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Research Article

Habitat Preferences and Spatiotemporal Distribution of Chinese Sturgeon (*Acipenser sinensis*) Revealed by Environmental DNA

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The Chinese sturgeon (*Acipenser sinensis*), a critically endangered anadromous species endemic to the Yangtze River, has undergone severe population decline due to habitat degradation and anthropogenic disturbances. To improve monitoring under low-abundance conditions, this study applied environmental DNA (eDNA) analysis using species-specific primers and SYBR Green quantitative PCR to investigate the spatiotemporal distribution of *A. sinensis* in the Yangtze River Estuary and adjacent coastal waters in May and August 2024. eDNA was detected at all sampling sites, with higher copy numbers generally observed in May, particularly in nearshore waters between Chongming and Changxing Islands. Generalized linear modeling indicated that eDNA concentrations were most closely associated with moderate temperatures, high dissolved oxygen, and slightly alkaline pH, while signal intensity declined under warmer and oxygen-poor conditions. Elevated eDNA values in May coincided with the timing of upstream release events, although the absence of prerelease baseline data prevents direct attribution to stocked individuals. Overall, the findings provide preliminary insights into seasonal habitat associations of *A. sinensis* in estuarine ecosystems and demonstrate the potential of eDNA as a sensitive and noninvasive tool for long-term monitoring and conservation assessment of endangered aquatic species.

Keywords: *Acipenser sinensis*; eDNA; habitat suitability; Yangtze River Estuary

1. Introduction

The Chinese sturgeon (*Acipenser sinensis*), an iconic migratory species endemic to China, is a nationally protected aquatic species and has become a key focus of ecological conservation due to its unique life history and critically endangered status [1]. Multiple pressures, including ecological changes in the Yangtze River Basin, overfishing, and habitat fragmentation caused by water conservancy facilities, have led to a sharp decline in the population of *A. sinensis*, resulting in a critical conservation situation [2–4]. Traditional monitoring approaches for *A. sinensis*, such as capture surveys, acoustic tagging, and individual tracking, can provide valuable individual-level information [5–7]. However, these methods are costly and may cause harm to the target species, particularly given the extremely small size of the remaining wild population, which makes efficient monitoring difficult to achieve [8]. Consequently, developing noninvasive, efficient, and sensitive monitoring techniques is critical for the conservation of *A. sinensis* [9].

In recent years, environmental DNA (eDNA) technology has emerged as a powerful noninvasive tool widely applied in the detection and monitoring of endangered aquatic organisms [10]. Unlike traditional methods, eDNA does not

rely on capturing individuals but instead detects genetic material released by target organisms into the water column, enabling sensitive detection of species presence [11]. Preliminary studies have applied eDNA to investigate the spatial distribution of *A. sinensis* [12, 13]. However, most studies have focused on detecting presence or describing ranges [14], and in-depth analyses of environmental drivers remain limited. In-depth analyses of environmental factors shaping the spatial distribution of *A. sinensis* eDNA remain limited, and eDNA has not yet been systematically applied to estuarine habitat monitoring of *A. sinensis*.

To address these gaps, this study applied eDNA methods in combination with water environmental factors to assess the potential habitat suitability of *A. sinensis* in the Yangtze River Estuary during May and August 2024. The findings provide preliminary evidence for the application of eDNA in monitoring the suitability of estuarine–nearshore habitats for large migratory fishes and offer a valuable reference for the conservation and release assessment of *A. sinensis*.

2. Materials and Methods

2.1. eDNA Surveys. Twelve sampling stations were established in the Yangtze River Estuary, China, including ten offshore stations (S1–S10) and two intertidal stations (CM1 and CM2) near Chongming Island (Figure 1). Sites were selected along known migration routes and in areas with historically high capture rates. Sampling was conducted during two fixed cruises in May and August 2024. This timing targets the main estuarine occurrence window for juvenile *A. sinensis* and improves detectability under low abundance [15]. It also avoids sampling immediately adjacent to release events, which helps ensure that signals reflect ambient distribution. At offshore sites, we collected water at 6 m using sterile Niskin-type samplers. Offshore station depths were greater than 10 m throughout the survey. The 6-m depth avoids surface films and ship wake, remains above the bottom boundary layer, where resuspension is common, and provides cleaner, more comparable samples for eDNA filtration. At intertidal sites, surface water samples were collected during ebb tide. All water samples were stored in presterilized bottles, placed in ice-packed coolers, and transported to the laboratory within 12 h.

2.2. DNA Extraction. Each 1-L water sample collected at each station was filtered through a 0.45 μm mixed cellulose ester (MCE) membrane (47 mm diameter) using a vacuum pump under sterile conditions, following the optimized workflow proposed by Tsuji et al. [16]. The filters were immediately preserved in sterile cryotubes at −20°C until extraction. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions, with final elution in 100 μL AE buffer.

Although no biological replicates were collected, three technical qPCR replicates were performed for each DNA extract to ensure detection reliability. To monitor potential contamination, several negative controls were included as follows: field blanks (500-mL ultrapure water filtered at each site), filter blanks (unused filters processed alongside samples), extraction blanks (extractions without filter material), and PCR no-template controls (NTCs).

2.3. PCR Amplification. eDNA of *A. sinensis* was specifically detected using species-specific primers developed by Yu et al. [17]: forward primer 5′-GGCAATTTTAATCTGGGG TTTCCA-3′, reverse primer 5′-TGGATGTTAGATATA TGTCCTTG-3′, and probe 5′-CAAGGTAGAACATTA CACACAACTGCTCG-3′. Each 20 μL reaction contained 10 μL of SYBR Premix Ex Taq II (Takara, Japan), 0.4 μM of each primer, 2 μL of DNA template, and nuclease-free water. The thermocycling program consisted of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. A melt-curve analysis (65°C–95°C, 0.5°C increments) was performed to verify amplification specificity. Each sample was analyzed in three technical replicates, and negative controls (NTCs, field blanks, filter blanks, and extraction blanks) were included in every run. Positive amplifications were further confirmed by Sanger sequencing.

To establish standard curves, a cloned plasmid containing the *A. sinensis* D-loop fragment was serially diluted tenfold from 10^6 to 10^0 copies per microliter. This span keeps low copy field samples within the calibrated range for quantification and preserves linear response across high and low templates. We report slope, efficiency, and R-squared and use only curves that meet accepted performance criteria. Each dilution was tested in 10 replicates. Amplification efficiency and R^2 were calculated from the standard curve. Following Klymus et al. [18], the limit of detection (LOD) was defined as the lowest concentration at which ≥ 95% of replicates yielded positive amplification, and the limit of quantification (LOQ) was defined as the lowest concentration at which the coefficient of variation (CV) of Cq values was < 35%.

2.4. Environmental Variables. In this study, in order to analyze the ecological characteristics of *A. sinensis* habitat, we collected data on a variety of environmental variables, which were measured with a YSI water quality analyzer during sampling.

All environmental variables were analyzed in R. Relationships between environmental factors and eDNA distribution were assessed using linear regression and correlation analyses.

2.5. Data Analysis and Modeling Methods. In order to evaluate the effects of environmental factors on eDNA concentration in *A. sinensis*, a generalized linear model (GLM) was used. eDNA copy number data were log10 transformed to satisfy the assumption of model normality. The GLM was constructed using water temperature, dissolved oxygen (DO), pH, salinity, and turbidity as independent variables and eDNA concentration as the response variable, and significant variables were screened based on stepwise regression. Model evaluation included estimated coefficients, standard errors, and p values. Analyses were performed in R (V4.4.1), and plots were generated with ggplot2 and ggpubr.

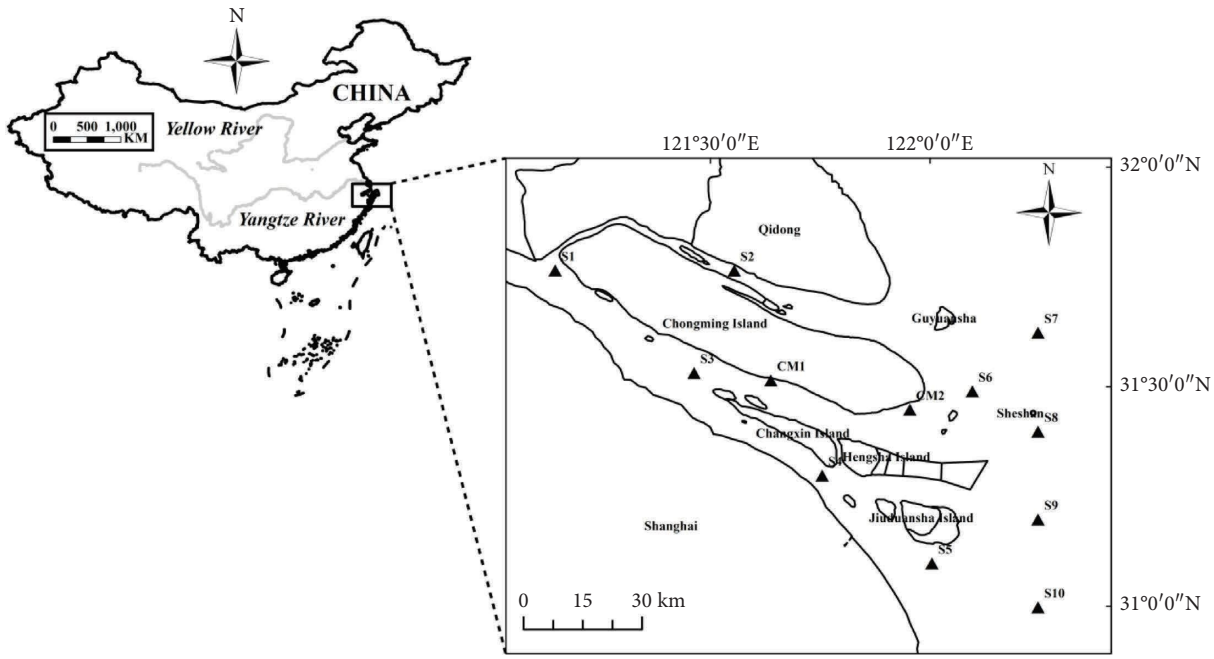

Figure 1: Yangtze Estuary study area and sample sites (triangle).

3. Results

3.1. A. sinensis eDNA Detection in the Yangtze River Estuary. Positive eDNA detections of *A. sinensis* were obtained from. l 12 sampling stations in both May and August, demonstrating broad spatial coverage across the estuarine region (Figure 2). In May, copy numbers ranged from 210 to 1097 copies/μL, with the highest values observed in the southern branch (S4–S6). Moderate levels (400–700 copies/μL) occurred near CM1 and CM2, while much lower values (< 300 copies/μL) were recorded at offshore sites close to Sheshan and the eastern estuary margin (Figure 2(a)). In contrast, August samples were lower overall, ranging from 85 to 420 copies/μL. Higher values were concentrated in nearshore waters adjacent to Hengsha Island and the southern flank of Changxing Island, whereas offshore sites showed only background levels (Figure 2(b)). One individual *A. sinensis* was captured at CM1 in May, corresponding to a site where eDNA copy numbers exceeded 800 copies/μL, showing consistency between the two detection approaches. All positive amplicons were confirmed by Sanger sequencing, and sequences were identical to the *A. sinensis* D-loop reference in GenBank, confirming the specificity of detection.

3.2. Environmental Changes in Different Months. Comparison of estuarine environmental conditions between May and August revealed pronounced seasonal variation across five key water quality parameters (Figure 3). Water temperature increased markedly from 20.0°C to 25.0°C in May (median: 22.7°C) to 28.1°C to 33.9°C in August (median: 31.2°C), reflecting typical summer warming. Salinity rose from 0.1 to 12.9 ppt in May to 0.1 to 15.0 ppt in August, with greater variability likely driven by reduced precipitation and enhanced seawater intrusion during summer. pH also increased slightly, ranging from 7.65 to 8.93 in May to 7.73 to 9.33 in August, indicating seasonal shifts in photosynthetic activity and organic matter decomposition. DO levels decreased from 7.20 to 8.78 mg^{-1} in May to 6.48 to 8.39 mg^{-1} in August, consistent with reduced oxygen solubility and enhanced oxygen demand at higher temperatures. Turbidity remained elevated in both months, ranging from 7.9 to 554.7 NTU in May and 9.3 to 299.1 NTU in August.

3.3. Relationships Between Environmental Variables and eDNA Concentration

3.3.1. Principal Component Analysis (PCA). PCA was performed to explore correlations among environmental variables (Figure 4). The first two principal components together explained 61.4% of the total variance. PC1 (38.1%) was positively associated with turbidity and latitude, while PC2 (23.3%) was positively associated with DO, salinity, and pH. Temperature was moderately aligned with PC1 in the opposite direction of turbidity, suggesting a negative correlation between these two factors. These patterns indicate that environmental variables in the estuary exhibited both seasonal and spatial covariation.

To reduce collinearity, variance inflation factors (VIFs) were calculated, and all predictors with VIF > 10 were excluded before model fitting. Based on the PCA loadings and collinearity screening, a GLM was constructed with log10-transformed eDNA copy number as the response variable and temperature, DO, pH, salinity, turbidity, longitude, and latitude as predictors.

The GLM indicated that temperature (estimate = −0.228, $p < 0.001$) and DO (estimate = +0.871, $p < 0.01$) were the variables most strongly associated with eDNA copy number. pH showed a weaker negative association (estimate = −1.015, $p < 0.1$), while longitude also contributed moderately ($p < 0.05$). Salinity, turbidity, and latitude were not retained

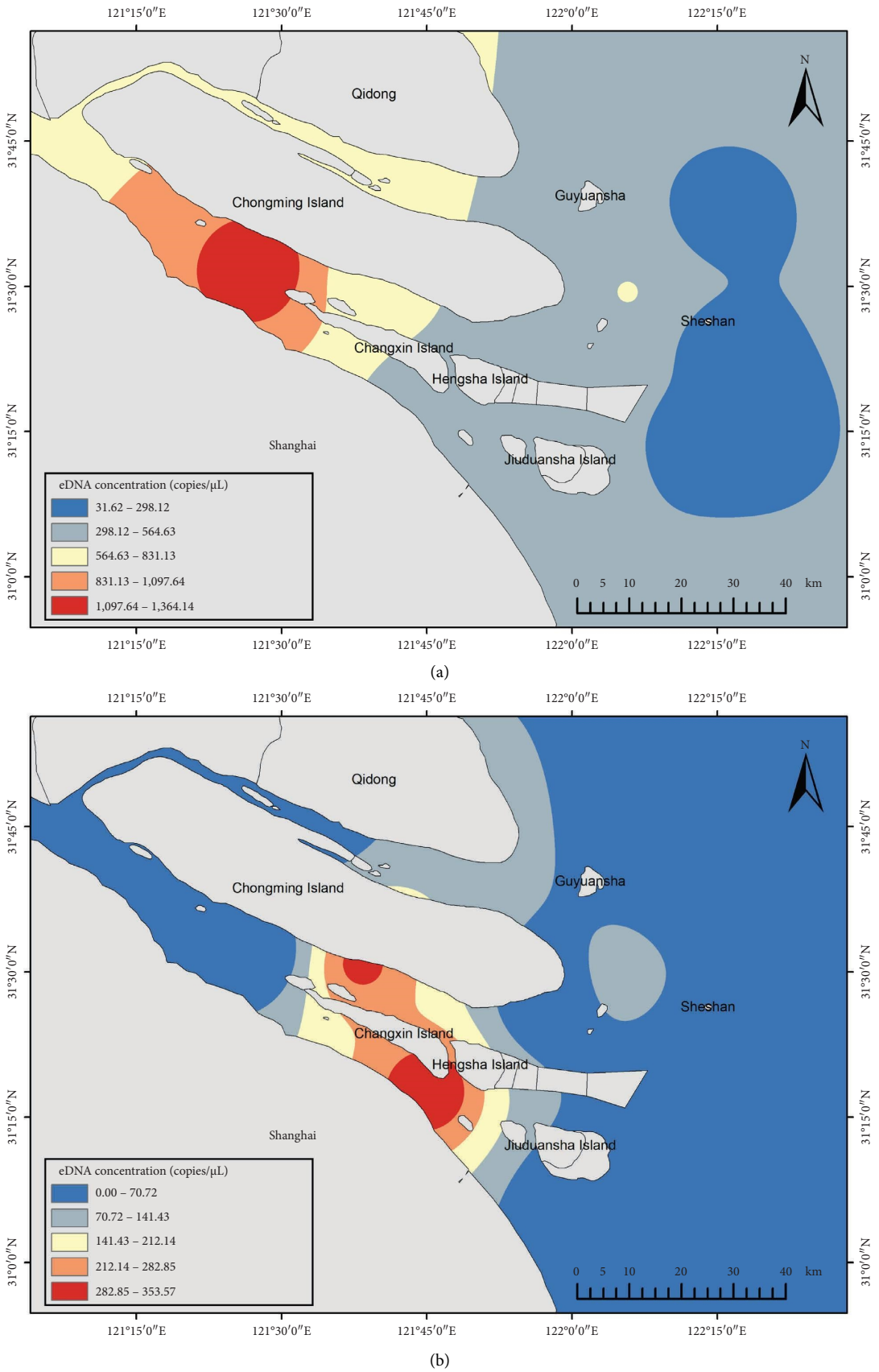

FIGURE 2: eDNA concentration in the Yangtze Estuary for May (a) and August (b).

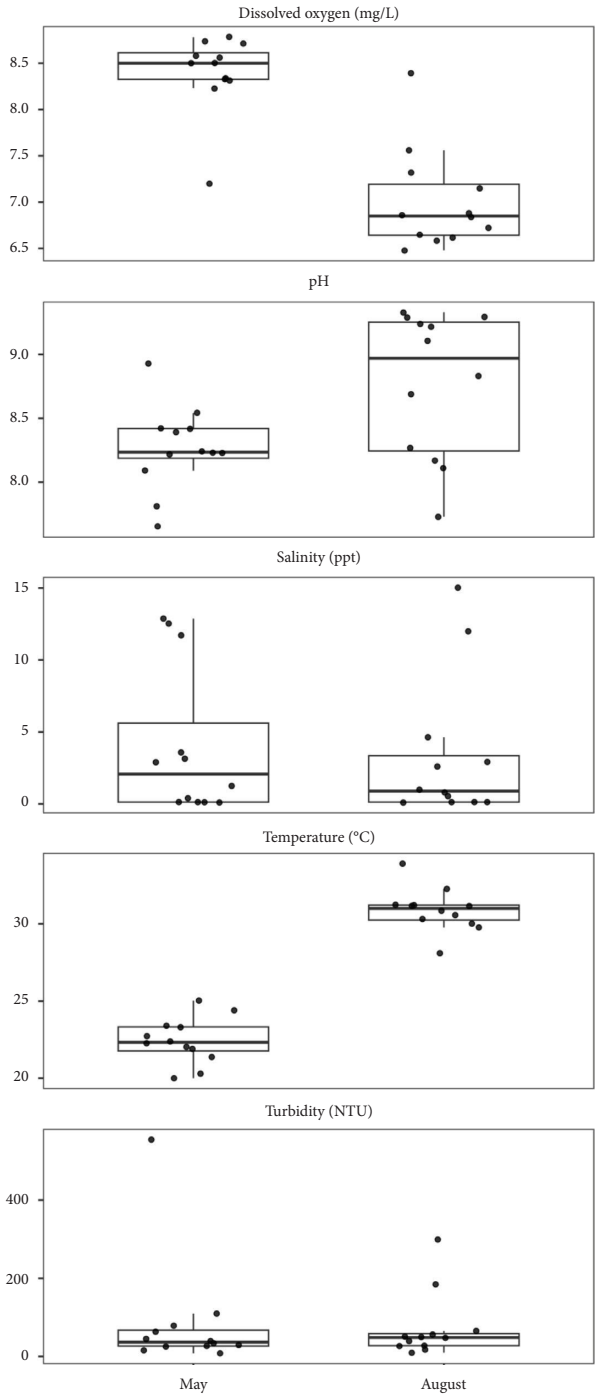

FIGURE 3: Seasonal comparison of key environmental variables in the Yangtze River Estuary (May and August).

as important predictors (Table 1). The final model explained 42.3% of the deviance, with an AIC of 163.7. Given the limited replication of water samples, these associations should be regarded as exploratory rather than conclusive.

3.3.2. Spatial Response Patterns of Significant Environmental Predictors on A. sinensis eDNA Concentration. Spatial interpolation maps were generated to illustrate general patterns of eDNA copy number relative to key environmental variables (Figure 5). In May, higher eDNA values were generally found in regions with moderate water temperatures (22°C–24°C), relatively high DO concentrations (> 8.0 mg^{-1}), and pH values between 8.2 and 8.6. These conditions were primarily located in the southern branch of the estuary and near the intertidal stations.

In contrast, in August, eDNA copy numbers were markedly lower across most stations. The lowest values were observed in areas where temperatures reached up to 33.9°C and DO dropped to 6.5–8.4 mg^{-1}, particularly in offshore waters. The spatial association with pH was less consistent during this period, with no consistent pattern across sites.

Because of the limited number of sampling sites, these interpolated maps should be interpreted as indicative rather than exact spatial distributions. Nonetheless, the results suggest that seasonal shifts in temperature and DO were the dominant factors associated with variation in *A. sinensis* eDNA signals across the estuary.

4. Discussion

4.1. Spatiotemporal Patterns and Source Attribution of A. sinensis eDNA. eDNA has been increasingly applied as a noninvasive tool for monitoring aquatic organisms in large rivers and estuaries [19], including endangered sturgeon species [20–22]. In this study, species-specific primers developed by Yu et al. were used with SYBR Green qPCR to investigate the spatiotemporal distribution of *A. sinensis* eDNA in the Yangtze River Estuary from May to August 2024. Positive detections were obtained from l station, consistent with surveys in 2022 and 2023 [23, 24]. Our results indicated that eDNA copy numbers were generally higher in May than in August. This between-month difference may reflect biological factors such as migration timing, foraging activity, or the occurrence of stocking events. The period of higher May signals coincided with upstream release activities reported in March 2024 [25], but because no prerelease baseline was available, these results cannot be directly attributed to stocking. In addition, downstream transport of genetic material has been observed in other systems [26], and increased DNA shedding during postrelease acclimation has been reported in freshwater pearl mussels and Japanese eel (*Anguilla japonica*) [27, 28]. These comparisons suggest that multiple processes could have contributed to the May signal.

Given the tidally dynamic nature of the Yangtze Estuary, tidal state (high vs. low tide) was considered as a categorical factor in statistical models [29]. However, no significant effect of tidal state on *A. sinensis* eDNA copy numbers was detected ($p = 0.39$). Similar results have been reported for other low-density wild populations [30], whereas stronger tidal influences are often observed in high-density aquaculture systems [31]. Overall, these results demonstrate that eDNA can reveal spatiotemporal variation in *A. sinensis* occurrence under low-abundance conditions. At the same time, the interpretation of seasonal changes must remain cautious, as the signals may be influenced by migration, release history, and hydrodynamic processes, rather than directly reflecting local abundance.

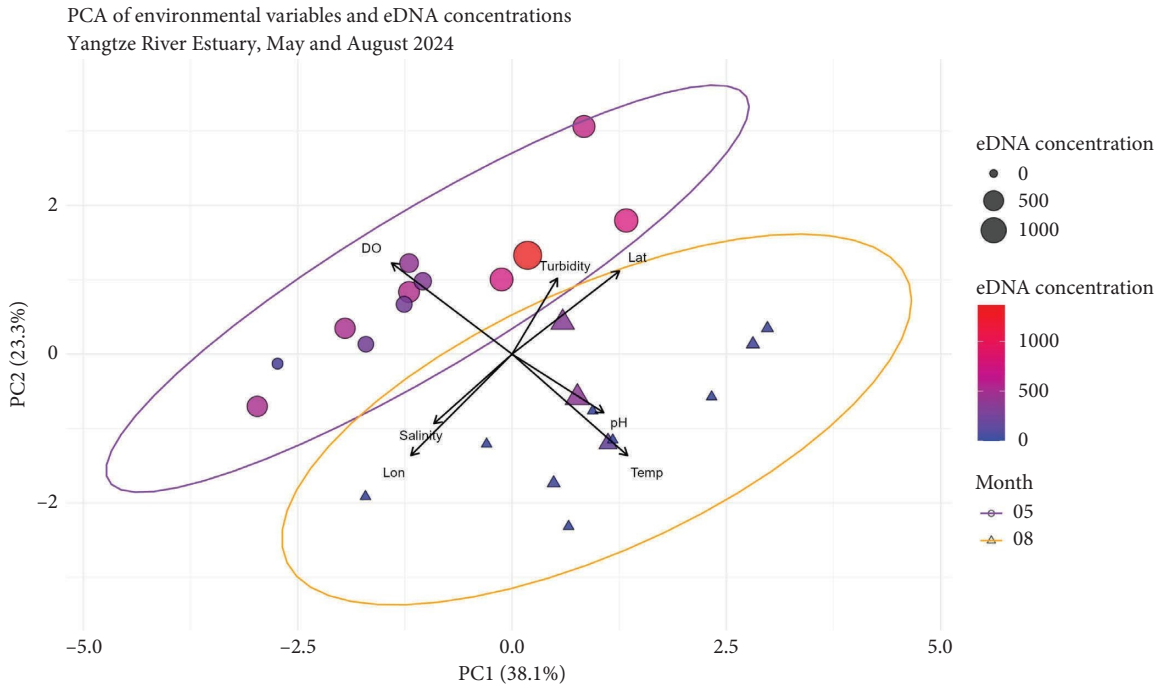

Figure 4: Principal component analysis (PCA) of environmental variables in the Yangtze River Estuary (May and August 2024). PC1 (38.1%) was mainly associated with turbidity and latitude, while PC2 (23.3%) was associated with dissolved oxygen, salinity, and pH. The eDNA concentration of *A. sinensis* is indicated by the color scale.

Table 1: Simplified GLM results for total eDNA concentration of *Acipenser sinensis* and environmental predictors at the Yangtze River Estuary during May and August 2024.

Variable	Estimate	Std. error	*t*-value	*P* value
Temp (°C)	−0.22815	0.05671	−4.023	< 0.001
DO	0.87124	0.32715	2.6631	< 0.01
Longitude (°E)	−1.31685	0.57493	−2.2904	< 0.05
pH	−1.01490	0.55294	−1.8354	< 0.1
Salinity	−0.07427	0.05444	−1.3642	0.186
Latitude (°N)	0.84640	1.03663	0.8164	0.422
Turbidity	0.00036	0.00204	0.1768	0.861

4.2. Environmental Drivers of eDNA Concentration. In 2024, eDNA surveys revealed that elevated copy numbers of *A. sinensis* were mainly detected in the western waters of Changxing Island and near Baozhen, within the core salt–freshwater mixing zone of the estuary. These areas were characterized by moderate temperatures (~22°C), slightly alkaline pH (8.2–8.5), and relatively low salinity and turbidity. By contrast, sites with higher temperatures, lower DO, or elevated salinity generally showed reduced eDNA signals. Previous research has indicated that spatiotemporal environmental variability strongly influences the occurrence of *A. sinensis* [32], and our results suggest that temperature and DO are the most important correlates of eDNA concentration in this system.

The GLM results identified temperature as the strongest negative correlate of eDNA concentration (estimate = −0.228, $p < 0.001$). Copy numbers were highest in May at sites with water temperatures of 20°C–22°C. Although most sturgeon species prefer 10°C–20°C [33–36], *A. sinensis* tolerates a broader thermal range, with optimal performance reported at 19°C–25°C [37]. This consistency suggests that seasonal temperature regimes in the Yangtze Estuary may influence the spatial variation of eDNA signals, but further validation with behavioral and physiological data is needed. DO showed a significant positive association with eDNA concentration (estimate = +0.871, $p < 0.01$). Higher eDNA values were consistently found at sites where DO exceeded 8.0 mg^{-1}. Although elevated DO may accelerate microbial degradation of eDNA [38], our results suggest that under field conditions, oxygen-rich environments are associated with stronger eDNA signals. Physiological studies have shown that oxygen availability is important for metabolism, feeding, and swimming activity in *A. sinensis* [39, 40], as well as in related species [41, 42]. In summary, temperature and DO were the main environmental variables associated with spatial differences in *A. sinensis* eDNA in the estuary. These associations are consistent with known physiological characteristics of the species, but should be interpreted cautiously because eDNA concentrations may also be affected by hydrodynamic transport, degradation processes, and the limited biological replication in this study. The sustained detection of *A. sinensis* eDNA across all stations highlights the utility of eDNA for monitoring estuarine habitats, but further studies are needed to disentangle the relative contributions of wild and stocked individuals.

4.3. Future Directions for eDNA Applications in Postrelease Monitoring of A. sinensis. This study demonstrates that eDNA is a useful tool for detecting *A. sinensis* under low-

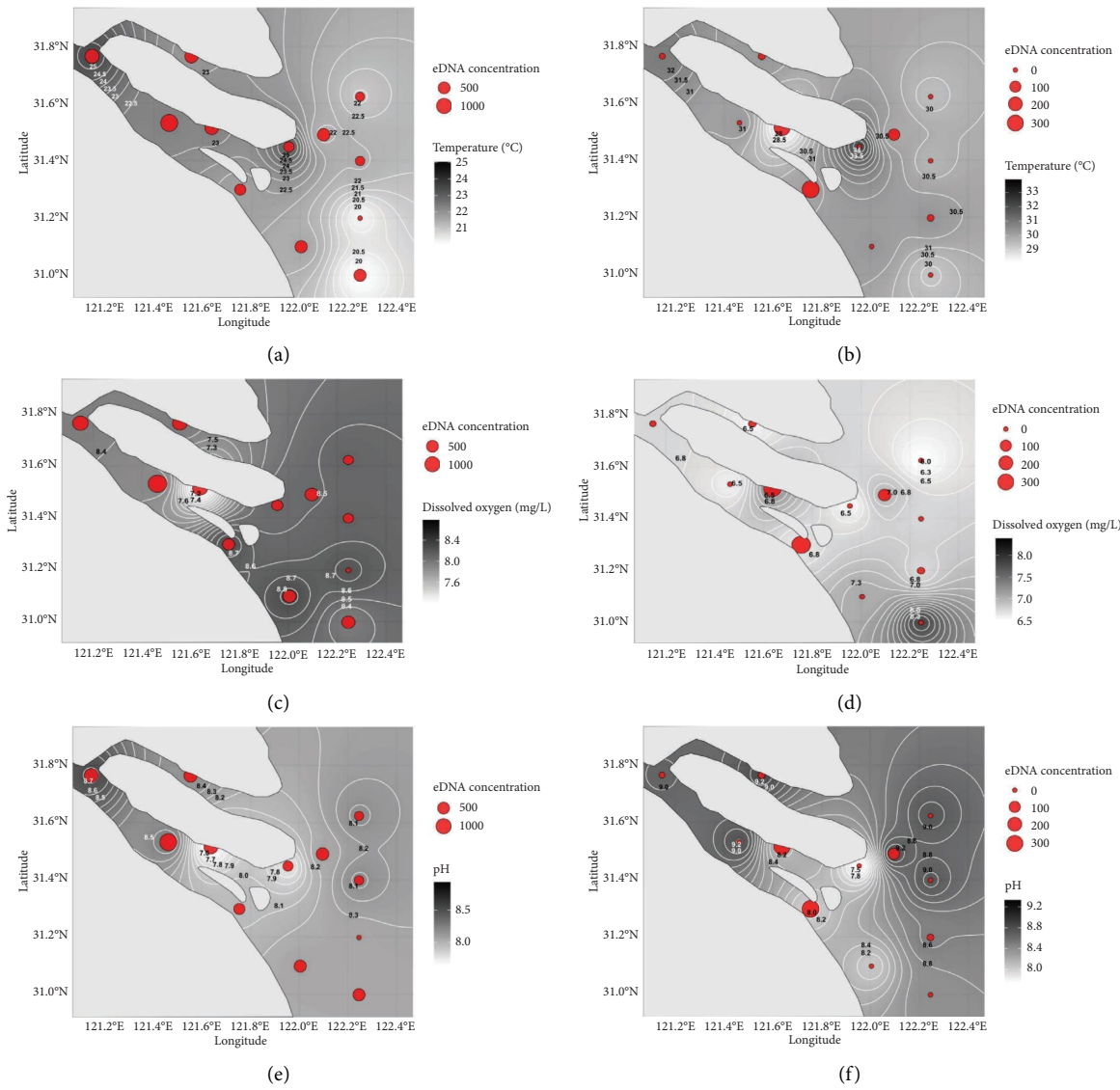

FIGURE 5: Spatial response patterns of significant environmental drivers on *A. sinensis* eDNA detections in May and August 2024 (a, c, e) water temperature, dissolved oxygen, and pH in May, respectively. (b, d, f) Water temperature, dissolved oxygen, and pH in August, respectively. Red circles indicate the relative concentration of *A. sinensis* eDNA at each sampling site. Interpolated layers were generated by inverse distance weighting (IDW).

abundance conditions, providing higher sensitivity and broader spatial coverage than traditional capture and tagging methods [43]. In May 2024, eDNA copy numbers were generally higher than in August, coinciding with the timing of large-scale upstream release events. Although the absence of prerelease baseline data prevents direct attribution, this temporal correspondence highlights the potential of eDNA as a noninvasive and rapid-response indicator for post-release monitoring.

Nevertheless, eDNA alone cannot provide individual-level information such as age structure, sex, or behavior [44]. To overcome this limitation, future monitoring should integrate eDNA surveys with complementary approaches, including acoustic telemetry and traditional capture methods, particularly in areas with consistently higher signals such as Baozhen and Changxing Island. Moreover, stratified vertical sampling and the inclusion of ecological variables (e.g., prey availability, chlorophyll concentration, and microbial community composition) would improve both model accuracy and ecological interpretation. Long-term, multiseason monitoring that combines these methods will provide a more robust assessment of the effectiveness of release programs and the habitat use of *A. sinensis* in the estuarine environment.

5. Conclusion

This study used eDNA to examine the spatiotemporal distribution of *A. sinensis* in the Yangtze River Estuary in 2024. eDNA signals were generally higher in May than in August, with stronger detections in nearshore waters around Chongming and Changxing Islands. Temperature and DO

were the environmental variables most closely associated with variation in eDNA copy numbers, suggesting that moderately warm and oxygen-rich waters may provide favorable conditions under our survey design.

The higher eDNA signals in May coincided with the timing of a release event in March 2024, but without prerelease baseline data, these results cannot be directly attributed to stocked individuals. Overall, the study highlights the potential of eDNA for monitoring *A. sinensis* under low-abundance conditions and provides a reference for future conservation and postrelease assessment efforts.

Data Availability Statement

Due to institutional confidentiality policies, the raw eDNA sequencing data generated in this study are not publicly available. Processed summary data and statistical outputs are available from the corresponding author upon reasonable request for academic purposes.

Ethics Statement

This study did not involve any direct handling or experimentation on live animals. All eDNA samples were collected from water bodies using noninvasive methods. No endangered or protected species were harmed or disturbed during the sampling process. Field sampling was conducted in accordance with national regulations and under permission from relevant local authorities where required.

Disclosure

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Xuzhe Gu and Guangpeng Feng. Methodology: Xuzhe Gu. Investigation: Xuzhe Gu, Tao Zhang, Gang Yang, Ju Yang, and Qingbo Zhang. Resources: Guangpeng Feng. Writing–original draft: Xuzhe Gu and Guangpeng Feng. Writing–review and editing: Xuzhe Gu, Bo Feng, Tao Zhang, Gang Yang, Ju Yang, Qingbo Zhang, and Guangpeng Feng. Visualization and validation: Xuzhe Gu and Bo Feng. Supervision, project administration, and funding acquisition: Guangpeng Feng.

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