

Intrinsic tracers reveal recent foraging ecology of giant Pacific bluefin tuna at their primary spawning grounds

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ABSTRACT: Pacific bluefin tuna *Thunnus orientalis* (PBFT) play important economic and ecological roles in the western Pacific Ocean. We currently lack basic information on PBFT foraging that would facilitate ecologically informed recovery strategies for this species. We used stable isotope analysis to investigate recent (previous ~1.5 yr based on isotopic turnover rate) trophic ecology of 261 giant (>180 cm), sexually mature PBFT entering their major spawning grounds off Taiwan. We performed amino acid-compound specific isotope analysis (AA-CSIA) on a subset of PBFT and select prey to assess the trophic position of PBFT in the western Pacific and to validate putative recent trans-Pacific migration from the eastern Pacific Ocean. Bayesian isotopic mixing model results suggested recent PBFT foraging off eastern Japan and in the Kuroshio-Oyashio transition region, with minimal inputs from the Sea of Japan and Taiwan waters. PBFT did not appear to feed primarily on zooplanktivorous forage fish (e.g. sardine, anchovy) but on higher trophic-level prey including mackerels, squids, and pomfrets. AA-CSIA confirmed a high trophic position (>5) of PBFT in this region and identified putative recent trans-Pacific migration of 2 individuals. This study identifies the prey base that sustains giant PBFT before migrating to spawning grounds off Taiwan and sets the stage for future studies comparing the movements and ecology of PBFT in the western Pacific Ocean.

KEY WORDS: *Thunnus orientalis* · Stable isotopes · AA-CSIA · Diet · Trophic

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INTRODUCTION

Pacific bluefin tuna (*Thunnus orientalis*; PBFT) are economically and ecologically valuable due to their high market value and their role as large predators in pelagic ecosystems. Fisheries exploitation and population declines due to overfishing (Dalton 2005, ISC 2014) necessitate basic information on the movements

and ecology of giant (>180 cm) spawning PBFT for more effective and holistic management. Here, we use stable isotope analysis (SIA) of PBFT to provide information on the recent regional foraging ecology of PBFT captured near Taiwan, the major spawning ground for giant, sexually mature individuals.

PBFT are widely distributed in the Pacific Ocean, seasonally inhabiting subarctic, temperate, and trop-

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ical waters in the North Pacific Ocean as well as temperate waters in the Southern Hemisphere around Australia and New Zealand (Collette & Nauen 1983, Bayliff 1994, Fujioka et al. 2015). Despite this broad range, the only 2 known PBFT spawning grounds are located in the western North Pacific Ocean; in the Sea of Japan and off the Nansei and Ryukyu Islands in waters east of Taiwan (Fujioka et al. 2015, Ohta & Yamada 2015). Differences in PBFT size have been documented between spawning grounds, with larger PBFT off the Ryukyu Islands (Chen et al. 2006, Okochi et al. 2016). This region is considered the major spawning ground, contributing >70% to PBFT recruitment around Japan (Itoh 2004, Kitagawa et al. 2010, Ohta & Yamada 2015). The timing of spawning also differs between regions, occurring in May–June off Taiwan and July–August in the Sea of Japan (Bayliff 1994). This timing difference, coupled with the presence of larger PBFT in Taiwan versus Sea of Japan spawning grounds, presents the possibility of different movement pathways into and out of these regions. However, movement information on large PBFT (> age 3) in and out of both spawning grounds is lacking (Fujioka et al. 2015).

Ecological information on giant PBFT, including primary prey and foraging habitat of spawners before entering spawning grounds, is also lacking. Understanding PBFT feeding habits facilitates understanding and predictions of multiple life history parameters. Stock biomass, growth rates, and somatic condition are linked to prey abundance, quality, and size (Golet et al. 2015, Shimose & Wells 2015). Heavy fishing and subsequent declines of forage fish have been described globally (Fréon et al. 2005, Tacon & Metian 2009, Cury et al. 2011), and understanding predator–prey relationships helps predict predator responses to such declines (Golet et al. 2007, Smith et al. 2011, Golet et al. 2015). The importance of predator–prey linkages has motivated PBFT diet studies, though most have focused on juveniles (Yokota et al. 1961, Pinkas et al. 1971, Uotani et al. 1990, Yamauchi 2011, Shimose et al. 2013, Madigan et al. 2015b, Shimose & Wells 2015). The only published diet information on adult PBFT in the western Pacific come from the Taiwan spawning grounds, where spawners fed largely on salps and crustaceans and stomach content index was low (Yamauchi 2011). There is no quantitative diet information from large PBFT in western Pacific foraging grounds, with only inferences of adults feeding on zooplanktivorous forage fish (e.g. anchovy *Engraulis mordax*) and flying squid *Todarodes pacificus* in the Sea of Japan and Tsugaru Strait, based on dominance of those prey during PBFT presence in the re-

gion (Shimose & Wells 2015). Movements of PBFT, and observed variability of these movements, have been linked to prey availability (Ito 1961, Polovina 1996), but such studies are hypothetical without identifying the prey base that sustains giant PBFT in the western Pacific Ocean.

Despite the range of tools to characterize tuna movements and foraging ecology, challenges exist for such studies of giant PBFT in the western Pacific. Electronic tags have greatly improved our understanding of juvenile PBFT movements in both the western (Kitagawa et al. 2000, 2004, 2009) and eastern (Boustany et al. 2010, Block et al. 2011, Whitlock et al. 2015) Pacific Ocean. However, it may be difficult to target large spawners before they enter spawning grounds. Feeding studies historically use traditional stomach content analyses, which require the collection of intact stomachs and taxonomic expertise for prey identification. Viscera, including the stomach, is commonly discarded at-sea by PBFT fisheries and are thus unavailable for sampling. Feeding studies provide only a snapshot of foraging and electronic tags provide only prospective data from the date of tag implantation, while prior movements of tagged fish remain unknown.

Certain intrinsic ‘tracers’ in tuna tissues can provide retrospective information on both the movements and diet of sampled individuals (Madigan 2015). SIA allows reconstruction of prior movements and feeding ecology in mobile marine predators by comparing isotopic values of predators with those of different regions and/or groupings of prey (e.g. Revill et al. 2009, Graham et al. 2010, Carlisle et al. 2012, 2015, Ramos & González-Solís 2012, Madigan et al. 2014). Isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) have been the most commonly used intrinsic tracers in pelagic marine fauna. In tunas, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses combined with Bayesian mixing models (e.g. Phillips & Gregg 2003, Moore & Semmens 2008) have led to novel insights on regional foraging patterns and seasonal diet shifts (e.g. Estrada et al. 2005, Logan et al. 2011, Varela et al. 2013). Importantly, such intrinsic chemical tracers require differences in regional chemistry to infer movements in migratory predators. Complementary approaches are especially useful when applying SIA to mobile marine predators, as the potentially confounding effects of migration and trophic ecology on predator isotopic values need to be isolated. Amino acid-compound specific nitrogen isotopic analysis (AA-CSIA) is particularly useful, as $\delta^{15}\text{N}$ values of source amino acids (unchanged through trophic transfer) and trophic amino acids (highly fractionated during tro-

phic transfer) allow differences in bulk $\delta^{15}\text{N}$ values to be attributed to migration, trophic shifts, or both (Dale et al. 2011, Seminoff et al. 2012, Madigan et al. 2014, 2015, Choy et al. 2015). Finally, lab-controlled studies have provided parameters of trophic discrimination and turnover rates of ^{13}C and ^{15}N in PBFT (Madigan et al. 2012b), informing interpretations of wild data. This study on captive PBFT provided lab-derived diet-tissue discrimination factors (DTDF) and constrained the timeframe represented by PBFT muscle isotopic values (~1.5 yr; Madigan et al. 2012b).

Here, we use SIA and AA-CSIA to reconstruct the recent (~1.5 yr based on isotopic turnover rates in PBFT muscle) foraging of PBFT before entering their major spawning ground. We separate isotopically distinct ecoregions in the western Pacific Ocean and characterize potential PBFT prey in these regions, and apply a Bayesian mixing model to first identify the recent foraging grounds of PBFT on Taiwan spawning grounds. We use these results to subsequently categorize prey from the predominant foraging grounds into trophic groupings and apply a second mixing model to ascertain the trophic position of giant PBFT in the western Pacific. AA-CSIA is used to validate trophic mixing model results and to identify recent trans-Pacific migration in 2 PBFT entering the Taiwan spawning grounds. These results set the stage for future studies comparing spawning ground dynamics and long-term variability in the migratory pathways and foraging habits of spawning PBFT in the western Pacific Ocean.

MATERIALS AND METHODS

Sampling and analysis of tissues

Muscle tissue was sampled from 261 PBFT captured by long-line fisheries in Taiwan from April to June, 2011 to 2014. Samples were taken from whole fish landed at various fishing ports in Taiwan which had been captured <500 km from eastern Taiwan. Fork length (FL; measurement from the tip of the rostrum to the fork of the tail; cm) was recorded for all fish. White muscle tissue was taken from the hypaxial dorsal musculature, stored in cryovials, and immediately frozen at -5°C . Muscle tissue was subsequently frozen at -80°C for 24 h, lyophilized for 72 h, and homogenized using a Wig-L-Bug® grinder (Sigma Aldrich®). Bulk tissue SIA of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were performed at the University of Hawaii using an on-line C-N analyzer (Costech Model 4010) coupled

with an isotope ratio mass spectrometer (Thermo-Finnigan Delta XP/ConFlo IV). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are reported as per mille (‰) relative to Vienna Pee Dee Belemnite (V-PDB) and atmospheric nitrogen, respectively. Replicate reference materials of known carbon and nitrogen isotopic composition (glycine and tuna muscle homogenate, both characterized with NIST certified reference materials) were analyzed between every 10 samples. Accuracy and precision were <0.2‰. All $\delta^{13}\text{C}$ values were arithmetically lipid-normalized based on mass C:N ratios using species-specific lipid normalization algorithms for Atlantic bluefin tuna *Thunnus thynnus* as reported in Logan et al. (2008). Linear regression was used to test for correlation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with PBFT length, and 95% prediction bounds computed for linear fits (MatLab vR2015a).

Muscle tissue samples were obtained from prey items for comparison with PBFT $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Pelagic fish and cephalopod samples were collected off eastern Japan during research cruises in 2012 and 2013 using surface neuston and midwater ORI trawls. Recently landed pelagic forage fish and squids were also sampled at fisheries landings ports on the east coast of Taiwan. For all fish, white muscle was removed from the dorsal musculature; for squids, a section of mantle was taken. All samples were prepared for SIA and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the University of Hawaii as described above.

Amino acid-compound specific isotopic analyses (AA-CSIA)

We selected 8 PBFT across a range of sizes (203–249 cm SL) and $\delta^{15}\text{N}$ values (13.2–14.7‰) for AA-CSIA to assess trophic position of giant PBFT in the western Pacific Ocean. For trophic position comparisons, we performed AA-CSIA on 3 prey species of high seasonal abundance and of potential importance to PBFT diet: flying squid *Todarodes pacificus*, sardine *Sardinops melanostictus*, and juvenile jack mackerel *Trachurus japonicus*. An additional 2 PBFT that were inferred to be recent migrants from the eastern Pacific Ocean based on aberrantly high bulk muscle $\delta^{15}\text{N}$ values were analyzed to confirm or refute recent trans-Pacific migration. For AA-CSIA, 5–15 mg of homogenized muscle tissue were hydrolyzed and then derivatized to produce trifluoroacetic amino acid esters (Popp et al. 2007, Hannides et al. 2009, Sherwood et al. 2011). The $\delta^{15}\text{N}$ values of individual amino acids were determined using a mass spectrometer (Thermo Scientific™ Delta V) inter-

faced (ThermoFinnigan GC Combustion-III) to a gas chromatograph (ThermoFinnigan Trace GC). All samples were analyzed at least in triplicate, and measured $\delta^{15}\text{N}$ values were normalized to the known nitrogen isotopic composition of internal references norleucine and aminoadipic acid co-injected with each sample (Dale et al. 2011). We used source amino acids glycine, serine, and phenylalanine (Gly, Ser, and Phe) and trophic amino acids alanine, valine, leucine, isoleucine, proline, and glutamic acid (Ala, Val, Leu, Iso, Pro, and Glu) to calculate a weighted average, based on measurement error for each amino acid, of $\delta^{15}\text{N}$ values for each sample group following Sherwood et al. (2011). Weighted averages of 9 amino acids were used for trophic position estimates to reduce the potential uncertainty contributed by variability of Glu and Phe trophic discrimination (Bradley et al. 2015). For western Pacific Ocean resident PBFT (PBFT with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values consistent with western Pacific prey base) and prey, we calculated weighted averages of source and trophic amino acids to estimate trophic position ($\pm\text{SD}$) following Chikaraishi et al. (2010). For 2 putative trans-Pacific migrants, weighted mean of sources amino acids was compared to that of residential PBFT and western Pacific prey and to eastern Pacific resident PBFT (Madigan et al. 2014) to determine if putative migrants more closely reflected western Pacific or eastern Pacific source amino acid $\delta^{15}\text{N}$ values. The standard deviation of $\delta^{15}\text{N}$ values derived from multiple analyses averaged 0.5‰ ($\pm 0.4\text{‰}$) and ranged from 0 to 2.4‰. See Popp et al. (2007), Sherwood et al. (2011) and Hannides et al. (2009) for more detailed description of AA-CSIA methods.

Mixing model estimates of regional diet and trophic level

Clustering of PBFT for mixing models

Both regional and trophic mixing models (approaches discussed below) were first run for all 261 PBFT. PBFT were then grouped by size to quantitatively examine diet differences of 2 PBFT size groups using the mixing model approach. To generate statistically meaningful groups, PBFT were separated into 2 clusters based on bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using Ward's minimum variance method. PBFT clusters based on isotopic differences were comprised of PBFT of significantly different sizes ([mean \pm SD] 210 ± 15 cm versus 241 ± 6 cm; 1-way ANOVA, $p = 0.01$). All mixing models used the Bayesian mixing

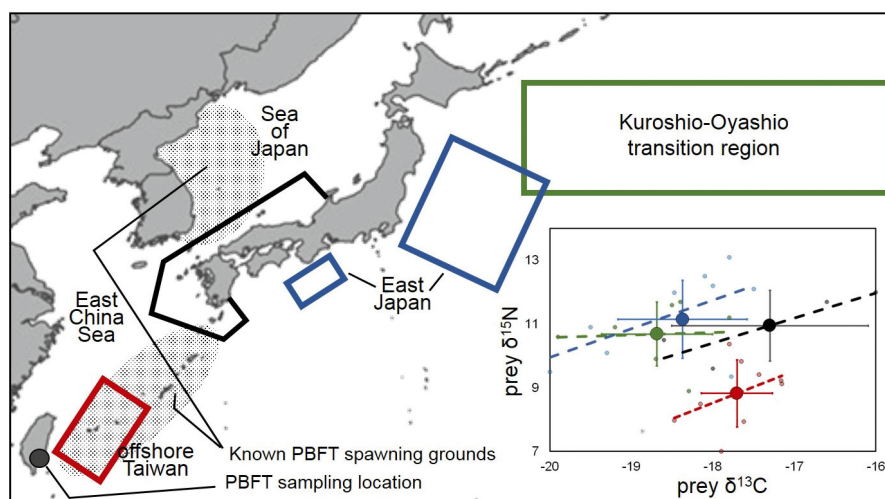
model MixSIR (Moore & Semmens 2008) with uniform priors and 10^6 iterations.

Regional mixing model

To quantify inputs of prey by foraging region, we first grouped prey by 4 broad sampling regions. Since large bluefin tunas in other ocean basins are known to feed on a wide variety of fish and cephalopods (Shimose & Wells 2015), we included data from any fish and cephalopod species of sizes that could be consumed by large PBFT. Prey included zooplanktivorous fish, squids, and larger teleost prey (e.g. mackerels, skipjack tuna). Prey were categorized into 4 broad marine ecoregions (Fig. 1) based on foraging regions for smaller PBFT in the western Pacific spawning grounds of adults: the Kuroshio-Oyashio transition region (hereafter 'Kuroshio-Oyashio'), waters off east Japan (hereafter 'East Japan'), the Sea of Japan/East China Sea, and Taiwan spawning grounds. There are oceanographic differences between regions which were expected to propagate to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of prey, and differences of regional prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were assessed graphically. Prey were sampled directly from East Japan and offshore Taiwan; these data were supplemented by prey values from the literature (Takai et al. 2000, Yasue et al. 2013). Prey values for the Sea of Japan and the Kuroshio-Oyashio transition region were taken from the literature (Gould et al. 1997, Takai et al. 2000, Tanaka et al. 2008). A mean ($\pm\text{SD}$) value for each region was computed weighing each prey species equally.

The 4 regional prey groups—Kuroshio-Oyashio, East Japan, the Sea of Japan, and eastern Taiwan—were used as diet inputs for PBFT captured off Taiwan. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\pm\text{SD}$) from each region, as calculated above, were used as prey inputs. Since isotopic mixing model results are sensitive to the selected DTDF, we used 2 different DTDFs for all mixing models. The first is a lab-derived, species-specific DTDF calculated from PBFT kept in captivity for ~8 yr: $\Delta^{15}\text{N} = 1.9 \pm 0.4$, $\Delta^{13}\text{C} = 1.8 \pm 0.3$ (Madigan et al. 2012b). Since diet $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can influence consumer DTDFs, we also applied a second, diet-based DTDF. This DTDF was calculated from a regression for fish from an extensive review of the literature that is based on fish diet (calculated DTDF for western Pacific PBFT: $\Delta^{15}\text{N} = 1.9 \pm 0.5$, $\Delta^{13}\text{C} = 1.1 \pm 0.5$) (Caut et al. 2009). Mixing models were run with both DTDFs to assess agreement between both approaches.

Fig. 1. Sampling areas for Pacific bluefin tuna and prey in the western Pacific Ocean. All Pacific bluefin tuna ($n = 261$) were collected off Taiwan. Prey were collected from offshore Taiwan and East Japan, and taken from published studies for the Sea of Japan and the Kuroshio-Oyashio transition region. Prey for each region includes sardine, anchovy, squid, mackerel, and other known prey taxa of Pacific bluefin tuna. Inset shows mean isotopic composition (colored circles) \pm SD (error bars) of prey for each region, and linear fit (dashed colored lines) to data for each region



Trophic mixing model

Regional mixing model results showed that PBFT fed primarily in the Kuroshio-Oyashio and East Japan region (see 'Results'), so we separated prey in these regions into trophic groups to assess the relative trophic position of PBFT. This trophic mixing model was used to assess the trophic position of PBFT in the western Pacific and test the null hypothesis that bluefin feed primarily on small schooling fishes (e.g. sardine and anchovy), as has been suggested elsewhere (Madigan et al. 2015b). For the 2 ecoregions determined as high-use (Kuroshio-Oyashio and East Japan), prey trophic position estimates were taken from FishBase (www.fishbase.org), which are calculated from published diet studies. Prey were separated into 2 groups: trophic position ≤ 3 (zooplanktivorous forage prey, e.g. sardine, anchovy, saury, myctophids) and trophic position > 3 , which were generally large, more omnivorous prey taxa (e.g. mackerels, pomfrets, large squids). These 2 prey groups, from the Kuroshio-Oyashio and East Japan regions, were used to assess the relative inputs of low versus high trophic level prey in the diets of western Pacific PBFT. Results were compared with AA-CSIA to obtain a comprehensive estimate of the foraging habits and trophic position of PBFT in the western Pacific Ocean.

When reporting results for all mixing models, the proportion of a given input (%) refers to the median estimate value. Parenthetical values report the 95% credible interval for mixing model estimates. Results for all fish and for 2 clusters are presented for both regional and trophic mixing models.

RESULTS

Mean (\pm SD) FL of 261 sampled PBFT was 225.3 cm (± 18.7) and individual PBFT lengths ranged from 172.8 to 254.5 cm. Measured lengths corresponded to a mean estimated age of 15.1 yr (± 5.0 yr), ranging from 7.1 to 26 yr assuming a maximum age of 26 yr (Shimose et al. 2009). PBFT mean $\delta^{15}\text{N}$ was 13.7‰ (± 0.6), ranging from 11.6 to 15.9. The overall mean $\delta^{13}\text{C}$ value after arithmetic lipid-correction (Logan et al. 2008) was -17.2 ‰ (± 0.5), ranging from -19.2 to -15.3 ‰. $\delta^{15}\text{N}$ values of 2 PBFT (FL 191 and 252 cm) were distinctively high (15.7 and 15.9‰; > 1 ‰ higher than next highest values) (Fig. 2). Source amino acid $\delta^{15}\text{N}$ values and trophic position (7.00‰ and 4.5) of the 2 PBFT with aberrantly high $\delta^{15}\text{N}$ values were similar to PBFT from the eastern Pacific Ocean (7.16‰ and 4.3) and source amino acid $\delta^{15}\text{N}$ values were highly dissimilar from other Taiwan PBFT (-1.99 ‰; Fig. 3).

PBFT $\delta^{13}\text{C}$ values correlated positively with $\delta^{15}\text{N}$ values (Fig. 2a), and positive correlations of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with PBFT length were slight but significant (linear regression; $p < 0.05$; Fig. 2b). Once corrected for both PBFT-specific and diet-determined DTDFs (Caut et al. 2009, Madigan et al. 2012b), PBFT $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values overlapped with the isotopic composition of East Japan and Kuroshio-Oyashio prey (Fig. 4). Only a few individuals showed values consistent with prey isotopic values from the Sea of Japan (and only when using DTDFs from Caut et al. 2009).

We obtained prey from 69 prey samples in East Japan and 54 prey samples in Taiwan, and obtained additional values from the literature (Table 1). Prey

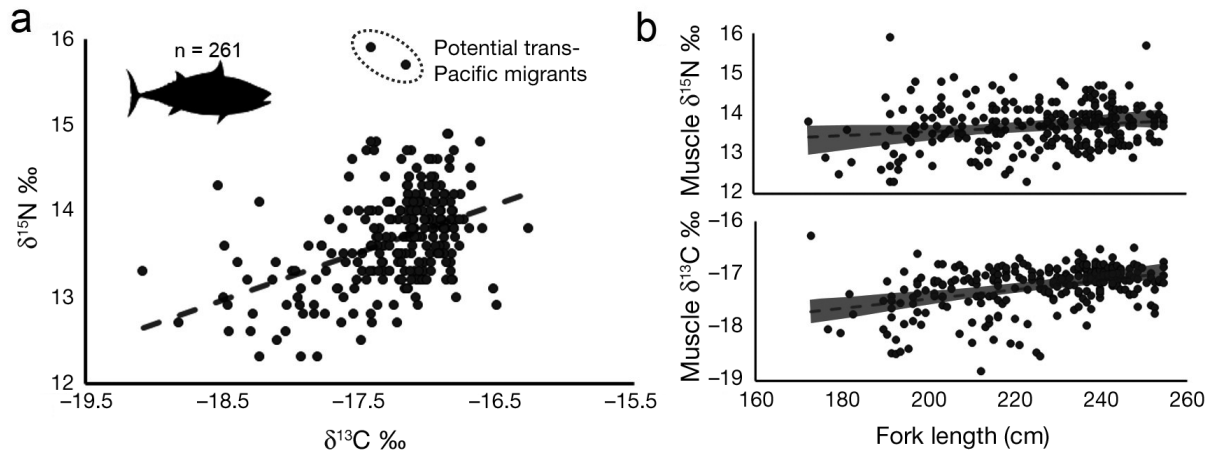


Fig. 2. Observed trends of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in 261 giant Pacific bluefin tuna (PBFT) captured off Taiwan. (a) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were positively correlated ($p < 0.01$). Note, 2 putative trans-Pacific migrants showing aberrantly high $\delta^{15}\text{N}$ values, similar to PBFT from the eastern Pacific Ocean (see 'Discussion'). (b) Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed a slight increase ($p < 0.05$) with fish size. Shaded grey regions in (b) show 95% prediction bounds for mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values based on PBFT size

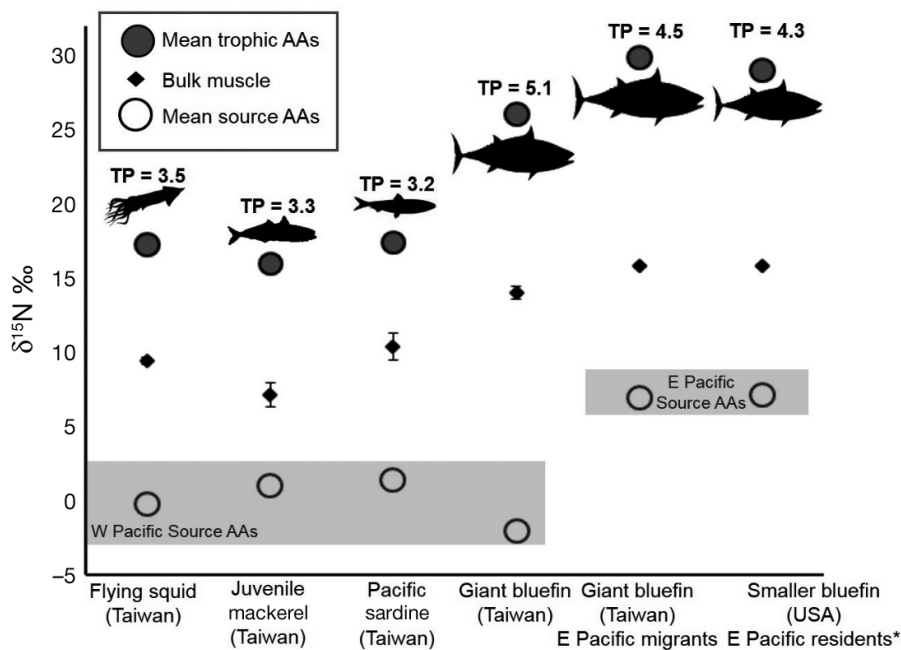


Fig. 3. Amino acid-compound specific isotopic analysis confirms high trophic position of Pacific bluefin tuna (PBFT) off Taiwan. Prey species were analyzed to assess trophic position relative to PBFT. Sardine and juvenile jack mackerels (zooplanktivorous) and flying squid (zooplanktivorous and/or piscivorous) showed similar source amino acid (AA) (glycine, serine, and phenylalanine) $\delta^{15}\text{N}$ values (open circles) and variable trophic AA (alanine, valine, leucine, isoleucine, proline, and glutamic acid) $\delta^{15}\text{N}$ values (closed circles). SD for all weighted mean source and trophic AA values was $< 1.0\%$ (not visible due to y-axis scale). Weighted mean trophic position of PBFT was 5.1, confirming high trophic level feeding suggested by bulk SIA and Bayesian mixing models. The source AA $\delta^{15}\text{N}$ values and trophic position of 2 Taiwan giants with high bulk muscle $\delta^{15}\text{N}$ values (black diamonds) were highly similar to eastern Pacific bluefin (far right), confirming that they had recently migrated from the eastern Pacific before moving into spawning ground off eastern Taiwan. *Data for smaller bluefin sampled in the USA (far right) are from Madigan et al. (2014)

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the 4 marine ecoregions (offshore Taiwan, Sea of Japan and East China Sea, East Japan, and Kuroshio-Oyashio) were similar within regions and dissimilar across regions (Table 1, Fig. 1) with the exception of East Japan and Kuroshio-Oyashio, which showed higher overlap (Table 1, Fig. 1 inset). East Japan and the Kuroshio-Oyashio collectively showed relatively low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values (Fig. 1, Table 1). In comparison, Sea of Japan prey showed higher $\delta^{13}\text{C}$ values and offshore Taiwan prey had intermediate $\delta^{13}\text{C}$ and lower $\delta^{15}\text{N}$ values (Table 1). Linear regression fit to prey from each region showed a positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in all regions (Fig. 1 inset).

Regional mixing model results suggested predominant recent feeding (previous ~ 1.5 yr) of Taiwan-captured PBFT in East Japan and Kuroshio-Oyashio waters (Fig. 5a). DTDFs from Madigan et al. (2012b) resulted in 100% (99–100%) of prey inputs from East Japan and Kuroshio-Oyashio collectively, and DTDFs from Caut et al. (2009) resulted in estimates of 84% from these regions with 16% (12–19%) from Sea of Japan; neither

Table 1. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for prey species in the western North Pacific Ocean used in this study. Mean isotopic values for regional prey groupings used in mixing models shown in bold. A dash (–) for a given species standard deviation (SD) indicates that value was not available from the published data. All values are in ‰

Region / Prey type	Species	$\delta^{13}\text{C}$ (SD)	$\delta^{15}\text{N}$ (SD)	Source
East Japan				
Pacific sardine	<i>S. melanostictus</i>	–17.8 (–)	10.5 (–)	Yasue et al. (2013)
Northern anchovy	<i>E. mordax</i>	–17.8 (1.2)	9.4 (0.7)	Yasue et al. (2013)
Northern anchovy	<i>E. mordax</i>	–18.1 (–)	11.1 (–)	Yasue et al. (2013)
Round herring	<i>E. teres</i>	–18.1 (–)	10.4 (–)	Yasue et al. (2013)
Neon flying squid	<i>O. bartramii</i>	–17.5 (0.2)	12.1 (0.3)	Takai et al. (2000)
Armhook squid	<i>G. borealis</i>	–17.8 (0.2)	13.1 (0.2)	Takai et al. (2000)
Squid	<i>O. borealijaponica</i>	–18.0 (0.4)	12.2 (0.3)	Takai et al. (2000)
Pacific mackerel	<i>S. japonicus</i>	–19.5 (0.6)	10.9 (0.5)	This study
Sand lance	<i>Ammodytes</i> spp.	–19.3 (0.6)	10.1 (0.2)	This study
Jack mackerel	<i>T. japonicus</i>	–18.1 (0.3)	12.5 (0.3)	This study
Squid	Miscellaneous	–20.0 (0.8)	9.5 (0.6)	This study
Mean		–18.4 (0.8)	11.1 (1.2)	
Kuroshio-Oyashio				
Pacific saury	<i>C. saira</i>	–18.7 (0.7)	9.9 (1.2)	Gould et al. (1997)
Lanternfish	<i>Myctophidae</i> spp.	–19.9 (0.3)	10.6 (0.3)	Gould et al. (1997)
Northern anchovy	<i>E. mordax</i>	–18.3 (0.8)	8.9 (0.8)	Tanaka et al. (2008)
Pacific pomfret	<i>B. japonica</i>	–19.2 (0.5)	10.9 (1.3)	Gould et al. (1997)
Luminous flying squid	<i>E. luminosa</i>	–17.8 (0.3)	11.2 (1.1)	Takai et al. (2000)
Neon flying squid	<i>O. bartramii</i>	–18.4 (0.2)	11.7 (0.4)	Gould et al. (1997)
Squid	Miscellaneous	–18.5 (0.4)	11.6 (1.9)	Gould et al. (1997)
Mean		–18.7 (0.7)	10.7 (1.0)	
Sea of Japan				
Northern anchovy	<i>E. japonicus</i>	–18.0 (0.5)	9.6 (0.4)	Tanaka et al. (2008)
Japanese flying squid	<i>T. pacificus</i>	–18.6 (0.5)	10.5 (0.4)	Takai et al. (2000)
Spear squid	<i>L. bleekeri</i>	–16.6 (0.5)	11.7 (0.6)	Takai et al. (2000)
Stubby squid	<i>R. pacifica</i>	–16.0 (0.2)	12.0 (0.5)	Takai et al. (2000)
Mean		–17.3 (1.2)	11.0 (1.1)	
Taiwan				
Bigeye scad	<i>S. crumenophthalmus</i>	–17.2 (0.4)	9.2 (0.6)	This study
Bullet mackerel	<i>A. rochei</i>	–17.7 (0.5)	9.8 (0.9)	This study
Skipjack tuna	<i>K. pelamis</i>	–17.6 (0.4)	7.9 (1.2)	This study
Red bigeye scad	<i>P. macracanthus</i>	–17.8 (0.4)	10.4 (0.5)	This study
Redtail scad	<i>D. kurroides</i>	–17.4 (0.4)	9.4 (2.0)	This study
Kawakawa	<i>E. affinis</i>	–17.9 (0.4)	7.0 (0.9)	This study
White-finned flyingfish	<i>C. arcticeps</i>	–17.2 (0.1)	9.1 (0.2)	This study
Moonfish	<i>M. maculata</i>	–18.2 (1.1)	8.5 (1.9)	This study
Squid	Miscellaneous.	–18.5 (0.1)	8.0 (0.6)	This study
Mean		–17.7 (0.4)	8.8 (1.1)	

approach suggested measurable PBFT feeding on prey in Taiwan waters (Fig. 5). Regional mixing model results using 2 clusters of isotopically similar PBFT (Fig. 6a) showed similar regional use between clusters (Fig. 6b), though larger PBFT showed slightly higher Kuroshio-Oyashio foraging than smaller PBFT (Fig. 6b).

Prey trophic groups in East Japan and Kuroshio-Oyashio were well differentiated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which supported trophic-based groupings for analyses (Fig. 7a). DTDF-corrected PBFT $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

values overlapped highly with larger prey from both the Kuroshio-Oyashio and East Japan (Fig. 7a). Trophic mixing models revealed higher use of large prey by both clusters of PBFT (73–94 %; Fig. 7b). Smaller PBFT used more forage fish prey from East Japan and Kuroshio-Oyashio (21–27 %) than did larger PBFT (2–6 %) (Fig. 7b).

AA-CSIA suggested high trophic level feeding by PBFT in the western Pacific Ocean (Fig. 3). Results showed estimated trophic positions of 3.2, 3.3, and 3.5 for sardine, juvenile mackerel, and flying squid,

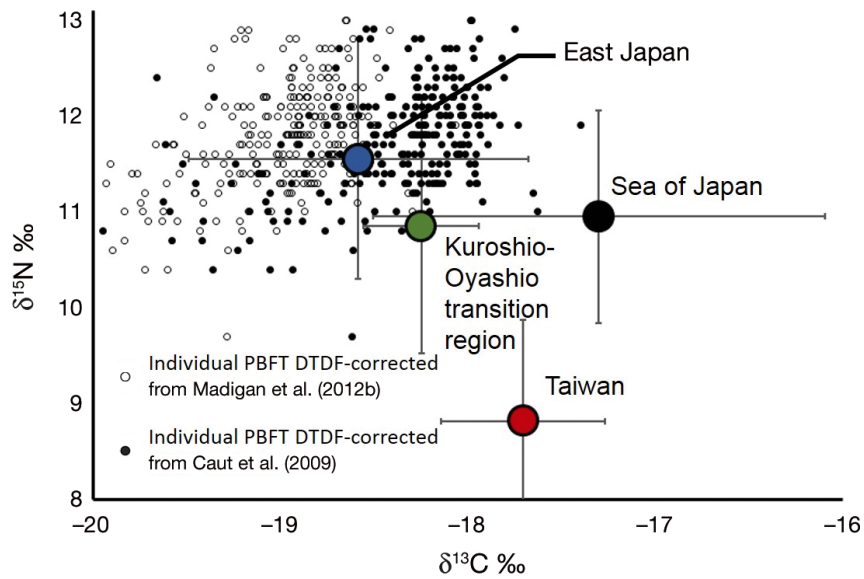


Fig. 4. Pacific bluefin tuna (PBFT) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (DTDF-corrected) overlaid on biplot of regional prey values. PBFT $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were DTDF-corrected (PBFT $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values – $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) using DTDF values from 2 different studies: Madigan et al. 2012b (open circles) and Caut et al. 2009 (filled circles). Both correction factors resulted in high overlap of tuna isotopic values with those of prey from East Japan and the Kuroshio-Oyashio transition region. DTDF: diet-tissue discrimination factor

respectively (Fig. 3). Mean trophic position of PBFT in Taiwan was 5.1, suggesting feeding approximately 2 trophic levels above zooplanktivorous forage fish (e.g. sardine).

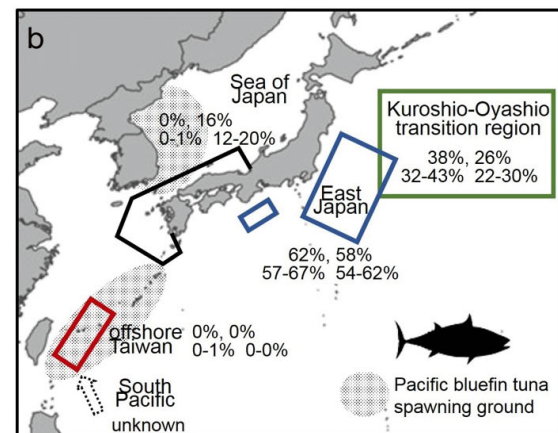
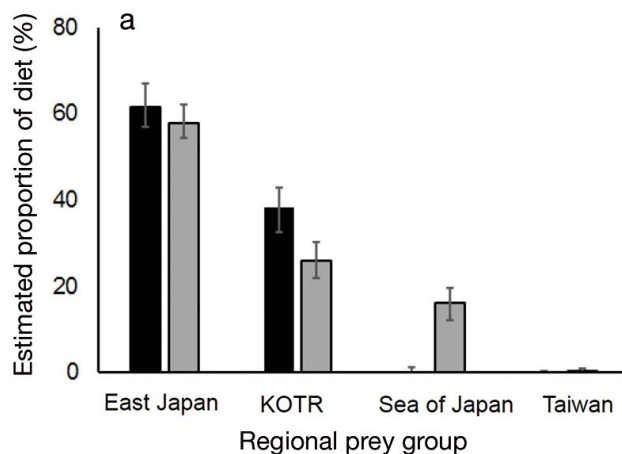


Fig. 5. Isotopic mixing model results for giant Pacific bluefin tuna (PBFT), with prey grouped by marine ecoregion. (a) Mixing model results using prey from 4 ecoregions (KOTR: Kuroshio-Oyashio transition region) as model inputs. Results are shown for 2 separate model runs (black and grey bars) using diet-tissue discrimination factors (DTDFs) from 2 different studies. (b) Map showing mixing model results. Percentages show results using DTDFs from Madigan et al. (2012b) (first value) and Caut et al. (2009) (second value) and associated 5–95% credible intervals. PBFT arriving in Taiwan waters showed high foraging off East Japan and in the Kuroshio-Oyashio transition region. Two different spawning grounds are indicated in (b); it is unknown whether previous movements of fish in the Sea of Japan spawning ground differ from fish entering spawning grounds off Taiwan. Note, potential migration of fish from the South Pacific, where giant PBFT are found but migrations are poorly understood

DISCUSSION

We used intrinsic chemical tracers in 261 PBFT from Taiwanese spawning grounds to ascertain pre-capture regional foraging ecology. Bulk isotopic analyses, when complemented by compound-specific isotopic data, demonstrated that the majority of large PBFT in the Taiwan fishery originate from similar foraging regions (East Japan and Kuroshio-Oyashio). Giant PBFT off Taiwan are high trophic-level predators, relying more on larger prey (mackerels, flying squids, pomfrets) than on smaller, zooplanktivorous forage fish (e.g. sardines, anchovy). This is the first documentation of putative westward trans-Pacific migrants entering PBFT spawning grounds. Although this recent migration was only detectable in 2 individuals, this finding demonstrates the utility of combined SIA and AA-CSIA to quantify exchange of PBFT between the eastern and western Pacific Ocean.

Basic relationships of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within PBFT and across PBFT size were similar to large fish in other systems (e.g. Das et al. 2000, Revill et al. 2009). Significant relationships between $\delta^{13}\text{C}$ and

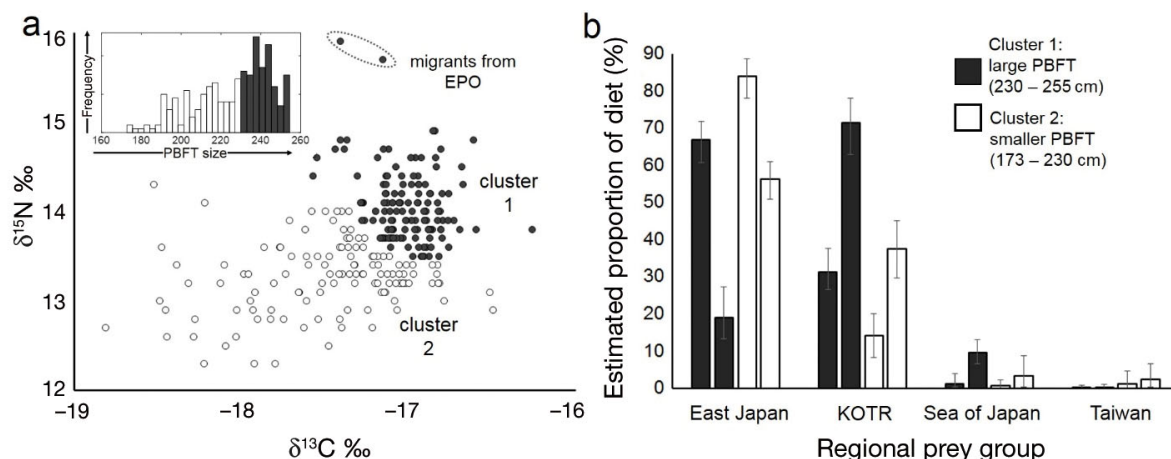


Fig. 6. Regional diet inputs into 2 clusters of Pacific bluefin tuna (PBFT) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data. (a) Cluster analysis separated fish into 2 groups with relatively high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Cluster 1) and lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Cluster 2). Cluster 1 was comprised of overall larger fish (a, inset). (b) Bayesian mixing model estimates using 2 diet-tissue discrimination factors (DTDFs) showed differences in regional use by cluster. Note differences in Kuroshio-Oyashio transition region (KOTR) and East Japan prey inputs for larger PBFT versus smaller PBFT. Error bars show 5–95% credible intervals of mixing model estimates

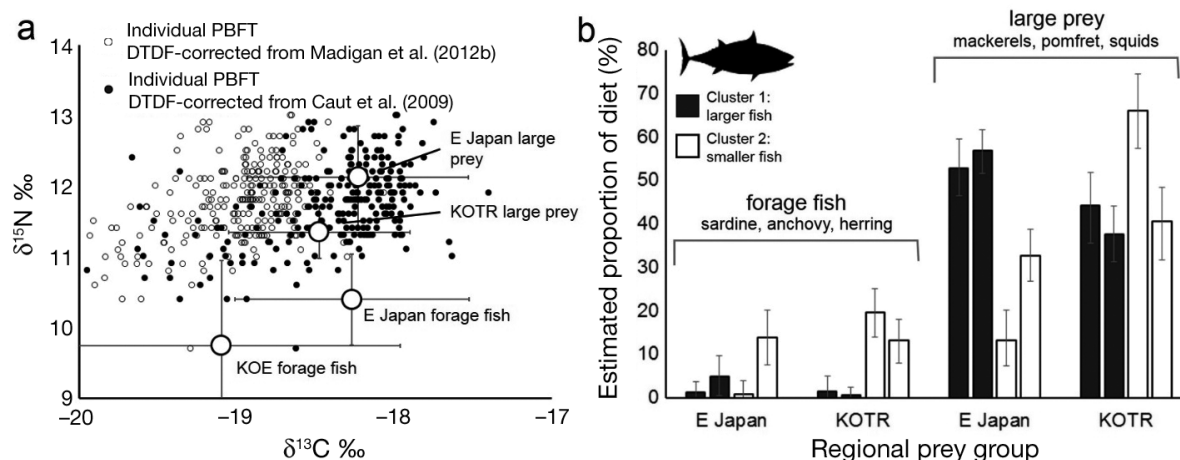


Fig. 7. Relative inputs of forage fish (sardine, anchovy, herring) versus higher trophic level prey (mackerels, ommastrephid squids, pomfrets) in giant Pacific bluefin tuna (PBFT) diet. Use of only 2 ecoregions (East Japan and Kuroshio-Oyashio transition region, KOTR) allowed for analysis of relative inputs of lower versus high trophic level prey. (a) Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\pm 1\text{SD}$) of forage fish and larger prey for East Japan and Kuroshio-Oyashio (large white circles). Overlaid are diet-tissue discrimination factor- (DTDF)-corrected (PBFT) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using DTDF values from 2 different studies (Caut et al. 2009, filled circles; Madigan et al. 2012b, open circles). (b) Large prey dominated the diets of both PBFT groups (Cluster 1 and Cluster 2), though Cluster 2 (smaller PBFT) showed higher inputs of forage fish in both regions

$\delta^{15}\text{N}$ values, PBFT size and $\delta^{13}\text{C}$ values, and PBFT size and $\delta^{15}\text{N}$ values (Fig. 2) support the notion of a transition to larger, higher trophic level prey as PBFT increase in size. Many large pelagic predators show similar trophic changes with size (ontogenetic feeding upshifts), indicated by SIA (Estrada et al. 2006, Phillips & Eldridge 2006, Graham et al. 2007, Carlisle et al. 2015). However, this contrasts with PBFT (and other predators) in the eastern Pacific Ocean, where several predators showed no isotopic increase with size (Madigan et al. 2012a). This may indicate fundamental differences between western and eastern

pelagic food webs, with potentially longer pelagic food web length in the western Pacific due to its relatively lower primary productivity than in the eastern Pacific upwelling system (e.g. Sommer et al. 2002). In contrast, large predators may largely subsist on highly abundant forage prey in the eastern Pacific upwelling pelagic ecosystem (Madigan et al. 2012a).

Isotopic similarity of prey from East Japan and Kuroshio-Oyashio regions (Fig. 1 and Table 1) was not surprising, as these regions overlap spatially and oceanographically. However, the Kuroshio-Oyashio extends eastward into the open Pacific, where juve-

nile PBFT and PBFT migrating from the eastern Pacific have been shown to aggregate and feed (Kitagawa et al. 2004, 2009, Boustany et al. 2010). Differences in the prey species available in the Kuroshio-Oyashio, including saury, ommastrephid squids, and pomfrets (Gould et al. 1997, Takai et al. 2000) justified separating this group from East Japan. The low $\delta^{15}\text{N}$ values of prey from offshore Taiwan waters are likely due to the largely oligotrophic Kuroshio Current, as nitrogen fixation associated with oligotrophic marine systems has been shown to cause low $\delta^{15}\text{N}$ baselines (e.g. Liu et al. 1996, Hannides et al. 2009). In contrast, seasonal upwelling off eastern Japan (Nakada & Hirose 2009) likely drives occasional high prey $\delta^{15}\text{N}$ values in that region, as upwelled nitrate has been shown to produce much higher $\delta^{15}\text{N}$ baseline values than nitrogen fixation (Lorrain et al. 2015). Overall, inter-region prey differences and tight clustering of PBFT isotope values (Fig. 4) were sufficient for clear mixing model results with narrow credible intervals (Fig. 5).

Mixing model results indicate the extent of foraging based on SIA of PBFT muscle tissue, which reflects the last ~1.5 yr of foraging based on published turnover rates (Madigan et al. 2012b). Muscle may represent a longer foraging timeframe in these giant PBFT, as turnover rates of large predators should scale allometrically with predator size (e.g. Carlisle et al. 2012); we thus assume 1.5 yr as a conservative estimate of time-integrated retrospective foraging patterns. In this temporal context, mixing model results suggested that spawners arriving in the Taiwan spawning ground have foraged almost exclusively in East Japan and Kuroshio-Oyashio waters (Fig. 5). This is in good agreement with catch data and with the hypothetical movements of 'spawner' PBFT presented in Fujioka et al. (2015). Mixing model results indicating minimal inputs from the Sea of Japan suggest that PBFT westward of Japan do not migrate into the Ryukyu spawning grounds, and likely utilize the spawning ground within the Sea of Japan. Speculatively, this indicates that fisheries harvesting mature PBFT in the Sea of Japan selectively remove fish that would eventually spawn in that specific spawning ground. Isotopic data from large PBFT in the Sea of Japan, which were unavailable for this study, would test this hypothesis. The fact that Ryukyu spawners are larger than those in the Sea of Japan (Chen et al. 2006, Okochi et al. 2016) suggests that younger spawners using the Sea of Japan spawning ground may eventually use the Taiwan spawning ground as larger fish. However, potential differences in pre-spawning for-

aging patterns between the groups are possible and remain unexamined.

The low percentage of SIA-detected trans-Pacific migrants in this dataset (0.8%) is open to multiple interpretations. Westward migrants from the eastern Pacific may provide an extremely low number of individuals to the overall spawning population; east-west migrants may forage for sufficient time (≥ 1.5 yr) in the western Pacific to eliminate the eastern Pacific isotopic fingerprint; or sexually mature, recent migrants from the eastern Pacific may preferentially utilize the Sea of Japan spawning ground. In previous electronic tagging studies (Boustany et al. 2010), westward trans-Pacific migration by age class 3 to 5 PBFT showed use of the Tsugaru Strait and entry into the Sea of Japan, though none of these fish migrated to the spawning grounds within the Sea of Japan (A. M. Boustany pers. comm.). The smaller size structure of spawners in the Sea of Japan coupled with the relatively young age (reported age class 3 to 5 yr) at which PBFT move from the eastern to the western Pacific (Boustany et al. 2010) makes use of the Sea of Japan by younger PBFT, including recent trans-Pacific migrants, a plausible hypothesis. Larger PBFT have been captured in the eastern Pacific in recent years (2013 to 2015) between ages 3 to 6 years (D. J. Madigan et al. unpubl. data). As all of these fish are smaller than PBFT generally found in the Taiwan fishing ground (Chen et al. 2006, Okochi et al. 2016), it is likely that these PBFT utilize the Sea of Japan upon return to the western Pacific. Isotopic sampling in the Sea of Japan would ascertain whether recently arrived trans-Pacific migrants constitute a more substantial proportion of spawners in that region.

While large (>160 cm) PBFT are present in the South Pacific off Australia and New Zealand, little is known of their large-scale movements once in that region (Smith et al. 2001, Fujioka et al. 2015). Migrations of large PBFT from the South Pacific Ocean to spawning grounds in the western North Pacific have not been observed, and electronic tagging studies of large PBFT near New Zealand have not revealed movement to the northern hemisphere (G. Shillinger pers. comm.). It is of course possible that these large PBFT migrate north to western North Pacific spawning grounds. As we did not sample PBFT or potential prey in South Pacific waters, we could not directly assess the possible contribution of South Pacific prey to giant PBFT isotopic values in the Taiwan spawning ground. However, published muscle SIA values are available for large (153 ± 28 [SD] cm, $n = 17$) southern bluefin *Thunnus maccoyii* (Revill et al. 2009). As southern bluefin forage with PBFT around New

Zealand (Smith et al. 2001), these values may be a reasonable proxy for expected South Pacific PBFT isotopic values. While $\delta^{15}\text{N}$ values were similar (14.3 ± 0.2 [SD] ‰) to PBFT analyzed here, $\delta^{13}\text{C}$ values were much lower (-20.2 ± 0.5 ‰) (Revell et al. 2009) than we observed (Fig. 2). This provides some evidence that PBFT isotopic values were not consistent with South Pacific foraging, but migrants from that region cannot be ruled out. Further study is clearly needed to understand potential movements of PBFT between the North and South Pacific Ocean.

Reliance of giant PBFT on large prey in the western Pacific should be considered in studies that aim to link giant PBFT residency and/or movement patterns to specific prey. However, while SIA analyses provide integrated, long-term estimates of trophic ecology, SIA-based trophic analyses rarely provide species-specific prey information. Large prey shared similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values regionally, likely due to similar trophic ecology (Fig. 7a). However, it is possible that large PBFT exploitation of larger prey is specific to particular prey items. For example, giant PBFT may largely target mackerels and squids, and feed minimally on pomfrets. Such prey-specific diet information requires traditional diet studies, which may be appropriate as complementary work to understand potentially strong linkages between giant PBFT predators and specific prey. Madigan et al. (2015b) demonstrated such linkages in the eastern Pacific Ocean, where PBFT seem to specialize on sardines and anchovy more than other tunas. A switch to larger teleost prey with increasing predator size is consistent with other bluefin species, as larger Atlantic bluefin feed on herring, mackerel, hake, pomfrets, and bluefish (Matthews et al. 1977, Chase 2002, Estrada et al. 2005, Butler 2007, Shimose & Wells 2015) and southern bluefin on Cape bonnetmouth and large squids (Young et al. 1997).

AA-CSIA provided crucial support to trophic mixing model estimates. Mixing model estimates rely on adequate prey representation, appropriate DTDF selection, and prey groupings that are statistically and ecologically sound (Post 2002, Bond & Diamond 2011). Since exhaustive sampling of prey in the pelagic environment is impossible, there always exists the possibility of unintentional exclusion of representative prey taxa. However, our mixing model results suggesting PBFT feeding more on higher trophic level prey than on zooplanktivorous forage fish (Fig. 7b) were supported by the independent AA-CSIA approach, with a trophic position estimate of 5.1 for PBFT (Fig. 3). The utility of AA-CSIA was further demonstrated by parsing out source and trophic

amino acid $\delta^{15}\text{N}$ values in 2 putative migrants. The 2 trans-Pacific migrants showed higher bulk $\delta^{15}\text{N}$ values than other Taiwan PBFT (Fig. 2a), which is often interpreted as an indication of higher trophic position. By quantifying source and trophic amino acid $\delta^{15}\text{N}$ values, AA-CSIA revealed a lower trophic position (4.5) that is more similar to PBFT in the eastern Pacific Ocean (4.3; Fig. 3). When such analyses are possible, AA-CSIA provides invaluable support and clarity to bulk SIA-based inferences.

Results and conclusions in this study have inherent caveats typical of many studies applying SIA to wild migratory predators. A regional mixing model contains the implicit assumption that all regions used by PBFT have been characterized. Even when migratory patterns are well constrained geographically, confidently representing all possible regions is not realistic for wide-ranging marine predators. Isotopic characterization of region will also be limited by data availability, and prey representation may differ between regions. Here, East Japan and Kuroshio-Oyashio are represented by more prey taxa than the Sea of Japan, which is more highly influenced by isotopic values of individual prey species (Table 1). Low $\delta^{13}\text{C}$ values of prey in the Sea of Japan largely drove inferences of low use of this area by these PBFT. Given the limited data for the Sea of Japan, this result will best be tested by future studies that explicitly explore fine-scale movements of PBFT (e.g. using electronic tagging technology). Prey sampling was not exhaustive in any region, and bluefin likely consume more prey species than are represented by any region. The assumption here, as with similar approaches in the SIA literature (e.g. Carlisle et al. 2012, Madigan et al. 2015a), is that regional prey reasonably represent other trophically similar prey available in the region. While quantitative mixing model results likely vary slightly from actual PBFT diet, the patterns observed (high foraging in East Japan and Kuroshio-Oyashio) would be maintained even with more exhaustive sampling, given the above assumption. Finally, DTDF selection has a demonstrably large impact on mixing model estimates (e.g. Caut et al. 2009, Hussey et al. 2014). Assumptions will always be necessary when applying lab-derived DTDFs to wild data. Studies in wild fish have revealed intra-specific variability, and suggest avoiding use of DTDFs derived from meta-analysis (e.g. the commonly applied $\delta^{15}\text{N}$ DTDF value of 3.4‰) and using lab-derived DTDFs when possible (Wyatt et al. 2010). While most studies of large marine predators must use DTDFs from smaller animals as proxies due to challenges of captive studies, here we were able to apply a species-

specific DTDF from an 8 yr study of the same fish species (Madigan et al. 2012b). In conjunction with results from an estimated DTDF based on diet effects (Caut et al. 2009), we report mixing model results using DTDFs of a specificity rarely possible in ecological SIA studies of wild pelagic predators.

Pacific bluefin tuna are highly exploited in both oceans, while basic life history information is still lacking. Results here provide insight into the general movements and foraging ecology of giant PBFT that utilize the major spawning ground of the species. However, more comparative work is needed. Migratory patterns between the Sea of Japan and Taiwan spawning regions should be compared; these can be combined with promising otolith microchemistry studies that can identify PBFT to their native nursery grounds (Rooker et al. 2001). The foraging ecology examined here reveals higher trophic functioning of giant PBFT than previously thought, but is relevant only to the years of study (2011 to 2014); trophic downshifts are plausible in the case of high sardine or anchovy abundance. Trophic dynamics fluctuate and we encourage long-term studies to examine PBFT trophic variability as it relates to environment and prey abundances. The implications of this study raise new questions about the dynamics of giant PBFT around their spawning grounds and the species and fishery will benefit from further comparative work aimed at answering these questions.

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