

Original Research Articles

Otolith Morphology Reveals Population Connectivity of *Siniperca kneri* (Garman, 1912) in the Xijiang River

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Morphological and morphometric analyses of otoliths are widely used for intraspecific comparisons and fish stock identification. This study examines the stock structure of *Siniperca kneri* (Garman, 1912), an endemic and economically important carnivorous fish in China, using otolith shape analysis in the Xijiang River. Specimens were collected from three sites in the middle and upper reaches of the river. Traditional shape indices and elliptic Fourier descriptors were used to evaluate otolith morphology after statistically removing the effect of fish size (allometry). After applying corrections for multiple comparisons, no significant differences were found in any of the eight shape indices among groups. Canonical analysis of principal coordinates (CAP) based on elliptic Fourier coefficients also revealed no clear morphological differentiation among the sites. The initial discriminant analysis yielded a raw classification success rate of 61.1%, but a more robust leave-one-out cross-validation reduced the accuracy to 55.6%, indicating substantial morphological overlap. These results suggest that *S. kneri* in the study area likely constitutes a single phenotypic stock with limited spatial structuring. The absence of strong stock differentiation may be attributed to relatively homogeneous environmental conditions and the potential effectiveness of fish passage facilities that could maintain ecological connectivity. However, these conclusions are tempered by the study's limitations in sample size. These findings underscore the importance of preserving river connectivity and highlight the urgent need for further studies utilizing otolith microchemistry and genetic tools to validate these findings and support effective conservation management.

INTRODUCTION

Organisms develop diverse morphological characteristics in different environments as a result of long-term adaptation.¹ This relationship between morphological variability and the environment is a key area of biological research. Otolith morphology, in particular, is widely used for comparative studies among different stocks of aquatic animals. For instance, multivariate morphometrics have been employed to analyze the shape indices of lapillus and asteriscus from nine populations of *Schizothorax o'connori*, revealing distinct stock differentiation.¹ Similarly, otolith shape indices have been used to identify species and validate the existence of *Coilia brachygnathus*.² These studies highlight the significance of otolith morphology in understanding environmental adaptation and population dynamics. Both genetic and environmental factors influence otolith morphology.^{3,4} Fish living in similar aquatic envi-

ronments often exhibit homogenized otolith morphology due to shared ecological conditions.⁵⁻⁷ Conversely, in hydrologically distinct environments, otoliths often display significant morphological variation, potentially due to differences in water temperature, flow velocity, and habitat complexity.⁸⁻¹⁰ Otoliths not only record fish age but also retain ecological information reflecting the environmental conditions experienced throughout their life history.¹¹ Otolith shape analysis, encompassing morphometric, traditional morphological, and geometric morphometric approaches, has been widely employed to identify nursery grounds, delineate fish stocks, and reconstruct life history patterns. Among the three otolith types, sagittae are often preferred in such studies due to their larger size, clear shape features, and accessibility.¹² The integration of otolith size and shape indices has been shown to enhance the resolution of stock discrimination.

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Big-eye Mandarin Fish (*Siniperca kneri*, Garman, 1912) is an economically significant, carnivorous freshwater fish endemic to China and widely distributed in the Xijiang River. Over the past few decades, the construction of large-scale hydropower and water conservancy projects along the Xijiang River has profoundly altered river connectivity and flow regimes, leading to reduced water levels, altered current velocities, and habitat fragmentation in the downstream reaches. These changes have caused a sharp decline in fishery resources, including the wild populations of *S. kneri*, which face significant pressure from both habitat degradation and overfishing. Although ecological restoration measures, such as the implementation and optimization of fish passage facilities, have been gradually introduced in recent years, their long-term effectiveness remains under evaluation.¹³ In this context, understanding how dam-induced habitat changes affect the population structure of *S. kneri* is crucial.¹⁴ Specifically, it is essential to determine whether geographical isolation caused by river fragmentation has led to population differentiation and whether there is ongoing gene flow between upstream and downstream groups.¹⁵⁻¹⁷ Maintaining the functional connectivity of the river through effective ecological corridors may help mitigate complete stock isolation.

Previous research on *S. kneri* has predominantly centered on reproductive biology, feeding ecology, and morphological variation.¹⁸ With a limited focus on stock delineation influenced by river regulation. To address this gap, we conducted an otolith shape analysis of *S. kneri* from multiple sites in the middle and upper reaches of the Xijiang River. By integrating morphological data with environmental context, this study aims to assess potential population differentiation resulting from dam-induced habitat changes, evaluate the spatial structure of fish stocks in relation to ecological connectivity, and provide a scientific basis for future conservation and fisheries management strategies.¹⁹

MATERIALS AND METHODS

STUDY AREA AND SAMPLE COLLECTION

Fish were captured by local fishermen using gillnets in the middle and upper reaches of the Xijiang River in November 2022 (Figure 1). Efforts were made to ensure that fish obtained from each location were from the same age/cohort by selecting individuals with similar lengths. The fish were stored in ice before being transported to the laboratory for processing. Each sample was measured for length (L, 0.01 cm) and weight (W, 0.1 g). Sagittal otoliths were then extracted using plastic forceps, washed, and cleaned in distilled water before being dried. Only sagittae with no damage or crystallization were used in this study. Age determination was based on the analysis of sagittae, which were read twice by experienced readers. If the two readings differed, a third reading was performed to ensure consistency. The coefficient of variation (CV) was calculated to assess the precision of age readings. Detailed information

on the age, CV, sample size, and length-weight data from the three sampling sites is provided in Table 1.

IMAGE ACQUISITION

Left sagittae were placed with the distal surface down, showing the sulcus up and the rostrum pointing to the right. Digital images of otoliths were captured using a stereomicroscope (Motic SM171, Motic, China) with a digital camera (MotiCam S6, Motic, China). Images were captured at a resolution of 1280 × 1024 pixels and saved as TIFF files. To ensure accurate measurements, a scale bar was included in each image for subsequent calibration (scaling). High-contrast digital images were obtained using reflected light against a dark background.

OTOLITH MORPHOMETRY

Otolith shape indices were calculated for each sagitta using digital images processed with Image-Pro Plus software. The otolith outline was automatically extracted by converting the image to a binary format through thresholding, effectively separating the bright otolith from the dark background. As a critical quality control step, each automatically generated outline was visually inspected and manually corrected or excluded if it was inaccurately traced due to imaging artifacts or uneven lighting, thereby ensuring the reliability of the morphological data. Morphometric measurements, including otolith area (A), perimeter (P), feret length (FL), feret width (FW), maximum feret length (Fmax), minimum feret length (Fmin), maximum radius (Rmax), and minimum radius (Rmin), were used to calculate eight shape indices: roundness (RO), format-factor (FO), circularity (CI), rectangularity (RE), ellipticity (EL), radius ratio (RA), feret ratio (FE), and aspect ratio (AS) (Table 2).

STATISTICAL ANALYSIS

All statistical analyses were performed using R (V.4.2.2) and SPSS (V.26.0), with a significance level (α) of 0.05. Data are presented as Mean ± Standard Deviation (Mean ± SD). Allometric correction: Otolith shape is often correlated with fish size (allometry). To ensure that observed differences were due to group effects rather than fish length, we corrected all eight shape indices for allometry. A linear regression was performed for each shape index against length (L). The standardized residuals from these regressions, which represent size-independent shape variation, were used for all subsequent statistical analyses.

Shape Index Analysis: The normality and homogeneity of variances for the size-corrected shape index residuals were checked using the Shapiro-Wilk and Levene's tests, respectively. For data meeting parametric assumptions, a one-way analysis of variance (ANOVA) was used. For data that did not meet these assumptions, a non-parametric Kruskal-Wallis H test was employed. To control the false discovery rate (FDR) arising from multiple comparisons across the eight indices, the Benjamini-Hochberg (BH) procedure was applied to all p-values. Principal Components

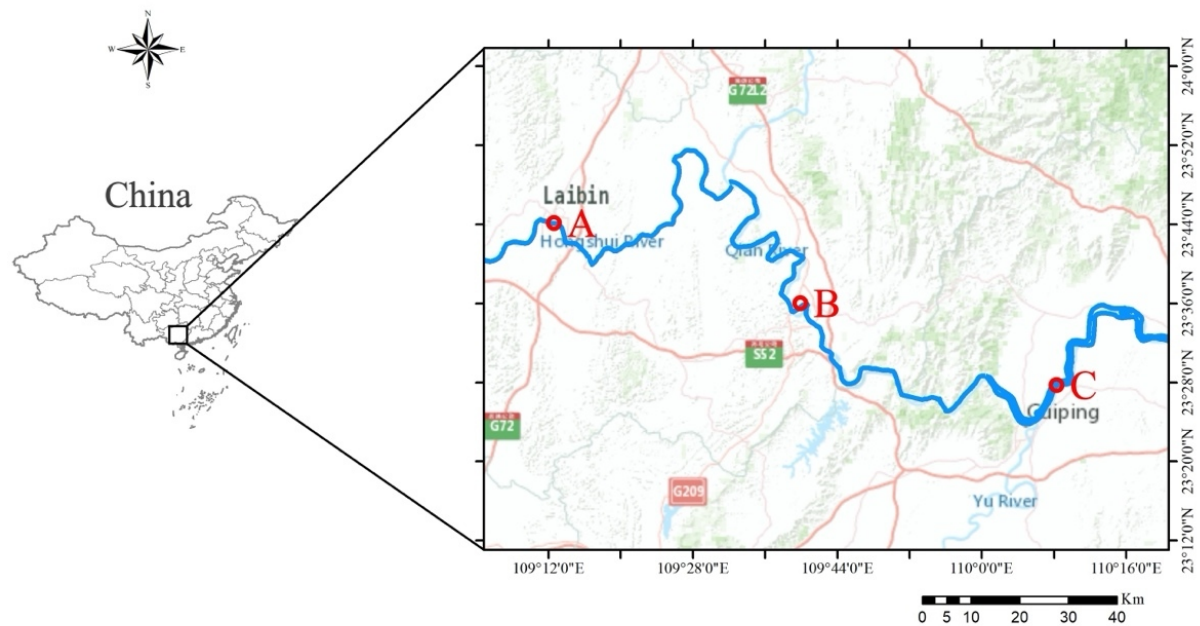


Figure 1. Map of *S. kneri* sampling areas

Table 1. Basic statistics from three sampling sites

Locations	Age (years)	CV (%)	Sample Size (n)	L (Mean±SD) cm	W (Mean±SD) g
A	3-4	5.2	17	19.63±3.38	91.21±56.8
B	3-4	4.8	23	18.32±4.65	123.44±57.62
C	3-4	4.5	14	21.41±3.44	186.67±72.05

Note: Data represent mean ± standard deviation. CV values were calculated to assess the consistency of age readings.

Table 2. Otolith shape indices calculated from the morphometric measurements

Size Parameters	Shape Indices	Formulae
Otolith area (A)	Roundness	$4A/\pi FL^{20}$
Otolith perimeter (P)	Format-factor	$4\pi A/P^2$
Otolith length (FL)	Circularity	P^2/A
Otolith width (FW)	Rectangularity	$A/(FL \times FW)$
Maximum Feret (F_{max})	Ellipticity	$(FL - FW)/(FL + FW)$
Minimum Feret (F_{min})	Radius ratio	R_{max}/R_{min}
Maximum radius (R_{max})	Feret ratio	F_{max}/F_{min}
Minimum radius (R_{min})	Aspect ratio	FL/FW

Note: Units are mm for P, FL, FW, Radius, and mm² for A

Analysis (PCA) was used to reduce the dimensionality of the shape indices and visualize overall morphological patterns. A linear discriminant analysis (LDA) was performed to assess the classification success among the three sites. To obtain a robust estimate of classification accuracy, a leave-one-out cross-validation (LOOCV) was conducted.

Elliptic Fourier Analysis (EFA): The ShapeR package in R was used to perform EFA. Otolith outlines were extracted and normalized to be invariant to size, rotation, and starting point. The outlines were described using 12 harmonics,

which were found to reconstruct the otolith contour with over 98% accuracy. A canonical analysis of principal coordinates (CAP) was applied to the matrix of Fourier coefficients to visualize the morphological differentiation among the three sites, and its significance was tested using a permutation test with the vegan package.^{21,22}

Table 3. One-way ANOVA results of different sites

Shape indices	Original p-value	Adjusted p-value
Format-factor	0.048	0.192
Circularity	0.042	0.192
Rectangularity	0.356	0.888
Radius ratio	0.762	0.888
Roundness	0.775	0.888
Ellipticity	0.857	0.888
Feret ratio	0.888	0.888
Aspect ratio	0.857	0.888

RESULTS

SHAPE INDEX ANALYSIS

ANOVA AND KRUSKAL-WALLIS H TEST ON SIZE-CORRECTED INDICES

All analyses were performed on the size-corrected residuals of the eight shape indices. After applying the Benjamini-Hochberg FDR correction, no shape index showed a significant difference among the three sites (all adjusted p-values > 0.05) (Table 3). The results indicate that once the effect of fish length is removed, there is no detectable difference in otolith shape indices among the populations from sites A, B, and C.

PRINCIPAL COMPONENTS ANALYSIS (PCA)

Principal components analysis of the eight size-corrected otolith shape indices showed that the first two principal components had eigenvalues greater than 1, explaining a cumulative 75.2% of the total variance (Table 4). A scatter plot of the first two principal components (Figure 3) shows extensive overlap among the individuals from the three sites, indicating a lack of clear separation based on overall otolith shape.

DISCRIMINANT ANALYSIS WITH CROSS-VALIDATION

Linear discriminant analysis was performed on the size-corrected shape indices. The overall raw classification rate was 61.1%. However, a more robust leave-one-out cross-validation (LOOCV) yielded an overall accuracy of 55.6% (Table 5), which is only slightly better than the random classification rate for the three groups (33.3%).²³ The confusion matrix details the classification results, showing high misclassification rates for all groups, particularly for group B. The scatter plot of the first two canonical discriminant functions (Figure 4) further illustrates the extensive overlap among the three groups, confirming the low discriminatory power of otolith shape indices.

ELLIPTIC FOURIER ANALYSIS

PERMUTATION TEST ON FOURIER COEFFICIENTS

A permutation test based on the full set of Fourier coefficients found no significant overall difference in otolith shape among the three sites (F-value = 0.747, p = 0.746) (Table 6), confirming the results from the shape indices.

MEAN SHAPE RECONSTRUCTION AND CAP ANALYSIS

Using an Elliptic Fourier Descriptor (EFD) analysis with 12 harmonics, the reconstruction of the average otolith shape for each group showed no visually discernible differences (Figure 5). The canonical analysis of principal coordinates (CAP) also failed to separate the three sites (Figure 6). The plot of the first two canonical axes, which together explained 100% of the variation among the predefined groups, showed that the mean canonical values for each site were clustered near the origin with overlapping standard errors. This result reinforces the conclusion of high morphological similarity among the three populations.

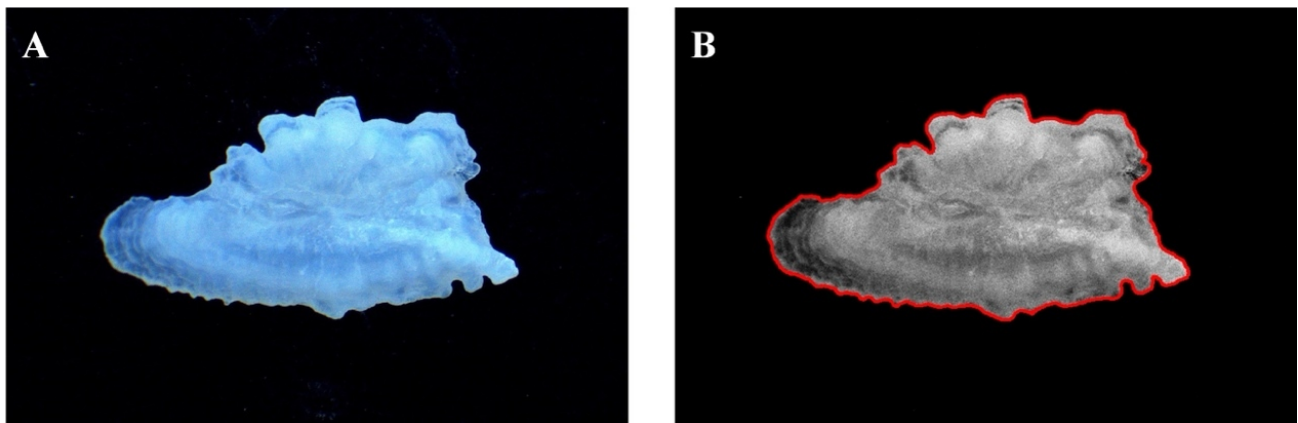
DISCUSSION

OTOLITH SHAPE AS AN INDICATOR OF STOCK STRUCTURE

The present study, using both traditional shape indices and elliptic Fourier analysis, found no significant evidence of stock differentiation for *S. kneri* among three sites in the Xijiang River. After rigorously correcting for allometric effects, which can confound shape comparisons, all statistical tests pointed towards a phenotypically homogeneous population. The low cross-validated classification accuracy (55.6%) from the discriminant analysis further underscores the extensive morphological overlap. This lack of differentiation suggests a high degree of connectivity among the sampled populations. This finding could be attributed to several factors. Firstly, the hydrological conditions and key environmental variables (e.g., temperature, flow regime) might be relatively uniform across the study reach, leading to similar otolith growth patterns. Secondly, and perhaps more importantly, functional ecological corridors may persist despite river regulation.^{8,19,21,24,25} The presence of ad-

Table 4. Loadings and eigenvalues of the first two principal components of shape indices of *S. kneri* at different sites

Otolith shape indices	Principal components	
	1	2
Roundness	-0.434	-0.063
Format-factor	-0.021	0.958
Circularity	0.020	-0.954
Rectangularity	0.224	0.589
Ellipticity	0.984	0.062
Radius ratio	0.745	-0.396
Feret ratio	0.973	0.050
Aspect ratio	0.980	0.067
Eigenvalue	3.670	2.347
Variance explained (%)	45.876	29.336
Cumulative percentage (%)	45.876	75.213

**Figure 2. Left sagitta of *S. kneri* showing the original photograph (A) and the outline photograph (B)**

equate fish passage facilities could facilitate movement and gene flow between upstream and downstream sections, preventing the genetic and morphological isolation that often results from dam construction.

STUDY LIMITATIONS AND FUTURE DIRECTIONS

Several limitations of this study must be acknowledged, as suggested by the peer review process. First, the sample sizes for each site ($n = 14$ to 23) are relatively small and unbalanced. This may have limited the statistical power to detect subtle morphological differences if they exist. Future studies should aim for larger and more balanced sampling designs, ideally informed by an a priori power analysis. Second, our inference that homogeneous conditions contribute to the lack of differentiation is speculative without direct, site-specific environmental data (e.g., temperature profiles, flow velocity, water chemistry). Correlating environmental variables with otolith morphology would provide more substantial evidence. Finally, while otolith shape is a valuable tool, it may not be sensitive enough to detect recent or cryptic population structure. Therefore, we strongly recommend that future research on *S. kneri* in the Xijiang River

incorporate more direct methods for assessing connectivity (such as otolith microchemistry²⁶⁻²⁹ to reconstruct migratory histories and population genetics (e.g., microsatellites or SNP analysis) to quantify gene flow and population structure directly.

EFFECTS OF DAM CONSTRUCTION AND RIVER CONNECTIVITY

Large-scale water conservancy projects are known to fragment aquatic habitats and potentially lead to the isolation of fish stocks. Otolith morphology can serve as a sensitive indicator of such divergence. Contrary to expectations, our results did not reveal distinct otolith shapes between populations upstream and downstream of dams. This suggests that, at present, river fragmentation has not led to detectable phenotypic divergence in the otoliths of *S. kneri*.^{20, 30} One plausible explanation is that the time since dam construction has been insufficient for morphological differences to evolve and become fixed. Alternatively, as mentioned, the fish passage facilities in place may be successfully mitigating the barrier effect, allowing for sufficient individual exchange to maintain a single, interbreeding

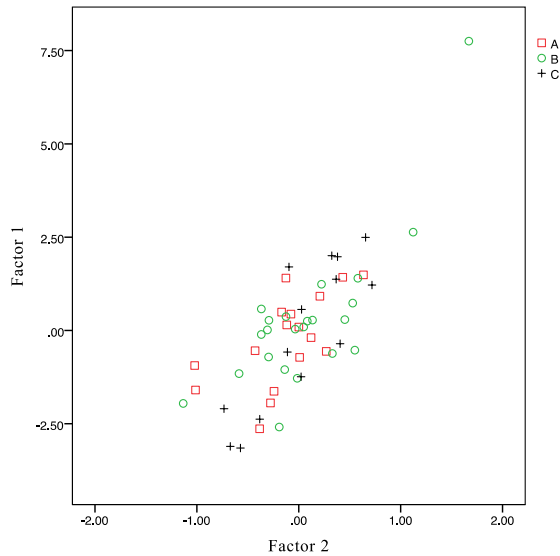


Figure 3. Scatter plot of principal component analysis of otolith shape index for *S. kneri*

population. However, it is crucial to interpret this finding with caution. Otolith shape reflects an integration of both genetic and environmental influences throughout a fish's life, and the absence of shape difference does not definitively rule out underlying genetic structuring or demographic independence.

CONCLUSION

This study employed otolith shape analysis to evaluate the stock structure of *Siniperca kneri* (Garman, 1912) in a dam-regulated section of the Xijiang River. After correcting for size-related effects, our results consistently showed no significant morphological differentiation among the three sampled populations. This suggests the presence of a single phenotypic stock, possibly maintained by ongoing ecological connectivity through effective fish passage systems and relatively homogeneous environmental conditions. However, given the limitations of our sample size and the absence of direct environmental data, these conclusions should be considered preliminary. This study provides a valuable baseline but also underscores the critical need for a multidisciplinary approach, integrating genetic and microchemical analyses, to definitively assess population

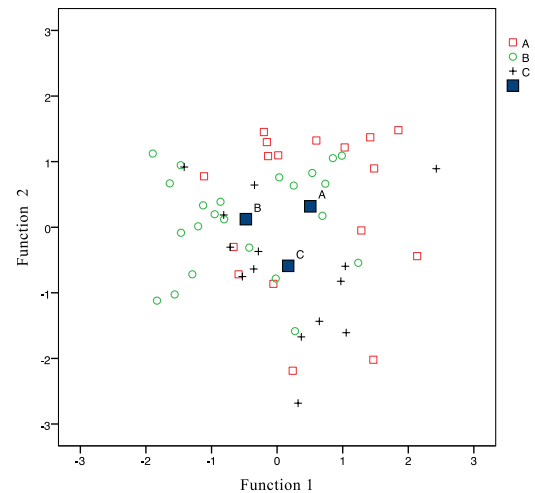


Figure 4. Scatter plot of scores based on the first two canonical discriminant functions

structure and inform robust conservation and management strategies for this economically important species.

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AUTHORS' CONTRIBUTION

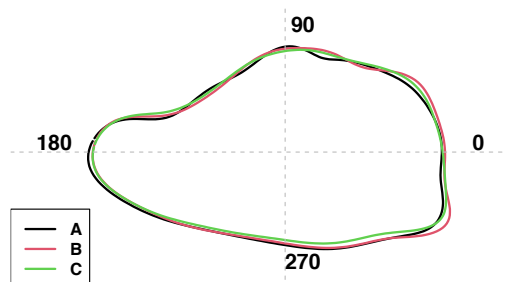
Weijie Cui: Writing – review & editing, Conceptualization, editing, Writing – original draft, Software. Zhongtang He: Writing – review & editing, Conceptualization. Fengling Zhang: Data curation, Software, Formal analysis. Li Xu: Writing – review & editing, Resources. Yang Zhou: Writing – review & editing, Supervision, Resources, Conceptualiza-

Table 5. Classification results from the leave-one-out cross-validated discriminant analysis of otolith shape indices for *S. kneri* stocks.

Sites	Size	Accuracy%	Predicted result		
			A	B	C
A	17	58.8	10*	3	4
B	23	52.2	7	12*	4
C	14	57.1	2	4	8*
Discriminant rate			55.6%		

Table 6. Comparing otolith shape among three sites using one-way ANOVA

Method	Df	Var	F-value	P-value
Fourier				
Model	2	0.064	0.747	0.746
Residual	51	2.174		

**Figure 5. Mean otolith shape based on the Fourier reconstruction for each group**

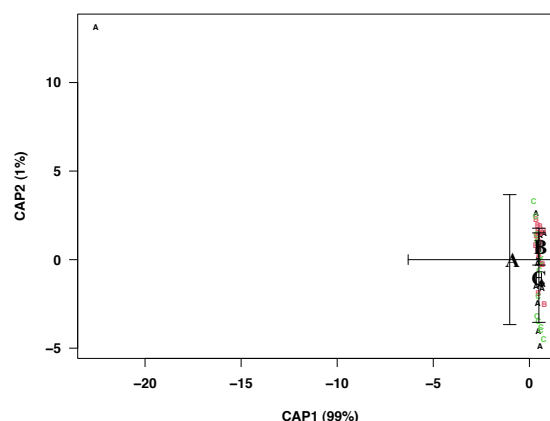
tion. Shengqi Su: Writing – review & editing, Methodology. Tao He: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization.

COMPETING OF INTEREST – COPE

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL CONDUCT APPROVAL – IACUC

No approval of research ethics committees was required for this study as the fish samples were collected from the commercial catches of the fishers.

**Figure 6. Otolith shape from three sites using canonical analysis of principal coordinates with the Fourier coefficients. Canonical scores on the first two discriminating axes CAP1 and CAP2 are shown. Black letters represent the mean canonical value for each sites (A, B and C). Interval surrounding the mean canonical values presents one standard error (mean $\pm$ 1SE)**

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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