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ORIGINAL ARTICLE

# The development of early monitoring tools to detect aquatic invasive species: eDNA assay development and the case of the armored catfish *Hypostomus robinii*

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## Abstract

Introductions of invasive non-native species (and their subsequent impacts) are recognized as a major threat to native flora and fauna. This is especially true in island ecosystems such as the tropical island of Martinique. In 2018, one such aquatic invasive species, the suckermouth catfish *Hypostomus robinii* (Loricariidae), was reported for the first time in two of the islands rivers. *H. robinii* is a popular freshwater aquarium fish and native to tropical and sub-tropical South America. Since its initial discovery, a growing number of populations have been found, suggesting a larger distribution of this species through Martinique's hydrographic network. Here, we developed a novel survey technique (utilizing environmental DNA) and conducted a widespread survey across the island to assess the distribution of this invasive species. We were able to detect *H. robinii* in 22% of sites surveyed (18 out of 83) via our eDNA-based assay. The presence of these fish was confirmed using traditional trapping at 14 of these sites. Additionally, we used occupancy modeling to investigate the impact of different environmental covariates on the detection efficiency of the novel assay and the potential impacts of false positives and negatives. We highlight a decrease in the detection probability when water volume filtered increases. That said, the eDNA-based method proves a useful tool for the detection of this invasive fish species and monitoring its spread for management purposes.

**KEYWORDS**
biodiversity hotspot, eDNA detection, *Hypostomus robinii*, invasive species, Martinique

Thomas Dubreuil and Thomas Baudry are co-first authors.

## 1 | INTRODUCTION

Although they occupy <0.8% of the total Earth surface, freshwater ecosystems provide habitat for at least 6% of known species, with many more likely still to be discovered (Michelet, 2017). Almost one-third of this freshwater biodiversity faces extinction, largely due to habitat loss, introduction of aquatic invasive species (AIS), pollution and over-harvesting (IUCN, 2020; Muralidharan, 2017). The main introductory pathway for AIS is arguably shipping (and the discharge of ballast water in particular); however, releases from aquaculture, and/or the pet trade have also shown to be sources of invasive species in specific cases (Molnar et al., 2008; Strecker et al., 2011). Regardless of the pathway of introduction, AIS often facilitates profound negative impacts on native biodiversity due to predation, competition and/or the spread of pathogens (Grandjean et al., 2017; Momot, 1995; Remon et al., 2016). They have even been accredited as likely sources of spreading human zoonotic diseases such as the West Nile Virus, Dengue Fever, Chikungunya Virus, and Dirofilariasis (Conn, 2014). Therefore, understanding AIS can have direct implications in human health and should stand at the forefront of policy meaning they are well suited to being applied to the 'One Health' concept which is being explored in recent years (Conn, 2014). The impacts of AIS are even more important in island ecosystems which often harbor more vulnerable native populations due to their isolation (Towns et al., 2006). As a first step to even attempt to assess, manage, mitigate and contain these invasions, the early detection of AIS is indispensable (Ficetola et al., 2008; Harper et al., 2018).

One such AIS, originally from the islands of Trinidad and Tobago (Boeseman, 1960), is *Hypostomus robinii*. Belonging to the Loricariidae family (Order Siluriformes), *H. robinii* can reach more than 320 mm in size with a weight of almost 350 g (Figure 1). The species is also often referred to as the 'armored catfish,' due to its body coverage of bony plates (Nico et al., 2009). Despite its distinct appearance, genetic analysis is often required to confirm species identification due to the sheer diversity associated with this family (Brandão et al., 2018; Matamoros et al., 2016). This particular species is, however, extremely popular in the aquarium trade, and as such, there have been many documented releases into wild river systems (Cook-Hildreth et al., 2016; Matamoros et al., 2016). Some studies have even documented the ecological and economic impacts this species has had in regions outside of the fish's native home range (Orfinger & Goodding, 2018; Pound et al., 2011). The species is known to breed extensively in many of these new habitats, with females releasing upwards of 200 eggs during a spawning event (Deacon, 2015). Further, these eggs hatch after only 10 days. Together, these traits make *H. robinii* an ideal invader, able to quickly colonize entire watersheds in short time periods (Deacon, 2015).

In Martinique, the armored catfish (*H. robinii*) was introduced through the pet trade via releases from aquarists, as the species was sold in pet shops until its recent classification as a level two order of invasive alien species in 2019 (NOR: TREL1934054A). The species was initially observed to inhabit two rivers in 2018, when a large-scale study targeting the Australian red-claw crayfish *Cherax quadricarinatus* was undertaken (Baudry et al., 2020). Following this first record, the presence of *H. robinii* has been confirmed upstream in these same rivers following electrofishing surveys led by the Direction de l'Environnement, de l'Aménagement et du Logement (DEAL), and the Office De l'Eau of Martinique (ODE). Locally, its presence is considered as a critical threat for the native biodiversity, particularly for native fish species such as *Sicydium* sp, which are suspected to share the same or similar diet. However, the true spread of *H. robinii* remains unknown, and without such baseline data, any attempts of management and mitigation would likely not work from the onset. Traditional survey methods (e.g., electrofishing campaigns,

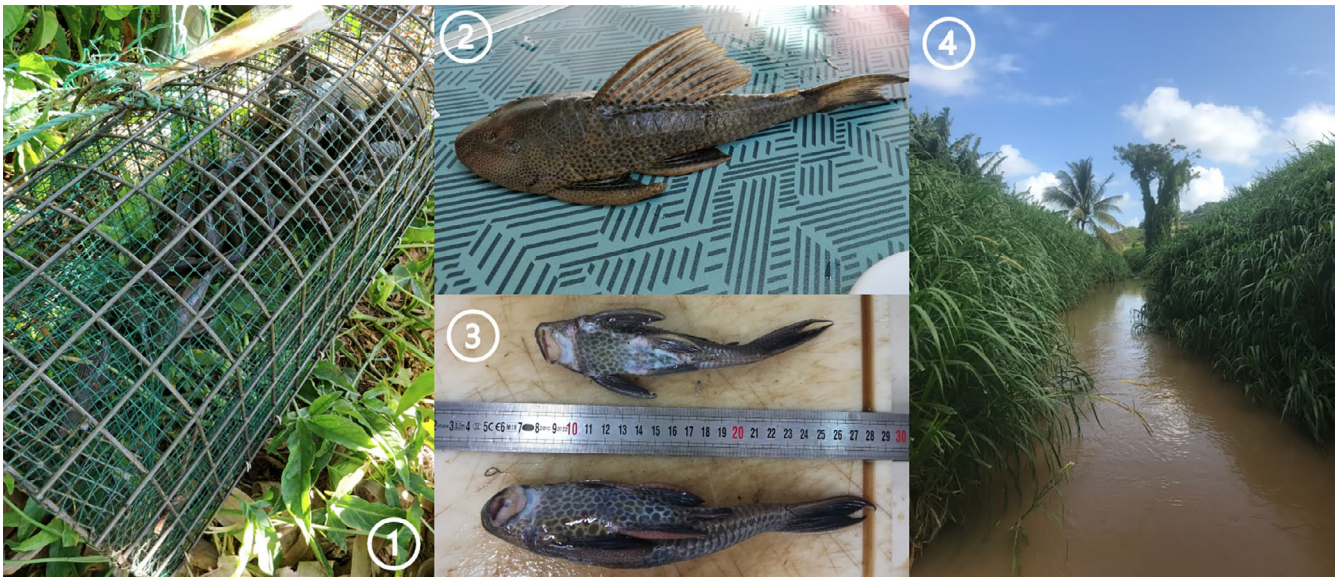


**FIGURE 1** Highlighting (1) The trap used for sampling, (2 and 3) Pictures of *Hypostomus robinii* occurring in Martinique and (4) "Brasserie Lorraine" sampling site

kick sampling, and the use of nets) are very useful to ascertain the general trends of many fish species in river systems; however, they are not without their own shares of problems (Radinger et al., 2019). For example, they require large sampling efforts are time consuming, ecologically invasive, and often lead to an underestimation of species distribution, especially when population densities are low (Hänfling et al., 2016; Manfrin et al., 2019; Robinson et al., 2018). In recent years, the use of environmental DNA (eDNA) has become increasingly popular as it is often reported as being equally if not more reliable, faster, and cheaper when attempting to monitor the distribution of a wide range of aquatic organisms (Baudry et al., 2021; Troth et al., 2020; Wang et al., 2021).

In this study, we developed and validated a species-specific eDNA-based detection protocol to monitor *H. robinii* through real-time quantitative PCR (qPCR). Following in silico, in vitro, and in situ validations of our assay, a large-scale monitoring campaign combining both eDNA detection and traditional fishing was performed at 83 sites across Martinique island. Here we present the most accurate and up-to-date occurrence data for this AIS in the river systems across Martinique and compare our novel eDNA-based monitoring approach to more conventional methods to assess reliability. Finally, we discuss the impacts, the armored catfish may have on local biodiversity and the scope for the possibility of eradicating the species from this biodiversity hotspot.

## 2 | MATERIAL AND METHODS

### 2.1 | Sampling protocol

Sampling was performed at 83 sites, across 53 streams on Martinique Island, and two closed water systems. Sampling was performed on sites where fish were known to be present or absent, and at unknown locations. All 83 sites were sampled following a standardized eDNA protocol (see below and Baudry et al., 2021) and further assessed using trapping to compare the efficiency of both methods.

For environmental DNA, a 4 L water sample was collected from the river using a Nalgene™ plastic bottle. A surveyor would walk a transect from one side of the bank to the other collecting as they went. This sample was then immediately filtered using a filtration unit (Nalgene™) and vacuum pump (Nalgene™ Repairable Hand-Operated PVC Vacuum Pumps with Gauge, 10 inHg vacuum; Cowart et al., 2018). Water samples were filtered until saturation using nitrocellulose filters (Sartorius 47 mm diameter and 0.45 µm pore size) and the volume filtered was recorded. The filter was then removed and stored (folded in quarters) in 1.5 ml microcentrifuge tubes with 1 ml of absolute ethanol as conservation buffer. Three biological replicates (i.e. three independent filters) were collected at each sampling site. For each sample, a fresh pair of powder-free nitrile gloves were worn and the equipment (tweezers) was decontaminated between each sampling site with 50% bleach and autoclaved after each sampling day. A field blank was also collected at each sampling site, allowing us to assess for any potential cross-contamination between locations. For this, 1000 ml of distilled water was filtered and stored as described above. All samples were kept in a cooler box until they were returned to the laboratory and placed at −20°C until DNA extraction (Appendix S1A).

### 2.2 | DNA sequencing

Due to the morphological similarities associated with members of the *Hypostomus* genus, species identification was confirmed via DNA sequencing prior to the development of the assay (Jardim de Queiroz et al., 2019). Two specimens, captured in two different sites in Martinique, were analyzed. DNA was extracted using the DNeasy® Blood & Tissue Kit following the manufacturer's recommendations. A fragment of the cytochrome c oxidase I (COI) was amplified by PCR using universal primers (H15149/L14841) previously designed by Kocher et al. (1989). The protocol followed was as in Chucholl et al. (2015): 2 min 30 s at 95°C for the initial denaturing step, followed by 35 cycles of 45 s at 95°C; 1 min at 48°C and 1 min at 72°C. The final elongation step was 10 min at 72°C. Amplified products were sequenced on an ABI PRISM 3130xl automated sequencer (PE Applied BioSystems). Both the forward and reverse primers were utilized for sequencing, and the resulting fragments aligned and edited with Sequencher® (version 5.4, 2016). The taxonomic assignment was then assessed with BLASTn 2.10.1 (Madden, 2002) comparing COI sequences to the non-redundant database of NCBI (Version August 2020). The newly generated sequences were deposited on NCBI GenBank under the accession numbers MZ066382 and MZ066383.

### 2.3 | qPCR assays

After confirmation of the species (see Results), species-specific primers and probe, targeting an 88 bp fragment within the COI region were designed for *H. robinii*. The forward primer 5′- CTCAGGGGTTGAAGCGGGA −3′, reverse primer 5′- GTCAACTGAAGCTCCTGCA −3′ and a specific 6-FAM MGB labeled probe 5′- ACCCACCCCTCGCTGGAAATTTA −3′ were constructed using sequences generated from specimens collected by the authors and from sequences previously deposited on GenBank (Appendix S1B). The species-specific assay was designed using the Geneious Pro R10 Software (https://www.geneious.com; Kearse et al., 2012) and following the method outlined in Brys et al. (2020). Sequences from the targeted species (*H. robinii*), along with closely related species, and an exhaustive list of co-occurring fish species also present in Martinique were used to increase the assays specificity *in silico* (Appendix S1B) via the primer-blast tool from NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Furthermore, in vitro testing, aimed at confirming primer specificity was also undertaken using DNA extracted from *H. plecostomus* and *Ancistrus sp.* (both belonging to the Loricarideae family), *Poecilia reticulata* (a fish occupying the same ecological niche in Martinique), and

*Phoxinus phoxinus*, a European fish species. Despite all efforts to ensure a single species target, the Loricariidae genus includes many subspecies, which are genetically very close. Although we found that our assay was specific to *H. robinii* (see results below), there may well be cross amplification with a closely related species not tested against in this study. That said, any Loricariidae would be classified as an AIS in Martinique, so this was not deemed as a major issue.

## 2.4 | qPCR protocol

The eDNA samples were extracted from filters following the method outlined in Baudry et al. (2021) (Appendix S1A). qPCR was then undertaken on all samples as follows. Optimization of the qPCR assay was initially undertaken utilizing a temperature gradient from 59°C to 63°C to determine the optimal annealing temperature. Similarly, different primers and probe concentrations, ranging from 0.5 μM to 10 μM of final concentration were investigated. qPCR reactions were as follows: 12.5 μl of TaqMan® Environmental Master Mix 2.0 (Life Technologies, Applied Biosystems), 2.5 μl of the forward primer (final concentration of 5 μM), 2.5 μl of the reverse primer, 1 μl of probe (final concentration of 5 μM), 1.5 μl of DNA-free water and 5 μl of DNA template (extracted from either individuals, eDNA samples or DNA-free water). Amplifications were run on a Roche LightCycler® 480 II quantitative thermocycler as follows: activation at 95°C for 10 min, then a repetition of 55 cycles of 1 min 15 s including 15 s at 95°C and 1 min at 61°C.

To determine potential contamination and assess the efficiency of qPCRs, four negative controls and four standards (i.e., DNA extracted from the targeted species with a known concentration) were added to each plate alongside the eDNA samples. These dilution standards, ranging from 4.25 to $2.59.10^{-4}$ ng/μl, were obtained doing a serial dilution from an initial concentration (17 ng/μl) following the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin et al., 2009). This further allowed us to calculate the relative DNA concentration in positive eDNA samples. For each site, six technical replicates were performed (using two technical replicates per filter). If one replicate showed a signal with a Ct below 42 the site was considered as positive for this AIS (Bedwell & Goldberg, 2020). Any signal higher than 42 was considered as a 'false positive' (Agersnap et al., 2017).

## 2.5 | Limit of detection and limit of quantification

Due to the variability in eDNA concentration across each of the samples (and following the MIQE guidelines), it was necessary to determine the LOD (limit of detection) and LOQ (limit of quantification) (Bustin et al., 2009). According to Mauvisseau et al. (2019), LOD corresponds to the concentration when one replicate out of 10 is positive with a signal below 45 Ct and LOQ is the concentration when nine replicates out of 10 are positive with signal below 45 Ct. Dilution series of known concentration using DNA extracted from *H. robinii* from Martinique was then performed. LOQ and LOD were determined using 10 replicates of standards, from $4.15.10^{-3}$ to $2.53.10^{-7}$ ng/μl.

## 2.6 | Trapping

Trapping consisted of the deployment of two large fish traps at each site (80 cm length × 25 cm width × 25 cm height with mesh of 1 cm × 1 cm and a single cone-shaped inlet; Figure 1). The traps were baited with mango to maximize fish capture after a prospection alongside the riverbank to find an index of presence (Culp & Glozier, 1989; Merilä, 2015). Traps were placed in flat streams or pits in the afternoon and collected the next morning after approximately 16 h of deployment. Each site was sampled three times. The AIS captured were measured to the nearest mm, weighed to the nearest g, and anesthetized using pure alcohol and clove oil following the protocol described by CQEEE (2015). Catch Per Unit Effort (CPUE) was calculated by dividing the number of fish caught by all traps by the number of fishing replicates. Results are reported in Table 1.

## 2.7 | Statistical analysis

To illustrate species distribution, QGIS 3.4.15 was used to generate maps (QGIS Team Development, 2016). The Martinique map was retrieved on the IGN© database and the streams on BDCarthage® and BD Topo®. Occupancy modeling using a Bayesian approach developed in Griffin et al. (2019) was used to assess potential false negative and false-positive errors (i.e., due to sample collection, laboratory experiments, biotic and abiotic factors). First, the presence or absence probability of the species was randomly set by the model as covariates. The random parameters, implemented in the model, allow for the determination of the following probabilities from both our field and lab data: probability of eDNA presence in the sample (defined as $\theta_{11}$, for true positive, and $\theta_{10}$, for false positive) and probability of positive qPCR replicate, identified as $p_{11}$, for true positive, thus species eDNA presence, and $p_{10}$, for false positive. This analysis was run using the following R Shiny application (https://seak.shinyapps.io/eDNA/; Griffin et al., 2019).

To quantify observation errors during the sampling plan and to allow for an estimate of the impact of environmental factors on *H. robinii* eDNA detection, Bayesian Monte Carlo Markov Chain (MCMC) algorithm was utilized (Dorazio & Erickson, 2018). pH, oxygen concentration, temperature, altitude, oxygen saturation, conductivity, and volume of water filtered were tested. This analysis was run with R 4.0.2 with the R package ednaoccupancy, designed to be used in MCMC algorithm (Dorazio & Erickson, 2018). The model was

**TABLE 1** Sampling location with field information (altitude, coordinates, river name, and total volume filtered), eDNA detection results (qPCR positive proportion considering the six technical replicates and the mean cycle threshold (Ct) for positive samples)

| Sampling location | Altitude (m) | Coordinates (° ′ ″) | Stream | Total volume filtered (ml) | qPCR positive proportion | Mean Ct | Catch Per Unit Effort (CPUE) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2018 (Baudry, unpublished data) | This study |
| Pont de Bassignac | 120 | 14°43′54″N; 60°59′4″O | Galion | 1150 | 0 | A.D | 0 | 0 |
| Fonds Galion - Sainte Luce | 160 | 14°44′7″N; 61°0′57″O | Galion | 2400 | 0 | A.D | | 0 |
| Usine du Galion | 20 | 14°43′7″N; 60°57′15″O | Galion | 600 | 1 | 38.28 | | 0.50 |
| Vauclin Pont N6 | 7 | 14°32′56″N; 60°50′42″O | Vauclin | 300 | 0 | A.D | | 0 |
| Pacquemar Pont N6 | 10 | 14°31′21″N; 60°50′16″O | Pacquemar | 450 | 0 | A.D | | 0 |
| Dormante | 20 | 14°29′9″N; 60°57′42″O | Oman | 800 | 0 | A.D | 0 | 0 |
| Station Epuration Marin | 20 | 14°28′12″N; 60°51′17″O | Mastor | 600 | 0 | A.D | | 0 |
| Ponton Savane | 10 | 14°23′50″N; 60°51′57″O | – | 900 | 0 | A.D | | 0 |
| Fontane | 20 | 14°35′1″N; 60°52′27″O | Simon | 450 | 0 | A.D | | 0 |
| Pont Madeleine | 10 | 14°29′46″N; 60°54′18″O | Petite Rivière Pilote | 950 | 0 | A.D | 0 | 0 |
| Desmartinières | 180 | 14°31′15″N; 60°54′41″O | Petite Rivière Pilote | 1000 | 0 | A.D | 0 | 0 |
| Domaine La Mauny | 30 | 14°30′12″N; 60°54′20″O | La Mauny | 1000 | 0 | A.D | | 0 |
| Guénot - Rivière Pilote | 20 | 14°29′36″N; 60°53′44″O | Grande Rivière Pilote | 1000 | 0 | A.D | 0 | 0 |
| Lowinski | 40 | 14°31′0″N; 60°52′55″O | Grande Rivière Pilote | 1200 | 0 | A.D | 0 | 0 |
| Jossaud | 50 | 14°31′31″N; 60°52′52″O | Grande Rivière Pilote | 800 | 0 | A.D | | 0 |
| Petit Bourg | 10 | 14°32′51″N; 60°57′36″O | Coulisses | 1600 | 0 | A.D | 0 | 0 |
| Parcours Sportif Saint-Esprit | 70 | 14°33′29″N; 60°56′24″O | Coulisses x Cacaos | 1550 | 0 | A.D | 0 | 0 |
| Duchâtel Cacaos | 40 | 14°34′26″N; 60°55′58″O | les Cacaos | 650 | 0 | A.D | | 0 |
| Pont D17 Saint-Esprit | 30 | 14°33′34″N; 60°56′4″O | Roussane | 1750 | 0 | A.D | | 0 |
| La Favorite | 80 | 14°37′59″N; 61°2′6″O | Jambette | 1000 | 0 | A.D | | 0 |
| Haut Case Navire | 20 | 14°37′17″N; 61°6′5″O | Case Navire | 2100 | 0 | A.D | 0 | 0 |

(Continues)

TABLE 1 (Continued)

| Sampling location | Altitude (m) | Coordinates (° ′ ″) | Stream | Total volume filtered (ml) | qPCR positive proportion | Mean Ct | Catch Per Unit Effort (CPUE) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2018 (Baudry, unpublished data) | This study |
| Ecole Fond Lahaye | 20 | 14°37′52″N; 61°6′33″O | Fond Lahaye | 2250 | 0 | A.D | | 0 |
| Maison de la Canne | 10 | 14°32′2″N; 61°1′10″O | Vatable | 1800 | 0 | A.D | | 0 |
| Trenelle 1 - Trenelle | 0 | 14°31′51″N; 60°57′56″O | Trenelle | 1200 | 0 | A.D | | 0 |
| Trenelle 2 - Abandon | 0 | 14°31′39″N; 60°58′11″O | Abandon | 1000 | 0 | A.D | | 0 |
| Didier - Après tunnel | 260 | 14°39′38″N; 61°5′52″O | Duclos | 3600 | 0 | A.D | | 0 |
| Didier - Avant tunnel | 150 | 14°38′45″N; 61°5′37″O | Dumauzé | 3350 | 0 | A.D | | 0 |
| Ozanam Maniba | 30 | 14°38′55″N; 61°8′6″O | Case Pilote | 2400 | 0 | A.D | | 0 |
| AMEP Moutte | 60 | 14°38′12″N; 61°2′42″O | Monsieur | 2400 | 0 | A.D | | 0 |
| Quartier Rivière l'Or | 200 | 14°39′58″N; 61°3′42″O | Monsieur | 1400 | 0 | A.D | | 0 |
| Balheu | 30 | 14°38′56″N; 61°0′59″O | Longvilliers | 850 | 0.66 | 35.93 | | 10.66 |
| Pont N8 | 10 | 14°33′53″N; 60°57′55″O | Manche | 1000 | 0.16 | 38.21 | | 0 |
| Quartier Rivière Lézarde 2 | 180 | 14°42′31″N; 61°1′58″O | Lézarde | 3800 | 0.75 | 35.01 | | 0.83 |
| Habitation Saint-Etienne | 110 | 14°41′32″N; 61°0′57″O | Lézarde | 2700 | 0.83 | 34.87 | 0 | 3.33 |
| Route des Gués | 130 | 14°40′42″N; 61°1′19″O | Blanche | 1200 | 0.83 | 38.20 | 0 | 12.66 |
| Quartier Hotel Plaisir | 220 | 14°41′12″N; 61°3′2″O | Blanche | 1400 | 0 | A.D | | 0 |
| Gué de la Désirade | 40 | 14°39′58″N; 60°59′45″O | Lézarde | 1100 | 0.66 | 34.83 | 1.2 | 7.00 |
| Soudon / Nasse Caraibes | 30 | 14°38′54″N; 60°59′24″O | Lézarde | 1500 | 0.66 | 34.34 | 2.11 | 6.83 |
| Ressource | 0 | 14°36′17″N; 60°59′53″O | Lézarde | 1500 | 0.50 | 33.35 | | 2.83 |
| Moulin à eau | 30 | 14°39′47″N; 60°58′8″O | Petite Rivière | 950 | 0.66 | 33.70 | | 13.33 |
| Rivière Caleçon | 30 | 14°36′49″N; 60°57′49″O | Caleçon | 1700 | 0.66 | 33.73 | | 0 |
| Brasserie Lorraine | 10 | 14°37′12″N; 60°58′28″O | Petite Rivière | 1100 | 0.66 | 33.94 | 4.5 | 19.00 |
| Saint Maurice | 60 | 14°40′11″N; 61°0′1″O | Petite Lézarde | 900 | 0.66 | 34.18 | 0 | 1.16 |
| Denel | 130 | 14°43′7″N; 60°59′47″O | Tracée | 1800 | 0 | A.D | | 0 |
| Fonds Desforts | 20 | 14°42′48″N; 60°58′3″O | Tracée | 1800 | 0.16 | 38.83 | | 0.83 |
| Case Dujon | 10 | 14°48′30″N; 61°0′6″O | Sainte-Marie | 1400 | 0.83 | 35.57 | | 7.00 |
| Marigot D15c | 60 | 14°49′11″N; 61°2′9″O | Marigot | 1150 | 0 | A.D | | 0 |

Continues

TABLE 1 (Continued)

| Sampling location | Altitude (m) | Coordinates (° ′ ″) | Stream | Total volume filtered (ml) | qPCR positive proportion | Mean Ct | Catch Per Unit Effort (CPUE) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2018 (Baudry, unpublished data) | This study |
| Habitation Charpentier | 30 | 14°48′13″N; 62°1′40″O | Charpentier | 800 | 0 | A.D | | 0 |
| Seguineau | 0 | 14°49′31″N; 61°2′35″O | Lorrain | 2900 | 0 | A.D | 0 | 0 |
| Ravine Lorrain | 50 | 14°48′9″N; 61°3′3″O | Lorrain | 1700 | 0 | A.D | 0 | 0 |
| Fonds Gens Libres | 30 | 14°49′39″N; 61°4′12″O | Grande Anse | 1200 | 0 | A.D | | 0 |
| Amont Mangatal | 10 | 14°42′54″N; 61°10′7″O | Carbet | 2100 | 0 | A.D | 0 | 0 |
| Campbeilh | 80 | 14°43′1″N; 61°9′41″O | Carbet | 1800 | 0 | A.D | 0 | 0 |
| Cascade du Carbet | 140 | 14°42′48″N; 61°9′4″O | Carbet | 2700 | 0 | A.D | 0 | 0 |
| Maison Rousse | 200 | 14°43′46″N; 61°8′28″O | Carbet | 3850 | 0 | A.D | | 0 |
| Grand Fond | 390 | 14°43′35″N; 61°6′59″O | Carbet | 5800 | 0 | A.D | | 0 |
| Canal Beauregard | 180 | 14°43′10″N; 61°9′49″O | Carbet | 1800 | 0 | A.D | | 0 |
| Rivière Céron | 10 | 14°50′19″N; 61°13′29″O | Céron | 3700 | 0 | A.D | 0 | 0 |
| Case Petit | 112 | 14°48′31″N; 61°13′2″O | Prêcheur | 1200 | 0 | A.D | | 0 |
| Route de Depaz | 120 | 14°45′27″N; 61°9′49″O | Roxelane | 2300 | 0 | A.D | | 0 |
| Trois Ponts | 60 | 14°45′25″N; 61°9′43″O | Clitandre | 1000 | 0 | A.D | | 0 |
| Renault Saint-Pierre | 70 | 14°45′15″N; 61°9′50″O | Rivières des écrevisses | 1200 | 0 | A.D | | 0 |
| Cœur Bouliki | 300 | 14°42′0″N; 61°4′8″O | Rivière Blanche | 2650 | 0 | A.D | 0 | 0 |
| Fond Marie-Reine | 320 | 14°45′52″N; 61°7′12″O | Capot | 2350 | 0 | A.D | | 0 |
| Mackintosh | 350 | 14°46′41″N; 61°7′0″O | Capot | 2400 | 0 | A.D | 0 | 0 |
| Fond Labour | 290 | 14°48′24″N; 61°5′55″O | Capot | 2800 | 0 | A.D | | 0 |
| Vallée Capot | 20 | 14°50′18″N; 61°5′28″O | Capot | 3000 | 0 | A.D | | 0 |
| Stade Grand'Rivière | 80 | 14°52′30″N; 61°8′52″O | Grande Rivière | 6300 | 0 | A.D | 0 | 0 |
| Route de Macouba | 90 | 14°52′21″N; 61°8′53″O | Rivière de Macouba | 5900 | 0 | A.D | | 0 |
| Bourg Basse Pointe | 20 | 14°52′7″N; 61°6′54″O | Basse Pointe | 4400 | 0 | A.D | | 0 |
| Fonds Saint-Jacques | 70 | 14°47′50″N; 61°0′27″O | Saint-Jacques | 2700 | 0 | A.D | | 0 |
| Trace des Jésuites | 314 | 14°44′47″N; 61°5′9″O | Lorrain | 4800 | 0 | A.D | | 0 |
| Saut Babin | 200 | 14°48′43″N; 61°7′18″O | Capot | 4500 | 0 | A.D | | 0 |
| EPLEFPA Robert | 0 | 14°39′24″N; 60°56′2″O | Cacao | 1500 | 0 | A.D | | 0 |
| Beauséjour | 60 | 14°33′5″N; 60°55′18″O | Beauséjour | 900 | 0 | A.D | | 0 |
| Duchatel St Pierre | 40 | 14°34′44″N; 60°56′30″O | Saint Pierre | 600 | 0.33 | 37.86 | | 1.33 |

(Continues)

TABLE 1 (Continued)

| Sampling location | Altitude (m) | Coordinates (° ′ ″) | Stream | Total volume filtered (ml) | qPCR positive proportion | Mean Ct | Catch Per Unit Effort (CPUE) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2018 (Baudry, unpublished data) | This study |
| Monnerot | 20 | 14°36′36″N; 60°52′59″O | Beauregard | 2700 | 0 | A.D | | 0 |
| Grands Fonds - François | 12 | 14°36′50″N; 60°55′18″O | Desroses | 1500 | 0 | A.D | | 0 |
| Trois Bras | – | 14°50′46″N; 61°11′51″O | Trois Bras | 7500 | 0 | A.D | | 0 |
| Anse Couleuvre | – | 14°50′30″N; 61°12′17″O | Couleuvre | 5400 | 0 | A.D | | 0 |
| Rue Jambette | 10 | 14°37′9″N; 61°2′10″O | Jambette | 750 | 0 | A.D | 0 | 0 |
| EM Anse Madame | 10 | 14°37′9″N; 61°6′1″O | Case Navire | 3300 | 0 | A.D | 0 | 0 |
| Manzo | 40 | 14°35′41″N; 60°56′20″O | – | 1000 | 0 | A.D | 0 | 0 |

*Note:* A.D. in 'Mean Ct' column represents an absence of detection. CPUE represents the number of specimens caught per event per trap as a confirmation of *Hypostomus robinii* presence. CPUE was highlighted for 2018 (Baudry, unpublished data), the first observation of *Hypostomus robinii* in traps, targeting originally another aquatic invasive species, and for this study. All present sites have not prospected during 2018.

fitted as described by the authors and MCMC chains were run for 10,000 iterations.

## 3 | RESULTS

### 3.1 | Sequencing to confirm species identification

From the two fishes caught at the two different sites, sequences of respectively 320 bp length (Brasserie Lorraine) and 312 bp (Soudon) from a fragment of the COI gene were obtained. Both returned matches to *Hypostomus robinii* with 99.6% similarity (closet Accession Number: DQ133770.1).

### 3.2 | Validation and specificity of eDNA assay using qPCR

The primers and probes designed in this study were found to be species-specific to the invasive *H. robinii* collected in Martinique rivers. DNA from non-target species, *H. plecostomus*, *Poecilia reticulata*, *Ancistrus* sp. and *Phoxinus phoxinus*, was not amplified. In situ positive and negative controls performed as expected during the study: samples collected at sites where *H. robinii* was known to occur showed an efficient amplification and inversely, samples collected where this AIS is known to be absent did not amplify. Optimum qPCR yields were observed when running qPCR protocol under the following setting: 5 μM of probe and primers final concentration and 61°C of temperature annealing. Following Mauvisseau et al. (2019), the LOD corresponds to $3.24.10^{-5}$ ng.μl$^{-1}$ at 37.53 Ct and the LOQ to $2.59.10^{-4}$ ng.μl$^{-1}$ at 37.24 ± 0.66 Ct.

### 3.3 | Distribution of *Hypostomus robinii*

No amplification occurred in any of the control samples (i.e. distilled water filtered before any of the eDNA samples were taken at each site). This indicates, no cross-contamination occurred between our sampling locations.

Via the use of eDNA, *H. robinii* was detected at 18 of the sites surveyed, corresponding to one closed water system (an ornamental pond) and 12 different rivers (Figure 2). The AIS presence was further confirmed using traditional trapping in 14 of these 18 sites (Figure 2). All sites that showed a negative signal with eDNA-based monitoring were also negative using traditional trapping. Interestingly, we were not able to trap these fish in some of the eDNA positive sites, despite previous visual observation of the AIS. For example, in the river La Manche (Pont N8 site), no *H. robinii* were trapped while the site was positive by eDNA detection. However, the AIS has been seen during a previous observation in other fishers traps and collected upstream (Duchâtel Saint-Pierre site; Figure 2). In the Lézarde watershed, where nine sites were eDNA positive, 403 fishes were collected, with sizes ranging from 71 to 312 mm.

### 3.4 | Modeling

Results of the eDNA occupancy model, which was undertaken following the protocols from Griffin et al. (2019), are reported in Table 2. The probability of occurrence (ψ) was 0.185, which is below the expected value set by the model of 0.5. False-positive probability for each sample ($\theta_{10}$), and for each qPCR replicate ($p_{10}$), were 0.0650 and 0.0133, respectively, which again are below the expected values, i.e., 0.11 and 0.1. At the same time, the true-positive probability for each sample ($\theta_{11}$) was equal to 0.999 which was overestimated (expected value 0.88). However, for each qPCR replicate, the true-positive probability ($p_{11}$) was 0.723, which is below the expected value (0.9).

None of the environmental covariates assessed were found to have a significant effect on *H. robinii* presence or detection. This may highlight the high tolerance of this fish to various environmental conditions. However, the total volume filtered was found to be at the margin of statistical significance ($F = 3.38$, $p = 0.06$). In fact, the highest probability of detection was found when the total filtered volume was comprised between 600 and 2000 ml, 600 ml corresponding to the lowest volume filtered and 75% of detection probability. After 2000 ml filtered, detection probability rapidly decreased below 70% (Figure 3). Other parameters (conductivity, pH, oxygen concentration and saturation, altitude, and temperature) showed no impact on the fish presence, maybe again indicating the species' high tolerance regarding water quality in the streams (Figure 3).

## 4 | DISCUSSION

The first record of the aquatic invasive species *H. robinii* in Martinique Island was in 2018, inhabiting only two river systems. However after a more extensive survey effort, further 12 rivers have been shown to be positive for this species (along with one closed water system on the island). To ensure our findings were accurate, we designed the novel eDNA-based survey tool following the 'gold standard' validation steps recently outlined by Thalinger et al. (2021). Indeed, the assay was found to be species-specific following in silico, in vitro and in situ validation. Additionally, we had no amplification occurring at sites where *H. robinii* was known to be absent and, inversely, a positive detection was observed where the species were fished, highlighting the reliability of our assay *in situ*. Finally, our designed assay showed a high sensitivity with relatively low *Limits of Detection* and *Limits of Quantification*, in accordance with other studies on fish eDNA. For example with the Topmouth Gudgeon (*Pseudorasbora parva*) (Davison et al., 2019) and/or for *Pseudobarbus swartzi* and *Sandelia capensis* (Castañeda et al., 2020).

To compare the new eDNA-based method with more traditional survey techniques, trapping sessions were performed in tandem with water filtration at all sites. The eDNA-based assay appeared to be a more sensitive tool. For example, across the 83 sites surveyed, 18 were positive using eDNA and only 14 with trapping (77% of the positive eDNA sites). There were no instances where sites were positive with trapping only. Such results are comparable to an increasing number of similar studies for other targeted fish species. For example, Schmelzle and Kinziger (2016) found that when using an eDNA-based method, 72% of sampling sites showed the presence of *Eucyclogobius newberryi* while only 39% showed presence with trapping. Further, Hinlo et al. (2017) showed the presence of *Cyprinus carpio*, *Misgurnus anguillicaudatus*, and *Perca fluviatilis* in 73% of its sampling sites when utilizing eDNA, while only on average 40% of them when using fyke nets. However, such a marked difference between methods is not surprising. Indeed, trapping by its nature will only capture species close to the survey site specifically, while eDNA will detect the presence of species upstream—sometimes at very large distances (Deiner & Altermatt, 2014; Schumer et al., 2019). Further, the efficiency of more traditional survey methods (such as trapping) is based on fish activity, food availability, turbidity, predation pressure, breeding, and/or intra-interspecific competition (Prchalová et al., 2010 and references therein). eDNA-based methods in contrast appear to be more universal in their detection capabilities—for example being efficient and applicable all year round (Troth et al., 2021).

Measuring the environmental parameters when sampling the eDNA meant we were also able to apply occupancy modeling to assess the covariates impacts on the presence/occurrence of *H. robinii* at specific sites. Interestingly, the results highlighted that there were no ecological preferences apparent for this species, a result likely due to the AIS adaptability and its low requirements regarding water quality. However, we did highlight that the eDNA-based method efficiency and reliability were impacted slightly by the total water volume filtered. Higher probabilities of detection were observed at lower volumes. This is in contrast to some studies which have found the opposite to more commonly occur i.e. detection probabilities associated with eDNA sampling increased with the volume sampled (Cantera et al., 2019; Sepulveda et al., 2019). For example, Cantera et al. (2019) highlights optimal detection rates between 34 and 340 L, while our optimal detection rates are far lower (600–2000 ml giving above 75% of likelihood to detect *H. robinii*). That said, we are not suggesting eDNA assays should filter smaller volumes in general—this may well be a species-specific finding. For example, the result may be explained if we look at the habitat preference of *H. robinii* and catfish more generally. Catfish often prefer deeper, slow-flowing water, which by their nature also tend to be turbid and loaded with suspended matter. Such water has been known to clog filters and explains our lower volumes able to be filtered in this study. Turbidity is further amplified by the behavior of this AIS in particular as they erode the banks of the rivers during nesting (Orfinger & Goodding, 2018).

That said, the behavior and habitat preferences of this species were known before we undertook this survey effort and assay development and so our protocol was optimized to obtain the best yields possible when working in environments with high levels of suspended solids. For example, we utilized 0.45 μm pore size nitrocellulose filters to reduce filter clogging (Hunter et al., 2019). Further, we ensured we filtered the water in the field directly after sampling to avoid any possible degradation of DNA, due to the high

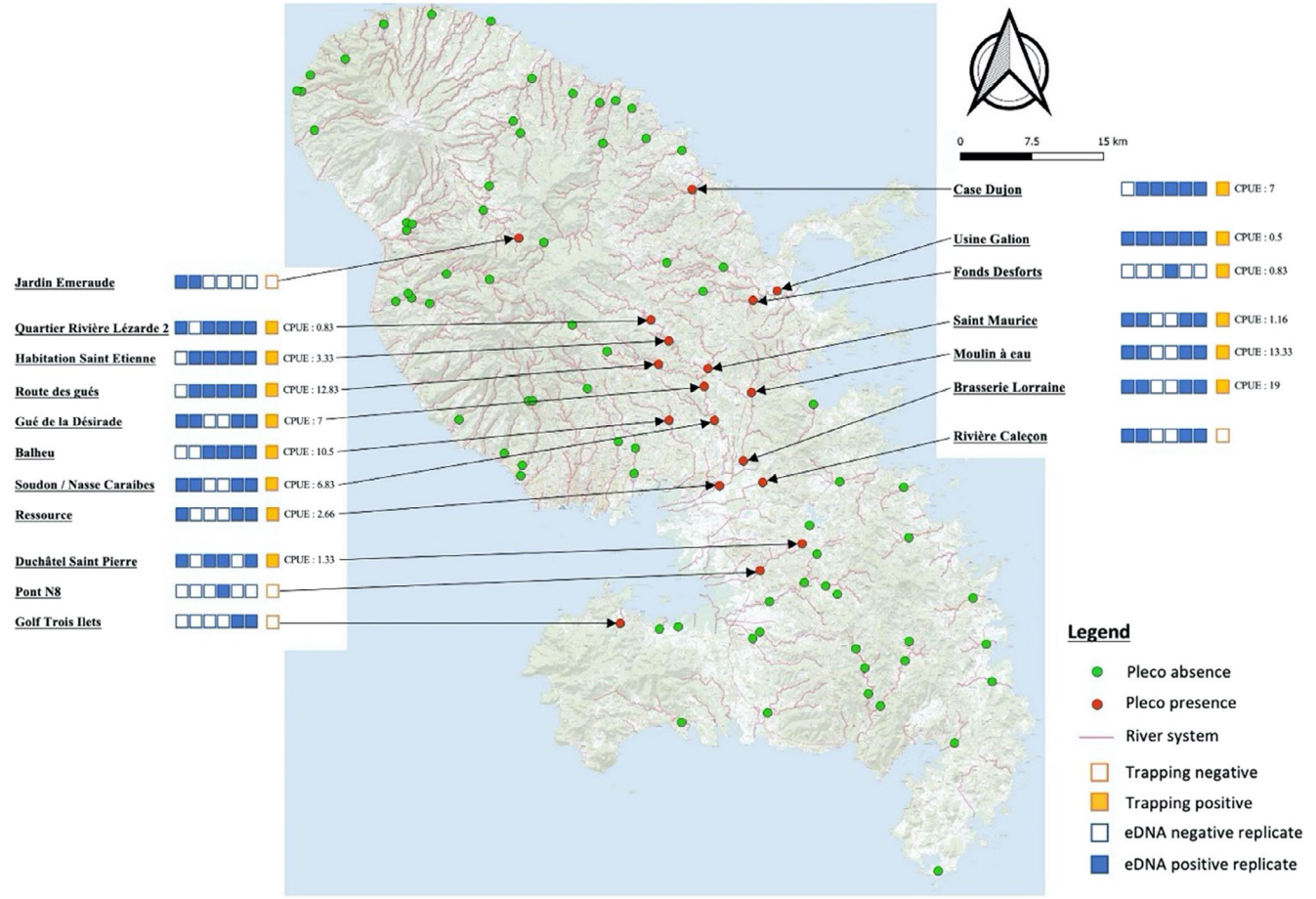


**FIGURE 2** FIGURE *Hypostomus robinii* site presence among the 83 sampling sites surveyed through Martinique using our novel species-specific eDNA-based method. Presence of the armored catfish is noted with a red point (for eDNA tested sites) and positive qPCR replicates (among the six qPCR replicates performed) by site are represented with blue-framed blue squares (blue-framed white square corresponding to negative qPCR replicate). Each sampling site was additionally surveyed by a more traditional trapping method and results are highlighted by an orange-framed orange square (if *H. robinii* was captured), with the associated Catch Per Unit Effort (CPUE), or orange-framed orange square (if no capture)

**TABLE 2** Observed and expected values of the different covariates' probability using Griffin et al. (2019) model

| Covariates | 2.5 credible interval | Mean | 97.5 credible interval | Expected value |
|---|---|---|---|---|
| Occurrence probability ($\psi$) | 0.108 | 0.185 | 0.276 | 0.5 |
| Sample true-positive probability ($\theta_{11}$) | 0.862 | 0.975 | 0.999 | 0.88 |
| Sample false-positive probability ($\theta_{10}$) | 0.0001 | 0.0160 | 0.0650 | 0.11 |
| qPCR replicate true-positive probability ($p_{11}$) | 0.525 | 0.626 | 0.723 | 0.9 |
| qPCR replicate false-positive probability ($p_{10}$) | 0.0001 | 0.003 | 0.0133 | 0.1 |

temperature and UV (Goldberg et al., 2011). This is particularly the case in tropical ecosystems where such parameters are higher than in temperate regions.

That said, despite our attempts to ensure the assay was validated to the highest degree, our models indicated that the probability of false positives for each sample was above the expected value. The high levels of suspended solids witnessed at the survey sites may also help explain this result. Increased suspended soils have been linked to increased inhibition in qPCR assays (Hunter et al., 2019). Although this issue is likely inherent for all eDNA assays, we cannot ignore this result and as such care should always be taken when interpreting the data without the physical sighting of the specimen in question. Further, the models also rely on the catch data presented which can be skewed by the randomness of the AIS activity. For example, catfish are nocturnal (Celestino et al., 2017; Mazzoni et al., 2015; Rodriguez-Santiano et al., 2002) hence why

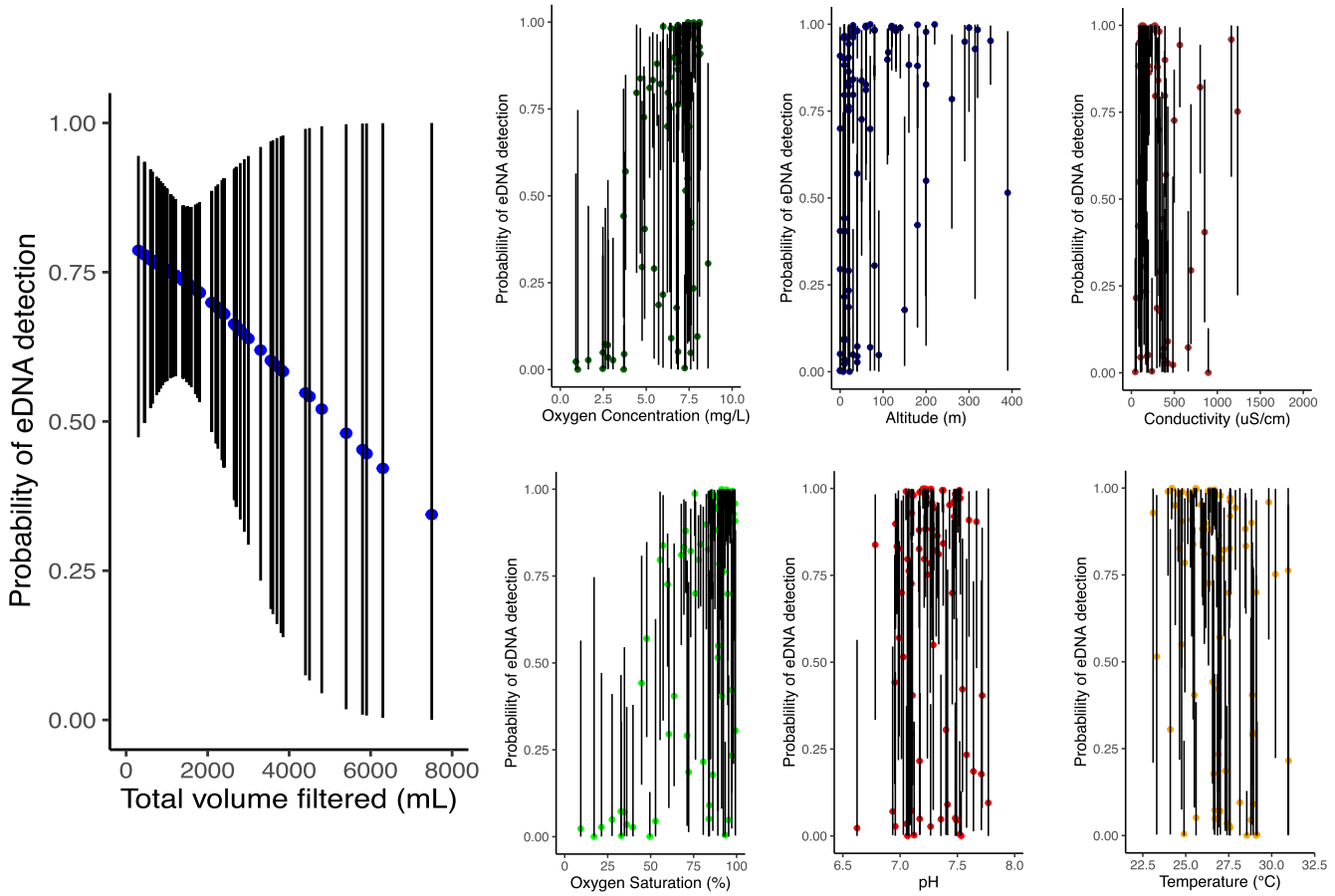


**FIGURE 3** Influence of total volume filtered, oxygen concentration and saturation, altitude, conductivity, pH and temperature on the detection probability of *Hypostomus robinii* by our eDNA-based assay in Martinique, following the site occupancy modelling treatment. The colored dots represent the mean values of the dataset, for each station, and the bars represent the confidence intervals

we left the traps overnight. However, in future surveys, physical torch surveys might also be employed to ascertain the abundances of these fish at the sites identified via our eDNA surveys.

Our results now highlight that *H. robinii* is widespread across Martinique. Specifically, an entire watershed, the Lezarde, appears almost completely colonized by *H. robinii*, with nine out of 11 sites showing positive for the species—further 403 fish were collected at these same sites via trapping. Sadly, such a high density was not an outlier, with the catch per unit effort indicated above 10 for four sites including one where it was closer to a mean of 20 individuals caught by trap per night (Table 1). Such widespread occurrence and high densities are alarming as this AIS is known to compete for food with local species such as *Eleotris perniger* and *Sicydium sp.* (Lim et al., 2002). As well as impacting the environment via increased bank erosion (Hoover et al., 2014), increased turbidity, impacts on the biogeochemical cycles (Coat et al., 2009) and introduction of non-native parasites. Indeed, two specific parasites, *Trinigyrus hypostomatis* (Hanek et al., 1974) and *Unilatus unilatus* (Mizelle & Kritsky, 1967) have been shown to be present in the same species across Trinidad and Tobago and so will likely be present in Martinique to. Interestingly, as *H. robinii* was detected in more eutrophic areas, it can be assumed that this AIS is becoming acclimatized to polluted environments in Martinique, due to its oligophagous character. High densities of individuals could then also possibly lead to phosphorus sequestration and a phase shift occurring to algal-dominated river systems (Capps & Flecker, 2013a, 2013b; Rubio et al., 2016)—a state which would likely be hard to recover from. Such a dramatic change in the ecosystem would certainly result in a rapid decline in the abundance of native flora and fauna, possibly as they are more sensitive to eutrophication and biogeochemical cycles. Further, although this AIS is known to be territorial in nature, the species has been known to migrate upriver by as much as 150 m a year (Mazzoni et al., 2018). This gives us an indication of how *H. robinii* can colonize a new environment at such a rapid rate.

With the detrimental impacts of the AIS clear, eradication efforts should be scaled up, along with continued monitoring of the current population and effectiveness of management and mitigation strategies aimed at removing this species from Martinican river systems. Our new eDNA-based assay will hopefully help in this effort and facilitate early detection of the species in otherwise pristine environments. However, to date, only one successful eradication effort has been documented for a member of the Locariidae family, that of *Pterygoplichthys disjunctivus*, and this was undertaken by hand with a spear with the removal of 28 fish (Hill & Sowards, 2015). Here we caught more than 400 at some of our sites and it is unlikely

we caught them all. Therefore, focus may have to be on limiting the spread of *H. robinii*, rather than eradication. The use of our assay could certainly assist in the monitoring stages of this management strategy. Further stopping new invasions will also be key to minimize the impact this species has on this valuable and vulnerable ecosystem. Indeed, a first step in this direction is already underway with the ban on the sale of fish from the Loricariidae family in pet shops, following their classification in the level two order of invasive alien species (NOR: TREL1934054A).

## 5 | CONCLUSION

Here, we developed and optimized a promising species-specific molecular tool, with great ease of implementation in the field (even if laboratory treatments require some expertise) making it a more efficient method than conventional ones, for the early detection of *H. robinii* in Martinique. Even if the impacts of this species are not well documented, the AIS certainly appears to compete with native species and possibly also alter the biochemical nature of the ecosystem directly. Early identification and monitoring of *H. robinii* will be essential to control the impact this species has on Martinique's fragile ecosystem, with the goal of preserving native species where at all possible. Finally, this study has made it possible to develop an effective species-specific detection tool for *H. robinii*, applicable in Martinique, but also in all tropical zones where this invasive species finds ecological niches favorable to its establishment.

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### CONFLICT OF INTEREST

None declared.

### AUTHOR CONTRIBUTIONS

Study design: TD, TB, and FG. Fieldwork, sampling collection, and trapping: TD and TB. Primers design: QM. Laboratory work: TD, TB, and CD. Data analyses: TD, TB, QM, and FG. Manuscript writing TD, TB, QM, MS, and FG. Funding acquisition: AA, CC, and FG. All co-authors provided comments and helped to revise the manuscript.

### DATA AVAILABILITY STATEMENT

Appendix A: Protocol for eDNA extraction from filters. Appendix B: List of closely related and/or co-occurring species and related GenBank accession numbers utilized during the in silico design and validation steps of the assay.

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