Trophic plasticity of the invasive redbelly tilapia (Coptodon zillii) in China inferred from DNA metabarcoding analysis

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Abstract

The redbelly tilapia (Coptodon zillii) is one of the most dangerous invasive alien fishes in the world. In order to better understand the feeding patterns of invasive populations in different habitats and seasons, and to reveal the driving force of differences in dietary composition among populations, we used DNA metabarcoding technology to analyze the dietary composition of 23 specimens from five different water bodies (two rivers and three reservoirs) in southern China, and 60 specimens from Shuikou Reservoir in four seasons (spring, summer, fall, and winter). The results showed that samples from five different water bodies and four seasons in Shuikou Reservoir were annotated to a total of 22 and 37 phyla of food categories, respectively. Generalist trophic strategies were dominant in C. zillii populations. There was significant spatial heterogeneity in the diet composition, with higher levels of trophic diversity in riverine populations. Water temperature, dissolved oxygen, and conductivity were important environmental factors driving changes in prey taxa of populations in different habitats. Dietary composition of populations in Shuikou Reservoir showed significant seasonal heterogeneity, with summer being the season with the highest level of trophic diversity. Total nitrogen, turbidity degree, pH and permanganate index were the important environmental factors driving the prey taxa changes of populations in different seasons of Shuikou Reservoir. Our results indicated that C. zillii are omnivorous, they have a wide range of recipes in both rivers and reservoirs in southern China, and show high trophic plasticity in different habitats and at different seasons of the year.

Materials and methods

Study area

The five sampling sites selected in this study were the water bodies that were seriously invaded by *C. zillii* in China, and they represented most of the invaded areas of *C. zillii* in China, which were, from north to south, Qiandao Lake (QDH), Shuikou Reservoir (SKSK), Letan Reservoir (XJ), Dongjiang River (DJ) and Nandujiang River (NDJ), of which QDH, SKSK, and XJ represented the reservoir (lentic habitat), while DJ and NDJ represent river (lotic habitat), and the information of the sampling sites is shown in Fig. 1.

Qiandao Lake, located in Chun'an county, Zhejiang Province, is a valley-type reservoir in the upper reaches of the Qiantang River. The reservoir has an average depth of 30 m and a water area of 580 km^2. It's a subtropical reservoir, which is the northernmost part of the invasive range of *C. zillii* in China. The origin of *C. zillii* in Qiandao Lake is unknown, but as early as 1987, Nile tilapia was cultured in hapas in this reservoir by aquatic bureau of Chun'an county (Zhou, 1988). We hypothesized that the *C. zillii* were probably unintentionally introduced into hapas by mixing with Nile tilapia fry, and then escaped from the hapas into the reservoir.

Shuikou Reservoir, located in Yanping District, Nanping City, Fujian Province, is a river-type reservoir in the middle reaches of the Minjiang River. It covers an area of about 99.6 km^{2}with an average water depth of about 65 m. Since the impoundment of the reservoir in March 1993, *C. zillii* have been farmed in earthen ponds around the reservoir on a large scale. They escaped into the reservoir due to the poor management of farmers. The number of*C. zillii* in the reservoir increased year by year and a large population was established since 2010 (He et al. 2013).

Located in Xincheng County, Laibin City, Guangxi Zhuang Autonomous Region, Letan Reservoir is a large hydropower station on the lower reaches of the Hongshuihe River in the Pearl River Basin. The reservoir has an average water depth of 112 m and a total capacity of 9.5×10^8 m^3. In 2007,*C. zillii* was introduced and cultured in hapas in the upper reaches of the Hongshuihe River. Gradually, they escaped from the hapas and established populations throughout the upper reaches of the Hongshuihe River. The *C. zillii* in the Letan Reservoir may have spread from the upper reaches.

The DJ sampling site was located in Huizhou City, Guangdong Province, in the middle reaches of the Dongjiang River, which is one of the main streams of the Pearl River system. The Dongjiang River has a total length of 562 km and a watershed area of 35,340 km^2.*C. zillii* was first cultured in Guangdong province after its introduction from Thailand in 1978, and due to the lack of management, they soon escaped into the natural water bodies. Owing to the well-developed network of the Pearl River system in Guangdong province, *C. zillii* established its population in the Dongjiang River very quickly. In recent years, *C. zillii* has expanded rapidly and it has become an absolutely dominant species in the Dongjiang River (Gu et al. 2020).

The NDJ sampling site was located in Ding'an County, Hainan Province, in the lower reaches of the Nandujiang River, which is the largest river in Hainan Province, with a main stream length of 334 km and a watershed area of 7,033 km^2. The culture of *C. zillii* in Hainan Province started in 1986, but they were quickly abandoned due to poor farming performance. Consequently, they escaped into natural water bodies and established populations in the Nandujiang River (Gu et al. 2016). In recent years, *C. zillii* has spread rapidly and it has become a dominant species in the Nandujiang River (Gu et al. 2020).

To obtain the water environment and climate information of the five sampling sites (Table S1), we downloaded nine water environment parameters from the China National Environmental Monitoring Centre (https://www.cnemc.cn/), including water temperature (TEMP), dissolved oxygen (DO), pH, conductivity (COND), turbidity degree (TD), permanganate index (PI), ammonia nitrogen (AN), total phosphorus (TP) and total nitrogen (TN). And four types of climatic data were downloaded from the Public Meteorological Service Center of the China Meteorological Administration (https://www.weather.com.cn), including daily mean temperature, daily maximum temperature, daily minimum temperature, and daily precipitation sum.

Field sampling and laboratory procedures

Sample collection and preparation

Field sampling was conducted in 2 phases, with phase 1 from October to November 2022 at 5 sampling sites, including QDH, SKSK, XJ, DJ, and NDJ. 110 (QDH), 63 (SKSK), 68 (XJ), 51 (DJ) and 55 (NDJ) of live specimens were trapped using gillnets with 6 mesh sizes (mesh sizes of 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, and 8 cm, respectively). The purpose of the first phase is to assess dietary differences caused by geographical differences and investigate the effects of associated environmental features on feeding habits (Table S1). In phase 2, from March 2023 to February 2024, different sizes of specimens were trapped with gillnets (same mesh size as in phase 1) at SKSK in the middle of each month. The number of live samples collected per month ranged from 80 to 100, for a total of 1,079 specimens. In order to accurately assess the effect of season on feeding traits, we divided the sampling period in SKSK into 4 seasons: spring (March to May), summer (June to August), fall (September to November) and winter (December to February). The collection of live specimens at the above 2 stages was entrusted to experienced local fishermen and both were licensed by the fisheries department of the sampling site. Each trapping was conducted at dawn, which corresponded to the peak feeding time of *C. zillii* .

At each sampling event, once the live fish samples were collected, sex was first determined from the morphology of the genital pore. While it was immediately immersed in an overdose of anesthetic (MS-222) solution (0.4 mg/ml) for 15 min, followed by measurement of standard length (SL) using digital calipers (to the nearest 0.1 cm), and body weight using an electronic balance (to the nearest 0.1 g). At the end of the measurements each individual was dissected from the digestive tract, the whole stomach (esophagus to just below the pyloric valve) was excised. The degree of stomach fullness was determined according to the method of Yin (1995), and the degree of fullness was categorized into 6 grades (grades 0 to 5) (Table S2). If the stomach fullness degree was graded from 4 to 5, all stomach contents (200 to 500 mg) were carefully removed with forceps, preserved in a 1.5 mL centrifuge tube, and immediately put on dry ice to stop its digestion process and preserve the DNA in the stomach contents. If the stomach fullness degree was graded from 0 to 3, no further sampling was done. In stage 1, we collected 23 samples of stomach contents, of which the number of samples from all sampling sites was 5, except for QDH, where the number of samples was 3. In phase 2, we collected 60 samples of stomach contents, with the number of samples collected at each month being five. Information on the standard length, body weight, and sex of the samples from each sampling site is shown in Tables S1 and S3.

Analysis of basic dietary data

To determine the adequacy of the amount of sequencing data for each sample, and the sample size for each population, the species rarefaction curve and species accumulation boxplot were calculated and plotted using the vegan package and the ggplot2 package in R software (Version 4.0.3). Fractions that could not be identified in each sample (less than 97% sequence homology in Nucleotide BLAST on Genbank) were excluded from analysis.

According to Deagle et al. (2019), the importance of each prey species in the diet was estimated by the frequency of occurrence (FO) and relative read abundance (RRA), respectively. FO is the number of samples containing a particular food group as a percentage of the total number of samples and is calculated as follows: $FO = \frac{N}{T}$x100, where N is the number of stomachs in which the prey items of one particular category are found and T is the total number of stomachs with food in the sample. RRA is the percentage of the number of sequences of a food category to the total number of sequences in that sample, reflecting the relative biomass, and is calculated as follows: $RRA = \frac{1}{N}\sum_{j=1}^{N}\frac{Si,j}{\sum_{i=1}^{T} Si,j}$x100, where T is the number of food categories, N is the total number of valid samples, $S_{i,j}$ is the sequence number of food category i in sample j . Feeding strategies (generalized or specialized) were analyzed by plotting the relationship between FO and RRA. When a prey taxon has a low FO but a high RRA, it indicates specialized feeding on a specific prey by *C. zillii* , whereas when the FO is high but the RRA is low, it represents a generalized feeding strategy.

A bar chart of RRA of species was plotted based on the results of species annotation at the phylum level using Perl 5.26.2 software. The top 20 species in terms of RRA of prey were selected, and the rest of the species were set as others in the bar chart. Species codes for stomach contents at the phylum level are shown in Table S4.

Shannon-Wiener index (H'), Pielou's evenness index (J) and niche breadth index (B) (Levins 1968) was used to describe the diet diversity of *C. zillii* . The formula was calculated as follows:

$H' = -\sum_{i=1}^{S}(pi \times lnpi)$, $J = -\frac{\sum_{i=1}^{S}(pi \times lnpi)}{\ln S}$, $B = \frac{1}{\sum_{i=1}^{S}(pi)^2}$, where H' is the Shannon-Wiener index of prey diversity in the diet and p is the proportion of prey items of one particular food category i to the total number of prey categories found. S denotes the total number of prey items. The H' and J were calculated using QIIME (The Quantitative Insights Into Microbial Ecology) software (Version 1.9.1) (Caporaso et al. 2010) to assess dietary diversity within a single sample. Differences in prey diversity between populations were analyzed for significance by the Kruskal-Wallis rank sum test, and box plots were drawn using the ggplot2 package of R software (Version 4.0.3).

Based on the species annotation results and the abundance information of the feature sequences of all the samples, the information of the feature sequences of the same classification was combined and processed to obtain the species abundance information table (Profiling Table). The phylogenetic relationships between the feature sequences were also utilized to calculate the Weighted unifrac distance and Bray-Curtis distance between samples using QIIME software (Version 1.9.1) (Caporaso et al. 2010). The Weighted unifrac distance and Bray-Curtis distance matrix heatmap were plotted using Perl 5.26.2 software. Dietary differences between samples, populations or seasons were assessed by non-metric multi-dimensional scaling (NMDS) based on Weighted unifrac distances in the ade4 and ggplot2 packages of the R software (version 4.0.3). To further analyze the significance of dietary differences between populations or between seasons, a permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis distance was performed in the vegan package of R software (version 4.0.3). And significant p -values were calculated during 999 random permutations. In order to search for species with statistical differences among recipes of different populations, a histogram of the distribution of LDA values was plotted using the linear discriminant analysis (LDA) effect size method in the online software Galaxy module (http://galaxy.biobakery.org/). And species with LDA scores greater than a set value (the default setting was 4) were statistically different between groups.

To assess dietary overlap between populations, we further calculated the Schoener overlap index (α) (Schoener 1970). This overlap measure ranges from 0 (no overlap) to 1 (complete overlap in resource use), and values above 0.6 are considered biologically significant according to Wallace and Ramsey (1983). The formula was calculated as α=1-0.5($\sum_{i=1}^{n} |P\text{xi} - P\text{yi}|$); where α is the measure of the relative amount of dietary overlap, varying between 0 (no overlap) and 1 (complete overlap), P_{xi} represents the proportion of food category i in the diet of the population x , P_{yi} is the proportion of food category i in the diet of the population y , and$n$ is the number of food categories.

Effects of environmental, geographic and climatic variables on trophic patterns of different populations

Screening for environmental and climatic variables

To remove possible high levels of multicollinearity between the environmental and climatic factors, the linear correlations between the factors were examined using the Variance Inflation Factor (VIF) method. Factors with VIF >10 were removed, thus excluding highly autocorrelated environmental and climatic factors and retaining those with the greatest influence on diet composition.

Spearman's correlation analysis

Spearman's correlation coefficients between prey taxa and environmental factors were calculated and tested for significance using the corr. test function of the psych package in R software (version 4.0.3). The results were then visualized using the pheatmap function of the pheatmap package.

Analysis of multiple linear regression models

Multiple linear regression models were used to screen for environmental factors that had significant effects on the H' , J , and$B$. The explanatory variables were standardized, and the response variables were tested for normal distribution, and those that did not meet the normal distribution were log-transformed or inverted. Multiple linear regression analysis was completed in SPSS 22.0 software.

Redundancy analysis (RDA)

In order to further illustrate the response of different prey items to various environmental factors, CANOCO 4.5 software was used to analyze the effect of environmental factors on prey items of populations in various habitats or different seasons by means of gradient analysis. Firstly, the RRA data of prey species of each sample were transformed by Hellinger transformation, and then detrended correspondence analysis (DCA) was performed. The sorting method was decided according to the lengths of gradient (LGA) in the first axis of the analyzed results. RDA was used when the LGA was less than 3, and canonical correspondence analysis (CCA) was used when the LGA was greater than 4. Both are used when the LGA was between 3 and 4 (Ter Braak and Smilauer 2002). RDA was chosen because the LGA in the first axis in the DCA analysis was 3.57 (between different habitats) (Table S5) and 3.21 (between different seasons in SKSK) (Table S5), respectively.

During the RDA analysis, RRA data for prey species were used as the response variable and environmental factors were used as predictor variables. Predictor variables were not transformed because the significance of the RDA results did not depend on the parameter distribution assumptions of the predictor variables (Ter Braak 1986). Rare species (i.e., species ranked after the 20th percentile in RRA in the diet for each population) were included in the analysis because they contribute to the breadth of taxa consumed by *C.zillii* , inform our findings of species sensitive to invasion, and removal may have affected the results (Poos and Jackson 2012). Monte Carlo permutation tests (n = 5000) were used to assess significance between the RDA ordination axes for prey species and environmental variables, and the first two axes were used to plot the two-dimensional ordination of sample-environmental factors.

Mantel test

Finally, we investigated whether the Bray-Curtis distance for dietary differences, and dietary overlap between pairs of populations were related to geographic distance between them. Using the Mantel test, we first developed pairwise geographic distance matrices for all sampling points based on GPS coordinates and compared them against pairwise matrices of Bray-Curtis distances (Table S9), and Shoener's overlap index values (Table S10), respectively, by completing 10,000 permutations in the ade4 R package (v1.7-15).

Results

Feeding intensity

Stomach fullness of five population samples from two habitats showed that DJ population had the highest Fullness Index (64.71%) and SKSK population had the highest Empty Index (60.32%) (Table S6). There was a significant seasonal difference in stomach fullness of SKSK population (Table S6), and the seasonal pattern of fullness index was: spring (87.68%) > summer (56.84%) > fall (32.22%) > winter (31.40%), indicating that the feeding intensity was significantly higher in spring and summer than in fall and winter.

DNA sequencing

The average number of effective tags of stomach contents samples from five populations from two habitats ranged from 92286 to 130330, and the percentage of effective tags reached 86.89% to 97.65% (Table S7). The average number of effective tags of stomach contents samples from four seasons of SKSK ranged from 79908 to 99437, and the percentage of effective tags reached 91.17% to 94.49% (Table S7). The quality of sequencing data of all samples met the requirements for analysis.

As the sequencing depth increased, the rarefaction curve of Shannon-Wiener index tended to flatten out for each sample (Fig. S1), indicating that the sequencing data volume was asymptotically reasonable, and that more data volume did not have a significant effect on the Shannon-Wiener index. As the sample size increased, the position of the boxplots increased sharply, indicating that a large number of species were found in the samples, but the boxplots did not reach a plateau for either the five population from different habitats or the samples from different seasons in SKSK (Fig. S2), suggesting that the sample size used for the study was insufficient.

Prey item composition

475 OTUs were delineated in samples of the five population from 2 habitats (Table S7), with a total of 22 phyla annotated (Table 1). 2,590 OTUs were delineated in samples of the four seasons from the SKSK (Table S7), with a total of 37 phyla annotated (Table 2). In addition, there were some OTUs that failed to be attributed to known taxonomic units. All prey annotated to the phylum level were grouped into 5 food categories: phytoplankton, protozoa, zooplankton, benthos, and detritus. The mean RRA of phytoplankton, protozoa, zooplankton, benthos, and detritus in the diet of the five populations from two habitats was 52.84%, 8.79%, 12.95%, 3.12%, and 13.49%, respectively (Table 1). The mean RRA of phytoplankton, protozoa, zooplankton, benthos, and detritus in the diet of seasonal samples from SKSK was 10.68%, 31.27%, 10.35%, 21.42% and 15.62%, respectively (Table 2). It can be inferred that *C.zillii* is omnivorous and the proportions of various food categories in its diet varied with habitat and season. The relationship between FO and RRA

showed (Table 1, Table 2, Fig. S3, Fig. S4) that generalist trophic strategies were dominant in the *C.zillii* populations.

Feeding habits variation with habitat

12, 11, 16, 11,17 phyla of food categories were identified in the stomach contents of DJ, NDJ, XJ, QDH, and SKSK populations, respectively. (Table 1, Fig. S3). Chlorophyta was the food category with the highest RRA in the NDJ, XJ, and QDH population. Arthropoda and Bacillariophyta were the food categories with the highest RRA in the DJ and SKSK population, respectively (Fig. S3).

The prey diversity indices (*H'* , *J*) in both XJ and NDJ populations were significantly ($P < 0.05$) higher than those in QDH and SKSK populations (Table 3, Fig. S5). The Niche breadth index (B) in XJ population was significantly ($P < 0.05$) higher than those in other four populations (Table 3).

Extremely significant ($P < 0.001$) Bray-Curtis distances were detected among six pairwise populations (Table S8). NMDS ordination showed that the coefficient of stress was 0.079 (Fig. 2A). PERMANOVA analysis showed that there were significant differences ($P < 0.05$) in diet composition among all paired populations (Table 4). In addition, Schoener's dietary overlap indices between populations ranged from 0.1116 to 0.5832 (Table S9), which were all less than the threshold (0.6). Histograms of the distribution of the LDA values showed that prey taxa causing significant differences in the diets of the five populations from the different habitats were Chlorophyta, Bacillariophyta, Cercozoa, Ciliophora, Annelida, Basidiomycota and Ascomycota (Fig. 3A).

Temporal variation of feeding habits

35 (spring), 31 (summer), 29 (fall), and 28 (winter) phyla of food categories were identified in the seasonal samples of the SKSK population (Table 2). Ciliophora was the food category with the highest RRA in summer, fall, and winter (Fig. S4). Bacillariophyta was the food category with the highest RRA in spring (Fig. S4). No significant ($P > 0.05$) differences in prey diversity indices (*H'* , *J* , *B*) were detected between seasons (Table 3, Fig. S5).

Significant Bray-Curtis distances were detected among four pairwise seasons ($P < 0.05$) (Table S10). NMDS ordination showed that the coefficient of stress was 0.2 (Fig. 2B). The PERMANOVA analysis showed that there were significant differences ($P < 0.05$) in diet composition between all paired seasons (Table 4). In addition, the Schoener diet overlap indices between seasons ranged from 0.4960 to 0.7390 (Table S11), and four pairwise diet overlap indices between seasons exceeded the threshold (0.6). Histogram of the distribution of the LDA values showed that prey taxa that caused the significant differences in the diets of samples among different seasons were Rotifera, Ciliophora and Mollusca (Fig. 3B).

Feeding patterns in relation to standard length

Generalized linear model analyses showed no significant effects ($P > 0.05$) of SL, population and its interactions on the presence and absence of the top 10 prey species in terms of RRA,*H'* , *J* , and *B* .

The driving factors of dietary differences between different habitats and seasons

A total of three environmental factors with VIF values less than 10 were detected in samples from two different habitats, i.e., TEMP (VIF=1.515056), DO (VIF=1.114976) and COND (VIF=1.594560). A total of six environmental factors with VIF values less than 10 were detected in seasonal samples from SKSK, i.e. pH (VIF=3.474507), COND (VIF=4.002988), TD (VIF=4.002742), PI (VIF= 7.432649), TP (VIF=3.664767) and TN (VIF=6.227738).

Spearman's correlation analysis showed that among the diet of five populations from two habitats (Fig. S6), Cercozoa showed extremely significant ($P < 0.01$) positive correlation with TEMP and extremely significant ($P < 0.01$) negative correlation with COND. Chlorophyta showed a significant ($P < 0.05$) positive correlation with DO. Ciliophora showed an extremely significant ($P < 0.01$) negative correlation with DO. Rotifera showed significant ($P < 0.05$) negative correlation with DO. Both Ascomycota and Basidiomycota showed extremely significant (P <0.01) positive correlations with COND. In the diet of seasonal samples from SKSK (Fig. S6), Bacillariophyta showed extremely significant (P <0.01) positive correlation with pH and TN. Rotifera showed extremely significant (P <0.01) negative correlation with TD and TN, and extremely significant (P <0.01) positive correlation with COND. Both Cercozoa and Euglenozoa showed extremely significant (P <0.01) negative correlation with pH. Euglenozoa showed significant (P <0.05) negative correlation with TD. Bacillariophyta showed significant (P <0.05) negative correlation with TD. Ciliophora showed significant (P <0.05) negative correlation with TN.

As for five populations from two different habitats, the multiple linear regression coefficients between prey diversity indices (H' and$J$) and TEMP as well as COND were extremely significant (P <0.01) (Table S12). The regression coefficient between$B$ and COND was extremely significant (P <0.01) (Table S12). As for seasonal samples from SKSK, the regression coefficient between J and TP was significant (P <0.05) (Table S13).

The first RDA ordination axis (F = 10.2, P = 0.002) and all RDA ordination axes (F = 4.7, P = 0.002) were significant for five populations from two different habitats (Table 5). The first two axes explained 42.29% (RDA1) and 34.45% (RDA2) of the information on the relationship between prey taxa and environmental factors, respectively (Fig. 4A). TEMP, DO and COND are important environmental factors that drive the changes of prey taxa in different habitats. Among them, COND (r^2 = 0.6580) was the environmental factor that had the greatest influence on the food composition, followed by TEMP (r^2 = 0.5108) and DO (r^2 = 0.3538) (Table 5). The first RDA ordination axis (F = 7.3, P = 0.004) and all RDA ordination axes (F = 3.3, P = 0.002) were significant for seasonal samples from SKSK. The first two axes explained 28.52% (RDA1) and 24.18% (RDA2) of the information on the relationship between prey taxa and environmental factors, respectively (Fig. 4B). TN, TD, pH and PI are the important environmental factors that drive the changes of prey taxa in different seasons of SKSK. Among them, TN (r^2 = 0.3735) was the environmental factor that had the greatest influence on the food composition, followed by TD (r^2 = 0.3285), pH (r^2= 0.1730) and PI (r^2 = 0.1074) (Table 5).

There was no significant Mantel correlation between the dietary overlap index and geographic distance between five populations from two different habitats (R^2=0.006313,P =0.8273) (Fig. S7). Likewise, there was no significant Mantel correlation between Bray-Curtis distance and geographic distance between populations (R^2=0.36626, P =0.0638) (Fig. S7).

Discussion

Trophic plasticity of *C. zillii* populations

Comparison of trophic plasticity in native and invasive populations

Trophic plasticity refers to the adaptability of an organism to reduce potential food competition by altering its diet (Mavraki et al. 2020). It is a form of trophic generalism, meaning that a species is able to feed on a broad food spectrum but only utilize a portion of it, depending on the environment it inhabits (Riera 2009). Trophic plasticity is considered to be one of the important factors contributing to the successful invasion of exotic fishes (Pettit-wade et al. 2015) and may allow them to colonize different environments, exploit new food resources, and outcompete many native fishes (Cathcart et al. 2019).

Based on previous studies of the dietary patterns, our results confirm that *C. zillii* are omnivorous, feeding on a wide variety of foods. Our study showed that the prey taxa of *C. zillii* in the invaded range differed from those in its native range. For example, in its native range, the diet of *C. zillii* in Lake Nasser, Egypt, includes detritus, plant tissues, green algae, diatoms, rotifers, branchiopods, copepods, invertebrates, and others (Shalloof et al. 2020). In Otamiri River, Nigeria, they feeds on algae, vegetative matter, detritus and aquatic invertebrate larvae (Agbabiaka 2012). In its invasive range, *C. zillii* feeds mainly on detritus, algae, macrophytes and diatoms in the Garmat Ali River, Iraq (Mohamed and Al-Wan 2020). *C. zillii* from Arm-Tigris River in Iraq is herbivorous, feeding on six food categories, including filamentous algae, plant particles and their seeds, organic matter, inorganic sediments, diatoms and fish eggs (Wahab 2021).

The feeding pattern of*C. zillii* from Shadegan wetland in Iran is vegetarian with low animal sources. Its gut contents include macrophytes, fish scales, fish eggs, branchiopods, copepods and periphyton species (Bavali et al. 2022). In the present study, the highest RRA of phytoplankton (52.84%) followed by detritus (13.49%), zooplankton (12.95%), protozoa (8.79%) and Zoobenthos (3.12%) were found in the diet of the five populations from two different habitats. A possible explanation for these different findings is that there are differences in the availability of prey species in different habitats. Therefore, to facilitate the comparison of the degree of trophic plasticity in native and invasive range, we used the prey species and proportions of *C. zillii* reported in the literature (Agbabiaka 2012; Wahab 2021; Mohamed and Al-Wan 2020; Shalloof et al. 2022) to characterize niche breadth index (*B*) of different populations. In its native range, the *B* of population from Nasser Lake in Egypt and Otamiri River in Nigeria were 5.1610 and 8.3171, respectively. In its invasive range, the *B* of population from Arm-Tigris River in Iraq, Garmat Ali River and Shadegan Wetland in Iran were 2.3607, 3.3825 and 3.7756, respectively. In the present study, the *B* of population from the two river habitats were 1.3718 (NDJ) and 2.2783 (DJ), and the *B* of population from the three reservoir habitats were 1.9356 (QDH), 2.0259 (SKSK) and 4.2474 (XJ), respectively. The results of the above studies showed that the *B* of the native populations were all greater than those of the invasive populations. Previous studies have shown that the *B* of species are influenced by a variety of abiotic and biotic factors, such as resource density and diversity, population densities, competitors and predators (Olsson et al. 2009). Moreover, ecological niche contraction usually occurs with increased interspecific competition (Bolnick et al. 2010). We suggest that invasive populations may use only the best food resources by reducing their spatial ecological niche in response to environmental pressures such as interspecific competition and predation in the new habitat, as compared to the native populations (MacArthur and Pianka 1966). On the other side, as a versatile predator with strong sense of territoriality, *C. zillii* may also reduce intraspecific competition by reducing overlap in resource use (Bolnick et al. 2007). All of these factors may contribute to the contraction of the trophic ecological niche of invasive populations. Limited evidence suggests that species can rapidly change their trophic ecological niche once they enter a new environment (Comte et al. 2016). Tran et al. (2015) found that the invasion of topmouth gudgeon (*Pseudorasbora parva*) led to a differentiation of trophic ecological niches, in part because they reduce their ecological niche width when they coexist with other species, thereby facilitating their coexistence in the invaded ecosystem.

Spatial variation in the trophic plasticity of invasive populations

Geographic and ecological habitats can play an active role in feeding strategies of fish by providing different food supplies (Garcia et al. 2018). Studies have shown that invasive freshwater fishes are able to flexibly adapt feeding strategies to prey availability under different habitat conditions (i.e., rivers vs. reservoirs) with a wide range of dietary plasticity (Marchetti et al. 2004). For example, trophic plasticity facilitates the invasion of bleak (*Alburnus alburnus*) in a variety of lentic (reservoirs) and lotic (rivers) habitats in the Iberian Peninsula (Almeida et al. 2017). The Eastern mosquitofish (*Gambusia holbrooki*) that invaded northwestern Turkey showed a generalized feeding strategy in lotic and lentic habitats (Saç 2023). In this study, the RRA of different categories of food in the diet of*C. zillii* populations in different habitats varied considerably. For example, the RRA of phytoplankton in the diet of NDJ population was 85.8948%, which was much higher than those of other populations. The RRA of protozoa in the diet of XJ and SKSK populations reached 17.4737% and 15.1579%, respectively, which was much higher than those of other populations. The RRA of zooplankton in the diet of DJ population was 43.7894%, which was much higher than those of other populations. The RRA of zoobenthos in the diet of SKSK population was 10.9474%, which was much higher than those of other populations, and no zoobenthos was detected in the diet of NDJ population. The RRA of detritus in the diet of QDH and XJ populations was 28.0702% and 26.1052%, respectively, which were much higher than those of the other populations. In riverine habitats (DJ and NDJ), *C. zillii* preferred phytoplankton and zooplankton followed by detritus and protozoa. In reservoir habitats, phytoplankton was the most consumed food followed by detritus, protozoa, and zoobenthos. These results suggest that *C. zillii* consumed different food resources in different habitats and showed a wide range of plasticity in dietary traits.

In general, river ecosystems are structurally more complex than reservoirs. Consequently, the nutrient resources that rivers can provide are usually more diverse (Terra and Arau ́jo 2011). Therefore, we expected a higher level of trophic diversity in river populations, and the results of this study largely support this hypothesis. In this study, the Shannon-Wiener index (*H'*) was used to characterize the food diversity of river and reservoir populations. According to Encina et al. (2004), low values indicate diets dominated by a small number of prey (specialist predators) and high values indicate extensive diets. Diets with values greater than 2 were considered high, while values less than 1 were considered low. We found that the highest *H'* was detected in NDJ population, followed by XJ population. The *H'* of the river populations (NDJ and DJ) were higher than those of the two reservoir populations (QDH and SKSK). And the *H'* of XJ population was higher than that of DJ population, but the difference between them was not significant (P >0.05). Meanwhile, the *H'* of two river populations and three reservoir populations were higher than 2 (*H'* = 2.5029 4.8544), indicating that *C. zillii* has a wide range of food spectrum in both river and reservoir habitats in China, and the trophic diversity level of the river populations was higher than that of the reservoir populations.

Seasonal changes in the trophic plasticity of invasive populations

Assessing seasonal trophic variation in invasive fish is critical because such differences may result in different impacts on invasive ecosystems throughout the year. Changes in seasonal trophic ecological niches of invasive fish can reflect changes in diet (e.g., utilization of more diverse prey).

And changes in the size of trophic ecological niche space reflect response of available resources to environmental drivers, e.g., differences in prey availability across seasons may result in seasonal shifts in ecological niches (Haubrock et al. 2021; Hedden et al. 2022). Seasonal variation in the diet of channel catfish (*Ictalurus punctatus*) has been reported in its native (Holland and Peters 1992) and introduced (Hedden et al. 2022) ranges, with a wider range of food items in spring. In the present study, the RRA of different food categories in the diet of the *C. zillii* population varied considerably between seasons. For example, the RRA of phytoplankton was highest in spring (24.9241%) and lowest in fall (3.22%). The RRA of protozoa and zooplankton were highest in fall at 47.9459% and 17.0708%, respectively. And the RRA of protozoa and zoobenthos were lowest in spring at 17.7915% and 12.1752%, respectively. The RRA of zoobenthos was highest in summer (34.8134%). These results suggest that*C. zillii* consumed different food resources in different seasons and showed a wide range of plasticity in dietary traits.

Water temperature in reservoirs change seasonally, and seasonal changes in water temperature are one of the main factors affecting the seasonal succession of food resources (including phytoplankton, zooplankton, protozoa, etc.) for invasive fish. Summer is the season with the highest average water temperatures in SKSK, and therefore, the nutrient resources that can be provided are usually more diverse. We predicted that the highest level of trophic diversity in SKSK would occur during the summer months, and the results of the present study largely supported this hypothesis. We found that the *H'* and B of summer were higher than those of the other seasons, and the B of winter was the lowest (2.6455). The *H'* of the populations in different seasons ranged from 3.8081 to 4.2230, which were higher than 2. This indicated that the population in SKSK had a wide range of food spectrum in different seasons and the level of trophic diversity in summer was higher than those in other seasons.

Dietary overlap and potential impacts between *C. zillii* and native omnivorous fishes

Species invasions alter interactions within and between communities, with potentially serious consequences for biodiversity and ecosystems (Pimm et al. 1991). A high degree of trophic plasticity allows invasive species to adjust their feeding behavior when ecological niches overlap with native species (Zengeya et al. 2011). Invasions of generalist predators often result in reduced abundance of native species (David et al. 2017). The global invasion of carp is facilitated by the high trophic plasticity, which often dominate aquatic ecosystems and threaten native aquatic taxa (e.g., fish, aquatic plants, etc.) through both top-down and bottom-up processes (e.g., predation and alteration of trophic levels and turbidity) (Weber and Brown 2009). The invasion of rainbow trout (*Oncorhynchus mykiss*) may lead to changes in ecological niches and declines in native fish populations through predation or competitive exclusion of food resources (Shelton et al. 2015). The invasion of *O. niloticus* and *C. rendalli* altered the structure of tropical freshwater food webs in artificial reservoirs (Lake Gatun and Lake Bayano) in central Panama (Sharpe et al. 2023). In this study, *C. zillii* was omnivorous, with a wide range of food spectrum and a high degree of trophic plasticity, and it was able to adjust its trophic position in response to food availability. We predicted that the invasion of *C. zillii* could change the structure and dynamics of food webs through various mechanisms (e.g., predation and competition), thereby affecting the dietary and trophic ecological position of native fishes. On the one hand, *C. zillii* may alter the energy supply of other fishes by feeding at the bottom of the food web (feed on phytoplankton), monopolizing and retaining sufficient basal food resources, even if they do not directly compete with them for food. This has been identified as one of the main invasion mechanisms for common carp (*Cyprinus carpio*), which also limit native fish biomass by monopolizing and retaining basal food resources in Australian rivers (Marshall et al. 2019). Nutrition of common fish (e.g., *Pelteobagrus fulvidraco* and *Pseudobagrus nitidus*) in QDH and SKSK is highly dependent on algal-feeding macroinvertebrates (Hu et al. 2019), and thus algal feeding by *C. zillii* may limit the food source for herbivorous invertebrates, thereby limiting the quantity and quality of food for native fish communities. On the other hand, omnivorous freshwater fishes are widespread in the food web of rivers and reservoirs in southern China, and when there is a dietary overlap between *C. zillii* and native fishes, *C. zillii* may pose a competitive threat to native omnivorous fishes in order to access limited food resources. Based on the published literature, we organized the diets of three common omnivorous fishes in southern China [*Xenocypris davidi* (Xu 1988), *Xenocypris argentea* (Xu and Liao 1984) and Prussian carp (*Carassius auratus gibelio*) (Zhang et al. 2020)], where *X. davidi* is indigenous to DJ, NDJ, and QDH, *X. argentea* is indigenous to SKSK, DJ, and QDH, and Prussian carp is indigenous to SKSK and QDH. We calculated the dietary overlap indices of *C. zillii* and these indigenous fish species according to the formula of Schoener (1970), and found that the dietary overlap indices (Schoener overlap index) of *C. zillii* with*X. davidi* , *X. argentea* , and *Carassius auratus gibelio* were 0.2151, 0.2759, and 0.4331, respectively. Although none of the overlap indices exceeded the threshold value (0.6), this already suggests that there is a partial dietary overlap between *C. zillii* and these native fishes. It is noteworthy that the proportion of phytoplankton in the diets of *C. zillii* and Prussian carp was very close to each other, which may have led to a high degree of ecological niche overlap between these two species. It can be hypothesized that in SKSK and QDH, *C. zillii* did not take advantage of vacant trophic ecological niches in these two aquatic ecosystems, but rather utilized similar food resources as Prussian carp to potentially displace it competitively.

Conclusion

For the first time, we made a direct comparison of the dietary patterns of Chinese invasive populations of *C. zillii* at a large geographic scale. The results revealed that *C. zillii* is an omnivorous fish that exhibits generalized feeding strategies in both river and reservoir habitats. In the aquatic ecosystems of southern China, populations of *C. zillii* showed high trophic plasticity in different spaces (rivers or reservoirs) and at different times (seasons). Aquatic environmental factors were key drivers of dietary differences between populations in different habitats and between populations in different seasons. We did not find significant correlations between individual standard length and dietary patterns. Although the existence of adverse effects of *C. zillii* on structure and dynamics of native food web has not been confirmed, we predict that *C. zillii* will pose a potential threat to the dietary and trophic ecological niches of native omnivorous fishes.

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