.0	0.3	0.0
megalopa larvae	2.2	0.1	0.1
Pisciformes			
Clupeiformes			
Engraulididae			
<i>Engraulis japonicus</i>	0.4	14.0	2.9
Perciformes			
Gobiidae	1.2	111.6	69.9
Unidentified fish	0.9	–	–

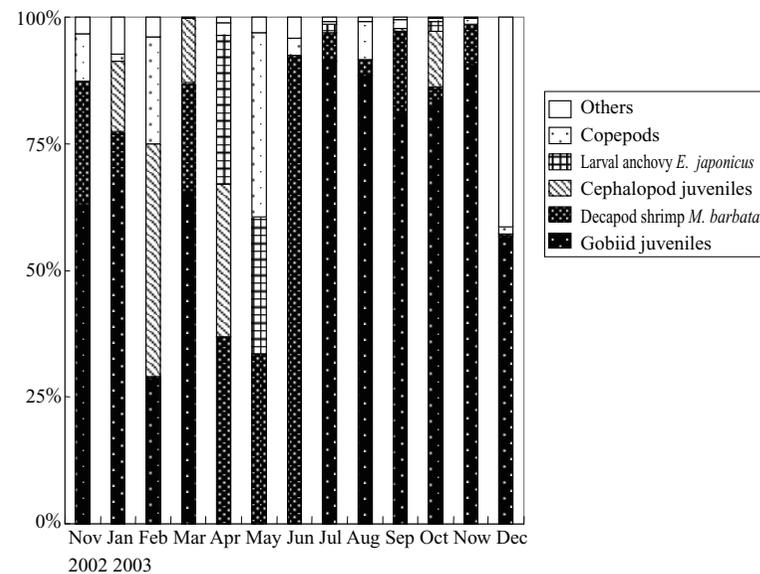


Fig. 2. Seasonal changes in feeding habits (%W) of *A. japonicum* collected from November 2002 to December 2003.

these specimens had similar or slightly higher $\delta^{15}\text{N}$ s compared to those of *A. japonicum*. This may be because the former specimens were much larger in size than those found in the stomach contents of *A. japonicum*.

There was a clear positive correlation between *A. japonicum* body size and $\delta^{15}\text{N}$ ($0+ : r = 0.67, n = 137, p < 0.001$; $1+\leq : r = 0.67, n = 133, p < 0.001$; Fig. 5a). The size dependence of $\delta^{13}\text{C}$ was obscure, although statistically significant, with the opposite pattern between age size groups ($0+ : r = -0.27, n = 137, p < 0.002$; $1+\leq : r = 0.33, n = 133, p < 0.001$; Fig. 5b). The $\delta^{15}\text{N}$ did not significantly differ between June and October (ANOVA, $F_{1,1} = 1.71, p = 0.199$) but the $\delta^{13}\text{C}$ was depleted in June compared to October (ANOVA, $F_{1,1} = 74.27, p < 0.001$; Fig. 4). Assuming that POM (i.e., phytoplankton) and EOM (i.e., benthic microalgae) are potential carbon sources for *A. japonicum*, its relative reliance on pelagic production was 34.3% in June and 7.8% in October. Its trophic level was 3.0 in June and 3.1 in October, with individual variations ranging from 2.6–3.5.

Seasonal changes in the isotopic signatures of *A. japonicum*

A. japonicum was separated into 0+ (small) and 1+ \leq (large) fish to examine seasonal changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 6). Its stable isotopic signatures showed a marked seasonal pattern (two-way ANOVA, $\delta^{13}\text{C}$ month: $F_{1,13} = 28.15, p < 0.001$; $\delta^{13}\text{C}$ age: $F_{1,13} = 2.12, p = 0.15$; $\delta^{15}\text{N}$ month: $F_{1,13} = 6.21, p < 0.001$; $\delta^{15}\text{N}$ age: $F_{1,13} = 310.76, p < 0.0001$). Both age classes enriched their $\delta^{15}\text{N}$ s from autumn to the following spring with increasing body size

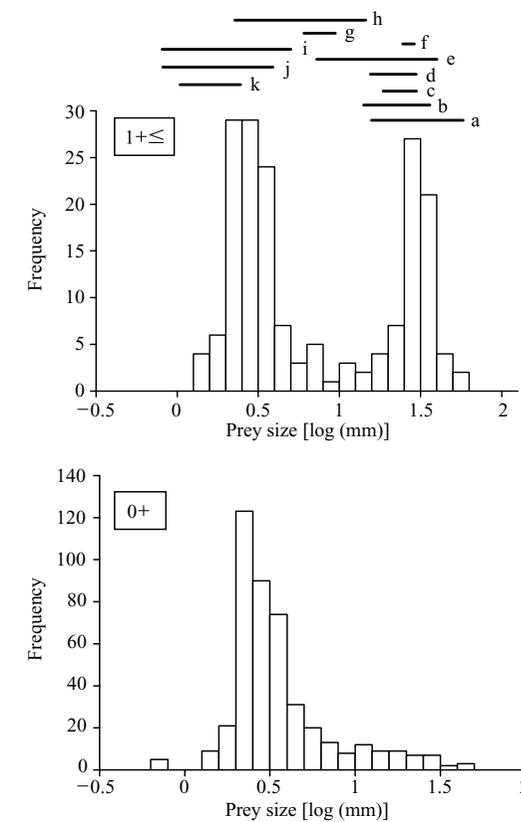


Fig. 3. Frequency distribution of prey organism size in stomach contents. Horizontal bars (a-k) represent the size ranges for each prey species. (a) Gobiid juveniles, (b) larvae of the anchovy *E. japonicus*, (c) cephalopod juveniles, (d) the pelagic shrimp *L. typus*, (e) the decapod shrimp *M. barbata*, (f) alpheidids, (g) euphausiids, (h) mysids, (i) megalopa larvae, (j) copepods, and (k) ostracods.

(Fig. 6a). However, only the 1+ \leq fish showed $\delta^{15}\text{N}$ depletion by 0.9‰ in June. During the following summer season, there were no clear differences in $\delta^{15}\text{N}$ between these two age classes (Fig. 6a). *A. japonicum*, whether its age was 0+ or 1+ \leq , showed substantial depletion of $\delta^{13}\text{C}$ from May to June (Fig. 6b). Similar to $\delta^{15}\text{N}$, there were no significant differences in $\delta^{13}\text{C}$ between the two age classes during the summer season.

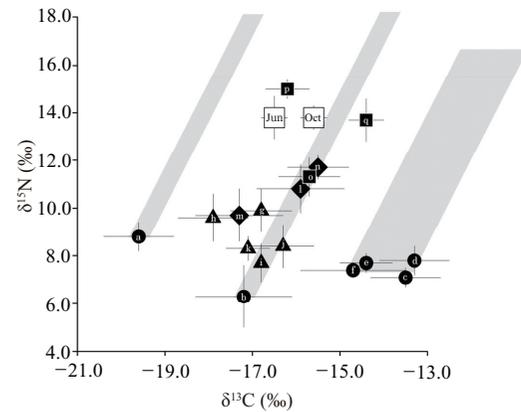


Fig. 4. Carbon and nitrogen isotopic signatures (mean \pm SD) of *A. japonicum* (open squares; collected in June and October), its prey organisms, and primary producers in the food web of Hoketsu Bay. Circles represent primary producers; triangles, zooplankton; diamonds, zoobenthos; and squares, fishes and squids. Shaded zones represent hypothetical trophic pathways starting from each primary producer (left: phytoplankton, middle: benthic microalgae, right: benthic macroalgae) with exclusive reliance on that producer. (a) POM, (b) EOM, (c) the green macroalga *C. fragile*, (d) the green macroalga *C. cylindricum*, (e) the brown macroalga *C. sinuosa*, (f) the brown macroalga *D. prolifera*, (g) pelagic gammarids, (h) calanoid copepods, (i) euphausiids, (j) the pelagic shrimp *L. typus*, (k) megalopa larvae, (l) benthic gammarids, (m) caprellids, (n) the decapod shrimp *M. barbata*, (o) juveniles of the anchovy *E. japonicum*, (p) the benthic goby *A. hexanema*, and (q) cephalopod juveniles.

Discussion

Demersal food web and carbon sources

Our stable isotopic approach revealed that the demersal food web dominated by *A. japonicum* in the Uwa Sea is supported by benthic as well as pelagic production (Fig. 4). In this study, benthic macroalgal production was not considered to be a primary carbon source for *A. japonicum*. In the coastal food web, particularly in shallow waters, macroalgal production is generally regarded as an important carbon source for benthic invertebrates and fishes (Jennings et al. 1997; Fredriksen 2003). If benthic macroalgae were incorporated into the multiple sources mixing model, it would be possible to conclude that *A. japonicum* relied on macroalgal production to some extent. This possibility, however, can be rejected for the following reasons. First, benthic macroalgae live only at depths of <10–30 m (Duarte 1991). At the study site, there are almost no macroalgae at depths of 10 m or more and they cannot grow in the deep-water habitat of *A. japonicum*. These two habitats are substantially separated (~1 km) from each other, limiting material transport between them. In addition, *A. japonicum* did not feed on epibenthic fauna on the macroalgae, except for

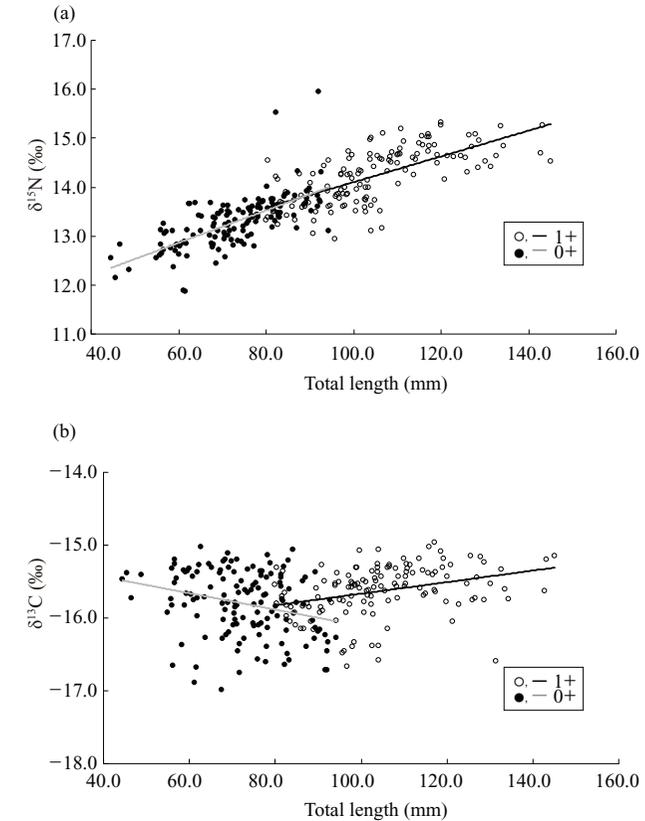


Fig. 5. Allometric relationships between the body size of *A. japonicum* and its (a) nitrogen and (b) carbon stable isotope ratios.

benthic gammarids and caprellids, which were minor food items. Second, in the Uwa Sea, which has a steep coastal slope, macroalgal distribution is confined to a narrow area of the shoreline (the proportional area of 10 m or shallower waters is 11%; Fig. 1b), thus limiting its contribution to total primary production in the coastal area. If the prey organisms of *A. japonicum* were to rely mainly on macroalgal production, the $\delta^{13}\text{C}$ of this fish would be lower in the autumn when the macroalgae were nearly gone than in the summer when they flourished. Contrary to this hypothesis, our results showed that the $\delta^{13}\text{C}$ decreased in the summer (Fig. 6b), indicating that macroalgal production contributed little to the demersal food web of the Uwa Sea.

For *A. japonicum*, the relative reliance on pelagic production was 34.3% in June and 7.8% in October. Considering that this fish lives in the deep aphotic layer, such low reliance, particularly in October, is surprising. In the conventional view of marine ecology, it is believed that benthic secondary production in the aphotic layer is supported by pelagic

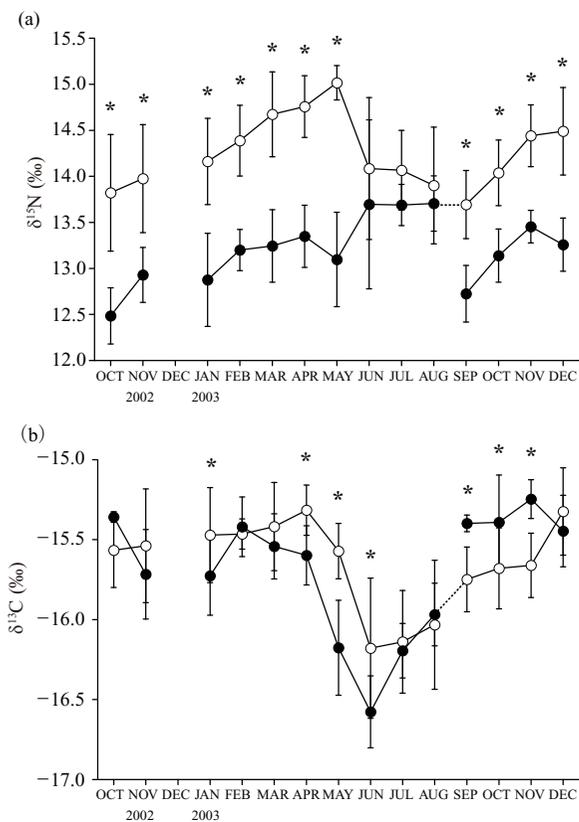


Fig. 6. Seasonal changes in (a) nitrogen and (b) carbon isotope ratios for 0+ (closed circles) and 1+ (open circles) *A. japonicum* fish. Note that the generation is replaced in September when recruitment occurs (Okuda et al 2005). Vertical bars indicate SD. Asterisks indicate that there was a significant difference between the two age classes in each month ($p < 0.05$ determined by the contrast method after two-way ANOVA).

primary production in the surface layer, the so-called “pelagic-benthic coupling” theory (Grebmeier et al. 1988; Johnson and Wiederholm 1992; Josefsen and Hansen 2003). Some mechanisms by which organic matter can be transported from the surface to the bottom layer have been proposed, e.g., settling of pelagic primary products and fecal pellets by zooplankton and active transport through vertical migration of zooplankton or zoobenthos (Turner 1977; Miquel et al. 1994; Schnetzer and Steinberg 2002).

Recent studies have used stable isotope analysis to show that demersal food webs are subsidized by benthic microalgal production (Takai et al. 2002; Yokoyama and Ishihi 2003; Nadon and Himmelman 2006; Doi et al. 2008). Takai et al. (2002) demonstrated that demersal fishes relied heavily on benthic production. However, benthic microalgae

are able to maintain high productivity in the Seto Inland Sea, which is relatively shallow (mean depth 38 m). By contrast, because the deep-water habitat of *A. japonicum* is entirely aphotic, it is unlikely that benthic microalgae grow in situ. In the demersal habitat, the isotopic signature of SOM ($\delta^{13}\text{C} = -20.6\text{‰}$; Okuda et al., unpublished data) was similar to that of our POM, suggesting that the demersal food web could potentially be coupled to pelagic production through sedimentation of POM. As possible mechanisms by which benthic products could be transported to the demersal food web, Takai et al. (2004) stressed the importance of resuspension of benthic microalgae along the shore and subsequent trophic transport mediated by animals migrating horizontally or vertically. In the Uwa Sea, by contrast, Fukumori (unpublished data) estimated that benthic microalgal production accounted for ~50% of the total primary production at a depth of 5 m and ~10% even at a depth of 40 m. It is plausible that benthic products could be transferred to higher consumers in the deep-water habitat through trophic interaction. Although we were not able to identify the underlying mechanisms by which the demersal food web is subsidized by benthic production, our study demonstrated that pelagic-benthic coupling can be ruled out in coastal ecosystems.

In the unique food web of the Uwa Sea, *A. japonicum* fed on a variety of prey organisms whose trophic levels ranged from low to intermediate and whose habitats ranged from the pelagic layer to the bottom layer. Associated with a wide variety of feeding habits, this fish showed great individual variation in trophic level, ranging from 2.4–3.3. This variation was closely related to its body size (Fig. 5a). The results accorded well with the body size constraint hypothesis that prey-predator interactions are limited by gape size (Nilsson and Bronmark 2000). This hypothesis generally applies to aquatic ecosystems, as reported in many studies that have found strong positive correlations between body size and $\delta^{15}\text{N}$, as a proxy of trophic level within or across various taxonomic groups of aquatic organisms (Gu et al. 1997; Badalamenti et al. 2002; Deudero et al. 2004; Jennings et al. 2001, 2002, 2007). Fish at an indeterminate growth stage have the potential to widely vary their feeding habits and thus their trophic level with ontogenetic growth, from being a planktivore after hatching to becoming a large piscivore. The ontogenetic shift in feeding habits found in *A. japonicum* suggests that this fish can play a wide variety of ecological roles in the coastal ecosystem and that its body size will be one of the important factors in determining its food niche width and thus its ecosystem function.

Trophodynamics from the stable isotopic view of a higher consumer

As shown in the stable isotopic food web map (Fig. 4), the higher consumer *A. japonicum*, whose trophic position is intermediate between the pelagic and benthic food chains, can play the ecological role of coupling two energy flows in the demersal food web. From the seasonal pattern of its stable isotopic signatures (Fig. 6), we could depict the trophodynamics of the demersal food web as reflecting seasonal changes in higher consumers’ feeding habits depending on the spatio-temporal dynamics of productivity at the base of the coastal food web. For *A. japonicum*, its $\delta^{13}\text{C}$ was substantially depleted from May to June. This season,

when *A. japonicum* begins to migrate from deep to shallow waters, is also when pelagic secondary production increases following the spring algal bloom in coastal ecosystems. The dietary analysis indicated that *A. japonicum* shifted its diet during this season from benthic to pelagic prey, particularly to copepods that strongly relied on pelagic production, and consequently markedly depleted its $\delta^{13}\text{C}$. Therefore, this $\delta^{13}\text{C}$ depletion indicates that the demersal food web was seasonally subsidized by the pelagic food web through a feeding shift by the demersal predator.

Although *A. japonicum* most frequently fed on copepods in May (Fig. 2), its $\delta^{13}\text{C}$ was most depleted in June (Fig. 6b). This delayed response is attributable to the time lag in isotopic turnover in somatic tissues after feeding. For some fish species that are similar in size to *A. japonicum*, the turnover time in somatic tissues has been estimated under captive conditions, e.g., 20–25 days for the Japanese temperate bass *Lateolabrax japonicus* (Suzuki et al. 2005) and 26 days for the sand goby *Pomatoschistus minutus* (Guelinckx et al. 2007). It is less likely that the substantial depletion in $\delta^{13}\text{C}$ for *A. japonicum* could be accounted for by seasonal changes in the isotopic signatures of basal food because there were no marked seasonal differences in the $\delta^{13}\text{C}$ of the primary producers.

In June, *A. japonicum*, particularly large adults, showed marked $\delta^{15}\text{N}$ depletion. This could be because it shifted its diet to copepods whose trophic level was lower than juvenile anchovies and decapod shrimp. This fish stores energy for reproduction prior to the breeding season (June–September) and reallocates it into the gonads, as indicated by its condition factor and fat-body ratio (Okuda et al. 2005). One might expect that such a physiological process caused the seasonal depletion in $\delta^{15}\text{N}$ for *A. japonicum*. However, lipid extraction generally has little effect on the $\delta^{15}\text{N}$ of muscle tissue (Ingram et al. 2007) and the $\delta^{15}\text{N}$ of the muscle tissue is more enriched than that of the eggs that will be released ($\Delta 1.4\text{‰}$ for perciformes inhabiting the Uwa Sea; N. Okuda, unpublished data), excluding this possibility. During the breeding season, in general, fish need substantial energy for reproduction. According to the optimal diet theory (Sih and Christensen 2001), there is no biological reason for *A. japonicum* to selectively feed on small zooplankton with the associated high handling cost, provided that larger prey are abundant. Why then did they feed more on small zooplankton than on large larval anchovies in the highly productive season?

One possible explanation is the marked decrease in the abundance of larval *E. japonicus* due to overfishing. Fishermen's anecdotes tell us that landed glowbellies often had mouths filled with *E. japonicus* in the old days (Akamatsu, personal communication). In the Uwa Sea, the transition from traditional manual fisheries to power-driven fisheries has led to a substantial collapse of the *E. japonicus* population over the last two decades (Fig. 7a; Sasaki 2005). Modern exploitative fishing may have accelerated the seasonal decline of larval *E. japonicus* abundance after its spring appearance (Fig. 7b). This may be the reason for the absence of larval anchovies in *A. japonicum* stomach contents in June and for its feeding shift to small zooplankton whose food quality is lower than that of the larval anchovy. Some predatory fishes have been found to reduce their trophic levels by shifting their

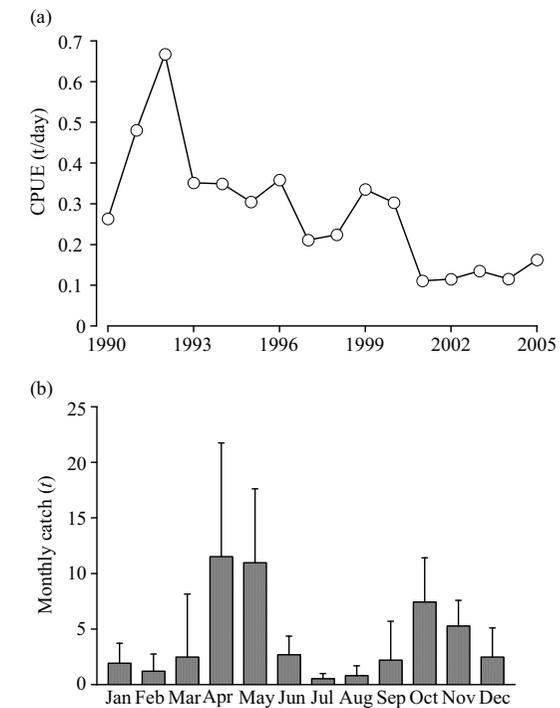


Fig. 7. (a) Annual changes in the catch per unit effort (CPUE) of the larval anchovy *E. japonicus* in the Uwa Sea. The CPUE was calculated as the annual catch divided by the total fishing days in each year. (b) Monthly catches (mean \pm SD) of larval anchovy *E. japonicus* in the Uwa Sea. Data are from 2002–2007.

diets to smaller prey with lower trophic levels. For example, using stable isotope analysis, Vander Zanden et al. (1999) demonstrated that a native predatory fish had substantially depleted $\delta^{15}\text{N}$ after the introduction of exotic predatory fishes in a North American lake. This occurred because the exotic predator decreased the availability of forage fish and therefore, the native predator shifted its diet from forage fish to zooplankton. In our study area, there are no records of invasion of exotic predatory fish that would compete with *A. japonicum*. Thus, overfishing may be an alternative anthropogenic factor resulting in a substantial decrease in the trophic level of *A. japonicum* in the Uwa Sea. Although the detailed mechanism for its lowered trophic level remains to be explored, it is expected to be similar to that of the invasion of exotic predators, that is, exploitative competition for forage fish between higher consumers and humans.

An increasing number of marine ecological studies have reported that fishing activities can have a substantial impact on aquatic ecosystems. In the southern Gulf of St. Lawrence, for example, heavy exploitation of fisheries resources shrunk the coastal food web through

feeding shifts by higher consumers (Savenkoff et al. 2007). Unfortunately, we have no direct evidence showing that the decline of larval anchovies has affected the trophic level of *A. japonicum*. However, the lack of forage fishes having energetically high quality must have negative effects on the life history and population growth of predatory fishes. To control the population of higher consumers that are typically important fisheries resources and play important roles in coastal ecosystems, ecosystem-based fisheries management should be established in which the dynamics of not only the target species but also the food web, including its prey species and primary producers, is considered. Our stable isotopic approach to depicting food web dynamics by focusing on higher consumers will contribute to the future development of ecologically sound fisheries management approaches.

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