Diet composition based on stable isotopic analysis of fecal samples revealed the preference of Black-faced Spoonbill (Platalea minor) for natural and artificial wetlands

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Abstract

Background: Black-faced spoonbill (BFS) is a global endangered species, distributed only in the coastal zones of East Asia. Xinghua Bay is one of the main wintering sites and migration stopovers of BFS in mainland China. However, with the reduction and degradation of natural wetlands, it is uncertain whether the constructed wetland can provide habitat for the endangered BFS. Research on diet of BFS will help to understand their preference between natural and artificial wetlands, and also provide reference for their conservation and habitat restoration. Methods: From December 2017 to February 2020, 45 potential food samples and 199 fecal samples of BFS were collected during six sampling period, of which Cyprinidae, Mugilidae, Portunidae, Gobiidae and Palaemonidae were collected from natural wetlands and Crucian (Carassius auratus) and Whiteshrimp (Litopenaeus Vannamei) were collected from artificial wetland. Their stable isotope values (δ13C, δ15N) were measured to obtain the food composition information of BFS. Results: In the early winter, the proportion of Palaemonidae in BFS's food was as high as 74.4%, while that of other food was only 3.0% to 6.0%. In the late winter, the food contribution of BFS was as follow: Portunidae 39.3% > Palaemonidae 26.1% > Cyprinidae 8.8% > Mugilidae 8.5% > Gobiidae 7.3% > Crucian 5.1% > Whiteshrimp 4.8%. The proportion of Portunidae exceeded that of Palaemonidae, and together with Palaemonidae, it has become the main food of BFS in late winter. Conclusion: The diet composition of BFS between the early and late winter was significantly different, which may be due to seasonal changes in food resources. Natural wetlands are the main feeding grounds of BFS, but artificial wetlands also provide them with supplementary feeding grounds and resting places. Aquaculture ponds play an important ecological function in maintaining the overwintering population of BFS in Xinghua Bay.

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Methods: From December 2017 to February 2020, 45 potential food samples and 199 fecal samples of BFS were collected during six sampling period, of which Cyprinidae, Mugilidae, Portunidae, Gobiidae and

Palaemonidae were collected from natural wetlands and Crucian (*Carassius auratus*) and Whiteshrimp (*Litopenaeus Vannamei*) were collected from artificial wetland. Their stable isotope values ($\delta^{13}C$, $\delta^{15}N$) were measured to obtain the food composition information of BFS.

Results: In the early winter, the proportion of Palaemonidae in BFS's food was as high as 74.4%, while that of other food was only 3.0% to 6.0%. In the late winter, the food contribution of BFS was as follow: Portunidae 39.3% > Palaemonidae 26.1% > Cyprinidae 8.8% > Mugilidae 8.5% > Gobiidae 7.3% > Crucian 5.1% > Whiteshrimp 4.8%. The proportion of Portunidae exceeded that of Palaemonidae, and together with Palaemonidae, it has become the main food of BFS in late winter.

Conclusion: The diet composition of BFS between the early and late winter was significantly different, which may be due to seasonal changes in food resources. Natural wetlands are the main feeding grounds of BFS, but artificial wetlands also provide them with supplementary feeding grounds and resting places. Aquaculture ponds play an important ecological function in maintaining the overwintering population of BFS in Xinghua Bay.

Key words

Aquaculture ponds, wintering grounds, dietary conversion, Palaemonidae, Portunidae

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Introduction

The Black-faced Spoonbill (*Platalea minor*) is one of the endangered waterbirds in the world, belonging to Ciconiiformes, Threskiorothidae (Jin J.F., 2009). Due to the population was far lower than the history number (Pickett et al., 2017), with the increase of protection, the population of BFS has been increasing in recent years (changed from less than 1000 indiv. in 2002 to 5222 indiv. in 2021. However, BFS is still listed as endangered, which is largely due to their narrow distribution area and the threat of living habits. BFS are distributed only in the coastal zones of East Asia, foraging and resting only in the intertidal zone or within 2-3 km of tidal areas. However, high intensity development of coastal zones in recent years had greatly reduced the area or quality of coastal wetlands (Yu & Swennen, 2004). According to Pickett's prediction, climate change and habitat loss together threaten the recovery of the BFS population such that, by 2050, the population declines of BFS might be apparent as a consequence of these cumulative impacts (Pickett et al., 2017).

It is a global phenomenon that natural wetlands were transformed into artificial wetlands, such as rice fields, artificial ponds or reservoirs (Li et al., 2013). Ornithologists have done a lot of research on whether artificial wetlands can replace natural wetlands as a suitable habitat for waterbirds. Some studies have shown that well-protected natural wetlands are more suitable as habitats for waterbirds than artificial wetlands with more species and higher density of waterbirds (Li et al., 2013). While other studies have shown that artificial wetlands can provide alternative habitats for the breeding, wintering and migration of waterbirds (Rajpar & Zakaria, 2013), however, newly built or poorly managed artificial wetlands cannot maintain a stable biological communitie due to the lack of stable food resources (Choi, Gan, Hua, Wang, & Ma, 2013). The BFS make use of artificial wetlands as resting ground and opportunistically choose the natural and artificial wetlands for foraging (Swennen & Yu, 2005), but their preference for the use of the two types of wetlands is not clear. It is difficult to directly determine the foraging habitat of BFS by conventional observation methods, because they mainly forage at dawn, dusk and night, and rest most of the day. The difference of birds' utilization of different habitats in the same geographical area is the result of the comprehensive effect of many environmental factors, and food resources are the decisive factors affecting the habitat utilization mechanism of birds. Therefore, based on the stable isotope technique, we used fecal samples to study the food compositon of BFS, attempting to reveal their preference for natural and artificial wetlands, as well as the survival status of BFS in Xinghua Bay.

In the past, most studies on the dietary composition of birds mainly used the technology of stomach content analysis (Lilliendahl & Solmundsson, 2006) and fecal microscopic analysis (Gasperin & Aurélio Pizo, 2009). However, both methods have some limitations, which may overestimate digestible resources. Moreover, the collection of stomach contents may harm individuals and is not suitable for rare and endangered birds. As for the fecal microscopic examination, the labor and time cost is high, the accuracy of food is very low due to the limication of the debris identification (Nielsen et al., 2017). By measuring the stable isotope values of bird tissues or feces and potential food sources, and establishing a Linear Mixed Model (such as Bayesian Model) between them, the food composition of birds can be obtained through the model. The stable isotopes commonly used in the diet research are carbon (^{13}C / ^{12}C) and nitrogen (^{15}N / ^{14}N). The fractionation effect of stable carbon isotopes is not obvious and the discrimination factor is small, which makes it an ideal element to study the food composition of animals. Stable nitrogen isotopes have stable and large trophic grade discrimination factors between food and their predator tissues, so they are often used for the analysis of food composition and trophic structure (J., T., A., & C., 2011). Generally, the tissues used in the study of bird's diet applying stable isotope technique include blood, muscle and feather (Boecklen, Yarnes, Cook, & James, 2011; J. H. X. Wang X., Zhang Y.N., 2015). As the BFS are endangered birds ("Catalog of Wildlife under Key State Protection,"), obtaining blood and muscle samples may cause great harm to individuals, and feces are the most nondestructive samples reflecting birds' diet. Moreover, the sample size of feces accumulates rapidly compared with blood, muscle or other tissues.

Ueng analyzed the stomach contents of more than 40 BFS which were poisoned in Chinese Taiwan (Ueng, 2006). This result showed that fish and shrimp were the main food of BFS, and that was confirmed by studies in other distribution area, such as the coasts of Chinese mainland, Honkong, South Korea, Japan, and Vietnam (Huang et al., 2021; Swennen & Yu, 2005; Takano, Takeshita, & Henmi, 2014). However, their research did not show the relative proportion of food composition of BFS, nor could they show the relative utilization preference of BFS for natural and artificial wetlands. Leong compared the stable isotope values at the beginning and end of chicks' feather of BFS breeding at the offshore sites of South Korea, and they found that the food of chicks mainly came from freshwater environment, and the proportion of freshwater food in the early stage was higher than that in the later stage (Jeong, Choi, Lee, & Lee, 2021). The food composition of BFS varies with different habitat types and distribution areas. Xinghua Bay in Fujian Province is an important migration stopover site and wintering ground for BFS, in where 323 indiv. were recorded in January 2020, exceeding 30% of the overwntering population in mainland China. In this paper, we collected the feces and potential food samples of BFS in Xinghua Bay through three winters, and analyzed the stable isotope ($\delta^{13}C$, $\delta^{15}N$) values of samples to study the relative preference of BFS for natural and artificial wetlands.

Meterials and Methods

Study site

Xinghua Bay is located in the middle coast of Fujian Province (25°14'˜25deg37'N, 119deg00'˜ 119deg37'E). It's the largest semi-enclosed bay in Fujian Province covering an area of about 620 km^2. It carried high diversity of waterbirds because of large area of shallow water wetland and rich food resources. In a single survey in Xinghua Bay, the largest wintering population of waterbirds reached 31275. The populations of Saunders's Gull (*Larus saundersi*), BFS, Kentish Plover (*Charadrius alexandrines*) and Eurasian Curlew (*Numenius arquata*) were accounted more than 1% of the global population, meeting the standards of international important wetland (Xia et al., 2017). Therefore, establishing a provincial waterbird nature reserve in Xinghua Bay had been approved in January 2022. A large number of waterbirds overwinter in

Xinghua Bay, using not only natural wetlands, but also a large area of aquaculture ponds that have been operated here for a long time.

Fig.1 Fecal and potential food sampling sites in Xinghua Bay. Figure made in ArcGIS for version 10.4.1

Sample collection

The BFS arrives at Xinghua Bay in early November and leaves at the end of March of the following year. Among them, deducting the autumn and spring migration, we divided the overwintering period into the early (November to December) and late parts (January to February). Samples were collected 6 times from December 2017 to February 2021, including 45 potential food samples and 199 fecal samples. The potential food samples were bought from local fishermen, and specified their fishing location to distinguish whether the food comes from natural or artificial wetlands. When collecting fecal samples, we observed the BFS's resting group for half an hour, and then we approached and carefully scraped the feces with plastic sheets and put them into 5 ml centrifuge tubes. In order to avoid pollution by impurities such as soil, we scraped only the middle part of the feces. BFS often mingle with other waterbirds to rest on the ridge of aquaculture ponds, mainly with Grey Herons (*Ardea cinerea*). Unlike other waterbirds, BFS like to gather in a single species, while herons spread and rest at equal distances in order to bask in the sun. More than this, their feces look very different. The feces of BFS are relatively small and there are solid blocks in the midlle of white liquid, while the feces of herons are in the shape of a large pool of liquid jet. Therefore, as long as we master the relative position and fecal differences, we can ensure that the collected fecal samples belonged to BFS. All samples were stored in a refrigerator at -20 .

Stable isotopic analysis (SIA)

After rinsed with distilled water, the food samples were marked and photographed. Then we measured their body length, width and weight. And the photos are handed over to experts for species identification. When taking the thumb-sized muscle samples, the fishes took the back muscle, the shrimps took the abdominal muscle, and the crabs took the leg muscle[13]. Then samples were soaked in 1 mol/L HCl for 2 hours to remove inorganic carbon from the sample surface, rinsed again with distilled water, and then dried in an oven at 60 for 12 hours. Due to the degreasing procedure may affect the true nitrogen isotope value of samples, it is necessary to divide the samples into two categories, one is to directly measure the $\delta^{15}N$ stable isotope, and the other is to measure after degreasing. Degreasing is done by soaking the samples in 0.25 mol/L NaOH solution for 2 hours.

After powdered, samples were analyzed using Stable Isotope Ratio Mass Spectrometer (EA-IRMS) at the Stable Isotope Center in Fujian Agriculture and Forestry University, and the brand of the instrument is Elementar (Germany) and Isoprime (UK). The formula for calculating the stable carbon and nitrogen isotope values is as follows:

$\delta X=(R_{sample}/R_{standard}-1)\times 1000$

Among them, X refers to ^{13}C or ^{15}N, R_{sample} refers to the stable isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the measured samples, $R_{standard}$ refers to the stable isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the international standard substance, carbon isotope values are calculated according to the standard PDB, and nitrogen isotope values are calculated according to the standard atmospheric nitrogen[10].

Data analysis

The mean and standard deviation of stable isotope values of samples were calculated, and the outliers that deviated from the mean by more than twice the standard deviation were screened out. The 14 potential food sources were divided into 7 groups, of which Cyprinidae, Mugilidae, Portunidae, Gobiidae and Palaemonidae were collected from natural wetlands and Crucian and Whiteshrimp were collected from artificial wetland.

The differences of $\delta^{13}C$ and $\delta^{15}N$ values between fecal samples and potential food samples were compared by One-Way ANOVA analysis. All isotope data were expressed as Mean$\pm$ standard deviation (Mean $\pm$ SE), and $p < 0.05$ was considered as significant difference.

The most commonly used model to study food composition by stable isotopic analysis is Bayesian mixing model. It can take into account the variable nondeterministic factors with relatively high accuracy and a wide range of application. We used the R package simmr, which can be used to process the mixing equations of stable isotope data within the framework of the bayesian mixing model (Parnell, Inger, Bearhop, & Jackson, 2010; Parnell et al., 2013). Fecal values were calibrated by the discrimination factors (δ^{13}C=1.15δ^{15}N=2.91birds in general (Caut, Angulo, & Courchamp, 2009).

Results

Stable isotope characteristics of BFS's feces and potential food samples

Fig. 2 The distribution of stable isotope values of BFS's feces and their potential food. Mixtures 1 represents the stable isotope values of BFS's feces in the early winter and Mixturs 2 represents the values in the late winter.

The δ^{13}C values of BFS's feces were mainly distributed within the ranges of the 7 groups of potential food, in which showed that the collected potential food can represent the diet composition of BFS overwintering in Xinghua Bay. The δ^{13}C values of 7 potential foods ranged from -26.55 difference ($F_{6,\,19}$=5.98, $p < 0.01$), while the δ^{15}N values ranged from 7.92significant difference ($F_{6,19}$=3.34, $p < 0.05$). The δ^{13}C values of potential foods from natural and artificial wetlands seperately ranged from -25.28-26.557.92difference in δ^{15}N values ($F_{1,24} = 9.56$, $p > 0.05$) but significant difference in δ^{13}C values ($F_{1,24} = 9.56$, $p < 0.01$) between natural and artificial wetlands. Therefore, δ^{13}C and δ^{15}N may be combined to indicate the feeding preference of BFS. The difference of fecal values between the early and late winter were significant (δ^{13}C: $F_{1,46}$=20.49, $p < 0.001$, δ^{15}N: $F_{1,46}$=71.25, $p < 0.001$). And the fecal δ^{13}C values were concentrated in the range of the δ^{13}C values of Cyprinidae, Mugilidae and Palaemonidae in the early winter, while concentrated in the range of the δ^{13}C values of Mugilidae, Portunidae and Gobiidae in the late winter, which means that BFS may have transformed their food habit in the whole overwintering period.

Diet composition inferred by SIA

Fig. 3 *(a)* represents the contribution rates of 7 potential foods in the early winter, and*(b)* represents the contribution rates in the late winter. The black horizontal lines in the square represent the median, the upper and lower sides represent the upper quartile and the lower quartile, the black vertical lines outside the square represent the maximum and minimum values, and the discontinuous black dots represent abnormal values.

The stable isotopic analysis showed that in the early winter, the Palaemonidae contributed the most to the diet of BFS with 74.4%, while the contribution rates of the other foods were smaller, ranging from 3.0% to 6.0%. In the late winter, the contribution rates of food are as follow: Portunidae (39.3 %)> Palaemonidae (26.1%) > Cyprinidae (8.8%) > Mugilidae (8.5 %) > Gobiidae (7.3%) > Crucian (5.1 %) > Whiteshrimp (4.8%) (Table 2). The combined contribution rate of Portunidae and Palaemonidae reached 65.4 %, and became the most important food of BFS in the late winter. The contribution rates of foods coming from natural wetlands were 93.4% in the early winter and 90.0% in the late winter, while those collected from artificial wetlands were 6.0% in the early winter and 9.9% in the late winter. These results showed that BFS mainly used natural wetlands for foraging gound while overwintering in Xinghua Bay, while the artificial wetlands can also provide a small part of food supply.

Discussion

The food composition of BFS overwintering in Xinghua Bay

Jin investigated the potential foods of BFS in the coastal wetlands of Xinghua Bay from 2007 to 2008, and estimated that their potential foods might include 19 species of fish and 6 species of shrimps. Among these potential foods, the Mugilidae and Gobiidae were the most abundant. In our results, fishes have only a low proportion among food of BFS, this showed that it is not enough to estimate the diet of BFS only through species of potential foods, because BFS may have some foraging strategy and habit which we don't know.

The low proportion of fishes may be due to reduction of fish resources in the costal wetland of Xinghua Bay. Over the past decade, the coastal development of Xinghua Bay has been strengthened, and many projects have been or are under construction, such as ports, wind farms and nuclear power plants. And the agricultural, domestic and industrial polutants are continuously reducing the quality of wetlandsdue to the weak self-purification ability. The eutrophication of Xinghua Bay coastal wetlands is very serious, and both inorganic nitrogen and organic phosphorus exceeds the standard. With the continuous deterioration of wetland quality and the long-term high-intensity fishing, the fishery resources along the coasts of Fujian Province are significantly depleted. In addition, the dams built in rivers in Fujian Province has affected the breeding activities of migratory fish (such as Gobiidae), which is also one of the reasons for the decline in fish sources.

Our results showed that the food composition of BFS have significant differences between the early and late winter. In the early winter, Palaemonidae are the only main food with a contribution rate coming to 74.4%; while in the late winter, the proportion of Palaemonidae decreased 48.3%, while that of Portunidae increase 33.3% and that of fishes increased 13.7%. BFS is an opportunistic predator (Swennen & Yu, 2005). The change of overwintering their diet is likely to reflect the change of relative abundance of food resources. Some studies have shown that the biomass of crabs in intertidal zone increases gradually from summer to winter, and reaches the peak in winter. On the other hand, the shrimps in the intertidal zone may migrate to deep-water areas in the late winter to avoid low temperatures. With the progress of winter, shrimps in the offshore area are decreasing, while the crabs might be increasing, which is likely to be the reason why BFS changes its feeding habits in Xinghua Bay.

Comparison of feeding habits of BFS in different wintering areas

Studies have shown that the feeding habits of BFS in different wintering areas were different. In Chinese Taiwan and Vietnam, Fishes and shrimps are the main food of BFS (Swennen & Yu, 2005). And in Hong Kong, fishes like *Mugiliformes* , *Cichliformes* and *Gobiiformes* are the main food of BFS (Huang et al., 2021). Our results based on stable isotopic analysis showed that Palaemonidae and Portunidae make a greater contribution to the food composition of BFS in Xinghua Bay, which is quite different from other wintering areas. The reasons may include the different composition of food resources in both natural and artificial wetlands, and the latter is mainly caused by different management methods.

There are large areas of aquaculture ponds in the Chiku Wetland in Taiwan and the Mai Po Wetland in Hong Kong, most of which are used to raise Mugilidae, Cyprinidae and Tilapia. By adjusting the appropriate water depth, these aquaculture ponds can provide foraging habitat for BFS. However, most of the ponds in Xinghua Bay are deep-water aquaculture, which can only be used by BFS when drained for fishing close to the Chinese Spring Festival. At other times, the overwintering BFS population is difficult to use the aquaculture ponds, but prefers to forage in the natural wetlands. In addition, due to the scarity of fish resources in the intertidal zone of Xinghua Bay, BFS here prefers to feed shrimps and crabs.

Relative utilization preference of BFS to natural and artificial wetland

The traditional extensive fish ponds in Haifeng, Guangdong Province attract a large number of waterbirds to spend the winter here every year, so this wetland is also an important wintering area for BFS. Zhen's research showed that maintaining the original ecological function of coastal wetlands can provide good habitat and rich food resources for BFS, while the intensive aquaculture ponds have been seperated from the tide, resulting the degradation of wetland function. As a result, the ecological habits of BFS were limited by the loss of habitat. According to Jin's field observation, the aquaculture ponds in Xinghua Bay are generally drained and cleaned in winter. At this time, the BFS stay in the ponds to rest during the day and rarely look for food. Only at dusk, BFS fly to the nearby tidal wetlands for food (Jin J.F., 2009). Our research supports Jin's observation that BFS mainly rest in the ponds and fly to the nearby tidal wetlands for food. But further, we use direct dietary evidence to prove that the foods of BFS in Xinghua Bay mainly come from natural wetlands rather than artificial wetlands.

Types of artificial wetlands that can be used by waterbirds include rice fields, salt ponds, aquaculture ponds and reservoirs. Different types of artificial wetlands provide different ecological functions for waterbirds due to different management modes. Due to the seasonal drainage of rice fields, such wetlands are often used by waterbirds such as Egrets and Herons as migration supply and wintering sites, while other waterbirds may use it as breeding habitats (Wood et al., 2013). Aquaculture and salt ponds can provide resting sites and alternative feeding grounds for shorebirds at high tide (Li et al., 2013). Since the management mode will deeply affect the carrying function of artificial wetlands on waterbird diversity, reasonable planning and management, especially the control of water depth, can effectively improve the supplementary function of artificial wetlands to natural wetlands. The Mai Po Nature Reserve in Hong Kong has adopted measures such as regulating the water level of ponds, regularly desilting the ponds, and pruning plants on the embankment to create suitable habitats for a large number of waterbirds. In Xinghua Bay, with the continous deteriaration of the quality of natural wetlands brought by the surrounding development, the exsisting aquaculture ponds should be retained and scientifically managed to alleviate the survival pressure of a large number of waterbirds wintering here, especially to protect the rare species such as BFS.

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Data Accesibility

The stable isotope value: Dryad https://doi.org/10.5061/dryad.zgmsbccdm.

Conflict of Interest

The authors have no conflict of interests to declare.

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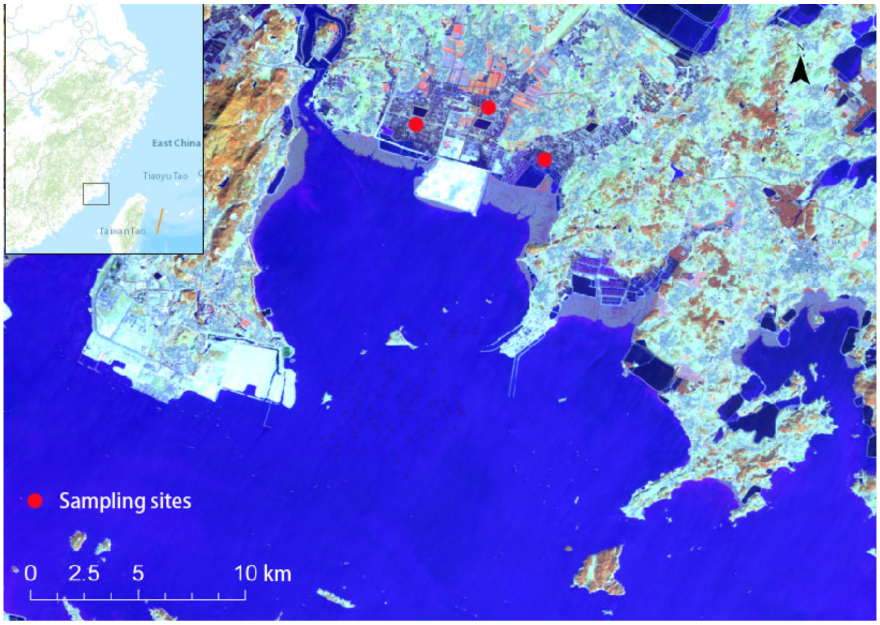
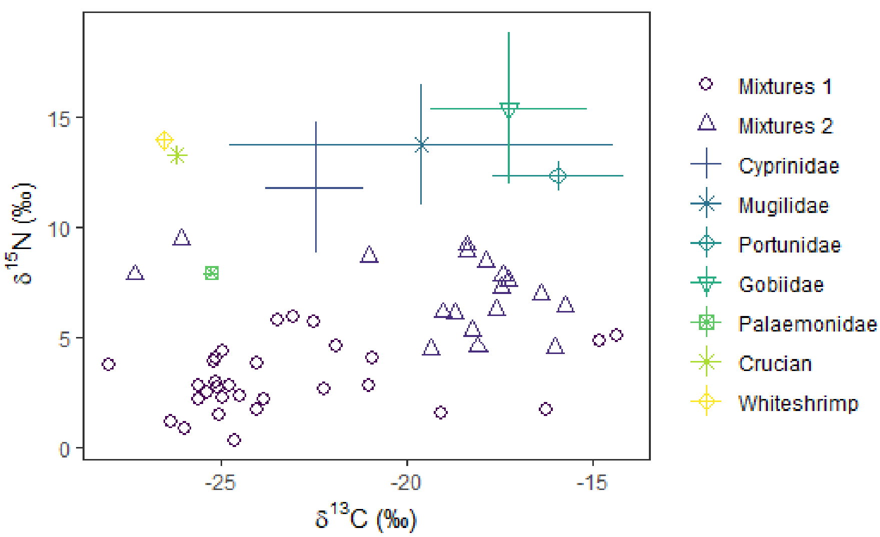
Table 1 Mean and standard deviation of food and fecal values after corrected

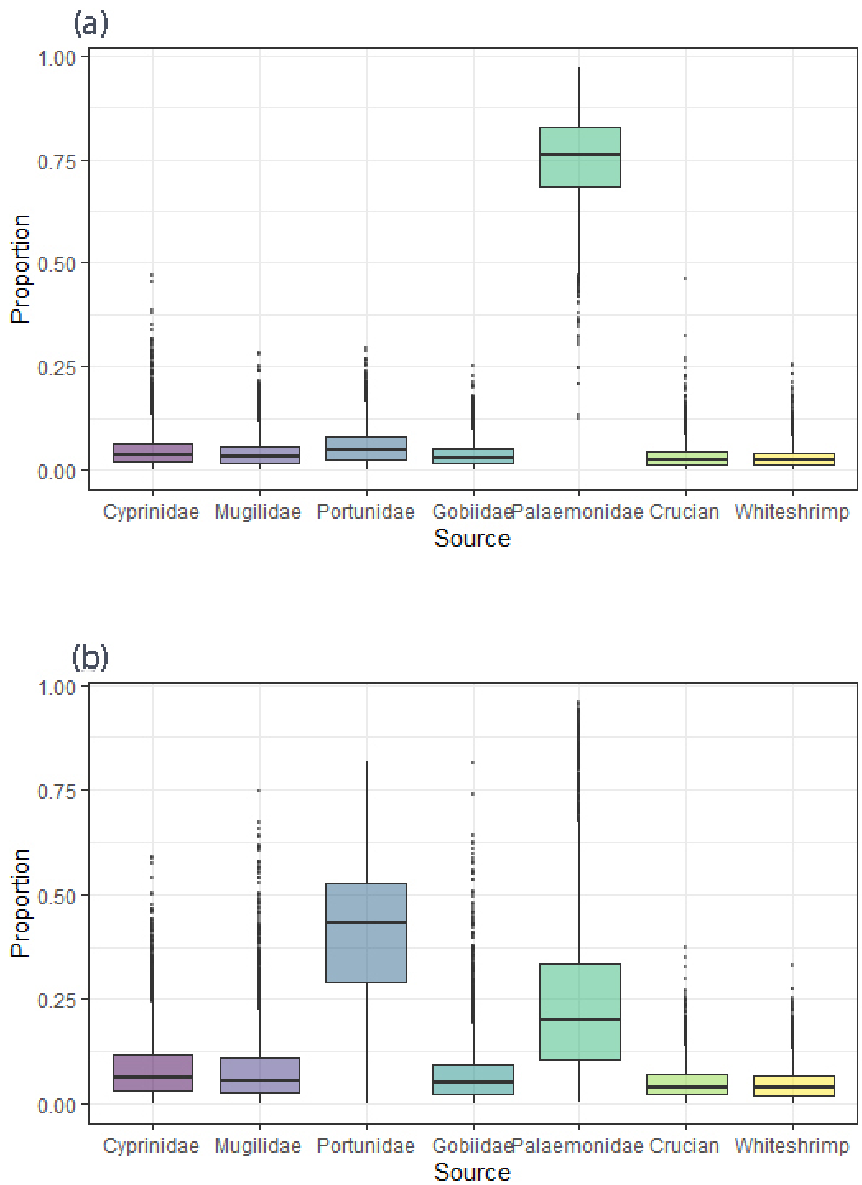
Source	Samples	Number	δ^{13}C/	δ^{15}N/
Natural wetland	Cyprinidae	3	-22.48±1.32	11.80±3.01
	Mugilidae	7	-19.62±5.15	13.76±2.75
	Portunidae	4	-15.95±1.76	12.34±0.67
	Gobiidae	5	-17.26±2.21	15.41±3.45
	Palaemonidae	3	-25.28±0.22	7.92±0.20
Artificial wetland	Crucian	2	-26.20±0.03	13.30±0.18
	Whiteshrimp	2	-26.55±0.01	13.98±0.01
BFS	Feces (early winter)	31	-23.28±3.30	3.08±1.50
	Feces (late winter)	26	-18.92±3.10	6.99±1.63

Table 2 Contribution rates of potential food of BFS in Xinghua Bay

Species	Contribution rates (early winter)	Contribution rates (early winter)	Contribution
	Mean (%) ± SD	95% CI	Mean (%) ±
Cyprinidae	5.1±0.05	0.5 - 18.6	8.8±0.08
Mugilidae	4.2±0.04	0.4 - 13.9	8.5±0.09
Portunidae	6.0±0.05	0.6 - 16.9	39.3±0.18
Gobiidae	3.7±0.03	0.4 - 11.6	7.3±0.07
Palaemonidae Crucian Whiteshrimp	74.4±0.11 3.0±0.03 3.0±0.03	49.1 - 91.4 0.4 - 11.8 0.4 - 10.6	26.1±0.22 5.1


East China
Sampling sites
0 2.5 5 10 km


Mixtures 1
Mixtures 2
Cyprinidae
Mugilidae
Portunidae
Gobiidae
Palaemonidae
Crucian
Whiteshrimp
δ15N (‰)
δ13C (‰)
0
5
10
15
-25
-20
-15


(a)
Proportion
1.00
0.75
0.50
0.25
0.00
Cyprinidae
Mugilidae
Portunidae
Gobiidae
Palaemonidae
Crucian
Whiteshrimp
Source
(b)
Proportion
1.00
0.75
0.50
0.25
0.00
Cyprinidae
Mugilidae
Portunidae
Gobiidae
Palaemonidae
Crucian
Whiteshrimp
Source