Spatial Variation in Feeding Habits and Carbon Source of Cutlassfish *Trichiurus japonicus* in the Western Seto Inland Sea, Japan

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**Abstract:** To identify spatial variations in the feeding habits of *Trichiurus japonicus* in the western Seto Inland Sea, Japan, we examined the carbon and nitrogen stable isotope ratios and the stomach contents of 256 specimens collected from four different sea areas. Stomach content analysis showed that the feeding habits of *T. japonicus* differed among sea areas, which may have been caused by spatial and temporal bias in the stomach contents. Additionally, trophic levels based on stable isotope analysis and the expected trophic niche by stomach content analysis did not agree. Greater sampling effort is needed to understand the feeding habits of fish considering spatio-temporal variations in the stomach contents of specimens. Using stable isotope analysis, we detected variations in the efficiencies of benthic and pelagic trophic carbon pathways, which were not detected by stomach content analysis, among sea areas. This result coincides with that of a previous study for primary production structure in the western Seto Inland Sea. Stable isotope analysis helps us effectively understand the feeding habits of fish with fewer efforts. These results will probably be useful in understanding the feeding ecology of *T. japonicus*.

**Key words:** *Trichiurus japonicus*; Feeding habits; Stable isotope; Carbon source

*Trichiurus japonicus* commonly inhabits the coastal area of the Japanese archipelago except the Ryukyu Islands and the northern part of Hokkaido Island. *T. japonicus* is a high trophic level consumer in coastal ecosystems and is one of the most important commercial species throughout East Asia (Nakamura et al. 1984; Rin 1993; Chakraborty et al. 2007). Catch volumes of *T. japonicus* have recently declined in the seas around Japan and in the East China Sea, and the fish stock is considered to be in poor condition (Park et al. 2001; Sanada et al. 2011). Although the western Seto Inland Sea is a popular fishery site for *T. japonicus* in Japan, the catch has been decreasing within the last few decades (Doiuchi et al. 2011; Sanada et al. 2011). Therefore, effective management and conservation strategies based on ecological knowledge (e.g., life history and feeding habits) are necessary for sustaining *T. japonicus* populations.

The quality and quantity of available prey has affected reproduction and growth of *T. japonicus*. Changes in the composition of available prey have considerable effects on fish population dynamics, including ontogenetic growth, sexual maturity, and reproductive success (Beverton and Holt 1957; Backiel and Le Cren 1978; Persson and De Roos 2006). Thus, an understanding of feeding habits is very important for resource management and conservation. *T. japonicus* shifts its diet from small crustaceans (e.g., copepods and mysids) to fish (e.g., *Engraulis japonicus*) during ontogenetic growth (Munekiyo and Kuwahara 1985); however, it reportedly feeds on various prey items...
(e.g., fish, shrimp, and squid) depending on spatial and temporal changes in prey composition (Chiou et al. 2006). The western Seto Inland Sea comprises areas with varying environmental characteristics and biotic communities, and feeding habits may vary depending on the difference in the characteristics of each sea area. Yoshida et al. (2010) showed the importance of accounting for differences in environmental and ecological characteristics among areas for the management and conservation of aquatic resources in the western Seto Inland Sea. Thus, it is important to understand the spatial variability in the feeding habits of *T. japonicus* among areas in the western Seto Inland Sea. However, few studies are available on this topic, except for some studies conducted in the Kii Channel in the eastern part of the western Seto Inland Sea (Sakamoto 1982; Doiuchi et al. 2011).

Stomach content analysis is the most commonly used technique for investigating feeding habits. Stomach content analyses provide detailed information on the feeding habits of focal species, but reflect only the transient diet (Vander Zanden et al. 1997). Therefore, substantial efforts must be expended to quantitatively evaluate feeding habits at the population and species levels. Aquatic ecologists have recognized stable isotope analysis as a useful, alternative tool for quantitatively evaluating feeding habits. Applying this analysis relies on the empirical rule that stable isotope ratios of carbon and nitrogen in animal tissue reflect those of their prey, plus a given fractionation factor through trophic transfer of organic matter from prey to consumer (DeNiro and Epstein 1978; Fry and Sherr 1984; Minagawa and Wada 1984; Gannes et al. 1997). Based on the principles of isotopic enrichment, it is possible to estimate the trophic positions of consumers and the relative importance of specific prey (or primary producers) to them. Moreover, the isotopic signatures of slow turnover tissues in consumers can provide time-integrated information on the long-term feeding habits (Hobson 1999; Vander Zanden and Vadeboncoeur 2002; Hamaoka et al. 2010). Stable isotope analyses can be used to quantitatively estimate trophically assimilated materials, but are not suitable for identifying specific prey species. The effectiveness of stable isotope analyses is improved when used in combination with conventional dietary analyses (Vander Zanden et al. 1997; Hamaoka et al. 2010). In this study, we examined the feeding habits of *T. japonicus* in four areas in the western Seto Inland Sea, and we used stable isotope analysis and supplementary stomach content data to reveal the trophic position and the carbon source of this species.

### Materials and Methods

**Study sites**

The western Seto Inland Sea is a semi-enclosed coastal sea surrounded by Honshu, Shikoku, and Kyushu Islands. The sea is divided by a number of islands and peninsulas into a number of wide sea areas called “Nada” in Japanese. The present study was conducted in four sea areas in the western Seto Inland Sea (Suo-Nada, Iyo-Nada, Aki-Nada, and the Bungo Channel) that have varying environmental characteristics (Fig. 1, Table 1).

**Specimens**

Specimens of *T. japonicus* were sampled from commercial catches (seine net fisheries) in each sea area (Suo-Nada: *n* = 56; Iyo-Nada: *n* = 82; Aki-Nada: *n* = 26; and the Bungo Channel: *n* = 116), from June to July in 2009 and 2010. Preanal lengths of the specimens were measured to the nearest 1 mm. Because populations of *T. japonicus* mainly comprised two size classes (Fig. 2), we separated the specimens into two groups: large fish (preanal length ≥ 200 mm) and small fish (preanal length < 200 mm) to statistically analyze trophic position and ontogenetic shifts in the feeding habits of *T. japonicus*. We collected small fish only in Iyo-Nada and the Bungo Channel. After measuring preanal length, we excised muscle tissue from the body, and the tissues were dried at 60°C for 24 h. We dissected specimens and preserved the collected stomach contents at −20°C until analyses could be conducted.

The main pelagic and benthic primary producers in the western Seto Inland Sea were phytoplankton and benthic microalgae, respectively. We collected particulate organic matter (POM) and epilithic organic matter (EOM) as proxies
Spatial Variation in Feeding Habit of Cutlassfish for phytoplankton and benthic microalgae from May to July in 2008 and 2009 from each sea area.

Twenty liters of seawater was taken from the surface to collect POM. In the laboratory, water samples were filtered onto precombusted GF/F glass filters (Whatman Ltd., Maidstone, UK) through a 100 μm mesh. Microscopic observations confirmed that POM almost entirely consisted of phytoplankton. The EOM samples were collected from subtidal zones in each sea area. We collected submerged pebbles from the bed along the shore and exfoliated EOM from

Table 1. Environmental characteristics of each region in the western Seto Inland Sea

<table>
<thead>
<tr>
<th>Sea area</th>
<th>Surface area (km²)</th>
<th>Mean depth (m)</th>
<th>Volume ($\times 10^8$ m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aki-Nada</td>
<td>744</td>
<td>39.9</td>
<td>297</td>
</tr>
<tr>
<td>Iyo-Nada</td>
<td>4009</td>
<td>55.7</td>
<td>2232</td>
</tr>
<tr>
<td>Suo-Nada</td>
<td>3805</td>
<td>24.1</td>
<td>917</td>
</tr>
<tr>
<td>Bungo Channel</td>
<td>2744</td>
<td>71.8</td>
<td>1970</td>
</tr>
</tbody>
</table>

Fig. 1. Map of the study area. Squares indicate sampling sites. (p) particulate organic matter, (e) epilithic organic matter, and (t) *Trichiurus japonicus*.

Fig. 2. Frequency histogram of *Trichiurus japonicus* of varying preanal lengths (mm).
the pebbles with a brush (after rinsing sediment from the stones with seawater). The EOM samples were trapped on precombusted GF/F glass filters. POM and EOM samples were dried at 60°C for 24 h.

**Stable isotope analysis**

Dried muscle samples were pulverized and immersed in chloroform:methanol (2:1) solution for 24 h to remove lipids for lipid correction, according to the method of Bligh and Dyer (1959).

POM and EOM were exfoliated from the glass filters and pulverized. The powdered samples were exposed to 12 N HCl vapor for 24 h to remove carbonates, then dried again at 60°C for 24 h. The dried samples were wrapped in tin capsules, and their carbon and nitrogen stable isotope ratios were measured using a mass spectrometer (ANCA-GSL, Sercon Inc., Crewe, Cheshire, UK).

The stable isotope ratios were expressed in \( \delta \) notation and defined as the ‰ deviation from the standard as follows:

\[
\delta^{13}C \text{ or } \delta^{15}N (\%e) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

Where \( R \) represents \(^{12}\text{C}/^{12}\text{C} \) or \(^{15}\text{N}/^{14}\text{N} \). The standards were Pee Dee belemnite limestone carbonate for \( \delta^{13}C \) and atmospheric nitrogen (N\(_2\)) for \( \delta^{15}N \). Analytical precision was 0.1‰ and 0.2‰ for \( \delta^{13}C \) and \( \delta^{15}N \), respectively.

We estimated the trophic level of *T. japonicus* and the proportional contribution of benthic production to *T. japonicus* in each sea area using a 2 end-member mixing model according to Post (2002) and from the stable isotope ratios of *T. japonicus*, POM, and EOM:

\[
\begin{align*}
\text{TL} & = 1 + \left( \delta^{15}\text{N}_{\text{sample}} - [\delta^{15}\text{N}_{\text{EOM}} \times \alpha + \delta^{15}\text{N}_{\text{POM}} \times (1 - \alpha)] \right) / 3.4 \\
\alpha & = \left[ \delta^{13}\text{C}_{\text{POM}} - \left( \delta^{13}\text{C}_{\text{sample}} - 0.8 \times (\text{TL} - 1) \right) \right] / \left( \delta^{13}\text{C}_{\text{POM}} - \delta^{13}\text{C}_{\text{EOM}} \right)
\end{align*}
\]

Where \( \delta^{13}\text{C}_{\text{sample}} \) and \( \delta^{15}\text{N}_{\text{sample}} \) are carbon and nitrogen stable isotope ratios in *T. japonicus*, respectively; \( \delta^{13}\text{C}_{\text{EOM}}, \delta^{15}\text{N}_{\text{EOM}}, \delta^{13}\text{C}_{\text{POM}}, \) and \( \delta^{15}\text{N}_{\text{POM}} \) are mean stable isotope ratios of EOM and POM, respectively; TL is the trophic level of *T. japonicus*; and \( \alpha \) is the proportional contribution of benthic production to *T. japonicus*. We assumed that the trophic enrichment factor was 0.8‰ for \( \delta^{13}C \) (DeNiro and Epstein 1978) and 3.4‰ for \( \delta^{15}N \) (Minagawa and Wada 1984).

**Stomach content analysis**

After thawing the frozen samples at room temperature, undigested prey items were isolated from *T. japonicus* gut content, identified to the lowest possible taxonomic level under a binocular microscope, and measured for wet weight. The relative abundance of prey was expressed as a percentage of each prey taxon to the total weight of the prey items of all stomachs in the entire sample (\( \%W \)).

**Results**

**Stable isotope analysis**

The results of stable isotope analyses for *T. japonicus*, POM, and EOM are shown in Tables 2 and 3. \( \delta^{13}C \) and \( \delta^{15}N \) of *T. japonicus* ranged from -19.9‰ to -15.2‰ and from 11.8‰ to 18.8‰, respectively (Table 3). The trophic levels of *T. japonicus* throughout the sea areas ranged from 2.6 to 4.7, and the contribution of benthic primary production ranged from 0.22 to 0.82 (Table 3, Fig. 3). The trophic levels of large fish were significantly higher than those of small fish in the Bungo Channel and Iyo-Nada (Mann–Whitney test; Bungo Channel: \( U = 1286, P < 0.001; \) Iyo-Nada: \( U = 1240, P < 0.001 \)). The difference between the benthic contribution to large and small fish was not significant in the Bungo Channel but was higher in large fish than in small fish in Iyo-Nada (Mann–Whitney test; Bungo Channel: \( U = 1528, P = 0.134; \) Iyo-Nada: \( U = 1240, P < 0.001 \)). The trophic level of large fish was highest in Iyo-Nada, followed by Aki-Nada and Suo-Nada. The trophic level of large fish differed significantly among sea areas (Kruskal–Wallis test, \( \chi^2 = 53.36, \) degree of freedom = 3, \( P < 0.001 \)); the median value in Suo-Nada was significantly lower than that in Aki-Nada and Iyo-Nada (Steel–Dwass test; Suo-Nada vs. Aki-Nada: \( t = 4.31, P < 0.001 \)).
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0.001; Suo-Nada vs. Iyo-Nada: $t = 7.52, P < 0.001$), and the median value in the Bungo Channel was significantly lower than that in Iyo-Nada (Steel–Dwass test; Bungo Channel vs. Iyo-Nada: $t = 4.24, P < 0.001$). The difference between the trophic levels in Suo-Nada and the Bungo Channel was not significant (Steel–Dwass test; Suo-Nada vs. Bungo Channel: $t = 2.33, P = 0.092$). Additionally, the difference between the trophic levels in Aki-Nada and Iyo-Nada was not significant (Steel–Dwass test; Aki-Nada vs. Iyo-Nada: $t = 2.38, P = 0.081$).

The contributions of benthic primary production to large fish differed significantly among sea areas (Kruskal–Wallis test, $\chi^2 = 147.8$, degree of freedom = 3, $P < 0.001$). The contribution in the Bungo Channel was significantly lower than that in other sea areas (Steel–Dwass test; Bungo Channel vs. Iyo-Nada: $t = 9.57, P < 0.001$; Bungo Channel vs. Suo-Nada: $t = 9.53, P < 0.001$; Bungo Channel vs. Aki-Nada: $t = 7.59, P < 0.001$). Differences between sea areas in the trophic level of small fish were not significant (Mann–Whitney test; Bungo Channel vs. Iyo-Nada: $U = 350, P = 0.330$). In contrast, the benthic contribution to small fish in the Bungo Channel was higher than that in Iyo-Nada (Mann–Whitney test; Bungo Channel vs. Iyo-Nada: $U = 471, P < 0.001$).

Table 2. Carbon and nitrogen stable isotope ratios (‰) of particulate organic matter (POM) in surface water and epilithic organic matter (EOM) among four regions in the western Seto Inland Sea

<table>
<thead>
<tr>
<th>Sea area</th>
<th>POM</th>
<th>EOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$\delta^{13}C$</td>
</tr>
<tr>
<td>Aki-Nada</td>
<td>3</td>
<td>22.2</td>
</tr>
<tr>
<td>Iyo-Nada</td>
<td>11</td>
<td>22.8</td>
</tr>
<tr>
<td>Suo-Nada</td>
<td>9</td>
<td>21.4</td>
</tr>
<tr>
<td>Bungo Channel</td>
<td>5</td>
<td>22.3</td>
</tr>
</tbody>
</table>

Fig. 3. The trophic levels of *Trichiurus japonicus* and proportional contributions of benthic primary production to fishes in the western Seto Inland Sea. White and black symbols indicate small and large fish, respectively; circles, Aki-Nada; triangles, Iyo-Nada; diamond, Suo-Nada; squares, Bungo Channel.
The diet of *T. japonicus* is shown in Table 4. Food items primarily comprised fish, crustaceans, and cephalopods. Fish, including unidentified species, dominated (>60% by mass) the diet of large fish throughout all sea areas. The ratio in the Bungo Channel was particularly high (95%). In Iyo-Nada, *Engraulis japonicus* was the most common food item (approximately 30%) after unidentified fish in the stomach contents of large fish, followed by cephalopods (approximately 17%). *E. japonicus* was a common food item (16.0%) for large fish in Suo-Nada. Species from the order Mysidae were often found (approximately 11.3%) when comparing the food items of large fish in Suo-Nada with those in other sea areas. Similarly, fish (63.9%) were the most common food item for large fish in
Aki-Nada (although we could not identify many of the fish obtained from the stomach contents), followed by crustaceans (19.8%) and cephalopods (16.5%). In the Bungo Channel, fish were the main food item for large fish. However, the most important food item for large *T. japonicus* in the Bungo Channel was smaller members of the same species (approximately 45% of total stomach contents). Cannibalism was observed only in the population in the Bungo Channel (24 of 56 large fish). A relatively large number of fish species (10) was observed in the stomach contents of large fish in the Bungo Channel (Carangidae, Sphyraenidae, Clupeidae, and others).

The stomach contents of small fish in Iyo-Nada almost entirely comprised juvenile *E. japonicus* (approximately 86%), whereas the stomach contents of small fish in the Bungo Channel mainly comprised small crustaceans (>70%; e.g., Mysidae, Euphausiacea, and unidentified crustaceans).

**Discussion**

Using stable isotope analysis to compare the feeding habits of *T. japonicus* among sea areas in the western Seto Inland Sea, we revealed that small fish in the Bungo Channel and Iyo-Nada were on the same trophic level (approximately 3.0), and they mainly relied on pelagic primary production. However, the stomach contents were clearly different between the sea areas. Small *T. japonicus* in the Bungo Channel mostly fed on pelagic primary consumers (e.g., Mysidae and Euphausiacea), whereas they mostly fed on juvenile *E. japonicus*, which is pelagic secondary consumer, in Iyo-Nada. This discrepancy between the trophic levels based on stable isotope analysis and expected trophic niche by stomach content analysis may have been caused by spatial and temporal bias in the stomach contents. *E. japonicus* is generally a migratory fish, and they move among sea areas in the western Seto Inland Sea or between the Pacific Ocean and the western Seto Inland Sea. Difference in the stomach contents between sea areas in this study may reflect this spatial bias. To understand the feeding ecology of *T. japonicas* by stomach content analysis, we need more spatio-temporal sampling. In contrast, stable isotope analysis indicated that the trophic level of small fish in Iyo-Nada was approximately 3, even if mainly secondary consumers were found in their stomach. This result suggests that small *T. japonicus* in Iyo-Nada also mainly fed on pelagic primary consumers from a long-term perspective.

Stable isotope analyses revealed that the reliance of large *T. japonicus* on benthic production in the Bungo Channel was lower than that in other sea areas. Furthermore, the stomach contents of large *T. japonicus* differed among sea areas. The stomach contents of large *T. japonicus* in the Bungo Channel mainly contained small *T. japonicus* and carangid species, whereas large *T. japonicus* in Iyo-Nada and Suo-Nada mainly fed on *E. japonicus*. However, the difference in the reliance on benthic production among sea areas may be not attributed to differences in the stomach contents among sea areas because trophic position of small *T. japonicus* in this study was isotopically similar to that of *E. japonicus* and carangid species, which were reported by Shibata et al. (2012) in the Bungo Channel.

Differences in the relative importance of benthic production to *T. japonicus* among sea areas are possibly the result of differences in landscape structure. Areas with high benthic primary producer productivity (e.g., shallow areas, tidal flats, and seaweed beds) are more abundant in Suo-Nada, Iyo-Nada, and Aki-Nada (Table 1; Environment Agency 1994); the abundance of benthic primary production is likely to support consumers and the coastal food web as a whole in these sea areas through trophic linkages (Takai et al. 2004). Furthermore, in a lake, the relative contribution of benthic production to a consumer declines as mean depth and lake size increases (Vandeboncoeur et al. 2002). The higher proportional contribution of pelagic production to large *T. japonicus* in the Bungo Channel than that in other sea areas may be attributed to the large euphotic zone in relation to the total area of shallow water.

Large *T. japonicus* in Suo-Nada, Iyo-Nada, and Aki-Nada, in which the dietary contribution of benthic production was high, mainly fed
on *E. japonicus*. It is generally believed that *E. japonicus* primarily rely on pelagic primary production because they feed on the planktonic organisms that inhabit pelagic water. Moreover, *E. japonicus* often feed on benthic microalgae, which are physically blown into the water column; consequently, the contribution of benthic production to the diet of *E. japonicus* is high in coastal areas (Tanaka et al. 2008). Furthermore, *E. japonicus* in Iyo-Nada, Suo-Nada, and Aki-Nada mostly rely on benthic production or detritus, whereas *E. japonicus* in the Bungo Channel rely more on pelagic production than that in other sea areas (Shibata et al. 2012). In our results, the high contribution of benthic production to *T. japonicus* in Iyo-Nada, Suo-Nada, and Aki-Nada may be attributed to the consumption of *E. japonicus* that have been influenced by the contribution of the benthic-demersal food chain.

In this study, we identified food resource characteristics of *T. japonicus* in the western Seto Inland Sea using stable isotope and stomach content analyses. Our results show that the feeding habits of *T. japonicus* differed among sea areas. This result may have been caused by spatial and temporal bias in the stomach contents. Furthermore, the trophic levels based on stable isotope analysis and expected trophic niche by stomach content analysis did not agree. Considering spatio-temporal variation in the stomach contents of specimens, greater sampling effort is needed to understand feeding habit of fish. Using stable isotope analysis, we did detect variations in efficiencies of benthic and pelagic trophic carbon pathways, which were not detected by stomach content analysis, among sea areas. This result coincides with that of a previous study (Takai et al. 2004, Shibata et al. 2012) for primary production structure in the western Seto Inland Sea. Stable isotope analysis helps us effectively understand feeding habit of fish with fewer efforts. The results of this study will probably be useful in understanding the feeding ecology of *T. japonicus*.

**Acknowledgments**

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**References**


西部瀬戸内海に生息するタチウオ Trichiurus japonicus の食性および炭素源の空間的変化

瀬戸内海西部に生息するタチウオ Trichiurus japonicus の餌資源特性を明らかにするため環境特性の異なる 4 海域から試料を集め、安定同位体比分析と胃内容分析によって T. japonicus の食性を海域間で比較した。その結果、T. japonicus の食性は海域間で異なっていたが、これは胃内容物の時間的、空間的なばらつきに影響されることが考えられる。安定同位体比に基づく栄養段階と胃内容物から推測される栄養ニッチは一致していないかった。そのため、胃内容物をもとめる炭素の食性解析にはより多くの採集努力が必要と考えられた。一方で、胃内容物分析では検出できなかった海域間での栄養炭素経路の違いを安定同位体比分析では検出することができた。この結果は西部瀬戸内海における基本生産構造に関する先行研究と一致しており、安定同位体比分析はより少ない採集努力で魚類の食性を効率的に理解することができる手法であると考えられた。