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Abstract Stable isotopes can provide useful knowledge about sources and processes within an ecosystem. The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were used to investigate trophic relationships of relatively commercially important 21 finfish species, cephalopods in Hiroshima Bay. Among 21 finfish species, the lowest mean $\delta^{15}N$ of 14.4‰ was recorded for and while the highest mean $\delta^{15}N$ of 16.8‰ was recorded for . The lowest and highest mean $\delta^{13}C$ were noted -17.6‰ for and , and -15.3‰ for and , respectively. Including with cephalopods, the highest mean $\delta^{13}C$ and $\delta^{15}N$ noted at -14.8‰ and 17.3‰ for . Information of stable isotope variation and trophic level in aquatic species of Hiroshima Bay can be used for monitoring and managing sustainable fisheries.

Keywords: Finfish, Hiroshima Bay, stable isotope analysis, trophic level

INTRODUCTION

Generally, stable isotope of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are used in ecological study to elucidate food web dynamics and distinguish the source of primary production (Fry, 2006). According to Minagawa and Wada (1984), the nitrogen difference in δ7) and the

$\delta^{13}C$ can also be used to distinguish between inshore and offshore feeding pattern (France, 1995). Furthermore, trophic position is important for the implementation of marine management indicators, such as the marine trophic index. In addition, variation in the trophic position of various aquatic species can be used as a key to understand coexisting of all species based on their food preference.

Hiroshima Bay with averages 25.6 m in depth is located in the western part of the Seto Inland Sea in Japan. The bottom of the Bay is composed mainly of rocks and sand, some of which are covered by seaweeds. The mean annual sea-surface temperature is about 19 ℃, ranging from 9 ℃ in March to 29 ℃ in August. The salinity averages 29psu, fluctuating from 15psu in July to 33psu in January (Blanco Gonzalez et al. 2008). These variable hydrographic features with well-mixed year around result in continued nutrient regeneration and high levels of primary productivity. The high levels of primary

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Accepted on september 10, 2013 *E-mail: umino@hiroshima-u.ac.jp productivity have, in turn, supported a diverse ecosystem with a high biomass of aquatic species. The Bay is also known to be the most popular fishing ground in the Seto Inland Sea. For instance, two most common dominant fish found, in which are (Blanco Gonzalez et al., 2008; Umino et al., 2011) and (Ahmad-Syazni et al., 2012).

In this study, the stable isotopes of commercially important aquatic species that are found in Hiroshima Bay are determined. Information on the stable isotope and trophic ecology of the commercially important and dominant species such as black seabream, will be useful for managing sustainable fisheries. Additionally, comparison of those important and dominant fish species with other aquatic species will provide better understanding of their role in marine food web, as well as the factors that may influence their distribution in this basin.

MATERIAL AND METHODS

Hiroshima Bay is located in the western part of the Seto Inland Sea in Japan (Fig.1). Fish species and cephalopods were sampled during autumn in which it is the richest in ichthyofauna due to suitable water temperature (Shimizu et al., 2010). A total of 21 finfish species, oval squid, , cuttlefish, and octopus, were collected through line fishing in or near the Bay in autumn 2012 (Table 1).

White muscle for fish and mantle for cephalopods were removed and stored in -20 ℃ until further analysis. Prior to analysis, the muscle and mantle of each species was rinsed with distilled water to remove any excess of superficial debris. Muscle and mantle were homogenized and keep in methanol-chloroform in 2:1 ratios in about 1 hour for lipid extraction. Specimens were then dried at 60 ℃ until a constant weight had been reached. Dried specimens were grind into small pieces and stored for stable isotope analysis.

About 1 to 2 mg of ground tissue was used to determine δ^{13}C and δ^{15}N, in which the samples were

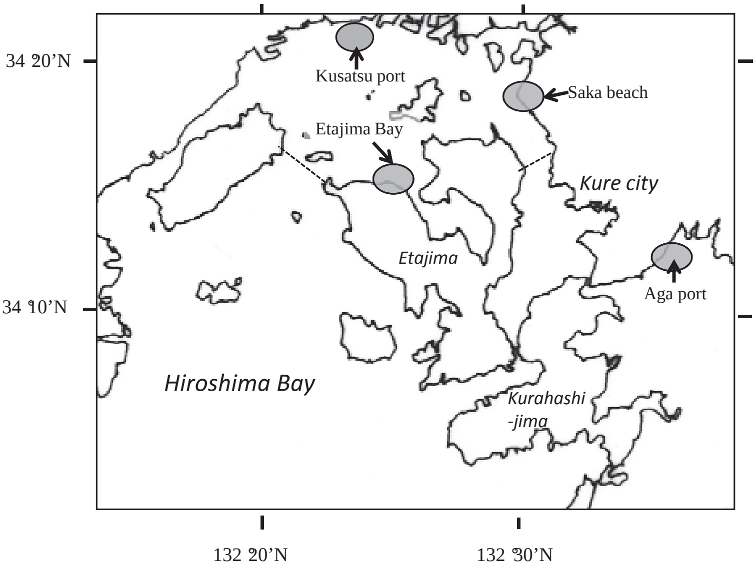

Fig. 1. Sampling sites in Hiroshima Bay. The area enclosed by the broken lines on the map is northern (internal) Hiroshima Bay.

Table 1. Mean ± standard deviation (SD) of $\delta^{15}N$ and $\delta^{13}C$ for 24 aquatic species in Hiroshima Bay.

Species codes	English name	Scientific name	Sampling location*	Sample size	Feeding habit	Sampling date	TL range (cm)	BW range (g)	$\delta^{13}C$ (‰)		$\delta^{15}N$(‰)	
									Means (SD)	Range	Means (SD)	Range
1	Yellowfin black seabream		SB	3	omnivore	10/24/2012	37.7 - 39.5	739 - 1043	-15.8 ± 0.2	-16.0 -15.7	16.1 ± 0.3	15.8 - 16.3
2	Black seabream		EB	3	omnivore	10/24/2012	18.3 - 36.7	101 - 724	-16.9 ± 0.5	-17.5 -16.6	15.5 ± 0.1	15.4 - 15.5
3	Red seabream		EB	3	omnivore	10/24/2012	11.6 - 18.9	27 - 98	-15.3 ± 0.4	-15.7 -15.0	16.2 ± 0.4	15.8 - 16.4
4	Japanese whiting		EB	3	omnivore	10/24/2012	13.1 - 16.4	17 - 31	-15.3 ± 0.3	-15.7 -15.1	16.4 ± 0.2	16.2 - 16.7
5	Black rockfish		EB	3	carnivore	10/24/2012	11.6 - 15.1	27 - 55	-17.4 ± 0.3	-17.7 -17.2	15.5 ± 0.3	15.2 - 15.8
6	Pearl-spot chromis		EB	3	omnivore	10/24/2012	10.8 - 11.2	21 -22	-17.6 ± 0.8	-18.5 -17.0	15. 6 ± 0.7	14.8 - 16.0
7	Largescale blackfish		SB	3	herbivore	10/18/2012	18.7 - 21.9	144 -188	-16.6 ± 0.7	-17.3 -15.8	14.7 ± 0.3	14.4 - 15.0
8	Jack mackerel/ Caranginae		SB	3	carnivore	10/19/2012	10.6 - 14.6	13 - 32	-16.7 ± 0.8	-17.6 -16.0	15.6 ± 1.3	14.4 - 17.0
9	Wrasse		EB	3	carnivore	10/24/2012	12.6 - 13.7	24 - 33	-16.3 ± 0.6	-17.0 -16.0	15.2 ±0.4	14.7 - 15.6
10	Multicolorfin rainbowfish		EB	3	carnivore	10/24/2012	17.9 - 21.4	68 - 117	-16.2 ± 0.4	-16.6 -15.8	15.9 ± 0.2	15.7 - 16.0
11	Whitespotted conger		EB, SB	3	carnivore	10/24/2012	32.7 - 43.5	40 - 118	-17.3 ± 1.3	-18.5 -15.9	16.5 ± 0.9	15.8 - 17.5
12	Big-eye sardine		SB	3	omnivore	10/24/2012	15.7 - 16.8	31 - 42	-15.6 ± 0.4	-16.1 -15.2	16.4 ± 1.0	15.7 - 17.6
13	Japanese stingfish		EB, KB	3	carnivore	10/24/2012	13.7 - 18.7	38 - 136	-15.7 ± 0.9	-16.6 -14.8	16.8 ± 0.7	16.2 - 17.5
14	Silver croaker		AP	3	carnivore	10/24/2012	24.5 - 25.5	181 - 265	-16.5 ± 0.7	-17.3 -16.0	16.4 ± 0.4	16.1 - 16.8
15	Grass Puffer		AP	3	carnivore	10/24/2012	9.7 - 10.7	12-21	-16.0 ± 0.4	-16.4 -15.8	14.9 ± 0.3	14.7 - 15.2
16	Largehead hairtail		AP	3	carnivore	10/24/2012	70.5 - 77.1	193 - 246	-16.1 ± 0.5	-16.7 -15.6	16.7 ± 0.1	16.6 - 16.8
17	Japanese halfbeak		EB, SB	3	plankton feeder	10/24/2012	15.7 -26.1	11-57	-17.6 ± 1.4	-19.2 -16.5	14.4 ± 1.2	13.1 - 15.4
18	Japanese horse mackerel		EB	3	carnivore	10/24/2012	14.7 - 16.2	35 - 43	-16.0 ± 0.1	-16.0 -16.0	16.1 ± 0.3	15.9 - 16.4
19	Japanese surfperch		EB, AP	3	plankton feeder	10/24/2012	11.8 - 16.3	23 - 80	-16.5 ± 0.3	-16.8 -16.2	15.0 ± 0.5	14.7 - 15.5
20	Japanese anchovy		SB	3	plankton feeder	10/18/2012	7.3 - 11.6	2.5 - 7.3	-16.4 ± 0.6	-17.1 -16.1	14.4 ± 1.0	13.7 - 15.6
21	Slender lizardfish		SB	3	carnivore	10/15/2012	30.2 - 31.7	173 - 197	-16.2 ± 0.5	-16.6 -15.7	16.2 ± 1.3	14.8 - 17.1
22	Oval squid		EB	5	carnivore	12/7/2012	15 - 20	150 - 280	-14.8 ± 0.6	-15.7 -14.2	17.3 ± 1.0	16.6 - 19.1
23	Cuttlefish		EB	5	omnivore	12/7/2012	-	250-300	-16.5 ± 0.2	-16.7 -16.3	13.8 ± 0.3	13.4 - 14.0
24	Common octopus		KP	5	carnivore	10/7/2012	-	750-800	-16.2 ± 0.1	-16.4 -16.0	17.1 ± 0.1	17.0 - 17.2

*Sampling location: EB = Etajima Bay, SB = Saka beach, KP = Kusatsu port, AP = Aga port combusted using Finnigan Conflo II open split interface through continuous flow to a Finnigan Mat 252 isotope-ratio mass spectrometer. Stable isotope abundance was measured by comparing the ratio of the two most abundance isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Stable isotope was expressed using the equation:

where X is ^{13}C or ^{15}N and R is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The values for $\delta^{15}N$ and $\delta^{13}C$ in 24 aquatic species were compared with the post-hoc Tukey's multiple comparisons after ANOVA analysis.

RESULTS AND DISCUSSION

Stable isotope signatures of the 21 finfish species and 3 cephalopods in Hiroshima Bay are generally well separated using both $\delta^{13}C$ and $\delta^{15}N$ (Fig.2). The $\delta^{15}N$ ranged from 13.8‰ at to 17.3‰ at (Table 1). Within finfish, and had the lowest mean values of $\delta^{15}N$ (14.4‰)

understanding the food web of fish inhabiting at Hiroshima Bay in near future.

Otherwise, δ^{15}N in several benthic food-chain such as , were recorded at 16.2 ± 0.4‰, 15.5 ± 0.3‰ and 15.9 ± 0.2‰ in Hiroshima Bay, compared to 17.4 ± 0.2‰, 19.2 ± 0.3‰ and 18.8 ± 0.9‰ at Hibiki-nada (Kagawa Prefecture) in Seto Inland Sea (Nakashima et al., 2007). Nakashima et al. (2007) mentioned that, the high δ^{15}N in their study is due to increase of organic matter with high δ^{15}N such as feed used for fish culture or the organic matter from eutrophic river. In addition, the δ^{15}N of , , , , and in the present study were ranged from 14.7‰ to 16.8‰. Similarly, Takai et al. (2002) revealed the δ^{15}N for several fishes in northern Hiroshima Bay such as , , , , and were ranged from 13.1‰ to 17.5‰. The value of 16.8 ± 1.5‰ for δ^{15}N of in the Kii Channel (Doiuchi et al, 2012) also recorded similar value as in the present study.

The δ^{13}C and δ^{15}N for in Hiroshima Bay were noted at -16.9 ± 0.5‰ and 15.5 ± 0.1‰, respectively. These values were in accordance with the previous study by Fujita et al. (2011) that δ^{13}C and δ^{15}N in the same species at Hiroshima Bay recorded at -15.9‰ and 16.6‰, respectively. Among Sparidae species, a post-hoc Tukey's multiple comparisons test revealed that no difference in the δ^{13}C between and (0.315). However both species were significantly more enriched in δ^{13}C than (0.020 and 0.004, respectively). Post-hoc Tukey's multiple comparisons test analysis also revealed that δ^{15}N for was significantly depleted compared to (0.05). Moreover, δ^{15}N for was not significantly differ neither nor (0.055 and 0.967, respectively).

In order to support findings of this research, the previous stomach content analysis using visual inspection of three Sparidae demonstrated that the three species had little overlapping prey categories within their diets (Shimamoto and Watanabe, 1994; Blanco Gonzalez et al., 2008). According to Blanco Gonzalez et al. (2008), fed mainly on bivalves, shrimp and seaweed, while Shimamoto and Watanabe (1994) mentioned that ate predominantly pisces and crustacean. Findings from the previous study on feeding preference of each species explained the different δ^{15}N and δ^{13}C between the two species. Thus, δ^{15}N and δ^{13}C, was suggested to prefer polychaets and bivalves in their diet. The preferences for a distinct prey category contribute to reducing the feeding overlap amongst the species; therefore, the stable isotope analysis can be used at least in part as a tool to differentiate them according to food preference.

Slight variation of δ^{15}N found in this study also can be explained by the complex food webs of inshore area in Hiroshima Bay where the variation of trophic levels is high, in which allow for additional δ^{15}N fractionations and more enriched δ^{15}N. Takai et al. (2002) suggested that, the diverse feeding of the fish during their stay in the Bay increased both of their δ^{13}C and δ^{15}N. These also support the δ^{13}C data, with France (1995) mentioned that the δ^{13}C from inshore food webs tend to be more δ^{13}C enriched than those from offshore environment. Hence, those report suggested that fishes in Hiroshima Bay posses complex food webs of inshore area due to the variation of trophic levels.

In conclusion, this study provides basic information of stable isotope value and trophic position of dominant fish species such as and in Hiroshima Bay. This study also suggested that the varied aquatic species poses different trophic status because of difference and complexity of their diet. The high variation of trophic status suggested a reflection of feeding habit and great variety of food organisms that might be one way in which those species are able to coexist.

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炭素窒素安定同位体比を用いた広島湾の海産生物24種の栄養段階の推定

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要　旨　本研究は広島湾に生息する魚類や頭足類などの栄養段階を炭素・窒素安定同位体分析を用いて明らかにした。分析した魚類の中で最もδ^{15}N値が低かったのはカタクチイワシとサヨリの14.4‰で, 逆に高かったのはカサゴの16.8‰であった。δ^{13}C値が低かったのはサヨリとスズメダイの -17.6‰で，高かったのはマダイとシロギスの -15.3‰であった。頭足類を加えると，アオリイカのδ^{15}N値とδ^{13}C値は最も高く，それぞれ17.3‰と -14.8‰であった。このような種間の栄養段階の違いは，食性や栄養源の違いを反映していると考えられた。本研究結果は，瀬戸内海でも屈指の漁場として知られている広島湾において，魚類資源の持続的利用を行うために有益な知見となるであろう。

キーワード：安定同位体分析，栄養段階，魚類，広島湾