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Eurasian otters prefer to prey on religious released non-native fish on the Qinghai-Tibetan Plateau

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Abstract

Religious wildlife release is prevalent worldwide, especially in Asia countries. It is one of the anthropogenic pathways to cause biological invasions. Religious fish release is common on the Qinghai-Tibetan Plateau, yet few studies have assessed the influences of religious fish release on local species. In Yushu, a city on the Qinghai-Tibetan Plateau, we interviewed local people, conducted fish trap surveys in local rivers, and examined the diet of Eurasian otters *Lutra lutra* using a fecal DNA metabarcoding approach. We found that fish release started at least in 1980–1990s in Yushu. Tibetan residents released fish in large amounts and released fish were usually exotic commercial fish purchased from market. Despite such long-term and intensive fish release activities, released fish were few in local rivers. On the other hand, Eurasian otters mainly prey on fish and released fish accounted for ~20% of relative read abundance of prey DNA in otters' diet, indicating their high preference on released fish. Our study suggested that religious fish release may provide additional food resources for otters, whereas otters, as a top predator in local rivers, may deplete non-native fish once they were released and, therefore, reduce the probability of colonization of released fish, although further studies are required to assess otters' impact. Our study revealed otters' diet in Yushu, providing basic information for local otter management and conservation. Furthermore, it represents a case showing that native predators prey on religious released animals, implying a probable direction for controlling invasive species through native predator conservation.

Key words: biological invasion, Buddhism, Eurasian otter, prey composition, religious wildlife release, scat DNA.

Human intentionally or unintentionally introduced numerous species to novel territory outside their natural range through various pathways, such as transport, trade, travel, tourism, and religious activities (Vitousek et al. 1997; Agoramoorthy and Hsu 2005; Hobbs et al. 2018), which may cause biological invasion and economic losses. Among these, religious wildlife release is a growing but generally neglected cause of biological invasion (Severinghaus and Chi 1999; Liu et al. 2012; Everard et al. 2019). Buddhism release activities are prevalent worldwide, particularly in Asian countries (Agoramoorthy and Hsu 2005; Shiu and Stokes 2008; Du et al. 2023). For example, in Yunnan province, China, Buddhists frequently released American bullfrogs *Lithobates catesbeianus* in rivers and lakes, which induced population establishment of the species (Liu et al. 2015), and threatened native frogs through competition and predation (Wu et al. 2005).

Qinghai-Tibetan Plateau, the largest and youngest plateau with the highest average elevation, is one of the biodiversity hotspots in the world (Mi et al. 2021). Most residents on the plateau are Tibetans who believe in Buddhism. Their religious/cultural tradition of *ahimsa* (no killing of wild animals) contributes to the survival of wildlife (Shiu and Stokes 2008; Wang et al. 2022a). On the other hand, religious wildlife release on the Plateau is prevalent, which may induce biological invasion (Chen and Chen 2010; Liu et al. 2012; Li et al. 2018), and pose threats to the unique and fragile plateau ecosystem. Fishes are commonly released and are usually imported from nonlocal regions. Religious fish release has resulted in species invasions on the Plateau. For example, in Lhasa, the capital of Tibet Autonomous Region, China, the crucian carp *Carassius auratus* had already established a viable population (Chen and Chen 2010; Fan et al. 2011; Li et al. 2018). Despite the environmental issues and economic losses caused by invasive species, few studies have yet assessed the influences of Buddhism release on local species on the Plateau.

Eurasian otters are top predators in freshwater ecosystems (Kruuk 2006). They mainly eat fish, although they could also be opportunistic or generalist predators that feed on amphibians, reptiles, mammals, and invertebrates when habitat instability increases (Krawczyk et al. 2016; Lanszki et al. 2016; Dou et al. 2023). Eurasian otters are regarded as relatively adventurous consumers to novel prey, for example, they

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prey on exotic red-swamp crayfish *Procambarus clarkii* in Mediterranean fresh waters (Barrientos et al. 2014). However, in other cases, Eurasian otters were reported to rarely prey on non-native fish even though they were abundant locally (Miranda et al. 2008; Balestrieri et al. 2013; Kumari et al. 2019).

For Eurasian otters on the Qinghai-Tibetan Plateau, whose habitat is the world's highest (Mason and Macdonald 1986; Kruuk 2006), contradictory factors may affect their diet and survival. On one hand, local residents rarely hunt otters or fish due to their no killing tradition, providing good conditions for the survival of otters. On the other hand, local fish communities may be influenced by the religious release of non-native fish, affecting the diversity and quantity of prey available to otters. Whether and how Eurasian otters adapt to a changing prey community caused by human activities on the Plateau remains unknown.

In this study, we investigated religious fish release, local fish community, and otter diet, trying to provide hints to the relationships between them. We conducted surveys in Yushu City, Qinghai Province, in the central Qinghai-Tibetan Plateau. We first interviewed residents to collect data on the frequency of religious fish releases and the species involved. Then we conducted fish surveys to determine whether released fish have successfully colonized local rivers. Finally, we examined Eurasian otter's diet using a fecal DNA metabarcoding approach, to determine whether they feed on released non-native fish and their prey preference. Our study reveals the diet of otters, providing basic information for local otter management and conservation, as well as providing a piece of puzzle to answer the broader question of otters' adaptation to human disturbances. Furthermore, our study represents a case showing that native predators prey on religious released animals, implying a probable direction for controlling invasive species through native predator conservation.

Material and Methods

Study area

We conducted field surveys in Yushu City, Qinghai Province, from 15 April to 20 May and from 8 November to 16 December in 2019, to avoid the flood season from late May to early October. The elevation of the study area ranges from 3530 to 3860 m (Wang et al. 2021). The average annual temperature is ~2.9 °C and the average annual precipitation is ~487 mm (Sun et al. 2019). The Batang River (43 ± 2 m wide) and Zhaqu River (25 ± 1 m wide) run through the city. Both rivers flow into the Tongtian River, the upper reach of the Yangtze River. Vegetation along the river is alpine meadow. Most parts of the rivers had been modified, with river banks being reinforced and instream boulders being removed. The total population in Yushu is around 46,600, and over 90% of residents are Tibetan (https://www.yushuzhou.gov.cn/html/2/7.html).

Household interviews

We conducted semi-structured interviews in villages along the Batang and Zhaqu rivers as well as the downtown area of Yushu. Our interview survey covered most villages along the two rivers. Only residents aged over 18 years old were interviewed. We selected interviewees opportunistically by walking through the village and we interviewed one family member per household. During the interview process, a Tibetan interpreter helped to translate between Mandarin and Tibetan. We collected the following information: 1) personal information such as sex, age, educational level, time of residence, and religious belief; 2) whether the interviewee had ever released fish; 3) if so, the released species, numbers, frequency, reasons, and sources; and 4) willingness to change fish release activities (details in the Appendix)

Fish surveys

We divided rivers into 1 km sections and conducted fish surveys in 30 sections in spring. In autumn, all the 30 sections were resurveyed except for one section. In each 1 km river section, we set four fishing traps (size: 32 cm × 24 cm × 5 m, mesh: 4 mm, entrance: 15 cm) at least 200 m apart, with bread as bait. The traps were set in the daytime (at least 6 h in the river) to avoid accidentally catching otters who are active at night (Han et al. 2021). We recorded the number of fish species and the total mass of each fish species. We also randomly selected 5 individuals for each species and measured their body mass. Once measured and recorded, fish were released at the location where they were caught.

Otter diet

Spraints collection

We conducted transect surveys along one side of the rivers in both seasons, during which we searched for otter spraints. Unfaded spraints (indicating their relative freshness) with a large volume were collected. In all, we collected 79 spraints from different sections of the rivers, with 41 spraints collected in spring, and 38 in autumn. Spraints were stored individually in a centrifuge tube and submerged in 95% ethanol for 24 h, then were dried with silicone beads, and finally stored at –20 °C in the lab.

DNA extraction, metabarcoding PCR, and sequencing

For each spraint, we took 200 mg content for DNA extraction, after removing fishbones, scales, and other solid objects from the spraint. We used DNeasy Blood & Tissue Extraction Kit (QIAGEN, Germany) following the standard protocol with a few modifications (Jang-Liaw 2021). We included a negative control (blank extraction) during each DNA extraction.

We used a specifically designed universal primer set, VertU V12S-U (VertU V12S-U F1: TYGTGCCAGCNRCCGCGGTYA, VertU V12S-U F2: GTGCCAGCNRCCGCGGTYANAC, VertU V12S-U R: ATAGTRGGGTATCTAATCCYAGT), which targets ~207 bp fragments of the mtDNA 12S genes of vertebrates (Wang et al. 2022b), to conduct metabarcoding PCR. We ran 3 replicates for each DNA sample. The amplifications were conducted in a total volume of 25 μL, including 0.1 μM each 12S-F/R primers, 2.5 μL DNA loading buffer, 0.5 μL dNTPs, 0.25 μL TransTaq® HiFi DNA Polymerase, and 1 μL extracted DNA. The PCR thermal cycling conditions started with an initial denaturation step of 30 s at 98 °C, followed by 35 cycles of 15 s at 98 °C, 15 s at 50 °C, 30 s at 72 °C, and a final step of 10 min at 72 °C. PCR products were purified, labeled with tag sequence, and pooled to form sequencing libraries (details could be found in Wang et al. 2022b). These libraries were sequenced on an Illumina HiSeq 2500 platform (Annoroad Gene Technology Co., Ltd., Beijing, China) with the paired-end 150 bp mode.

Local prey DNA reference database

To build a local prey DNA reference database, we collected tissue samples of any species that were captured during trap surveys. We collected small body parts such as fish fins and frog toes to diminish interference to the animals. In total, we collected tissue samples of 6 fishes, 2 amphibians, and 1 mammal. We used the same DNA extraction kit to extract DNA from prey tissue samples, following the standard protocol. We then amplified these DNA extractions using the same 12S primers, and Sanger sequenced them on an Applied Biosystems 3730XLDNA Analyzer [Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China]. In addition, we downloaded the complete mitochondrial genome sequences of ~7,000 vertebrate species from the NCBI nucleotide database as a supplement to the local reference.

Bioinformatics processing of sequences

Sequenced reads were filtered, sorted, trimmed, merged, and clustered into molecular operational taxonomic units (MOTUs) (Wang et al. 2022b). To detect main prey taxa cost-effectively, we set a 5% threshold within a sample and removed reads that were lower than the threshold (Drake et al. 2021; Wang et al. 2022b). Prey taxa were determined based on the similarity between MOTUs and sequences in the local DNA reference and NCBI database, following a series of identification criteria (Wang et al. 2022b).

Predator reads usually accounted for >30% of the total reads in a sample. The scat was assigned to a specific predator if only one predator species was detected. When multiple predators were detected, the scat was assigned to the one with >90% of the total predator reads (Harper et al. 2020). Samples were excluded when scats were not assigned to otters. Sequences of species that are unlikely prey of otters, including human, large mammals such as yak *Bos grunniens*, horse *Equus ferus caballus*, and Tibetan sheep *Ovis aries*, as well as other carnivores, were also removed in the following analyses.

Diet composition and prey preference of otters

Following previous studies (Deagle et al. 2019; Shao et al. 2021), we summarized otter diet composition based on the relative read abundance (RRA, i.e., proportional summaries of counts) of each prey taxon in all spraint samples. We did not use the weighted percent of occurrence (wPOO) to calculate diet composition and prey selection, because it is sensitive to potential contamination and usually overestimates low-abundance prey taxa (Deagle et al. 2019). The RRA of each prey was calculated as:

$$\mathrm{RRA}_i = \frac{1}{S}\sum_{k=1}^{s}\frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}} \times 100\%,$$

in which S is the number of samples, T is the number of prey items (MOTUs), $n_{i,k}$ is the number of sequences of prey item i in sample k. Because fish taxonomy on the Qinghai-Tibetan Plateau is still obscure (Feng et al. 2019; Tang et al. 2019), we summarized otter diet composition at the genus level and considered all non-native prey as a single group. To compare the taxon richness and composition of prey in two seasons, we used the Wilcoxon rank sum test and permutational multivariate analysis of variance (PERMANOVA) with 999 permutations.

Prey preference of otters was indicated by the Jacobs' index D (Jacobs 1974), which was calculated as:

$$Di = \frac{r_i - p_i}{r_i + p_i - 2r_ip_i},$$

where i is the prey item, r_i is the proportion of the RRA of prey taxon from DNA metabarcoding analyses, and p_i is the proportion of the number of prey taxon from fishing trap surveys. The value of the index ranges from −1 (highly selected) to +1 (highly avoided), and a value of 0 indicates no selection.

Results

Household interviews

We interviewed 60 Tibetan residents from downtown Yushu and 12 villages along the rivers, including 41 males and 19 females. All of them were Buddhists. The average age of interviewees was 48.6 ± 14.2 (range: 20–73). Except for one interviewee with missing educational information, 36 interviewees had no formal education, 14 finished elementary school education, 5 finished middle school, whereas only 4 interviewees had high school or higher degrees.

Thirty-seven interviewees had ever released fish and they reported 45 cases of fish release. They usually bought fish from local markets (n = 41), or occasionally from fishermen (n = 1). Released fish usually were common commercial fish sold on the market, including carps and catfishes. All of them were exotic fish in our study area. Among the 45 cases of release, only 1 case released fish fewer than 10 individuals, 17 released 10–100 individuals, 17 released 100–1000 individuals, 4 released more than 1,000 individuals, while 6 cases did not report the number of fish released. In an extreme case, 1 interviewee said that once he and friends crowdfunded 1,70,000 RMB (~14,500 USD) to release tens of thousands of fish. In terms of release frequency, 22 interviewees released 1–5 times per year, 4 released once every year, whereas others could not recall specific release times. The timing of release was quite random, although some interviewees prefer to release fishes on the 15th or 30th of every month of the Tibetan calendar (n = 6).

The purposes of fish release included saving life as an act of compassion (n = 24), praying for a better life (n = 18), and praying for sick family members (n = 12). In addition to fish, 31 interviewees had released cows, 5 had released sheep, 1 had released rabbits, and 1 had released chickens. These released animals were bought from local herders or in the local market. However, the interviewees most often released fishes because fish are cheaper, so with a same budget they could "save" more lives to release fish than to release cows or sheep.

Fish release in Yushu started at least in the 1980s. Although most interviewees were not aware of the start time, 8 of them recalled fish release in the 1980s and the 1990s when Han people brought exotic commercial fish to Yushu.

The Yushu government banned fish release in January 2019. However, only 23% (n = 14) of the interviewees were aware of this ban. One fish release in 2019 was reported in the interview survey, and we encountered a fish release during the transect survey along the Zhaqu River in April 2019. When asked whether they would continue fish release, 47 out of 57 interviewees indicated that they would not continue releasing fish if fish could not survive in local rivers; 51 out of 54 interviewees indicated that they would not continue if these fish have a bad impact on other animals; 50 out of 55 interviewees said that if the government or living Buddha ban fish release, they would not continue releasing fishes in the future.

Fish community

Since we could not identify all fish species by morphological traits during field surveys, we recorded fish data at the genus level. We captured 5 fish taxa, including *Schizopygosiss*, *Ptychobarbus*, *Triplophysa*, *Lefua*, and a non-native fish species *C. auratus* (Figure 1A). The dominant species were *Schizopygopsis*, followed by *Ptychobarbus*, *Triplophysa*, and *Lefua*. Only 2 individuals of *C. auratus* were captured in spring. The average body mass of the fishes were as follows: *C. auratus* (316.2 ± 59.3 g, n = 2), *Ptychobarbus* (129.8 ± 13.6 g, n = 65), *Schizopygosiss* (64.2 ± 3.0 g, n = 885), and *Triplophysa* (10.2 ± 0.6 g, n = 100). The average mass of captured native fishes per trap was 525.5 ± 83.4 g.

Otter diet composition

We successfully sequenced 77 out of the 79 spraints, and all of them were from otters. We generated 10.9 Gb sequence data with 8.39 million sequences in spring and 3.13 million sequences in autumn. We excluded sequences that were from otters (32.1%), as well as sequences with <95% identification or erroneous sequences from cross-contamination (6.6%). The remaining sequences (61.3%) were used in the subsequent analyses.

In all, 14 prey taxa were identified from spraints in 2 seasons (Figure 2). The most abundant prey was fish (97.9%), followed by amphibians (1%), birds (0.6%), and mammals (0.5%). In addition to native fish, we found 4 non-native fish species from spraints, including *C. auratus*, *Carassius carpio*, *Ctenophryngodon idell*, and *Misgurnus anguillicaudatus*.

Seasonal variation of otter diet

In spring, 98.3% of RRA in spraints were fish. *Triplophysa* was the most abundant prey (63.1%), followed by non-native fish (18.3%), *Schizopygosiss* (13.6%), and *Ptychobarbus* (3.3%). Otters preyed occasionally on amphibians (1.7%), whereas no birds or mammals were found in their spraints (Figure 2). In autumn, 97.6% of RRA in spraints were fish. *Schizopygosiss* was the most abundant prey (42.4%), followed by non-native fish (25.2%), *Triplophysa* (27.7%), and *Ptychobarbus* (2.3%). Otters preyed occasionally on birds (1.1%), mammals (0.9%), and amphibians (0.4%) (Figure 2).

Prey taxa richness was not different between the two seasons (w = 734.5, P = 0.947). However, composition of prey was different between the two seasons (F = 5.070. R^2 = 0.063, P = 0.02, Figure 3).

Prey preference of otters

The 5 fish taxa captured during trap surveys were the most abundant prey in otter spraints, representing 98.3% of prey sequences in spring and 97.6% in autumn. In both seasons, otters preferred *Triplophysa* and non-native fish, but avoided *Schizopygosiss*. Interestingly, they showed an opposite preference for *Ptychobarbus* in the two seasons (Figure 1B).

Discussion

Our study explored fish release activities, fish community composition in local rivers, and the diet of a top predator—Eurasian otter on the Qinghai-Tibetan Plateau. We found that although residents in Yushu frequently released large amounts of fish, which usually were exotic commercial fish purchased from the market, the density of non-native fish in local rivers was quite low. We also found that Eurasian otters in Yushu mainly prey on fish, and released fish accounted for

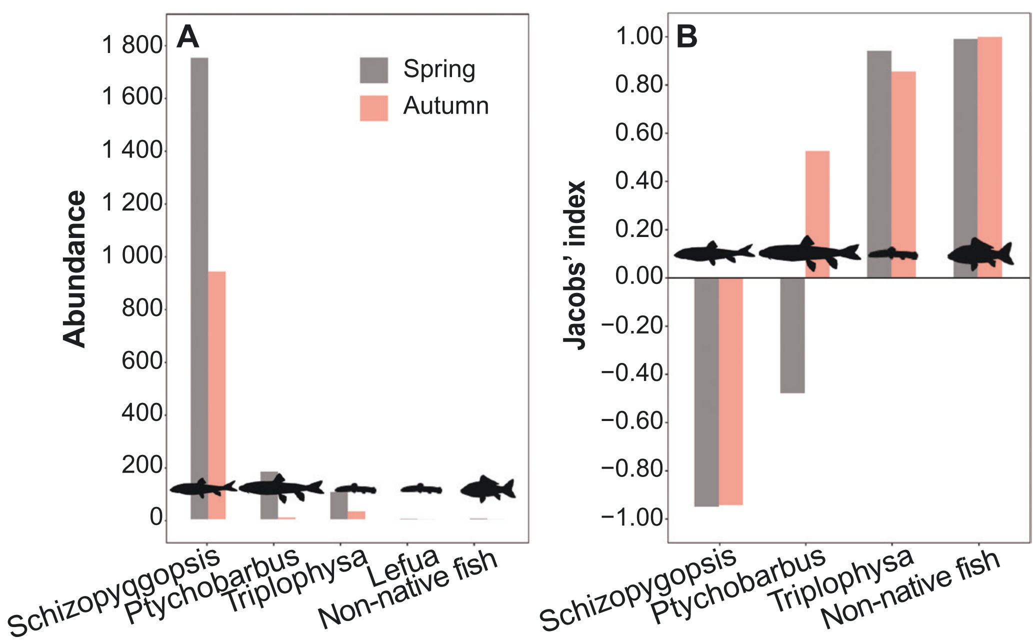

Figure 1 (A) Abundance of fish taxa from fishing trap surveys and (B) otter prey preference based on relative read abundance of prey taxon from DNA metabarcoding analyses and the number of prey taxon from fishing trap surveys in Yushu city, Qinghai-Tibetan Plateau.

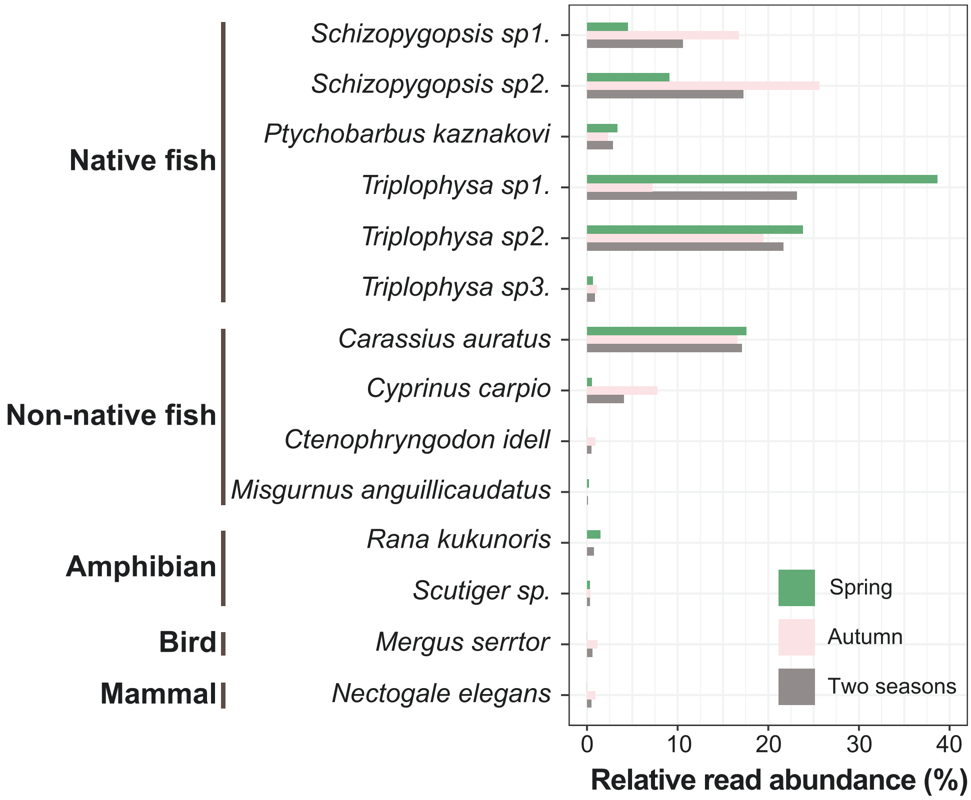

Figure 2 Relative read abundance of prey taxon in all spraints of otters in Yushu City, Qinghai-Tibetan Plateau.

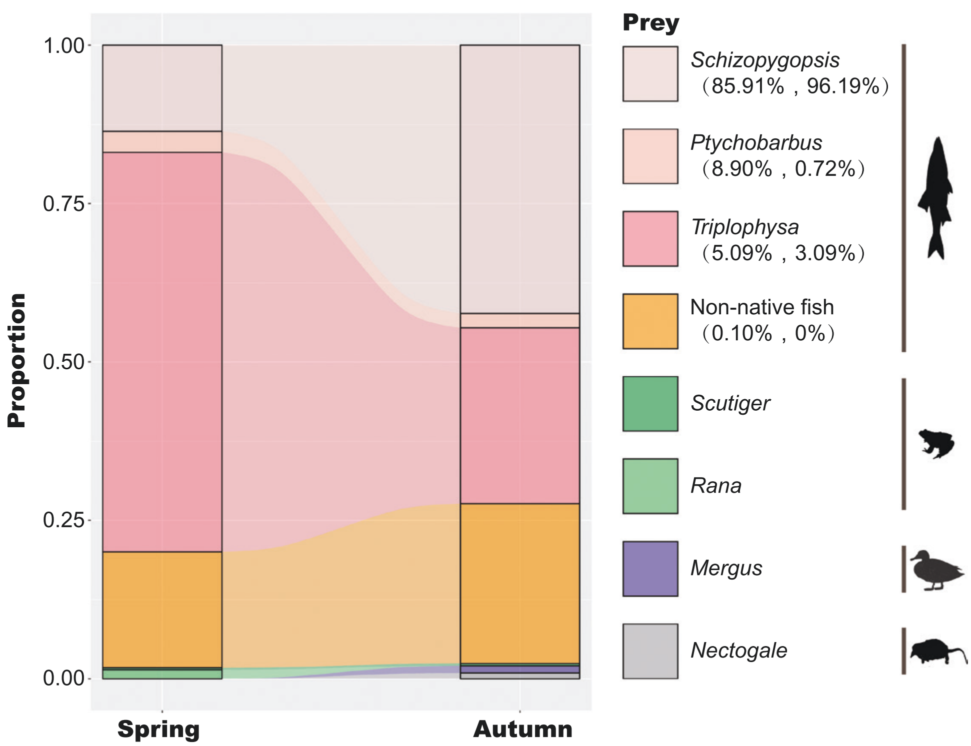

Figure 3 Diet variation between two seasons based on relative read abundance of prey taxon of otters in Yushu City, Qinghai-Tibetan Plateau. The percentages in parentheses indicate the proportions of prey taxon captured in fishing trap surveys in spring and autumn.

a substantial part in otters' diet. Eurasian otters showed a preference for released fishes.

From our interview survey, we may infer that due to religious/cultural traditions, residents in Yushu have constantly released large amounts of non-native fish to local rivers for the last 30–40 years. Such activities may greatly alter the composition of the local fish community (Marchetti et al. 2004). However, from our fish trap surveys, we may infer that the density of non-native fish was quite low in local rivers compared to native fish. One possible explanation is that non-native fish transported from lower elevations could not adapt to the harsh environment on the Plateau (Rahel and Olden 2008; Liu et al. 2019). However, non-native fish (e.g., *C. auratus*) have successfully invaded rivers in Lhasa, another city on the Plateau which has environmental conditions similar to Yushu (Chen and Chen 2010; Ding et al. 2014; Fan et al. 2016), indicating a possibility of fish invasion in Yushu. For example, in Chabalang Wetland in Lhasa, released non-native fishes account for 99.32% of total mass in 2009, and no native fishes were captured in 2013 (Ding et al. 2014). Another possible explanation to the low density of non-native fish in Yushu is that we failed to capture non-native fish in trap surveys. Traps are easy to use and do little harm to fish. However, their use may be limited in catching big-sized fish (Ruetz III et al. 2007). A more effective method, for example, electrofishing, needs to be applied to survey fish community thoroughly, despite the difficulty of getting a permit for electrofishing in this Buddhist region, especially after the launch of a 10-year ban on fishing in key waters of the Yangtze River Basin in January 2021.

Alternatively, we propose that Eurasian otters helped in controlling non-native fish in Yushu. We found that Eurasian otters had a steady feeding proportion (~20% RRA) on non-native fish in both seasons. Eurasian otters in Yushu act as adventurous consumers to exotic prey (Delibes and Adrián 1987; Barrientos et al. 2014), probably because the long-term repeated fish release gives them continuous exposure to non-native fish (Hughes and O'Brien 2001; Alexander et al. 2022). Furthermore, otters had a high preference for non-native fish in both seasons. One potential reason was that non-native fish transported from lower elevations may have low moving capability on the Plateau, where environmental conditions such as low temperature and low oxygen are less favorable to these fish (Zięba et al. 2010; Balestrieri et al. 2013). Therefore, otters may have a higher success rate preying on non-native fish. This is probably the same reason for otters prefer benthic fishes (Harper et al. 2020; Martínez-Abraín et al. 2020), for example, *Triplophysa*, in both seasons in Yushu. Otters' prey preference largely depends on the difficulty of catching prey in specific habitat, for example, otter prey more on benthic fish in open water, whereas they prey more on fast-swimming Cyprinids in winter with colder water (Erlinge 1968; Grant and Harrington 2015; Martínez-Abraín et al. 2020). Another reason may be that non-native fish have higher nutrition and/or energy (Ruff 2007), which needs to be studied further in the future.

Even though Eurasian otters help in controlling biological invasion in Yushu, the extent to which they play a role needs further studies. A key point, for instance, maybe the survival rates of non-native fish after releasing in rivers with and without otters. A controlled experiment in the field, or a large-scale survey covering sites with and without otters but having religious fish release on the Plateau, may help to answer this question.

Nevertheless, our results indicated that non-native fish provided additional food resources for Eurasian otters in Yushu, which may benefit their survival. However, non-native fish may have negative impacts on local fish communities, for example, spreading diseases or outcompeting local species (Gozlan et al. 2010; Reid et al. 2019). Our surveys revealed a high abundance of native fish in Yushu (525.5 ± 83.4 g per trap), which is even higher than that in Tangjiahe National Nature Reserve (98.9 ± 16.4 g per trap), a well-protected reserve with abundant fish (; Wang et al. 2021; Wang et al. 2022b). The high abundance of native fish in Yushu may benefit from the residents' religious tradition of no killing. In such circumstances, food resources may not be a limit to otters' survival. Therefore, the release of non-native fish may bring more losses than benefits to the river ecosystem on the Plateau.

Considering the potential negative impacts on local fish communities, we recommend completely ban fish release on the Plateau. However, despite the fact that fish release was banned by the Yushu government since January 2019, most residents were not aware of this ban and fish release still occurred in 2019. In our interview survey, most interviewees expressed their positive response to government policy or appeals from living Buddhas. Hence, publicity of the ban, as well as the threats that fish release may bring, should be promoted. Considering the powerful influences of religious leaders, for example, living Buddhas, on the Buddhist residents (Gong et al. 2012; Yeh 2013), the involvement of living Buddhas may change the tradition of fish release in this region effectively.

In conclusion, our study revealed long-term and intensive religious fish release activities in Yushu, a city on the Qinghai-Tibetan Plateau. Most fish released were non-native commercial fish purchased from the market. Nevertheless, the density of non-native fish in local rivers was quite low. We found that Eurasian otters in Yushu mainly preyed on fish, and non-native fish accounted for about one fifth of otter prey. The otters showed a high preference for non-native fish. We propose that religious fish release provides additional food resources for otters, whereas otters, in turn, may reduce the probability of invasion of released fish. More studies are required to further assess the role of otters in invasive species control.

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Conflict of interest

The authors declare that they have no competing interests.

Author Contributions

P.F. and L.Z. conceived and designed the study. L.Z., Q.W., X.H., and Z.D. conducted the field surveys. P.Z. designed the experimental procedures. Q.W., Z.W., and K.Z. conducted lab work. Q.W. and Z.W. analyzed the data. Q.W., H.X., L.Z., and P.F. wrote the manuscript. All authors read and approved the final version of the manuscript.

Data Accessibility

Original sequence data in this study have been uploaded to the NCBI Sequence Read Archive under BioProject accession number PRJNA981302.

Appendix Questionnaire of animal release in Yushu (English Version)

We are from Sun Yat-sen University/Shan Shui Conservation Center, and we would like to ask you about the animal release in this region. We appreciate your help. This survey is anonymous. The information you provide will only be used for research, and we will not divulge any information.

Date _________ Village __________ Latitude and longitude _________/__________

1. Would you like to be interviewed? ☐Yes ☐No

2. Information of interviewee

Age: Sex: Ethnic group: Religion: Educational level:

Job Category: Size of household: Lived here for a long time? ☐Yes ☐No

3. Information of the release event

* Reason for release: A. Family member being sick B. Death of family member

C. Life is hard D. Others: _____

1. Time: Site: Species: Numbers: Reason: Sources of released animals:
2. Time: Site: Species: Numbers: Reason: Sources of released animals:
3. Time: Site: Species: Numbers: Reason: Sources of released animals:
4. Time: Site: Species: Numbers: Reason: Sources of released animals:

Why do you release fish instead of other animals?

4. Historical change of fish release

When did fish release start? ____________________

Change of fish release in 10 years? ☐Increasing ☐Decreasing ☐No change ☐Be unaware of

5. Influence of fish release

Do you know what kind of fish are in the river? ☐Yes Which ones?: ☐No

Do you think fish release will affect native fish? ☐Yes ☐No ☐Be unaware of

If so, what are the influences? ____________________

Do you think fish release will affect other animals, such as otters? ☐Yes ☐No ☐Be unaware of

If so, what are the influences? ____________________

6. Willingness to change fish release activities

If released fish cannot survive in the river but die soon, will you continue releasing fish in the future? ☐Yes ☐No ☐Be unaware of

If released fish will have a negative effect on other animals, will you continue releasing fish in the future? ☐Yes ☐No ☐Be unaware of

If the Living Buddha say fish release should be stopped, will you continue releasing fish? ☐Yes ☐No ☐Be unaware of

If the government bans fish release, will you continue releasing fish in the future? ☐Yes ☐No ☐Be unaware of

Do you know that the government has banned exotic fish release? ☐Yes ☐No

Do you know when the ban starts? ☐Yes ☐No

NO. _______________ Name of interviewer _______________

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