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DNA-based monitoring of the alien invasive North American crayfish *Procambarus clarkii* in Andean lakes (Ecuador)

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Abstract

In 2013 the red swamp crayfish (*Procambarus clarkii*) was introduced in Lake Yahuarcocha, a tropical, high altitude lake located in the Andes of northern Ecuador. Abundance of this alien invasive species has increased to such extent that it is disturbing the autochthonous aquatic ecosystem. Little is known about its distribution throughout Ecuador. Monitoring is therefore important to assess the current distribution and to control future spread. A molecular method based on environmental DNA (eDNA) was developed and used to assess the distribution of *P. clarkii* at 21 sites in Lake Yahuarcocha, and three sites each at Lake Mojanda and Lake San Pablo in the Ecuadorian highlands. The molecular detection of crayfish from water samples were compared to catch-based distribution patterns obtained from traditional trapping. Results showed an eDNA detection accuracy of 86% in water samples, without any false positives. The results indicate that this budget-friendly method is easy to use and can provide important information on the occurrence of alien species in the tropics.

Keywords

Andes; eDNA-based monitoring; alien invasive species; *Procambarus clarkii*; early detection

Introduction

Global biodiversity is under threat, leading to regime shifts and measurable loss of ecosystem services (Hooper et al., 2012). Reducing the loss of biodiversity is essential for the future of our planet. Among the causes for rapid species decline are habitat fragmentation, introduction of alien invasive species, resource exploitation, and illegal trade (Fischer and Lindenmayer, 2007; Lenzen et al., 2012; Mathers et al., 2016; Tapia-Armijos et al., 2015). Alien invasive species are often directly linked to extinction of birds, fish and mammals (Clavero and García-Berthou, 2005). Although biological implications differ, the introduction of alien species may lead to competitive exclusion of native species and regime shifts of the ecosystem (Clavero and García-Berthou, 2005). Monitoring natural populations is a necessary step to establish conservation status, and it contributes to environmental management decisions (Martin et al., 2007).

Environmental DNA (eDNA) is DNA released by organisms in the environment, through feces, hair, skin, gametes, and other sources (Wilcox et al., 2013). Thus, eDNA can be extracted from environmental samples such as soil and water without direct access to the organism of interest or any part of it (Taberlet et al., 2012). The eDNA method and related molecular tools allow the monitoring of species distributions and can assess the presence or absence of alien invasive or endangered species without capturing organisms and disturbing the ecosystem (Agersnap et al., 2017; Barnes and Turner, 2016; Bohmann et al., 2014; Thomsen and Willerslev, 2015).

The eDNA method has been successfully applied to many species of various taxonomic groups and habitats (Herder et al., 2013). Once released into the aquatic environment, DNA is diluted and starts to degrade, since it is a chemically unstable compound (Barnes and Turner, 2016). Barnes and Turner (2016) summarized the factors affecting eDNA persistence into three categories: characteristics of DNA (such as its length or conformation), abiotic environment (such as oxygen, pH or salinity), and the biotic environment (such as the microbial community

or enzymes). Thus, eDNA can be detected in aquatic environments for 7 to 21 days, depending on the environmental conditions (Barnes and Turner, 2016; Dejean et al., 2012; Pilliod et al., 2013). Most research in aquatic environments has focused on large species such as amphibians and fish, who secrete more DNA through e.g. their mucous skin, than might be expected from, for example, macroinvertebrates (Ficetola et al., 2008; Takahara et al., 2013; Takahara et al., 2012). This has changed recently, with more effort being put in eDNA research on macroinvertebrates (Agersnap et al., 2017; Bista et al., 2017; Klymus et al., 2017; Niemiller et al., 2017).

Lake Yahuarcocha is located in the province of Imbabura in northern Ecuador within the Andes mountain range. Its freshwater biodiversity has suffered considerably in the course of the years (Escaleras, 2016; Portilla, 2015; Van Colen et al., 2017). One of the most influential effects has been the introduction of alien invasive species like the red swamp crayfish *Procambarus clarkii*, guppy *Poecilia reticulata* and carp *Cyprinus carpio* (Oluoch, 1990). These alien species have displaced native species through competition for resources, predation, and modification of the surroundings (Souty-Grosset et al., 2016; Valero et al., 2008; Yamamoto, 2010).

In the case of *Procambarus clarkii* little progress has been made in detection and control of the species. The species is known worldwide as a highly invasive species capable of modifying the ecosystems (Geiger et al., 2005) and has been observed in Ecuador since 1986-1989. There is little information on its distribution, status, habitat requirements and genetic variation in this region, all of which are important data needed to establish plans for monitoring and control of the species (Mora & Uyaguari, 2004).

The detection of alien species, and more specifically *Procambarus clarkii*, with eDNA was first studied by Tréguier et al. (2014) using qPCR. They tested 158 ponds in a natural park in France, using traditional traps and eDNA, and showed that eDNA had a slightly better detection efficiency (73%) compared to conventional trapping (65%) in the 78 ponds where the species

was found. However, the authors also mentioned that eDNA performed worse than traditional methods in ponds with low *P. clarkii* abundance, which limits efficient detection (Tréguier et al., 2014). In another study, Cai and colleagues (2017) used the methods described by Tréguier et al. (2014) to detect *P. clarkii* in rice paddies in Yunnan, China. They identified the invasive species consistently where it was physically present using eDNA. A recent study comparing eDNA sampling and extraction methods used to identify *P. clarkii* found that the species could be detected in field samples, but that results were highly dependent on the method used (Geerts et al., 2018). These promising results fostered the idea to develop a DNA-based method to assess the distribution of alien invasive crayfish under tropical climatic conditions, more specifically in Ecuador.

This is the first study where eDNA is used to detect alien invasive species in a tropical lake. The research used classic PCR, to allow the use of a simple and low-cost technique that can be applied universally. The objectives of the current study were to, firstly, use eDNA to detect the presence of the alien invasive species *P. clarkii* in a laboratory setting (aquaria) and in the field (Lake Yahuarcocha). Secondly, the effectivity of the monitoring of *P. clarkii* through eDNA based methods was compared against traditional capture based methods. And lastly, the reliability of the method was tested in two other Andean lakes.

Materials & Methods

The study organism *Procambarus clarkii*

Procambarus clarkii (Girard 1852) (Cambaridae; Decapoda) was introduced in the coastal region of Taura, Ecuador in the period 1986-1988 (Salvador & Leyton, 2000) for aquaculture purposes. A “mixed culture” project was started in the area of Taura (Guayaquil), similar to Louisiana (United States), where *P. clarkii* was grown in rotation with rice (Chien, 1978;

Halwart and Gupta, 2004). However, after three harvests of rice in Taura, the project was abandoned because the production of *P. clarkii* was not profitable (Mora & Uyaguari, 2004; Salvador & Leyton, 2000). The species probably escaped when brought to Imbabura by local sellers, or perhaps was intentionally introduced as a food source. In 2014, the Ecuadorian Ministry of Agriculture, Livestock and Fishing warned about the presence of this species in Lake Yahuarcocha. The local authorities set out to eradicate it by 2015, mostly by trapping, but the species still persists and is used by locals as food.

Study area

Lake Yahuarcocha

Research was performed in Lake Yahuarcocha located in the province of Imbabura (00º22'N 78°06'W, 2210 m a.s.l), in the Andes mountains of Northern Ecuador (Figure 1A). The water body is heart-shaped (260 ha), is fed naturally through runoff and three natural springs, and has a maximum depth of 7.1 m (Van Colen et al., 2017). Traps and eDNA sampling were used for the detection of *P. clarkii* at 21 sampling points. Crayfish traps were placed in sets of 1-4 traps per sampling site (Figure 1A). Sites were chosen randomly based on information retrieved from previous studies (Escaleras, 2016; Portilla, 2015). Details of the sampling sites can be found in Appendix 2.1.

Three field sampling trips were organized (February-May, 2016) to test and optimize all methods. Results shown here are based on the fourth sampling campaign (June 15th, 2016), when techniques and work-flow had been optimized.

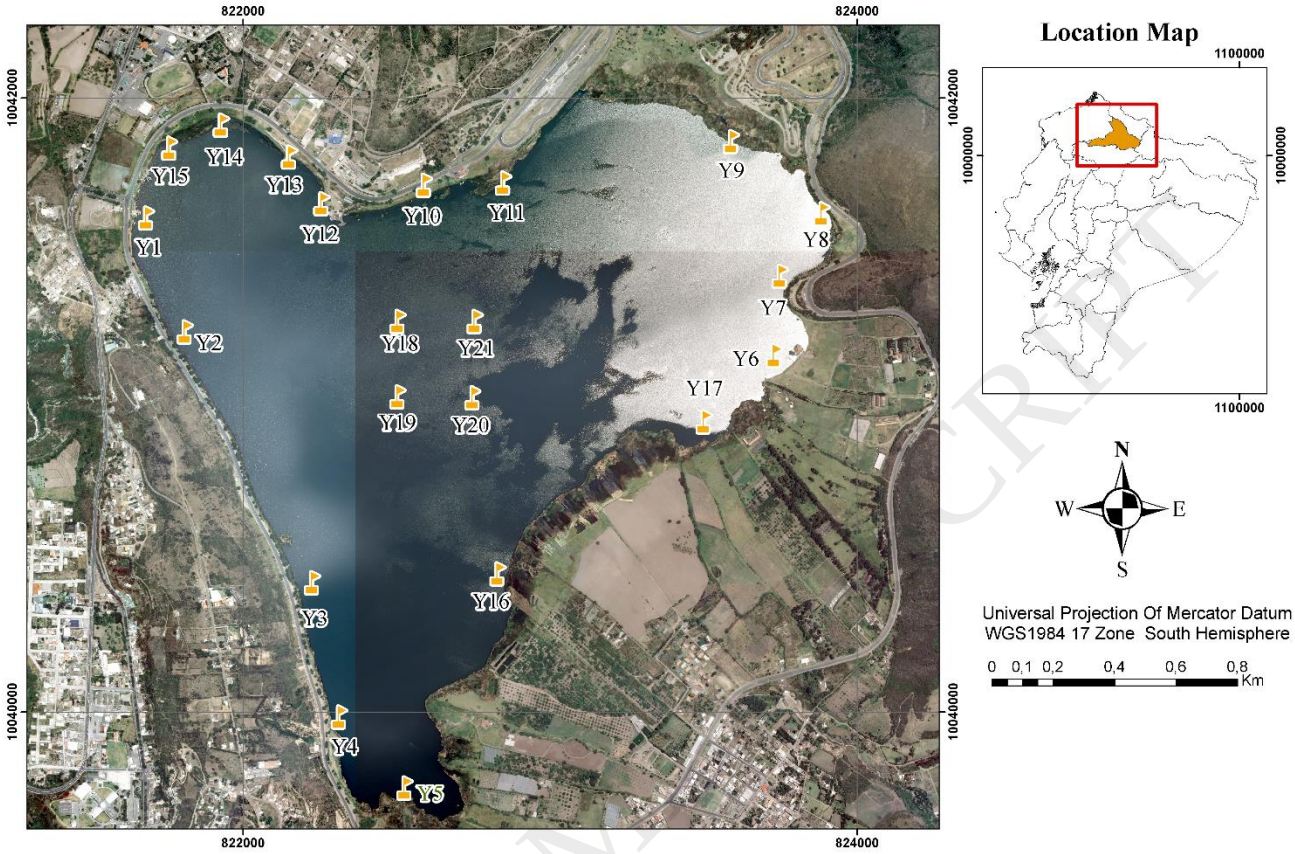
Lake San Pablo

Lake San Pablo (figure 1B) is located in the northern part of the Ecuadorian Andes, 20 km south from Lake Yahuarcocha at 2660 m a.s.l. (Imbabura province, 0°13’ N, 78°14° W). It covers a surface of 670 ha with a maximum depth of 35.2 m (Santander et al., 2006). Both eDNA sampling and traps were employed in three sampling points in Lake San Pablo (figure 1B; see Appendix table 2.2 for details on the sampling sites).

Lake Mojanda

The Mojanda volcano gave rise to three lakes. We focused on the largest, Caricocha, which we refer to as Lake Mojanda. This volcanic lake is found on the border between the Imbabura and Pichincha provinces at 3716 m a.s.l. (0° 08' N 78°15 W, figure 1C). It covers a surface of 30 ha, and has a depth of 109 m (Muylaert et al., pers. comm). eDNA sampling and crayfish traps were also employed at three sampling points in Lake Mojanda (figure 1C; see Appendix table 2.3 for details on the sampling sites).

A) **eDNA sampling sites in Lake Yahuarcocha (Ecuador)**



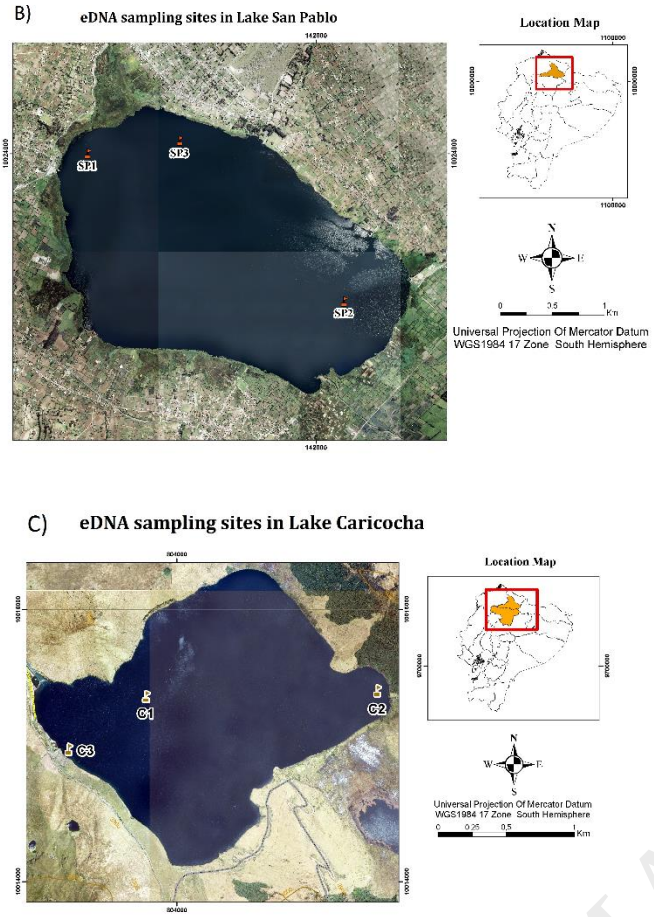


Fig. 1. Maps of the studied lakes A) Lake Yahuarcocha, B) Lake San Pablo, C) Lake Mojanda (originally called Caricocha). Flags show sampling points for *Procambarus clarkii* trapping and eDNA collection.

Methodology for the capture of *Procambarus clarkii*

Baited crayfish traps were used based on a model proposed by Hyatt (2004) for the capture of *P. clarkii* individuals in Lake Yahuarcocha. They were manufactured from 2.5 L plastic bottles, forming a cylinder of approximately 310 mm length and 100 mm width, and secured by clamps. One end featured a funnel with a 100 mm wide mouth. The opening of the funnel, the stem, was 25 mm in diameter. An anchor was added for stability. Bait, consisting of pieces of fish and beef was provided in each trap. The bait is commonly used by local fishermen to catch *P.*

clarkii and has been used in previous experiments. The traps were placed at less than 5 m distance from the shore depending on the conditions at each site. The period between the deployment and removal of the traps was 12 h overnight, from 18h00 to 06h00.

eDNA sampling

In order to avoid contamination, all the materials used to take eDNA samples (such as scissors and pliers) were sterilized by submersion in a solution of sodium hypochlorite (1/5 of commercial chlorine bleach) during 1 h; rinsed with distilled water, wrapped in aluminum paper, and dried at 80 °C for 2 h.

Aquarium samples

Lab based aquariums of 15 L of capacity were used to harbor *P. clarkii*. Crayfish were kept individually because of their territorial behavior. Afterwards, individuals were snap frozen and tissue samples were taken from the pereiopods for genomic DNA extraction.

Three *P. clarkii* individuals were placed in separate aquaria for 48 h. Two samples of 250 mL from each aquarium were filtered over a nitrocellulose filter (Porafil) of 0.45 µm pore size and 47 mm diameter with a vacuum pump fitted with a Nalgene filtration device (Thermo Scientific), in between sampling the device was rinsed with distilled water. After filtration, each filter was first cut into two parts with sterile scissors: each half was cut into several small pieces and collected in 1.5 mL Eppendorf tubes (one aquarium: four tubes). These were then stored at -20 °C, and DNA was extracted using the same protocol as the protocol described for field samples.

One negative laboratory control was used: per extraction, two 1 L bottles filled with sterile ultrapure water were left open in the filtration area each night before extraction. 300 mL of one control bottle was filtered over a nitrocellulose filter (Porafil: 0.45 µm pore size, 47 mm diameter) before the filtering of the field samples occurred (controlling for contamination of filtration area), 300 mL of the other bottle was filtered afterwards (controlling for contamination of filtration material and of the filtration area after handling other samples). Filters were processed as mentioned before: half filter per tube, two tubes (half filter) per control for two controls in total. In this way, we tested for contamination before and after filtering. These controls were extracted together with the field and aquarium samples. The DNA from extracted samples was resuspended in 20 µL of sterile ultrapure water. Extracted samples from the same control were pooled, resulting in a final volume of 40 µL.

Field samples

From Lake Yahuarcocha 21 points were sampled (Figure 1A). At each sampling point the sampling bottles were first rinsed with distilled water (autoclaved) before sampling took place. Two samples of 1000 mL were collected, over a range of 25 m: 200 mL was collected every 5 m and pooled to a final volume of 1000 mL. Contamination was avoided by wearing new gloves during each sampling. A cooling box was used for the shipping of the samples; the sampling bottles were covered with Ziploc bags to label each sampling point and to prevent contamination.

In the laboratory, the samples were filtered through sterile 0.45 µm pore size, 47 mm diameter nitrocellulose filters: 300 mL of water from each bottle (two per sampling point, 600 mL total). Each filter was processed as mentioned before. The membranes were divided equally into four

sterile 1.5 mL Eppendorf tubes that served for the process of extraction of DNA (4 halves of membranes = 1 sampling point).

Two 1 L bottles filled with sterile ultrapure water were taken into the field, where they remained closed during each sampling and put in the same cooling box with the other samples. Back at the lab, the content of the bottles was filtered and extracted using the same protocol as the field samples. These controls are further referred to as "negative field controls", because they can show if contamination happened in the field though handling of the bottles.

DNA extraction

Different environmental DNA extraction protocols were tested during the preparation phase. Based on in house results (and see Geerts et al., 2018), the MasterPureTM® extraction kit was chosen (see full protocol in Appendix). This method was used for all eDNA, control, and tissue samples. For each extraction, DNA from an Eppendorf tube filled with 20 µL of sterile ultrapure water was also extracted; this was the "negative extraction control".

To each tube (containing half filter or tissue sample), 1.3 µL Proteinase K (50 µg / µL) diluted in 400 µL Tissue and Cell Lysis Solution was added, followed by vortexing for 5 min. Each sample was incubated at 65 °C for 40 min, vortexing every 10 min. The liquid was transferred to a new sterile 1.5 mL Eppendorf tube and the pellet (filter) was discarded. Subsequently, 1.3 µL of RNase was added to each sample and vortexed for 5 min, followed by incubation at 37 °C in a warm water bath for 30 min. After the lysis, the samples were placed in the freezer for 15 min. MCP Protein Precipitation Reagent, 233 µL, was added to the samples and was vortexed for 1 min. The cell debris was pelleted (10000 x g, 4 °C, 10 min), and the supernatant transferred to a new sterile 1.5 mL tube. Five hundred µL isopropanol (cold) was added and each tube was turned by hand 40 times. To pellet the DNA, samples were centrifuged at 4 °C

for 10 min (10000 x g). The isopropanol was carefully removed by pipetting and the pellet was then washed two times with cold ethanol 70%. Finally, ethanol was removed and samples were air dried at room temperature for 3 h. DNA originating from the same sampling point was collected after extraction in the same sterile 1.5 mL Eppendorf tube. DNA was resuspended in 40 µL of sterile ultrapure water.

Primer development and PCR

The primers used were designed by Treguier et al. (2014) for mitochondrial genes, which amplify a COI region of 65 bp (see Table 1). The primer pair was chosen based on previous in-house laboratory tests and other studies for their efficiency (Cai et al., 2017).

Three PCR replicates were analyzed with the pooled DNA of each sampling point. PCR was also performed on the controls: 2 field, 2 laboratory, 1 extraction control, a positive PCR control (consisting of previously extracted and tested *P. clarkii* DNA), and one PCR control (0.6 µL ultrapure water instead of DNA). PCR was performed on a Mastercycler nexus gradient (Eppendorf).

The Taqman® Environmental Master Mix 2.0 was used in all reactions. The PCR was prepared in volumes of 10 µL: 5 µL of Taqman® Environmental Master Mix, 3.6 µL of MilliQ water, 0.4 µL of each primer (10 µM) and 0.6 µL of DNA.

Amplicons (10 eDNA samples and 1 tissue sample) of the PCR reactions were purified from the gel using Wizard SV Gel and PCR clean (Promega), prepared using the Macrogen Single Pass Sequencing protocol, and 20 µL per sample was sequenced on an Applied Biosystems 3730XL sequencer (Thermo Fisher Scientific). Amplicon sequences (see 3. Amplicon sequencing results in Supplementary material) were compared to known sequences using NCBI

BLAST 2.6.1 (Altschul et al., 1997). 'Procambarus clarkii taxid 6728' was selected as the amplicon sequences are very short.

Table 1 Polymerase chain reaction (PCR) primer pair sequences, and annealing temperatures used. Amplicon size includes primer pair length. The annealing temperature was chosen after gradient PCR

Species	Primer name	Primer sequence 5'-3'	Primer design	Primer origin	Amplicon size	Annealing temperature
Procambarus clarkii	SPY-ProCla-F SPY-ProCla-R	CAGAAGCTAAAGGAGATAA AACTAGGGGTATAGTTGAGAG	Tréguier et al. (2014)	COI (Tréguier et al., 2014)	65 bp	56 °C

Samples were loaded (8 µL PCR product and 2 µL ladder) on an 1X agarose gel with a 100 bp ladder (Promega). DNA was visualized with a Blue light transilluminator (Thermo Fisher). If samples showed a 65 bp size band they were considered positive. Results were only taken into account if positive controls reacted positively and all negative controls reacted negatively.

Results

Traditional trapping

The capture of *Procambarus clarkii* individuals with traditional baited crayfish traps varied from 1 to 4 individuals per trap per 24 h. *P. clarkii* was caught at 14 out of the 21 sampling points (see table 2). *P. clarkii* was never caught at the four sampling points in the central deeper parts of Lake Yahuarcocha (Yah 18-21, see figure 1A). *P. clarkii* was not found at any sampling point of both Lake San Pablo and Lake Mojanda.

Detection of eDNA

Procamburus clarkii was consistently identified in aquarium samples; all samples showing positive PCR results (100% accuracy). Twelve of the 21 locations in Lake Yahuarcocha were positive (Table 2). Locations were characterized as positive when two out of three replicates showed positive PCR results. No location had just one positive replicate (see table 2.4 in Appendix). Extraction and PCR were only taken into account when all negative controls showed no bands. Controls did not show evidence of contamination; hence no samples were excluded. The sequenced amplicons aligned significantly with known sequences of *P. clarkii* (BLASTX 2.6.1) (6e-08 E value, and 69% identification). Positive eDNA results were associated with positive trapping samples. If traditional trapping is considered 100% accurate, the eDNA method showed a relative accuracy of 85.7% for the positive detection of *P. clarkii* in field samples. All eDNA samples showed negative results at both Lakes San Pablo and Mojanda. These results correspond to the trapping results, as no *P. clarkii* were found in the baited crayfish traps.

Table 2. Results of both traditional trapping and eDNA monitoring in Lake Yahuarcocha. A "Yes" in the eDNA results means at least two out of three PCR replicates showed positive results.

CODE	Traditional trapping	eDNA results
Yah 1	Yes	Yes
Yah 2	Yes	Yes
Yah 3	Yes	No
Yah 4	Yes	Yes
Yah 5	Yes	Yes
Yah 6	No	No
Yah 7	Yes	Yes
Yah 8	Yes	Yes
Yah 9	Yes	Yes
Yah 10	Yes	Yes
Yah 11	Yes	No
Yah 12	Yes	Yes
Yah 13	Yes	Yes
Yah 14	No	No
Yah 15	No	No
Yah 16	Yes	Yes
Yah 17	Yes	Yes
Yah 18	No	No
Yah 19	No	No
Yah 20	No	No
Yah 21	No	No
Total	14	12

Discussion

We set out to identify *P. clarkii* using simple PCR techniques on eDNA samples from Lake Yahuarcocha (Ibarra, Ecuador). The results showed that *P. clarkii* was effectively detected with an accuracy of 85.7% (12 out of 14 locations) where the species was found with traditional trapping. This invasive species was detected without contamination or false positives, both in filtered aquarium water and field samples. Compared to traditional trapping, the eDNA method was almost as efficient; 12 out of 14 locations where *P. clarkii* was detected showed positive reactions. Our detection efficiency was higher than the first study that used eDNA to detect *P. clarkii* (Tréguier et al., 2014). The authors identified the species, using qPCR, in 59% of the locations where it was known to be present.

The two negative results were probably due to low DNA concentrations either in the sample or at the sampling site, since the habitat composition of these locations did not differ from the other 12. The amount of individuals caught with traditional trapping was relatively low (1 to 4 individuals per 24 h), which might, by chance, result in low DNA concentrations at locations with less *P. clarkii*. No access to nanovolume spectrophotometry (such as Qubit™) prevented us from measuring the DNA concentration of each sample. Additionally, as we saw from the sampling results in the deeper parts of the lake (Yah 18-21, see figure 1A), eDNA does not easily migrate within the lake. This might be attributed to the lentic characteristics of Lake Yahuarcocha, which shows little to no mixing. Aside from the deeper parts of the lake, *P. clarkii* was also absent from three other locations in Lake Yahuarcocha (Yah 6, 14-15). These locations are nesting spots for ducks and herons (Riascos, pers. obs.), which might result in a higher predation pressure on *P. clarkii*. It is documented that local fish and avian predators start feeding on *P. clarkii* relatively quickly upon invasion of a new habitat (Correia, 2001), and that the crayfish responds by exhibiting both complex and generalized reactions to fish predators

(Gherardi et al., 2011; Martin, 2014). Our results suggest that *P. clarkii* are, perhaps actively, avoiding these sites. No other differences were observed between locations.

Environmental DNA has proven to be an effective and rapid method to detect single species, such as endangered or alien invasive species (Agersnap et al., 2017; Rees et al., 2014), as well as extensive invertebrate communities (Deiner et al., 2016; Elbrecht et al., 2017). However, these studies used more advanced molecular techniques like RT-qPCR and Next-Generation sequencing. An approach similar to ours, using low-cost PCR techniques, has many advantages since access to advanced biotechnological techniques is limited in many South American research institutes (and other nations of the Greater South).

Our method was tested on two other lakes, Mojanda and San Pablo. Both the traditional and eDNA method showed the same results; no *P. clarkii* was detected. Here we tested whether the method worked for other locations and whether it was sensitive to false positives. Our results also suggest that *P. clarkii* has not invaded these lakes yet. The absence of the species might be due to several factors: it might neither have reached or been introduced to these lakes yet; the species has not been able to find a suitable ecological niche in these lakes; or the density of *P. clarkii* individuals is too low for detection with both eDNA and traditional methods. Future research should ideally include a number of Andean lakes where *P. clarkii* is present, to reflect differences in habitat preference and difference in detection depending on environmental conditions.

Because the eDNA method did not perfectly match with the presence of *P. clarkii*, we recommend to combine it with occasional traditional trapping. Monitoring could rely on eDNA while traditional trapping avoids false negative results. Such result was not entirely unexpected; previous research had also shown mixed detection efficiencies for *P. clarkii* (Tréguier et al., 2014) and other species (Rees et al., 2014). However, a recent study also using the more sensitive RT-qPCR method (but see Bastien et al., 2008) on *P. clarkii*, showed a detection

efficiency of 100% (Cai et al., 2017). Further research is needed to improve detection and to optimize PCR based eDNA methods. Once optimization has been achieved, eDNA sampling should be faster and cheaper than traditional monitoring (i.e. setting traps and recovering them later), and several locations can be monitored simultaneously. Therefore, we propose that field monitoring of *P. clarkii* using eDNA should occur more often than physical trapping. If *P. clarkii* is detected, several traps should be set in order to locate the individuals. We have shown that detection is possible using simple extraction and PCR methods. We believe this is the most important strength of the eDNA method: many locations are monitored continuously without disturbing the local ecosystem at a relatively low cost. Taking a field sample of 1 L from several spots per location should suffice to prove the presence.

Identifying invasive species in new habitats requires a range of efforts, from fast identification in the newly invaded locations, and a swift response to remove the species, developing a legal framework and enforcement capacity, to working with local communities to avoid further dispersal. Equatorial regions, like Ecuador, complicate matters, since species may reproduce continuously, resulting in more propagules and a higher chance for invasion (Gherardi, 2006). By combining traditional trapping with the eDNA method, results are obtained faster, which might allow for the efficient planning and implementation of a specific management action.

Our study is one of the first to report a budget-friendly, quick and reliable eDNA-based method for the detection of *P. clarkii* using classic PCR and Sanger sequencing. Such know-how friendly techniques are accessible to any research group. In order to conserve the rich biodiversity present in equatorial ecosystems, we need quick, efficient and low-budget tools to successfully identify alien invasive species. We suggest that future research should broaden the scale of monitoring for *P. clarkii*, and other local invasive species such as guppies *Poecilia reticulata* and carp *Cyprinus carpio*, by sampling freshwater ecosystems across Ecuador and

South America to compare eDNA with traditional trapping results and recommend the use of eDNA as a fast warning method for newly-invaded habitats.

Conclusion

The application of molecular tools to detect alien invasive aquatic organisms in Ecuador, and by extension worldwide, is a fundamental part of biodiversity conservation. We have shown that low-budget eDNA methods using classic PCR and Sanger sequencing can be successfully applied to detect a highly invasive species. Future research should focus on validating the results in a wider geographical context by comparing traditional and eDNA methods.

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