Influences of Ocean Currents on the Diets of Demersal Fish Communities in the Western North Pacific Revealed by Their Muscle Carbon and Nitrogen Isotopic Compositions

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To study the influence of different ocean currents on the trophic spectra found in a sympatric fish community, we analyzed the radiocarbon contents (Δ14C) and stable carbon (δ13C) and nitrogen (δ15N) isotopic compositions in their bulk muscle tissues and the δ15N of individual amino acids in 26 species of demersal fish collected from off Tohoku (Pacific coast), northeastern Japan. The Δ14C values of the fish varied from –42 to +41‰, consistent with the Δ14C of the dissolved inorganic carbon in the cold Oyashio Current (typically about –50‰) and the warm Kuroshio Current (typically about +50‰). The trophic positions (TPs) of the fish estimated from the δ15N values of amino acids increased from 3.1 to 4.5 with increasing snout length. A negative correlation was observed between Δ14C and TP in gadiform fish and flatfish, suggesting that the Oyashio Current delivers a high TP diet to these fish groups. These results suggest that the trophic ecology of marine fish in the coastal western North Pacific is primarily controlled by the two major surface water currents, but is also significantly influenced by a combination of species-specific feeding and migration strategies.

Keywords: deep sea, demersal fish, off Sanriku, Oyashio–Kuroshio transition, trophic position, migration, amino acid nitrogen isotopic composition, radiocarbon

INTRODUCTION

Surface ocean currents play an important role in controlling primary production, ecosystem structures, and fisheries resources (Ganachaud and Wunsch, 2000; Toggweiler and Russell, 2008). The interfrontal regions in which different currents meet and mix are important sites for local fishery grounds, because their high primary production is supported by different temperature,
salinity, and nutrient profiles (Yatsu et al., 2013). The Sanriku region, in the western North Pacific, is one such region (Shiozaki et al., 2020), yielding the world’s largest fish catch of 22.4 million tons per year (FAO, 2018). In the Sanriku region, the cold Oyashio Current (of the northwestern subarctic gyre) meets the warm northward Kuroshio Current (of the western subtropical gyre) around middle latitudes. The warm Tsugaro Current, which originates in the Kuroshio Current, also contributes significantly to the water masses, especially nearshore on the Sanriku coast (Hanawa and Mitsudera, 1987; Itoh et al., 2016; Figure 1). Understanding ecosystem structures and functions is essential for sustainable fisheries and resource use, and yet fundamental information is still largely unavailable, including the sources of carbon and nitrogen in the benthic ecosystems off the Sanriku region.

Primary production in the surface ocean is supported by the uptake of dissolved inorganic carbon (DIC) and nitrogen by phytoplankton during their photosynthesis in the euphotic zone. Stable carbon (δ13C) and nitrogen (δ15N) isotopic compositions have been used as indicators of the diet sources and trophic positions (TPs), respectively, of marine organisms for the past four decades (e.g., Wada et al., 1987; Pinnegar and Polunin, 2000; Zintzen et al., 2013). Both the δ13C and δ15N values of primary producers, such as phytoplankton, are determined by the fractionations against 13C and 15N during the fixation from inorganic nitrogen (Nakatsuka et al., 1992), and are primarily characterized by local productivity. From lower to higher TPs, the δ15N value increases while δ13C remains unchanged (Wada et al., 1987; Sweeting et al., 2007).

14C is the radioactive isotope of carbon with a half-life of 5,730 years. It is produced in the earth’s upper atmosphere by cosmogenic radiation. The 14C content (Δ14C) in the DIC of ocean water reflects the source and/or age of its carbon. The Oyashio Current (Δ14CDIC typically about -50‰) is influenced by convective mixing with subsurface waters in the subarctic Pacific. By contrast, the DIC in the Kuroshio Current and the downstream Tsugaro Current (Δ14CDIC typically about +50‰) is well equilibrated with the contemporary atmospheric CO2 (Kubota et al., 2018; Larsen et al., 2018; Satoh, 2020; Figure 1). Furthermore, warm and low-salinity water masses (e.g., Oyashio Current) with higher Δ14CDIC dominate the surface layer, whereas cold and high-salinity water masses (e.g., Kuroshio Current) with lower Δ14CDIC dominate the deep layer in the western North Pacific (Ding et al., 2018). The Δ14CDIC value in the surface water is transferred to photosynthetic phytoplankton, where Δ14C is corrected for isotopic fractionation associated with CO2 assimilation using δ13C, assuming a mass dependent fractionation relationship between δ13C and δ14C (Stuiver and Polach, 1977). Therefore, the Δ14C of fish should reflect the Δ14CDIC of the local seawater and the species-specific migration history, which integrates the various Δ14CDIC values of different oceans.

The compound-specific nitrogen isotope analysis of amino acids (CSIA-AA) is a tool that has emerged in recent years and offers a significant advantage over the conventional bulk isotope analysis. This is because the TPs of marine organisms can be determined by the difference in δ15N between glutamic acid (trophic amino acid) and phenylalanine (source amino acid), with no requirement to characterize the δ15N value of phytoplankton (Chikaraishi et al., 2009). Fish TPs can potentially be used to explore how the Δ14C diversity found in a sympatric fish community is integrated with trophic transfer, or conversely, how the ecological niche of a fish species controls its carbon sources and Δ14C. Several studies have also demonstrated that the δ15N value of phenylalanine is a more precise proxy for the nitrogen sources (i.e., inorganic nitrogen, such as nitrate) than the bulk δ15N value, and can be used as a tracer for migratory fish (Volkshoort and McCarthy, 2014; Matsubayashi et al., 2020).

In the present study, we studied the trophic ecology of demersal fish in the north-west Pacific Ocean below 200 m. We focused specifically on determining the relationship between the mixing of different ocean currents and the trophic spectra found in the fish community off Sanriku. We hypothesized that Oyashio-dependent fish would show higher TP than Kuroshio-dependent fish because the former feeds on higher-TP foods sourced from the subarctic North Pacific (Matsubayashi et al., 2020). Demersal fish were targeted because they occupy a near-apical position in the hierarchy of the seafloor ecosystem, which integrates energy flow through the trophic pathway in the benthic food web. In addition to conventional bulk δ13C and δ15N analyses, we used Δ14C and amino acid δ15N values of fish to trace their carbon sources and TPs, respectively.

MATERIALS AND METHODS

Study Sites

In collaboration with the Tohoku Ecosystem-Associated Marine Sciences (TEAMS), we collected a variety of demersal fish using a trawl net at water depths of 200–500 m off the Sanriku coast during the research cruise of R/V Iwate-maru in 2012 to 2014 (Figure 1). A detailed description of this sampling is provided in Ohkouchi et al. (2016).

Sample Collection

Detailed information on the fish samples is provided in Supplementary Table 1. In total, 53 individuals from 26 fish species were used in the analysis (Supplementary Table 1). After the snout length (SnL), which was expected to affect the type of foods consumed, was measured, a small piece of muscle near the snout was excised, freeze dried, and defatted with methanol and dichloromethane (Ohkouchi et al., 1997). It should be noted that some species had relatively high carbon to nitrogen ratios (g/g, > 4.0), suggesting that our defatting procedure did not work for some of the samples (Supplementary Table 1). The samples were stored at -20°C until further processing.

Bulk Stable Isotope Measurements

We measured the carbon to nitrogen ratios (C/N, g/g) and the stable isotope ratios of carbon (δ13C) and nitrogen (δ15N) in the fish samples with a modified Flash EA1112 elemental analyzer connected to a Delta plus XP isotope ratio mass spectrometer with a Confo III interface (Thermo Finnigan, Bremen, Germany) (Ogawa et al., 2010). The δ13C and δ15N values were reported
The radiocarbon content ($\delta^{14}C$) of the bulk fish muscle (approximately 4 mg dry weight) was measured with a single-stage accelerator mass spectrometry (AMS) equipped with an elemental analyzer at the Atmosphere and Ocean Research Institute, the University of Tokyo, with analytical errors typically smaller than 3‰ (Yokoyama et al., 2019). $\Delta^{14}C$ values were reported after $\delta^{13}C$ correction with the following equation (Stuiver and Polach, 1977):

$$
\Delta^{14}C (\%e) = \delta^{14}C - 2(\delta^{13}C + 25)(1 + \frac{\delta^{14}C}{1000})
$$

where $\delta^{14}C$ was defined as the $^{14}C$ content of the sample relative to the international standard (HOx II oxalic acid) (Stuiver and Polach, 1977).

### Amino Acid $\delta^{15}N$ and TPs

The amino acids were extracted from the fish samples with HCl hydrolysis and then derivatized to N-pivaloyl-isopropyl esters (Pv/iPr) with the improved procedures described by Chikaraishi et al. (2010). In brief, the samples were hydrolyzed with 12 M HCl at 110°C for 12 h. Each hydrolysate was washed with n-hexane/dichloromethane (3/2, v/v) to remove any hydrophobic constituents (e.g., lipids). After the samples were defatted and dried under N$_2$ gas flow, they were derivatized sequentially with thionyl chloride/2-propanol (1/4, v/v) and pivaloyl chloride/dichloromethane (1/4, v/v). The Pv/iPr derivatives of the amino acids were extracted from the final fraction with n-hexane/dichloromethane (3/2, v/v) before gas chromatographic separation.

The $\delta^{15}N$ values of individual amino acids were determined with a Delta plus XP isotope ratio mass spectrometer, coupled to an Agilent 6890N, via a Conflo III interface with combustion (950°C) and reduction (550°C) furnaces (Thermo Finnigan). The Pv/iPr-derivatized amino acids were injected with a programmable-temperature vaporizing (PTV) injector (Gerstel, Germany). The PTV temperature was held at 50°C (initial temperature) for 0.3 min, increased from 50 to 350°C at a rate of 200°C min$^{-1}$, and held at 350°C for 18 min, and finally held at 350°C for 10 min. The flow rate of the carrier gas (He) was controlled with a constant flow mode at 1.4 mL min$^{-1}$. The gas chromatograph oven temperature was held at 40°C (initial temperature) for 3.0 min, increased at 15°C min$^{-1}$ to 110°C, increased at 3°C min$^{-1}$ to 150°C, increased at 6°C min$^{-1}$ to 220°C, held at 260°C for 18 min, and held at the final temperature for 5 min. The amino acids were separated on a column (HP-Ultra 2, 0.32 mm × 50 m, film thickness 0.52 µm, Agilent Technologies) before they were introduced into the IRMS (Chikaraishi et al., 2010). An isotopic
reference mixture of nine amino acids (i.e., alanine, glycine, leucine, norleucine, aspartic acid, methionine, glutamic acid, phenylalanine, and hydroxyproline), with δ¹⁵N values ranging from −26.6 to +45.6‰ (Indiana University, United States; SI Science, Japan), was analyzed every 5–6 injections to confirm the reproducibility of the isotope measurements. Three and two pulses of the reference cylinder N₂ gas were measured for calibration at the beginning and end of each run, respectively. The δ¹⁵N values of all the samples (>0.5 nmol N) were corrected using the regression line between the published δ¹⁵N values and the measured δ¹⁵N values for our internal amino acid standards (i.e., the above nine amino acid mixture, Ohkouchi et al., 2017). The analytical errors (1σ) of the standards were smaller than 0.8‰.

The TPs of the fish were calculated as follows:

$$\text{TP} = \frac{\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}} - 3.4}{7.6} + 1$$  \hspace{1cm} (3)

where δ¹⁵N_Glu and δ¹⁵N_Phe are the δ¹⁵N values for glutamic acid and phenylalanine in the samples, respectively; 3.4 is the initial offset (‰) of δ¹⁵N_Glu and δ¹⁵N_Phe found in phytoplankton; and 7.6 is the trophic discrimination factor offset (‰) between δ¹⁵N_Glu (8.0 ± 1.2‰) and δ¹⁵N_Phe (0.4 ± 0.5‰) (Chikaraishi et al., 2009). Thirty-five out of the 50 total TP data were published in our previous paper (Ohkouchi et al., 2016) (see Supplementary Table 1).

### Database Search

We browsed the biogeographic data for the studied fish species (latitude, longitude, and depth at which catches were recorded) in the open-source database Biological Information System for Marine Life (BISMaL).¹ The recorded latitude for each species was used to determine the distribution of the fish between the subarctic Oyashio Current (high latitude) and the subtropical Kuroshio Current (low latitude). The depth record of each fish species in BISMaL was used to examine the effect of water depth on the isotopic compositions of the fish.

### Statistical Analysis

A regression analysis and the Welch’s two-sample t-test were used to examine the significance of differences among sampling locations (water depth, latitude, and longitude), SnL, bulk δ¹⁴C, δ¹⁵N, and Δ¹⁴C (δ¹⁵C_Bulk, δ¹⁵N_Bulk, and Δ¹⁴C_Bulk, respectively), δ¹⁵N of phenylalanine (δ¹⁵N_Phe), and TPs for each fish sample. Some isotope data are not available (Supplementary Table 1). Based on the number of replicates available at the level of fish orders, we categorized the data into three groups: gadiform fish (the order Gadiformes); flatfish (the order Pleuronectiformes); and other demersal fish (the families Pterothrissidae, Synaphobranchidae, Sebastolobidae, Ereunidae, Cottidae, Psychrolutidae, Agonidae, Liparidae, and Zoarcidae). All statistical analyses and graphing were performed with R 3.5.3 (R Development Core Team, 2019). Statistical significance was set at α = 0.05.

¹https://www.godac.jamstec.go.jp/bismal/e/index.html

### RESULTS

Gadiform fish had significantly higher SnL than flatfish (Welch’s two-sample t-test, t = 3.06, df = 19.6, p = 0.006). We observed considerable variations in the bulk δ¹⁴C (δ¹⁵C_Bulk) (from −25.5‰ to −16.5‰, n = 49) and δ¹⁵N values (δ¹⁵N_Bulk) (+10.4 to +15.3‰, n = 52) of the fish, indicating that their carbon and nitrogen sources and their trophic positions varied widely (Figures 2, 3). In particular, the δ¹⁵C_Bulk values of the gadiiform fish (−19.9 ± 1.9‰, mean ± SD, n = 18) and other demersal fish (−18.9 ± 1.8‰, n = 20) were lower and more variable than those of the flatfish (−18.0 ± 0.9‰, n = 11). We found no significant relationship between sampling depth, latitude, or longitude and δ¹⁵C_Bulk (n ≥ 42, r² < 0.02, p > 0.41) or δ¹⁵N_Bulk (n ≥ 44, r² < 0.06, p > 0.10), except for the effect of longitude on δ¹⁵N_Bulk (n = 47, r² = 0.11, p = 0.04). The SnL showed significantly negative and positive correlations with δ¹⁵C_Bulk (n = 48, r² = 0.10, p = 0.03) and δ¹⁵N_Bulk (n = 51, r² = 0.19, p = 0.001), respectively (Figure 3).

The Δ¹⁴C_Bulk values of the fish ranged from −42 to +41‰ (+5.2 ± 17‰, mean ± SD, n = 51) (Figures 2, 3, 4) and were unrelated to SnL (n = 50, r² = 0.02, p = 0.28). We found no significant effect of sampling depth, latitude, or longitude on Δ¹⁴C_Bulk (n ≥ 44, r² < 0.04, p > 0.19). The correlations between Δ¹⁴C_Bulk and δ¹⁵C_Bulk (n = 47, r² = 0.04, p = 0.16) and between Δ¹⁴C_Bulk and δ¹⁵N_Bulk (n = 50, r² < 0.01, p = 0.83) were not significant (Figure 3). The lowest Δ¹⁴C_Bulk values were found in Laenonomia longipes (gadiform fish, threadfin hakeling, −41 to −22‰, n = 4), whereas the highest Δ¹⁴C_Bulk values were found for Dextesia rikuzienensis (flatfish, Rikuzen sole), Glytopocephalus stelleri (flatfish, Korean flounder), and Alcichthys elongatus (Elkhorn sculpin) (+35 to +41‰, n = 4) (Figure 4).

Among the gadiiform fish, the deep-layer-inhabiting L. longipes (n = 4) showed Δ¹⁴C_Bulk values 26.1–68.7‰ lower than those of the shallow-layer-inhabiting Physiculus japonicus (Japanese codling, n = 4) (Figure 4). Similarly, among the flatfish, the deep-layer-inhabiting Hippoglossoides dubius (flathead flounder, n = 2) showed Δ¹⁴C_Bulk values 14.9–44.4‰ lower than those of the shallow-layer-inhabiting D. rikuzienensis (n = 4). We detected intraspecies variations in Δ¹⁴C_Bulk as large as 39.6‰ for G. stelleri (n = 4) and 20.5‰ for D. rikuzienensis (n = 4) (Figure 4).

The fish δ¹⁵N values for glutamic acid (δ¹⁵N_Glu) ranged from +20.3 to +32.9‰ (+27.1 ± 2.9‰, mean ± SD, n = 55), whereas those for phenylalanine (δ¹⁵N_Phe) ranged from −3.6‰ to +5.5‰ (+1.8 ± 2.5‰, mean ± SD, n = 55) (Figure 5). δ¹⁵N_Phe and TP correlated with the δ¹³C_Bulk values (δ¹⁵N_Phe: n = 49, r² = 0.10, p = 0.03; TP: n = 49, r² = 0.12, p = 0.01), but not with the δ¹⁵N_Bulk values (n = 52, r² < 0.02, p > 0.32) (Figure 3). A marginally significant negative correlation was observed between TP and δ¹⁵N_Phe (n = 55, r² = 0.07, p = 0.06) (Figure 3). We found no significant effects of sampling depth, latitude, or longitude on δ¹⁵N_Phe (n ≥ 44, r² < 0.05, p > 0.15) or TP (n ≥ 44, r² < 0.07, p > 0.07). The TP of the fish ranged from 3.1 to 4.5 (3.8 ± 0.3, mean ± SD, n = 55) and correlated positively with SnL (n = 51, r² = 0.23, p < 0.001) (Figure 3). Synaphobranchus kaupii (Kaup’s arrowtooth eel, TP: 4.5, n = 2) and D. rikuzienensis (Rikuzen sole, TP: 3.1–3.3, n = 4) showed the highest and lowest TPs, respectively.
The intraspecies variations in TP were smaller than 0.4 units, and occurred in species such as Malacocottus zonurus (darkfin sculpin, TP: 3.7–4.1, \( n = 4 \)), P. japonicus ( gadiform fish, TP: 3.6–3.9, \( n = 4 \)), Gadus chalcogrammus ( gadiform fish, walleye pollock, TP: 3.8–4.0, \( n = 6 \)), and G. telleri (flatfish, Rikuzen sole TP: 3.6–3.8, \( n = 4 \)) (Figure 6). A significantly negative correlation between TP and \( \Delta^{14}C_{\text{Bulk}} \) was observed within the gadiform fish (\( n = 19, r^2 = 0.46, p = 0.001 \)) and flatfish (\( n = 12, r^2 = 0.49, p = 0.01 \)) (Figure 6).

**DISCUSSION**

**Overview**

Among the three fish groups investigated in this study (gadiform fish, flatfish, and other demersal fish), the flatfish had smaller SnL and showed lower TPs than the other groups. The relatively constant \( \delta^{13}C_{\text{Bulk}} \) values of the flatfish (–18 ± 0.9‰, \( n = 11 \)) suggest that their low migration rates only allow flatfish to consume locally provided foods. By contrast, the \( \delta^{13}C_{\text{Bulk}} \) values of the gadiform fish and other demersal fish with higher migration rates deviated from –18‰, suggesting that their carbon is derived not from local sources but from somewhere else. Furthermore, the larger SnLs and higher TPs of the gadiform fish and other demersal fish suggest that they integrate autochthonous and allochthonous production in the benthic ecosystems off Sanriku. However, \( \delta^{13}C \) cannot be used to determine from where allochthonous food sources originate because the baseline \( \delta^{13}C \) value (in phytoplankton) is largely dependent on the isotopic fractionation associated with DIC uptake (Nakatsuka et al., 1992).

The \( \Delta^{14}C \) signature can be used to distinguish the two locally dominant water masses off Sanriku: the Oyashio Current (\( \Delta^{14}C_{\text{DIC}} \) typically about −50‰) and the Tsugaru/Kuroshio Current (\( \Delta^{14}C_{\text{DIC}} \) typically about +50‰) (Kubota et al., 2018; Satoh et al., 2019). Even within the local area off Sanriku,
a difference of over 80% in the fish Δ¹⁴C_{Bulk} values was observed, and this variation is explained by the large Δ¹⁴C_{DIC} difference between the Oyashio and Kuroshio currents. We have calculated the Oyashio contribution to fish muscle using a two-endmember mixing model using Δ¹⁴C_{DIC} of the Oyashio (ca. −50‰) and Kuroshio (ca. +50‰) Currents (Supplementary Table 1). The Oyashio Current contributed to the southern (Kuroshio) fish (species 1–11) by 34 ± 15% (mean ± SD, n = 20), and to the northern (Oyashio) fish (species 12–25) by 52 ± 14% (mean ± SD, n = 31). These results suggest that the ocean off Sanriku is a unique region for fisheries, hosting the highly diverse fish community fueled by the Oyashio and Kuroshio currents. However, the endmember values are not well constrained, because Δ¹⁴C_{DIC} in modern surface seawater is a function of time.

We observed a relatively scattered distribution of fish δ¹⁵N_{Phe} values along the Oyashio–Kuroshio transition, which correlated positively with the δ¹³C_{Bulk} values (r = 0.309; Figure 3). Because the δ¹⁵N_{Phe} value reflects the δ¹⁵N value of nitrate in seawater (Matsubayashi et al., 2020), the variation in δ¹⁵N_{Phe} of up to 9‰ among these fish is controlled by the regional oceanographic environment and the δ¹⁵N of nutrients and/or the isotopic...
fractionation by in situ phytoplanktonic photosynthesis. We also found a wide trophic spectrum (i.e., a TP range over 1.4 units) in the local fish community, which correlated significantly with the $\Delta^{14}C_{\text{Bulk}}$ values of the gadiform fish and flatfish. We will get back to this point later in the section “Trophic Ecology of Fish Communities.”

One of the greatest advantages of this study is the $\Delta^{14}C_{\text{Bulk}}$ and $\delta^{15}N_{\text{Phe}}$ values in fish muscle being used as indicators of their carbon and nitrogen sources, respectively, without the need for data on their potential diets such as benthic invertebrates. In the following sections, we discuss the patterns and factors controlling $\Delta^{14}C_{\text{Bulk}}$ and TP in the fish in terms of their species-specific feeding and migration behavior.

**Gadiform Fish**

*Laemonema longipes* and *P. japonicus* have a similar life history: they spawn and hatch on the southeast coast of Japan (off the Izu Peninsula) and their larvae migrate northeast via the Kuroshio Current and settle along the Sanriku coast to grow (Kitagawa and Nagahora, 1983; Hattori et al., 2009). However, our data show that their TPs and $\Delta^{14}C_{\text{Bulk}}$ values are extremely different. The lower $\Delta^{14}C_{\text{Bulk}}$ values of *L. longipes* are consistent with their preference for deep water (Yamamura and Nobetsu, 2012) (catch-record depth: 318–656 m; Supplementary Table 1), where the Oyashio Current conveys $^{14}C$-depleted food sources. By contrast, *P. japonicus* prefers shallower water (Kitagawa and Nagahora, 1983) (catch-record depth: 20–561 m; Supplementary Table 1). The high $\Delta^{14}C_{\text{Bulk}}$ values of *P. japonicus* are attributable to the influence of the Tsugaru/Kuroshio Current, with $^{14}C$-enriched DIC in the Sanriku coastal seawater. *G. chalcogrammus* showed a wide range of $\Delta^{14}C_{\text{Bulk}}$ values (–8 to +15‰, n = 6) among the gadiform fish, possibly reflecting their migration from their spawning/hatching region (Funka Bay, Figure 1) to off Sanriku (Sakurai, 2007; Funamoto et al., 2014). The $\Delta^{14}C_{\text{DIC}}$ value in Funka Bay was +40 to +50‰ in 1988 (Tsunogai et al., 1993). Although no data are available after that time, the $\Delta^{14}C_{\text{DIC}}$ in Funka Bay during our sampling period (2012–2014) is expected to have been much lower than that in 1988 (~0‰) as a consequence of the continuous removal of bomb-produced $^{14}C$ to the biosphere through photosynthesis and to the geosphere through sedimentation for the past half century (Druffel et al., 2016).

*Laemonema longipes* and *P. japonicus* also showed variable $\delta^{15}N_{\text{Phe}}$ values, supporting the speculation cited above that they had migrated from south of Sanriku where phytoplankton $^{15}N$ values vary up to 5‰ (Matsubayashi et al., 2020). The TP values of *L. longipes* were approximately 0.5 units higher than those of *P. japonicus*, possibly because *L. longipes* prefers to feed on squids and mesopelagic fish, whereas *P. japonicus* feeds on amphipods, small shrimps, and large zooplankton, such as...


**Euphausia japonica** and **Neocalanus cristatus** (Yamamura and Nobetsu, 2012). **G. macrocephalus** also showed a higher TP than other gadiform species, probably because it consumes a variety of food items, including squids, octopi, krill, crabs, and other fish, such as the juveniles of **G. chalcogrammus**, as well as practicing intraspecies cannibalism (Hashimoto, 1974; Fujita et al., 1995).

**Flatfish**

The less variable $\delta^{13}C_{\text{Bulk}}$ values found in flatfish compared with the other fish suggest that their carbon sources are provided locally by small benthic animals on the seafloor, which is discussed later. Except for **H. dubius**, whose $\Delta^{14}C_{\text{Bulk}}$ values (-2.7 and +5.7‰, $n = 2$) were the lowest of all flatfish, the flatfish analyzed in this study is restricted to live in shallow waters (Amaoka, 2016), where the Kuroshio Current dominates and is enriched in $^{14}C$ in DIC (by about +50‰; Ding et al., 2018; Satoh, 2020). In other words, the $\delta^{13}C_{\text{Bulk}}$ values indicate flatfish do not migrate greatly, and the $\Delta^{14}C_{\text{Bulk}}$ values indicate flatfish rely on diets of organisms such as zooplankton and/or benthic animals that feed on phytoplankton with high $^{14}C$ values. The large intraspecies variation in $\Delta^{14}C_{\text{Bulk}}$ values detected in **G. stelleri** reflects its distribution at a wide range of water depths (catch-record depth: 125–540 m; Supplementary Table 1). Similarly, the low $\Delta^{14}C_{\text{Bulk}}$ values of **H. dubius** relative to those of the other flatfish species may be attributable to their preference for deep water (catch-record depth: 201 to 2021 m; Supplementary Table 1).

The large intraspecies variation in the $\delta^{15}N_{\text{Phe}}$ values of **G. stelleri** is consistent with the pattern found in its $\Delta^{14}C$. By contrast, **G. stelleri** showed small variations in its TP (3.6–3.8, $n = 4$), suggesting that its diet does not change markedly (it is constantly benthic animals; Hayase and Hamai, 1974). The flatfish had smaller SnL and lower TP values than the gadiform fish, probably because the food items of flatfish are predominantly benthic animals (Amaoka, 2016). For example, it was reported that 85% of the gut contents of **D. rikuzenius** in Sanriku consisted of small crustaceans, polychaetes, and ophiuroids (Fujita et al., 1995). An exception is **H. dubius**, whose TP was as high as those of gadiform fish such as **L. longipes**. This observation is consistent with the previous report that **H. dubius** eats benthic animals as well as other fish, such as the juveniles of **G. chalcogrammus** (Hayase and Hamai, 1974).

**Other Demersal Fish**

The wide range in $\Delta^{14}C$ values in **M. zonurus** may reflect its behavior, moving between various water depths
(Shinohara et al., 1992) (catch-record depth: 50–1,497 m; Supplementary Table 1). *Bothrocara tanakae* and *S. kaupii* are bottom-dwelling fish species with $\Delta^{14}C_{\text{Bulk}}$ values of $\sim 0\%$, suggesting that their diets are ultimately derived from both the Oyashio and Tsugaru/Kuroshio currents. The $\Delta^{14}C_{\text{Bulk}}$ value of *A. elongatus*, the highest among those of other demersal fish, may be explained by its habitat preference for relatively shallow water (Kitagawa, 1990; Fujita et al., 1995) (catch-record depth: 20–269 m; Supplementary Table 1), which is mainly affected by $^{14}$C-enriched DIC from the Tsugaru/Kuroshio Current. By contrast, the $\Delta^{14}C_{\text{Bulk}}$ value of *S. macrochir*, the lowest among those of other demersal fish, may reflect its feeding preference for benthic animals (Hattori et al., 2009b), which originate in $^{14}$C-depleted DIC in deep waters.

*Z. tonsum* displayed a relatively wide range of $\delta^{13}$N of the TP values, varying by up to 6% and 0.4 units, respectively, which is consistent with their large variation in $\Delta^{14}C_{\text{Bulk}}$. The TP of *S. kaupii* (TP = 4.52 and 4.53, n = 2), the highest among the fish studied, is consistent with the carnivorous behavior of this species (Gordon and Mauchline, 1996). In the other demersal fish, including deep-sea fish such as *S. kaupii* (catch-record depth: 250–2,935 m; Supplementary Table 1), *B. zestum* (catch-record depth: 0–1,728 m; Supplementary Table 1), and *B. tanakae* (catch-record depth: 300–789 m; Supplementary Table 1), there was no significant correlation between TP and $\Delta^{14}C_{\text{Bulk}}$. These results suggest that other factors integrate the variations in $\Delta^{14}C_{\text{Bulk}}$ into intermediate values ($-6.9$ to $+4.1\%$) near the seafloor.

**Trophic Ecology of Fish Communities**

The gadiform fish and flatfish with higher TPs had lower $\Delta^{14}C_{\text{Bulk}}$. Within these two commercially important fish orders, the gadiform fish plotted in the high-TP/low-$\Delta^{14}C_{\text{Bulk}}$ range, whereas the flatfish plotted in the low-TP/high-$\Delta^{14}C_{\text{Bulk}}$ range. These results suggest that gadiform fish utilize carbon derived from phytoplankton in the Oyashio Current, where $\Delta^{14}C_{\text{DIC}}$ is generally restricted to the range of $-78$ to $0\%$, whereas the carbon sources of the flatfish are derived from the Kuroshio Current, with high $\Delta^{14}C_{\text{DIC}}$ reaching $+33\%$ (Satoh, 2020; Figure 7). Furthermore, the diet consumed by gadiform fish from the Oyashio Current has a mean TP that is 0.34 units higher than that of the flatfish from the Kuroshio Current. This may be partly explained by the observation that the TPs of the large zooplankton (*Neocalanus cristatus* and *Neocalanus flemingeri*) dominant in the Oyashio region are high (2.6–2.7), whereas the TPs of the small zooplankton (*Paracalanus aculeatus*...
and *Paracalanus parvus* dominant in the Kuroshio region are low (2.2–2.3) (Matsubayashi et al., 2020). Moreover, the $\delta^{13}C_{\text{Bulk}}$ results show different migration rates for the gadiform fish and flatfish (Figure 7). These results collectively support our hypothesis that the Oyashio/bottom waters provide high-TP foods to the gadiform fish, whereas the Kuroshio/surface waters provide low-TP foods to the flatfish. It should be noted that Bradley et al. (2015) found that the trophic discrimination factor offset ($h$) in equation 3 is lower than 7.6‰. However, they compared the offset against data from stomach content analysis. Therefore, their assessment would not be compatible with equation 3, because the TP value estimated by stomach contents is not necessarily consistent with that estimated by CSIA-AA (Ishikawa, 2018). If this was the case for the present study, our fish TP values might be lower than their functional TPs.

The interpretation of the $\Delta^{14}C_{\text{Bulk}}$ values of demersal fish may be confounded by the possibility that some microbes are depleted in $^{14}C$ ($\Delta^{14}C \leq -100‰$) by the chemosynthetic assimilation of $^{14}C$-depleted DIC (Hansman et al., 2009) or by the heterotrophic assimilation of aged organic carbon (Cherrier et al., 1999; McCallister et al., 2004). These microbial activities can provide $^{14}C$-depleted organic carbon to organisms at higher TPs, although the degree to which these microbes contribute to the diets of demersal fish is poorly quantified. Furthermore, a significant perturbation of $\delta^{13}C_{\text{Bulk}}$ and $\delta^{15}N_{\text{Bulk}}$ values would be expected if this were the case. Therefore, in this study, we assumed that the demersal fish and their prey are primarily supported by fresh organic carbon derived from primary production in the surface current systems (via benthic–pelagic coupling; Purinton et al., 2008) and that the influence of $^{14}C$-depleted microbial biomass and aged organic carbon to the $\Delta^{14}C_{\text{Bulk}}$ values of demersal fish is minimal.

Although our dataset is limited in terms of the number of replicates per species, we obtained several findings as mentioned above. Quantitative and intensive samplings and analyses are essential in future research to examine what controls the variations in $\Delta^{14}C_{\text{Bulk}}$ and TP, from the individual scale to the community scale. No age information was available for the fish analyzed in this study. Given that the larger fish disperse more deeply (Macpherson and Duarte, 1991), a positive correlation between body size and $\Delta^{14}C_{\text{Bulk}}$ within a single species can be expected. Furthermore, the turnover time of fish muscle should be taken into account to calibrate the ontogenetic dietary shift (Yamamura et al., 2002). It should also be noted that consumption of more migratory prey would affect fish predator isotope values even if the predators themselves have relatively limited movements. Understanding trophic structures (e.g., the biomass distribution along trophic spectra: Kato et al., 2018, and the integrated TPs of a community: Ishikawa et al., 2017) is fundamental to constraining the carrying capacity of ocean ecosystems because both the resource productivity and the trophic transfer efficiency are limited on this planet (Pauly and Christensen, 1995).

In the Atlantic Ocean, the population dynamics of demersal fish and benthic invertebrates are reversely synchronized, suggesting that the fisheries resources are strongly regulated by the trophic cascade (Frank et al., 2005). Furthermore, the benthic-pelagic coupling plays an important role in biological production on the continental shelf (Trueman et al., 2014). These studies suggest that fish migration history is quite important not only for fisheries management, but also for understanding of the trophic structure in the coastal ocean ecosystems. In this context, researchers have employed $\delta^{13}C$ and $\delta^{15}N$ analyses to draw “isoscapes” in the Atlantic, on which fish isotopic signatures are overlaid to track their migration history (e.g., Graham et al., 2010). Our results will contribute to this emerging research field by combining radiocarbon and CSIA-AA and applying their insights to ocean currents and fish trophic ecology in a variety of oceanographic settings.

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**FIGURE 7** | Trophic ecology of demersal fish in the Sanriku ecosystems inferred from the present study.
The structure of the fish community off the Sanriku ecosystem, including the resource abundance, species composition, and TPs, has changed significantly during the last half century (Yonezaki et al., 2015). This change has been ascribed to the climatic and oceanographic regime shifts that have occurred multiple times in the North Pacific within this period (Hare and Mantua, 2000; Yatsu et al., 2008). The ecosystem dynamics are projected to fluctuate in response to the increasing variability in the global climate, especially in the coastal area, where most of the commercially important fish species spend at least part of their life histories (Attrill and Power, 2002). Our results strongly suggest that the fish migration rates off Sanriku are responsible for their reliance upon the Oyashio and Tsugaru/Kuroshio currents and for the wide range of the trophic spectra they display (Figure 7). This may be one reason that the Sanriku region is a hot spot for local fisheries (FAO, 2018). In other words, it is possible that these hot-spot ecosystems are vulnerable to environmental changes, including biodiversity loss through overfishing and ocean current modification through global warming. We conclude that isotopic evidence has significant potential utility in predicting of the distribution of fisheries resources in the ocean.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Tohoku Ecosystem-Associated Marine Sciences Project.

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AUTHOR CONTRIBUTIONS

NOg, YC, KF, YY, TN, and NOh designed the project. MY collected and identified the fish samples. NOg, YC, YM, and YY made the isotope measurements. NI analyzed the data and wrote the first draft. All authors participated in the discussion to finalize the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.641282/full#supplementary-material

Supplementary Figure 1 | Results of the two-endmember mixing model.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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