

# Otolith microchemistry and chemoscape matching accurately reconstruct spawning ground use in riverine fish

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## ABSTRACT

Freshwater ecosystems face escalating degradation worldwide, undermining ecological functions and services. Spawning habitats in large river systems are vital for sustaining fish populations, yet remain poorly characterized due to limitations in tagging-based approaches. This study reconstructed the spatial use of spawning grounds by *Clupisoma yunnanensis* in the upper Nu-Salween River through integration of otolith microchemical signatures with high-resolution water geochemical maps (chemoscapes). Otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios were quantified via electron probe microanalysis, while corresponding water chemistry profiles were established using inductively coupled plasma mass spectrometry. Quantitative relationships between otolith and ambient element ratios were modeled using a random forest algorithm to reconstruct fish spawning ground distribution. Environmental niche analysis was employed for validation. Results demonstrated pronounced core-to-edge gradients in otolith elemental composition alongside marked spatiotemporal variation in riverine water chemistry, reflecting individual habitat trajectories across a heterogeneous chemical landscape. Downstream areas were identified as dominant spawning grounds with 98% predictive accuracy, and niche-based classification showed 83% concordance with conventional ecological data. This work establishes a novel integrated biogeochemical framework, combining otolith microchemistry, chemoscape modeling, and machine learning, for precise reconstruction of essential spawning habitats. The approach elucidates the reproductive

ecology of *C. yunnanensis* and provides a scalable tool for habitat conservation in data-scarce freshwater systems worldwide.

**Keywords:** Otolith microchemistry; Water trace element; Nujiang River; Fish habitat uses; Fish conservation

## INTRODUCTION

Anthropogenic disturbances and climate change continue to drive global habitat degradation and biodiversity loss at an accelerating pace (Grill et al., 2019; Palmeirim et al., 2024; Tilman et al., 2017). Freshwater biota are disproportionately affected relative to terrestrial and marine taxa, exhibiting more rapid declines in both abundance and diversity (Dudgeon et al., 2006; Shivanna, 2022). These organisms underpin essential ecological functions and ecosystem services (Dudgeon et al., 2006; Lynch et al., 2023; Yao et al., 2025), including nutrient cycling, energy transfer, and provisioning of crucial food sources for human communities, thereby contributing directly to ecosystem resilience and socio-ecological sustainability (Khalili Tilami & Sampels, 2018; Lynch et al., 2023). Despite their ecological and functional importance, comprehensive understanding of habitat utilization by freshwater species remains constrained, particularly for mobile vertebrates such as fish. Challenges arise due to their cryptic behavior, dynamic movement across

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heterogeneous landscapes, and the technical difficulty of underwater observation, which collectively limit the resolution of ecological data necessary for effective conservation efforts.

Recent advances in habitat use reconstruction, including acoustic telemetry, stable isotope profiling, environmental DNA (eDNA) metabarcoding, and remote sensing, have enhanced the capacity to track freshwater species beyond traditional sampling and direct underwater observation (Sandford et al., 2020; Sirot et al., 2017). However, two fundamental obstacles persist. First, reliance on invasive tagging technologies, such as passive integrated transponder (PIT), acoustic, or dart tags, can significantly alter physiological performance and natural behavior, particularly during energetically costly phases like reproduction and migration. Empirical studies have documented tag-induced reductions in swimming performance, foraging efficiency, and predator avoidance, thereby compromising ecological validity and survival outcomes (Thorstad et al., 2013; Verhelst et al., 2022). Despite ongoing miniaturization efforts, tag-associated stress remains a confounding factor in behavioral studies and may distort inferences regarding natural habitat use and population dynamics. Second, prevailing methodologies often lack temporal granularity needed to resolve continuous or stage-specific habitat transitions. While traditional sampling and environmental DNA metabarcoding can effectively detect species presence, they fail to capture individual movement trajectories or residency durations (Verhelst et al., 2022; Wang et al., 2021). Similarly, stable isotope analysis, particularly  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in otoliths or somatic tissues, offers valuable insights into trophic ecology over extended periods but lacks the resolution to detect short-term habitat shifts (Sirot et al., 2017). Consequently, critical knowledge gaps remain regarding habitat connectivity and the complete life-history utilization of freshwater environments by mobile taxa.

*Clupisoma yunnanensis* is an endemic species of the Nujiang River with significant economic and ecological value. It serves as a vital protein source and contributes to household income in local communities due to its abundance and harvestability (Yang et al., 2022). Functionally, this migratory catfish facilitates longitudinal nutrient cycling and energy flow through the riverine system and occupies a central position in aquatic food webs due to its broad dietary niche, thereby helping maintain ecosystem stability (Kurasawa et al., 2024; Perälä et al., 2024). However, the species now faces intensifying anthropogenic threats (Yang et al., 2022). For example, regional warming in the Nujiang River has accelerated ( $>0.02^\circ\text{C}/\text{year}$  from 1980 to 2018; Yang et al., 2021), potentially disrupting reproductive timing and decoupling spawning activity from food resource availability (Opdal et al., 2024; Tao et al., 2018). The riverine fish assemblage has also shifted with the introduction of 18 non-native species (Yang et al., 2022), which may displace native taxa via interspecific competition and predation due to the significant spatial niche overlap (Britton, 2023; Gallardo et al., 2016). Concurrently, critical spawning grounds are increasingly degraded by sediment extraction, water abstraction, and chemical pollution. Most concerning is the potential construction of hydropower dams, which could block essential migratory corridors and prevent completion of essential life history processes such as spawning (Huang & Li, 2024; Tao et al., 2023).

Calcified biological structures, such as otoliths, vertebrae, and mollusk shells, provide a powerful, non-invasive archive

for reconstructing habitat use histories of freshwater species. Notably, these structures grow continuously, capturing trace elements from the surrounding environment in chronological sequence and preserving a chemical record of exposure across developmental stages (Becker et al., 2005; Ding et al., 2024; Feitosa et al., 2020; Gauthier et al., 2024). In particular, fish otoliths form concentric growth layers, analogous to tree rings, that incorporate elemental ratios (e.g., Sr:Ca, Ba:Ca) reflective of ambient water chemistry. These elemental signatures enable retrospective inferences about natal origins, migration routes, and spatial habitat use (Campana & Thorold, 2001; Hüsey et al., 2021; Reis-Santos et al., 2023; Thomas & Swearer, 2019; Xuan et al., 2023). Applications in species such as *Hippoglossus hippoglossus* have demonstrated the utility of otolith microchemistry in life-history reconstruction without disrupting behavior or fitness (Gauthier et al., 2024).

Despite its promise, fine-scale reconstruction of freshwater habitat use from otolith microchemistry remains constrained by two principal limitations. First, conventional proxies such as Sr:Ca and Ba:Ca are effective in delineating migratory patterns across chemically distinct environments (e.g., between freshwater, estuarine, and marine systems) but often lack the resolution needed to identify critical habitats within a single freshwater system. Within such chemically homogeneous systems, the spatial variability of Sr:Ca and Ba:Ca is typically low, limiting discriminatory power (Reis-Santos et al., 2023; Thomas & Swearer, 2019; Vu et al., 2022). Second, the use of otolith microchemistry for intra-basin habitat reconstruction, particularly in potamodromous species in rivers, requires direct matching of otolith elemental profiles with high-resolution water chemistry maps (i.e., chemoscapes) (Burns et al., 2020). This approach is theoretically robust, especially when multiple elements are integrated and spatial variation is prioritized over temporal fluctuations (Morissette & Sirois, 2021; Rowles et al., 2022; Tang et al., 2024). However, the practical implementation of this strategy remains limited by the scarcity of fine-grained, multi-element chemoscapes in most riverine systems, constraining its widespread adoption.

This study evaluated the reliability and capacity of otolith microchemistry integrated with riverine chemoscapes to reconstruct spawning ground utilization by *C. yunnanensis*, an ecologically and economically important native fish in the upper Nu-Salween River. High-resolution chemoscapes were generated from 598 water samples collected across 85 sites over two consecutive years, with geochemical variability primarily driven by spatial rather than temporal factors (Tang et al., 2024). Otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios were quantified using electron probe microanalysis (EPMA), and these elemental fingerprints were linked to aqueous baselines through a random forest (RF) model to infer spawning ground distribution. Model performance was evaluated by comparing microchemistry-derived spawning grounds with traditionally documented sites using environmental niche analysis that integrated hydrological discharge and temperature profiles. This framework addressed two objectives: quantification of the accuracy of chemoscape-guided otolith microchemistry for fine-scale fish habitat reconstruction in exclusively freshwater river systems; and identification of key spawning grounds of *C. yunnanensis* in the upper Nu-Salween River. The results establish an integrative analytical pipeline for resolving reproductive habitat

use in data-limited freshwater systems and provide actionable guidance for conservation planning, including spatial prioritization and protected area design.

## MATERIALS AND METHODS

### Study area

The Nu-Salween River, the longest free-flowing international river in Asia, originates in the Tanggula Mountains of the Qinghai-Xizang Plateau and flows through China, Myanmar, and Thailand before discharging into the Andaman Sea (He et al., 1999; Tao et al., 2023). Its watershed spans approximately 325 000 km<sup>2</sup>, with a 3 200 km mainstream. The Chinese section, known as the Nujiang River, covers around 136 000 km<sup>2</sup> and descends over 4 800 m in elevation. This system exhibits pronounced environmental heterogeneity and supports exceptional ichthyofaunal diversity, including 85 native freshwater fish species such as *C. yunnanensis* and *Schizothorax nukiangensis* (Ding et al., 2023b; Wang et al., 2025; Yang et al., 2022). Substantial spatial variation in water chemistry has also been documented throughout the catchment (Tang et al., 2024). Downstream tributaries, particularly the Mengboluo River (6 594 km<sup>2</sup>) and Nating River

(8 207 km<sup>2</sup>), represent regional biodiversity hotspots, supporting 39 and 52 native fish species, respectively (Yang et al., 2022; Zhou et al., 2016). Given its ecological significance, hydrological complexity, and geochemical variability, the Nu-Salween River provides an ideal system for evaluating fish habitat use through microchemical approaches.

### Fish collection and otolith section preparation

Fish specimens were collected from May 2020 to May 2022 at three sites within the Nujiang River system: the mainstream at Dalandi (DLD, E99°1'10.8", N24°28'16.3") and two tributaries, the Mengboluo River (MBLR, E99°15'10", N24°25'15.4") and the Nating River (NTR, E98°56'42.6", N23°30'27.8") (Figure 1). Each specimen was measured for total and standard length (mm) and body mass (g), followed by bilateral extraction of lapilli. Otoliths were first ultrasonically cleaned in deionized water to remove organic material, then air-dried, weighed, and photographed.

Otolith sectioning proceeded in three stages. First, otoliths were embedded in labeled square silica gel molds (20 mm×20 mm×20 mm, length×width×height) containing a transparent epoxy matrix prepared at a 4:1 mass ratio of resin to hardener (approximately 2 g total mass). Embedded blocks were degassed under vacuum for 10 min to remove entrained air

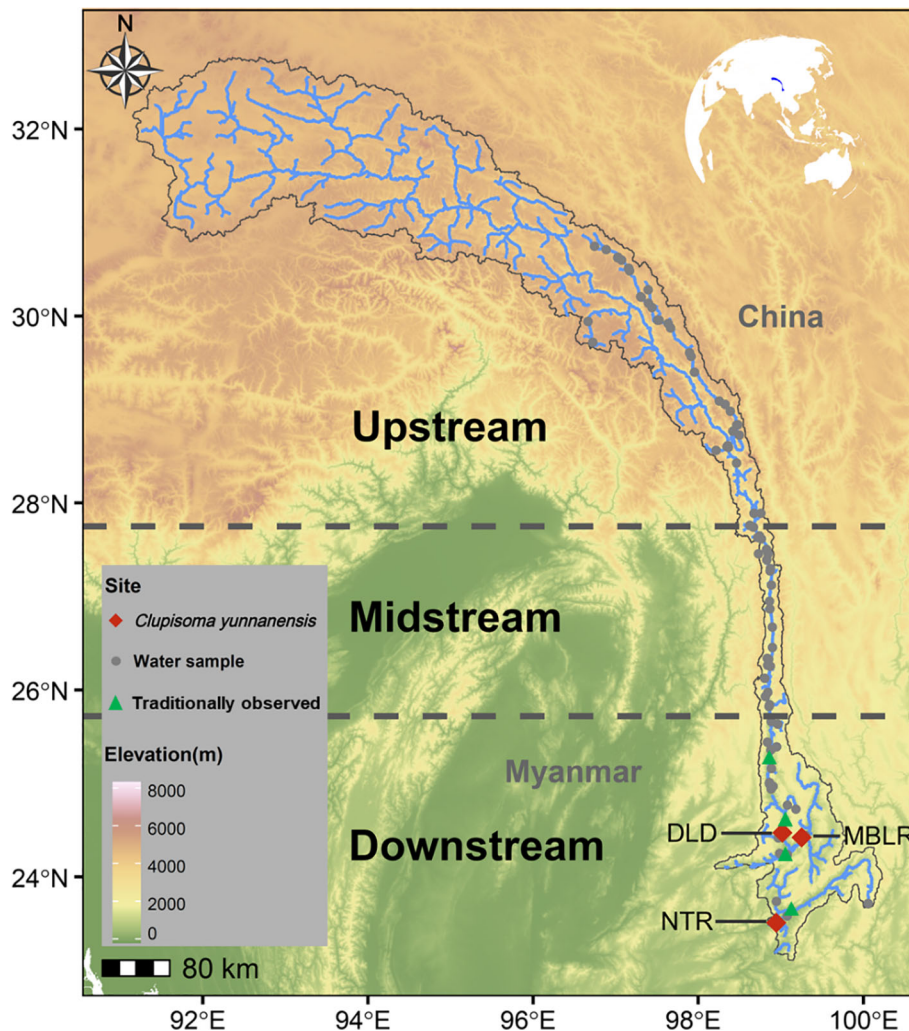


Figure 1 Sampling locations in the upper Nu-Salween River basin. The study area (Nujiang River section) is divided into upstream, midstream, and downstream regions (demarcated by gray dashed lines). Fish sampling sites are indicated by red diamonds (DLD = Dalandi mainstream site; MBLR = Mengboluo River tributary; NTR = Nating River tributary), while water chemistry sampling locations and traditionally observed fish sampling sites are shown as gray dots and green triangles, respectively.

and subsequently cured on a heating plate for 1 h. Second, cured blocks were longitudinally sectioned using a low-speed precision cutter (TechCut 4, Allied, USA) and sequentially ground using sandpaper (600–10 000 grit) until the otolith core was exposed and visible under microscopic examination. Third, semi-processed sections were mounted in cylindrical silicone molds (20 mm inner diameter) and polished from the opposite surface until core and annular growths were clearly resolved. Final polishing was performed with aluminum oxide solution to remove residual surface abrasions. Prepared sections were observed and photographed with a microscope.

#### Otolith elemental analysis and chemoscape construction

Reconstruction of fish habitat use via microchemical tracing requires paired elemental profiles from otoliths and spatially resolved aqueous geochemistry. Previous studies have indicated that otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios represent robust tracers of fish movement, as these elements derive primarily from the aquatic environment and are minimally influenced by physiological processes (Ding et al., 2023a; Hüßy et al., 2024). Application of this multi-element framework has enabled successful delineation of the spawning grounds of *Tinca tinca* in the St. Lawrence River, achieving 69% classification accuracy based on an RF model (Morissette et al., 2021). Accordingly, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios were measured in otoliths from core to edge using EPMA (JXA-8230, Japan) operated at 15 kV accelerating voltage and  $1.5 \times 10^{-8}$  A beam current. Analytical parameters included a 5 s dwell time and a 5  $\mu\text{m}$  spot scan radius. Calcite ( $\text{CaCO}_3$ ) and strontium tamarite ( $\text{SrTiO}_3$ ) (Institute of Mineral Resources, CAGS) served as calibration standards. Riverine chemoscapes were sourced from a contemporaneous dataset (Tang et al., 2024), generated from two years of water sampling across 85 sites in the upper Nu-Salween River (Figure 1). Dissolved elemental concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS; NexION 2000B, PerkinElmer, USA), providing the spatial baselines required for otolith-to-water elemental matching.

#### Data analysis

Following EPMA of otolith trace elements (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca), outliers were excluded based on Ca concentration profiles across the otolith from the core to edge (256 points per specimen), given the highly stable Ca concentration in otoliths (~30%). All elemental concentrations were standardized as (element/Ca)  $\times 1000$ . Trace elements at each analysis point were independently determined, reflecting ambient environmental conditions during different life stages. To evaluate water chemistry differences between natal and capture environments, microchemistry from the otolith core (first 10 points) and edge (last 10 points) was compared using Mann-Whitney U tests due to non-normal data distributions (Morissette et al., 2021). Linear regression was used to establish quantitative relationships between otolith edge microchemistry (final edge point) and corresponding water chemistry. These relationships were then applied in linear mixed-effects models (LMMs) using the 'lme4' package (Bates et al., 2015) to predict site-level monthly water chemistry and capture intra-annual variation. The resulting predictions were converted to high-resolution otolith-based chemoscapes using the previously defined regressions. These reconstructed otolith chemoscapes were used to train an RF classification model ('randomForest' package; Breiman et al., 2024). A total

of 5 000 trees were generated using 75% of the data for model training and 25% for validation. Predictor variable importance was assessed using the 'varImpPlot' function. Otolith core microchemistry was used to classify natal origins, as all sampled individuals were estimated to be between 0 and 2 years of age.

To refine spawning ground predictions, environmental niche overlap analysis was conducted using the 'ecospat' package (Di Cola et al., 2017), comparing RF-predicted sites with traditionally observed spawning areas (Figure 1; Yang et al., 2022). Global monthly average discharge and water temperature data from 2020 to 2022 were obtained from the European Centre for Medium-Range Weather Forecasts (ECMWF) and the "FutureStreams" dataset, respectively. The 'raster' package was used to extract discharge and water temperature values at predicted and traditionally observed spawning grounds (May–August) based on latitude and longitude coordinates (Bosmans et al., 2022; Harrigan et al., 2024). Environmental niche overlap between predicted and observed spawning grounds (Figure 1) was calculated for each subbasin (i.e., upstream, midstream, and downstream), and the resulting overlap indices were used as weighting coefficients to adjust the predicted spawning ground distribution by re-scaling the relative distribution percentages at each chemoscape site. All analyses were conducted in R (R Core Team, 2024).

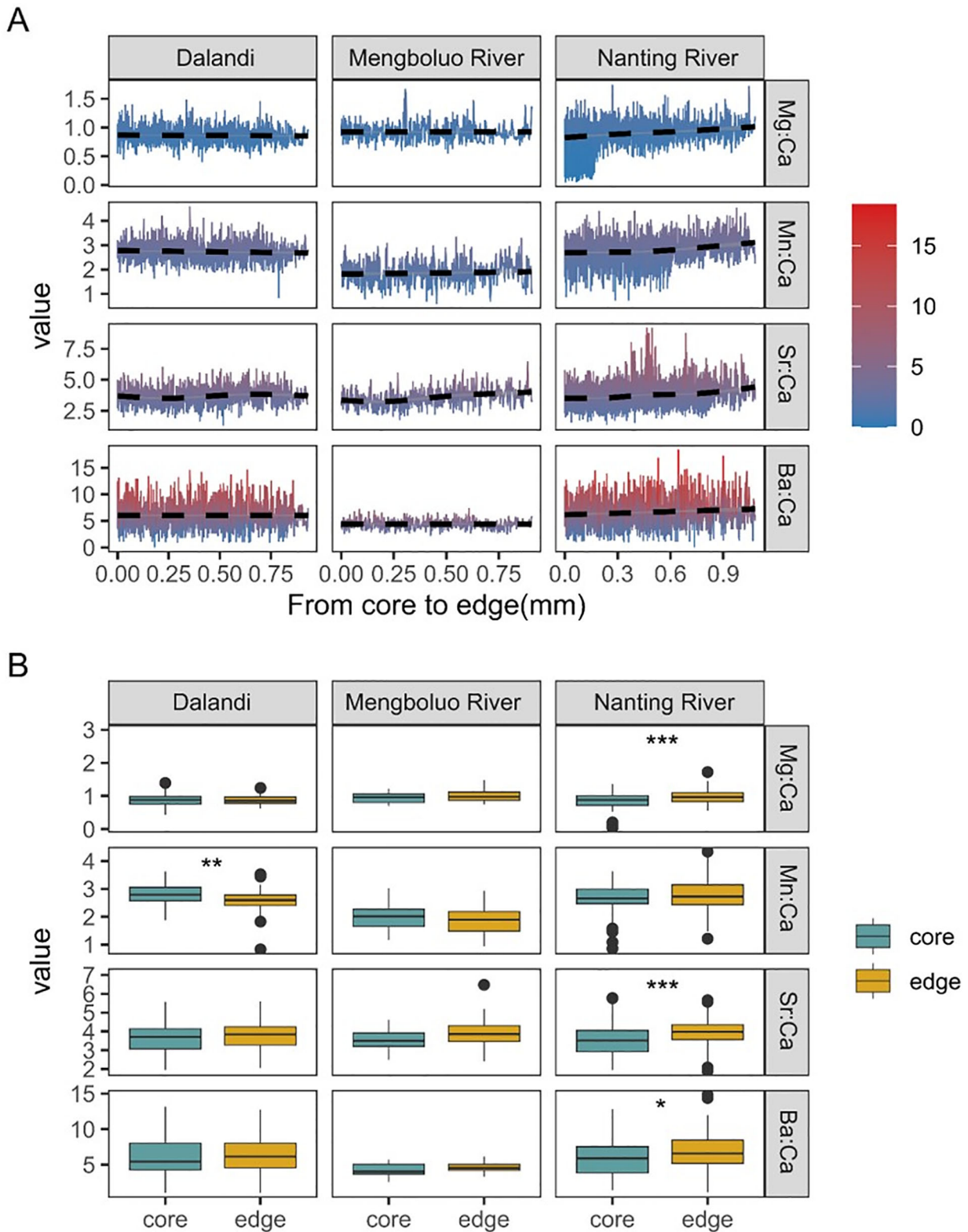
## RESULTS

#### Otolith microchemistry patterns

A total of 57 *C. yunnanensis* individuals were analyzed, comprising seven from DLD, two from MBLR, and 48 from NTR. Otolith elemental profiles displayed heterogeneous core-to-edge patterns across sampling sites (Figure 2). In DLD specimens, elemental ratios ranged as follows: Mg:Ca (0.39–1.49  $\times 1000$ ), Mn:Ca (0.82–5.00  $\times 1000$ ), Sr:Ca (1.28–6.06  $\times 1000$ ), and Ba:Ca (0.86–14.66  $\times 1000$ ), with Mn:Ca exhibiting significantly elevated values at the core relative to the edge ( $P < 0.05$ ). MBLR specimens displayed comparable ranges: Mg:Ca (0.54–1.67  $\times 1000$ ), Mn:Ca (0.58–3.36  $\times 1000$ ), Sr:Ca (1.73–6.48  $\times 1000$ ), and Ba:Ca (2.16–7.40  $\times 1000$ ), although without significant core-edge differences. The NTR population exhibited the broadest elemental variation: Mg:Ca (0.045–1.75  $\times 1000$ ), Mn:Ca (0.71–4.54  $\times 1000$ ), Sr:Ca (1.36–9.19  $\times 1000$ ), and Ba:Ca (0.94–18.19  $\times 1000$ ), with significantly higher edge values detected for all elements except Mn:Ca ( $P < 0.05$ ).

#### Site classification using otolith microchemistry

The RF model constructed with otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios yielded high classification accuracy, with an overall rate of 97.83% (range: 96.73%–98.63%) (Supplementary Figure S1). Linear regressions revealed significant correlations between observed and predicted water chemistry values for all four elements at monthly resolution ( $R > 0.65$ ,  $P < 0.001$ ) (Supplementary Figure S2). To expand the training dataset, LMMs were applied to estimate monthly water chemistry across sampling sites (Supplementary Figure S3). Strong positive associations were evident between otolith microchemical signals and corresponding water chemistry (Supplementary Figure S4). Among the four element ratios, variable importance for site classification was ranked as follows: Sr:Ca, Mg:Ca, Ba:Ca, and Mn:Ca (Supplementary



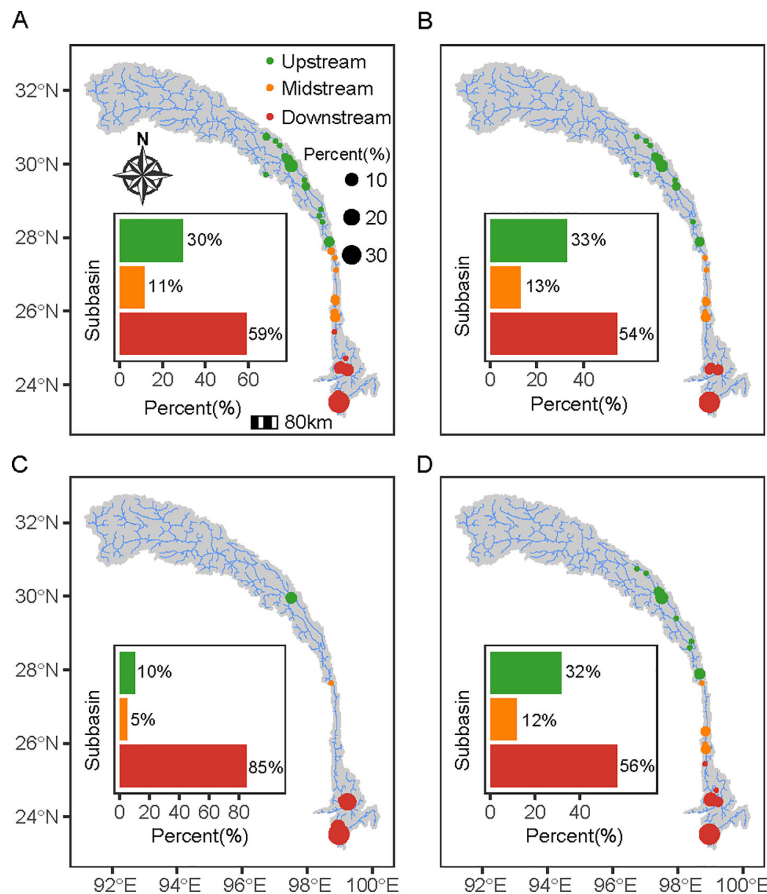
**Figure 2** Microchemistry patterns (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca ratios) in *C. yunnanensis* Otoliths across three sampling sites. (A) Core-to-edge transect profiles showing elemental deposition patterns; (B) Statistical comparison between core and edge values with significance levels indicated (\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ ).

Figure S5).

#### Spawning ground identification and validation

RF classification of otolith core microchemistry in *C. yunnanensis* identified the downstream Nujiang River as the principal spawning region (Figure 3). Predicted natal origin distributions varied by capture site. Among DLD-captured fish,

otolith core points were allocated as follows: 32.86% upstream (12 sites), 12.86% midstream (six sites), and 54.29% downstream (four sites). The two MBLR specimens exhibited strong downstream affinity, with 85% of core point assignments linked to four downstream sites, and only 10% and 5% assigned to single upstream and midstream sites,



**Figure 3** Spawning ground distribution of *C. yunnanensis* reconstructed from otolith microchemistry profiles of individuals collected at different sites: (A) Overall; (B) Dalandi (DLD); (C) Mengboluo River (MBLR); (D) Nanting River (NTR). Dot sizes and bar graphs represent the relative percent of otolith core assignments per chemoscape site and basin, respectively.

respectively. Individuals collected from NTR showed 56.52% downstream allocation (six sites), 31.88% upstream (11 sites), and 11.59% midstream (four sites). Across all samples, NTR sites accounted for the highest percentage of otolith core point allocations (overall: 38.36%, DLD: 40%, MBLR: 40%, NTR: 36.23%). Notably, the 25% allocation of otolith core points to MBLR in one of the two MBLR-captured individuals suggests natal origin within this tributary.

Environmental niche analysis based on discharge and water temperature revealed significant regional differentiation among microchemistry-inferred spawning grounds (Figure 4). Downstream regions showed substantial overlap (83%) with traditionally observed spawning habitats, whereas niche overlap was markedly lower for upstream (11%) and midstream (44%) sites (Supplementary Table S1). Applying environmental niche constraints to the model output further refined spawning ground predictions, indicating that 91.26% of spawning activity occurred downstream, with 8.74% in midstream zones and none in upstream areas. The highest-probability spawning sites were located in NTR (59.22%), MBLR (11.65%), and DLD (11.65%).

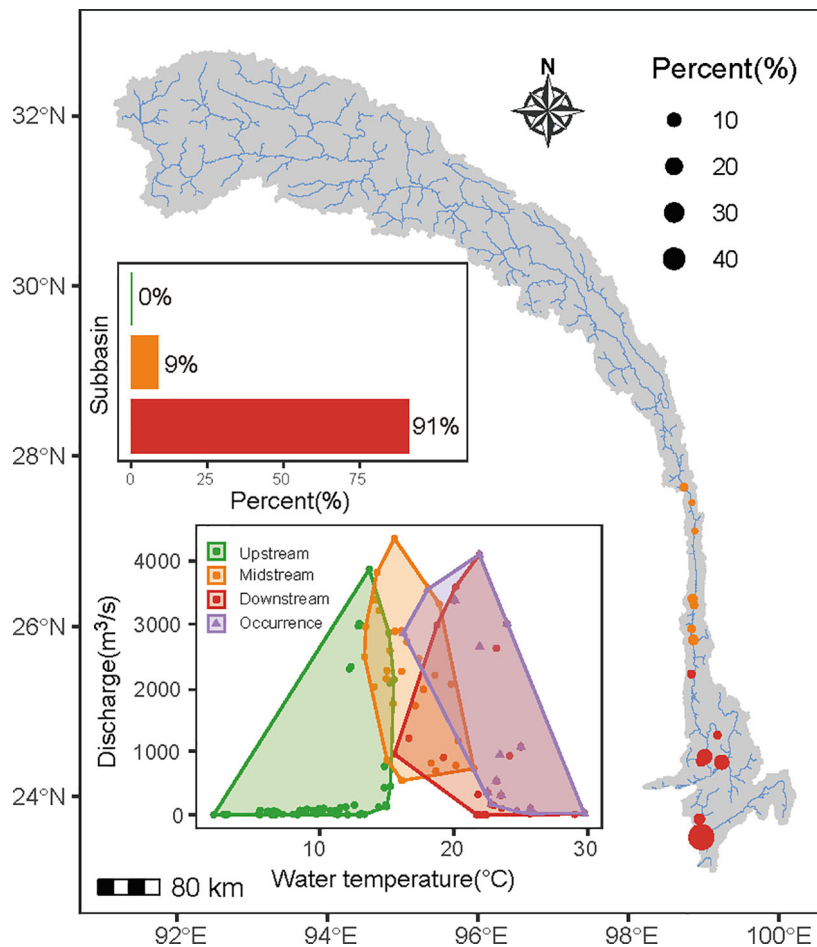
## DISCUSSION

High-resolution reconstruction of habitat use by freshwater taxa in large river networks has remained elusive due to constraints associated with invasive tagging techniques and the limited availability of fine-scale chemoscapes required to contextualize elemental records archived in calcified structures. This study addressed these limitations through the

integration of otolith microchemistry with chemoscapes and machine learning to resolve spawning ground distribution of *C. yunnanensis* in the upper Nu-Salween River. The RF framework achieved 98% classification accuracy, identifying downstream reaches—especially the NTR—as core spawning areas. Independent validation using environmental niche analysis demonstrated 83% concordance between microchemistry-inferred spawning grounds and historically documented occurrence sites. Beyond resolving reproductive habitat use in an endemic riverine fish, this integrative framework highlighted the capacity of calcified tissue microchemistry, combined with chemoscape mapping and data-driven modeling, to identify critical habitats at fine spatial resolution in chemically subtle freshwater systems, offering a practical strategy to inform conservation planning amid accelerating freshwater biodiversity loss.

## Reliability of otolith microchemistry for reconstructing fish habitat use

The reliability of otolith microchemistry for reconstructing fish habitat use is grounded in three core principles: elemental incorporation in otoliths reflects ambient water chemistry experienced throughout the life cycle of fish; spatial variability in water chemistry exceeds temporal fluctuations in river systems; and multi-element approaches enhance spatial resolution beyond the limitations inherent to single-element methods. First, otolith elemental signatures record environmental exposure across ontogeny, enabling retrospective habitat reconstruction (Campana, 1999; Thomas & Swearer, 2019). In *C. yunnanensis*, this mechanism was



**Figure 4** Spawning ground distribution of *C. yunnanensis* reconstructed from otolith microchemistry profiles by calibrating based on environmental niche overlap analysis. The convex hull diagram illustrates niche overlap between each basin and traditionally observed sites (occurrence). Dot sizes and bar graphs represent the relative percent of otolith core assignments per chemoscape site and basin, respectively, with the upstream region excluded due to its low niche overlap with occurrence sites.

evident through pronounced core-to-edge variation in otolith trace element ratios (Figure 2), consistent with the premise that ambient water is the primary source of elemental uptake (Doubleday et al., 2013; Elsdon et al., 2008). The present study demonstrated strong positive correlations between otolith and water chemistry across all four target elements in the Nu-Salween River (Supplementary Figure S3), consistent with patterns observed in other large river systems (Fortin et al., 2024; Valentine et al., 2024). For example, Valentine et al. (2024) reported robust otolith-water relationships across multiple fish species in the Upper Mississippi River.

Second, the dominance of spatial over temporal variability in riverine water chemistry is essential for accurate habitat reconstruction, as elemental incorporation is primarily governed by environmental conditions rather than physiological processes (Hüssy et al., 2024; Tang et al., 2024). This environmental sensitivity is well-established for key geochemical tracers: Sr:Ca ratios typically increase with salinity and act as oceanographic tracers, while Ba:Ca ratios decrease and function as terrestrial indicators, as demonstrated in Mekong pangasiid catfish (Elsdon et al., 2008; Hüssy et al., 2021; Vu et al., 2022). Similarly, Mn:Ca ratios reflect hypoxic exposure, with elevated Mn levels marking early life residence in oxygen-depleted environments, as shown in arapaima and cod (Hüssy et al., 2021). Although Mg incorporation may correlate with temperature-dependent metabolic activity in some taxa (Hüssy et al., 2024), the

absence of seasonal trends in otolith Mg:Ca profiles of *C. yunnanensis* (Figure 2A) suggests minimal physiological influence. The observed preservation of spatial geochemical signatures indicates that elemental profiles in this species reliably encode environmental heterogeneity rather than endogenous variability.

Third, multi-element approaches enhance spatial resolution in chemically homogeneous systems by amplifying subtle geochemical signals (Morissette et al., 2021; Strohm et al., 2017). In this study, an RF model trained on otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca achieved high site classification accuracy (Supplementary Figure S4), highlighting the strength of non-linear machine learning techniques in extracting ecological signals from complex, multi-dimensional datasets—beyond the capabilities of linear discriminant methods such as LDA or QDA (Jones et al., 2017; Mercier et al., 2011). Although single-element markers like Sr:Ca can effectively trace cross-ecosystem migrations of fish (Rohtla et al., 2023), their spatial resolution declines in low-contrast, homogeneous environments, such as rivers, where spatial geochemical variability is subtle (Cheruvilil et al., 2013). Multi-element signatures resolve this limitation by enhancing classification performance (Morissette et al., 2021; Strohm et al., 2017). For example, the same four-element otolith profile (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) has been successfully applied to resolve the natal origins of invasive tench (*T. tinca*) in the St. Lawrence River (Morissette et al.,

2021). Together, these findings support the reliability of otolith microchemistry for reconstructing *C. yunnanensis* spawning ground utilization, consistent with the three fundamental principles and in agreement with traditional field observations.

#### Spawning ground utilization in *C. yunnanensis*

Spawning ground reconstruction based on converging evidence identified the downstream Nujiang River—particularly NTR—as the primary reproductive area for *C. yunnanensis* (Figures 3,4; Supplementary Figure S6). Otolith core microchemistry assigned the highest proportion of natal origin to NTR, followed by MBLR and DLD. While otolith elemental profiles (e.g., Sr:Ca, Ba:Ca) reflect environmental exposure, they do not directly capture reproductive constraints such as flow regime and thermal conditions (Elsdon et al., 2008; Thomas & Swearer, 2019). Integration of discharge and water temperature data through environmental niche analysis revealed an 83% overlap between microchemistry-derived spawning grounds and historically observed sites (Figure 4; Supplementary Table S1), underscoring the relevance of hydrological parameters as key determinants of spawning suitability (Ding et al., 2023a; Paumier et al., 2020). Additionally, the presence of sexually mature individuals in the NTR during the spawning season provided direct biological confirmation (Supplementary Figure S6). Collectively, these lines of evidence converge to designate the NTR and surrounding downstream tributaries as core spawning regions.

Secondary areas showed more variable patterns. The MBLR site likely serves as a spawning ground for a subset of the population, while the DLD site appears to function primarily as a migratory corridor. Evidence of homing behavior, typical among migratory fish species (Bett & Hinch, 2016), was reflected in the alignment between capture and inferred natal sites for individuals from NTR and MBLR (Figure 3C, D). However, the MBLR data are limited by low sample size ( $n=2$ ), precluding definitive conclusions. Spawning outside natal tributaries may reflect responses to population competition or anthropogenic disturbance (Brown et al., 2018). Historical records confirm spawning occurs in tributaries rather than the mainstem habitats despite extensive adult migrations (~270 km) (Yang et al., 2022). The absence of historical evidence for mainstream spawning, combined with the position of DLD along a known migration corridor, suggests its primary role is transit or access to unmonitored tributaries outside the current chemoscape framework. Consequently, while MBLR is likely a functional secondary spawning area, its relative importance and the extent of homing fidelity require verification through larger sample sizes and validation using traditional sampling, environmental DNA, or electronic tagging to clarify the status of potential but unconfirmed spawning sites (Verhelst et al., 2022).

This pronounced spatial concentration of spawning activity in the downstream region is likely shaped by ecological suitability. Successful reproduction in *C. yunnanensis* requires a combination of thermal thresholds, flow regimes conducive to migration and egg development, and appropriate substrate composition (Ding et al., 2023a). Under present hydrological and thermal conditions, these requirements are most consistently met in the downstream reaches and associated tributaries. In contrast, midstream reaches host only occasional spawning events, suggesting that these regions function as marginal habitats used under demographic or competitive stress (Bett & Hinch, 2016). Furthermore, the

complete absence of upstream reproductive activity suggests the existence of critical ecological barriers, such as unsuitable temperature profiles, excessive flow velocities, or a lack of accessible tributaries with appropriate spawning substrates. Comparatively, NTR exhibited a relatively higher probability of supporting spawning than MBLR, likely reflecting abiotic suitability and relative refuge from anthropogenic pressures such as pollution or invasive species. Consequently, the spatial distribution of spawning grounds reflects the intersection of species-specific reproductive requirements, the spatial heterogeneity of riverine environments, and gradients of anthropogenic disturbance.

#### Challenges and future directions

Despite its analytical power, otolith microchemistry remains constrained by several methodological and ecological challenges. First, high-resolution chemoscape development requires extensive, long-term water sampling across multiple hydrological seasons, imposing significant logistical and financial burdens, particularly in large, remote basins where access is intermittent. Such spatial and temporal coverage is essential, as incomplete geochemical baselines increase the risk of origin misclassification. Second, robust habitat inference depends on spatial heterogeneity in water chemistry exceeding temporal fluctuation, an assumption that does not universally apply across all freshwater systems (Morissette & Sirois, 2021; Tang et al., 2024). In chemically homogeneous rivers or environments characterized by pronounced seasonal fluctuations—such as floodplain systems with alternating wet-dry regimes—the signal-to-noise ratio may be insufficient for reliable habitat discrimination. Third, although ambient water represents the dominant source of otolith elemental incorporation, dietary inputs can contribute substantially to otolith composition (Doubleday et al., 2013; Walther & Thorold, 2006; Woodcock & Walther, 2014), introducing potential confounding effects. For instance, Sr:Ca ratios, often used as salinity proxies, can vary among prey taxa across habitats, thereby biasing environmental reconstructions when trophic contributions are unaccounted for. In the Nu-Salween River, pronounced spatial heterogeneity in water chemistry appears sufficient to outweigh dietary noise, as evidenced by the high classification accuracy achieved by the RF model (98%). Nevertheless, future investigations should explicitly quantify the relative contributions of water and dietary sources to otolith chemistry across ontogenetic stages. Integration of dietary information through combined otolith-stable isotope analyses may significantly improve habitat assignment beyond water-based models alone. Additionally, comparative studies of microchemical proxies in other calcified structures—such as fish eye lenses for early developmental signals or vertebrae for adult habitat use—offer promising complementary avenues for reconstructing lifetime movement trajectories and refining freshwater conservation strategies.

#### Conservation implications

Fine-scale reconstruction of spawning habitats for *C. yunnanensis* yields directly actionable guidance for river management and biodiversity protection. Identification of NTR as a primary spawning area elevates this tributary to a high conservation priority and supports implementation of stringent protection measures. Management actions should focus on preserving key habitat features, including natural flow regimes, thermal stability, and appropriate gravel substrates, which necessitates strict regulation of extractive activities such as

sand mining and water abstraction. Beyond specific-site recommendations, the analytical framework itself represents a powerful and scalable tool for conservation planning. By enabling non-invasive, high-resolution mapping of critical habitats in data-limited systems, this approach facilitates spatial planning based on empirically derived habitat use rather than indirect environmental surrogates, thereby strengthening the scientific foundation of priority area design. Furthermore, this framework can be applied proactively to assess potential anthropogenic impacts, allowing quantitative assessment of how climate-driven hydrological shifts or hydropower development may alter the spatial distribution and viability of spawning grounds.

## CONCLUSIONS

This study achieved high-resolution reconstruction of spawning ground utilization in *C. yunnanensis* across the upper Nu-Salween River by integrating otolith microchemistry with spatially explicit water chemoscapes. Three principal findings emerged: (1) significant core-to-edge variations in otolith Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios indicated movement across geochemically heterogeneous habitats throughout ontogeny; (2) a four-element RF model classified spawning origins with 98% accuracy, identifying downstream areas—particularly NTR—as the primary spawning area; and (3) environmental niche analysis revealed 83% overlap between microchemistry-derived spawning grounds and traditionally observed sites, providing independent validation of the approach. The integrated framework developed here, combining biogeochemical tracers, chemoscape mapping, and machine learning, overcomes key limitations of traditional tagging-based methods by offering a non-invasive, data-rich alternative for inferring habitat use. These results not only define concrete conservation targets for *C. yunnanensis* within the Nu-Salween basin but also present a transferable protocol for mapping critical habitats in other freshwater species across data-deficient river systems globally.

## SCIENTIFIC FILED SURVEY PERMISSION INFORMATION

Field surveys were conducted in accordance with the Fisheries Law of the People's Republic of China. Collections of fish specimens and tissues were conducted according to the Laboratory animal—Guideline for ethical review of animal welfare (Chinese National Standard GB/T 35892-2018).

## DATA AVAILABILITY

The data from this study will be made available in Science Data Bank at <https://doi.org/10.57760/sciencedb.28816>.

## SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHOR' CONTRIBUTION

B. L.T.: writing—original draft, methodology, investigation, data curation. J. T.: writing—review & editing, project administration, supervision, funding acquisition, conceptualization. L.Y. D.: methodology, investigation, data curation. Y.T.Z.: investigation, data curation. T.Q. D.: investigation, data curation. J.S.T.: project administration, supervision, methodology. A.V.: methodology, writing—review & editing. C.Z.D.: writing—review & editing, project administration, supervision, funding acquisition, conceptualization.

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