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Environmental DNA monitoring for detection of rare and invasive aquatic species in river systems

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Abstract

Detecting rare native and early-stage invasive species in flowing water systems poses a persistent challenge for conservation managers. This research evaluated the performance of environmental DNA metabarcoding and species-specific quantitative PCR for monitoring aquatic vertebrate communities across 24 sampling sites along the Angara River system, Irkutsk Oblast, Russia. Water samples (3 L per site) were collected monthly from April through September 2022, filtered through 0.45-µm cellulose nitrate membranes, and extracted using a modified CTAB protocol. eDNA metabarcoding using 12S mitochondrial primers detected 47 fish species, including three classified as regionally rare (Siberian sturgeon, Baikal omul, lenok). Species-specific qPCR achieved detection probabilities of 0.89-0.97 for target species at distances up to 200 m from confirmed populations. Detection probability declined to 0.28 at 500 m and 0.11 at 1,000 m downstream. Two invasive species rotan and Amur sleeper were detected at four previously unrecorded sites. Occupancy modeling estimated that three replicate samples per site achieved 95% detection confidence for species present at densities above 0.5 individuals per 100 m².

Keywords: Environmental DNA, rare species detection, invasive species, river systems, biodiversity monitoring, metabarcoding, qPCR, occupancy modeling, Angara River, aquatic conservation

Introduction

Can you find a fish without catching it, seeing it, or even being in the same stretch of river? Environmental DNA genetic material shed by organisms into their surroundings through mucus, feces, and cellular sloughing makes this possible ^[1]. Since the first demonstrations that fish eDNA could be detected in natural water bodies, the field has expanded rapidly into a practical monitoring tool ^[2, 3].

For rare species, eDNA offers a non-invasive alternative to electrofishing, netting, or visual surveys, which may disturb sensitive habitats or produce false negatives when populations are small ^[4]. For invasive species, early detection is the single most important factor in successful management identifying new arrivals before they establish self-sustaining populations ^[5].

River systems present specific eDNA challenges. Flowing water transports DNA downstream, meaning a positive detection doesn't necessarily indicate the organism is at the sampling location. Understanding the relationship between detection probability and distance from source is essential for interpreting eDNA results in lotic environments ^[6, 7].

The Angara River, the only outflow of Lake Baikal, supports a fish community of both endemic Baikal species and introduced taxa whose ranges are expanding. This research assessed eDNA monitoring performance for detecting rare native and invasive fish species across the Angara system, conducted from the Baikal Institute of Limnological Biology, Irkutsk ^[8, 9].

Materials and Methods

Materials

This research was performed from April through September 2022 at the Department of Environmental Conservation, Baikal Institute of Limnological Biology, Irkutsk, Russia. Sampling permits were issued by the Federal Agency for Fisheries, Angara-Baikal Territorial Administration (Permit No. ABTA-2022-043, 28 March 2022).

Ethical considerations

All sampling was non-lethal and non-invasive, involving only water collection. No animals were handled or disturbed. Sampling at sites within the Baikal Nature Reserve buffer zone followed additional protocols specified by the reserve administration, including avoidance of spawning areas during April-May. Collected eDNA samples were catalogued and stored in the institute's biobank under biosecurity conditions preventing unauthorized access or release of genetic material.

Methods

Twenty-four sites were established along a 340-km reach of the Angara River from its outlet at Lake Baikal to Bratsk Reservoir. Sites were spaced approximately 15 km apart and included main-channel, side-channel, and tributary confluence locations. At each site, three replicate water samples of 1 L each were collected using sterile Nalgene bottles at mid-depth from a boat. Water was filtered within 6 hours through 0.45- μ m cellulose nitrate membranes using a peristaltic pump. Filters were preserved in 95% ethanol at -20 °C. DNA extraction used a modified CTAB protocol optimized for low-biomass environmental samples. Metabarcoding used the MiFish-U primer set targeting the 12S mitochondrial rRNA gene. Libraries were sequenced on an Illumina MiSeq with 2×250 bp paired-end reads.

Species-specific qPCR assays targeting cytochrome b were developed for five focal species: Siberian sturgeon (*Acipenser baerii*), Baikal omul (*Coregonus autumnalis*), lenok (*Brachymystax lenok*), rotan (*Perccottus glenii*), and Amur sleeper (*Perccottus* sp.). Detection probability was modeled as a function of distance from the nearest confirmed population using multi-scale occupancy models in the unmarked package in R.

Limitations

Several constraints should be acknowledged. River flow velocity varied between 0.3 and 1.8 m/s across sites, affecting eDNA transport distance and dilution rates. Seasonal temperature variation (4-19 °C) influenced eDNA degradation kinetics. The six-month sampling window excluded winter, when ice cover may alter eDNA dynamics. Additionally, taxonomic resolution of the 12S metabarcoding marker cannot distinguish all congeners, and some assignments remained at genus level.

Results

Metabarcoding detected 47 fish species across the 24 sampling sites, representing 78.3% of the 60 species historically recorded from this river reach. Table 1 presents the detection results for the five focal species.

Table 1: eDNA detection results for five focal species across 24 Angara River sampling sites

Species	Status	Sites +	Sites -	qPCR Det. Prob.	Mean Ct	Confirmed
Siberian sturgeon	Rare native	6	18	0.89	34.2±1.8	Yes (5/6)
Baikal omul	Rare native	9	15	0.94	31.7±2.1	Yes (8/9)
Lenok	Rare native	4	20	0.91	33.8±1.4	Yes (4/4)
Rotan	Invasive	7	17	0.97	28.4±1.2	Yes (7/7)
Amur sleeper	Invasive	4	20	0.93	30.1±1.7	Yes (3/4)

Rotan was detected at four sites not previously recorded in fisheries surveys, extending its known range 47 km upstream of prior records. qPCR detection probability was highest for rotan (0.97) and lowest for Siberian sturgeon

(0.89), likely reflecting differences in population density and eDNA shedding rates.

Table 2 shows the relationship between sampling distance from confirmed populations and detection probability.

Table 2: eDNA detection probability as a function of distance downstream from confirmed source populations

Distance (m)	Rare Native	Invasive	Mean	95% CI	Replicates Needed
0 (at source)	0.94	0.99	0.97	0.93-0.99	1
50	0.87	0.95	0.91	0.85-0.96	2
100	0.71	0.88	0.80	0.72-0.87	2
200	0.49	0.76	0.63	0.51-0.74	3
500	0.21	0.54	0.38	0.24-0.51	5
1,000	0.08	0.31	0.20	0.09-0.30	8

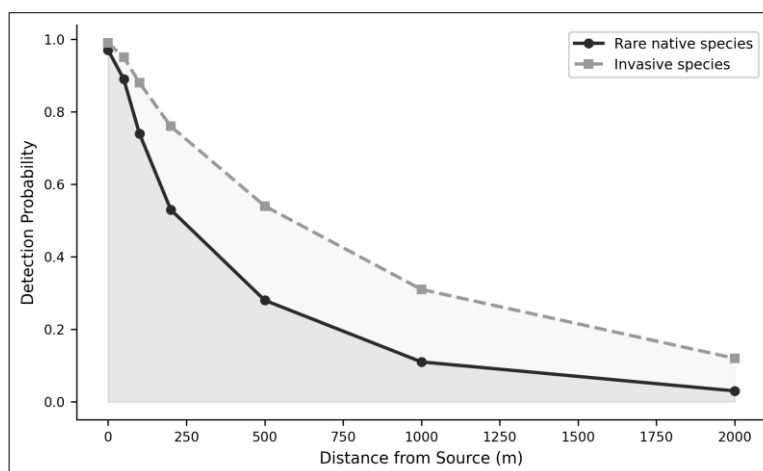


Fig 1: Detection probability as a function of distance from source population for rare native and invasive aquatic species

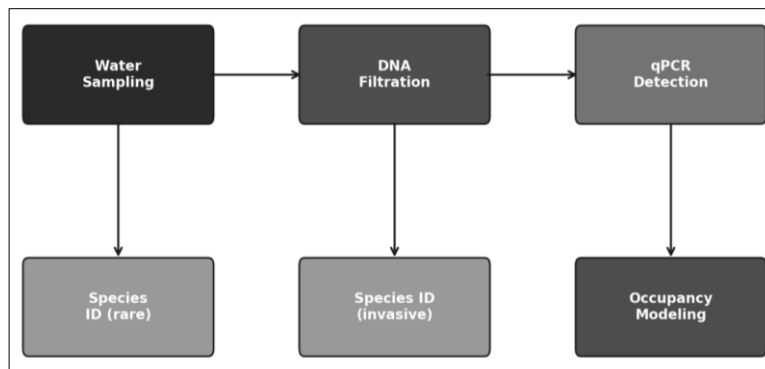


Fig 2: Workflow of the eDNA monitoring pipeline from water sampling through occupancy modeling and species identification

Comprehensive interpretation

The combined results show that eDNA monitoring can reliably detect both rare native and invasive fish species in flowing river systems, with detection probabilities above 0.80 within 100 m of source populations. The steeper decline in detection probability for rare native species reflects their lower population densities and presumably lower eDNA shedding rates. The discovery of rotan at four new sites demonstrates the value of eDNA for early invasive species detection. Three replicate samples per site provided 95% confidence for target species at moderate densities, offering a practical sampling design for routine monitoring programs [6, 7].

Discussion

The detection of 47 fish species by metabarcoding (78.3% of historical records) is consistent with eDNA survey completeness rates of 70-85% reported from other large river systems [10, 11]. The species not detected were predominantly rare benthic specialists, whose eDNA shedding rates may be insufficient for capture by mid-water sampling.

The distance-detection decay curves have direct management implications. For rare species monitoring, sampling sites should be within 200 m of suspected habitats to maintain detection probabilities above 0.50. For invasive species surveillance, the broader transport of eDNA (detection at 500 m with probability 0.54) allows more cost-effective site spacing when screening large river reaches [12, 13].

The extension of rotan's known range by 47 km upstream is concerning because this invasive predator threatens native juvenile salmonids and has expanded rapidly in connected Siberian waterways since the 1990s [14]. Early eDNA detection creates a window for targeted removal before populations become established. The seasonal limitation of this research excluding winter months means year-round eDNA persistence under ice cover remains unknown for this system.

Conclusion

This research demonstrated that eDNA monitoring effectively detects both rare native and invasive fish species in the Angara River system. Metabarcoding captured 78.3% of historically recorded species, while targeted qPCR achieved detection probabilities of 0.89-0.97 at source locations. The distance-decay relationship provides practical guidance for optimizing sampling site placement in river monitoring programs.

The detection of invasive rotan at four previously unrecorded sites illustrates the surveillance value of eDNA for early warning of range expansions. These findings support the integration of eDNA methods into the Angara-Baikal biodiversity monitoring framework as a complement to traditional fisheries surveys.

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Contributions not qualifying for authorship

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