

Temporal variation in the diet of Yangtze finless porpoise calls for conservation of semi-migratory fish

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Abstract

1. Identifying the feeding patterns of large-bodied predators is necessary to predict their potential effects on food-web dynamics and to inform species conservation. In contrast to diet information from stomach contents, diet estimates obtained using stable isotopes in blood and muscle can reveal temporal variation in diets.
2. Here, we used an extensive stable isotope dataset and Bayesian stable isotope mixing model to evaluate the trophic role of the Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*), a critically endangered species distributed in the middle and lower catchments of the Yangtze River. We analysed blood samples from 24 individuals captured during March 2017 in Poyang Lake (which adjoins the Yangtze River), and muscle samples from 8 individuals stranded in the lake over a 1-year period (2012–2013). We additionally genotyped prey from faecal samples taken from 13 individuals captured in March 2017.
3. We found that as a generalist apex predator, this finless porpoise consumes semi-migratory and non-migratory fish prey. However, relative proportions of these two prey resources were different between dietary analyses based on blood samples compared to muscle samples, reflecting recent and longer-term consumption patterns, respectively.
4. Non-migratory fish contributed mainly to the diet of predators in the early and middle of March. In contrast, semi-migratory fish comprised the majority of the prey composition over a more extended period (e.g. 2–3 months), and the difference may be linked to changes in the fish abundance influenced by seasonal factors.
5. Our findings provide insights into the trophic role of this critically imperilled but little studied species, demonstrate how stable isotope analyses can elucidate finless porpoise feeding ecology, and emphasise the importance of semi-migratory fish to conserving this porpoise population.

KEYWORDS

apex predator, cetacean, foraging ecology, stable isotopes, trophic coupling

1 | INTRODUCTION

Decreases in the number of large predators have occurred globally, with potentially serious consequences for ecosystems (Worm et al., 2002). Understanding foraging patterns of large-bodied predators is necessary for predicting their potential effects on food web dynamics and is an important empirical incentive for conserving large predators (Myers & Worm, 2003). Typically, these predators are highly mobile within a large territory and can increase spatial connectivity between habitats and ecosystems, which is hypothesised to stabilise food webs through predator-driven linkage complexity (Xu et al., 2016). These effects can then cascade down food chains to influence primary producer abundances and distributions, resulting in changes in community structure and ecosystem function (Aufderheide et al. 2013). Freshwater dolphins are among the world's most threatened mammals, owing to their small population size due to the effects of extensive human activities and accompanying habitat damage. They are apex predators in some of the world's large river systems, yet a comprehensive investigation of their foraging strategies remains intractable (Smith & Reeves, 2012).

As a large-bodied apex predator, the Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) is the only freshwater porpoise endemic to the middle-lower reaches of the Yangtze River and two connected lakes, Poyang Lake and Dongting Lake (Wang, 2009). The species has been classified as Critically Endangered by the IUCN Red List of Threatened Species because of rapid population decreases (Mei et al., 2014). The depletion of food resources is one of the most important threats faced by the Yangtze finless porpoise in its local habitat; its prey fishes have been over-exploited by net and trap fishing since 1954 (Mei et al., 2012, 2014). Large-bodied apex predators in marine ecosystems can respond to local food limitation by migrating on a large scale, following the movement of their prey. As Yangtze finless porpoises live in freshwater, they cannot follow their prey using large-scale migration. They are constrained by the loss of hydrobiological and biological linkages between most lakes distributed along the mainstream in the middle and lower Yangtze River caused by land reclamation, and dam and dike construction during mid-20th century (Wang, 2009). Therefore, an adequate exploration of the feeding ecology of the Yangtze finless porpoise can help us to understand present foraging strategies of the porpoise population limited to small-scale movements, which is necessary for the well-informed management of both this endangered species and its prey (Wang et al., 2019).

Yangtze finless porpoises are often described as opportunistic predators in that rather than preying on particular taxa, the porpoises consume prey in proportion to prey availability (Kasuya, 1999). Recently, at least 20 potential prey species were identified using DNA-based stomach content analysis from stranded porpoises collected from different habitats across the main stem of the Yangtze River, Poyang Lake, and Dongting Lake (Yang et al., 2019). However, stomach content analysis records recent consumption (i.e. over a few days) and may not reflect the entire diet of the dolphin (Pierce & Boyle, 1991).

Foraging strategy variation at different timescales potentially regulates consumer-resource relationships, particularly for opportunistic predators such as Yangtze finless porpoises that are unable to migrate to follow prey. The dietary composition of these porpoises is influenced by seasonal variation in the abundance of dominant prey species (Deng et al. 2013; Hu et al., 2011; Zhang et al., 2013). Therefore, it is possible that the porpoises can form a foraging strategy of opportunistically feeding on particular prey that are abundant over a limited period (short term), in contrast to prey that are consumed in greater abundances when averaged over longer periods (long term). This temporal variation in dietary composition has been confirmed in harbour porpoises (*Phocoena phocoena*) and bottlenose dolphins (*Tursiops truncatus*) (Gimenez et al., 2017; Jansen et al., 2013), but it is not clear for the Yangtze finless porpoise. In addition, large-bodied apex predator populations can vary in their use of habitat and food resources because of ontogeny, sex, and individual specialisation. Accordingly, understanding comprehensively the diet of Yangtze finless porpoises, both in the short and long term, should be considered to understand the interactive dynamics of the apex predator–multispecies prey relationship. This can provide more targeted conservation efforts through food web interactions from a time-integrated viewpoint.

While there are several studies on short-term trophic strategies using stomach contents or faecal analysis, no study has directly assessed how lake populations of porpoise vary in the use of different prey types such as semi-migratory and non-migratory fish. Semi-migratory fish form schools during the breeding season and show potamodromous migration, whereas non-migratory fish usually spend their entire lives in one area. Investigating the proportion of semi-migratory and non-migratory fish in the porpoises' diet is a vital way to analyse temporal variance porpoise reliance on alternative prey. Here, we analysed stable isotopes in whole blood (i.e. short term) and muscle (long term), and DNA in faeces collected from free-ranging Yangtze finless porpoises. These analyses were used to assess variation in the use of semi-migratory and non-migratory fish prey by the Yangtze finless porpoise population inhabiting Poyang Lake, China. Specifically, we asked the following questions: (1) Do porpoises use semi-migratory and non-migratory fish prey to differing degrees? (2) Does foraging composition vary at different times and/or differ between sexes or sizes? By answering these questions, we also aimed to provide further information regarding the importance of conserving alternative fish prey resources for Yangtze finless porpoises.

2 | METHODS

2.1 | Sample collection

Poyang Lake, one of the lakes adjoining the main stem of the Yangtze River, is currently habitat for approximately 450 wild Yangtze finless porpoises (Huang et al., 2020). Although the number of porpoises in Poyang Lake has remained stable in the past decade (Mei et al., 2019),

fishery resources are being gradually reduced by intensified fishing activities and widespread illegal fishing (Wang et al., 2019).

Eight stranded adult finless porpoises collected from Poyang Lake, Jiangxi Province, China, between 2012 and 2013 were selected for this study (Appendix S1: Table S1). The carcasses were relatively fresh, necropsied, and sampled within 24 hr. Frozen muscle samples (preserved at -20°C) were analysed for this study. Whole blood samples were collected from 24 free-ranging Yangtze finless porpoises (Appendix S1: Table S1) living in Poyang Lake between 16 and 22 March 2017, while a catch-and-release operation was determining population status in this area. After the porpoises were caught and sent to an examination platform, blood was drawn from the vein in the fluke using disposable syringes, placed in sterilised 1.5-ml Eppendorf tubes, and then preserved in liquid nitrogen until stable isotopic analysis. Stable isotope analyses of the whole blood and muscle provide short-term and long-term foraging information, respectively, because the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of whole blood in marine mammals reflect the nutrients absorbed approximately 1–2 weeks prior to collection (Kurle, 2002), whereas the muscle tissue can reflect the assimilated diet for several months (Hobson et al., 1996; Tieszen et al., 1983).

To obtain diet information to use as priors in stable isotope mixing models, faecal samples were collected from the Yangtze finless porpoises captured in this survey. Disposable soft plastic tubes 4 mm in diameter were inserted into the anus of the animals, allowing any faecal material to be carried along the tubes. We obtained faecal samples from 13 individuals (Appendix S1: Table S1). Faecal materials were also preserved in liquid nitrogen.

During this catch-and-release survey, half-day electrofishing was implemented to collect potential prey species in the area where the porpoises were caught. Fish prey samples were selected based on a similar size as the length of the prey that appeared in the stomach contents of the Yangtze finless porpoise. The prey samples were stored at -20°C prior to analysis.

2.2 | Stable isotope analysis

Whole blood (200 μl) samples collected from 24 porpoises in March 2017 were vacuum freeze-dried and ground into a fine powder. Additionally, approximately 2-g samples of dorsal muscle tissue from eight stranded porpoises (collected in 2012–2013) and from prey fish collected in March 2017 were rinsed in distilled water, dried for 48 hr at 55°C , and finely ground into a homogenous powder using a sterile mortar and pestle. Since the C: N ratios of the muscle samples were <3.6 , lipid extraction was not needed in this study (Post et al., 2007). Then the powder was analysed in a continuous flow isotope ratio mass spectrometer (Flash 2000 EA-HT DELTA V Advantage, Thermo Fisher Scientific, Inc., USA). Stable isotope abundance is expressed in standard δ notation (IAEA Stable Isotope Laboratory, Vienna, Austria) relative to carbonate Pee Dee Belemnite and atmospheric nitrogen. Replicate assays of standards routinely inserted within the sampling sequence indicated analytical

measurement errors of $\pm 0.2\text{‰}$ and $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively (Xu et al., 2016).

2.3 | Faecal analysis

About 180 mg of faecal samples were extracted for DNA analysis using the QIAamp Fast DNA Stool Mini Kit (Qiagen Inc., Wuhan, China). We then conducted the DNA amplification and next-generation sequencing of 13 samples at the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) following the protocol for the dietary analysis of Yangtze finless porpoises (Yang et al., 2019).

2.4 | Data analysis

Nine of the most abundant potential prey species (as identified from DNA-identification of stomach contents in stranded individuals (Yang et al., 2019) in our electrofishing catch were treated as prey sources in our analysis. The mean isotopic composition and the respective standard deviation of the adult and juvenile porpoises were calculated for Yangtze finless porpoises and their prey. According to age-length formula, one porpoise with body length less than 130 cm was considered as a juvenile individual (Gao & Zhou, 1993). Statistical analysis of stable isotopes was performed using SPSS Statistics 19. We used multivariate analysis of variance (MANOVA) to compare $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in blood and muscle between sexes in different tissues (muscle and whole blood). Before the construction of Bayesian stable isotope mixing models (BSIMM), the prey fishes were divided into two groups, semi-migratory species (which migrate to the upper reaches of the major lakes or rivers to spawn) and non-migratory species (Liang et al., 1981). Stable isotope values for each group were assessed for normality using the Shapiro–Wilk test. MANOVA was used to test significant differences between semi-migratory and non-migratory fishes by assessing stable isotopes simultaneously with a post hoc test for pairwise differences. The BSIMM analysis assumes that sources differ significantly in stable isotope signatures. The level of significance was set at $\alpha = 0.05$.

We used a Monte Carlo simulation of mixing polygons to apply the point-in-polygon assumption in stable isotope mixing models (Smith et al., 2013). The method iterates convex hulls of the distributions of the proposed dietary sources and trophic enrichment factors (TEFs) and calculates the mixing polygons that have the consumers' isotopic signature. Owing to a lack of TEF data for the Yangtze finless porpoise, we used TEFs from previously published literature: $\Delta^{13}\text{C} = 1.05 \pm 0.33$ (‰) from whole blood samples of Killer Whales (*Orcinus orca*) and $\Delta^{15}\text{N} = 2.73 \pm 0.58$ (‰) from muscle samples of fin whales (*Balaenoptera physalus*) (Borrell et al., 2012; Caut et al., 2011; Healy et al., 2018). The mixing polygon simulation is then visualised with a mixing region, which is calculated by testing a grid of values for the point-in-polygon (Smith et al., 2013).

We used proportions of prey identified as operational taxonomic units (OTUs) in faecal samples as informative priors for potential prey

sources in the BSIMM. The faeces data were analysed on the free online Majorbio I-Sanger Cloud Platform (<http://www.i-sanger.com>). All raw reads were demultiplexed, quality-filtered using Trimmomatic and merged by FLASH according to the criteria stated by (Yang et al., 2019). Filtered sequences were clustered into OTUs and picked at 97% similarity value by the UCLUST algorithm (Whon et al., 2018). After excluding OTUs with low numbers (<10 sequences per OTU), representative sequences of each OTU were compared with GenBank. Closely matched sequences were downloaded from GenBank in accordance with the descriptions page of BLAST, and these sequences with one representative sequence of prey species were aligned by DNAMAN. Only representative sequences matched with 99% sequence similarity can be regarded as an available identification, although some OTUs may have a 99% match to several species because of short length of the sequences (250 bp) (Yang et al., 2019).

Finally, MixSIAR was used to estimate the proportional contribution of prey species to the diet of the porpoises (Stock et al., 2018). The parameters in the Markov Chain Monte Carlo were specified as chain length 50,000, burn-in 25,000, and thin by 25 to ensure that the three chains converged. From this estimated *posterior* distribution, we can then calculate the mean/median, standard deviation, and Bayesian credible intervals for the contribution of potential prey sources. MixSIAR can include fixed and random effects as covariates that explain the variability in mixture proportions and calculate relative support for multiple models via information criteria (Stock et al., 2018). Thus, to assess effects of time-scale (based on blood vs. muscle), and porpoise sex and length on estimated dietary proportions of semi-migratory and non-migratory fish prey, we fit seven alternative models to the data: (1) a null mode (no fixed effects); (2) time-scale dependent model (with muscle and blood factor fixed effects); (3) sex model; and (4) size model. From Model 5 to Model 7, the tissue type was combined with sex, length, or both as fixed factors and covariates. We then evaluated the relative support for each model using

information criteria, that is, leave-one-out cross validation (LOO) and widely applicable information criterion (WAIC). The LOO and WAIC are methods for estimating pointwise out-of-sample prediction accuracy from a fitted Bayesian model using the log-likelihood evaluated at the posterior simulations of the parameter values (LOO/WAIC) and standard errors for the difference in LOO/WAIC between two models (dLOOic). The relative support for each model using Akaike weights was also calculated (Hewson, 2016; Vehtari et al., 2017).

3 | RESULTS

The $\delta^{13}\text{C}$ values measured in the whole blood samples of the adult porpoises were lower than the values measured in the muscle samples, while the $\delta^{15}\text{N}$ values of the whole blood samples of the adult porpoises were higher than those of the muscle samples (Table 1). In terms of whole blood samples, we did not find a significant difference between sexes in $\delta^{13}\text{C}$ (male: -26.20 ± 0.41 ; female: -26.17 ± 0.53 ; $F_{1,22} = 0.34$, $p = 0.855$) and $\delta^{15}\text{N}$ (male: 17.28 ± 0.71 ; female: 17.43 ± 0.38 ; $F_{1,22} = 0.419$, $p = 0.524$). However, $\delta^{15}\text{N}$ of muscle samples of males (17.20 ± 1.03) were significantly higher than females (15.22 ± 0.90 ; $F_{1,6} = 8.210$, $p = 0.029$) but there was no difference in $\delta^{13}\text{C}$ isotopes (male: -25.75 ± 1.00 ; female: -25.23 ± 2.31 ; $F_{1,6} = 0.133$, $p = 8.210$). For the prey species, *Hemiculter bleekeri* exhibited the lowest levels of $\delta^{13}\text{C}$ (-26.62 ± 1.32), in contrast to *Acheilognathus macropterus* (-23.79 ± 1.41). In addition, *Coilia brachygnathus* showed the highest level (16.76 ± 0.9) for $\delta^{15}\text{N}$, while *Pseudobrama simoni* showed the lowest level (12.12 ± 1.5 ; Table 1). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly different in the muscle of prey species (Kruskal-Wallis, $\delta^{13}\text{C}$: $F_{8,9} = 24.57$, $df = 8$, $p = 0.02$; $\delta^{15}\text{N}$: $F_{8,9} = 62.28$, $df = 8$, $p < 0.001$).

A total of 25,325 raw sequences were retrieved from the faecal samples. After quality filtering, representative sequences of seven

Species	Tissue	n	$\delta^{13}\text{C}$ (‰) mean \pm SD	$\delta^{15}\text{N}$ (‰) mean \pm SD
Predator				
Yangtze finless porpoise	Whole blood	20	-26.23 ± 0.44	17.25 ± 0.58
Yangtze finless porpoise	Muscle	8	-25.42 ± 1.84	15.96 ± 1.34
Prey				
<i>Coilia brachygnathus</i>	Muscle	9	-26.53 ± 1.14	16.76 ± 0.90
<i>Pseudobrama simoni</i> *	Muscle	10	-24.45 ± 2.85	12.12 ± 1.50
<i>Cyprinus carpio</i>	Muscle	10	-25.86 ± 1.55	15.17 ± 0.27
<i>Pelteobagrus nitidus</i> *	Muscle	8	-26.37 ± 0.72	14.27 ± 0.29
<i>Carassius auratus</i>	Muscle	10	-25.09 ± 2.08	15.59 ± 0.96
<i>Hemiculter bleekeri</i> *	Muscle	10	-26.62 ± 1.32	14.43 ± 0.62
<i>Silurus asotus</i>	Muscle	10	-25.10 ± 1.83	14.89 ± 1.04
<i>Acheilognathus macropterus</i>	Muscle	10	-23.79 ± 1.41	16.48 ± 0.54
<i>Squalidus argentatus</i> *	Muscle	10	-25.81 ± 1.11	15.62 ± 0.40

TABLE 1 Isotopic composition of blood and muscle samples from Yangtze finless porpoises in Poyang Lake, Yangtze River, and of muscle samples from potential prey fish species. Asterisks indicate semi-migratory fish species; the remaining fishes are non-migratory

OTUs were identified by comparison with the Genbank. Six different prey taxa were identified unambiguously to species from the seven OTUs. A total of 31 prey occurrences were identified, and the most frequently occurring prey items were *C. brachygnathus* (41.9%), *H. bleekeri* (12.9%), and *P. nitidus* (12.9%; Appendix S1: Figure S1).

Before grouping the prey fishes into two prey source groups of semi-migratory fish and non-migratory fish, we constructed two BSIMMs using an uninformative prior (equal contribution), and an informative prior with faecal sample results, respectively at species levels (Appendix S1: Figure S2). Since the stable isotopes of fish prey were showing a significant difference between species, the BSIMMs showed a large uncertainty at the species level for diet proportions (Appendix S1: Figure S2). But these exploratory models show that some semi-migratory and non-migratory fish prey contributed relatively high proportions to the diet. MANOVA showed a significant difference between isotopic signatures of semi-migratory and non-migratory fish (Figure 1).

The mixing polygon simulation approach showed that all individuals were within the 90% probability region of possible mixing polygons (Figure 2c). For muscle tissue samples, about 60% of individuals fell within the 95% probable mixing region (Figure 2f), which was sufficient to allow use of the proposed mixing model.

Model comparison, including the null model, time-scale dependent model, sex model, size model and combination of these factors (Table 2), indicated that the model with a fixed effect of Tissue had the lowest LOOic and received 60% of the Akaike weight, indicating a 60% probability it is the best model (expected predictive performance on new data, out of the models considered, given the data observed). Posterior diet proportion estimates for the Yangtze finless porpoise of this model indicate that proportion of semi-migratory

prey is more important, especially for muscle tissue, which indicates the long-term foraging strategy (Figure 3).

The models with Tissue + Sex and Tissue + Length cannot be ruled out (24% and 20% weight, respectively). The proportion (median with confidence intervals) of non-migratory and semi-migratory fish prey in the Tissue + Sex model showed that male individuals relied more on semi-migratory prey than non-migratory prey (Table 3). Posterior diet proportion estimates in the model with Tissue + Size as the fixed effects showed that, for both short and long terms, semi-migratory fish prey increased with body length (Figure 4).

4 | DISCUSSION

Studies on the diets of apex predators, especially endangered species, are essential to understanding their trophic interactions and the conservation strategies necessary to support them. Prior studies of the Yangtze finless porpoise showed them to be opportunistic predators that feed in proportion to prey availability. Here, we demonstrated that the porpoises use semi-migratory and non-migratory fish prey to differing degrees, which indicates that there was dietary variance in timescales which may be influenced by season.

In our study, we found a genuine difference between the long- (2–3 months) and short-term (1–2 weeks) diets of Yangtze finless porpoises in Poyang Lake. For the short-term diet, higher proportions of *C. brachygnathus*, *H. bleekeri*, and *P. nitidus* were ingested by Yangtze finless porpoises in the early and middle of March. In contrast, the longer-term diet outcome based on muscle samples in our study reveals that the predominant prey species were *P. simoni*, *P. nitidus*, and *H. bleekeri*, whereas *C. brachygnathus* only accounts for relatively lower

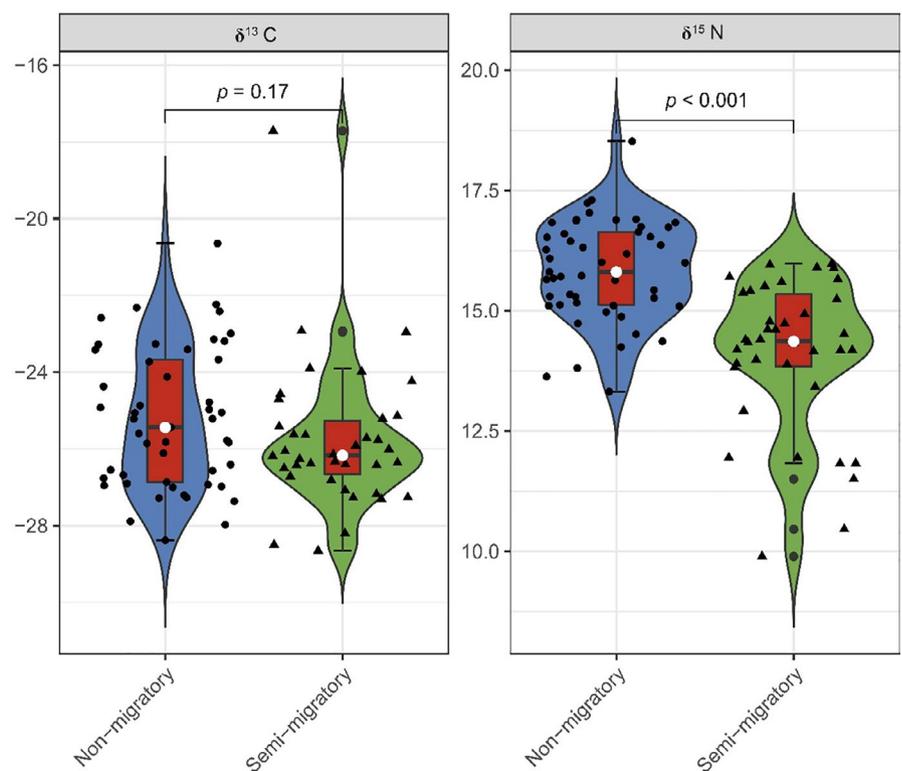


FIGURE 1 Multivariate ANOVA of carbon and nitrogen isotopic composition between non-migratory and semi-migratory prey fish, with post hoc test showing the significant difference in $\delta^{15}\text{N}$

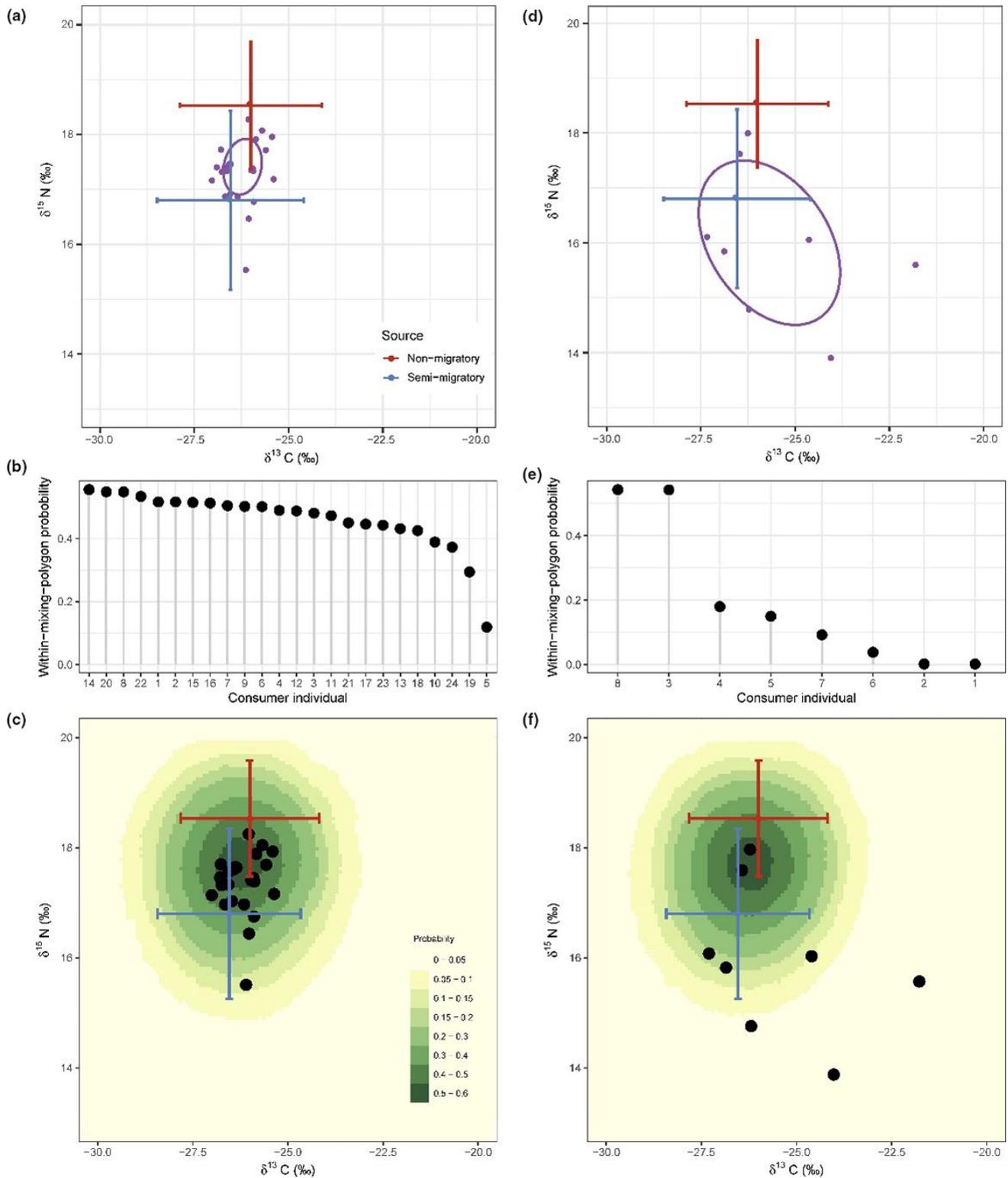


FIGURE 2 Stable isotope biplot and point-in-polygon assumption test. Yangtze finless porpoises tissue data are shown as black dots. (a–c) For blood and (d–f) for muscle tissue: (b) and (e) show within-mixing polygon probability of samples, while (c) and (f) show them in the isospaces for blood and muscle tissue samples, respectively. Non-migratory and semi-migratory prey fish data have been adjusted by means and SDs of trophic discrimination factors (TDFs). Error bars indicate ± 1 SD, the combined prey + TDFs SD calculated as the square root of quadratic sum, under the assumption of independence

contribution to the porpoises' assimilated energy compared to diets in March. Furthermore, *P. simoni*, *P. nitidus*, *H. bleekeri*, and *Squalidus argentatus* are all semi-migratory fish: reproductive adults spawn in the

upper reaches of rivers or lakes with flowing water, and juveniles grow in the lower reaches of major lakes (Liang et al., 1981). In our study, the proportion of these fish in the long-term diet of porpoises was

higher than that of non-migratory fish. Conversely, the percentage of semi-migratory fish was much reduced in March because of the increased contribution of *C. brachygnathus*, a non-migratory fish.

Our observed differences between long-term and short-term diets in Yangtze finless porpoise probably reflects seasonal variation in the abundances of fish prey. The most common fish in the diet (*C. brachygnathus*, *P. simoni*, *P. nitidus*, *H. bleekeri*) are also the most abundant and widely distributed fishery resources in Poyang Lake (Hu et al., 2011). However, *C. brachygnathus* is the dominant species in the winter in Poyang Lake (Hu et al., 2011), and thus was likely to be the primary food for porpoises during the period of the capture–release project in

March. Furthermore, potamodromous *P. simoni* usually migrate from lakes and the lower reaches of rivers to the spawning ground in the upper reaches of the major lakes or rivers from May to September (Li, 1981). They may be a significantly prey species for groups of porpoises during that time. Hence, changes in fish abundances across seasons may lead to large changes in porpoise diets. These patterns may provide further support for the view that Yangtze finless porpoises should be considered opportunistic feeders (Yang et al., 2019).

Our study indicates that porpoise diets vary according to length, possibly reflecting individual differences in foraging (Kelly, 2000). Of course, the small sample size of juvenile porpoises (<5) increases

TABLE 2 Fitted Bayesian model comparison. The model with a fixed effect of Tissue had the lowest leave-one-out cross-validation information criterion (LOOic) and received 60% of the Akaike weight, indicating a 60% probability it is the best model (expected predictive performance on new data, out of the models considered, given the data observed). The model with Tissue + Sex and Tissue + Length cannot be ruled out (24 and 20% weight, respectively). dLOOic is the difference in LOOic between each model and the model with the lowest LOOic

Model ID	Model	LOOic	se_LOOic	dLOOic	se_dLOOic	Akaike weight
2	Tissue	99.8	25.3	0	NA	0.517
5	Tissue + Sex	101.3	25	1.5	0.7	0.244
6	Tissue + Length	101.7	25	1.9	1.3	0.200
7	Tissue + Sex +Length	105	26.2	5.2	1.5	0.038
3	Sex	124.5	26.7	24.7	6.1	0
4	Length	127.3	26.9	27.5	5.6	0
1	Null	130.9	29.1	31.1	8.3	0

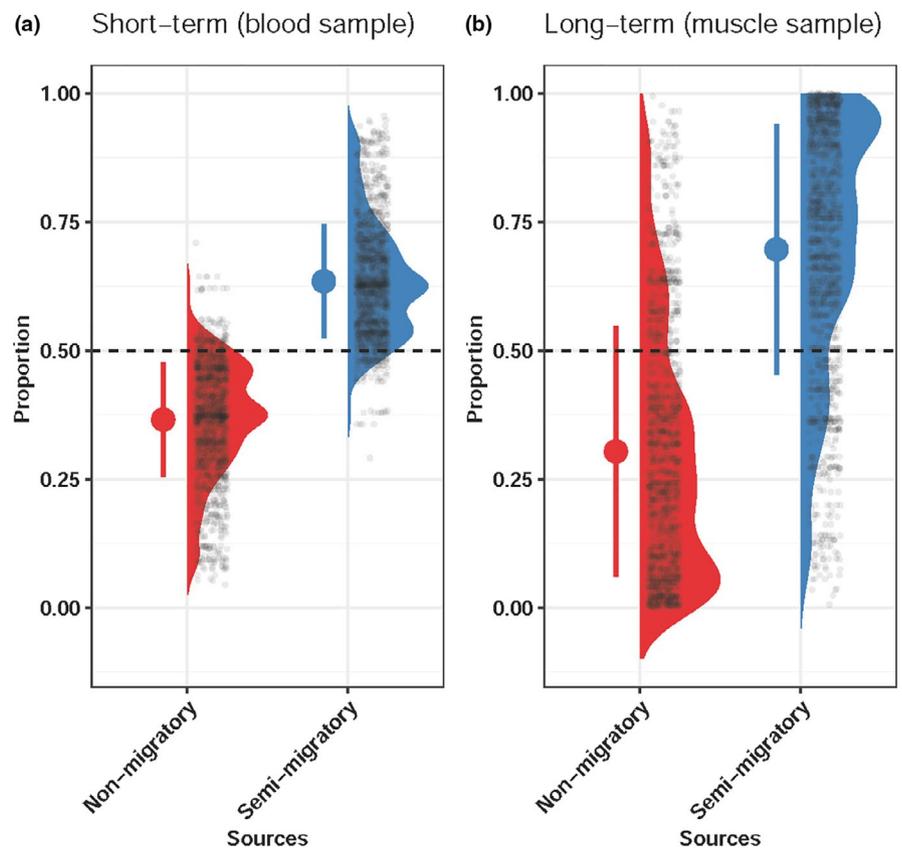


FIGURE 3 Posterior diet proportion estimates for the Yangtze finless porpoise. On the left (a) are the results using the blood sample, and on the right (b) are the results using the muscle sample. Horizontal dashed lines represent 50% contribution level

uncertainty in diet assessments based on stable isotope mixing models (Inger et al. 2010). On the other hand, we found no evidence that diets differed between males and females, although By contrast, a higher trophic level in the food web because of a higher $\delta^{15}\text{N}$ than females (O'Brien, 2015). Devoting additional effort to sample collection remains a priority for future work to clarify ontogenetic diet shifts in this species.

4.1 | Management suggestions

Based on the apparent dietary differences observed above, the following management guidance is provided. Firstly, given the high contribution of *C. brachygnathus* to the diet of the Yangtze finless porpoise in early and middle March, which is just prior to the commencement of the Chinese annual fishing ban (from March 20 to

June 20); we advise extending the regulation of the fish harvest specific to *C. brachygnathus* in Poyang Lake into the first half of March. Also, we advocate a complete ban on the use of seine nets in March because illegal fishing gear has been the main threat to *C. brachygnathus* populations, and the widespread use of seine nets can also increase the rates of accidental death of porpoises when animals in starvation are forced to prey on netted fish and can be fatally entangled (Mei et al., 2019). Secondly, we found that semi-migratory fish are probably the main source of assimilated diets of porpoises over the longer-term. We also call attention to the lack of conservation or protection for semi-migratory fishes in Poyang Lake. In addition, the interaction between the Yangtze River and Poyang Lake has undergone fundamental changes over the past few decades due to extensive and intensive sand mining activities in Poyang Lake since 2001 (Lai et al., 2014), coupled with reduced rainfall due to climatic variability and changing hydrological conditions in the Yangtze River (Feng et al., 2012; Liu et al., 2013). Significant shrinkage of lake water levels and surface area, and increased duration of extremely low water events (Feng et al., 2012; Hervé et al., 2011; Min and Lasheng 2012) have affected aquatic habitat and are partly responsible for the decline in the lake's semi-migratory fishery resources. Currently, the local government is planning a water gate spanning the lake's outflow channel to Yangtze River in order to regulate the water level to prevent the recurrence of the dramatic decline of Poyang Lake (Lai et al., 2014). We suggest that related authorities should examine the extent to which the water gate will affect the growth and migration of semi-migratory fish during the environmental impact assessment of building the water gate.

Targeted conservation efforts for the Yangtze finless porpoise require better data for porpoise diets to clarify fisheries regulations

TABLE 3 Proportion (median with confidence intervals) of non-migratory and semi-migratory fish prey in the model with Tissue and Sex as the fixed effects. CI, confidence interval

Tissue	Sex	Prey type	Median	2.5% CI	97.5% CI
Blood	Male	Non-migratory	0.46	0.27	0.64
Blood	Male	Semi-migratory	0.54	0.36	0.73
Blood	Female	Non-migratory	0.50	0.32	0.64
Blood	Female	Semi-migratory	0.50	0.36	0.68
Muscle	Female	Non-migratory	0.05	0.01	0.15
Muscle	Female	Semi-migratory	0.95	0.85	0.99
Muscle	Male	Non-migratory	0.04	0.01	0.18
Muscle	Male	Semi-migratory	0.96	0.82	0.99

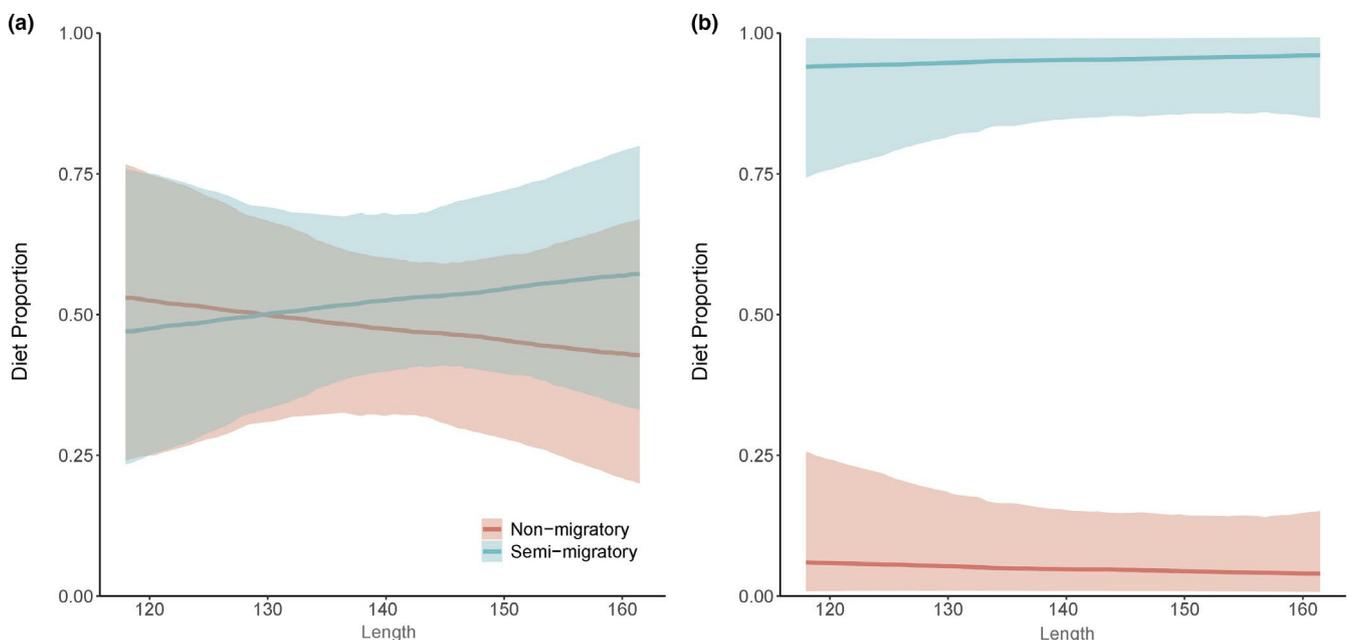


FIGURE 4 Posterior diet proportion estimates with a size increase of non-migratory and semi-migratory fish prey in the model with Tissue and Size as the fixed effects. (a) Blood samples and (b) muscle tissue samples

needed to protect their prey. Consequently, to promote understanding of feeding habits of Yangtze finless porpoises and suggest more accurate protective strategies, we need to investigate and compare further their dietary composition at short and long timescales as well as in different habitats.

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CONFLICT OF INTERESTS

All the authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All sequences generated by next-generation sequencing from 13 faecal samples of wild alive Yangtze finless porpoises in Poyang Lake were submitted to GenBank under the accession number PRJNA533287.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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