bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1

2

3 # Using eDNA to biomonitor the fish community in a tropical oligotrophic lake

4

5

6 Martha Valdez-Moreno[1¶], Natalia V. Ivanova[2*¶], Manuel Elías-Gutiérrez[1], Stephanie L.

7 Pedersen[2], Kyrylo Bessonov[2], Paul D.N. Hebert[2]

8

9 [1] – El Colegio de la Frontera Sur, Unidad Chetumal, Av. Centenario Km 5.5, Chetumal,

10 Quintana Roo, Mexico

11 [2] – Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph,

12 Guelph, ON N1G 2W1, Canada

13

14 *Corresponding author:

15 E-mail: nivanova@uoguelph.ca (NVI)

16

17 [¶]These authors contributed equally to this work.

18

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

19 # Abstract

20 Environmental DNA (eDNA) is an effective approach for detecting vertebrates and plants,
21 especially in aquatic ecosystems, but prior studies have largely examined eDNA in cool
22 temperate settings. By contrast, this study employs eDNA to survey the fish fauna in tropical
23 Lake Bacalar (Mexico) with the additional goal of assessing the possible presence of invasive
24 fishes, such as Amazon sailfin catfish. Sediment and water samples were collected from eight
25 stations in Lake Bacalar on three occasions over a 4-month interval. Each sample was stored in
26 the presence or absence of lysis buffer to compare eDNA recovery. Short fragments (184-187
27 bp) of the cytochrome *c* oxidase I (COI) gene were amplified using fusion primers and then
28 sequenced on Ion Torrent PGM and S5 before their source species were determined using a
29 custom reference sequence database constructed on BOLD. In total, eDNA sequences were
30 recovered from 75 species of vertebrates including 47 fishes, 15 birds, 7 mammals, 5 reptiles,
31 and 1 amphibian. Although all species are known from this region, 6 fish species represent new
32 records for the study area, while 2 require verification. Sequences for five species (2 birds, 2
33 mammals, 1 reptile) were only detected from sediments, while sequences from 52 species were
34 only recovered from water. Because DNA from the Amazon sailfin catfish was not detected, we
35 used a mock eDNA experiment to confirm our methods were appropriate for its detection. We
36 developed protocols that enabled the recovery of eDNA from tropical oligotrophic aquatic
37 ecosystems, and confirmed their effectiveness in detecting diverse species of vertebrates
38 including an invasive species of Amazon catfish.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

39 # Introduction

40 Environmental DNA (eDNA) has gained popularity for biomonitoring, especially for the
41 detection of invasive species and for baseline surveys of animal and plant communties [1,2]. This
42 eDNA derives from cells shed into the environment as mucus, urine, feces, or gametes [2–7].
43 More than 120 articles have now considered eDNA, including a special issue on the topic [8].
44 The rapid adoption of eDNA-based bioassessments reflects two factors: improved access to the
45 reference sequences [9,10] required to link the short reads from eDNA to their source species,
46 and the availability of high-throughput sequencers that can generate large volumes of data at
47 modest cost.

48 Conventional biomonitoring programs require extensive effort with sampling repeated over time
49 and space [11]. Because these methods utilize nets and electrofishing, they are invasive and can
50 damage habitats [3,12,13]. Furthermore, they rarely reveal all species in an environment, and
51 each specimen that is collected must be identified to a species, requiring access to taxonomic
52 experts [14,15]. Given these limitations, eDNA-based methods are an attractive approach for the
53 quantification of biodiversity required for conservation and management [8,16].

54 Most prior eDNA studies have focused on the detection of invasive fish species [2,3,17] or rare/
55 endangered aquatic species [18,19] although they have also been used to examine the abundance
56 and biomass of fish species [20,21]. Early results revealed complexities in data acquisition and
57 interpretation that have provoked studies to improve sampling and DNA extraction while also
58 minimizing methodological biases [16,22,23].

59 Varied protocols have been used to preserve eDNA, but they usually involve the immediate
60 storage of samples at low temperature [21], precipitation [24], the addition of lysis buffers to

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

61 filters [25], and the inclusion of surfactants [26]. While eDNA has traditionally been assumed to
62 rapidly degrade, this is not always the case. For example, Deiner et al. [27] employed long-range
63 PCR (>16 kb) to recover whole mitochondrial genomes from the six fish species in a mock
64 community, and from 10 of 12 species represented in water samples from a stream. Prior studies
65 have also demonstrated that eDNA metabarcoding is an effective alternative to traditional
66 biomonitoring as it has revealed the species composition of fish communities in various
67 ecosystems including a species-rich coastal sea in Japan [28] and a river in Indiana, USA [29]. In
68 fact, in both cases, it revealed species that were overlooked by other sampling methods.

69 Although most past eDNA studies have examined temperate ecosystems, there is increasing
70 interest in the use of this method in the tropics [30–32]. The present study sought to develop an
71 eDNA protocol for biomonitoring the fish fauna of oligotrophic tropical Lake Bacalar and
72 adjacent habitats, and to verify its capacity to detect an invasive fish, the Amazon sailfin catfish,
73 *Pterygoplichthys* sp., which represents a serious threat in this region.

74 Material and Methods

75 Study site

76 Lake Bacalar, the largest freshwater habitat in the Yucatan Peninsula, is renowned for its striking
77 blue color, the clarity of its water, and for the world's largest occurrence of microbialites. With a
78 length of more than 10 km [33–35], it occupies a larger fault basin [36] (50 km long, 2 to 3 km
79 wide). With sediments derived from karst limestone, it represents the world's largest freshwater
80 lens system [37]. Lake Bacalar is not directly connected to the sea, but it shows a high rate of
81 groundwater flow [38]. The northern part is connected to a complex system of lagoons including

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

82 Laguna Chile Verde and Laguna Guerrero. The southern part of the lake has an indirect
83 connection to the sea via the Hondo River that enters Chetumal Bay. There are four cenotes
84 (sinkholes) in the lake whose depths range from 50-90 m.

85 Water temperatures in Lake Bacalar range from 25-28 °C, while the Secchi transparency
86 averages 10.3 m. The lake is unstratified [33,39] with a water temperature of 27°C or higher
87 throughout the year. Its surface waters are slightly alkaline (pH=7.8) with a conductivity of 1220
88 µS/cm [39] and HCO_3 values higher than in marine environments [33].

89 All samples were taken from the littoral zone at a maximum depth of 0.5 m, except La Unión (2
90 m) and Xul-Ha (4 m). Eight sampling sites were examined along the main axis of Lake Bacalar
91 and associated systems in the Hondo River (Fig 1). Samples were collected in December 2015,
92 January 2016, and April 2016.

93 **Fig 1. Sampling locations.**

94 Sampling

95 Eight localities (one with three points) (Fig 1) were sampled and most were examined on more
96 than one occasion, producing a total of 14 sampling events. Three sites (Cocalitos, Huay Pix and
97 Xul-Ha) were sampled in December 2015, four sites in January 2016 (Alvaro Obregón Viejo,
98 Huay Pix, La Unión and Pedro A. Santos), and seven sites in April 2016 (Cacao, Cocalitos, Huay
99 Pix, Juan Sarabia, La Unión, Pedro A. Santos and Xul-Ha).

100 All sampling bottles and equipment were handled with gloves to minimize contamination with
101 human DNA. At each site, three replicate samples of water and sediment were taken. Each 1L
102 water sample was placed in a sterilized CIVEQ® bottle, while each sediment sample was placed

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

103 in a separate (100 cm^3) Ziplock bag. Each water and sediment sample was collected using a new
104 turkey baster. Sediment samples were obtained from the upper 1 cm. All samples were
105 immediately placed on ice, and then transported to the lab in Chetumal for processing.

106 Processing water samples

107 Water samples were filtered within 7 hours of collection. Prior to processing, all lab surfaces and
108 materials were sterilized with 10% bleach, followed by 70% ethanol; gloves were changed
109 between each sample. Each sample was split into two 0.5 L subsamples that were filtered
110 through separate 0.22 µm filters. One of these filters was stored with 1 ml of PW1 solution with
111 grinding media while the other filter was placed with grinding media in a dry tube covered by
112 aluminum foil. This approach resulted in six samples per locality for each collection event (Fig
113 2). All filters were stored at -18°C before being transported on ice from Chetumal to Guelph
114 where DNA extraction was undertaken. The interval between filtration and DNA extraction was
115 always less than 48 hours.

116 **Fig 2. Experimental design for the water and sediment samples.**

117 Processing sediment samples

118 During the first collection trip, three replicate sediment samples (20 cm^3 each) were collected at
119 each site. Each sample was split into two 10 cm^3 subsamples; 2 ml of 5M GuSCN buffer was
120 either used as a preservation buffer or was added during lysis. For the second and third collection
121 events, one pooled sample (60 cm^3) was collected at each site and split into two replicates of 30
122 cm^3; 6 ml of 5M GuSCN buffer [40] was used as a preservation buffer or was added during lysis
123 step (Fig 2). Sediments were kept at -18°C before transportation on ice to Guelph.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

124 ## DNA extraction from water samples

125 Prior to DNA extraction, all lab surfaces and pipettors were sterilized using 10% bleach,
126 followed by 70% ethanol. Pipettors were repeatedly cleaned with 10% bleach, followed by 70%
127 ethanol during extraction and gloves were frequently changed. Centrifuge rotors, adapters, and
128 tube racks were washed with diluted ELIMINase (Decon Labs) (1:10) and rinsed with deionized
129 water.

130 DNA was extracted from the filters from the water samples using a PowerWater DNA extraction
131 kit (MoBio) following a modified lysis protocol (tubes were incubated at 65°C prior to the
132 grinding stage) with minor modifications to avoid cross-contamination between samples; all
133 steps requiring 650 µl were reduced to 625 µl. Columns were incubated at 56°C for 15 min prior
134 to DNA elution in 100 µl of PW6 buffer and quantified using Qubit 2.0 and HS dsDNA kit.

135 ## DNA extraction from sediment samples

136 All lab surfaces and equipment were decontaminated as described above. The DNA extraction
137 protocol employed ProK digestion in the presence of chaotropic salts followed by the use of
138 magnetic beads to bind and then release DNA. Sera-Mag SpeedBead Carboxylate-Modified
139 Magnetic Particles (Hydrophilic) from GE Healthcare were employed to bind/release DNA.
140 They were prepared as described in [41] in a polyethylene glycol/NaCl buffer (0.1% Sera
141 Magnetic Particles (w/v) in 18% PEG-8000, 1M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA
142 pH 8.0, 0.05% Tween 20). Extraction was initiated by adding a 1/5 volume of 5M GuSCN lysis
143 buffer [40] to non-treated samples and a 1/10 volume of ProK (20 mg/ml). Each tube was
144 vortexed and incubated for 2 hours at 56°C on an orbital shaker, then held for 30 min at 65°C
145 before being centrifuged at 2000 g for 2 min. DNA was extracted from 500 µl of the resultant

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

146 lysate after its transfer to a 2 ml Eppendorf LoBind tube. 500 µl of prepared magnetic beads was
147 added to each lysate, and a pipettor was used to mix the beads and lysate before incubation at
148 room temperature for 10 min. Tubes were then placed on a magnet (DynaMag) and incubated
149 until the supernatant was clear. Beads were thoroughly washed once with 1 ml of PWB buffer
150 [42] and twice with 1 ml of 70% ethanol. Magnetic beads were then dried at room temperature
151 for 10-15 min before DNA was eluted into 100 µl of 10 mM Tris-HCl, pH 8.0 and quantified
152 using Qubit 2.0 and HS dsDNA kit.

153 PCR amplification

154 Prior to PCR amplification, each DNA extract from sediments was diluted 1:10 with molecular
155 grade water while extracts from water were used without dilution.

156 In order to increase species recovery and overcome possible PCR bias introduced by UMI-
157 labelled fusion primers [43] we followed a two-step PCR approach [43] with the first round
158 employing conventional primers, where the diluted PCR product serves as a template for a
159 second round of PCR with fusion primers containing sequencing adapters and UMI-tags.

160 A 184-187 bp segment of the barcode region of COI was amplified with two primer sets
161 (AquaF2/C_FishR1, AquaF3/C_FishR1) known to be effective for vertebrates (Table 1). The
162 PCR reactions employed the master mix described in [44] and Platinum Taq. The first round of
163 PCR employed the following thermal regime: 94°C for 2 min, 40 cycles of 94°C for 40 s,
164 annealing at 51°C for AquaF2 or 50°C for AquaF3 for 1 min, and 72°C for 1 min, with a final
165 extension at 72°C for 5 min. Following the first round, PCR products were diluted 2-fold with
166 molecular grade water, and 2 µl was transferred to a new tube for a second round of PCR to
167 create amplicon libraries tagged with unique molecular identifiers (UMIs) with fusion primers

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

168 containing Ion Xpress™ MID tags and Ion Adapters (Table 4). The same UMI-tag was applied
169 to all three replicates for each treatment from a particular collection event (Fig 6). The PCR
170 regime for the second round consisted of 94°C for 2 min, 20 cycles of 94°C for 40 s, annealing at
171 51°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were
172 visualized on an E-Gel96 (Invitrogen, Thermo Fisher Scientific).

173 **Table 1. Primers used for eDNA amplification**

Region/Primer name	Direction	Primer sequence	Reference
COX1			
AquaF2	F	ATCACRACCATCATYAAYATRAARCC	[44]
AquaF3	F	CCAGCCATTTCNCARTACCARACRCC	this study
C_FishR1		**Cocktail primers (FR1d: FishR2; 1:1)**	[45]
FR1d	R	ACCTCAGGGTGTCCGAARAAYCARAA	
FishR2	R	ACTTCAGGGTGACCGAAGAATCAGAA	
NGS-fusion			
IonA	F	CCATCTCATCCCTGCGTGTCTCC[GACT][IonExpress-MID][specific sequence]	Ion Torrent, Thermo Fisher Scientific
trP1	R	CCTCTCTATGGGCAGTCGGTGAT[specific sequence]	Ion Torrent, Thermo Fisher Scientific

174

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

175 Libraries for AquaF2/C_FishR1 and AquaF3/C_FishR1 were purified separately using magnetic
176 beads prepared as described in [41], quantified using Qubit 2.0 and HS dsDNA kit, and
177 normalized to 13 pM prior to sequencing. Most amplicon libraries were sequenced on an Ion
178 PGM using the 400 Template and Sequencing kits following manufacturer's instructions with
179 either a 316 or 318 chip. Water samples from April 2016 were also analyzed on an Ion S5 using
180 the Chef for templating and an Ion 520 and 530 kit with a 530 chip. Fig 7 summarizes the
181 treatment for each water or sediment sample. HTS data (FASTQ files) are available in the
182 European Nucleotide Archive (ENA) under submission number: PRJEB25950.

183 Detection of an invasive fish

184 We conducted a mock eDNA experiment to ensure our methods could detect species of
185 *Pterigoplichthys*. Two of these fishes were kept in a 10 L aquarium for 10 days at 25°C. One
186 liter of water was collected and two 0.5 L subsamples were filtered through separate 0.22 µm
187 filters; filters were stored with buffer and no buffer as described above.

188 Bioinformatics workflow

189 We created a reference dataset (DS-EBACALAR) on BOLD with sequences from 3,534
190 Mexican fishes representing 576 BINs, including 219 records from Lake Bacalar and
191 surrounding habitats (Hondo River, and adjacent lakes – Huay Pix, Xul-Ha). This reference
192 barcode library included sequences for 53 of the 57 fish species (Table 2) known from these
193 habitats (see Fig 1). As well, four other datasets with all public data on BOLD for Amphibia,
194 Mammalia, Aves, and Reptilia were constructed. These five datasets were employed as a basis
195 for assigning a source species to each OTU recovered from the present eDNA analyses.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

196 The following procedure was used to process the raw NGS reads: Cutadapt (v1.8.1) was used to
197 trim primer sequences; Sickle (v1.33) was used for size filtering (sequences from 100-205 bp
198 were retained), while Uclust (v1.2.22q) was employed to recognize OTUs based on the >98%
199 identity and a minimum read depth of 2 thresholds. The Local Blast 2.2.29+ algorithm was then
200 used to compare each OTU to the reference sequences in five datasets: DS-EBACALAR Bacalar
201 Fish Dataset for eDNA Detection (3,534 sequences), and public BOLD data for Amphibia, Aves,
202 Mammalia, and Reptilia represented by the following datasets: DS-EBACAMPH (11,018
203 sequences), DS-EBACAVES (28,914 sequences), DS-EBACMAMM (39,890 sequences), DS-
204 EBACREPT (5,424 sequences). Raw Blast output results were parsed using a custom-built
205 python script OTUBlastParser.py, and concatenated using ConcatenatorResults.py available at
206 https://gitlab.com/kbessonov/OTUExtractorFromBLASToutput; processed results in tab-
207 delimited format were imported to MS Excel, filtered by min score of 250, and 97-100% percent
208 identity range. Filtered results werefurther processed and visualized in Tableau software 10.2.
209 All human reads were excluded from the final results and species counts.

210 Species accumulation curves were calculated in EstimateS software available at
211 http://purl.oclc.org/estimates [46], with the following settings: 1000 randomizations; do not
212 extrapolate rarefaction curves; classic formula for Chao 1 and Chao 2. Results were exported to
213 Microsoft Excel and visualized in Tableau software 10.4.

214 Sequences with 97-100% identity to fish species represented in the DS-EBACALAR dataset
215 were mapped onto a neighbor-joining Taxon ID tree generated in BOLD for these taxa, and
216 visualized in iTOL [47]. Results

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

217 The eDNA analyses recovered sequences from 75 species of vertebrates including 47 fishes
218 (Table 2), 15 birds, 7 mammals, 5 reptiles and 1 amphibian, most previously known from this
219 region. Although substantially more species were recovered from the water than from the
220 sediments (Table 3, Fig 3), five species were only recovered from sediments – racoon (*Procyon*
221 *lotor*), pig (*Sus scrofa*), meso-american slider turtle (*Trachemys venusta*), brown jay (*Psilorhinus*
222 *morio*), and great blue heron (*Ardea herodias*). However, all fish species detected in sediment
223 samples were also recovered from the water samples (Table 3, S1 Table).

224 **Fig 3. Number of species detected versus the number of samples analyzed for water,**
225 **sediments, and combined.** Species accumulation curves were calculated in EstimateS software
226 with 1000 randomizations and classic formula for Chao 1 and Chao 2.

227 **Table 2. Fishes that were detected in eDNA, known from the literature and those with**
228 **barcodes.**

Species list	Reference known from the study area	Sequence recovered from eDNA	Barcode sequence available
Achirus lineatus	[48]		+
Anchoa parva	[49,50]		N/A
Anchovia clupeoides	[49]		+
Astyanax aeneus	[50–53]	*	+
Astyanax mexicanus		*	+
Atherinella alvarezi	[49,52]		+
Atherinella sp.	[48,49]	*✓	+

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Atherinomorus stipes	[50]		+
Bagre marinus	[48]		+
Bardiella ronchus		*✓	+
Bathygobius curacao	[49]		+
Bathygobius soporator	[50]	*	+
Belonesox belizanus	[48,50,51,54]	*	+
Bramocharax caballeroi		*	+
Caranx hippos	[48]		+
Centropomus undecimalis	[48]		+
Chriodorus atherinoides	[49,52]		+
Cribroheros robertsoni	[48,54]	*	+
Cryptoheros chetumalensis	[48,53–55]	*	+
Ctenogobius fasciatus		*✓	+
Cyprinodon artifrons	[49–52,54]		+
Cyprinodon beltrani /simus		*?	+
Dormitator maculatus	[49,50]		+
Dorosoma anale		*✓	+
Dorosoma petenense	[48,50,54]	*	+
Engraulidae		*✓	+
Eugerres plumieri	[48,54]	*	+
Floridichthys polyommus	[49,50,52,54,56]	*	+
Gambusia sexradiata	[48,49,51,52,54]	*	+
Gambusia yucatana	[48,49,51,54]	*	+

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Gobiomorus dormitor	[48,49,52,54]	*	+
Gobiosoma sp.		*✓	+
Heterandria bimaculata	[48–50,54]	*	+
Hyphessobrycon compressus	[48,49,54]	*	+
Hyporhamphus roberti	[49,52]		N/A
Ictalurus meridionalis	[48]		+
Jordanella pulchra	[48,49,54,56]	*	+
Lachnolaimus maximus		*	+
Lophogobius cyprinoides	[49,53,54,56]	*	+
Lutjanus griseus	[48]		+
Mayaheros urophthalmus	[53–55]	*	+
Megalops atlanticus	[48,49,54]	*	+
Mugil cephalus	[49]		+
Oligoplites saurus		*✓	+
Ophisternon sp.	[48,49,54]	*	+
Oreochromis mossambicus	[48,50]	*	+
Oreochromis niloticus	[48]	*	+
Parachromis friedrichsthalii	[48,51,52,54]	*	+
Petenia splendida	[48,53–55]	*	+
Phallichthys fairweatheri	[48,49,52,54]	*	+
Poecilia mexicana	[48,51,54]	*	+
Poecilia orri	[48,52,54]		+

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Poecilia petenensis	[48,49,52,54]	*	+
Pterygoplichthys sp.		**X**	+
Rhamdia guatemalensis	[48,50]	*	+
Rhamdia laticauda	[48,49,54]	*	+
Rocio octofasciata	[48,51,54]	*	+
Sciades assimilis	[48,49,51,52,56]	*	+
Sphoeroides testudineus	[48,54]		+
Strongylura notata	[49,52,54,56]	*	+
Strongylura timucu	[48]	*	+
Thorichthys aureus	[48]		N/A
Thorichthys aff. meeki	[49,56]		+
Thorichthys meeki	[48,51–54]	*	+
Trichromis salvini	[48,52,53,55]	*	+
Urolophus jamaicensis	[49,50]		N/A
Vieja fenestrata		***?**	+
Vieja melanura	[48]	*	+
Xiphophorus maculatus	[48,49,51]	*	+
Number of taxa	**57**	**48**	**65**

229 + – Species with DNA barcode present in the BOLD dataset; * – detected in eDNA; ✓ –
230 potentially new record or species for the study area; ? – questionable presence; X – detected in
231 mock experiment; N/A – no barcode sequence available.

232

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

233 **Table 3. Number of species recovered from the sediment and water samples.**

	Total	Sediments only	Sediments + water	Water only
Actinopterygii	47	0	14	33
Amphibia	1	0	1	0
Reptilia	5	1	1	3
Mammalia	7	2	1	4
Aves	15	2	1	12
Total:	***75***	***5***	***18***	***52***

234

235 The three fish orders with highest number of detected species were Cichliformes (12 species),
236 Cyprinodontiformes (11 species), and Gobiiformes (5 species) (S2 Table). Although
237 *Pterygoplichthys* sp. was not recovered from water and sediment samples, it was recovered from
238 the mock eDNA study (S1 Table and S2 Table).

239 In total, 272,610 reads obtained by the PGM from the water and sediment samples showed a
240 close match to a vertebrate species in one of the five reference libraries. The three sites with the
241 most fish species recovered were Alvaro Obregón Viejo, Cocalitos and Xul-Ha (Fig 4, Table 4),
242 with 24-25 fish species per site. On average, the sediment and water samples from a particular
243 site on a particular date generated eDNA sequences for 45% (mean =20.9; range= 15-25) of the
244 47 fish species detected in the survey (Table 4). Although nearly 2/3 of these reads derived from
245 sediments (177,077 reads versus 95,533 from water), more species of vertebrates were recovered
246 from the water samples (Fig 4). In fact, no species were recovered from the sediment samples
247 taken at three sites (Cocalitos, Huay Pix, Xul-Ha) in December 2015 or from those preserved
248 without buffer from Alvaro Obregón Viejo in January 2016. Although water samples revealed

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

249 more species, there was evidence of temporal shifts in species recovery and DNA concentration
250 with the lowest value in December 2015 (Fig 4, 5A). Best results were obtained with filtered
251 water rather than sediments for both treated and untreated samples (Fig 3, 4, 5A).

252 **Table 4. Number of vertebrate species detected at each of the eight sampling sites**

Sampling site No	**Sampling site**	**Actinopterygii**	**Amphibia**	**Aves**	**Mammalia**	**Reptilia**
1	Pedro A. Santos / 18.920052°; -88.170100°	18		2	1	
2	Cocalitos / 18.651469°; -88.408948°	24	1	5		1
3	Xul-Ha / 18.560084°; -88.446216°	25		1		2
4a	Huay Pix / 18.514639°; -88.426750°	19		3		
4b	Huay Pix / 18.528561° -88.429242°	15		1		
4c	Huay Pix / 18.516144°; -88.436876°	19		1	1	1
5	Juan Sarabia/ 18.493471°; -88.478857°	19		1	1	
6	Alvaro Obregón Viejo / 18.299259°; -88.597250°	24		6	2	1
7	Cacao / 18.186488°;-88.694636°	23		1	2	
8	La Unión / 17.894676°; -88.870059°	23		4	4	2

253

254 **Fig 4. Number of vertebrate species recovered in eDNA from each sampling event at each**
255 **collection site.**

256

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

257 **Fig 5. Species recovery and DNA concentration for each sampling event and treatment.** A –
258 number of vertebrate species recovered for each sampling event and treatment (only PGM data
259 for fishes are shown, circle size indicates average read count per sampling event). B – DNA
260 concentration of sediment and water samples (one replicate per sampling event) measured on
261 Qubit. Whiskers correspond to data within 1.5x of interquartile range (IQR).

262 With two exceptions, negative PCR and DNA extraction controls did not produce any vertebrate
263 reads aside from those deriving from humans (S2 Table). Two reads of *Trichromis salvini* were
264 recovered from a negative control on the S5, and two reads of *Mayaheros urophthalmus* were
265 obtained from a negative control on the PGM. Because both cases involved a species common in
266 samples from the same run, they likely reflect tag-switching due to a misread UMI [57,58] or
267 cross-contamination. Given their rarity, it is unlikely such analytical errors significantly
268 impacted our overall conclusions.

269 Comparison of results from two sequencing platforms

270 Two sequencing platforms (PGM, S5) were used to analyze the same set of water samples from
271 April 2016 and from the mock experiment. The PGM generated 57,689 reads that matched
272 vertebrates while the S5 generated 1,106,574 vertebrate reads. Reflecting its 20-fold higher
273 sequence count, the S5 recovered more fish species than the PGM (41 vs. 34). However, large
274 shifts in the relative number of read counts for the component species were also detected. For
275 example, *Trichromis salvini* comprised 59.1% of the S5 reads, but just 5.9% of those from PGM
276 (Fig 6).

277 **Fig 6. Percentage of total read coverage for each species recovered in water samples from**
278 **April 2016 and mock experiment, analyzed with Ion Torrent PGM and S5 instruments.**

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

279 To verify the accuracy of the identifications assigned to the sequences recovered from eDNA, we
280 generated a Neighbor-Joining tree in BOLD for detected fish species and mapped corresponding
281 top-hit process IDs (Fig 7).

282 **Fig 7. Top hit process IDs mapped on the Neighbor-Joining tree for fish taxa visualized in**
283 **iTOL.** Blue – species previously reported from the Lake Bacalar region; red – species new to
284 Lake Bacalar); orange – species complexes lacking resolution with COI: 1 – *Bramocharax-*
285 *Astyanax* complex, 2 – *Cyprinodon simus/beltrani*; green – *Pterygoplichthys* from mock eDNA
286 experiment.

287 Discussion

288 Although eDNA has often been thought to degrade rapidly, factors such as temperature,
289 alkalinity, and trophic state [31,59] affect its stability. For example, cold temperatures, low UV-
290 B levels, and high pH slow eDNA degradation [59], while acidity promotes it [59,60]. The
291 overall probability of eDNA detection also depends on its production which may vary by
292 species, by season, by density, and diet, and its loss from the study system via water discharge or
293 diffusion [59].

294 Because techniques for eDNA analysis are still being optimized, some contradictory results have
295 been reported. For example, a laboratory study showed that eDNA degradation increased with
296 rising temperature, particularly in water samples from an oligotrophic lake [31]. By contrast,
297 Robson et al [32] evaluated effects of high water temperature and fish density on the detection of
298 invasive Mozambique tilapia in ponds via eDNA protocols and found that increased water

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

299 temperatures did not affect degradation rates. However, they did detect increased rates of eDNA
300 shedding at 35°C.

301 The present study represents the first time that the effectiveness of eDNA has been tested in an
302 oligotrophic tropical lake, where high temperatures (>27°C year round) should speed DNA
303 degradation. However, its water is slightly alkaline (pH 7.8) [39] which may aid eDNA
304 preservation, perhaps explaining the better recovery of eDNA from water samples than
305 sediments (Fig 2, 5A). The recovery of species from sediments were low (0-8 species per site)
306 despite the higher DNA concentration in most samples treated with buffer (Fig 5B). The low
307 recovery may reflect the presence of inhibitors or nuclease activity although best practices were
308 followed to minimize DNA loss [61,62]. In particular, samples were transferred onto ice
309 immediately following collection, and DNA was promptly extracted from the samples.

310 Most prior eDNA studies have examined vertebrates in temperate freshwater streams [63], lakes
311 [64] or sea water [65,66]. However, Lopes et al. [30] recently demonstrated that eDNA surveys
312 were effective in revealing anurans in streams in the Brazilian Atlantic forest, while Robson et
313 al. [32] developed eDNA protocols that employ qPCR to detect invasive fish in tropical
314 environments. Our results further validate the effectiveness of eDNA surveys in these settings.

315 There are three major approaches for of eDNA detection: the use of qPCR to detect one or a
316 small number of target species [67,68], shotgun sequencing of aquatic metagenomes [69] and
317 PCR-based metabarcoding [28,29,63–66]. Metabarcoding studies cannot deliver information on
318 species composition without a reliable reference database, such as that available for Mexican
319 freshwater fishes which includes records for 93% of the 70 species known from Lake Bacalar
320 and associated wetlands [48,51–54,70]. The present study has affirmed the effectiveness of

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

321 eDNA analysis as a tool for rapidly assessing the species composition of fish communities. The
322 extraction of eDNA from just 42 liters of water collected on three dates at 14 sampling points
323 revealed 41 of 57 of previously recorded species and added 6 potentially new species to the
324 fauna of the Lake Bacalar region. The present results mirror those obtained in a study which used
325 eDNA to examine the composition of fish communities a coastal sea [28]. The analysis of 94
326 water samples obtained by sampling 47 sites in six hours revealed 40 of the 80 species known
327 from 14 years of underwater surveys, as well as 23 new species.

328 The present study recovered sequences records from six fish species new to the Lake Bacalar
329 region (*Bardiella ronchus*, *Ctenogobius fasciatus, Gobiosoma* sp., *Dorosoma anale, Oligoplites*
330 *saurus* and one Engraulidae). Two of the new species (*B. ronchus*, *O. saurus*) detected in Juan
331 Sarabia and Alvaro Obregón Viejo are known from the adjacent Chetumal Bay, but have not
332 previously been reported from inland waters (Valdez-Moreno, pers. obs.). The new gobiid, *C.*
333 *fasciatus*, was detected in four localities (Xul-Ha, Pedro A. Santos, Cocalitos, Huay Pix), but was
334 overlooked in prior field surveys because of its morphological similarity to other gobids in Lake
335 Bacalar, especially *Lophogobius cyprinoides*. The other two new records require confirmation –
336 the presence of a *Gobiosoma* in Huay Pix (51 reads) and a member of the family Engraulidae in
337 Pedro A. Santos (>7000 reads).

338 A few sequences (13) of *Lachnolaimus maximus*, a commercially fished marine species, were
339 recovered from Huay Pix, but the presence of its DNA was almost certainly mediated by human
340 activity as fish were prepared for consumption.

341 As reported previously, species in two characid genera (*Astyanax* and *Bramocharax*) are difficult
342 to discriminate using DNA barcodes as they show low divergences between species and genera

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

343 [51,71]. Moreover, in a study utilizing three mitochondrial genes (Cyt b, 16S, COI) and a single
344 nuclear gene (RAG1), *Bramocharax* was found to be polyphyletic, with species in this genus
345 being sisters to different clades of *Astyanax*, making *Astyanax* paraphyletic [72]. In fact, all
346 *Bramocharax* species grouped with sympatric *Astyanax* lineages (or even with allopatric
347 *Astyanax* populations), with less than 1% divergence. In our dataset, *Astyanax aeneus*, *Astyanax*
348 *mexicanus*, and *Bramocharax caballeroi* also formed an intermixed cluster in the Taxon ID tree
349 generated (see the dataset DS-EBACALAR in www.boldsystems.org). Among the species
350 detected with eDNA, *Astyanax mexicanus* was the least certain as it was only represented by 7
351 reads from the S5 with an average identity of 0.97 (S2 Table), which may be low quality reads.

352 Although *Dorosoma anale* was only represented by 13 reads from water at La Unión station, this
353 species is known to occur at sites in northern Belize close to the Hondo River (Valdez-Moreno
354 pers. obs.).

355 The presence of two other species (*Cyprinodon beltrani* (two PGM reads) and *Vieja fenestrata*
356 (five S5 reads) was less certain. *C. beltrani* is native to Chichancanab lagoon, and cannot be
357 distinguished from *C. simus* with DNA barcodes (see DS-EBACALAR in
358 www.boldsystems.org), while *V. fenestrata* is native to the Papaloapan River [52] so its presence
359 in the study area is unlikely.

360 Our results also revealed good eDNA recovery for other vertebrates, including rare species, such
361 as *Tamandua mexicana,* which was represented in both PGM and S5 sequences from a river
362 sample (Juan Sarabia). Another interesting record involved *Oreothlypis peregrina*, a migratory
363 warbler that is known to overwinter in the Yucatan Peninsula, but that is very morphologically
364 similar to other species.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

365 Biodiversity in freshwater ecosystems in undergoing losses as a result of landscape
366 transformation, pollution, and biological invasions. In fact intentional or accidental introduction
367 of invasive alien species is the third leading cause of global biodiversity loss [73]. In 2008 it was
368 estimated that nearly 40% of the freshwater fish species in North America, including Mexico,
369 were threatened by invasive species [74]. The Lake Bacalar water basin has been recognized as
370 one of the hydrological basins with high priority for conservation by the Mexican National
371 Commission for the Use and Knowledge of the Biodiversity (CONABIO) [75]. Fortunately, most
372 wetlands and lakes in this region are relatively pristine, excepting the Hondo Riveron the border
373 between Mexico and Belize, which has been heavily impacted by the discharge of organic waste
374 and pesticides, by vegetation clearing, and by the introduction of invasive such as tilapia [76],
375 and the Amazon sailfin catfish (*Pterygoplichthys pardalis)*, which was recently detected [77,78]
376 near La Unión [79]. The impact of this declining water quality and rising incidence of invasive
377 species on the native fish fauna needs to be carefully monitored and eDNA-based studies could
378 provide a cost-effective way to meet this need

379 Conclusions

380 We developed field sampling protocols and a HTS pipeline which enabled the efficient recovery
381 of eDNA from several tropical aquatic ecosystems. Water samples consistently revealed more
382 vertebrate species than sediment samples although about 10% of the species were only recovered
383 from sediments.

384 eDNA sequences were recovered from 75 species of vertebrates including 47 species of fishes,
385 with six new records for the Lake Bacalar region and two other species whose detection is likely
386 to be due to human activity. Sequences were also detected from another 28 vertebrate species

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

387 including 15 birds, 7 mammals, 5 reptiles, and 1 amphibian, all species known from the
388 watershed.

389 This study indicates that eDNA can aid conservation and monitoring programs in tropical areas
390 by improving our capacity to map occurrence records for resident and invasive species. There
391 remains a need to convince both regulatory agencies and the public that this approach can
392 provide the detailed information on species composition needed to underpin conservation policy
393 for tropical aquatic ecosystems.

394 # Acknowledgments

395 We thank Jose Angel Cohuo, Miguel Valadez, and Rosaura Castro from the Instituto
396 Tecnológico de Chetumal and Fernando Cortés Carrasco from El Colegio de la Frontera Sur for
397 help with collecting and processing samples. DNA extraction and NGS were carried out at the
398 Centre for Biodiversity Genomics (CBG), University of Guelph through funding to PDNH from
399 Ann McCain Evans and Chris Evans. We thank Thomas Braukmann for designing the
400 bioinformatics pipeline for processing the raw data. This paper represents a contribution from the
401 Chetumal node of the Mexican Barcode of Life (MEXBOL) network. MVM and MEG thank
402 CONACYT for support through the Sabbatical Stays Program (Grants 261790 and 262267).

403 # Author contributions

404 Conceived and designed the experiments: MVM, MEG.
405 Coordinated the collection of material and performed field experiments: MVM, MEG.
406 Performed the lab experiments: NVI, SLP.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

407 Analyzed the data: NVI, MVM, MEG, SLP.

408 Designed illustrations: NVI, MVM.

409 Designed *OTUBlastParser.py* and *ConcatenatorResults.py* Python scripts: KB.

410 Wrote the paper: MVM, NVI, MEG, SLP, PDNH.

411 All authors have read and approved the final manuscript.

412 Data accessibility for BOLD datasets:

413 dx.doi.org/10.5883/DS-EBACALAR

414 dx.doi.org/10.5883/DS-EBACAMPH

415 dx.doi.org/10.5883/DS-EBACAVES

416 dx.doi.org/10.5883/DS-EBACREPT

417 dx.doi.org/10.5883/DS-EBACMAMM

418 References

419 1. Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, et al.
420 Environmental DNA metabarcoding: Transforming how we survey animal and plant
421 communities. Mol Ecol. 2017;26: 5872–5895. doi:10.1111/mec.14350

422 2. Barnes MA, Turner CR. The ecology of environmental DNA and implications for
423 conservation genetics. Conserv Genet. 2015;17: 1–17. doi:10.1007/s10592-015-0775-4

424 3. Thomsen PF, Willerslev E. Environmental DNA – an emerging tool in conservation for

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

425 monitoring past and present biodiversity. Biol Conserv. 2014;
426 doi:10.1016/j.biocon.2014.11.019

427 4. Mächler E, Deiner K, Spahn F, Altermatt F. Fishing in the water: effect of sampled water
428 volume on environmental DNA-based detection of macroinvertebrates. Environ Sci
429 Technol. 2016;50: 305–312. doi:10.1021/acs.est.5b04188

430 5. Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. Environmental DNA. Mol Ecol.
431 2012;21: 1789–1793. doi:10.1111/j.1365-294X.2012.05542.x

432 6. Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, et al.
433 Persistence of environmental DNA in freshwater ecosystems. PLoS One. 2011;6: 8–11.
434 doi:10.1371/journal.pone.0023398

435 7. Schmelzle MC, Kinziger AP. Using occupancy modelling to compare environmental
436 DNA to traditional field methods for regional-scale monitoring of an endangered aquatic
437 species. Mol Ecol Resour. 2016;16: 895–908. doi:10.1111/1755-0998.12501

438 8. Goldberg CS, Strickler KM, Pilliod DS. Moving environmental DNA methods from
439 concept to practice for monitoring aquatic macroorganisms. Biol Conserv. 2014;
440 doi:10.1016/j.biocon.2014.11.040

441 9. Hebert PDN, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA
442 barcodes. Proc Biol Sci. 2003;270: 313–21. doi:10.1098/rspb.2002.2218

443 10. Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System
444 (www.barcodinglife.org). Mol Ecol Notes. 2007;7: 355–364.

445 11. Hajibabaei M. The golden age of DNA metasystematics. Trends Genet. 2012;28: 535–

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

446 537. doi:10.1016/j.tig.2012.08.001

447 12. Lodge DM, Turner CR, Jerde CL, Barnes MA, Chadderton L, Egan SP, et al.
448 Conservation in a cup of water: estimating biodiversity and population abundance from
449 environmental DNA. Mol Ecol. 2012;21: 2555–2558. doi:10.1111/j.1365-
450 294X.2012.05600.x

451 13. Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, et al.
452 Critical considerations for the application of environmental DNA methods to detect
453 aquatic species. Methods Ecol Evol. 2016;7: 1299–1307. doi:10.1111/2041-210X.12595

454 14. Sigsgaard EE, Carl H, Møller PR, Thomsen PF. Monitoring the near-extinct European
455 weather loach in Denmark based on environmental DNA from water samples. Biol
456 Conserv. 2014;183: 46–52. doi:10.1016/j.biocon.2014.11.023

457 15. de Carvalho MR, Bockmann FA, Amorim DS, Brandão CRF, de Vivo M, de Figueiredo
458 JL, et al. Taxonomic impediment or impediment to taxonomy? A commentary on
459 systematics and the cybertaxonomic-automation paradigm. Evol Biol. 2007;34: 140–143.
460 doi:10.1007/s11692-007-9011-6

461 16. Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. The detection of
462 aquatic animal species using environmental DNA – a review of eDNA as a survey tool in
463 ecology. Journal of Applied Ecology. 2014. doi:10.1111/1365-2664.12306

464 17. Comtet T, Sandionigi A, Viard F, Casiraghi M. DNA (meta)barcoding of biological
465 invasions: a powerful tool to elucidate invasion processes and help managing aliens. Biol
466 Invasions. 2015;17. doi:10.1007/s10530-015-0854-y

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

467 18. Bergman PS, Schumer G, Blankenship S, Campbell E. Detection of adult green sturgeon
468 using environmental DNA analysis. PLoS One. 2016;11: 1–8.
469 doi:10.1371/journal.pone.0153500

470 19. Eva B, Harmony P, Thomas G, Francois G, Alice V, Claude M, et al. Trails of river
471 monsters: Detecting critically endangered Mekong giant catfish *Pangasianodon gigas*
472 using environmental DNA. Glob Ecol Conserv. 2016;7: 148–156.
473 doi:10.1016/j.gecco.2016.06.007

474 20. Doi H, Inui R, Akamatsu Y, Kanno K, Yamanaka H, Takahara T, et al. Environmental
475 DNA analysis for estimating the abundance and biomass of stream fish. Freshw Biol.
476 2016; 30–39. doi:10.1111/fwb.12846

477 21. Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z. Estimation of fish biomass
478 using environmental DNA. PLoS One. 2012;7: 3–10. doi:10.1371/journal.pone.0035868

479 22. Deiner K, Walser J-C, Mächler E, Altermatt F. Choice of capture and extraction methods
480 affect detection of freshwater biodiversity from environmental DNA. Biol Conserv. 2014;
481 doi:10.1016/j.biocon.2014.11.018

482 23. Turner CR, Barnes MA, Xu CCY, Jones SE, Jerde CL, Lodge DM. Particle size
483 distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol Evol. 2014;5:
484 676–684. doi:10.1111/2041-210X.12206

485 24. Ficetola GF, Miaud C, Pompanon F, Taberlet P. Species detection using environmental
486 DNA from water samples. Biol Lett. 2008;4: 423–5. doi:10.1098/rsbl.2008.0118

487 25. Wegleitner BJ, Jerde CL, Tucker A, Chadderton WL, Mahon AR. Long duration, room

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

488 temperature preservation of filtered eDNA samples. Conserv Genet Resour. 2015;7: 789–
489 791. doi:10.1007/s12686-015-0483-x

490 26. Yamanaka H, Minamoto T, Matsuura J, Sakurai S, Tsuji S, Motozawa H, et al. A simple
491 method for preserving environmental DNA in water samples at ambient temperature by
492 addition of cationic surfactant. Limnology. 2017;18: 233–241. doi:10.1007/s10201-016-
493 0508-5

494 27. Deiner K, Renshaw MA, Li Y, Olds BP, Lodge DM, Pfrender ME. Long-range PCR
495 allows sequencing of mitochondrial genomes from environmental DNA. Methods Ecol
496 Evol. 2017; doi:10.1111/2041-210X.12836

497 28. Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, et al. Environmental DNA
498 metabarcoding reveals local fish communities in a species-rich coastal sea. Sci Rep.
499 2017;7: 40368. doi:10.1038/srep40368

500 29. Olds BP, Jerde CL, Renshaw MA, Li Y, Evans NT, Turner CR, et al. Estimating species
501 richness using environmental DNA. Ecol Evol. 2016;6: 4214–4226.
502 doi:10.1002/ece3.2186

503 30. Lopes CM, Sasso T, Valentini A, Dejean T, Martins M, Zamudio KR, et al. eDNA
504 metabarcoding: a promising method for anuran surveys in highly diverse tropical forests.
505 Mol Ecol Resour. 2017;17: 904–914. doi:10.1111/1755-0998.12643

506 31. Eichmiller J, Best SE, Sorensen PW. Effects of temperature and trophic state on
507 degradation of environmental DNA in lake water. Environ Sci Technol. 2016;
508 acs.est.5b05672. doi:10.1021/acs.est.5b05672

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

509 32. Robson HLA, Noble TH, Saunders RJ, Robson SKA, Burrows DW, Jerry DR. Fine-tuning
510 for the tropics: application of eDNA technology for invasive fish detection in tropical
511 freshwater ecosystems. Mol Ecol Resour. 2016;16: 922–932. doi:10.1111/1755-
512 0998.12505

513 33. Gischler E, Gibson MA, Oschmann W. Giant Holocene freshwater microbialites, Laguna
514 Bacalar, Quintana Roo, Mexico. Sedimentology. 2008;55: 1293–1309.
515 doi:10.1111/j.1365-3091.2007.00946.x

516 34. Castro-Contreras SI, Gingras MK, Pecoits E, Abert NR, Petrash D, Castro-Contreras SM,
517 et al. Textural and geochemical features of freshwater. Palaios. 2013;29: 192–209.
518 doi:10.2110/palo.2013.063

519 35. Centeno CM, Legendre P, Beltrán Y, Alcántara-Hernández RJ, Lidström UE, Ashby MN,
520 et al. Microbialite genetic diversity and composition relate to environmental variables.
521 FEMS Microbiol Ecol. 2012;82: 724–735. doi:10.1111/j.1574-6941.2012.01447.x

522 36. Bauer-Gottwein P, Gondwe BRN, Charvet G, Marín LE, Rebolledo-Vieyra M, Merediz-
523 Alonso G. Review: The Yucatán Peninsula karst aquifer, Mexico. Hydrogeol J. 2011;19:
524 507–524. doi:10.1007/s10040-010-0699-5

525 37. Moore YH, Stoessel RK, Easley D. Fresh-water/sea-water relationship within a
526 groundwater system, northeastern coast of the Yucatán Peninsula. Gr Wat. 1992;30: 343–
527 350.

528 38. Perry E, Velazquez-Oliman G, Marin LE. The hydrogeochemistry of the karst aquifer
529 system of the northern Yucatan Peninsula, Mexico. Int Geol Rev. 2002;44: 191–221.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

530 doi:10.2747/0020-6814.44.3.191

531 39. Pérez L, Bugja R, Lorenschat J, Brenner M, Curtis J, Hoelzmann P, et al. Aquatic
532 ecosystems of the Yucatan Peninsula (Mexico), Belize, and Guatemala. Hydrobiologia.
533 2011;661: 407–433. doi:10.1007/s10750-010-0552-9

534 40. Ivanova NV, Fazekas AJ, Hebert PDN. Semi-automated, membrane-based protocol for
535 DNA isolation from plants. Plant Mol Biol Report. 2008;26: 186–198.

536 41. Rohland N, Reich D, Rohland and Reich, Rohland N, Reich D. Cost-effective, high-
537 throughput DNA sequencing libraries for multiplexed target capture. Genome Res.
538 2012;22: 939–946. doi:10.1101/gr.128124.111

539 42. Ivanova N V., Dewaard JR, Hebert PDN. An inexpensive, automation-friendly protocol
540 for recovering high-quality DNA. Mol Ecol Notes. 2006;6: 998–1002. doi:10.1111/j.1471-
541 8286.2006.01428.x

542 43. Berry D, Ben Mahfoudh K, Wagner M, Loy A. Barcoded primers used in multiplex
543 amplicon pyrosequencing bias amplification. Appl Environ Microbiol. 2011;77: 7846–9.
544 doi:10.1128/AEM.05220-11

545 44. Ivanova N V, Clare EL, Borisenko A V. DNA barcoding in mammals. In: Kress J,
546 Erickson D, editors. Analytical protocols In: DNA barcodes, methods in molecular
547 biology. Humana Press; 2012. pp. 153–182. doi:10.1007/978-1-61779-591-6

548 45. Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN. Universal primer cocktails for fish
549 DNA barcoding. Mol Ecol Notes. 2007;7: 544–548.

550 46. Colwell RK. EstimateS: Statistical estimation of species richness and shared species from

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

551 samples. Version 9. User's guide and application. [Internet]. 2013. Available:
552 http://purl.oclc.org/estimates

553 47. Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and
554 annotation of phylogenetic and other trees. Nucleic Acids Res. 2016;44: W242-5.
555 doi:10.1093/nar/gkw290

556 48. López-Vila JM, Valdéz-Moreno ME, Schmitter-Soto JJ, Mendoza-Carranza M, Herrera-
557 Pavón RL. Composition and structure of the ichthyofauna in the Hondo River, Mexico-
558 Belize, using a harpoon. Rev Mex Biodivers. 2014;85: 866–874. doi:10.7550/rmb.35806

559 49. Schmitter-Soto JJ. Catalogo de los peces continentales de quintana roo [Internet]. 1996.
560 Available: http://www.nativefishlab.net/library/textpdf/20569.pdf

561 50. Schmitter-Soto JJ. Distribution of continental fishes in northern Quintana Roo, Mexico.
562 Southwest Nat. 1999;44: 166–172.

563 51. Valdez-Moreno M, Ivanova N V., Elías-Gutiérrez M, Contreras-Balderas S, Hebert PDN.
564 Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. J
565 Fish Biol. 2009;74: 377–402. doi:10.1111/j.1095-8649.2008.02077.x

566 52. Miller RR. Peces dulceacuícolas de México. México, D.F.: Comisión Nacional para el
567 Conocimiento y Uso de la Biodiversidad, Sociedad Ictiológica Mexicana A. C., El colegio
568 de la Frontera Sur y Consejo de los Peces del Desierto México-Estados Unidos; 2009.

569 53. Neil SJ. Field studies of the behavioral ecology and agonistic behavior of *Cichlasoma*
570 *meeki* (Pisces: Cichlidae). Environ Biol Fishes. 1984;10: 59–68. doi:10.1007/BF00001662

571 54. Schmitter-Soto JJ, Gamboa-Pérez HC. Composición y distribución de peces continentales

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

572 en el sur de Quintana Roo, Península de Yucatán, México. Rev Biol Trop. 1996;44: 199–
573 212.

574 55. Gamboa-Pérez H and Schmitter-Soto J.J. Distribution of cichid fishes in the littoral of
575 Lake Bacalar, Yucatan Peninsula. Environ Biol Fishes. 1999;54: 35–43.

576 56. Miller RR. Composition and derivation of the freshwater fish fauna of Mexico. An la Esc
577 Nac Ciencias Biológicas. 1986;30: 121–153.

578 57. Palmer JM, Jusino MA, Banik MT, Lindner DL. Non-biological synthetic spike-in
579 controls and the AMPtk software pipeline improve mycobiome data. PeerJ. 2018;6:
580 e4925. doi:10.7717/peerj.4925

581 58. Esling P, Lejzerowicz F, Pawlowski J. Accurate multiplexing and filtering for high-
582 throughput amplicon-sequencing. Nucleic Acids Res. 2015;43: 2513–24.
583 doi:10.1093/nar/gkv107

584 59. Strickler KM, Fremier AK, Goldberg CS. Quantifying effects of UV-B, temperature, and
585 pH on eDNA degradation in aquatic microcosms. Biol Conserv. 2014;
586 doi:10.1016/j.biocon.2014.11.038

587 60. Seymour M, Durance I, Cosby BJ, Ransom-Jones E, Deiner K, Ormerod SJ, et al. Acidity
588 promotes degradation of multi-species environmental DNA in lotic mesocosms. Commun
589 Biol. 2018;1: 4. doi:10.1038/s42003-017-0005-3

590 61. Prosser S, Martínez-Arce A, Elías-Gutiérrez M. A new set of primers for COI
591 amplification from freshwater microcrustaceans. Mol Ecol Resour. 2013;13: 1151–1155.
592 doi:10.1111/1755-0998.12132

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

593 62. Elías-Gutiérrez M, Valdez-Moreno M, Topan J, Young MR, Cohuo-Colli JA. Improved
594 protocols to accelerate the assembly of DNA barcode reference libraries for freshwater
595 zooplankton. Ecol Evol. 2018;8: 3002–3018. doi:10.1002/ece3.3742

596 63. Vences M, Lyra ML, Perl RGB, Bletz MC, Stanković D, Lopes CM, et al. Freshwater
597 vertebrate metabarcoding on Illumina platforms using double-indexed primers of the
598 mitochondrial 16S rRNA gene. Conserv Genet Resour. 2016;8: 323–327.
599 doi:10.1007/s12686-016-0550-y

600 64. Sato H, Sogo Y, Doi H, Yamanaka H. Usefulness and limitations of sample pooling for
601 environmental DNA metabarcoding of freshwater fish communities. Sci Rep. 2017;7:
602 14860. doi:10.1038/s41598-017-14978-6

603 65. Port JA, O'Donnell JL, Romero-Maraccini OC, Leary PR, Litvin SY, Nickols KJ, et al.
604 Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA.
605 Mol Ecol. 2016;25: 527–541. doi:10.1111/mec.13481

606 66. Andruszkiewicz EA, Starks HA, Chavez FP, Sassoubre LM, Block BA, Boehm AB.
607 Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS
608 One. 2017;12: e0176343. doi:10.1371/journal.pone.0176343

609 67. Dougherty MM, Larson ER, Renshaw MA, Gantz CA, Egan SP, Erickson DM, et al.
610 Environmental DNA (eDNA) detects the invasive rusty crayfish *Orconectes rusticus* at
611 low abundances. Journal of Applied Ecology. 2016. doi:10.1111/1365-2664.12621

612 68. Klymus KE, Richter CA, Chapman DC, Paukert C. Quantification of eDNA shedding
613 rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

614 *Hypophthalmichthys molitrix*. Biol Conserv. 2015;183: 77–84.
615 doi:10.1016/j.biocon.2014.11.020

616 69. Cowart DA, Murphy KR, Cheng C-HC. Metagenomic sequencing of environmental DNA
617 reveals marine faunal assemblages from the West Antarctic Peninsula. Mar Genomics.
618 2018;37: 148–160. doi:10.1016/J.MARGEN.2017.11.003

619 70. Espinoza Pérez H, Gaspar Dillanes MT, Fuentes Mata P. Listado faunísticos de México.
620 III. Los peces dulceaciucolas Mexicanos. México, D. F.: Universidad Nacional Autonoma
621 de Mexico; 1993.

622 71. Rossini BC, Oliveira CAM, Melo FAG de, Bertaco V de A, Astarloa JMD de, Rosso JJ, et
623 al. Highlighting *Astyanax* species diversity through DNA barcoding. PLoS One. 2016;11:
624 e0167203. doi:10.1371/journal.pone.0167203

625 72. Ornelas-García C, Domínguez-Domínguez O, Doadrio I. Evolutionary history of the fish
626 genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica
627 reveals multiple morphological homoplasies. BMC Evol Biol. 2008;8: 340.
628 doi:10.1186/1471-2148-8-340

629 73. Sarukhán J, Whyte A, editors. Millenium ecosystem assessment. Ecosystems and human
630 well-being: biodiversity synthesis [Internet]. Ecosystems. Washington, DC: Island Press;
631 2005. Available:
632 https://www.millenniumassessment.org/documents/document.356.aspx.pdf

633 74. Jelks HL, Walsh SJ, Burkhead NM, Contreras-Balderas S, Diaz-Pardo E, Hendrickson
634 DA, et al. Conservation status of imperiled North American freshwater and diadromous

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

635 fishes. Fisheries. 2008;33: 372–407. doi:10.1577/1548-8446-33.8.372

636 75. Arriaga Cabrera L, Aguilar Sierra V, Alcocer Durán J, Jiménez Rosenberg R, Muñoz
637 López E, Vázquez Domínguez E, editors. Regiones hidrológicas prioritarias: fichas
638 técnicas y mapa (escala 1:4,000,000) [Internet]. Mexico: Comisión Nacional para el
639 Conocimiento y Uso de la Biodiversidad; 1998. Available:
640 https://books.google.ca/books?id=b116YgEACAAJ

641 76. Schmitter-Soto JJ, Ruiz-Cauich LE, Herrera RL, González-Solís D. An index of biotic
642 integrity for shallow streams of the Hondo River basin, Yucatan Peninsula. Sci Total
643 Environ. 2011;409: 844–852. doi:10.1016/j.scitotenv.2010.11.017

644 77. Wakida-Kusunoki AT, Luis Enrique Amador-del Ángel. Aspectos biológicos del pleco
645 invasor *Pterygoplichthys pardalis* (Teleostei: Loricariidae) en el río Palizada, Campeche,
646 México. Rev Mex Biodivers. 2011;82: 870–878.

647 78. Alfaro REM, Fisher JP, Courtenay W, Ramírez Martínez C, Orbe-Mendoza A, Escalera
648 Gallardo C, et al. Armored catfish (Loricariidae) trinational risk assessment guidlines for
649 aquatic alien invasive species. Test cases for the snakeheads (Channidae) and armored
650 catfishes (Loricariidae) in North American inland waters. Montreal, Canadá: Commission
651 for Environmental Cooperation; 2009. pp. 25–49.

652 79. Schmitter-Soto JJ, Quintana R, Valdéz-Moreno ME, Herrera-Pavón RL, Esselman PC.
653 Armoured catfish (*Pterygoplichthys pardalis*) in the Hondo River basin, Mexico-Belize.
654 Mesoamericana. 2015;19: 9–19.

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

655 # Supporting Information

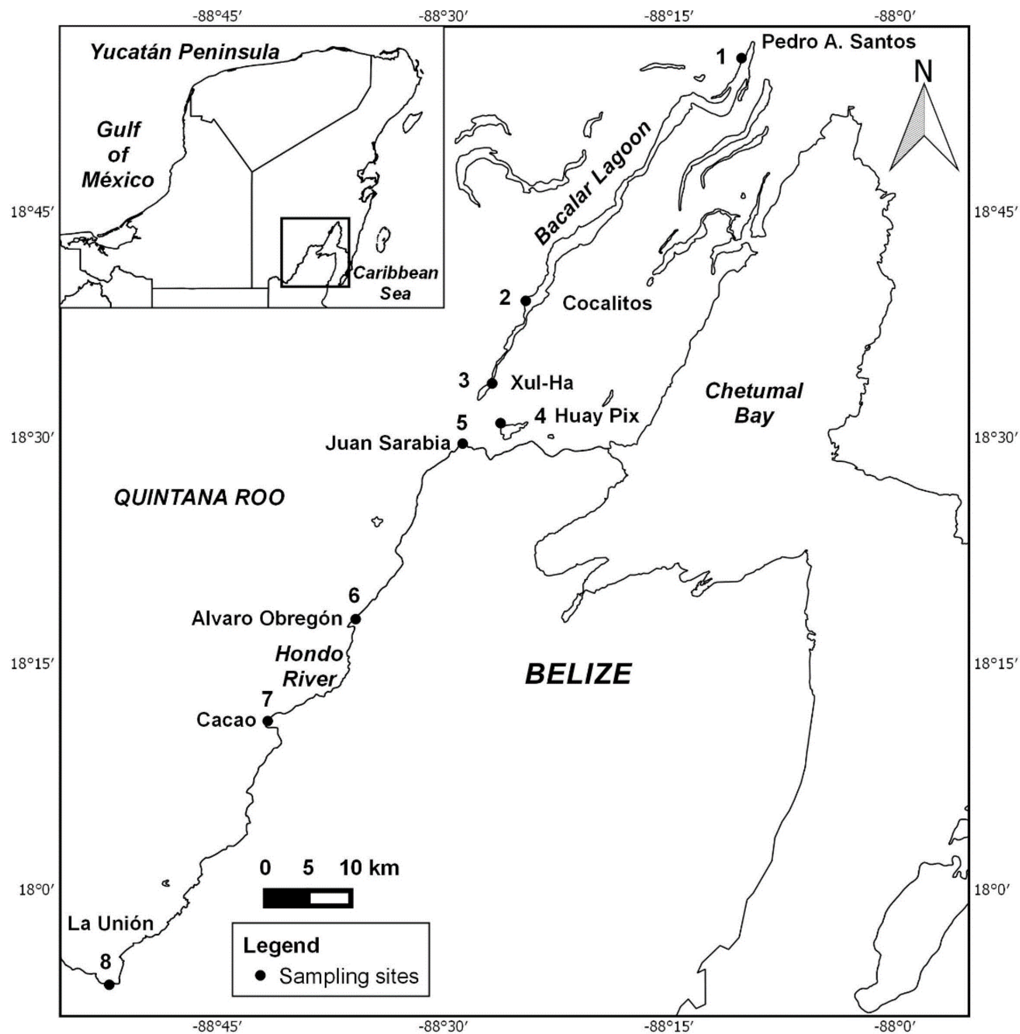
656 **S1 Table. Supplementary Table 1.** Summary of coverage for vertebrate reads for all collection
657 events and for positive controls.

658 **S2 Table. Supplementary Table 2.** Taxonomy, top hit Process IDs, average overlap, average
659 score, coverage, and average identity for vertebrate species detected by eDNA in environmental
660 samples, positive and negative controls.

661

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

662 Fig 1.



663

664

665

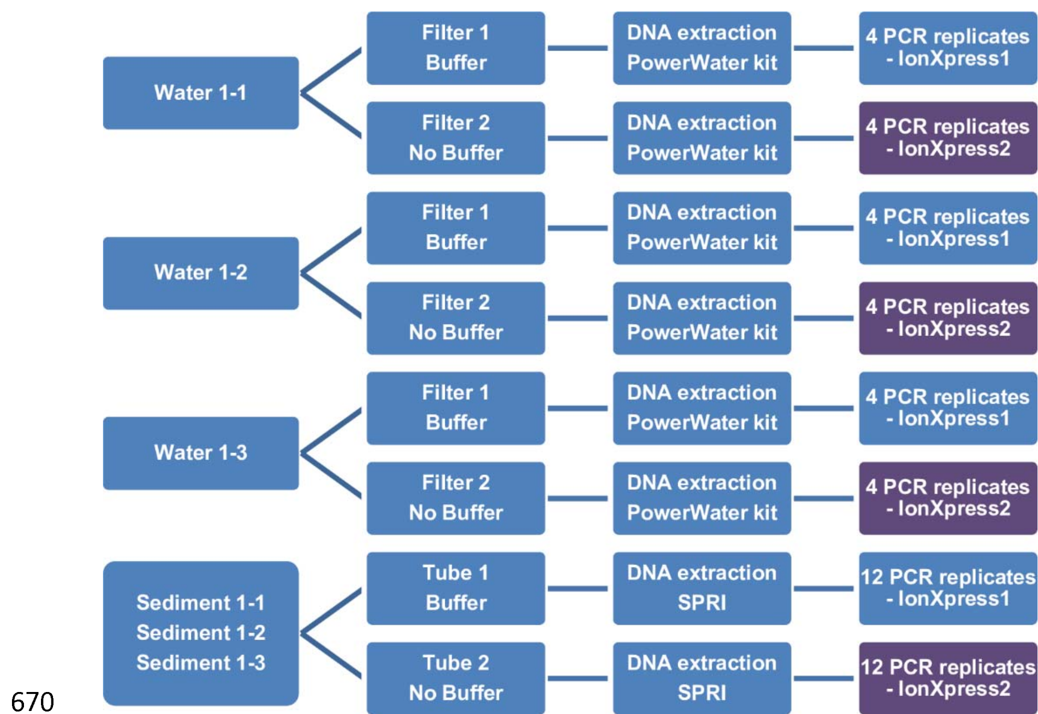
666

667

668

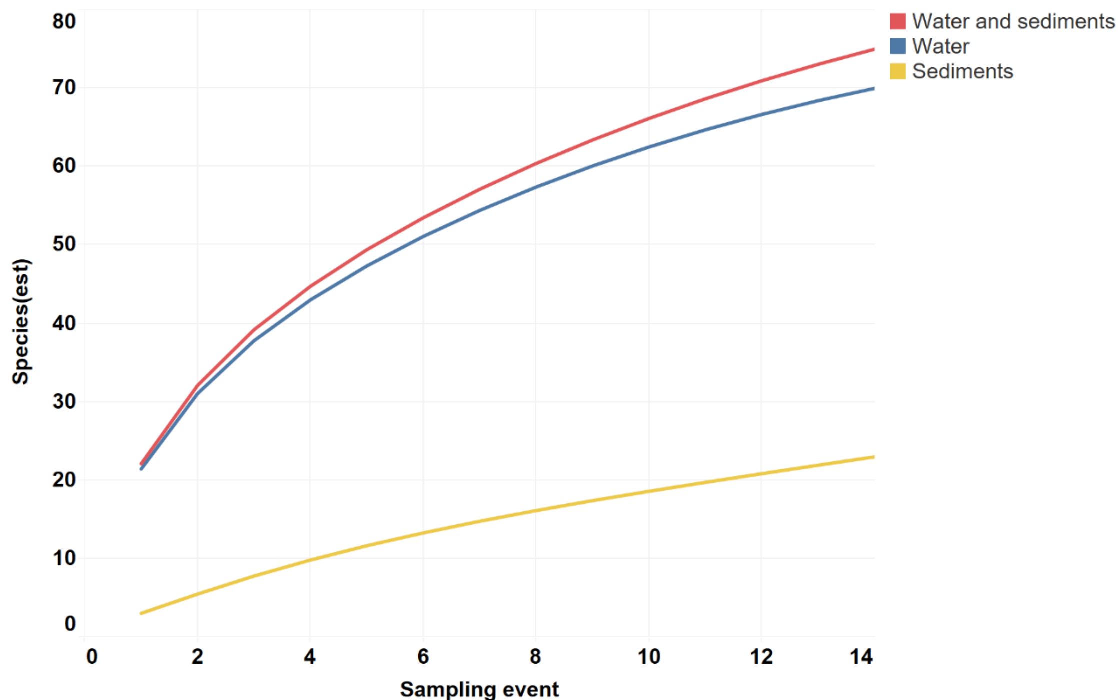
bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

669 Fig 2.



670

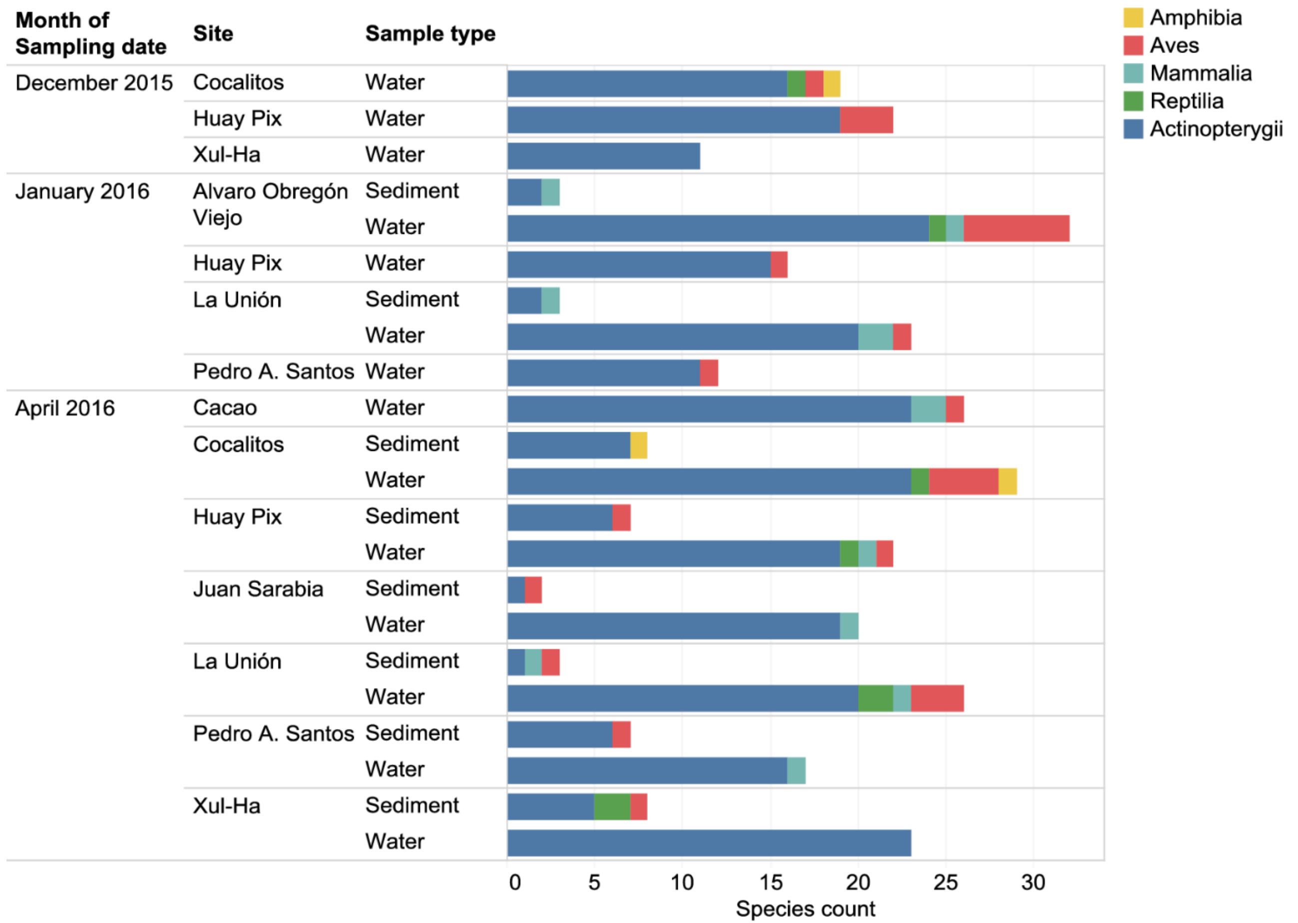
671 Fig 3.



672

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

673 Fig 4.



674

675

676

677

678

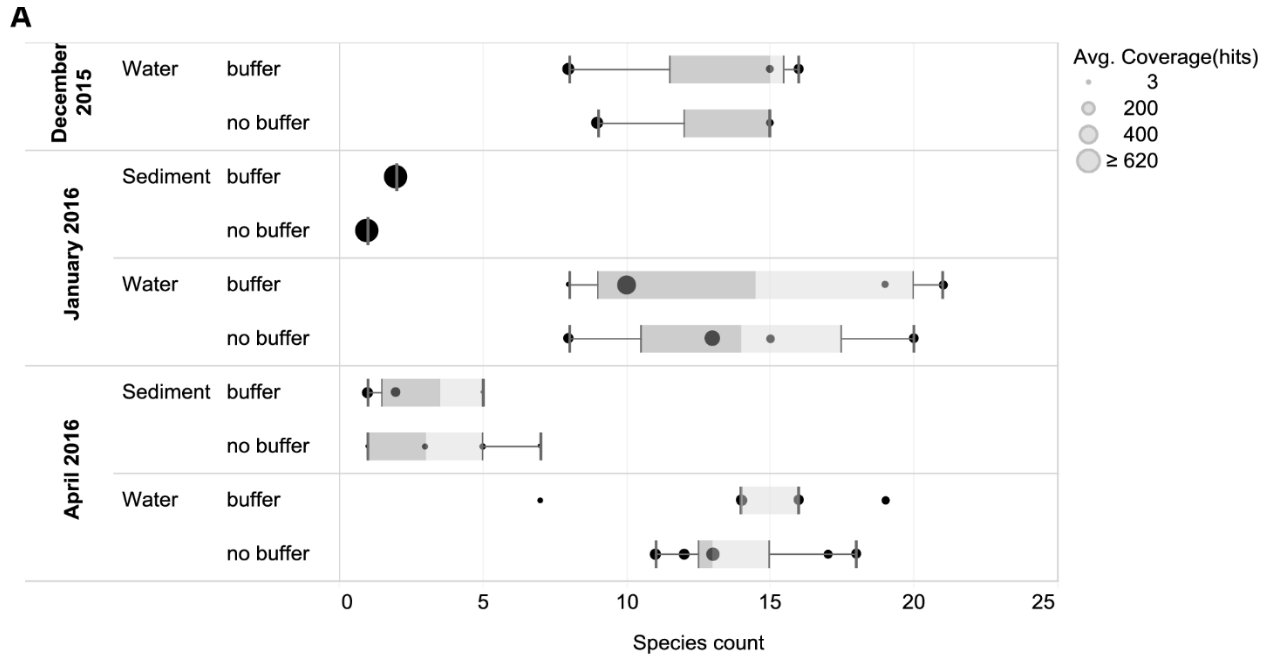
679

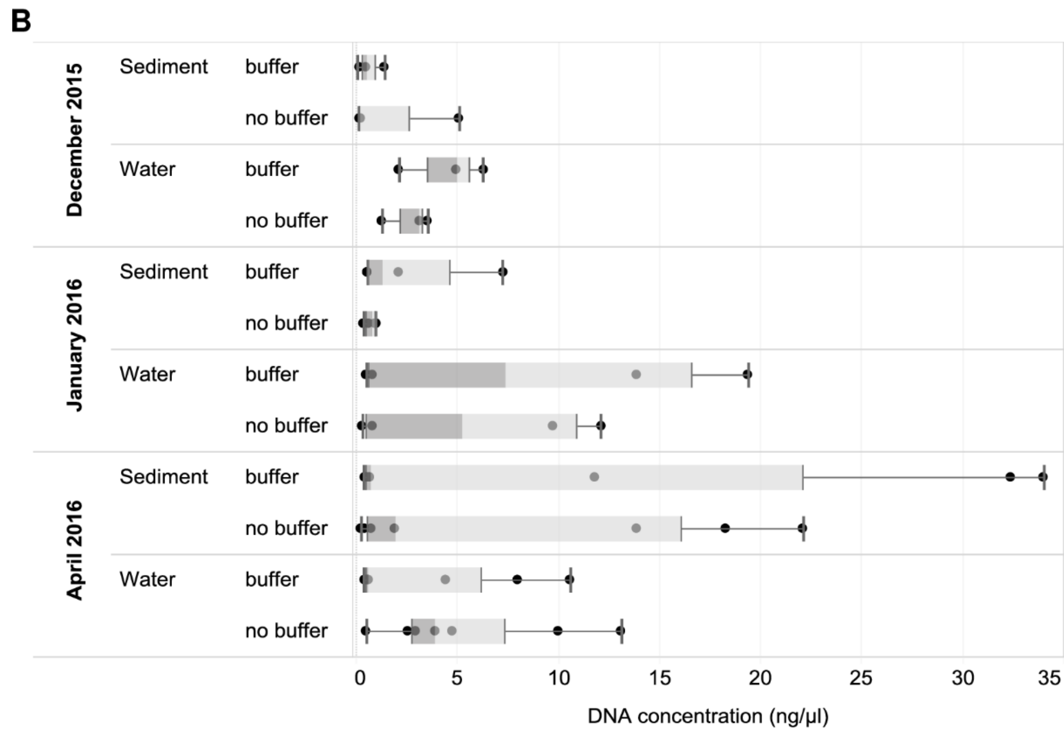
680

681

bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

682 Fig 5.





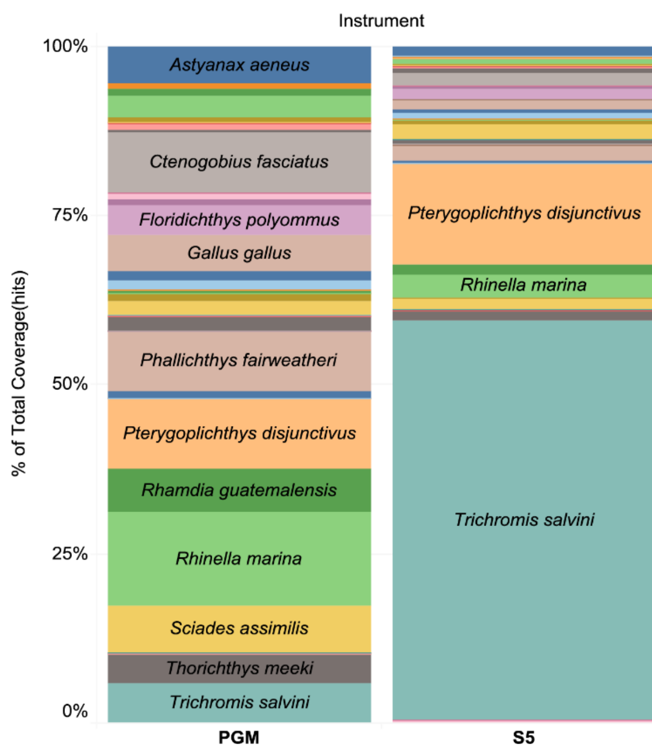
683

684

685

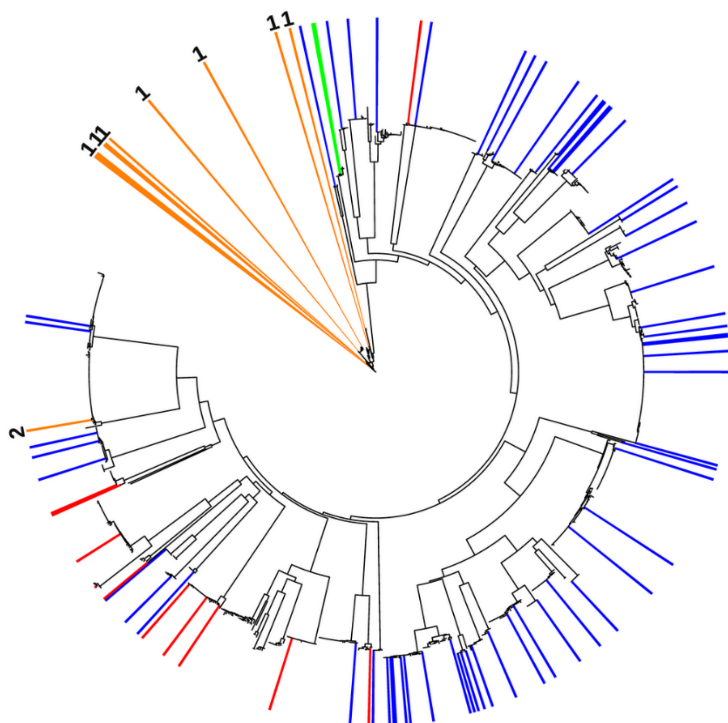
bioRxiv preprint doi: https://doi.org/10.1101/375089; this version posted July 23, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

686 Fig 6.

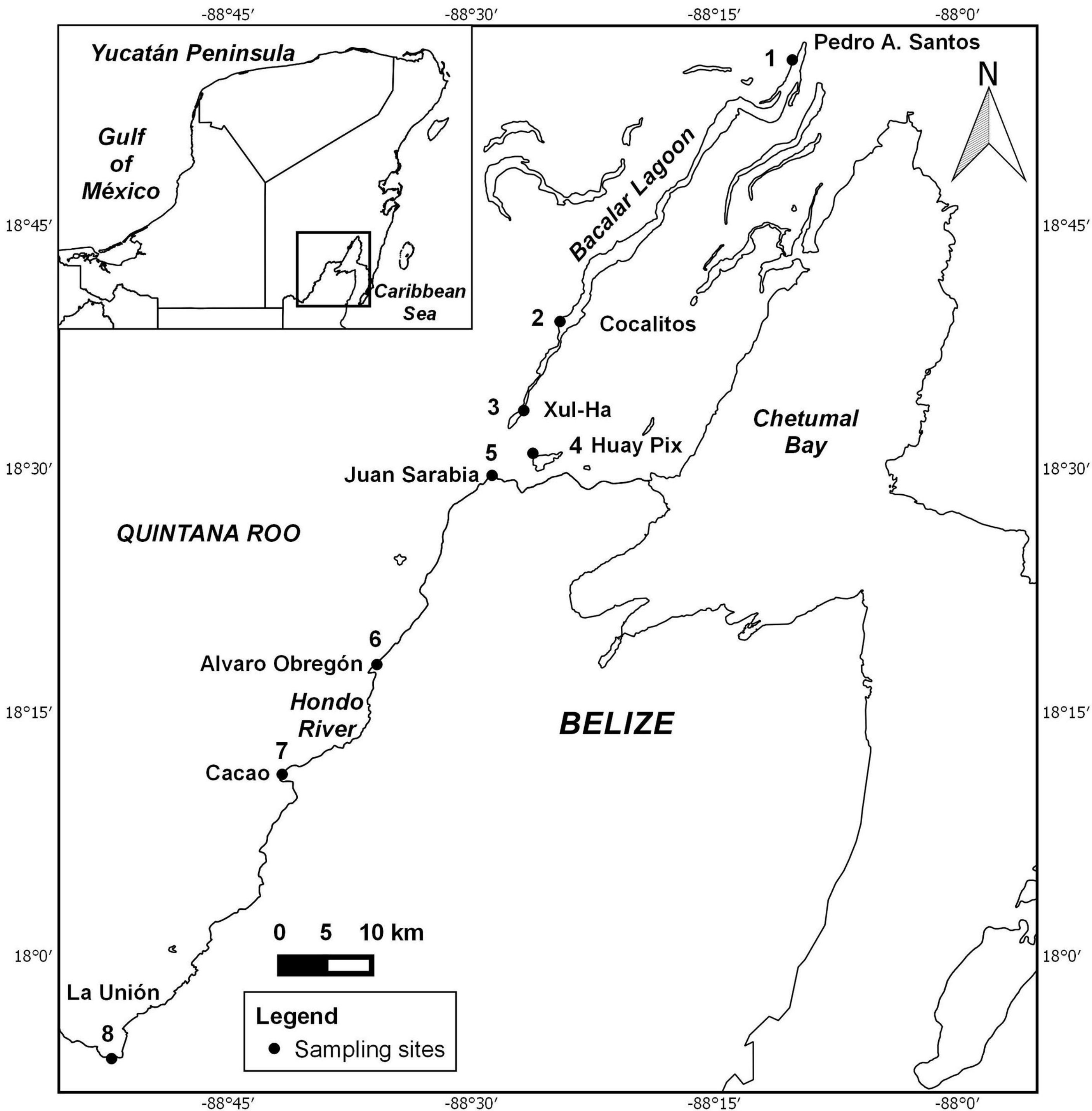


687

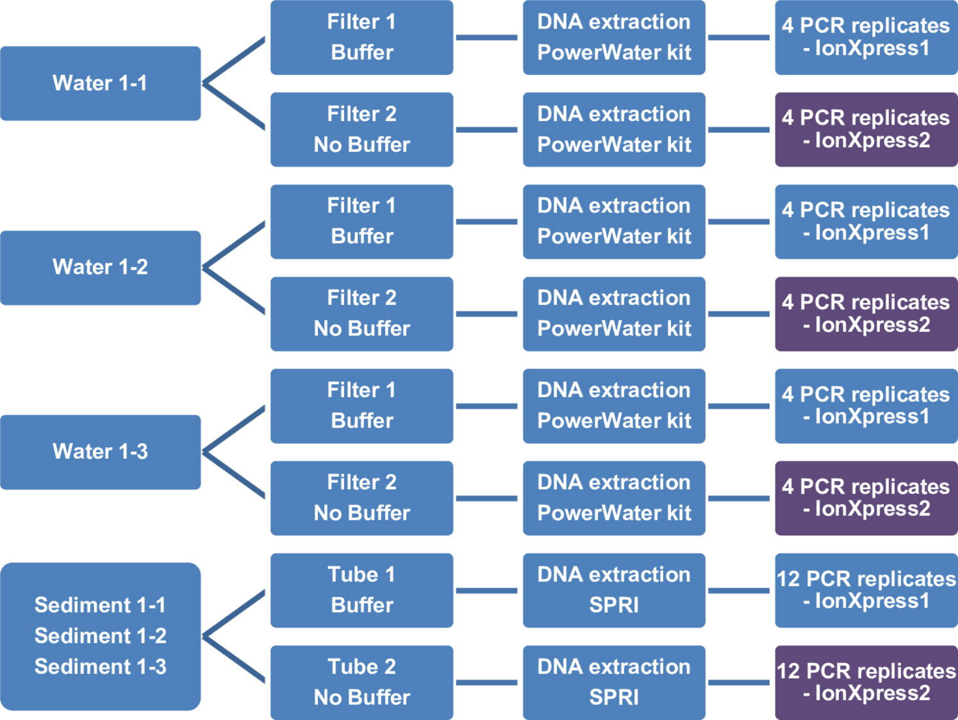
688 Fig 7.

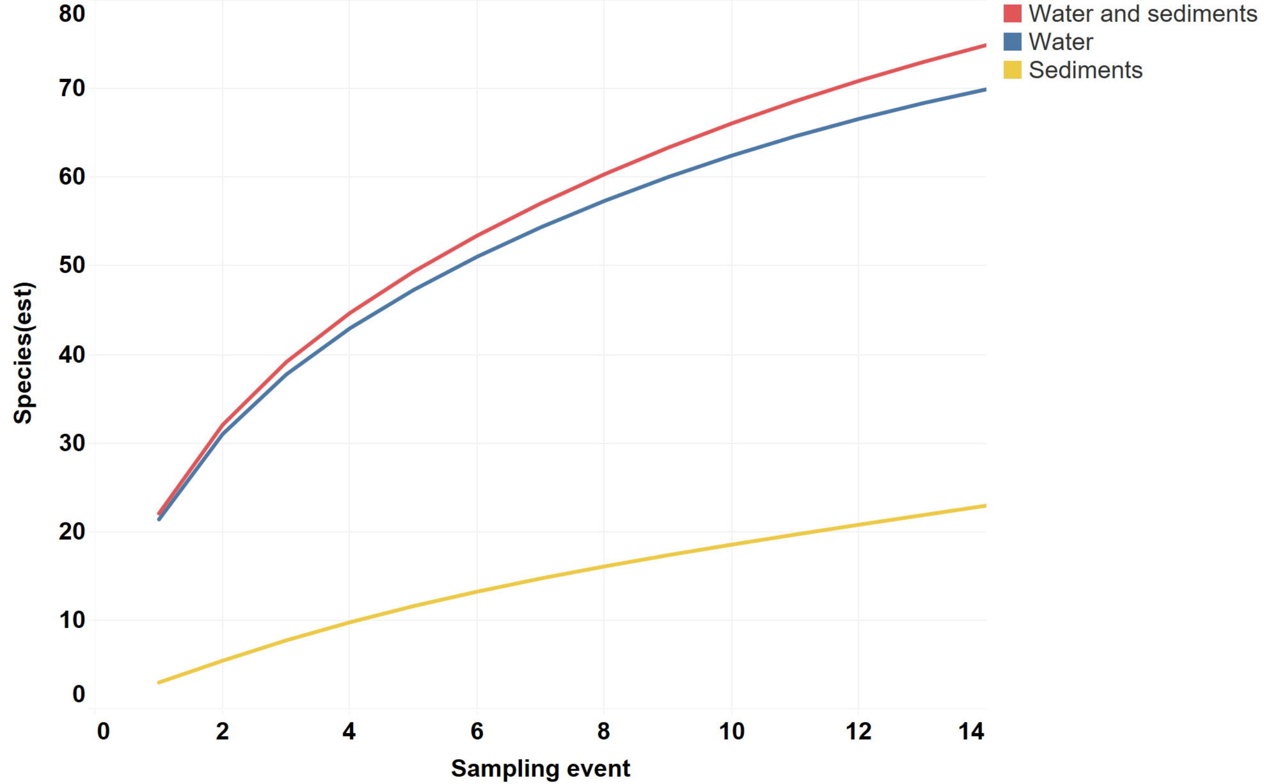


689

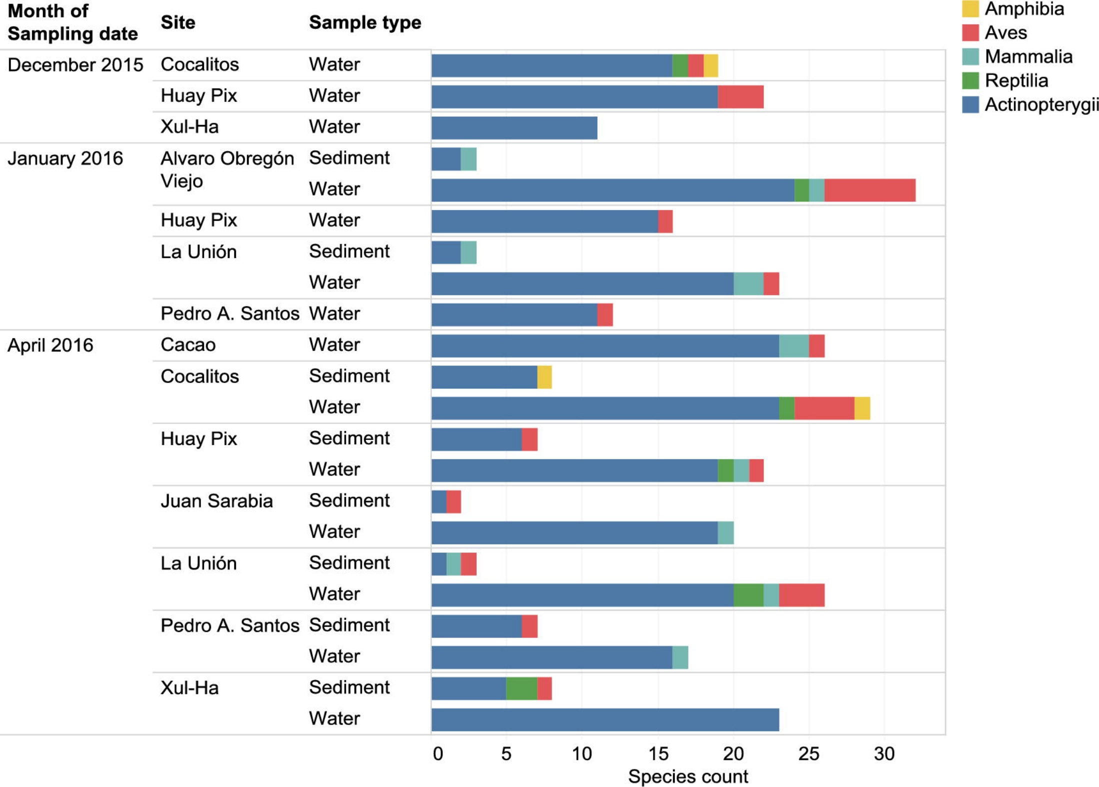


-88°45′
-88°30′
-88°15′
-88°0′
18°45′
18°30′
18°15′
18°0′
Yucatán Peninsula
Gulf of México
Caribbean Sea
1
Pedro A. Santos
N
Bacalar Lagoon
2
Cocalitos
3
Xul-Ha
4
Huay Pix
5
Juan Sarabia
Chetumal Bay
QUINTANA ROO
6
Alvaro Obregón
Hondo River
BELIZE
7
Cacao
0 5 10 km
La Unión
8
Legend
Sampling sites


Water 1-1
Filter 1
Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress1
Filter 2
No Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress2
Water 1-2
Filter 1
Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress1
Filter 2
No Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress2
Water 1-3
Filter 1
Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress1
Filter 2
No Buffer
DNA extraction
PowerWater kit
4 PCR replicates
- IonXpress2
Sediment 1-1
Sediment 1-2
Sediment 1-3
Tube 1
Buffer
DNA extraction
SPRI
12 PCR replicates
- IonXpress1
Tube 2
No Buffer
DNA extraction
SPRI
12 PCR replicates
- IonXpress2



Water and sediments
Water
Sediments
Species(est)
Sampling event
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14



Month of Sampling date
Site
Sample type
December 2015
Cocalitos
Water
Huay Pix
Water
Xul-Ha
Water
January 2016
Alvaro Obregón Viejo
Sediment
Water
Huay Pix
Water
La Unión
Sediment
Water
Pedro A. Santos
Water
April 2016
Cacao
Water
Cocalitos
Sediment
Water
Huay Pix
Sediment
Water
Juan Sarabia
Sediment
Water
La Unión
Sediment
Water
Pedro A. Santos
Sediment
Water
Xul-Ha
Sediment
Water
0
5
10
15
20
25
30
Species count
Amphibia
Aves
Mammalia
Reptilia
Actinopterygii

A

Avg. Coverage(hits)
3
200
400
≥ 620

December 2015
Water
buffer
no buffer

January 2016
Sediment
buffer
no buffer
Water
buffer
no buffer

April 2016
Sediment
buffer
no buffer
Water
buffer
no buffer

0 5 10 15 20 25

Species count

B

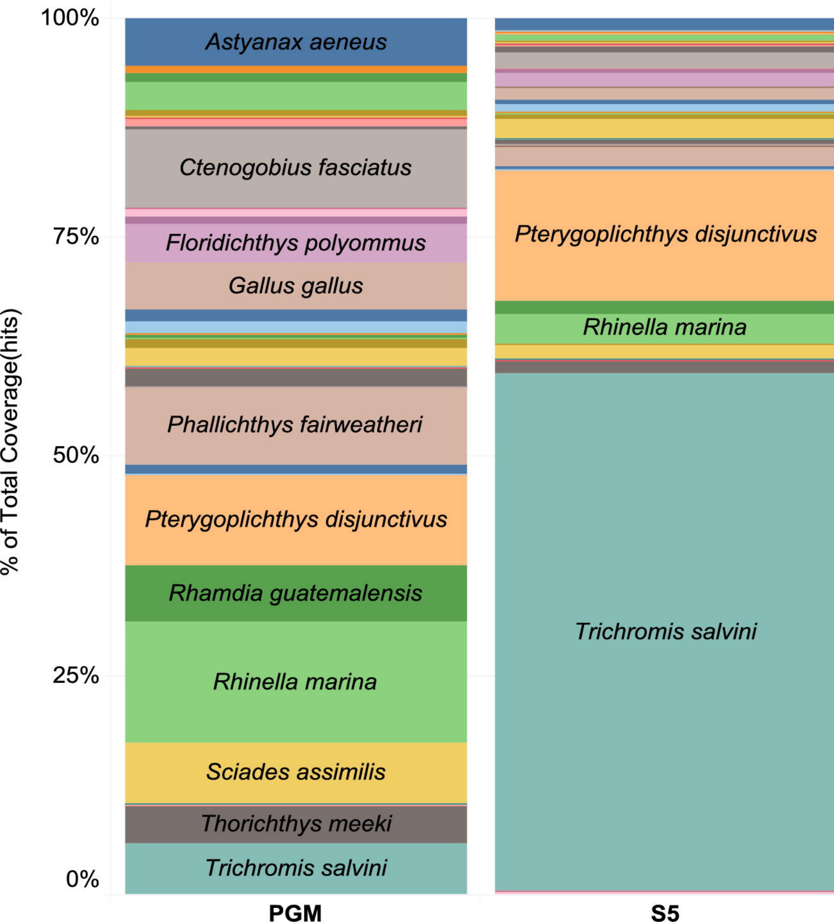
December 2015
Sediment
buffer
no buffer
Water
buffer
no buffer

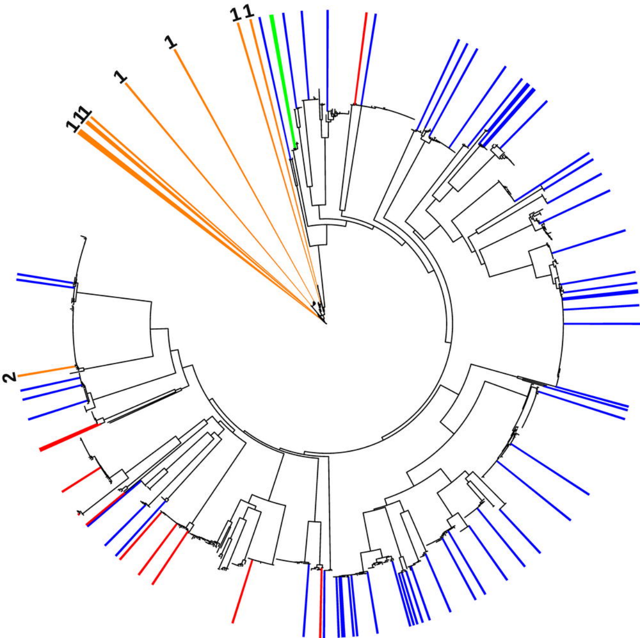
January 2016
Sediment
buffer
no buffer
Water
buffer
no buffer

April 2016
Sediment
buffer
no buffer
Water
buffer
no buffer

0 5 10 15 20 25 30 35

DNA concentration (ng/µl)


Instrument
100%
75%
50%
25%
0%
% of Total Coverage(hits)
Astyanax aeneus
Ctenogobius fasciatus
Floridichthys polyommus
Gallus gallus
Phallichthys fairweatheri
Pterygoplichthys disjunctivus
Rhamdia guatemalensis
Rhinella marina
Sciades assimilis
Thorichthys meeki
Trichromis salvini
Pterygoplichthys disjunctivus
Rhinella marina
Trichromis salvini
PGM
S5


1
1
1
1
111
2