Using environmental DNA from sediment samples to detect invasive alligator gar (*Atractosteus spatula*) in an artificial pond

M Azis, N Andayani and A E Maryanto

Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: andayani@ui.ac.id

Abstract. The early detection and assessment of aquatic species distribution is important in studying conservation and management. However, due to high costs and ineffective conventional methods, this is difficult in developing regions. Molecular genetic studies have led to advanced technology to allow researchers to monitor the presence of trace DNA levels found in the environment. In this study, we show that sediment can be used as an environmental DNA sample to detect the presence of an invasive species, the Alligator gar (*Atractosteus spatula*). Samples were processed directly using the FastDNA Spin Kit for soil, followed by other downstream applications, including PCR and sequencing. Amplified DNA fragments and sequence analysis revealed successful identification of the Alligator gar. Despite the environmental conditions, which tend to be warmer than typical eDNA samples, the high DNA concentrations in sediment samples allowed the reliable detection of this invasive species.

Keywords: Alligator, sediment, eDNA, pond

1. Introduction

Environmental DNA (eDNA) refers to mitochondrial or nuclear DNA released by feces, urine, skin cells, or other tissues [1]. In aquatic environments, eDNA is distributed by water currents and other hydrological processes, and it can remain stable for several days to months, depending on the environmental conditions of the waters. eDNA is used as a means of identifying the existence of a species without capturing or monitoring animals in their habitats [2]. It is possible that eDNA sequences are present at high concentrations in the environment, but their potential for studying organismal communities has not been explored widely [3]. Despite the potential for long-term stability, eDNA molecules are particularly susceptible to DNAse degradation caused by bacteria and fungi [4]. However, eDNA can be shielded from nuclease activity when it binds to large, charged organic molecules contained in sediments, such as clay [5].

The Alligator gar (*Atractosteus spatula*) is native to areas such as Indonesia, but it is able to survive and develop beyond its natural habitats, where it has become invasive in southern portions of the United States extending into Mexico [6]. It can grow to a length of 3 m and can weigh about 140 kg, making it the largest species in the Gar family [7]. Alligator gars are opportunistic carnivores and sit-and-wait predators. They feed on almost anything they can find, including fish, ducks, turtles, small mammals,
and carrion [8]. Females generally live longer than males and are larger, with life spans ranging from 26 to 50 years in the wild [7]. Due to their size, feeding behavior, and life expectancy, they are the top predators in aquatic environments once they become established. In this study, we examined sediment as an eDNA source to determine whether Alligator gar DNA fragments are preserved in the sediment and whether they can be used for reliable species detection, which would provide an excellent means of monitoring population size changes and invasion success among habitats.

2. Materials and method
Experiments were performed in an artificial pond, sized 1.6 × 3.4 × 0.8 m. The pond composition consists of several vertebrate species such as small fishes and freshwater turtle. Environmental monitoring was performed weekly over one month to determine variations in humidity, temperature, pH, and light intensity. Approximately 1 g of sediment samples were collected using a mini shovel to scoop up layers of sediment that settled at the bottom of the pond. Immediately after collection, samples were centrifuged (14,000 g for 5 min) and the water was discarded. DNA was extracted from the samples using the FastDNA SPIN Kit for Soil. The sample was eluted in a final volume of 100 µL. As a positive control for polymerase chain reaction (PCR) and sequencing protocols, DNA was extracted from a tissue (fin) sample of Alligator Gar using Thermo Scientific GeneJET Genomic DNA Purification Kit, following the manufacturer’s protocols. In addition, to ensure that the tissue was completely homogenized, we included a bead-beating step using 10 beads and 180 µL of digestion buffer for 2 min. Isolated DNA was visualized using agarose gel electrophoresis.

PCR amplification was performed with ecoPrimerF 5'-ACTGGGATTAGATACCCC-3' and ecoPrimerR 5'-TAGAACAGGCTCCTCTAG-3' to amplify an approximately 130-bp fragment of hypervariable regions in the mitochondrial 12S ribosomal RNA (rRNA) gene. PCRs were conducted in a 25-µL total volume, containing 1.5 µL of nuclease-free water, 8 µL of template DNA, 20 pmol of each primer, and 12.5 µL of GreenTaq DNA polymerase. The amplification was repeated using a multi-tube approach. The PCR program included a pre-denaturation step at 95 °C for 3 min, 28 cycles of denaturation at 95 °C for 30 s, and annealing at 56 °C for 30 s. PCR products were verified on 1.5 % agarose gels stained with 5 µL of peqGreen. The PCR products were sequenced by Macrogen (Seoul, Korea). The results were compared against the National Center for Biotechnology Information (NCBI) nucleotide database.

3. Results and discussion
DNA extraction results revealed high DNA concentrations in the sediment samples. Only the sample from the third week had an A260/280 ratio below 1.8, indicating low purity [9] (table 1). The highest concentration and the cleanest band were visualized from the sample from week 4 (table 2); hence this sample was selected for sequencing (table 3).

PCRs amplified the sediment and positive control samples. Sequencing revealed a 115-bp fragment from the sample, and a 112-bp fragment from the positive control (table 3). The obtained sequences were 70 % matches to the mitochondrial 12S rRNA complete sequence from A. spatula (JF912040.1) and Atractosteus tropicus (JF912038.1) on the NCBI database (table 4). We found from these study that the fragment of 12 rRNA of mitochondrial DNA has ability to identify the presence of eDNA from our species target A. spatula. These finding should be useful for further analysis such as for quantifying the biomass of A. spatula in freshwater using eDNA approach. The target gene is also very short and should be easy to amplified from degraded DNA from environment.

We showed that our eDNA sample from sediment can be used to detect the presence of A. spatula in an artificial pond. The extracted DNA had high concentration and purity. As the environment can retain traces of the species that inhabit it [10], sediment samples allowed for the reliable detection of the targeted species A. spatula. The main challenge is determining how to isolate the eDNA, since it is susceptible to degradation once exposed to the environment. A study on quantitative eDNA analysis
with river flow distances found no correlation between river flow distances and eDNA concentration [11]. This indicates that eDNA does not accumulate in the lower region, but undergoes deposition or degradation due to physical and chemical factors, such as currents, temperature, acidity (pH), and exposure to UV radiation. Moreover, the use of this method requires several factors, such as the volume of secretions, size, and density of the organism, which could affect the amount of DNA in sediment samples. eDNA samples are typically short fragments and can persist for a long time under dry, cold, alkaline conditions, in the absence of light [3]. DNA from extinct vertebrates has been amplified from 10,000-year-old dry cave sediments [12]. In addition, eDNA molecules that have been deposited in sediments can last longer than water-soluble eDNA molecules [13]. Hence, we used sediment samples that were not directly influenced by the water current or UV radiation, and were bound to clay, which helped to preserve the DNA samples. Clay minerals, such as montmorillonite, can absorb DNA molecules heavier than their own size, because they have negatively charged surfaces. In addition, humic acid tends to be resistant to degradation and is able to bind with eDNA molecules in the sediments, causing the eDNA to last longer [14].

The application of this method allows researchers to detect species much more efficiently than conventional survey techniques. Since sampling is not difficult, it should be a potent procedure to survey large-scale environments. In addition, this allows better accuracy than the manual methods of environmental sampling. The main advantages of this approach are that they are noninvasive [3],

### Table 1. Concentration of extracted DNA.

| Sample       | ng/µL | A260   | A280   | 260/280 (purity) |
|--------------|-------|--------|--------|------------------|
| Sediment week 1 | 204.37 | 4.087  | 2.267  | 1.80             |
| Sediment week 2 | 117.53 | 2.351  | 1.244  | 1.89             |
| Sediment week 3 | 141.08 | 2.822  | 1.643  | 1.72             |
| Sediment week 4 | 199.73 | 3.995  | 2.120  | 1.88             |
| Tissue (fin)   | 11.48  | 0.230  | 0.115  | 2.00             |

### Table 2. Environmental condition of the artificial pond.

| Week | Humidity (%) | Temperature (°C) | pH | Light intensity (LUX) |
|------|--------------|------------------|----|-----------------------|
| 1    | 81           | 31.1             | 8  | 7.98                  |
| 2    | 89           | 27.9             | 7  | 11.48                 |
| 3    | 88           | 29.7             | 7  | 7.98                  |
| 4    | 75           | 30.5             | 7  | 7.9                   |

### Table 3. Amplicon

| Sample        | Sequence (5’-3’) | Length (bp) |
|---------------|------------------|-------------|
| Sediment week 4 | CTCTAACCTTAGTCCTTGTGATATTTTJAGCAAACCGCCAGGGGTTGCCGAAACCCATCTGTTGGTGTATCTTTATTCCAAAAA | 115 |
| Tissue (fin)   | CTTAACCTAAGTGATATGATACGAATATCATCGCTCAGCCAGGGTCTACTAGCGCTACTGTATCTATTCTTTGGGTGTTAGGATACCCACCTAGGGGATCTGTTCTAAAGTAG | 112 |
Table 4. Sequence producing significant alignments (sediment sample).

| Description                                                                 | Max score | Total score | Query cover (%) | E value  | Ident (%) |
|------------------------------------------------------------------------------|-----------|-------------|-----------------|----------|-----------|
| *Atractosteus tropicus* mitochondrion, complete genome                       | 37.4      | 125         | 83              | 8e-05    | 70        |
| *Atractosteus tropicus* isolate 202 12S rRNA gene, complete sequence; mitochondrial | 37.4      | 37.4        | 83              | 8e-05    | 70        |
| *Atractosteus tropicus* isolate 201 12S rRNA gene, complete sequence; mitochondrial | 37.4      | 37.4        | 83              | 8e-05    | 70        |
| *Atractosteus spatula* mitochondrial DNA, almost complete genome            | 37.4      | 60.3        | 60              | 8e-05    | 74        |
| *Atractosteus spatula* isolate 283 12S rRNA gene, complete sequence; mitochondrial | 33.7      | 33.7        | 70              | 0.001    | 70        |
| *Atractosteus spatula* isolate 001 12S rRNA gene, partial sequence; mitochondrial | 26.5      | 26.5        | 58              | 0.15     | 71        |
| *Atractosteus tropicus* carnitine palmitoyltransferase mRNA, partial cds    | 21.1      | 21.1        | 9               | 6.3      | 100       |
| *Atractosteus tropicus* isolate Atrop17 23S ribosomal RNA gene, partial sequence | 21.1      | 21.1        | 9               | 6.3      | 100       |
| *Atractosteus spatula* clone ASP21 microsatellite sequence                  | 21.1      | 21.1        | 9               | 6.3      | 100       |
| *Atractosteus spatula* clone Atsp108 microsatellite sequence               | 21.1      | 21.1        | 9               | 6.3      | 100       |

have higher efficiency than traditional methods (such as visual monitoring), and are easy to standardize across sampling personnel [15]. Further, this eDNA method can become a standard tool to survey communities within diverse ecosystems. Because of these advantages, this method should be employed by researchers in Indonesia, a nation rich in biodiversity but lacking in the technology needed to study this diversity. By conducting this research, we hope there will be further research in Indonesia that uses eDNA to advance tools in conservation biology, ecology, and molecular biology.

4. Conclusion
Environmental DNA from sediment can be extracted and successfully amplified the specific target of alligator gar fish. Despite the environmental conditions, which tend to be warmer than typical eDNA samples, the high DNA concentrations in sediment samples allowed the reliable detection of targeted species.

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