Note

Spatial variation of carbon and nitrogen stable isotope ratios in Japanese anchovy, *Engraulis japonicus*, in the eastern Seto Inland Sea, Japan

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Abstract: The isotopic variations in the populations of the Japanese anchovy, *Engraulis japonicus*, in three sea areas of the eastern Seto Inland Sea were examined. The carbon and nitrogen isotope ratios for Japanese anchovy differed among the sea areas. The results showed much greater isotopic variations in the populations in Harima-Nada and Kii Channel than Osaka Bay. Moreover, clustering analysis showed that the populations in Kii Channel and Harima-Nada consisted of two subgroups that are isotopically different, suggesting that the populations consist of groups of different feeding history. The spatial isotopic variations of the anchovies in our results may help us to understand the migration behavior of the Japanese anchovy in the eastern Seto Inland Sea.

Key words: Japanese anchovy; Stable isotope; Spatial variation; Seto Inland Sea

Migration is one of the important life-history traits for many fish species. Many fishes migrate among habitats throughout their life for various reasons, including feeding, predation avoidance, reproduction and ontogenetic changes in their resource requirements (Dingle and Drake 2007). The migration greatly affects not only individual fitness but also their population structure. Thus, understanding migration is a key issue in the biological conservation of fish populations. However, it is difficult to directly track movement of fishes underwater. Therefore, a biological assay is necessary to ascertain the migration route among habitats (Hobson 1999).

The Japanese anchovy, *Engraulis japonicus*, is one of the most important commercial fish species in Japan. Japanese anchovy is thought to comprise several main stocks around Japan, and intrapopulation differences in reproductive characteristics exist due to the prolonged spawning season and large spawning areas extending from inshore to offshore regions (Funamoto et al. 2004; Takasuka et al. 2005). Therefore, it is thought that the stocks comprise many regional and seasonal populations (Tanaka et al. 2010). The Seto Inland Sea, which is one of the main habitats and spawning areas for Japanese anchovy (Zenitani et al. 2007), is connected to the Pacific Ocean via channels. It is a semi-closed sea, famous for high productivity of fishes. It is believed that several local populations of Japanese anchovy inhabit the Seto Inland Sea (Kono and Zenitani 2008). These local populations migrate among the sea areas partitioned by islands and capes within the Seto Inland Sea and between the Seto Inland Sea and the Pacific Ocean to meet their resource
requirements. However, their migration behavior within the Seto Inland Sea remains unclear.

Stable isotope analysis has been used to study the movements of various fish species (Cunjak et al. 2005; Carlisle et al. 2012). This technique is based on the premise that primary producers tend to exhibit distinctive isotopic signatures that are propagated through the food web (Hobson 1999). The Seto Inland Sea has various marine environments in terms of geographical and spatial variations in anthropogenic nitrogen loading (Miller et al. 2010). The Japanese anchovy migrates between spawning and feeding habitats. Because the isotopic signatures of muscle tissues of subadult and adult clupeoid fishes can require over several months for equilibration to a constant diet (Miller 2007), the tissues should have the integrated information of the isotopic base lines of each area. Therefore, if a population comprises individuals of several different feeding histories associated with their migration, it is expected that the variation in the isotopic signatures in the population exhibit a larger isotopic variation.

We analyzed the nitrogen and carbon stable isotope ratios (δ¹⁵N and δ¹³C, respectively) in the muscle tissues of the Japanese anchovy from three areas in the eastern Seto Inland Sea to examine the variations of the isotopic signatures in each sea areas. If a population comprises individuals of several different feeding histories, the isotopic signatures may be divided into several groups.

**Materials and Methods**

The specimens of anchovy were sampled from commercial catches (seine net fishery) in Harima-Nada, Osaka Bay and Kii Channel in the eastern Seto Inland Sea (Fig. 1, Table 1) in autumn (September–October) in 2008. We excised muscle tissues from the body of each fish for the stable isotope analysis. Tissues were frozen at −20°C until the analysis.

For stable isotope analysis, tissue 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. The drying of samples was repeated at 60°C for 24 h. The dried samples were wrapped with tin capsule, and the carbon and nitrogen stable isotope ratios were measured using a mass spectrometer (ANCA-GSL; Sercon, UK).

The stable isotope ratios were expressed in δ notion and defined as the per mill (‰) deviation from the standard as follows:

![Fig. 1. Sampling areas and the three sea areas referred to in this study. Shaded areas represent sampling areas of Japanese anchovy in the each sea areas.](image-url)
$\delta^{13}C$ or $\delta^{15}N$ ($\%$) = ($R_{\text{sample}}/R_{\text{standard}} - 1$) × 1000
where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard substances were Pee Dee Belemnite limestone carbonate for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Analytical errors of reproducibility were ±0.3‰ for both $\delta^{15}N$ and $\delta^{13}C$.

We used the model-based clustering method with the Mclust software package (Fraley and Raftery 2006) for R (R Development Core Team 2012) to identify sub-groups in the anchovy populations based on the values of stable isotope ratios. This method fits the observed frequency distribution of these values to a series of alternative models incorporating one or mixtures of several Gaussian distributions. The Mclust software estimates the optimal number of clusters based on Bayesian information criteria (BIC). In this software, higher BIC values indicate stronger evidence for the optimal number of clusters (Fraley and Raftery 2006). In the present study, we assumed that the volumes and the shapes of all clusters were variable.

**Results**

The results of stable isotope analyses for Japanese anchovy in each sea area are shown in Tables 2. The $\delta^{13}C$ in Harima-Nada and Osaka Bay ranged from $-16.4\%$ to $-14.5\%$ and from $-15.9\%$ to $-14.4\%$, respectively. The $\delta^{13}C$ and $\delta^{15}N$ of Japanese anchovy showed wide variations within each area (Table 2), and exhibited larger variations in Kii Channel (quartile deviation: $\delta^{13}C = 0.53\%$ and $\delta^{15}N = 0.58\%$) and Harima-Nada (quartile deviation: $\delta^{13}C = 0.55\%$ and $\delta^{15}N = 1.26\%$) than in Osaka Bay (quartile deviation: $\delta^{13}C = 0.24\%$ and $\delta^{15}N = 0.39\%$). For Kii Channel and Harima-Nada, the clustering analysis based on the isotopic values showed that the model with two components had the highest BIC value (Kii Channel: -376.7, Harima-Nada: -229.0), indicating that the populations are divided into two sub-groups (Fig. 2 and 3). The clustering analysis showed that the model with one component had the highest BIC value for Osaka Bay ($-63.4$), indicating that the population of Osaka Bay comprised one group.

In Harima-Nada, the $\delta^{15}N$ and $\delta^{13}C$ of the sub-group 1 ranged from 16.5% to 19.5% and from $-16.4\%$ to $-14.9\%$, respectively. The $\delta^{15}N$ and $\delta^{13}C$ of the sub-group 2 ranged from 20.1% to 24.3% and from $-15.3\%$ to $-14.5\%$, respectively. The $\delta^{15}N$ of the sub-group 2 was higher than those of sub-group 1. Although the range of $\delta^{13}C$ in sub-group 1 overlapped with those in sub-group 2, the mean $\delta^{13}C$ ($-15.0\%$) of sub-group 2 was higher than those of sub-group 1 ($-15.6\%$). In Kii Channel, $\delta^{15}N$ and $\delta^{13}C$ of the sub-group 1 ranged from 10.6% to 18.0% and from $-19.9\%$ to $-16.6\%$.

### Table 1. Sampling collection data and body length of *Engraulis japonicus* for stable isotope analysis

<table>
<thead>
<tr>
<th>Sea area</th>
<th>Date</th>
<th>n</th>
<th>Total length range (mm)</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osaka Bay</td>
<td>2-Oct-08</td>
<td>19</td>
<td>82.0</td>
<td>69.0</td>
<td>93.0</td>
<td></td>
</tr>
<tr>
<td>Harima-Nada</td>
<td>22-Oct-08</td>
<td>49</td>
<td>86.0</td>
<td>76.3</td>
<td>102.8</td>
<td></td>
</tr>
<tr>
<td>Kii Channel</td>
<td>10-Sep-08</td>
<td>77</td>
<td>89.2</td>
<td>74.5</td>
<td>142.1</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Carbon and nitrogen stable isotope ratios of *Engraulis japonicus* collected in Osaka Bay, Harima-Nada and Kii Channel

<table>
<thead>
<tr>
<th>Sea area</th>
<th>n</th>
<th>$\delta^{15}N$ (%)</th>
<th>$\delta^{13}C$ (%)</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Quantile deviation</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Quantile deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osaka Bay</td>
<td>19</td>
<td>16.4</td>
<td>-15.1</td>
<td>15.4</td>
<td>17.3</td>
<td>0.39</td>
<td>0.24</td>
<td>-15.9</td>
<td>-14.4</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Harima-Nada</td>
<td>49</td>
<td>18.9</td>
<td>-15.3</td>
<td>16.5</td>
<td>24.3</td>
<td>1.26</td>
<td>0.55</td>
<td>-16.4</td>
<td>-14.5</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Kii Channel</td>
<td>77</td>
<td>16.0</td>
<td>-16.8</td>
<td>10.6</td>
<td>18.0</td>
<td>0.58</td>
<td>0.53</td>
<td>-19.9</td>
<td>-16.2</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>
respectively. The $\delta^{15}N$ and $\delta^{13}C$ of the sub-group 2 ranged from 15.5‰ to 17.3‰ and from −17.0‰ to −16.2‰, respectively. The $\delta^{15}N$ and $\delta^{13}C$ of the sub-group 1 was lower than those of sub-group 2.

Discussion

Higher $\delta^{13}C$ values were observed in Harima-Nada and Osaka Bay than in Kii Channel. This result is consistent with the results of previous studies in the studied areas. High $\delta^{13}C$ values were found in anchovies sampled from the inner part of Seto Inland Sea, such as Osaka Bay (−15.9‰: Mishima and Hoshika 2002), Hiroshima Bay (ca. −16.5‰ to −14.5‰: Takai et al. 2002) Hiuchi-Nada (−16.6‰: Nakashima et al. 2007). On the other hand, anchovies sampled from Kii Channel showed relatively low $\delta^{13}C$ values (−18.5‰ to −16.0‰: Doiuchi et al. 2011). The spatial trend in $\delta^{13}C$ values in the eastern Seto Inland Sea may reflect difference in the extent of contribution of benthic carbon production to the anchovies among sea areas as previously suggested by Shibata et al. (2012).

The median $\delta^{15}N$ of Japanese anchovy was highest in Harima-Nada, followed by Osaka Bay and Kii Channel. The $\delta^{15}N$ of particulate organic matter and zooplankton tend to become higher toward the inner part of Seto Inland Sea compared with the Pacific Ocean because of the influence of anthropogenic nitrogen inputs (Miller et al. 2010), whereas the $\delta^{15}N$ baseline in the Pacific Ocean tends to be lower because of the influence of $^{15}N$-depleted inorganic nitrogen fixed in the Kuroshio water (Liu et al. 1996). The spatial trend of the $\delta^{15}N$ of the
anchovies in our study may reflect the difference in the $\delta^{15}$N baselines of each sea areas.

In this study, the Japanese anchovy in Kii Channel and Harima-Nada exhibited larger isotopic variations within the population than those in Osaka Bay. Moreover, the results of clustering analysis showed that the anchovy populations in Kii Channel and Harima-Nada consisted of two isotopically different sub-groups. These results suggest that groups of different feeding history exist within the population. The sub-group 2 in Harima-Nada showed $\delta^{15}$N values (20.1‰ to 24.3‰) higher than those reported for piscivorous fishes in Harima-Nada (e.g., sea bass Lateolabrax japonicus; 19.9‰, Nakashima et al. 2007). Lowest $\delta^{13}$C (−19.9‰) and $\delta^{15}$N (10.6‰) were found in sub-group 1 in Kii Channel, which were lower than those of Japanese anchovy sampled in the inner part of Kii Channel in autumn ($\delta^{13}$C: ca. −17‰ to −15‰, $\delta^{15}$N: ca. 13‰ to 18‰) (Yasue et al. 2013). This discrepancy between our results and previous reports and the large isotopic variations observed in Harima-Nada and Kii Channel may be attributable to the migration of the anchovy population from other areas that have isotopically different baselines.

In this study, the carbon and nitrogen isotope ratios for Japanese anchovy differed among three sea areas in the eastern Seto Inland Sea. Moreover, our results showed much greater isotopic variations in the populations in Harima-Nada and Kii Channel than Osaka Bay. Recently, a technique based on the isotopic patterns across landscapes, termed “isoscales” has been recognized as a useful tool to infer animal origins and migration (Hobson and Norris 2008). Such an integrated technique and the spatial isotopic variations in our results may help us understand the migration behavior of the Japanese anchovy.

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References


東部瀬戸内海に生息するカタクリワシ *Engraulis japonicus* における炭素・窒素安定同位体比の空間的変化

濱岡秀樹・曾我部篤・大森浩二

瀬戸内海東部の3つの海域で採取されたカタクリワシ *Engraulis japonicus* 個体群の炭素・窒素安定同位体比を調査した。その結果、カタクリワシの炭素・窒素安定同位体比は海域間で異なっていた。大阪湾に比べ播磨灘と紀伊水道の個体群内で大きさなばらつきが見られた。また、クラスタリング解析によってこれらの安定同位体比の分布は2つのサブグループに分けられることが明らかとなった。この結果は播磨灘および紀伊水道のカタクリワシ個体群の中に異なる摂餌履歴をもつ2つの集団が存在することを示している。本研究で見られた東部瀬戸内海におけるカタクリワシの安定同位体比の空間的な変化は将来的にカタクリワシの回遊を理解するうえで役立つだろう。