TECHNICAL NOTE

Development of a TaqMan qPCR protocol for detecting *Acipenser ruthenus* in the Volga headwaters from eDNA samples

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Abstract

A recent eDNA-metabarcoding study assessing fish diversity in the Upper Volga catchment did not detect sterlet at any of the sampled stretches, despite recent sightings that suggest its presence. We designed a TaqMan qPCR protocol to test for sterlet in selected eDNA samples from that study. In-silico and in-vitro tests confirm the protocol's high sensitivity and specificity to sturgeon taxa and potentially paddlefishes. Using this assay, sterlet were not detected in 26 eDNA samples from the Volga headwaters, agreeing with the metabarcoding results.

Keywords Sterlet · Environmental DNA · Sturgeon · Freshwater monitoring · Real-time PCR · Endangered species

Assessing presence and distribution of freshwater species using environmental DNA (eDNA) is developing into a powerful conservation management tool (Deiner et al. 2017; Ruppert et al. 2019). Taxon-specific qPCRs are widely used to assess fish presence (e.g. Carim et al. 2016; Jensen et al. 2018; Roy et al. 2018) and can be more sensitive than metabarcoding (Harper et al. 2018). The sterlet *Acipenser ruthenus* is listed as vulnerable by the IUCN with a decreasing population trend. Main threats involve habitat fragmentation and loss and overexploitation (IUCN 2019). Using an eDNA-metabarcoding approach, Lecaudey et al. (2019) identified 23 fish species in the Upper Volga catchment. However, the sterlet, a species of conservation interest in this area (Schletterer 2006; Schletterer et al. 2018), was not detected.

To evaluate the metabarcoding results, we designed a TaqMan-qPCR assay to detect sterlet from environmental

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samples. Other sturgeon qPCR protocols (Farrington and Lance 2014; Bergman et al. 2016; Pfleger et al. 2016; Yusishen et al. 2018) focused on North American species and were optimized on the respective local fish community. We designed TaqMan primers (AruF: 5′-TCTACCGTCACC CAGGTCAT-3′; AruR: 5′-CGCCTGTTAAGGTTGTGT TCTTTT-3′) and probe (AruPr: 5′-FAM-GAGAGGTAC AGCTCTCTTG-MGB-Q500-3′) in the 16S rRNA gene using the DECIPHER package (Wright et al. 2014; Wright 2015). We utilized the reference database of Lecaudey et al. (2019) containing 150 sequences of 45 native and invasive fishes, covering 19 Palearctic fish families, including sterlet and Russian sturgeon *A. gueldenstaedtii* (Supplemental 1). Primers and probe were quality-checked with MULTIPLEPRI-MERANALYZER (Thermo Fisher Scientific). For in-silico assay testing, we performed a PRIMERBLAST search (Ye et al. 2012) yielding most significant hits with *Acipenser* sp. (up to 1 mismatch), with best hits on the target species, *A. ruthenus* (no mismatch). There were two additional hits (2 mismatches in the forward primer) for beluga (*Huso huso*) and American paddlefish (*Polyodon spathula*). Both of these latter species do not occur in our study area (IUCN 2019). Furthermore, sterlet is the only *Acipenser* species currently occurring in the Upper Volga (Schletterer et al. 2018), therefore, a positive signal would indicate this species' presence. Primer and probe concentrations were optimized via a qPCR reaction-series using sterlet tissue extract (approx. 20 ng/ μl) as template, varying both primer concentrations from 200 to 800 nM (in 200 nM steps) and probe concentrations

Table 1 Details on the locations of eDNA samples tested for sterlet with the newly developed protocol

River	Location	GPS coordinates	Sampling date	Number of eDNA samples tested
Volga	Staritsa	56° 30′ 44.1″ N 34° 55′ 33.2″ E	13.08.2017	3
Volga	Rzhev	56° 15′ 31.7″ N 34° 19′ 12.2″ E	13.08.2017	8[a]
Volga	Tver Migalovo	56° 50′ 53.7″ N 35° 46′ 40.9″ E	17.08.2017	3
Tudovka	Molodoy Tud	56° 25′ 16.9″ N 33° 36′ 28.4″ E	13.08.2017	4
Tvertsa	Mel'nikovo	56° 56′ 35.0″ N 35° 47′ 00.1″ E	18.08.2017	8[a]

Details on sampling protocol are given in Supplemental 4

[a]Locations with recently documented sterlet presence (Supplemental 5). Therefore, all available eDNA samples were surveyed

from 100 to 400 nM (in 100 nM steps). Final qPCR reactions consisted of 10 µl 2x TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific), 800 nM of forward and reverse primer, respectively, 200 nM of probe, 2 µl template and ddH$_2$O up to a final volume of 20 µl. A standard curve was generated for assay efficiency evaluation (from 10^9 to 10^0 copies/µl, six replicates each), showing a limit of quantification at 10^4 copies/µl, a limit of detection at 10^3 copies/µl, an R^2-value of 0.998 and a reaction efficiency of 99.6% (Supplemental 2). For in-vitro testing, we ran qPCRs on tissue extracts from four sturgeons (*A. ruthenus, A. sturio, A. gueldenstaedtii* and *A. baerii*), and eight teleost species from six families (*Anguilla anguilla, Ballerus ballerus, Ballerus sapa, Leuciscus aspius, Cobitis* sp., *Salmo trutta, Silurus glanis, Thymallus thymallus*). Cycling conditions consisted of an initial denaturation of 95 °C for 10 min followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min, performed on a Corbett Rotor-Gene RG-3000 (Qiagen) in standard speed mode. Amplification was observed for *Acipenser* species only. For in-situ verification, we sampled five fish tanks of a local hatchery and pet shop harboring three different *Acipenser* species (Supplemental 3). All fish-tank eDNA samples showed positive amplification in all PCR replicates and Sanger sequencing confirmed amplification of only target species.

Finally, we assayed 26 samples from five locations collected by Lecaudey et al. (2019) (Table 1, Supplemental 4). In the Upper Volga, sterlet is present in the Ivankovskoe and Uglich reservoirs (Supplemental 5). Formerly common in the Volga headwaters (Grazianov 1907), the sterlet is reported by anglers to be rare upstream of Tver. In August 2005, sterlet was documented 50–100 km upstream of Rzhev (Schletterer 2006) and in 2016, they were stocked in the Tvertsa River (Supplemental 5).

No amplification was observed from any eDNA samples corroborating the results of Lecaudey et al. (2019). The non-detection could be caused by seasonal migrations during summer months (Kubala et al. 2019, Sorokin et al. 2002), orvery low abundances ("rarely caught andin single instances"—Sorokin et al. 2002), that may have led to eDNAconcentrations below the detection threshold of the assay. One sampling replicate consisted of 250 ml of filtered water and replicates were kept separate for analyses. This volume falls below generally recommended sample volumes (e.g. Wilcox et al. 2018) and might have led to a very low sterlet DNA concentration in the individual extractions (elution volume 100 µl each). We therefore recommend a larger filtered water volume (> 1 l) per sampling replicate when applying this protocol for a scarce species such as sturgeon in large lowland rivers.

The main intention of this assay was to detect sterlet in the headwaters of the Volga, excluding cross-amplification from teleosts and lampreys. In-silico and in-vitro tests show that it will also detect other Palearctic *Acipenser* species and potentially the two *Huso* species. This assay might also be suitable for detecting other Palearctic sturgeon species, but would need further testing for specificity and sensitivity.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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