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ORIGINAL ARTICLE

Environmental DNA reveals the ecology and seasonal migration of a rare sturgeon species in the Ural River

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Abstract

Environmental DNA (eDNA) technology is an essential tool for monitoring living organisms in ecological research. The combination of eDNA methods with traditional methods of ecological observation can significantly improve the study of the ecology of rare species. Here, we present the development and application of an eDNA approach to identify rare sturgeons in the lower reaches of the Ural River (Zhaiyk) (~1084 km). The presence of representatives of the genus Sturgeon was detected at all sites in spring (nine sites) and autumn (ten sites) while they were absent during the summer period, consistent with their semi-anadromous ecology. Detection in spring and autumn indicates the passage of spring and winter forms to the lower and upper spawning grounds, respectively. This study confirms the difficulties of species-specific identification of Eurasian sturgeon and provides the first documented eDNA detection of specimens of the genus Sturgeon in the Ural River. It also provides a biogeographic snapshot of their distribution, experimentally confirming their seasonal migrations in the lower reaches of the river. The successful detection of sturgeon motivates further eDNA surveys of this and other fish species for accurate species identification and population assessment, opening up prospects for the management of these threatened species.

KEYWORDS
Caspian sturgeons, conservation, eDNA isolation, environmental DNA, spawning, Ural River

1 | INTRODUCTION

Sturgeons (Acipenseriformes) are ancient fish dating back to the early Jurassic (approx. 200 MYBP) (Bemis et al., 1997). All 27 species are listed on the IUCN Red List as vulnerable to critically endangered (IUCN 2023), with 23 under CITES control. Between 1970 and 2016, global sturgeon specimens decreased by around 91% (Secretariat, 2022; Yarushina et al., 2009). Factors like intensive fishing, poaching, habitat loss, and pollution have greatly affected sturgeon stocks worldwide (IUCN 2023) (Rosenthal et al., 2006). Sturgeons, with their long lifespan, serve as flagship species reflecting ecosystem quality. Their fate is critical in European rivers, where the Atlantic sturgeon became extinct in the 1960s in the Danube basin (Jarić et al., 2009). Ship sturgeon population was functionally extinct by 2002 (Jarić et al., 2009). Sterlet is the only sturgeon species in the Upper Danube, with only 20 individuals recorded in 2020. Russian sturgeon, stellate sturgeon, and beluga are restricted by dams, and their extinction is predicted in the Lower Danube.

Conservation efforts like the Action Plan for the Conservation of the Sturgeons (Bloesch et al., 2006) and the "Sturgeon 2020" program aim to protect sturgeons in the Danube. The LIFE-Sterlet Project (2015–2022) and LIFE-Boat 4 Sturgeon (2022–2030) focus on strengthening populations through hatchery breeding. Despite global IUCN Red List inclusion, only three sturgeon species in the Republic of Kazakhstan's Red Data Book—ship sturgeon, Syr Darya shovelnose, and Siberian sturgeon—are categorized as endangered or extinct. Historically, Kazakhstan hosted eight sturgeon species, ranging from vulnerable to critically endangered (Table 1), IUCN Red List of Threatened Species (IUCN 2023). The Ural–Caspian basin has five noncommercial sturgeon species: Russian and Persian sturgeons, beluga, stellate, and ship sturgeons, along with one potamodromous species—sterlet.

The Caspian Sea and rivers flowing into it are the last of the preserved natural habitats and hence in need of conservation; they are also the habitats in which most of the worldwide sturgeon populations on the verge of extinction (Rosenthal et al., 2006) are currently found. Most sturgeons, as migratory fish, spend some periods of their life in rivers after migrating from the sea between early spring and late autumn; beluga do not stop migrating, even in winter. Spawning begins at the onset of spawning temperatures from May to June, and their juveniles move downstream shortly thereafter (Kulikov et al., 2018). Rivers are convenient for studying sturgeon biology and ecology, as well as for counting migrating individuals during the spawning period. The Ural River is the only river in the Caspian basin with unregulated flow in the lower and middle reaches, which currently has preserved—albeit not sufficiently qualitative—natural spawning grounds. Another unique feature of this river is that it is inhabited by roughly 11 anadromous and potamodromous endangered fish species (The Red Data Book, IUCN 2023). The Ural River is the third longest river in Europe, with an average annual water flow of 380 m^3/s near the Kushum village, flowing through the territory of two countries with different cultural, political, and environmental heritage.

Currently, the number of populations have reduced to critical values, biological parameters of individuals have reached a minimum, and only single spawners pass for spawning. As a result, no annual juvenile sturgeon is observed in the river. The spawner catch for artificial reproduction is also one of the reasons for the absence of sturgeon. Winter forms have not been found in the spawning populations of the Ural River since the mid-1990s, which disrupted the intraspecific differentiation in populations, except for a small amount of winter stellate sturgeon. According to various studies, the effectiveness of natural reproduction of sturgeons in the river Ural has now been reduced to zero, past the point of no return (Kulikov et al., 2018). The Persian sturgeon has also disappeared from catches since 1990. Since 2008, the spawners of the ship sturgeon have not been found in the river. The last sturgeon of its juveniles was recorded in 2007, and migrations of beluga and sturgeon juveniles only occurred until 2010. Currently, there are irregular reports of sturgeon of juvenile stellate sturgeon and sterlet by single specimens because single anadromous spawners pass to

TABLE 1 Representatives of *Acipenseridae* family in Kazakhstan.

Scientific name	Common name	Location	IUCN status	Status in Kazakhstan
Huso huso (Linnaeus, 1758)	Beluga	Ural-Caspian basin	Critically Endangered (CR)	–
Acipenser gueldenstaedtii Brandt, 1833	Russian sturgeon	Ural-Caspian basin	Critically Endangered (CR)	–
Acipenser persicus Borodin, 1897	Persian sturgeon	Ural-Caspian basin	Critically Endangered (CR)	–
Acipenser baerii Brandt, 1869	Siberian sturgeon	Irtysh river basin	Critically Endangered (CR)	Endangered (Category II)
Acipenser stellatus Pallas, 1771	Stellate sturgeon	Ural-Caspian basin	Critically Endangered (CR)	–
Acipenser nudiventris Lovetsky, 1828	Ship sturgeon	Ural-Caspian, Aral-Syrdarya, and Ili-Balkhash basins	Critically Endangered (CR)	Extinct (Category I) (Aral and Ili populations)
Acipenser ruthenus Linnaeus, 1758	Sterlet	Ural-Caspian, Tobol and Irtysh rivers basins	Vulnerable (VU)	–
Pseudoscaphirhynchus fedtschenkoi Kessler, 1872	Syr Darya shovelnose sturgeon	Syr Darya river basin	Critically Endangered (CR)	Extinct (Category I)

spawning grounds. During 2010–2016, 220 specimens of juvenile stellate sturgeon were reported to be migrating (156 in 2014 and 64 in 2016). In 2018, only 56 specimens of sterlet and stellate sturgeon juveniles were caught. Moreover, during the period from 2007 to 2021 in the West Kazakhstan region, only a single sterlet fry was registered between 2010 and 2012 (Kadimov et al., 2018). To reduce the anthropogenic load on the Ural–Caspian basin, it is necessary to consolidate the integrated management of water resources of all the Caspian littoral countries by applying the principles outlined by the Integrated Water Resources Management and Ramsar Convention (Rosenthal et al., 2006). In June 2007, in Orenburg (Russia), the First International Ural River Basin Workshop (NATO Advanced Research Workshop) was organized with participation of researchers from Russia and Kazakhstan, FAO, the Secretariat of the Ramsar Convention on Wetlands (RAMSAR), the International Association for Danube Research, and many others. As an outcome of the conference, a resolution "Rescue of Sturgeon Species by Means of Transboundary Integrated Watershed Management in the Ural River Basin" was adopted.

To understand the biology of sturgeons, and for many production processes in Kazakhstan, telemetry methods are applied by using sensors to tag sturgeons grown in hatcheries (Sergaliev et al., 2020). In the early 2000s, studies of Ural sturgeon were conducted using high-tech satellite and acoustic tagging, emphasizing beluga as an object of profitable fishing at the time of the survey. However, these works have faced many problems, including insufficient salinity of the Caspian Sea, radio-frequency interference, the stress for incubated sturgeons, and frequent poaching nets in the northern Caspian Sea and Ural River delta, despite the ban on sturgeon fishing in the sea. Furthermore, these studies are expensive and only applicable when the number of sturgeons is sufficient for the study. Modern means of monitoring water areas, such as unmanned aerial vehicles (UAVs) and satellite imagery, have recently been proposed as methods to optimize the protection regime on the river. In addition, to restore natural reproduction, patent works have been developed for the creation of artificial spawning grounds for sturgeons.

An important aspect of effective management of rare species is noninvasive environmental monitoring using the molecular method of environmental DNA analysis, successfully applied and superior to the traditional fish survey (Berger et al., 2020; Boivin-Delisle et al., 2020; Deeg et al., 2023; Dejean et al., 2012; Meulenbroek et al., 2022). The success of this method can be attributed to the fact that it is rapid and accurate, noninvasive and sensitive, relatively inexpensive and less labor-intensive than other methods, facilitating the detection and management of specific species—including rare ones (Anderson et al., 2018; Jerde et al., 2011; Piggott et al., 2020; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012; Wilcox et al., 2013, 2016)—as well as identification of entire communities (Araujo et al., 2023; Kelly et al., 2019; Li et al., 2018; Thomsen, Kielgast, Iversen, Moller, et al., 2012). It has also been used as a tool for the relative quantitative assessment of target species (Agersnap et al., 2017), fish communities reflecting internal ecological interactions (Yang et al., 2023), and restoring shifts in their spatial structure throughout lotic ecosystems (García-Machado et al., 2021). Despite the vast amount of research in the field of fish eDNA, there is a clear gap in the detection of rare sturgeon species. Dejean et al. (2011) were the first to use the sturgeon—namely the Siberian sturgeon—in an experiment on the persistence of eDNA in the aquatic environment.

The geography of eDNA studies of rare species of *Acipenseridae* is currently expanding, providing information on the identification, distribution, and relative abundance of populations. For example, in North America, green sturgeon DNA has been found at sites of their known presence in the Sacramento River (Bergman et al., 2016), as well as outside its established range (Anderson et al., 2018). The Gulf sturgeon and extremely rare Alabama sturgeon have been found to be able to overcome dams during migration in the Mobile River Basin (Pfleger et al., 2016). A follow-up study of the Alabama sturgeon recommended using a convenient precipitation method due to small volumes and additional sampling instead of water filtration. As a result, logistics are simplified, increasing the spatial and seasonal coverage of rare species. Moreover, additional samples of benthic water can be included; sturgeons are bottom-dwelling animals, so the likelihood of obtaining false negatives is reduced (Janosik et al., 2021). Conventional and quantitative PCR eDNA assays have also been developed and tested for lake and Atlantic sturgeons protected in Canada and the United States (Bronnenhuber & Wilson, 2013; Hernandez et al., 2020; Plough et al., 2021; Yusishen et al., 2018). Analysis of eDNA in the diets of piscine predators can be used to reveal information on the ecology of lake sturgeon larvae. For example, a study showed the predominance of their numbers on sandy transects rather than on gravel ones, reflecting the survival strategy for juveniles (Waraniak et al., 2017). In China, eDNA was used to monitor the spatio-temporal distribution of Chinese sturgeon in the Yangtze (Xu et al., 2018), where changes in eDNA concentrations were correlated with breeding seasons (Yu et al., 2021). In addition to targeted detection, *Acipenseridae* species in fish communities have been detected through metabarcoding (Stoeckle et al., 2017; Yu et al., 2021). In a tracer experiment, Fremier et al. (2019) used in situ injections of eDNA from white sturgeon not native to streams with different hydrology and geomorphology. As a result, these authors recommended increasing sampling in low-slope areas where eDNA is retained and removed to the benthic zone.

Despite the ongoing development of species-specific primers for sturgeons, their reliable species identification remains questionable. The difficulty in their genetic determination lies in the high degree of similarity between different species of Eurasian sturgeon (for example, only one base differentiates *Acipenser stellatus* from *A. ruthenus*) and consequently their frequent interspecific hybridization (Ludwig et al., 2002; Meulenbroek et al., 2022), leading to the appearance of various mitochondrial haplotypes and maternal mtDNA inheritance (mtDNA). A recent study of sturgeon eDNA conducted in Iran by Jafroudi et al. (2023) using primers developed by Waraniak et al. (2017) also highlighted the challenges of distinguishing Caspian sturgeons.

Specific primers are being developed for the unique mitochondrial haplotypes of the target species within this family. Thus, the existing test systems for distinguishing native (*A. sturio* and *A. oxyrinchus*) and non-native (*A. baerii*, *A. gueldenstaedtii*, *A. ruthenus*, and *A. stellatus*) sturgeon species in Danish marine waters have proven to be efficient in both in silico and in vivo sampling. However, these primers are not able to distinguish among Ponto-Caspian species, namely the Siberian and Russian sturgeons, and sterlet (Knudsen et al., 2022). Schenekar et al. (2020) developed an assay for *A. ruthenus* and possibly *Huso*, but this also amplified other non-target sturgeons. According to the approach proposed by Farrington and Lance (2014), positive detection of North American species using common markers, in combination with the absence of positive detection by species-specific markers, makes it possible to determine the occurrence of other sturgeon species with an overlapping range. As an alternative to species-specific detection, metabarcoding using universal "teleo" primers amplifying the 12S mtDNA fragment successfully detected Danube sturgeons (Meulenbroek et al., 2022; Pont et al., 2023). This study proposes an efficient eDNA isolation technique and demonstrates the effective use of eDNA as a tool for detecting and obtaining a snapshot of the seasonal distribution of rare and endangered Ural-Caspian sturgeons in the Lower Ural River (~1084 km).

2 | METHODS

2.1 | Study area and eDNA sampling

The main habitat of sturgeons is the lower reaches of the river to Uralsk city. We selected 9–10 sites along the river for our study (Figure 1). The map was generated in ArcGis 10.4 (https://www.esri.com/en-us/arcgis/products/arcgis-online/overview) and SASPlanet (https://sasplanet.geojamal.com/) software on the basis of the generated database. For layers of land, lakes and rivers, the database developed at the Institute of Geography of the Republic of Kazakhstan was used. The points were fixed by coordinates (Tables S1–S3), formatted in the application, and transferred to SASPlanet program. Then the obtained image was taken out of SASPlanet program and transferred to ArcGis 10.4 program, where a separate surface layer was created using layer parameters from the database and NextGIS portal (https://nextgis.com/datasets/). Sampling sites were grouped to cover the upper and lower regions of the Lower Ural River; the average distance between the upper sites was 110.5 and 7 km between the lower sites. Water sampling was carried out from April 22 to October 4, 2021. The sampling period was divided into three seasons: spawning migration of spring forms (the month of April represents the peak of the run), after the spring run (July is the month

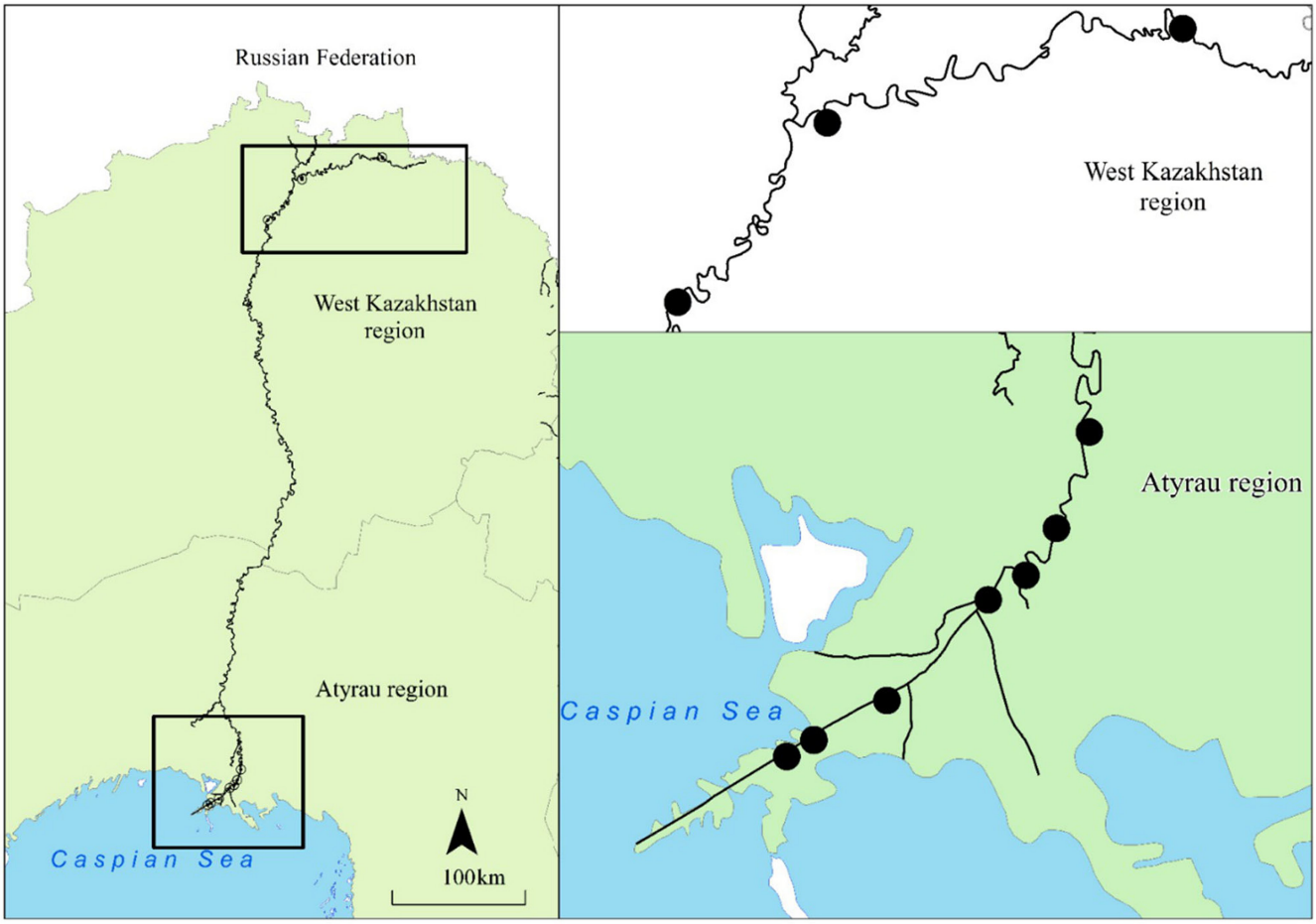

FIGURE 1 Locations of sampling points of samples collected in this study for sturgeon eDNA monitoring during April–October 2021.

with no adults or juveniles in the river), and spawning migration of winter forms (the beginning of October represents the end of the autumn run).

A total of 29 water samples were collected from inflatable and power boats at sites in the Lower Ural River (Tables S1–S3). Sampling was carried out from upstream to downstream. In the upper section of the river, three samples for each season (nine in total) were taken at the Yanvartsevo, Uralsk, and Kushum gauging stations (West Kazakhstan region). In the lower section, six samples were taken in spring and seven samples were taken both in summer and autumn (20 in total) at the mouth of the river at intermediate stations and fishing grounds along the Golden Arm main channel: Hillocks, Water Intake, Institute, Balykshy, Lower Dam, Seventh station, and Peshnoyskaya (Atyrau region). A 2-L sample of the surface water was taken at each site using sterile dark plastic bottles (sterility in this context means the absence of DNA of the target species), either by wading or from a boat with measures taken to avoid contamination following (Jerde et al., 2011). Extraction from summer and autumn environmental water samples was performed within 4–7 days from sampling, while spring samples were frozen immediately and stored at −20°C until DNA extraction several months later. This work resulted in an eDNA collection of 29 samples from spring, summer, and autumn collections.

2.2 | eDNA isolation and targeting amplification

We proposed a method for the direct isolation of eDNA from river water. The principle of the method is based on the use of hexadecyltrimethylammonium bromide (CTAB) cationic detergent, which precipitates DNA from an aqueous solution with a low concentration of inorganic salt. Precipitation of free eDNA was performed from filtered samples of river water by mixing the samples with CTAB buffer to a final concentration of 0.02%, followed by filtration. This method allows for the isolation of free eDNA from high-volume, highly diluted aqueous samples comprising nucleic acids. For this, 500 mL of river water samples were mixed with 20 mL of a 0.5% (w/v) aqueous CTAB solution and filtered through a cellulose nitrate membrane filter (0.22 μm) (Merck Millipore) using a glass vacuum filtration distillation apparatus (Microyn) and a vacuum pump. The membranes were stored at −20°C in 15 mL Falcon tubes until further DNA extraction. The filters with free eDNA, proteins, and other components deposited on them were also stored until further DNA extraction and purification. Specifically, a small fragment (5 × 5 mm) from each filter was placed in 2-mL tubes and further processed according to the protocol. DNA isolation from filters was performed using two protocols to compare their efficiency (Tables S2 and S3). The first protocol is based on the use of 1% CTAB solution in the presence of 1 M NaCl (Kalendar et al., 2021, 2023) and treatment with chloroform. The second protocol uses SDS as the main component for DNA extraction from the filter, followed by chloroform treatment. The DNA precipitate was dissolved in 200 μL of 1× TE solution (0.1 mM EDTA, 10 mM Tris–HCl, pH 8.0), and total DNA concentration was determined using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific Inc.). The obtained eDNA was visualized on a 1% agarose gel using the ChemiDoc XRS+ Gel Imaging System (Bio-Rad Laboratories, Inc).

For amplification, universal primers for the internal transcribed spacer (ITS) region and *Acipenser* species-specific primers were used. The sequence and source of primer pairs used are listed in Table 2. PCR reactions were performed in a 25-μL reaction mixture. Each reaction mixture contained about 25 ng of template DNA, 1× Phusion HF Buffer with 1.5 mM $MgCl_2$, 0.2 μM each primer, 0.2 mM each dNTP, and 0.25 μL Phusion HS II Hot Start DNA Polymerase (2 U/μL) (Thermo Fisher Scientific Inc., Waltham, MA, USA). PCR amplification was carried out in a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific Inc.) under the following conditions: initial denaturation step at 98°C for 90 s, followed by 40 amplifications at 98°C for 5 s, extension at 60°C for 30 s, and 72°C for 10 s, followed by a final extension at 72°C for 2 min.

For negative and positive controls, we used eDNA samples obtained from the fish aquarium water, as well as a mixture of DNA for different species of plants and fungi. A mixture of DNA was used as a positive control, and sterile water was used as a negative control.

PCR products were separated by electrophoresis at 90 V for 2 h in 1.5% agarose gel with 1× TAE buffer. A Thermo Scientific (100–10,000 base pairs) GeneRuler DNA Ladder Mix (#SM0332) was used as a standard DNA ladder. The PCR products were visualized with the ChemiDoc XRS+ Gel Imaging System after staining with ethidium bromide. DNA sequencing was performed using an ABI3700 capillary sequencer (Applied Biosystems Thermo Fisher Scientific) and the Sanger method (BigDye Terminator chemistry). The obtained sequences were visualized and analyzed using FastPCR (Kalendar et al., 2017) and NCBI Blast software.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of eDNA extraction methodology from river water samples

We used our adapted protocol for extraction of free eDNA directly from river water on filters after sample filtration. In addition, we used protocols using saline CTAB and SDS methods (Kalendar et al., 2021) to isolate DNA (Supplementary Material) from the filter surface. To assess the performance of the protocols used to extract DNA from the filter surface, the criteria were the extraction frequency (%) and the characteristics of the DNA samples (Table S2). The evaluation of the efficiency of these methods showed that the highest concentration and purity of isolated eDNA were obtained using protocols based on CTAB salt solution and SDS (Kalendar et al., 2021). As a positive control for the presence of genomic DNA in eDNA samples, amplification was performed with universal nuclear ribosomal ITS primers for 18-23S rRNA genes of plant, fungi, and animal organisms. The universal PCR with ITS primers showed the presence of a target

TABLE 2 Primers used for amplification of eDNA samples, including sequences of general primers for detection of representatives of the genus *Acipenser*.

Scientific name	Locus	Primer ID	Sequence (5′-3′)	Expected size of products (bp) (NCBI reference)	References
Acipenser spp. (*A. baerii*, *A. gueldenstaedtii*, *A. ruthenus*, and *A. stellatus*)	mitochondrial Control Region (CR)	Acibae_CR_F02	CAGTTGTATCCCCATAATCAGCC	214	Knudsen et al. (2022)
		Acibae_CR_R03	TTATTCATTATCTCTGAGCAGTCGTGA		
Acipenser spp. (*A. baerii*, *A. gueldenstaedtii*, *A. ruthenus*, and *A. stellatus*)	mitochondrial cytb gene	Acibae_cytb_F11	TTCCACCCGTACTTCTCATAC	180 (KF153104) to 216	
		Acibae_cytb_R16	GGCGTAGGCGAAGAGAAAGTA		
Acipenser spp. (*A. baerii*, *A. gueldenstaedtii*, *A. ruthenus*, and *A. stellatus*)	16S rRNA	AruF	TCTACCGTCACCCAGGTCAT	104 (KF153104)	Schenekar et al. (2020)
		AruR	CGCCTGTTAAGGTTGTGTTCTTTT		
Universal primers for the ITS region	18S-23S rRNA	ITS1	GGAAGTAAAAGTCGTAACAAGG	593 (LC749799) to 672 (CP034379)	Yang et al. (2018)
		ITS4	TCCTCCGCTTATTGATATGC		

amplification product of the expected size (1.8–2 kb). ITS sequences were detected in all analyzed eDNA specimens and control samples, indicating that the quantity and quality of the extracted total DNA were sufficient for detection of the tested species (Figure 2a).

3.2 | Amplification of eDNA samples isolated from river water with primers common to the genus Acipenser

The next step was to perform targeted amplification of eDNA samples using genus-specific primers. We used genus-specific primers aimed at distinguishing Eurasian members of the genus Acipenser occurring in the waters of the Ural River and the Caspian Sea, from other species of the genus, particularly North American sturgeon (Table 2). The primers used were designed to amplify mitochondrial DNA control region (CR) and cytochrome b (cytb), which distinguish representatives of *Acipenseriformes* to the genus *Acipenser* (and possibly *Huso huso*), as well as a set of universal ITS primers for detection of rRNA fragments of all eDNA specimens as a positive control for the presence of DNA in the samples.

The analysis of summer eDNA samples with *Acipenser* genus-specific primers showed no amplification of sequences of the desired size. In contrast, spring and autumn samples revealed target products of expected sizes (Figure 2). DNA isolated from sturgeon (Russian sturgeon) was used as a positive control. Thus, all 19 river samples collected in spring and autumn were effective for the isolation of representatives of the sturgeon genus, demonstrating positive identifications.

3.3 | Targeted detection of species-specific nuclear DNA markers in eDNA specimens isolated from river water

A combination of species-specific nuclear primers designed to detect beluga (*Huso huso*), sterlet (*Acipenser ruthenus*), and their interspecific hybrid bester (*H. huso* × *A. ruthenus*) was used for targeted amplification from the eDNA samples (Table 3). However, we did not identify these fish species in the studied samples. The maximum spawning migration is known to occur in April. Fry cannot be detected in July and August since the juveniles roll into the sea by early summer. This absence was confirmed by traditional methods and eDNA metabarcoding (Lecaudey et al., 2019), as well as a TaqMan qPCR protocol for *Acipenser ruthenus* (Schenekar et al., 2020) conducted in August in the upper Volga River of the Caspian Basin. One could assume the presence of a potamodromous (riverine) species of sterlet in the river, but (Bokova, 2016) noted that it rarely enters the Ural River. On the other hand, the detection of sturgeon eDNA in early October confirms the arrival of winter representatives of populations. For example, although recently questioned, the passage of a small number of winter starred sturgeon individuals in the autumn was recorded. Consequently, September and October may

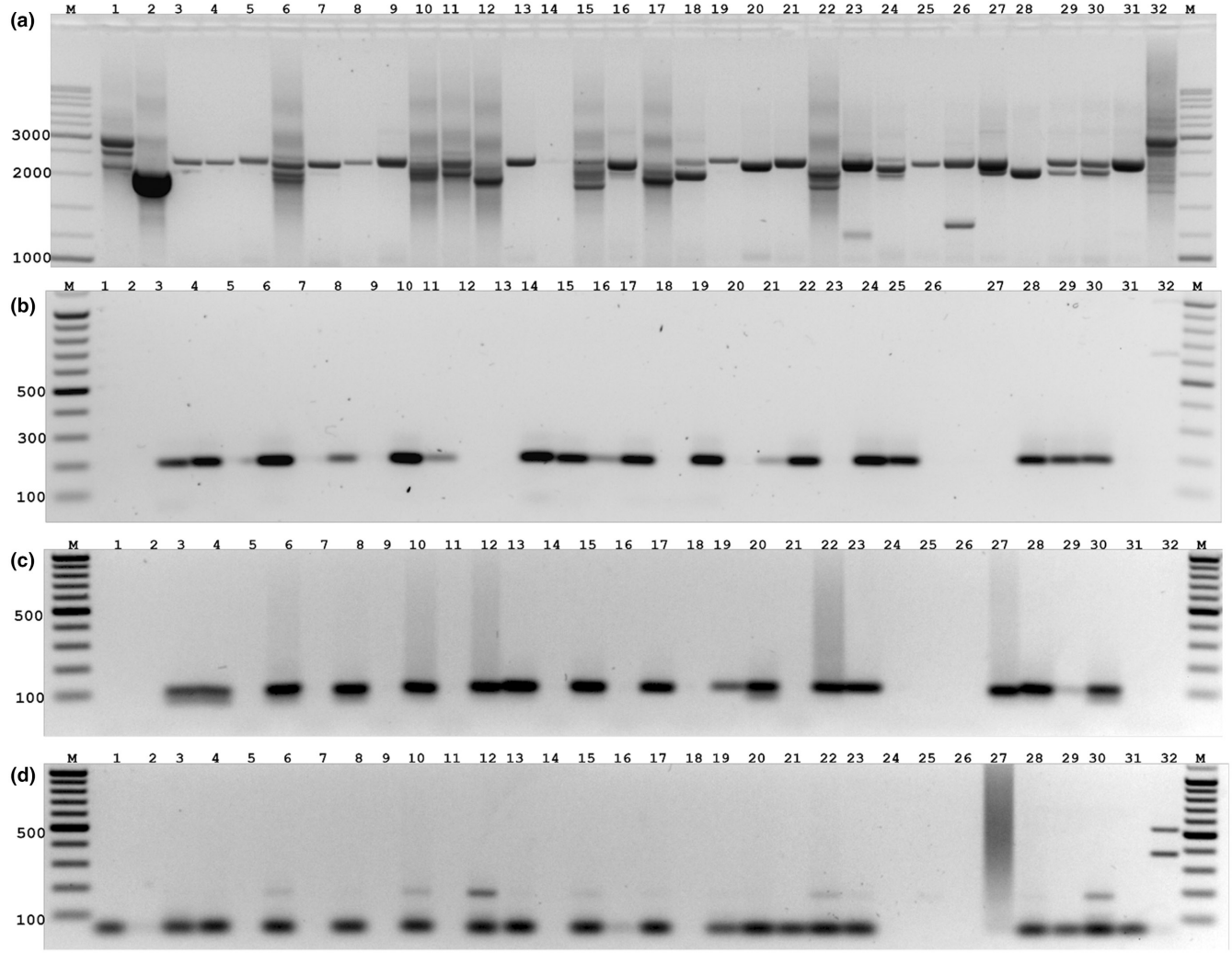

FIGURE 2 PCR amplification for genus-specific primers and for monitoring the presence of intact DNA in samples. (a) Monitoring the presence of intact DNA in samples using universal nuclear ribosomal internal transcribed spacer primers for 18-23S rRNA genes for the detection of plant, fungal, and animal organisms. Target amplification product of the expected size between 1.8 and 3 kb. (b) Amplification results of all samples with *Acipenser* genus-specific primers for mitochondrial DNA control region (CR) (primers: Acibae_CR_F02 and Acibae_CR_R03). Target amplification product of the expected size 214 bp. (c) Amplification results of all samples with *Acipenser* genus-specific 16S rRNA primers (AruF and AruR). Target amplification product of the expected size 104 bp (*Acipenser ruthenus*, KF153104). (d) Amplification results of all samples with *Acipenser* species-specific (*A. baerii*, *A. gueldenstaedtii*, *A. ruthenus*, and *A. stellatus*) primers for mitochondrial DNA cytochrome b (cytb) gene (Acibae_cytb_F11 and Acibae_cytb_R16). Target amplification product of the expected size 180–216 bp. eDNA samples (3–31): 1—negative control of eDNA from the fish aquarium water; 2negative control of eDNA from the fish aquarium water; 3—1c (autumn water); 4—1a (spring water); 5—1b (summer water); 6—2c (autumn water); 7—2b (summer water); 8—3c (autumn water); 9—3b (summer water); 10—4c (autumn water); 11—4b (summer water); 12—5c (autumn water); 13—2a (spring water); 14—5b (summer water); 15—6c (autumn water); 16—6b (summer water); 17—7c (autumn water); 18—7b (summer water); 19—8c (autumn water); 20—3a (spring water); 21—9c (autumn water); 22—10c (autumn water); 23—4a (spring water); 24—8b (summer water); 25—9b (summer water); 26—10b (summer water); 27—5a (spring water); 28—6a (spring water); 29—7a (spring water); 30—8a (spring water); 31—9a (spring water); 32—DNA mixer as negative or positive control. For negative and positive controls, we used eDNA samples obtained from the fish aquarium water, as well as a mixture of DNA for different species of plants and fungi. A mixture of DNA was used as a positive control, and sterile water was used as a negative control.

also be an optimal period to collect eDNA samples for the purpose of monitoring Ural sturgeons. In general, the successful detection of sturgeons in the lower reaches of the river raises the possibility of their representatives migrating downstream to the middle reaches of the Ural, Ilek, and Sakmara tributaries until the upper reaches of the river in the Orenburg region of the Russian Federation, where the most productive sturgeon spawning grounds are located.

Environmental factors such as temperature and lotic geomorphology, as well as the condition of the eDNA and extraction methods, are known to influence the efficiency of eDNA technology (Barnes et al., 2014; Fremier et al., 2019; Kirtane et al., 2023; Naef et al., 2023). Consequently, we anticipated degradation of DNA due to high summer water temperatures (28–29°C) and the long time before sample filtration (4–7 days including transportation,

TABLE 3 Sequences of species-specific primers for detection of beluga and sterlet and their bester hybrid.

Scientific name	Locus	Primer ID	Sequence (5′–3′)	Expected size of products (bp)	Reference
Huso huso	Sodium bicarbonate cotransporter 3-like (LOC117399294) (NCBI Reference Sequence: XM_059022892)	153_HHp	GATCTGAACATCAGCCACTGC	153	Havelka et al. (2017)
		153_uni	TACTGTGCCTGTATGTCTCC		
		153_HHn	GATCTGAACATCAGCCACTGG	153	
		153_uni	TACTGTGCCTGTATGTCTCC		
Acipenser ruthenus	Potassium channel subfamily T member 2 (LOC117404818) (NCBI Reference Sequence: XM_059031815)	247_Arp	TAAGGGTCCATGCATGCAG	247	
		247_uni	TTTTAGCTGCACCGTGGC		
		247_Arn	TAAGGGTCCATGCATGCCT	247	
		247_uni	TTTTAGCTGCACCGTGGC		

instead of the recommended 24 h after sampling). However, the use of PCR with universal ITS primers showed sufficient concentration of isolated total eDNA for PCR. Moreover, the quantitative and qualitative properties of genomic DNA from summer specimens exceeded those of spring and autumn samples, indicating rich species diversity and resistance of eDNA to degradation in the environment, namely high temperature and long storage (up to 1 week) of river samples before filtration. Estimating the distribution of individuals on temporal and spatial scales requires experimental confirmation. The exact location of the target species is quite difficult to determine, and many studies have yielded varying results. Thus, while Pont et al. (2018) suggest the possibility of DNA drift over distances up to 130 km, Xu et al. (2018) suggest that eDNA of low-density species is unlikely to drift hundreds of kilometers in flowing waters using sampling sites 30–50 km apart. Berger et al. (2020) also demonstrated that target eDNA for fish is detectable 40–50 km downstream of the source. Furthermore, recent quantitative eDNA metabarcoding studies in the St. Lawrence River (Canada) indicated a strong local detection signal for coho salmon at a resolution of 10 to 100 m from the source population, with relative amounts of eDNA mixtures up to 13%, sufficient to cluster the fish community (Laporte et al., 2022). Previous results from metabarcoding analyses of eDNA samples from the transect of the aforementioned river (1300 km long) also highlighted the possibility of capturing changes in fish species composition of lotic ecosystems despite potential eDNA transport (García-Machado et al., 2021). The upper and lower groups of sampling points in our study were 723 km apart, with an intermediate distance of 91–130 km between the inner points in West Kazakhstan Province and 3–16 km in Atyrau Province. Hence, our positive detections from these sites will not be able to indicate the exact location of individuals; we only know that at the time of sampling, they were distributed along the entire length of the river. A series of quantitative analyses using qPCR or NGS analysis will be required to determine their exact location (Laporte et al., 2022). Regarding the timing, while the beginning of the sturgeon spawning migration period can be considered to the accuracy of days with the initial detection of target eDNA, the last eDNA detection signal indicating the end of spawning or juvenile rays is unclear, also requiring experimental confirmation. To this end, researchers are evaluating the degradation rate of free eDNA (Kirtane et al., 2021) and quantifying fish abundance using a mass balance model of eDNA concentration (Sassoubre et al., 2016). Although Yates et al. (2019) state that under natural conditions, organism abundance is correlated with eDNA concentration by approximately 50%. It is known that under mesocosm conditions, eDNA can be detected for up to 2 or 3 weeks (Dejean et al., 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012), or even 58 days according to one study (Strickler et al., 2015). Target sturgeon eDNA was detected in low numbers (two replicates among 25 eDNA samples) for a single individual from the reservoir within a week after the individual was removed (Meulenbroek et al., 2022; Strickler et al., 2015). For marine systems, a period of up to 48 h has been suggested for optimal processing before eDNA starts to degrade (Collins et al., 2018). The lack of successful identification in our study based on biallelic nuclear DNA markers of *H. huso*, *A. ruthenus*, and bester can be explained by the fact that these DNA markers are effective for testing species by eDNA, the source of which is usually the remains of fish eggs in water bodies (Havelka et al., 2017). Nuclear marker copy number in cells is known to be significantly inferior to mitochondrial DNA copy number; hence, species detection using nuclear markers may be inefficient. On the other hand, the copy number of nuclear ITS sequences exceeds that of CytB genes by up to 150-fold. Therefore, high copy number ITS sequences allow for increased sensitivity of eDNA detection, and with the use of smaller volumes of water samples for analysis (Minamoto et al., 2017). In this study, ribosomal gene sequences also showed sufficient amplification of the nuclear ITS regions of all samples. It is known that the detection of rare species requires the most sensitive protocol to avoid false negative results (Sanches & Schreier, 2020). In this study, we used the liquid-phase extraction methods based on eDNA precipitation from a salt-free aqueous solution with CTAB detergent on a membrane, followed by extraction with CTAB salt solution and chloroform. This resulted in an excellent yield of membrane-bound eDNA. Previous work also confirms the advantage of CTAB protocols over commercial kits (Renshaw et al., 2015; Turner et al., 2014), despite their common use (Shu et al., 2020; Tsuji et al., 2019), including in

sturgeon detection (Janosik et al., 2021). The main reason for the loss of DNA in commercial kits is the inhibition of PCR by organic contaminants during extraction of genomic DNA from the sample (Eichmiller et al., 2016). The dilution of DNA solutions used in this process further inhibits PCRs and contributes to an even lower detection of target DNA (Takahara et al., 2015). Regarding the volume, our 500-mL river samples showed successful detection of target sturgeon DNA, indicating sufficient eDNA of these rare fishes in the samples and the efficiency of our proposed method. The mesocosm experiment of Thomsen, Kielgast, Iversen, Wiuf, et al. (2012) and the studies of Janosik et al. (2021) in a lotic system also demonstrate the sufficiency and convenience of small water samples (15 mL) for both searching for eDNA of endangered species and increasing the range of coverage of target species. However, contrary reports also exist; according to Shaw et al. (2016), 2 L of water samples are insufficient for detection of rare species by metabarcoding with prior isolation by commercial kits, requiring even 5 L of water samples for 100% detection rate. Increasing the volume of filtered water is particularly relevant for maximizing biodiversity coverage, for example, in marine ecosystems (Bessey et al., 2020), whereas small volumes are quite effective when searching for target species in lotic ecosystems, as our study confirms.

Our studies represent the first known case of documented presence of sturgeons in the Ural River using eDNA methods. Moreover, the presence of sturgeons in all spring and autumn samples may indicate their sufficient abundance, although species affiliation remains questionable. As identification of sturgeon species by classical species-specific detection is currently challenging, total eDNA analysis with high-throughput sequencing may be a solution (Meulenbroek et al., 2022; Yang et al., 2023). Thus, our experimental studies contribute to the methodology of free eDNA analysis and expand the potential for monitoring sturgeon fishes of the Ural River basin creating prerequisites for location mapping and quantitative assessment of population status using eDNA.

4 | CONCLUSION

Our analysis of river samples and the proposed method of eDNA isolation and purification from river samples is a highly efficient, sensitive, and relatively inexpensive method for detecting rare species in environmental samples. This study presented the first biogeographic analysis of sturgeon distribution and evidence of seasonal migration of their representatives in the Lower Urals. We have shown that the presence of fish species of interest was detected in all spring and autumn samples detected, but not in summer, consistent with sturgeon ecology.

AUTHOR CONTRIBUTIONS

A.Z. and G.A. conceptualized the project. R.K. and G.A. developed the methods and software. R.K., G.A., A.T., A.Z., and O.K. performed the experiments and analyzed the data. R.K., G.A., and A.Z. wrote the paper. R.K., G.A. prepared the figures; R.K., G.A., and A.Z. acquired funds for the project. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have declared that no competing interests exist.

DATA AVAILABILITY STATEMENT

Data are available in the article's Supplementary Material.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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