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Broad Diet Composition and Seasonal Feeding Variation Facilitate Successful Invasion of the Shimofuri Goby (*Tridentiger bifasciatus*) in a Water Transfer System

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Abstract: The diet composition of an invasive population of Shimofuri goby (*Tridentiger bifasciatus*) was investigated bimonthly during the period from September 2015 through August 2016 in Nansi Lake, a storage lake of the East Route of the South-to-North Water Transfer Project, China. The diet consists of a broad spectrum of prey items, including mollusca (*Bellamya* sp. and *Physa* sp.), aquatic insects (Odonata sp., Chironomidae sp., and Cirolanidae sp.), other macroinvertebrates (Nematoda sp. and Rhynchobdellida sp.), shrimp (*Palaemon modestus* and *Gammarus* sp.), fish (*Rhinogobius giurinus* and *Tridentiger bifasciatus*), fish eggs, and detritus. Dominant diets shifted from Rhynchobdellida sp. and unidentified digested food in July to *P. modestus* during September and November, and then shifted to both *P. modestus* and *R. giurinus*. Additionally, cannibalism was observed in March before spawning season of the goby, during which large males (SL > 70.0 mm) predated on small-sized ones. We suggest that broad spectrum of prey items and apparent seasonal shifting of dominant diets in the invasive goby fish, which might be an important mechanism favoring its successful invasion in water transfer system.

Keywords: alien species; diet composition; food preferences; *Tridentiger bifasciatus*; water diversion

1. Introduction

Invasion of fish is a significant threat to biodiversity and the ecosystem in inland waters [1]. Invasive success of a species is determined by its ability to tolerate and overcome various pressures of the new environment, which typically include the resource availability of the system. Successful invaders are often characterized by high foraging efficiency and resource use [2]. Trophic interactions are a major way in which the invaders influence native species in the ecosystems through predation and competition [3]. Direct predation is a predominant mechanism through which invaders can dramatically decrease the population of indigenous species or even cause their extinction [4,5]. For example, the invasion of Nile perch (*Lates niloticus*) in Lake Victoria caused the extinction of 60% of the endemic fish species [6]. Competition for food resources is another critical mechanism that contributes to the decline of native species [7]. Analyses of diet and trophic strategies of invasive predators are a primary approach for prediction of their potential impacts.

As one of the important pathways of freshwater invasions [8–10], inter-basin water transfers (IBWTs) decrease the strength of biotic interactions among indigenous species, and then increase the risk of predation and competition by invasive species in the recipient system [11–13]. The South-to-North

Water Transfer Project of China is one of the world's largest IBWTs [14]. Associated with water diversion of the East Route of the South-to-North Water Transfer Project (ESNT), the invasion of a goby species, Shimofuri goby (*Tridentiger bifasciatus*), was observed in water storage lakes along the ESNT [15].

The invasive mechanism of Shimofuri goby and its ecological impacts on the recipient ecosystem have become major concerns, with a rapid increase in the abundance of the fish and a dramatic decline in the populations of some native species [16]. Shimofuri goby is native to estuaries along the west Pacific coast from Hokkaido to Hong Kong, including the Yangtze estuary [17]. Its appearance in the storage lakes of the ESNT was first reported in catches of commercial shrimp traps in Nansi Lake in May 2015, and was further observed in the other storage lakes, including Luoma Lake, Dongping Lake and Hongze Lake [15]. Spawning season (from April to June) overlaps with the water diversion period (from October to May) of the ENST that water diversions may entrain mass early life-history stage individuals, probably originated from the Yangtze Estuary, into the ENST system [15]. Due to its low economic value, this goby is not a target exploited by local fishermen, and according to our monitoring, Shimofuri goby has established population successful and has quickly increasing in its abundance in these storage lakes. Our previous study indicates that opportunistic life history traits such as short lifespan and high fecundity, equilibrium trait (males guarding nest) and life history traits plasticity exhibited between lake and estuary habitats may facilitate its invasions [18].

Invasion of this species has also been reported in the San Francisco Estuary, USA, with its first appearance in 1985 [19], and it has since widely colonized the brackish and freshwater bodies [20]. The invasive population of Shimofuri goby in the San Francisco Estuary predates mainly on benthic invertebrates, consuming seasonally abundant prey resources (especially amphipods, *Corophium* sp.). It has been suggested that this feeding habit facilitates their adaptation to the highly fluctuating environment of the San Francisco Estuary [21]. In the present study, we investigated diet composition of the invasive population of Shimofuri goby in Nansi Lake of the ESNT bimonthly during September 2015 through August 2016. Our major objective was to identify diet composition and seasonal feeding variation of this species contributing to the invasion success. The results were then integrated with those of the invasive populations of this species in the San Francisco Estuary to provide insights into the feeding ecology that favors species establishment in new areas and potential impacts on indigenous species.

2. Materials and Methods

2.1. Study Area and Fish Sampling

Specimens of Shimofuri goby collected in the Nansi Lake for life history traits analyzed in our previous study [18] were used for diet composition analysis in the present study. Nansi Lake (34°27′ N–35°20′ N, 116°34′ E–117°21′ E) is a storage lake of the ESNT belonging to the Huai River watershed. With an area of 1153 km^2 and an average water depth of 1.4 m, it is the largest freshwater lake in northern China [22]. The lake was divided into an upper part and a lower part by a sluice dam, the Erji Dam (Figure 1).

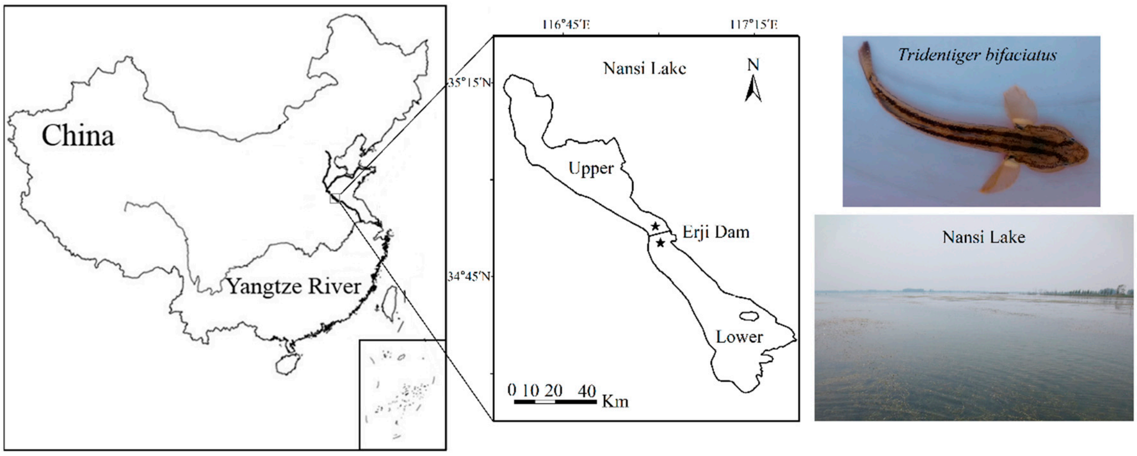

Figure 1. Nansi Lake, a storage lake of the East Route of the South-to-North Water Transfer Project, China, showing sampling locations for the Shimofuri goby (*Tridentiger bifasciatus*).

Shimofuri gobies were sampled bimonthly in two areas of Nansi Lake from September 2015 to August 2016 using benthic fyke nets (15-m long, 0.6-m wide, and 4-mm mesh size) (Figure 1). When sampling, the nets were deployed at 06:00–08:00 am for 24 h, and specimens were collected in the next morning. Sampling was carried out once every two months, in September and November 2015, and January, March, May, and July 2016, respectively. For each sample, a total of 100 benthic fyke nets were deployed with 50 nets in each of the upper and lower parts of the lake, respectively (Figure 1). Specimens collected in a sample day were pooled, anesthetized with M-222 and immediately stored in ice. Details of fish sampling were described in a previous study [18].

2.2. Diet Analysis

Standard length (SL, 0.1 mm) measured, and body weight (BW, 0.001 g) was weighed for each fish. The Shimofuri goby has an S-shaped digestive tract without a true stomach [16]. Diet compositions were determined in the foregut for each fish, which is defined as the part of the gut before the first bend [16]. The gut contents were removed and analyzed using a stereo microscope. Prey items were identified as the lowest recognizable taxa, counted, and weighed (0.0001 g) [23].

The importance of diet items was analyzed using the frequency of occurrence (%F) and percentage of biomass (%W). Frequency of occurrence was calculated by the equation: $\%F = f_i/\sum f \times 100$, where f_i is the number of guts containing the prey item I and $\sum f$ is the total number of non-empty guts. Percentage of biomass was calculated by the equation: $\%W = w_i/\sum w \times 100$, where w_i is the total weight of prey item i and $\sum w$ is the total weight of prey items consumed by fishThe index of relative importance (IRI) and percent index of relative importance (%IRI) of a diet item were calculated using the following equations [23]: $\mathrm{IRI} = \%\mathrm{W} \times \%\mathrm{F}$, and $\%\mathrm{IRI} = \mathrm{IRIi}/\sum \mathrm{IRI} \times 100$, where IRIi is the index of relative importance of prey item i.

Trophic niche breadth was calculated using Levin's standardized niche breadth (BA) [24] as: $\mathrm{BA} = \left[\frac{1}{\sum Pi^2} - 1\right]/(n-1)$, where P_i is the proportion (in weight) of food item i in the diet and n is the number of food items. BA ranges from 0, when a species consumes only one type of food, to 1, when it consumes similar food items. The Costello (1990) graphical method modified by Amundsen et al. (1996) [25] (p. 3) was used to illustrate the importance of diet items by plotting frequencies of occurrence (%F) and relative prey-specific abundance of food items. Relative prey-specific abundance of a food item (Pi) was calculated as the percentage of biomass of the food item (i) to the biomass of all the food items for consumers with the food item i presented [25]. The importance of the food items increases along the diagonal from the lower left to the upper right corner of the graph, with dominant prey at the upper end and rare or unimportant prey at the lower end [25].

Statistical analyses were performed using the vegan package in the R Programming Environment 3.5.3 (R Project for Statistical Computing, http://www.r-project.org/).

3. Results

A total of 200 fish were analyzed for gut contents. Standard lengths varied among months, with an increasing trend from July (mean value of 37.0 mm) to March (mean value of 72.3 mm), and slightly decrease in May (mean value of 50.4 mm) (Table 1). Body weights exhibited the same trend, increasing from July to March and then decreasing in May (Table 1). Among them, two individuals in November and seven in July had empty guts and were excluded from the analysis. The diet items were grouped into the following eight categories: mollusca (*Bellamya* sp. and *Physa* sp.), aquatic insects (Odonata sp., Chironomidae sp. and Cirolanidae sp.), other macro-invertebrates (Nematoda sp. and Rhynchobdellida sp.), shrimp (*Palaemon modestus* and *Gammarus* sp.), fish (*Rhinogobius giurinus* and *T. bifasciatus*), fish eggs, detritus, and unidentified digested food (Table 1). *Palaemon modestus* had the highest occurrence frequency and high relative prey-specific abundance. *Rhinogobius giurinus* had a high contribution to the diet biomass of some individuals with an occurrence frequency (23.3%) lower than *that of P. modestus* and higher than that of other food items. Chironomid larvae, fish eggs, *Bellamy* sp., *T. bifasciatus*, and *Gammarus* sp. were observed with high biomass contribution in some individuals, but the occurrence frequency was low (<20%) (Figure 2). All the other food items had low occurrence frequency and biomass contribution (Figure 2).

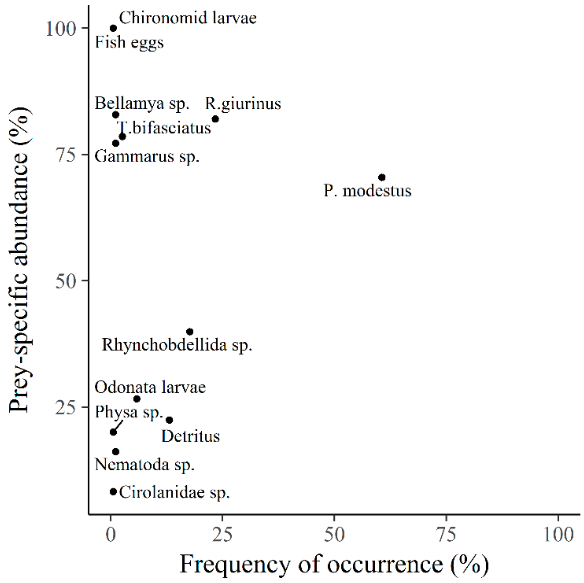

Figure 2. Percentage of prey-specific abundance and frequency of occurrence of prey items of the Shimofuri goby (*Tridentiger bifasciatus*) in Nansi Lake, China.

In July, a major part of the diet was digested and unidentifiable. Among the identified food items, Rhynchobdellida sp. had the highest contribution, followed by *Gammarus* sp. (Table 1). In September and November, the diet was dominated by *P. modestus* with a %IRI of 90.7% and 88.7%, respectively. In January, March, and May, the diets were dominated by *P. modestus* and *R. giurinus*, with *R. giurinus* having a %IRI value that increased with time (Table 1). The cannibalism phenomenon was observed in March for five individuals (occurrence frequency 14.3%) (Table 1). These predators were all male, with SLs ranging from 70.2 to 95.3 mm. In May, we observed one male (46.9 mm SL) with its gut full of eggs, which was similar to the appearance of *T. bifasciatus* eggs but not verified.

Trophic niche breadth was typically lower in September (0.13), November (0.18), and May (0.21), and relatively higher in July (0.51), January (0.41), and March (0.50) (Table 1).

Table 1. Mean (SD) and range of standard length (SL) and body weight (BW), and importance of prey item categories measured by frequency of occurrence (%F), percentage of biomass (%W), and percent index of relative importance (%IRI) for invasive Shimofuru goby (*Tridentiger bifasciatus*) collected in different months in the Nansi Lake, China.

		September			**November**			**January**			**March**			**May**			**July**		
Number of Fish			**35**			**37**			**17**			**35**			**41**			**26**	
SL (mm)	Mean ± SD		52.9 ± 7.2			51.9 ± 10.7			60.2 ± 7.6			72.3 ± 6.7			50.4 ± 7.6			37.0 ± 4.6	
BW(g)	Mean ± SD		2.869 ± 1.128			3.202 ± 2.371			4.562 ± 1.729			8.869 ± 2.757			2.275 ± 1.239			0.925 ± 0.408	
Prey items		%F	%W	%IRI	%F	%W	%IRI	%F	%W	%IRI	%F	%W	%IRI	%F	%W	%IRI	%F	%W	%IRI
Mollusca					2.70	0.19	0.01							4.88	4.40	0.50			
Bellamya sp.					2.70	0.19	0.01							2.44	4.25	0.24			
Physa sp.														2.44	0.15	0.01			
Aquatic insects		17.14	4.12	1.38	13.51	6.63	1.59	5.88	4.60	0.53	2.86	0.14	0.01						
Chironomid larvae		2.86	0.11	0.01															
Odonata larvae		11.42	3.56	0.80	13.51	6.63	1.59	5.88	4.6	0.53	2.86	0.14	0.01						
Cirolanidae sp.		2.86	0.45	0.03															
Other Macroinvertebrate		5.71	0.53	0.06	2.70	0.25	0.01							46.34	1.47	1.58	23.07	38.28	23.76
Nematoda sp.		5.71	0.53	0.06	2.70	0.25	0.01										7.69	3.91	0.63
Rhynchobdellida sp.														46.34	1.47	1.58	57.69	34.38	41.78
Shrimp		62.86	73.24	90.23	72.97	68.67	88.70	76.47	57.4	86.73	74.29	37.43	48.04	68.29	38.23	60.57	11.54	18.75	5.82
Palaemon modestus		62.86	73.24	90.74	72.97	68.67	88.70	76.47	57.4	86.73	74.29	37.43	59.32	68.29	38.23	60.73	3.85	7.29	0.59
Gammarus sp.																	7.69	11.46	1.86
Fish		14.29	17.35	4.86	24.32	22.03	9.48	17.65	33.57	11.71	48.57	61.57	51.66	29.27	54.58	37.07	3.85	5.47	0.57
Rhinogobius giurinus		14.29	17.35	4.89	24.32	22.03	9.48	17.65	33.57	11.71	42.86	35.34	32.31	29.27	54.58	37.16	3.85	5.47	0.44
Tridentiger bifasciatus											14.29	26.23	8.00						
Fish egg														2.44	4.41	0.25			
Detritus		37.14	4.76	3.46	5.41	2.22	0.21	11.76	4.44	1.03	20.00	0.86	0.30	2.44	0.54	0.03			
Unidentified digested food																	69.23	37.50	69.85
Unidentified digested food																	69.23	37.50	54.69
Niche breadth			0.13			0.18			0.41			0.50			0.21			0.51	

4. Discussion

Our results showed that diets of the Shimofuri goby included eight categories from detritus to fish in Nansi Lake; and in association with the size increase, the fish diet shifted from small-sized macroinvertebrate to large-sized shrimp, and then further to large-sized fish. These results revealed a broad spectrum of prey items and apparent seasonal shifts of dominant diets in the invasive Shimofuri goby population in Nansi Lake, which might be an important mechanism that favored the successful invasion of this species.

Our previous study indicated that combined opportunistic and equilibrium life-history traits facilitate successful invasions of the Shimofuri goby in water transfer systems [18]. In this study, the invasive goby population in the lake was found to feed multi-kinds prey items; specifically, some prey items (e.g., Chironomidae sp., *Bellamya* sp., and fish eggs) had a relatively high contribution to the diet of some individuals despite their low occurrence frequency, which indicates high variability in feeding between individuals. Meanwhile, the invasive population of the Shimofuri goby in the San Francisco Estuary also exhibited a wide range of food items [16]. A broad spectrum of prey items is widely reported in invasive gobiids [26–29]. Thus, the broad spectrum of prey items may be is a significant characteristic of the Shimofuri goby that favors successful invasion. A share of unidentified digested food was observed in July, which was probably due to the high digestion rates of juveniles caught in fyke nets [30]. Our previous study showed gender could not be determined by naked-eye from July to October [18], their diet compositions may vary between males and females during the spawning season, which will be studied in future research.

Seasonal shifting of dominant diets of the fish was revealed, which reflects the Shimofuri goby could change its preferred diets according to its ontogenic stages and/or availability of prey resources in the environment. Our previous study showed that the Shimofuri goby in Nansi Lake has a 1-year lifespan; it spawns during April and June and dies after spawning [18]. The Shimofuri population investigated in this study from September 2015 to July 2016 belonged to a 1-year class born in 2015. The SLs of the analyzed individuals with a mean value of 37.0 mm in July, increased to a mean value of 52.9 mm in September, and a mean value of 60.2 mm in January (Table 1). The goby predominantly predated on *Rhynchobdellida* sp. in July, which shifted to a diet with *P. modestus* September. The importance of *R. giurinus* in the diets increased gradually and became as important as a diet with *P. modestus* from January. Such transition from small-sized to large-sized prey in accordance with the growth of the individuals will benefit the efficient exploitation of nutrition for growth and gonad development, which is consistent with the optimal foraging theory [31]. Seasonal diet variations were also observed in the goby invasive population in the San Francisco Estuary, which mainly reflected seasonal variations in prey resource availability [16]. This phenomenon was observed in many invasive fish species, particularly invasive gobiids such as the round goby *Neogobius melanostomus* [32].

We observed cannibalism of the Shimofuri goby before the spawning season (March) as the larger individuals (SL > 70 mm) predated upon the smaller-sized ones [18]. Cannibalism is very common among piscivorous fishes, especially those that feed on diets containing various proportions of aquatic insects, crustaceans, mollusks, and fish [33–35]. This case was consistent with the diet spectrum of the Shimofuri goby in Nansi Lake, and interestingly, all individuals with cannibalism were male. A previous study reported that male Shimofuri gobies have nest-guarding behavior [16]. For many nest-guarding fish, especially gobies, males usually court and compete more intensely to attract females for mating [35]. When fighting behavior is frequent and lasts restively long among males, death and predation of conspecifics sometimes occur [36]. Thus, we suggest that competitive behavior for attracting females may cause cannibalism before spawning. Additionally, cannibalism observed in this study was different from that observed in the San Francisco Estuary, where the males (SL > 52 mm) ate their nests of unfertilized or dead eggs during the spawning season [16]. This cannibalism may be considered a strategy for providing additional energy associated with nest guarding behavior [37], which has been reported to increase larval survival rate in the San Francisco Estuary [16]. We also observed one male (46.9 mm SL) with its gut full of eggs during spawning in

Nansi Lake, which might be similar to cannibalism in the San Francisco Estuary. With the discussion mentioned above, we propose that further studies are required to fully understand the ecological mechanism of cannibalism observed in the Shimofuri goby.

The native goby *R. giurinus* is one of the most dominant preys of the Shimofuri goby in Nansi Lake. Predation has been recognized as a direct interaction that influences the population recruitment of native species [4,5]. Additionally, the spawning seasons (from April to June) of the two gobies are highly overlapped [38], which indicates potential competition for spawning grounds and a disadvantage to population recruitment [39]. Moreover, *P. modestus* and *R. giurinus* were the main prey items of some resident piscivorous fishes, for example, *Pelteobagrus fulvidraco*, *Siniperca chuatsi*, and *Channa argus*. There is a high likelihood of competition for food between the Shimofuri goby and these native species, which may impact their abundance through bottom-up trophic cascade processes. Further studies should be conducted to investigate the trophic interactions of the invasive Shimofuri goby with the native species. Additionally, annual water diversion of the ENST will continually entrain mass early life-history stage individuals into the recipient lakes [18], which may provide high propagule pressure to accelerate invasion process, thus, artificial barriers will be required to construct to prevent the drifting dispersal.

5. Conclusions

The Shimofuri goby has a broad spectrum of prey items and apparent seasonal shifting of dominant diets in different invaded habitats, which facilitates it to widely use resources, contributing to its integration into local ecosystems. This species also exhibits life history plasticity among non-native populations [18] and has high tolerance to different water temperatures and salinities to enable its adaptation to various environmental conditions [40]. These characteristics may account for the high invasion risk of the Shimofuri goby. Direct predation upon local prey resources and competition with some resident piscivorous fishes imply a risk of potential ecological impact. Further study of the population dynamics of this invasive goby and its population interactions with other species in the recipient environment will be required to fully understand its ecological role.

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