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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 *Engraulis japonicus* and *Hyporhamphus sajori* while the highest mean $\delta^{15}N$ of 16.8‰ was recorded for *Sebasticus marmoratus*. The lowest and highest mean $\delta^{13}C$ were noted -17.6‰ for *Chromis notata* and *H. sajori*, and -15.3‰ for *Pagrus major* and *Sillago japonica*, respectively. Including with cephalopods, the highest mean $\delta^{13}C$ and $\delta^{15}N$ noted at -14.8‰ and 17.3‰ for *Sepioteuthis lessoniana*. 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 $\delta^{15}N$ between a consumer and its diet, in which can be understood as trophic fractionation is approximately 3.4‰. Otherwise, the carbon usually less fractionated at source production (Peterson and Fry, 1987) 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°C, ranging from 9°C in March to 29°C 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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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 *Acanthopagrus* fish found, in which are *A. schlegelii* (Blanco Gonzalez et al., 2008; Umino et al., 2011) and *A. latus* (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, *A. schlegelii* 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, *Sepioteuthis lessoniana*, cuttlefish, *Sepia esculenta* and octopus, *Octopus vulgaris* 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°C 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 $\delta^{13}C$ and $\delta^{15}N$, in which the samples were

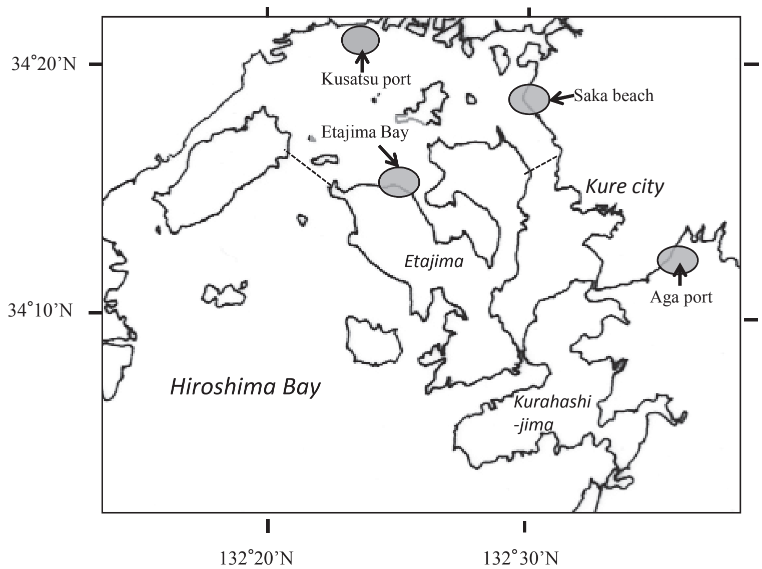

Fig. 1. Sampling sites in Hiroshima Bay. The area enclosed by the broken lines on the map is northern (internal) Hiroshima Bay.

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	*Acanthopagrus latus*	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	*Acanthopagrus schlegelii*	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	*Pagrus major*	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	*Sillago japonica*	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	*Sebastes inermis*	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	*Chromis notata*	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	*Girella punctata*	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	*Decapterus maruadsi*	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	*Halichoeres tenuispinis*	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	*Halichoeres poecilopterus*	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	*Conger myriaster*	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	*Etrumeus teres*	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	*Sebasticus marmoratus*	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	*Pennahia argentata*	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	*Takifugu niphobles*	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	*Trichiurus japonicus*	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	*Hyporhamphus sajori*	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	*Trachurus japonicus*	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	*Ditrema temmincki temmincki*	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	*Engraulis japonicus*	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	*Saurida elongata*	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	*Sepioteuthis lessoniana*	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	*Sepia esculenta*	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	*Octopus vulgaris*	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:

$$\delta X = \left[\frac{\text{R sample}}{\text{R standard}} - 1 \right] \times 1000$$

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 *S. esculenta* to 17.3‰ at *S. lessoniana* (Table 1). Within finfish, *Engraulis japonicus* and *Hyporhamphus sajori* had the lowest mean values of $\delta^{15}N$ (14.4‰), while *Sebasticus marmoratus* poses highest $\delta^{15}N$ (16.8 ± 0.7‰). Variation in trophic position can be explained by their prey preference (Table 1). For instance, *E. japonicus* eat mainly zooplankton (Islam and Tanaka, 2009), while *S. marmoratus* prefer fishes and crab as their main prey items (Fujita and Kohda, 1996). Bulman et al. (2001) explained that the fish with a high $\delta^{15}N$ generally had a high proportion of fish in their diet or they ate other species (e.g., polychaetes) with a high $\delta^{15}N$. Furthermore, in general, the larger fish whether within or between species had a higher $\delta^{15}N$ than smaller fish. This is due to the fact that the larger sized fish, the more the opportunity for feeding on large prey and selecting from a greater variety of prey species (Davenport and Bax, 2002). Meanwhile, according to Davenport and Bax (2002), $\delta^{13}C$ can be used to distinguish them according to primarily benthic or pelagic feeding modes. Hence, result of this study will provide basic information in

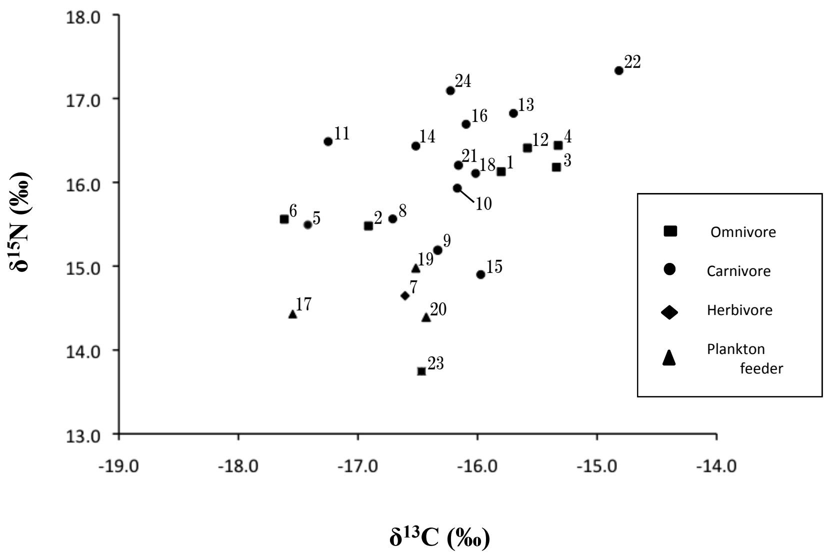

Fig. 2. Stable isotope signatures of the 24 aquatic species in Hiroshima Bay. Superscript number on the symbols are corresponding with species codes in Table 1.

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

Otherwise, δ^{15}N in several benthic food-chain such as *Pagrus major*, *Sebastes inermis and Halichoeres poecilopterus* 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 *S. inermis*, *Trachurus japonicus*, *Ditrema temmincki temmincki*, *Halichoeres tenuispinnis*, *H. poecilopterus* and *Trichiurus japonicus* 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 *S. inermis*, *Trachurus japonicus*, *D. temmincki temmincki*, *H. tenuispinnis*, *H. poecilopterus* and *Trichiurus japonicus* were ranged from 13.1‰ to 17.5‰. The value of 16.8 ± 1.5‰ for δ^{15}N of *Trichiurus japonicus* 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 *A. schlegelii* 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 *A. latus* and *P. major* (p=0.315). However both species were significantly more enriched in δ^{13}C than *A. schlegelii* (p=0.020 and 0.004, respectively). Post-hoc Tukey's multiple comparisons test analysis also revealed that δ^{15}N for *A. schlegelii* was significantly depleted compared to *P. major* ($p<0.05$). Moreover, δ^{15}N for *A. latus* was not significantly differ neither *A. schlegelii* nor *P. major* (p=0.055 and p=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), *A. schlegelii* fed mainly on bivalves, shrimp and seaweed, while Shimamoto and Watanabe (1994) mentioned that *P. major* 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, *A. latus* 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 *A. schlegelii* and *A. latus* 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 $\delta^{15}N$ 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。$\delta^{13}C$ 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 $\delta^{15}N$ 値と $\delta^{13}C$ 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