Trophic niche variation and niche overlap between invasive mrigal carp and native mud carp in southern China

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Abstract

The ecological impacts of freshwater invasive fishes can develop through elevated interspecific competition where they share similar resources to native species, but with this potentially mitigated by high resource availability. The trophic ecology of invasive mrigal carp *Cirrhinus mrigala* and native mud carp *Cirrhinus molitorella* were assessed in the Pearl River, Southern China, through application of DNA metabarcoding (based on the 18S rDNA V4 region) on samples collected across the basin in both the wet and dry seasons between 2019 and 2021, enabling assessment of their trophic niche variation and niche overlap versus prey diversity across time and space. Both fishes were revealed to have diets comprising of items including algae, ciliates, arthropods, plants and rotifers, with the extent of their niche overlap varying according to both the year and season of sampling, but being highest during the 2021 dry season. Whilst there was a positive association between prey diversity (described by the ecological opportunity) and population niche width (PNW), this was not significant, with the negative relationships between PNW and interspecific niche overlap suggesting interspecific interactions were constraining the ability of the species to increase their niche sizes during periods of limited resource diversity, and indicating the importance of resource availability in determining the extent to which invasive and native species will compete for resources. These results thus demonstrate that the dynamic nature of the trophic interactions of these fishes, indicating the importance of considering temporal and seasonal variability within invasion risk screening processes and impact assessments.

Introduction

Biological invasions are a substantial driver of environmental change and economic damage at global scales (Crystal-Ornelas and Lockwood 2020, Haubrock et al. 2021, Leroy et al. 2022), with freshwater ecosystems being especially susceptible to invasion (Gallardo et al. 2016). Ecological impacts from freshwater invaders include the adverse consequences of elevated inter-specific competition that can result from the invading population (Gozlan et al. 2010). The strength and direction of ecological impacts from elevated competition is then dependent on the extent to which the invader and native species exploit similar prey resources, coupled with prey resource availability (Tran et al. 2015, Jackson et al. 2016). High convergence in resource use of native species and the invader on limiting prey resources could result in intense asymmetric competition, where the weaker competitor will potentially be competitively excluded from their trophic niche through having to exploit alternative prey to maintain their energetic requirements (Huxel 1999, Britton et al. 2018, Britton 2022).

The range of prey resources exploited by a consumer species can be described through their trophic niche width (Roughgarden 1972), where the diversity of available prey resources that could contribute to the trophic niche is referred to as the ecological opportunity (Sánchez-Hernández et al. 2021). The ecological opportunity of a population has been assumed to be constrained by its competitors (Van Valen 1965), but is also affected by the effects of seasonality in rivers (e.g. differences in prey availability in wet versus dry and summer versus winter) (Xenopoulos and Lodge 2006, Wang et al. 2009, Costa-Pereira et al. 2017). The extent to which two competitior populations can coexist is also influenced by the extent of their niche overlap, where in periods of low prey availability, increaed sharing of resources could result in detrimental conseqeucnes for one or both the populaions (Chesson 2000, Chesson 2018, Pastore et al. 2021). Conversely, periods of increased ecological opportunity could reduce the extent of interspecific niche overlap (Pastore et al. 2021) and/ or enable trophic niche expansion (Van Valen 1965, Sjodin et al. 2018, Herrmann et al. 2021). The resultant increase in population trophic niche width (PNW) can arise either by an expansion in individual trophic niche width (INW) or individual specialisation (IS). For INW (the within-individual component (WIC) of PNW), all individuals became more generalized in their resource use, while for IS, niche width increases as specialization increases the extent of inter-individual niche partitioning (affecting the between individual component (BIC) of PNW) (Roughgarden 1972, Sjodin et al. 2018, Baker et al. 2022). The niche variation hypothesis predicts that population niche expansion is achieved mainly through individual specialization, irrespective of variation in the WIC (Van Valen 1965, Maldonado et al. 2017, Sjodin et al. 2018), with this having some empirical support (Bolnick et al. 2007, Costa et al. 2008, Maldonado et al. 2017, Costa et al. 2023).

Testing how the availability of prey resources (as diversity and/ or abundance) affects trophic niche sizes and overlaps of co-existing fishes can take advantage of freshwaters invaded by non-native fish. This is because these freshwater systems provide scenarios for studying novel trophic interactions through the presence of the invasive fish within a food web otherwise comprised of native species; these relatively large-bodied consumer species are also able to be sampled with some ease, coupled with well recognised methods for describing their diet composition (Britton 2018). In the Pearl River basin, southern China, the introduction in 1982 of the non-native mrigal carp *Cirrhinus mrigala* and its subsequent invasion has resulted in its co-existence with its native congener mud carp *Cirrhinus molitorella* across a wide geographical range (Yu et al. 2019). Both species are ilyophagous, with the combination of their feeding in soft sediments and sharing of similar habitats raising concerns over their extent of resource sharing. Indeed, while mud carp is a dominant native fish species in freshwaters across southern China (Bowen et al. 2006, Zhang et al. 2020), the mrigal carp has become an abundant invader (Yu et al. 2019). As the specific dietary composition of these co-existing species has yet to be fully understood then they provide the opportunity for investigating their competitive interactions via testing effects of ecological opportunity on PNW and interspecific niche overlap, and PNW in relation to changes in INW and BIC. With testing the diet composition and trophic interactions of ilyophagous fishes being challenging and often resulting in taxonomic ambiguity (Bowen et al. 2006), the development of DNA metabarcoding now provides a powerful tool for more precise dietary analyses (Roslin and Majaneva 2016, Ando et al. 2022, Villsen et al. 2022b). Its application relies on the relative read abundance (Deagle et al. 2019) and the standardised DNA-based diversity analysis (Alberdi and Gilbert 2019), and it has been applied to a wide variety of taxa, including fish (Villsen et al. 2022a, Villsen et al. 2022b), shrimp (Siegenthaler et al. 2019), bat (Alberdi et al. 2020), and rodents (Lopes et al. 2020, Shaner and Ke 2022).

Accordingly, DNA metabarcoding is applied here to reveal the trophic interactions of native mud carp and invasive mrigal carp across the Pearl River basin during both wet and dry seasons between 2019 and 2021. By combining the metabarcoding with eDNA analysis of water samples, fish diet composition was also tested in relation to the available prey (i.e. the ecological opportunity) (Wang et al. 2019, Carraro et al. 2020). The metabarcoding results also enabled testing of the trophic niche overlap between the two fishes, determination of how WIC and BIC vary for each species in relation to PNW (i.e. whether increased PNW is through increased individual specialization or generalization), and how this was affected by individual fish condition. Finally, the results of these analyses were used to assess the management implications regarding both invasion risk screening and ecological impact assessment.

Materials and Methods

Fish and water sample collection

Samples of mud carp and mrigal carp were collected in dry (November 2019, January 2021) and wet seasons (July/September 2019, July 2021) in the West River, North River, East River and the central delta of the Pearl River, China (Fig. 1). To provide representative samples across the length range of fish present, fish were captured using a combination of gill nets, cage traps, and cast nets following Zhang et al. (2022b). Following their capture, fish were euthanized using an anaesthetic overdose, held on ice, and transported to the laboratory, where they were identified to species and measured (standard length, nearest mm; total weight 0.1 g), before their gut was dissected. Where the gut was > 50 % full, the gut contents were collected, with the contents of the fore-, mid- and hind-gut removed by applying gentle pressure on the gut. During each sampling event, 1 litre of river water was also collected (from ≤ 1 m from the riverbed), which was filtered using precombusted Whatman® glass fibre filter (GFF; diameter: 47 mm; pore size: 0.7 µm) and then used for environmental DNA (eDNA) detection. The GFF had been combusted for 4 hours at 450 °C before filtering. Both the extraction of gut contents and filtering of water filtration was completed in sterile conditions to avoid contamination, with all samples then frozen in liquid nitrogen. The water temperature at the bottom ≤ 1 m from the riverbed was measured using the Aqua TROLL 400 instrument (In-Situ®, Fort Collins, USA). Daily river discharge gauged by the hydrometric stations nearby the sampling sites were collected from the national water regime and precipitation website. Before statistical analysis, daily discharge was averaged during the period from one week prior to the sampling date until one week after.

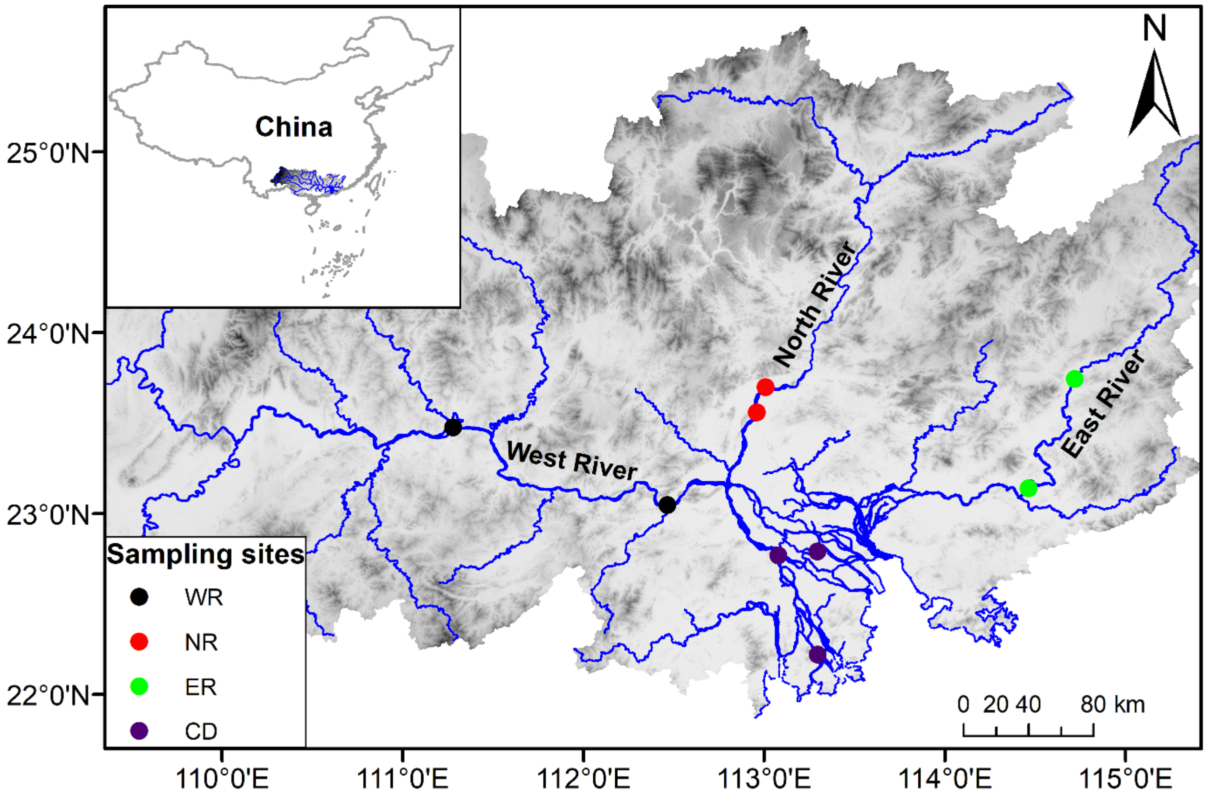

Fig. 1

Sampling sites in the West River (WR), North River (NR), East River (ER) and the central delta (CD) of the Pearl River, China.

Metabarcoding

Total genomic DNA of the gut contents for each individual and the eDNA from the filtered water were extracted using the DP812 TGuide S96 Magnetic Soil/Stool DNA Kit (TIANGEN®, Beijing, China). The V4 region of the 18S rDNA was amplified using the primer with barcode TAReuk454FWD1 (5'-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3') and TAReukREV3 (5'-ACTTTCGTTCTTGAT(C/T)(A/G)A-3') (Stoeck et al. 2010). All PCR products were purified, quantified and homogenized to get a sequence library. The library concentration was quantified using Qubit 3.0 (Invitrogen, Carlsbad, USA) and the quality control were performed by Qsep 400 fragment analyser (BiOptic, Taiwan, China). The criterion of qualified library is a concentration > 1 ng/µl in a range of 380 bp ~ 630 bp fragments. Qualified libraries were then sequenced on Illumina Novaseq 6000.

Raw data of paired sequences were demultiplexed and denoised using DADA2 methods following the workflows in QIIME2 (Bolyen et al. 2019). The features produced by denoising are amplicon sequence variant (ASV). The ASVs with reads number < 10 or being present in only a single sample were removed as these could have resulted from contamination. The taxonomy of the ASV was classified using a pre-trained Naive Bayes classifier of the Silva v138 database (Robeson et al. 2020). The ASVs with taxonomy confidence ≥ 0.85 and their relative read abundance (i.e. their proportional representation) were used in subsequent data analyses. To avoid the contamination of the mud carp/mrigal carp DNA in the gut content DNA, ASVs were removed that were assigned under the order of Teleostei. The unassigned ASVs or ASVs that were assigned to the kingdom of bacteria, the phylum of Acanthocephala, Apicomplexa, Nematozoa or Platyhelminthes (parasites), were also removed, as they were the non-targeted taxa.

Statistical analysis

To assess spatial and temporal differences in ecological opportunity and diet composition between the two fishes, the permutational multivariate analysis of variance (PERMANOVA) (Anderson 2017) was applied. It was conducted on the Bray-Curtis distance matrix of organism composition in the river and individual dietary compositions after applying a Hellinger transformation to the relative read abundance of the ASVs. The PERMANOVA was completed using the *adonis2* function in the `vegan` package, with 999 permutations. To identify which taxa contributed most to the differences between groups (e.g. between the fishes, and fish versus water), an indicator species analysis was applied using the *signassoc* function in the `indicspecies` package in R (Cáceres and Legendre 2009). This analysis tested the null hypothesis that the relative abundance of one taxa in samples from one group is not greater than those in samples of the other (Cáceres and Legendre 2009).

Niche width is measured as the diversity of prey items consumed (Bolnick et al. 2007) and was quantified as INW (WIC), BIC, and PNW. The INW, related to α diversity, was estimated as the Hill number with $q = 1$ (^1D) using the `hilldiv` package (Alberdi and Gilbert

2019). The ^1D in the sampling water were also calculated as an indicator of ‘ecological opportunity’ (i.e. the extent of prey available for ingestion). Spatial and temporal differences in ^1D (positive continuous data) was analysed by fitting generalized linear models (GLM) in Gamma distribution using `lme4` package. In the model, the terms of sampling region, site, year, season, sampling sources and their interaction terms were set as fixed factors. Difference in ^1D between groups were determined using the *emmeans* function in `emmeans` package (Lenth 2021). The WIC, BIC (related to β diversity) and PNW (related to γ diversity) were estimated for each population with ≥ 2 individuals captured and analysed during each sampling campaign (specified to site and date). The WIC was calculated as mean INW among individuals for each population. The BIC was firstly estimated as the average of the pairwise individual dissimilarities for each population based on ^1D (BIC_D) using the *pair_dis* function in `hilldiv` package (Alberdi and Gilbert 2019). Based on the individual specialization index (IS) proposed by Bolnick et al. (2002), the BIC was secondly estimated as individual specialization (BIC_V) and calculated as $BIC_V = 1 – IS$ following Bolnick et al. (2007). IS was calculated using the *PSicalc* function in the `RInSp` package (Bolnick et al. 2007, Zaccarelli et al. 2013). It is an average of proportional similarity between individual and population’s diet, thus lower values suggest decreasing similarity and high individual specialization (Bolnick et al. 2002). The PNW was calculated as ^1D and standardized with different sample sizes at 90% sample coverage using the function *EstimateD* from the package `iNEXT` (Hsieh et al. 2016). Spatiotemporal and interspecific variation in PNW, BIC_D, and BIC_V were then analysed using linear models. Interspecific niche overlaps were calculated at both individual and population levels as the Sørensen-type overlap by running the *pair_dis* function in `hilldiv` package

(Alberdi and Gilbert 2019). At the individual level, niche overlap was averaged across individual pairs to derive an average value for each individual. Spatial and temporal variation in niche overlap was then analysed using linear models.

Effects of river discharge and water temperature on ecological opportunity, then on interspecific niche overlap, WIC, BIC, and PNW were analysed by structural equations modelling (SEM). To minimise the difference in the magnitude of variances between variables, river discharge, ecological opportunity, WIC, and PNW were natural log transformed before testing. Due to the significant positive correlations between river discharge and water temperature (Pearson's correlation: 0.44; $P = 0.01$), and between BIC_D and BIC_V (Pearson's correlation = 0.86, $P < 0.001$), river discharge and BIC_D were retained but water temperature or BIC_V were removed for the SEM. The SEM was performed in the `lavaan` (Rosseel 2012) and `lavaanPlot` packages in R, where model fit was assessed using a combination of Chi-square tests ($P > 0.05$ indicated the absence of significant discrepancy between data and model), the comparative fit index (CFI; 0.90 – 1, good fit; < 0.90, poor fit), and the root mean square error of approximation (RMSEA; ≤ 0.05, close fit; ≥ 0.08, poor fit) (Rosseel 2012). The relationships between ecological opportunity, inter-specific niche overlap and PNW were further analysed by building linear models. The niche variation hypothesis was also tested by regressing WIC, BIC_D, and BIC_V respectively on PNW and species in linear models. Pairwise Pearson correlations among ecological opportunity, inter-specific niche overlap, WIC, BIC_D, BIC_V and PNW were also estimated. The normality and the homogeneity in variance of the residuals of linear models, and GLM models were checked using the `DHARMa` package (Hartig

2022). The importance of fixed factors in these models was determined using the individual marginal R^2 (i.e. the variance explained the individual fixed factor) by running the `glmm.hp` package (Lai et al. 2022). All calculations and statistical analyses were completed in R 4.2.3 (R Core Team 2023).

Results

Fish diet composition and ecological opportunity

A total of 188 samples were analysed (98 mud carp, 71 mrigal carp and 19 water samples; Table S1). Across these samples, there were 3722 ASVs retained that were classified into 55 phyla, 109 classes, 160 orders, 188 families and 296 genera. The ASVs indicated that mud carp and mrigal carp had broad diets that mainly comprised of algae, ciliates, arthropods, plants, rotifers and fungi (Fig. 2; Fig. S1). These phyla contributed to accumulated read abundances of consistently ≥ 0.79 in the fish diets during each sampling campaign (Fig. 2), while they contributed on average of 0.59 (range 0.34 – 0.85) in the water samples..

Across the fish and water samples, PERMANOVA indicated that the largest variation in taxa composition was the sample source (i.e. fish vs water), followed by the nested sampling season, site, region and year (Table 1). Comparisons of fish diet composition versus the water samples indicated that Rotifera were significantly more abundant in mud carp diets, Phragmoplastophyta were significantly more abundant in mrigal carp diets, but with Cryptophyceae, Mollusca, Chytridiomycota, and Protalveolata being more abundant in the water (Table S3, S4). Differences in diet composition between the two fishes were mostly explained by the nested sampling season, followed by the nested sampling site and fish length (PERMANOVA; Table S5). During the wet season in each site, there were higher relative abundances of Ciliophora, Dinoflagellata, Ascomycota, and Tardigrada in fish diets, while in the dry season there were more Arthropoda and Vertebrata (Table S6).

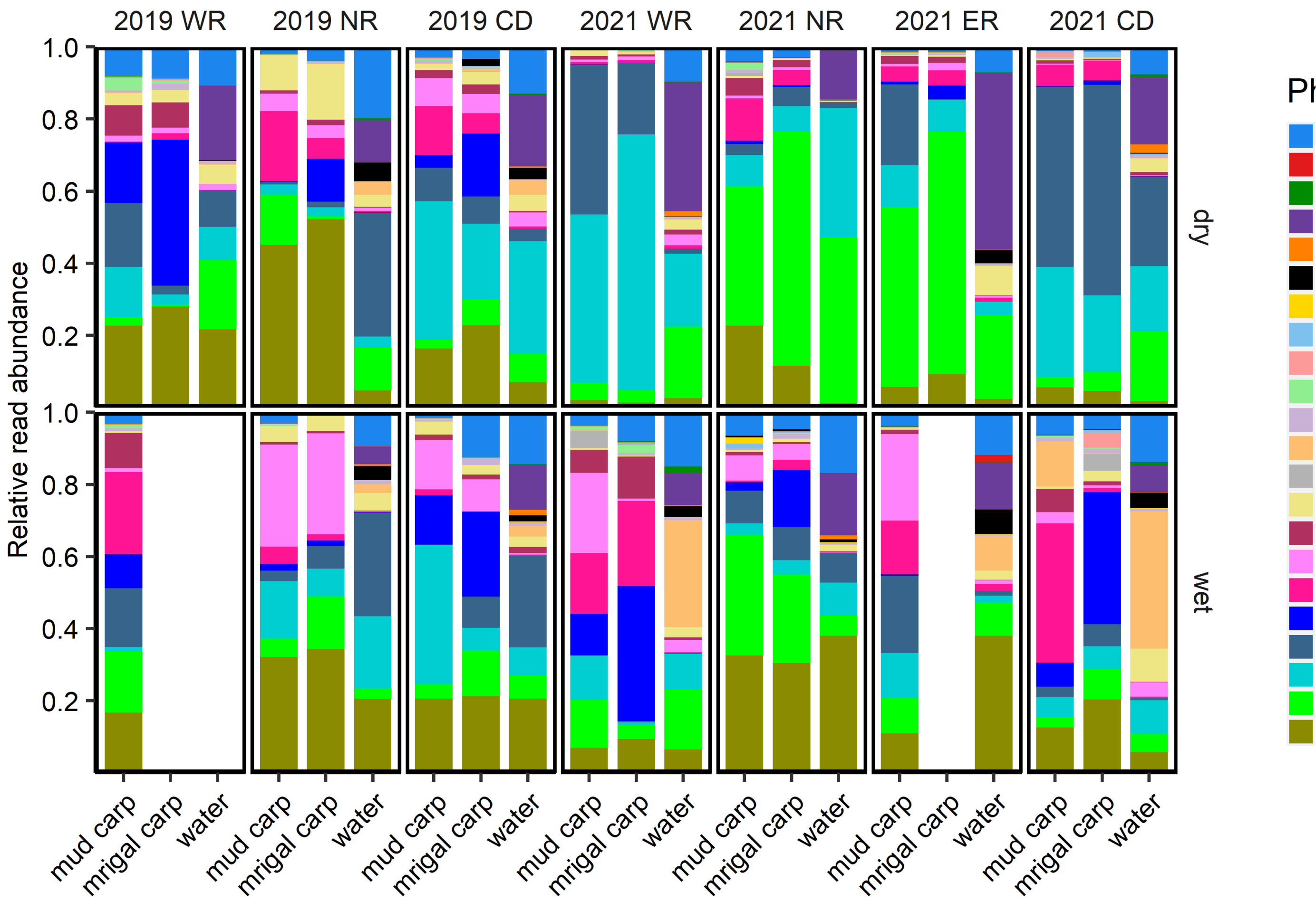

Fig. 2. Relative abundance of taxonomy at the phylum level in the gut contents of mrigal carp and mud carp, and the water sampled during the dry season and the wet season of 2019 and 2021 in the West River (WR), North River (NR), East River (ER), and the central delta (CD) of the Pearl River. Taxonomy with low relative abundance (< 0.015) were categorized as "Others".

Table 1. Permutational multivariate analysis of variance (PERMANOVA) results on variation of organism composition across sampling region, year, season, and sampling sources.

	df	Sum Sqs	R^2	F	*P* (>F)
region	3	3.85	0.08	10.71	<0.001 ***
region: site	5	5.00	0.11	8.35	<0.001 ***
region: site: year	3	2.94	0.06	8.17	<0.001 ***
region: site: year: season	11	8.01	0.17	6.08	<0.001 ***
region: site: year: season: source	38	12.62	0.27	2.77	<0.001 ***
Residual	127	15.21	0.32		
Total	187	47.62	1.00		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Spatiotemporal variation in trophic niche metrics

Differences in individual trophic niche width (INW) of mud carp and mrigal carp, and ecological opportunity were mostly significantly driven by sampling source, and the interaction between year and season (Table 2; Table S7). For both fishes, the INW was generally lower than the ecological opportunity provided by river water (mud carp INW: 23.2 ± 13.8; mrigal carp INW: 23.1 ± 13.4; river water: 51.6 ± 32.1) (Fig. 3; Table 2, S8). Between the two fishes, INW did not differ significantly in general ($t = 0.01$, $P = 0.996$), or in 11 of 12 samplings across regions, years, and seasons ($P > 0.05$; Table S8). The INW and ecological opportunity were generally higher in the wet season than in the dry season Table 2), with the seasonal difference larger and of higher significance in 2021 than in 2019 (Fig. S2; Table S9).

For population niche width (PNW), no significant interspecific difference was detected, with the interaction of year and season explaining most variation across the samples (LMM; Table S10-11). PNW was significantly lower in the dry than in the wet season of 2021, while no significant seasonal differences in 2019 were detected (Fig. S2). Variation in BIC_D and BIC_V were not significantly influenced by spatiotemporal variation and species (Table S11), despite the interaction between year and season explaining most of the variation (Table S10).

Mud carp and mrigal carp had varying interspecific trophic niche overlaps at both population (mean: 0.56 ± 0.22; range: 0.09 - 0.86) and individual levels (mean: 0.42 ± 0.19; range: 0.04 - 0.96). Variation in interspecific overlaps at both individual and population levels was mostly explained by the interaction of year and season (Table 3; Table S12). In 2021, niche overlap was higher in the dry season than in the wet season (population level: $P = 0.06$, individual level: $P < 0.05$), whereas there was no significant seasonal difference in niche overlap in 2021 (Fig. 4; Fig. S2). The nested sites in region also significantly contributed to variation in individual niche overlap (Table S12; Fig. S3).

Mechanisms underlying interspecific niche partitioning and population niche expansion

The structural equation model was a good fit to the data (Chi-square test ($P = 0.63$), CFI = 1 and RMSEA = 0.00), with the results suggesting a causal path from river discharge to PNW (Fig. 5). Increasing river discharge and water temperatures led to significant increases in ecological opportunity, which then resulted in significant decreases in interspecific niche overlap. This decrease in interspecific niche overlap drove a significant increase in BIC and non-significant increase in WIC, with both WIC and BIC contributing positively to the larger PNW (Fig. 5).

The nonsignificant positive correlations between ecological opportunity and PNW (Fig. 6a, Table S13), and the components of PNW (WIC, BIC_D, and BIC_V in Fig. S4), indicated that increasing ecological opportunity did not contribute increased PNW. The significant negative linear relationships between ecological opportunity and interspecific niche overlap does suggest the important role that increasing ecological opportunity has on interspecific niche partitioning (Fig. 6b, Table S13),. Interspecific niche overlap was not significantly correlated with PNW (Fig. 6c, Table S13). Although WIC, BIC_D, and BIC_V had positive linear relationships with PNW between fishes, these were not significant (Fig. 6d-f; Table S13).

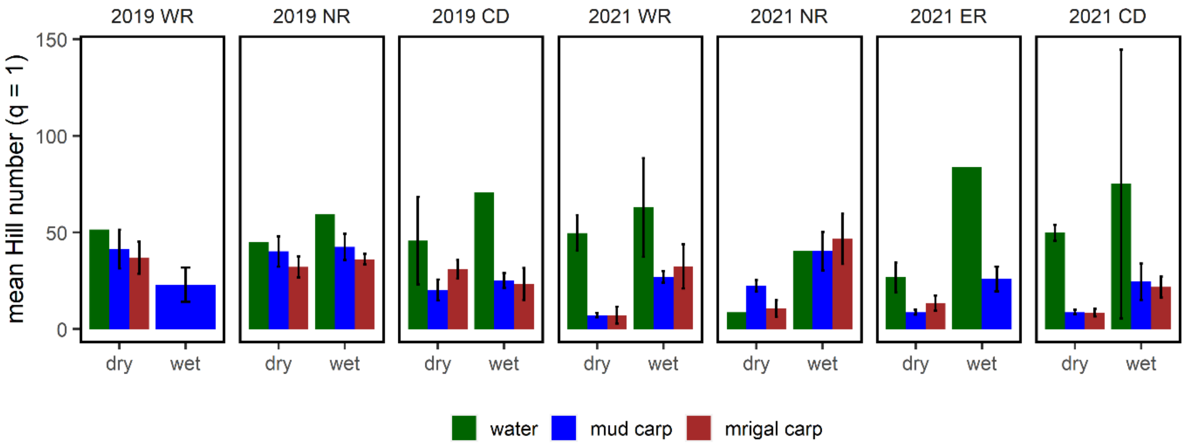

Fig. 3. The average value (mean ± SE) of Hill number (q = 1) in the river water, mud carp gut content, and mrigal carp gut content over dry and wet seasons in 2019 and 2021 across the West River (WR), North River (NR), East River (ER), and the central delta (CD).

Table 2. Effects of region, site, year, season, sampling source on variation in Hill number (q = 1).

Predictor	Estimate	Std. Error	t value	*P* (>|t|)
(Intercept)	3.52	0.13	27.10	<0.001***
region NR	0.13	0.17	0.74	0.46
region ER	-0.61	0.22	-2.81	<0.01**
region CD	-0.47	0.16	-3.06	<0.01**
region:site CD lanhe	0.26	0.19	1.38	0.17
region:site CD zuotan	0.37	0.16	2.36	0.02*
region:site ER heyuan	0.89	0.24	3.69	<0.001***
region:site NR qingyuan	0.20	0.21	0.94	0.35
region:site WR wuzhou	-0.32	0.21	-1.57	0.12
year 2021	-0.12	0.16	-0.75	0.45
season wet season	0.90	0.12	7.28	<0.001***
year:season 2019 wet	-1.00	0.19	-5.38	<0.001***
source mud carp	-1.03	0.15	-7.03	<0.001***
source mrigal carp	-1.04	0.15	-6.90	<0.001***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

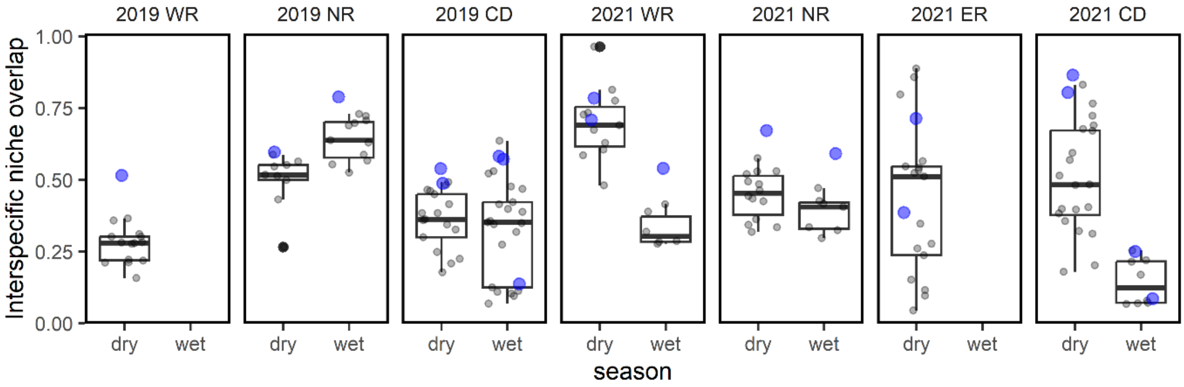

Fig. 4. Interspecific trophic niche overlap at individual level (small grey dots) and population level (big blue dots) over dry and wet seasons in 2019 and 2021 across the West River (WR), North River (NR), East River (ER) and the central delta (CD).

Table 3. Dependence of interspecific niche overlap at population and individual levels on region, year, season, and their interaction suggested by analysis of variance.

Response	Predictor	Df	Sum Sq	Mean Sq	F value	*P*
Population niche overlap	region	3	0.12	0.04	1.15	0.39
	year	1	0.01	0.01	0.15	0.71
	season	1	0.17	0.17	4.72	0.07
	region: site	5	0.14	0.03	0.81	0.58
	year: season	1	0.17	0.17	4.68	0.07
	Residuals	7	0.25	0.04		
Individual niche overlap	region	3	0.47	0.16	7.51	<0.001***
	year	1	0.08	0.08	3.81	0.05
	season	1	0.17	0.17	8.02	<0.01**
	species	1	0.03	0.03	1.48	0.23
	region: site	5	1.35	0.27	12.99	<0.001***
	year: season	1	0.80	0.80	38.48	<0.001***
	Residuals	142	2.94	0.02		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

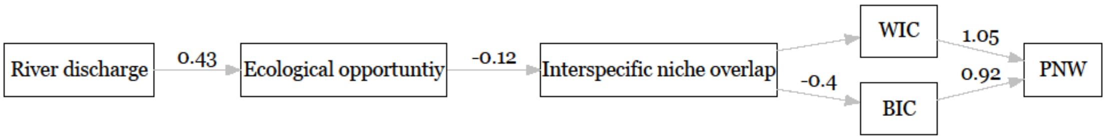

Fig. 5. Suggested pathway describing how variation in river discharge contributes to differences in population niche width (PNW), through changes in PNW's within individual components (WIC) and between individual components (BIC). The s regression coefficients with $P < 0.05$ as displayed.

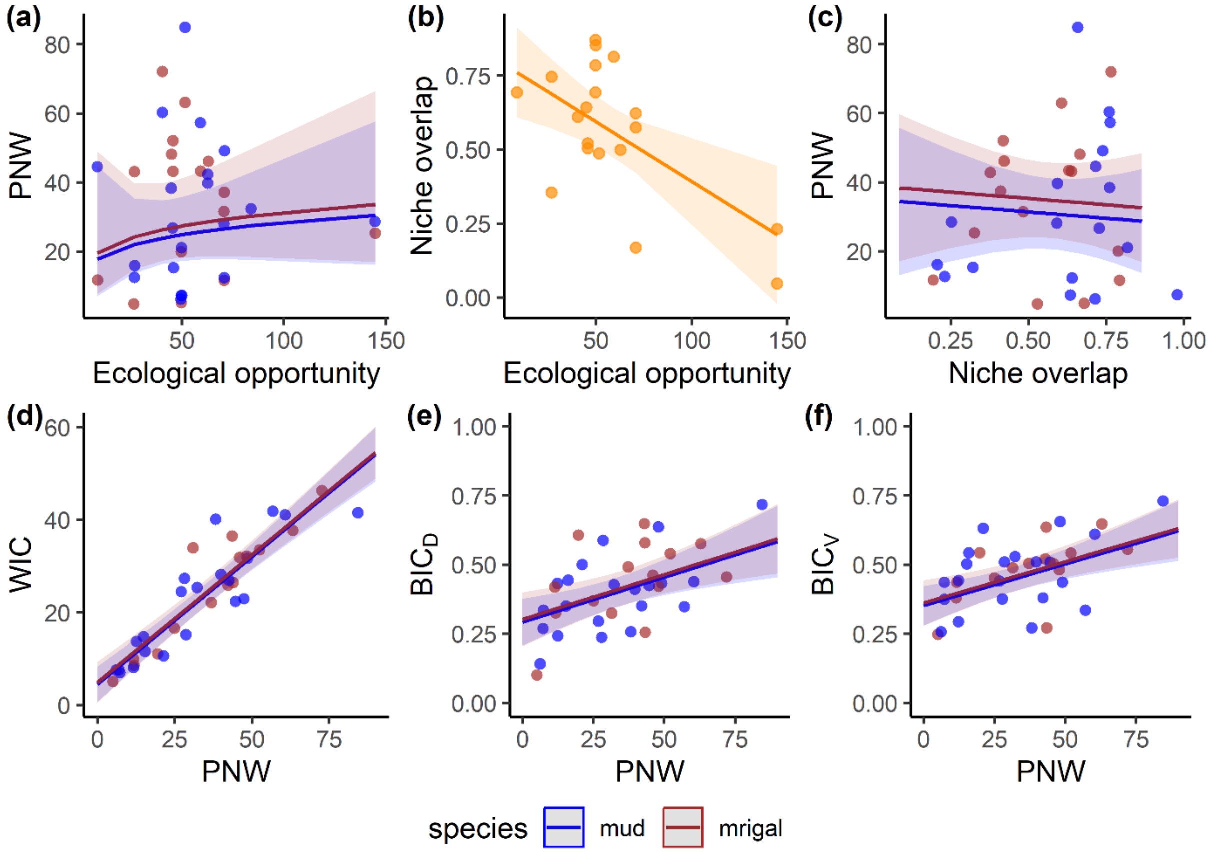

Fig. 6. Associations between ecological opportunity, population niche width (PNW) and inter-specific niche overlap (a-c) and mechanisms underlying PNW expansions (d-f) for mud carp (mud) and mrigal carp (mrigal). WIC: within-individual component; BIC_D, between individual component based on Hill number (q=1); BIC_V: BIC calculated from individual specialization.

Discussion

This assessment of the trophic relationships of invasive migral carp and native mud carp across two years and seasons for the three main tributaries of the Pearl River and its central delta using 18S DNA metabarcoding revealed differences in the fish diets from the total prey available as indicated by the eDNA, and some distinct temporal patterns between the diets of the two fishes. The interaction of year and season was the most important predictor driving variation in the dietary composition and trophic niche variation of the two fishes, with the ecological opportunity (which increased with increasing river discharge and water temperature) significantly influencing the extent of interspecific niche overlap, and was positively associated with PNW and its components (WIC and BIC). For each species, the increased PNW resulted from increasing in its components of WIC and BIC.

Spatiotemporal variation in dietary composition and niche metrics

The results relating to the taxonomy and abundance of prey for mud carp and mrigal carp in the Pearl River were novel, given dietary studies on these ilyophagous fishes usually provide limited taxonomic detail on prey (e.g. Bowen et al. 2006). The DNA metabarcoding results indicated that both species were mainly feeding on algae, ciliates, arthropods, plants, rotifers, and fungi, with their diets not only differing significantly from the organism composition of the river water, but also between the two species. For example, mud carp diet fed more on Rotifera while mrigal carp fed more on Phragmoplastophyta. However, there was no significant interspecific differences in either PNW or its components of WIC and BIC.

The interaction between year and season significantly influenced the fish diet composition, the INW, PNW and interspecific niche overlap, along with the ecological opportunity, with significantly higher seasonal differences in 2021 versus 2019. This could be due to an artefact of sampling, given differences in the sampling months in the dry season that potentially affected prey availability (November 2019 vs January 2021). For example, river discharge rates, sediment loadings and water temperature are lowest in January, whereas in November they are still relatively high as they remain affected by the wet season that lasts between July and September (Liu et al. 2022). The resulting differing influences of the wet season between the dry season sampling periods and 2019 and 2021 would thus result in some differences in the ecological opportunity of this fishes via phytoplankton biomass (Townsend and Douglas 2017) and aquatic insect resource pulses (Anderson et al. 2019). In January, the lower discharge which could also have suppressed the available habitat area e for the fishes by decreasing the drainage area and ecosystem size (Sabo et al. 2010), which when coupled with the lower ecological opportunity, could have been the driver of the elevated interspecific competition for resources, as indicated by the higher niche overlap (Porter and Dueser 1982, Shaner and Ke 2022; Zhang et al. 2022a).

Mechanisms underlying niche variation and interspecific niche partitioning

The ecological opportunity, being influenced by river discharge and water temperature fluctuations, had positive but non-significant influences on PNW and its components, suggested there might have been some effects of prey availability and diversity on changes in population niche sizes (Van Valen 1965, Costa-Pereira et al. 2019, Herrmann et al. 2021). The negative relationships between PNW (or its components) and niche overlap indicated the constraining effects of interspecific competition on PNW, where PNW and its components increased following a reduction in interspecific competition (the phenomenon of ecological release; Herrmann et al. 2021). Their association also suggested the consequences of population niche expansion on interspecific niche partitioning.

The positive association of PNW with its components of WIC, BIC_D, and BIC_V, suggested the importance of both individual niche expansion and individual specialization in population niche expansion. This is a dual expansion case of the niche variation hypothesis (Sjodin et al. 2018), which is also found in brook trout in lakes by Baker et al. (2022), despite strict individual specialization without individual generalization being commonly treated as the only realization of the niche variation hypothesis (Sjodin et al. 2018). Accordingly, the increase in WIC and PNW driven by the addition of new prey items to diets at individual and population levels, indicated that individuals possess substantial abilities to utilize alternative resources (Toscano et al. 2016, Costa-Pereira et al. 2019). However, there is a cost of individual generalization, i.e. a resource-acquisition trade-off between individual niche width and total consumption rate, which determined the ways of population niche expansion by switching from individual generalization when the cost is low to individual specialization when the cost is high (Sjodin et al. 2018). The increases in both WIC and BIC when PNW increased for the fishes suggested that the cost of individual generalization had driven individual specialization, but this was not high enough to constrain individual generalization. Conversely, individual specialization is not solely dependent on interspecific competition and ecological opportunity (Costa-Pereira et al. 2018), which is consistent with the correlation between the BIC_D/BIC_V, ecological opportunity, and interspecific niche overlap, but also on intraspecific competition (which was not addressed here).

The ecological opportunity also had strong influences on interspecific niche overlap between mud carp and mrigal carp. Under this influence, the niche overlap was higher under more limited prey availability, which can result in elevated interspecific competition (Shaner and Ke 2022) and competitive exclusion, with the overlap reducing as prey resource diversity increased, which can lead to enhanced probability of co-existence (Pastore et al. 2021). Although, we did not directly measure competitive differences, the other components determining species coexistence in modern coexistence theory suggested no significant effects of ecological opportunity on competitive differences (Chesson 2000, 2018; Pastore et al. 2021). Thus, in environments with varying ecological opportunity, niche overlap (rather than the competitive difference) was considered as likely playing a key role in driving the interspecific interactions of the two fishes here.

Management considerations

The results here highlight some important considerations for how invasive fishes can be managed in sub-tropical rivers. The results indicated that the extent of resource sharing between the two fishes was temporally variable and, although assessment was not made on how this resource sharing translated into ecological consequences for the fishes, increased resource sharing generally indicates elevated competition and the potential for detrimental impacts in at least one species (e.g. decreased growth rates; Britton 2018, 2022 and reproductive interference; Tarkan et al. 2012). That the extent of resource sharing was temporally variable, being driven primarily by year and season, indicates that impact assessments of invasive fishes in similar systems must consider seasonality in prey availability as a factor driving competitive interactions, with wet season likely to have reduced competitive interactions versus dry season due to higher prey availability. Thus, in considering both pre-introduction risk screening (e.g. Vilizzi et al. 2021) and post-introduction management decision-making (e.g. Britton et al. 2022), it must be considered that any impacts from species such as mrigal carp are not constant, but are dynamic and context dependent according to the differences in the seasonal availability of prey resources, with impacts likely to be elevated in dry seasons when availability is lower. Moreover, with mrigal carp being ilyophagous, it is unclear as to whether their invasive populations will be a driver of prey availability or be limited by the available prey in substrata, with this aspect of their invasion ecology requiring further work.

In summary, this work has generated novel insights into the niche variation and interspecific niche overlap of invasive mrigal carp and native mud carp across an extensive geographical range in the Pearl River, southern China. Through the demonstration that the trophic niche overlap of the two species were not fixed but dynamic according to seasonal variability in prey availability then the results demonstrate the importance of considering the temporal sampling of fish and prey resources within invasion risk and impact assessment processes.

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