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Innovative eDNA Approaches For Fish Biomass Estimation In Aquatic Environments

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Abstract

We conducted research utilizing environmental DNA (eDNA) derived from aquatic vertebrates to assess species presence, with a specific focus on estimating biomass. Our hypothesis posited that fish release DNA into the water proportionate to their biomass, enabling the use of eDNA concentration to estimate species biomass. A novel eDNA method was developed for estimating the Rohu fish, *Labeo rohita* (Hamilton, 1822) biomass through both laboratory and field experimentation. In aquarium settings, we observed a dynamic change in eDNA concentration initially, stabilizing after 6 days. Notably, temperature exhibited no significant impact on eDNA concentrations in aquarium environments. Positive correlations between fish biomass and eDNA concentration were identified in both aquarium and experimental pond settings. Subsequently, we applied this eDNA method to estimate Rohu fish biomass and distribution in a natural freshwater ecosystem. Our findings indicated modifying the distribution of fish eDNA concentration could be revealed by water temperature. Consequently, we propose that biomass data derived after eDNA concentration serves as a reliable indicator of the likely distribution of carp in natural environments. The measurement of eDNA concentration offers a non-invasive, straightforward, and rapid approach to biomass estimation. This method holds promise for informing management plans geared toward ecosystem conservation.

Keywords: environmental DNA (eDNA), Biomass estimation, Freshwater ecosystem.

Introduction

Understanding a species' distribution is crucial for comprehending its ecological dynamics, assessing extinction risks, and implementing effective conservation measures (Pollock et al., 2020). However, in aquatic ecosystems, the intricate microhabitat topology and vegetation often pose challenges to obtaining precise distribution estimates (Olden et al., 2010). Environmental DNA (eDNA) has forthwith emerged as a valuable tool for documenting aquatic vertebrate species distributions (Banerjee et al., 2021). The revealing of small,

species-specific DNA fragments in the waterbody holds the potential to enhance survey accuracy, reduce costs, and facilitate the identification of rare or invasive species (Senapati et al., 2019). Noteworthy applications include using eDNA to confirm the presence of bullfrog tadpoles (Everts et al., 2022), bighead and silver carp (Chapman et al., 2021), as well as frogs and salamanders (Walker et al., 2017) across various water bodies.

While eDNA has proven effective for illustrating species distribution through absence/presence data, understanding species biomass is equally vital for estimating ecosystem production (Rourke et al., 2022). Biomass, an ultimate biological parameter, presents challenges in accurate estimation, particularly for aquatic organisms like fish. Accurate biomass information is pivotal for the conservation of rare and endangered species and the effective management of population sizes (Barnes & Turner, 2016). The observation that terrestrial vertebrates' fecal DNA at the soil surface reflects relative biomass underscores the potential utility of eDNA (de Sousa et al., 2019). Moreover, this method was also successfully utilized for monitoring virus concentrations in lakes(Aldeguer-Riquelme et al., 2021). If aquatic vertebrates secrete eDNA into the water—whether from secretions (Andruszkiewicz et al., 2017), tissues (Sigsgaard et al., 2020), or feces (Stewart, 2019)—proportionally to their biomass, eDNA stands as a promising method for estimating species biomass by quantifying the amount of eDNA copies (Schneider et al., 2016) in water samples. This innovative approach opens new avenues for gaining insights into the biomass dynamics of aquatic ecosystems, offering valuable contributions to conservation efforts and population management strategies (Rees et al., 2014).

Our primary objective was to establish a non-invasive method using environmental DNA (eDNA) to estimate fish biomass, with the Rohu fish, *Labeo rohita* (Hamilton, 1822), serving as the model organism. Our approach encompassed a series of comprehensive experiments, including laboratory studies, experiments in a pond, and afterward a field survey conducted in a freshwater riverine ecosystem.

In the laboratory phase, we systematically examined the effect of temperature and time on eDNA concentrations. Utilizing this data, we established a model for estimating carp biomass based on the quantification of eDNA copies. Furthermore, the concentration of eDNA is influenced by both the release rate and breakdown rate, factors intricately linked to ambient temperature. Previous studies have noted detectability of fish eDNA of anuran tadpoles for 25 days at 8–11°C and 21 days at 17–26°C. Additionally, dissolved DNA pieces (approximately 400 bp) persisted in water for up to a week at 18°C. Nevertheless, the specific effects of varying temperature environments on eDNA concentrations remain unexplored.

Employing artificial ponds, we refined a methodology for assessing eDNA concentration in large water volumes and assessed the correlation between eDNA concentrations with carp biomass and or abundance. Lastly, we applied this method to account for the biomass of a naturally occurring carp population within a riverine system. We further examined the influence of environmental influences, such as habitat type and water temperature, on the distribution of carp eDNA.

This multifaceted approach aimed to not only develop a robust eDNA-based biomass estimation method but also to understand the intricate interplay between environmental factors and eDNA dynamics in a natural setting.

Materials and Methods

We focused on the *Labeo rohita* (Hamilton, 1822), as an ideal model organism for its global prevalence and ecological impact. Widely transported for ornamental, sport, and consumption purposes, Rohu carp introduces ecological disruptions, altering water quality, macrophyte abundance, and invertebrate diversity (Ghori et al., 2022). The species' impact is closely tied to population densities, crucial for understanding and managing its effects on aquatic ecosystems (Ali & Kaviraj, 2018; Rahman, 2015). Our study leverages these characteristics, making Rohu carp an insightful model organism within the global context of its distribution and ecological consequences (MILLER & CROWL, 2006).

Real-time quantitative PCR

We utilized real-time TaqMan® PCR on a StepOne-Plus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) for eDNA quantification. Short gene fragments of Mitochondrial cytochrome b (*MTCYB*) were amplified and quantified using specific primers: CpCyB_496F (5'-ACTAAGCCAACCCGGATCAC-3'), CpCyB_573R (5'- TGGCACGAGTCAGTTTCCAA-3'), and CpCyB_550p probe (5'-FAM-CACTTACACGATTCATCGCATTCCACATCC-TAMRA-3'). This primer is designed exclusively for Rohu carp, generating a 78 bp cytochrome b gene fragment. Primer specificity was tested against the sequences of 56 species from 19 families in the Kangsabati River, West Bengal, India, where our field study took place. The primers were meticulously designed to exhibit encountering a minimum of three discrepancies with non-target species. Given the coexistence of Indian indigenous-type carp and domesticated carp, belonging to the same

genetic cluster (Ito et al., 2014), we tailored the primers and probe to detect both carp types, addressing the ecological differences between them not clarified in their natural environment.
Quantitative real-time PCR (qPCR) was conducted in triplicate, and the mean value was used during assays. The limit of detection (LOD) for carp DNA using the qPCR assay was defined as one copy since one DNA copy was detected in at least two wells in each triplicate. In case any triplicate, yielded a negative outcome, it was attributed a value of zero. Details qPCR method are followed from Takahara et al (Takahara et al., 2012). Target sequence qPCR products were cloned into the pGEM-T Easy Vector, and a dilution series of the plasmid containing 3 x 10^1 to 3 x 10^4 copies were amplified as standards in triplicate in all qPCR assays. (Reference) Additionally, three wells of a no-template negative control were employed in all qPCR assays and exhibited no amplification.
The Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was employed for an *in-silico* specificity screen. The results indicated the potential amplification of two species in Kangsabati River (*Mylopharyngodon piceus* and *Opsariichthys uncirostris uncirostris*) utilising our primer set. Notably, *Mylopharyngodon piceus* is unlikely to reproduce in Kangsabati River, as recorded in surveys from 1953. The probe crafted in this investigation featured four deviations from the resembling sequence of *O. uncirostris uncirostris*. Additionally, qPCR amplicon sequencing directly confirmed that the amplified fragments indeed originated from carp DNA (see Results). Furthermore, attempts to amplify DNA extracted from tissues from closely connected and non-targeted species (*Carassius auratus langsdorfii*, *Carassius cuvieri*, and *Carassius buergeri grandoculis*) using these primers resulted in neither amplification nor amplification under the limit of detection (LOD). Therefore, our experimental system demonstrated a minimal tendency to overestimate the carp eDNA concentration.

Experiment 1: aquarium experiments
Juvenile common carp were hatched and raised at CIFRI, India, before being transported to a laboratory at the Centre for Aquaculture Research, Extension & Livelihood (CAREL), Vidyasagar University.
The carp were individually housed in 66 L plastic containers, with each container accommodating approximately 25 individuals. Continuous water filtration was maintained in these containers. The fish were fed a commercial diet three times a week and were kept at a temperature of 19 ± 1°C under a 12-hour light-dark cycle. All procedures adhered to current laws in India regarding experimental fish and were permitted by the safety management committee for trials conducted at CAREL.
We assessed the correlations among eDNA concentrations in the aquaria and three variables: water temperature, time, and fish biomass. Three separate aquarium experiments were carried out using plastic tanks (32 x 45 x 25 cm) filled with 8 L of aged tap water. Continuous aeration through a filter maintained water quality in the tanks. For each investigation, carp were by chance selected from the holding container and placed in the tanks.
They were provided with a diet free from any common carp materials, as confirmed by qPCR assay throughout all three days.
1. To assess temporal variations in eDNA concentrations, we measured the amount of eDNA replicates in the water at 1, 2, 3, 6, and 8 days following the introduction of carp to the tank. Tanks were kept with either 1 or 3 fish per tank, with each density replicated three times. The water temperature in the tanks was kept at 18 ± 1°C. At each designated time point, a 20-mL water sample was collected from each tank. Each fish's wet weight was measured at the experiment's conclusion (15.0 ± 3.0 g, n = 12, mean ± SD). Instantly post-collection, water samples were filtered using a centrifugal filter unit (Amicon Ultra-15, 30-kDa cutoff, UFC903096; Millipore, Billerica, MA, USA). The resulting sample solution was concentrated to a volume of 150 µL and stored in 1.5-mL microtubes (Eppendorf®) at -20°C. Subsequently, by using a DNeasy blood and tissue kit, eDNA from each sample solution was separated in an absolute volume of 100 µL. To verify the absence of carp eDNA in the water prior to the experiments, 2 tanks devoid of carp were established. A water sample gathered on day 6 underwent the same treatment as explained above.
2. To assess the impact of temperature, we kept fish (n = 3 fish per tank) at 7, 15, or 25°C (±1°C) with four tanks allocated to each treatment group. The temperature of the water in every tank was regulated using a heater with an integrated thermostat. On day 6, corresponding to the findings of the initial trial, a 45-mL water sample was collected from each tank. The wet weight of each individual of each carp was 15.5 ± 3.0 g (n = 36, mean ± SD). Subsequently, the water samples underwent concentration and extraction processes as listed earlier.
3. We examined the influence of carp biomass/abundance on eDNA concentration by introducing 1, 5, and 10 fish into a tank (with four tanks per treatment group). The aquatic temperature in the tanks was regulated at 18 ± 1°C. On day six, a 50-mL water sample was collected from each tank. Each carp had a wet weight of 15.8 ±

2.8 g (n = 64, mean ± SD). Subsequently, the water samples underwent concentration and extraction procedures as outlined earlier.

Experiment 2: pond experiment

To assess the correlations between carp biomass and abundance and eDNA concentrations in a natural setting, we introduced carp into 2 manmade ponds in Midnapore (22°25'N 87°18'E). The experiment spanned 21 days, conducted from November 30 to December 21, 2021. All our observational and field studies were conducted with permission from the Center for Aquatic Environments Research. Ponds A and B exhibited mean volumes of 47.5 and 40.2 m^3, with average in/outflow rates of 2.7 and 2.5 L min^{-1}, respectively. The water in the ponds was replenished at rates of 7.5% and 8.9% per day, separately. The environmental conditions in the ponds during the study period were recorded.

An isolated carp inhabited pond A for a year before the experiment, while pond B remained unoccupied during the same period. Initial eDNA analyses of water samples from each pond at day 0 revealed the presence of common carp DNA exclusively in pond A (refer to Results). Subsequently, one week before the experiment's initiation, a single carp was weighed and introduced into pond B (n = 1 fish per pond). On day 7, 2-L water samples were collected from the surface at three designated points in each pond. Additionally, two fin-clipped and weighed fish were added to each pond on this day (n = 3 fish per pond). By day 14, water samples were collected as before, and an additional 12 fish were weighed, marked, and six of them were introduced into each pond (n = 9 fish per pond). On day 21, samples of water were stored, and the lone unmarked fish in pond A was captured to measure its wet weight. The mean value for the 3 sampling spots per pond represented the eDNA present on the respective sampling day in each pond. The individual wet weight of carp in both ponds was 973 ± 569 g (n = 15, mean ± SD).

Followed by a 0.8-µm pore size filter we concentrated eDNA by filtering each water sample through either a 3.0-µm pore size filter or a pre-filter (12.0-µm pore size). Filtration was carried out using filter holders (Nalgene® NL300-4100) and a vacuum pump. Subsequently, filter discs containing the samples were transferred to DNA-free 50-mL conical tubes (BD Falcon™) using tweezers. The tubes were sealed and transported on ice in a cooling box to CAREL and stored at -20°C until the next day. Rigorous rinsing with purified water among filtration operations was managed to prevent cross-uncleanness. To assess the absence/presence of common carp eDNA in both ponds, a beginning qPCR assay was executed before the testing using the 0.8-µm filter.

The filter discs within each tube were immersed in 10 mL of distilled water and agitated on a rotary shaker at utmost speed for 12 minutes. Subsequently, the suspension underwent concentration via centrifugal filtration (Amicon Ultra-15, 5000 x g for 10–15 minutes). This process was iterated three times for each tube. The adequacy of carp eDNA extraction from the filter discs was affirmed as the pigmentation remnants from the filtration were effectively eliminated. The resulting sample solutions were concentrated to volumes of 350 µL and preserved at -20°C in microtubes size 1.5-mL (Eppendorf®). The eDNA within the sample mixtures was then extracted using the previously described method.

Field survey

Water samples were obtained from a freshwater lagoon, Kangsabati River (sampling sites, and coordinates are depicted in Fig 4) between 10:50 and 12:20 on February 8, 2022. To minimize water mixing during collection, the boat moved carefully from downstream to upstream sites. Inhabited by various cyprinid fish, including common carp for breeding, the lagoon's conditions included water temperature ranging from 5.9 to 10.7°C, conductivity from 0.009 to 0.018 S m^{-1}, dissolved oxygen from 10.7 to 13.3 mg L^{-1}, pH from 7.2 to 7.8, turbidity from 0.4 to 12.1 NTU, also chlorophyll a concentration from 0.0 to 6.2 µg L^{-1}. A multi-water profiler (6600EDS; YSI, Yellow Springs, OH, USA) monitored water chemistry. No permits were necessary for the conducted field studies.

We gathered 2-L water samples from the surface at 5 locations within the river. These samples were kept in DNA-free 4-L bottles and promptly moved to the test center.

The 2-L water samples underwent filtration using a 3.0-µm membrane filter. This specific pore-size filter was determined to be the most effective based on findings from the outdoor pond experiments (refer to Results). Every filter disc holding the sample was carefully folded with tweezers, encased in DNA-free aluminium foil, and immediately stored at -20°C until subsequent analysis.

To extract eDNA from common carp, the filter discs with the samples were carefully transferred into autoclaved 500-mL Nalgene® bottles using tweezers. Subsequently, the filter discs within the bottles were immersed in distilled water and agitated, following the procedure outlined in the pond experiment mentioned earlier. The resulting sample solution underwent concentration and extraction using the methods detailed earlier.

DNA sequencing

To verify the specificity of the previously mentioned primer set for the ground samples, qPCR amplicons from all sites showing positive qPCR results were subject to direct sequencing after treatment with ExoSAP-IT™.

Statistical analyses

We assessed the impact of temperature on eDNA concentration using a one-way analysis of variance (ANOVA, $\alpha = 0.05$). In both pond and aquarium experiments, the correlation between eDNA concentration and carp biomass per one L water sample was analyzed using a Type II regression, while the connection between the quantity of carp and biomass was evaluated using a Type I regression. For the field survey, a general linear model (GLM) (Venables & Ripley, 2002) was employed to examine the correlation between six environmental factors such as habitat type (shore or offshore), dissolved oxygen (DO), water temperature, conductivity, pH, and chlorophyll *a* concentration with eDNA concentration. The GLM factors were standardized and centered, and model selection followed a downward stepwise procedure based on the Akaike Information Criterion (Akaike, 1998, 2015). Before GLM analysis, a variance inflation factor (VIF) was used to assess factor co-linearity, with a maximum VIF of 4.4 suggesting no significant influence on the GLM results. To normalize ANOVA values, eDNA concentrations were log10-transformed based on the results of the Shapiro-Wilk normality test ($\alpha = 0.05$). ANOVA was conducted using IBM SPSS Statistics (version 20) while other statistical analyses were performed in R ver. 2.13.0 (Team, 2021).

Results

Experiment 1: aquarium experiments

The peak amount of eDNA copies was observed in tanks with 3 fish on day one and in tanks with 1 fish on day two (20,536 ± 6,807 and 3,698 ± 1,406 copies per 20-mL sample, mean ± SD) (Fig. 1a). Subsequently, the number of eDNA copies slowly declined, showing minimal change between days six and eight (Limit: 3 fish, 1,903–2,297; one fish, 296–235 copies). This indicated that the amount of eDNA copies reached a plateau on day 6. Consequently, eDNA concentration sampling in the following experiments was conducted on day 6. No carp eDNA was revealed in the negative control tanks with no carp.

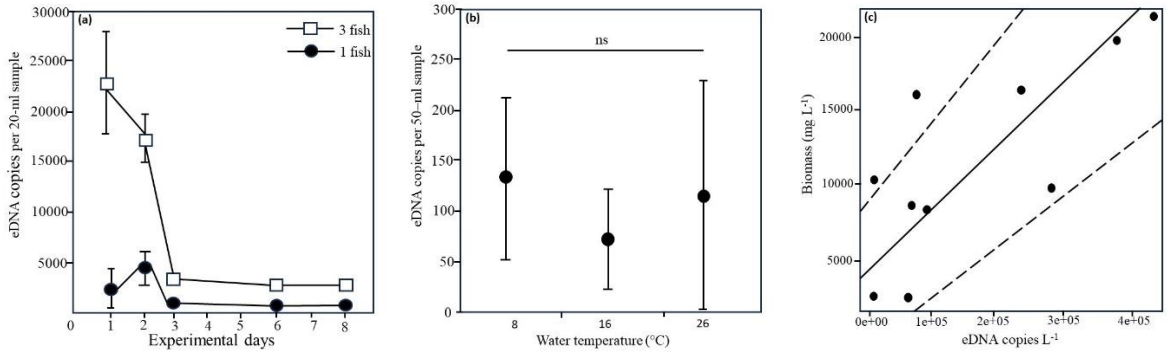


Figure 1 illustrates the associations among the concentration of environmental DNA (eDNA) from carp and three influencing factors, namely duration, water temperature, and biomass, as observed in aquarium experiments. (a) Depicts the time-dependent fluctuation in eDNA concentration at two different biomass levels (one or three fish per tank). The error bars indicate the ±1SD. (b) Examines the impact of temperature on eDNA concentrations six days after the introduction of fish to the tank. The notation "n.s." denotes no significant differences. The error bars represent ±1SD. (c) Demonstrates the correlation between eDNA concentration and carp biomass per 1-L water six days after introducing fish to the tank. The regression analysis showed statistical significance ($p<0.05$). The dotted lines represent the lower or upper limits of the 95% confidence intervals for the slope of the regression.

Water temperature exhibited no significant impact on the number of eDNA replicates ($F_{2,9}= 1.29$, $p = 0.31$; Fig. 1b). Conversely, a noteworthy positive correlation was found between the number of carp biomass and eDNA copies per one L ($y = 0.051x+2801$, $R^2= 0.59$, $p= 0.001$; Fig. 1c). This supports the utilization of eDNA for estimating carp biomass via a Type II regression model. Furthermore, the correlation between the carp number and biomass was notably positive ($y = 16776x – 626.30$, $R^2= 0.95$, $p<0.001$ in Supporting Information, Fig. S1a).

Experiment 2: pond experiment

Beforehand the research commenced (day 0), carp eDNA was identified in pond A (i.e., the pond with 1 fish for 1 year: 56 copies L^{-1} on the 0.8-µm filter) but not in pond B (i.e., the pond 0 fish earlier the experiment). A +ve relationship existed from days 7 to 21, between the number of carp biomass and eDNA copies per one L for samples treated using both 3.0-µm filters [pond A: 39±44 (1 carp $pond^{-1}$), 698±248 (3 carp $pond^{-1}$), 1,418 ± 1,112 (9 carp $pond^{-1}$); pond B: 297 ± 156 (1), 769 ± 546 (3), 1,978 ± 1,361 (9), mean ± SD, n = 3] and 0.8-µm filters [pond A: 22 ± 16 (1), 278 ± 151 (3), 1,1612 ± 1,021 (9); pond B: 24 ± 17 (1), 138 ± 147 (3), 812 ± 616 (9)]. The 3.0-µm filter (Fig. 2a) brings in marginally better results associated with the 0.8-µm filter, (Fig. 2b). The correlation between the biomass and number of carp was meaningful +ve ($y = 7.896x + 32.06$, $R^2 = 0.94$, $p < 0.001$ in Supporting Information, Fig. S1b).

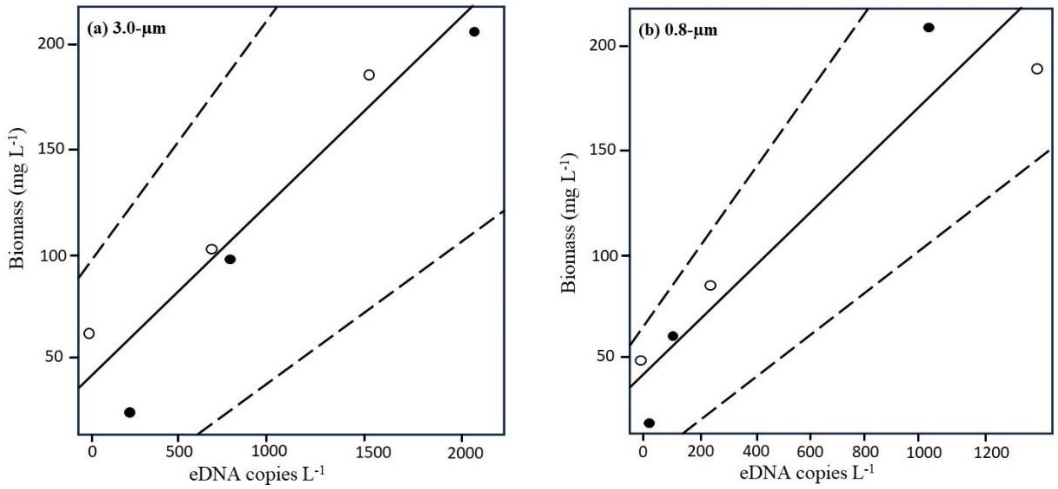
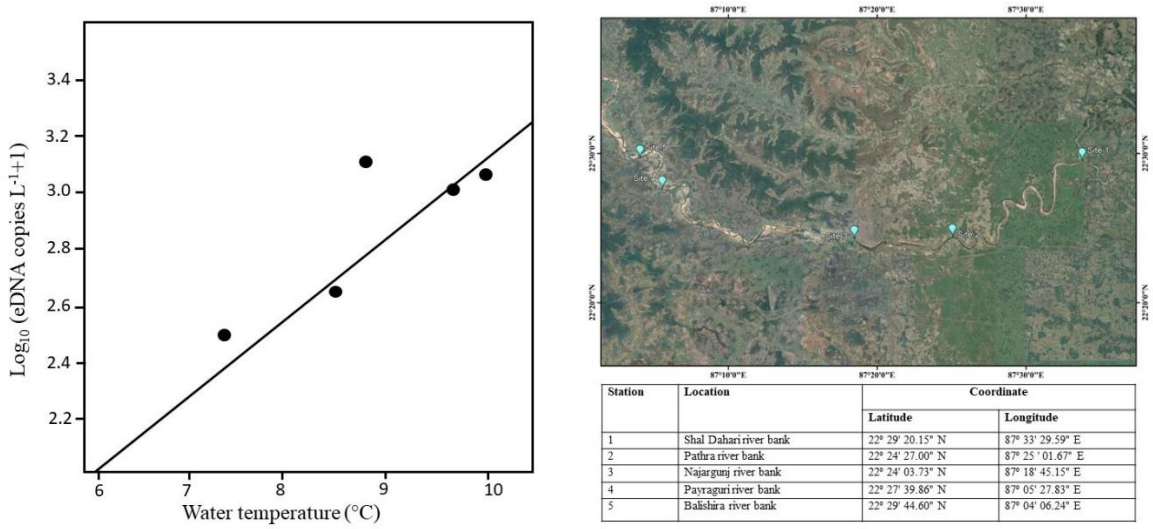


Figure 2 illustrates the connections between the concentration of environmental DNA (eDNA) originating from carp and their biomass in an outdoor pond experiment. The eDNA in the water samples was concentrated using two different pore-sized filters: (a) 3.0-µm and (b) 0.8-µm. For both filter types, the regression lines indicate a positive relationship between eDNA concentration and carp biomass per 1-L water, as revealed in the Results. The dotted lines on the graph represent the lower or upper limits of the 95% confidence intervals for the slope of the regression. The data points are differentiated by open and closed circles, representing information from ponds A and B, respectively.



Station	Location	Coordinate	
		Latitude	Longitude
1	Shal Dahari river bank	22° 29' 20.15" N	87° 33' 29.59" E
2	Pathra river bank	22° 24' 27.00" N	87° 25 ' 01.67" E
3	Najargunj river bank	22° 24' 03.73" N	87° 18' 45.15" E
4	Payraguri river bank	22° 27' 39.86" N	87° 05' 27.83" E
5	Balishira river bank	22° 29' 44.60" N	87° 04' 06.24" E

Figure 3 depicts the associations between the concentration of environmental DNA (eDNA) from fish and water temperature in the Kangsabati River. The regression line, analyzed through GLM, exhibits a statistically significant trend ($p<0.05$), as indicated in the Results).

Figure 4 depicts the 5 sampling sites and their coordinates.

Field survey

The correlation between the eDNA concentration of carp and the water temperature was highly positive ($p < 0.001$; Fig. 3). The estimated carp biomass varied from 0 to 278 mg L^{-1} (calculated from 0– 2,869 copies L^{-1} in eDNA application) at the 5 sites in the river. To confirm the specificity of the primer set, we precisely sequenced the qPCR amplicons, excluding the sites that were negative for the qPCR assay. Entire sequences from every qPCR amplicon at Kangsabati River were proved to be from common carp.

Discussion

We have devised a technique for assessing fish biomass by analyzing concentrations of environmental DNA (eDNA) in water samples, providing a rapid and non-invasive approach for monitoring freshwater fish populations. In our aquarium experiments, eDNA concentrations peaked immediately after introducing fish, likely due to increased DNA discharge during fish acclimation. Subsequently, eDNA concentrations stabilized by the sixth day, suggesting an equilibrium between DNA release and degradation. The positive correlation between eDNA concentration and fish biomass observed in outdoor pond and aquarium experiments supported the hypothesis that eDNA reflects target species biomass. The use of a 3.0-µm filter facilitated efficient eDNA collection from lentic environments, while smaller filters required pre-filtration and increased labour. In a natural river, eDNA concentration varied significantly among sites, with a strong positive correlation between eDNA concentration and water temperature. The absence of carp eDNA at certain sites, despite proximity to positive sites, indicated limited mixing and complete degradation at each location.

Temperature emerged as a major driver of carp eDNA distribution, suggesting a link between temperature-dependent carp behaviour and eDNA release. The study, conducted during winter, indicated that carp might prefer warmer habitats to maintain metabolism. While the current analysis focused on six environmental factors, future modelling should consider additional factors, such as predation, to enhance predictions of carp distribution.

This eDNA-based method offers advantages over traditional biomass estimation methods, enabling easier and faster monitoring of fish populations. Continuous eDNA sampling could predict microhabitats crucial for reproduction, feeding, and refuge. The approach holds promise for monitoring seasonal changes and supporting conservation management. Further research should expand field data and compare this method with existing estimation techniques for improved accuracy. Additionally, investigations into the impact of stressful environments on eDNA release could enhance our understanding and refine the method's applicability. Overall, this eDNA-based approach represents a valuable tool for monitoring fish populations and predicting infectious diseases like *Cyprinid herpesvirus 3* (Minamoto et al., 2012).

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Availability of data and material

All data generated or analysed during this study are included in this article.

References:

1. Akaike, H. (1998). Information Theory and an Extension of Information the Maximum Theory Likelihood and an Principle Extension of the Maximum Likelihood Principle. Biogeochemistry, 1998. https://doi.org/10.1007/978-1-4612-1694-0_15
2. Akaike, H. (2015). Information Theory and an Extension of the Maximum Likelihood Principle. In Trees - Structure and Function (Vol. 29, Issue 6). https://doi.org/10.1007/978-1-4612-0919-5_38

3. Aldeguer-Riquelme, B., Ramos-Barbero, M. D., Santos, F., & Antón, J. (2021). Environmental dissolved DNA harbours meaningful biological information on microbial community structure. Environmental Microbiology, 23(5). https://doi.org/10.1111/1462-2920.15510
4. Ali, S., & Kaviraj, A. (2018). Aquatic weed Ipomoea aquatica as feed ingredient for rearing Rohu, Labeo rohita (Hamilton). Egyptian Journal of Aquatic Research, 44(4). https://doi.org/10.1016/j.ejar.2018.09.004
5. Andruszkiewicz, E. A., Starks, H. A., Chavez, F. P., Sassoubre, L. M., Block, B. A., & Boehm, A. B. (2017). Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS ONE, 12(4). https://doi.org/10.1371/journal.pone.0176343
6. Banerjee, P., Dey, G., Antognazza, C. M., Sharma, R. K., Maity, J. P., Chan, M. W. Y., Huang, Y. H., Lin, P. Y., Chao, H. C., Lu, C. M., & Chen, C. Y. (2021). Reinforcement of environmental dna based methods (Sensu stricto) in biodiversity monitoring and conservation: A review. In Biology (Vol. 10, Issue 12). https://doi.org/10.3390/biology10121223
7. Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. In Conservation Genetics (Vol. 17, Issue 1). https://doi.org/10.1007/s10592-015-0775-4
8. Chapman, D. C., Benson, A. J., Embke, H. S., King, N. R., Kočovský, P. M., Lewis, T. D., & Mandrak, N. E. (2021). Status of the major aquaculture carps of China in the Laurentian Great Lakes Basin. In Journal of Great Lakes Research (Vol. 47, Issue 1). https://doi.org/10.1016/j.jglr.2020.07.018
9. de Sousa, L. L., Silva, S. M., & Xavier, R. (2019). DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. In Environmental DNA (Vol. 1, Issue 3). https://doi.org/10.1002/edn3.27
10. Everts, T., Van Driessche, C., Neyrinck, S., De Regge, N., Descamps, S., De Vocht, A., Jacquemyn, H., & Brys, R. (2022). Using quantitative eDNA analyses to accurately estimate American bullfrog abundance and to evaluate management efficacy. Environmental DNA, 4(5). https://doi.org/10.1002/edn3.301
11. Ghori, I., Tubassam, M., Ahmad, T., Zuberi, A., & Imran, M. (2022). Gut microbiome modulation mediated by probiotics: Positive impact on growth and health status of Labeo rohita. Frontiers in Physiology, 13. https://doi.org/10.3389/fphys.2022.949559
12. Ito, T., Kurita, J., & Yuasa, K. (2014). Differences in the susceptibility of Japanese indigenous and domesticated Eurasian common carp (Cyprinus carpio), identified by mitochondrial DNA typing, to cyprinid herpesvirus 3 (CyHV-3). Veterinary Microbiology, 171(1–2). https://doi.org/10.1016/j.vetmic.2014.03.002
13. MILLER, S. A., & CROWL, T. A. (2006). Effects of common carp (Cyprinus carpio) on macrophytes and invertebrate communities in a shallow lake. Freshwater Biology, 51(1). https://doi.org/10.1111/j.1365-2427.2005.01477.x
14. Minamoto, T., Honjo, M. N., Yamanaka, H., Uchii, K., & Kawabata, Z. (2012). Nationwide Cyprinid herpesvirus 3 contamination in natural rivers of Japan. Research in Veterinary Science, 93(1). https://doi.org/10.1016/j.rvsc.2011.06.004
15. Olden, J. D., Kennard, M. J., Leprieur, F., Tedesco, P. A., Winemiller, K. O., & García-Berthou, E. (2010). Conservation biogeography of freshwater fishes: Recent progress and future challenges. Diversity and Distributions, 16(3). https://doi.org/10.1111/j.1472-4642.2010.00655.x
16. Pollock, L. J., O'Connor, L. M. J., Mokany, K., Rosauer, D. F., Talluto, M. V., & Thuiller, W. (2020). Protecting Biodiversity (in All Its Complexity): New Models and Methods. In Trends in Ecology and Evolution (Vol. 35, Issue 12). https://doi.org/10.1016/j.tree.2020.08.015
17. Rahman, M. M. (2015). Effects of co-cultured common carp on nutrients and food web dynamics in rohu aquaculture ponds. Aquaculture Environment Interactions, 6(3). https://doi.org/10.3354/aei00127
18. Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. In Journal of Applied Ecology (Vol. 51, Issue 5). https://doi.org/10.1111/1365-2664.12306
19. Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. Environmental DNA, 4(1). https://doi.org/10.1002/edn3.185
20. Schneider, J., Valentini, A., Dejean, T., Montarsi, F., Taberlet, P., Glaizot, O., & Fumagalli, L. (2016). Detection of invasive mosquito vectors using environmental DNA (eDNA) from water samples. PLoS ONE, 11(9). https://doi.org/10.1371/journal.pone.0162493

21. Senapati, D., Bhattacharya, M., Kar, A., Chini, D. S., Das, B. K., & Patra, B. C. (2019). Environmental DNA (eDNA): A Promising Biological Survey Tool for Aquatic Species Detection. Proceedings of the Zoological Society, 72(3). https://doi.org/10.1007/s12595-018-0268-9
22. Sigsgaard, E. E., Torquato, F., Frøslev, T. G., Moore, A. B. M., Sørensen, J. M., Range, P., Ben-Hamadou, R., Bach, S. S., Møller, P. R., & Thomsen, P. F. (2020). Using vertebrate environmental DNA from seawater in biomonitoring of marine habitats. Conservation Biology, 34(3). https://doi.org/10.1111/cobi.13437
23. Stewart, K. A. (2019). Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. In Biodiversity and Conservation (Vol. 28, Issue 5). https://doi.org/10.1007/s10531-019-01709-8
24. Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. PLoS ONE, 7(4). https://doi.org/10.1371/journal.pone.0035868
25. Team, R. C. (2021). R: A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing.
26. Venables, W. N., & Ripley, B. D. (2002). Statistics Complements to Modern Applied Statistics with S. In Modern Applied Statistics with S.
27. Walker, D. M., Leys, J. E., Dunham, K. E., Oliver, J. C., Schiller, E. E., Stephenson, K. S., Kimrey, J. T., Wooten, J., & Rogers, M. W. (2017). Methodological considerations for detection of terrestrial small-body salamander eDNA and implications for biodiversity conservation. Molecular Ecology Resources, 17(6). https://doi.org/10.1111/1755-0998.12667