Northern Australia Environmental Resources Hub

National Environmental Science Programme

Environmental DNA detection of snakehead species (*Channa* spp.) in the Barron River catchment, north Queensland

Report

Cecilia Villacorta-Rath and Damien Burrows

TropWATER Centre for Tropical Water and Aquatic Ecosystem Research

James Cook University Australia

© James Cook University, 2021

*Environmental DNA detection of snakehead species (*Channa *spp.) in the Barron River catchment, north Queensland* is licensed by the James Cook University for use under a Creative Commons Attribution 4.0 Australia licence. For licence conditions see creativecommons.org/licenses/by/4.0

This report should be cited as:

Villacorta-Rath, C., & Burrows, D. 2021. *Environmental DNA detection of snakehead species (*Channa *spp.) in the Barron River catchment, north Queensland*. Report 21/19, Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville.

Front cover photographs: eDNA sampling in the Barron River catchment, north Queensland (photo: Biosecurity Queensland).

This report is available for download from the Northern Australia Environmental Resources (NAER) Hub website at nespnorthern.edu.au

The Hub is supported through funding from the Australian Government's National Environmental Science Program (NESP). The NESP NAER Hub is hosted by Charles Darwin University.

ISBN: 978-1-922684-00-4

March, 2021

Printed by Uniprint

Contents

Acronyms & abbreviations iii
Acknowledgements iv
Executive summary 1
1. Introduction 2
2. Methodology 3
    2.1 Field collection of eDNA samples 3
    2.2 Environmental DNA extractions 4
    2.3 *Channa argus* assay design 5
    2.4 Detection of species-specific eDNA by quantitative PCR (qPCR) 7
    2.5 Verification of positive detections 7
3. Results 8
4. Discussion 9
References 11

List of tables

Table 2.1. Sampling sites for the direct water collection samples in the Barron River catchment, north Queensland. 3

Table 2.2. Primer information for *C. argus* eDNA assay targeting a region of the 16S rRNA gene. 6

Table 3.1. Summary of snakehead and tilapia eDNA detections in the Barron River catchment, north Queensland. Positive eDNA detections are indicated in **bold**. 8

List of figures

Figure 2.1. Sampling sites for the direct water collection samples in the Barron River catchment, north Queensland. 4

Acronyms & abbreviations

eDNA............. environmental DNA

NESP............... National Environmental Science Program

PCR .... polymerase chain reaction

qPCR............. quantitative real-time polymerase chain reaction

TropWATER ... Centre for Tropical Water and Aquatic Ecosystem Research

Acknowledgements

We would like to thank Biosecurity Queensland and Tropical River Consulting for carrying out the eDNA field sampling. We acknowledge the Traditional Owners of the lands and waters where the field and laboratory work was conducted.

Executive summary

Biosecurity Queensland engaged the Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) to conduct environmental DNA (eDNA) analysis of water samples collected in the Barron River catchment during December 2020, following a report on presence of snakehead (*Channa* spp.) from a recreational fisher. Snakeheads are a group of predatory fish species from southeast Asia that have been introduced and become established in a number of other countries, including the southern coastline of Papua New Guinea. They are considered a serious threat to native aquatic biodiversity in Australia, should they become established here. Biosecurity Queensland and Tropical River Consulting carried out an electrofishing survey in two sections of the Barron River catchment (that failed to detect snakeheads) and collected water samples at six sites for eDNA analysis. Environmental DNA was extracted from water samples, purified and screened for presence of seven invasive species using species-specific quantitative PCR (qPCR) assays targeting: *C. striata*, *C. asiatica*, *C. diplogramme*, *C. melasoma*, *C. micropeltes*, *C. argus* and *C. maculata*. Additionally, samples were screened for two other exotic tilapia species known to be present in the Barron catchment – *Tilapia mariae* and *Oreochromis mossambicus* – as a positive control. We found presence of tilapia eDNA at all sites, however, no snakehead species eDNA was detected in the field samples, supporting the results of the electrofishing survey. Based on this, we conclude that it is highly unlikely that snakeheads would be present at the surveyed sites.

1. Introduction

The Australian tropics face a large number of invasive pest species that can have destabilising effects on native communities (Shine, 2012). Preventing the spread of a pest depends on successfully detecting them at the onset of an invasion and eradicating them rapidly (Hulme, 2006). Over the past ten years, environmental DNA (eDNA) has been increasingly used for detection of aquatic and semi-aquatic species of management concern in temperate and tropical areas (Cristescu & Hebert, 2018). This technique is based on the principle that all organisms shed genetic material into their environment via physiological processes (Jerde et al, 2011). Capture and extraction of DNA from environmental samples (i.e. water, soil, snow, etc.), followed by a targeted polymerase chain reaction (PCR) allows for species detection with high confidence (Ficetola, Miaud, Pompanon, & Taberlet, 2008). Consequently, eDNA detection could constitute an effective early warning system for invasive species detection (Jerde et al, 2011) or could help in the assessment of eradication programs (Rees, Maddison, Middleditch, Patmore, & Gough, 2014).

Snakehead fish, *Channa* spp., represent a major threat to Australian biodiversity (Hitchcock et al, 2012). This fish is native to southeast Asia, from India to Indonesia, and invaded southern Papua New Guinea coastal villages approximately 20 years ago (Burrows & Perna, 2009). Snakeheads are large predatory fish that can also survive without water for extended periods and disperse via land (Lee & Ng, 1994). If these exotic fish are translocated into mainland Queensland, they could spread through the Cape York Peninsula and threaten native birds, reptiles and fish through competition and predation (Hitchcock et al, 2012).

During late 2020, Biosecurity Queensland received a report on presence of snakehead in the Barron River from a recreational fisher. Biosecurity Queensland and Tropical River Consulting carried out electrofishing activities in two sections of the Barron River catchment to survey for invasive snakeheads. Additionally, water samples from six sites located between the electrofishing areas were collected for eDNA analysis. Here we report the results from eDNA sampling carried out by the Biosecurity Queensland and analysed by TropWATER.

2. Methodology

2.1 Field collection of eDNA samples

Environmental DNA kits, including materials for eDNA sample collection and a field protocol, were sent to Biosecurity Queensland prior to field collection. Field collection was carried out by Biosecurity Queensland staff using the eDNA kits and following TropWATER's eDNA collection protocol. Samples were collected at six sites in the Barron River catchment (Table 2.1, Figure 2.1). At each site, five field samples and one field blank were collected. Each field sample consisted of 300 mL of water decanted into a jar pre-loaded with 100 mL Longmire's preservative buffer (Longmire et al, 1997), making a final volume of 400 mL. The field blank consisted of decanting 300 mL of laboratory-grade water into a jar containing 100 mL Longmire's preservative buffer.

Table 2.1. Sampling sites for the direct water collection samples in the Barron River catchment, north Queensland.

Site	Collection date	Latitude	Longitude	No. samples collected
Hickory Road Bridge	16/12/2020	-16.802952°	145.589725°	5
Oak Forest Road Bridge	16/12/2020	-16.803899°	145.562478°	5
Barron River Bridge	16/12/2020	-16.815890°	145.634369°	5
Barron River adjacent Station	16/12/2020	-16.821550°	145.641310°	5
Little Road Bridge	16/12/2020	-16.800425°	145.612649°	5
River Street	18/12/2020	-16.802721°	145.580301°	5

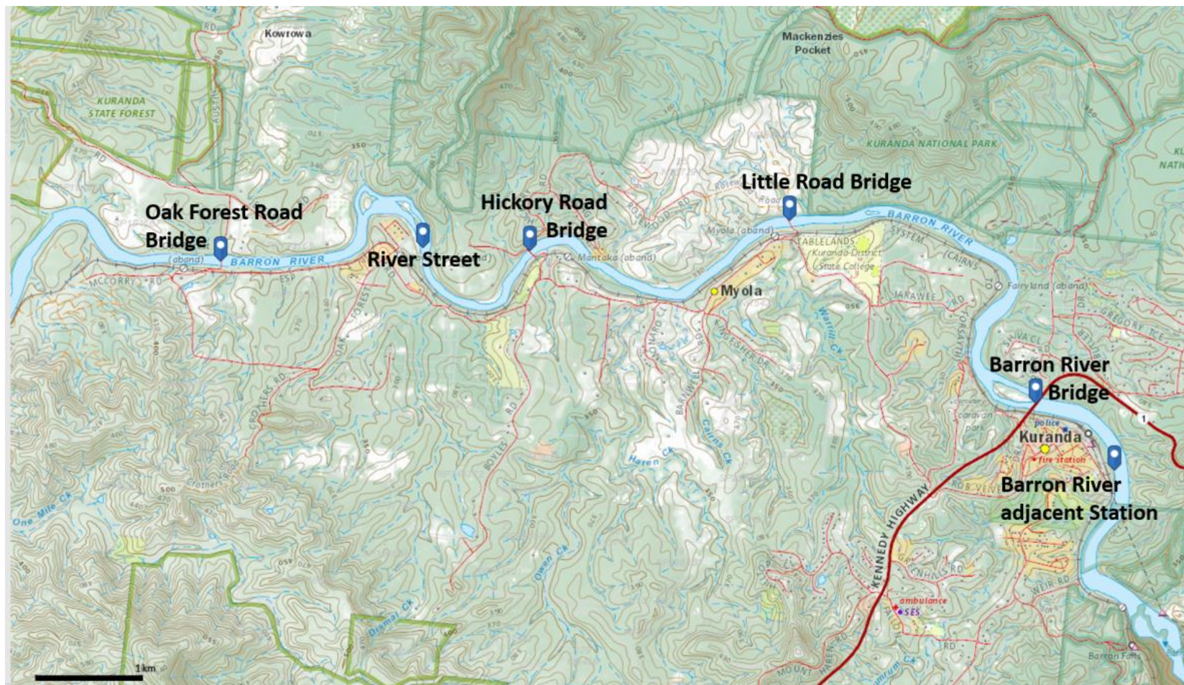

Figure 2.1. Sampling sites for the direct water collection samples in the Barron River catchment, north Queensland.

2.2 Environmental DNA extractions

Before extraction commenced, bench top surfaces and floors in a dedicated eDNA laboratory were decontaminated with 10% *v/v* bleach, as per standard operating procedure. Upon the arrival of samples to the laboratory, each individual field sample from all sites was decanted into five aliquots of 20 mL for eDNA extraction. Therefore, a total of 100 mL of each sample was screened for eDNA of the target species.

We followed a DNA precipitation method protocol described in Edmunds and Burrows 2020. Briefly, we added 20 mL isopropanol, 5 mL sodium chloride 5M and 10 µL glycogen to the 20-mL aliquots of water and Longmire's solution and incubated samples at 4°C overnight. We then centrifuged this solution (6,750 g; 10 min; 22°C), discarded the supernatant, dissolved the pellet in 120 µL lysis buffer (guanidinium hydrochloride and TritonX) and pooled all five tubes from each sample together into one single tube (total of 600 µL lysis buffer). Tubes were then frozen overnight at -20°C. Subsequently samples were thawed, vortexed and lysed for four hours at 50°C. After sample lysis we added polyethylene glycol (PEG) precipitation buffer and 5 µL glycogen and incubated the samples overnight at 4°C. Finally, samples were centrifuged (20,000 g; 30 min; 22°C), the supernatant was discarded and the pellet was washed twice with 70% ethanol before resuspending it in 100 µL elution buffer. Subsequently, a DNA purification was performed using the DNeasy PowerClean Pro Cleanup Kit (Qiagen). A negative extraction control was added to each batch of eDNA extractions to ensure that no contamination was introduced during laboratory procedures (Goldberg et al, 2016).

2.3 *Channa argus* assay design

An eDNA assay targeting a fragment of the *16S* ribosomal RNA (rRNA) gene of *Channa argus* was developed. A total of seven *C. argus 16S* rRNA sequences were downloaded from the NCBI GenBank® database using the Geneious Prime software (v. 2020.02). *Channa argus* sequences were aligned using the MUSCLE algorithm on Geneious Prime to generate a consensus sequence. Additionally, sequences of Australian fish, following Edmunds & Burrows (2019a), were downloaded and aligned to the *C. argus* consensus sequence. Aligned sequences were visually inspected and candidate forward and reverse primer regions were selected based on: (1) ≥ 4 base pair (bp) mismatches with *C. argus*; (2) ≥ 2 bp mismatches in the 3' end region of each primer; (3) melting temperature (T_m): 60 – 64˚C with < 4˚C difference between primer pair; (4) GC content: 35-65%; (5) amplicon size: 80 – 350 bp; (6) self-dimer T_m < 30°C; (7) hairpin T_m < 30°C; (8) overall self-complementarity < 8; and (9) self 3' complementarity < 8. Based on these conditions, one primer pair candidate was identified (Table 2.2), with the targeted *16S* section being: AGCGCAATCACTTGTCTTTTAAATGAAGACCCGTATGAATGGCATAACGAGGGCTTAACTGTCTCCTTTTTCAAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGAGGATAACAACATAAGACGAGAAGACCCTATGGAGCTTTAGACACCAGAGTAGCCCTTGTCAAACACCCCCAGTAAAAGGGCAAAACCAAAAGGACCCTA.

In-silico validation of the selected primer pair consisted of assessing the cross-amplification using the NCBI Primer-BLAST tool. Five species of the *Channa* genus were identified as potentially amplified by the selected primer: *C. argus*, *C. striata*, *C. micropeltes*, *C. maculata* and *C. asiatica*. Additionally, three non co-occuring fish species were amplified by the selected primer pair: *Symphodus ocellatus, Parazanclistius hutchinsi* and *Lepisosteus osseus*.

The limit of detection (LOD) of the developed assay was estimated using a 12-fold serial dilution of double-stranded synthetic DNA fragments (gBlocks™ Integrated DNA Technologies Pty Ltd, New South Wales, Australia) synthesized to match the target fragment. Dilutions ranged from 1.8 x 10^7 to 1.8 x 10^{-5} DNA copies/µL. A total of 24 technical replicates per dilution were used, and the LOD was set at the lowest standard with 95% or greater detection (Klymus et al, 2019). qPCR assays were run on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC) in white 384-well plates, sealed with optical films (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC). Each qPCR assay consisted of 3 µL of template DNA and 7 µL of master mix (5 µL PowerUp SYBR Green Master Mix; 0.5 µL forward primer at 10 µM; 0.5 µL reverse primer at 10 µM; 1 µL MilliQ® water). Thermal cycling conditions were as follows: initial denaturation and activation at 95°C for 2 min then 45 cycles of 95°C for 15 secs and 65°C for 1 min. A subsequent melt curve analysis was performed to generate dissociation curves by transitioning from 65°C to 95°C at 0.15°C sec^{-1}. Mean plate efficiency of the assays was 90.3%, mean slope -3.6 and R^2 0.99. Based on the serial dilutions, the LOD of the assay was estimated to be 1.8 DNA copies per reaction.

Finally, an *in-vitro* primer specificity was determined by attempting to amplify genomic DNA from potentially co-occurring frogs (n = 5 species), turtles (n = 6 species) and fish (n = 31 species) within Australia, following Edmunds & Burrows (2019a). All qPCR assay reactions and thermocycling conditions followed those of the LOD estimation.

Table 2.2. Primer information for C. argus *eDNA assay targeting a region of the 16S rRNA gene.*

Primer	Nucleotide sequence (5' – 3')	Length (bp)	T_m (°C)	GC content (%)	Self-complementarity	Self 3' complementarity	Amplicon size (bp)
2111F	AGCGCAATCACTTGTCTTTTAAATGAA	27	60.9	33.3	8.00	6.00	
2295R	TAGGGTCCTTTTGGTTTTGCCC	22	61.0	50.0	4.00	0.00	206

2.4 Detection of species-specific eDNA by quantitative PCR (qPCR)

All sites were screened for presence of snakeheads as well as Mozambique tilapia (*Oreochromis mossmabicus*) and spotted tilapia (*Tilapia mariae*). Detection of each species-specific eDNA by quantitative real-time PCR (qPCR) consisted of using three different qPCR assays specifically designed to detect spotted and Mozambique tilapia (Edmunds and Burrows 2019b), *C. striata*, *C. asiatica*, *C. diplogramme*, *C. melasoma*, *C. micropeltes*, *C. argus* and *C. maculata* (Edmunds & Burrows 2019a; this study).

All qPCR assays were run on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC) in white 384-well plates, sealed with optical films (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC). Environmental DNA detection of each species was carried out using four technical replicate of each sample and three no template control (NTC) samples.

Environmental DNA detection each qPCR assay consisted of 6 µL of template DNA and 14 µL of master mix (10 µL PowerUp SYBR Green Master Mix; 1 µL forward primer at 10 µM; 1 µL reverse primer at 10 µM; 2 µL MilliQ® water). Thermal cycling conditions for snakeheads detection were as follows: initial denaturation and activation at 95°C for 2 min then 45 cycles of 95°C for 15 secs and 65°C for 1 min. A subsequent melt curve analysis was performed to generate dissociation curves by transitioning from 65°C to 95°C at 0.15°C sec^{-1}. Finally, thermal cycling conditions for tilapia eDNA detection were as follows: initial denaturation and activation at 95°C for 2 min then 45 cycles of 95°C for 15 secs and 65°C for 1 min. A subsequent melt curve analysis was performed to generate dissociation curves by transitioning from 60°C to 95°C at 0.15°C sec^{-1}.

All plates were analysed with a common fluorescence threshold (0.2) using QuantStudio™ Design and Analysis Software (version 1.4.2; Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC) before export and subsequent analyses in Microsoft Excel (version 15.41). A field site was considered to be positive for each species detection if at least one of the total technical qPCR replicates for that site (4 * the number of samples for each particular site) met the following criteria: 1) amplification curve crossed florescence threshold within 40 cycles, 2) dissociation temperature within 99.7% confidence interval of each species genomic DNA standards, 3) corresponding equipment controls, field blanks, and extraction blanks were not contaminated.

2.5 Verification of positive detections

qPCR product of all positive detections were Sanger sequenced at the Australian Genome Research Facility Ltd (AGRF), Brisbane, to verify that the DNA corresponded to each of the target species.

3. Results

Presence of tilapia (*O. mossambicus* and *T. mariae*) was detected at all sampled sites, while snakeheads were not detected in any of the samples at any site (Table 3.1). Positive detections were verified by Sanger sequencing to confirm that they were true detections. All field and extraction control samples were verified to be devoid of the target species eDNA by qPCR. Therefore, all qPCR assays are accurate reflections of collected site-specific eDNA.

*Table 3.1. Summary of snakehead and tilapia eDNA detections in the Barron River catchment, north Queensland. Positive eDNA detections are indicated in **bold**.*

		Field samples		qPCR analysis	
Site	**Species**	**No. positive detections**	**% positive detections**	**No. positive detections**	**% positive detections**
Hickory Road Bridge	Tilapia	**5**	**100**	**17**	**85**
	Snakeheads	0	0	0	0
Oak Forest Road Bridge	Tilapia	**5**	**100**	**17**	**85**
	Snakeheads	0	0	0	0
Barron River Bridge	Tilapia	**5**	**100**	**20**	**100**
	Snakeheads	0	0	0	0
Barron River adjacent Station	Tilapia	**5**	**100**	**17**	**85**
	Snakeheads	0	0	0	0
Little Road Bridge	Tilapia	**5**	**100**	**20**	**100**
	Snakeheads	0	0	0	0
River Street	Tilapia	**5**	**100**	**17**	**85**
	Snakeheads	0	0	0	0

4. Discussion

Environmental DNA is a sensitive tool for monitoring species that persist at low abundances, which is the case of early incursions of invasive species (Dejean et al, 2012; Smart et al, 2015). Following a report on presence of snakehead in the Barron River during November 2020, we screened water samples collected at six sites on the Barron River catchment for presence of seven snakehead species: *C. striata*, *C. asiatica*, *C. diplogramme*, *C. melasoma*, *C. micropeltes*, *C. argus* and *C. maculata*. As a positive control, we also screened samples for presence of two tilapia species, which are known to exist in the sampling area. A high percentage of samples at all sites (85–100%) were positive for tilapia eDNA; however, there was no evidence of snakehead eDNA presence at any site.

In flowing streams, eDNA detectability not only depends on shedding rates and population abundance of the target organism, but also on physical processes such as eDNA transport, retention, resuspension and decay (Barnes, Turner, & Turner, 2016). Recent studies increasingly show that eDNA transport distance and retention in a system are highly influenced by water discharge and physical-chemical characteristics of a stream (i.e. susbtrate type) (Shogren et al., 2017; Shogren, Tank, Egan, Bolster, & Riis, 2019). Additionally, eDNA decay is mainly driven by the biotic (i.e. bacterial community) and abiotic factors (e.g., temperature, pH, UV) surrounding it (Nielsen, Johnsen, Bensasson, & Daffonchio, 2017). Studies on several taxa show that eDNA decays rapidly and can be reliably detected up to eight days after been shed (Eichmiller, Best, & Sorensen, 2016; Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016; Villacorta-Rath et al., 2020). In freshwater systems, eDNA degrades faster than in marine systems (reviewed by Collins et al., 2018) and its half-life ranges between 0.7 hours (Seymour et al., 2018) and 23.9 hours (Sansom & Sassoubre, 2017). Therefore, any positive eDNA detection found in the present study means that the target species has likely occupied the area up to one week prior to sample collection.

The eDNA technique is increasingly been recognised as a sensitive tool for detection of exotic species in recently invaded areas, with equally or higher detection efficiency than traditional techniques, including electrofishing (Blackman et al, 2020; Smart et al, 2015). This is because traditional methods are more efficient when a species is present at moderate abundances, which is not the case of an early incursion (Magnuson et al, 1994). Despite the power of eDNA over traditional methods to detect species that occur at low abundance, in some cases eDNA can also result in false negative detections (Furlan et al, 2019). Therefore, it is crucial to determine an appropriate sampling effort in order to get reliable eDNA results (Furlan et al, 2019). In the present study, we processed 100 mL of water from five replicate samples at each site (total of 500 mL at each site) along the Barron River catchment. We have previously determined that precipitating eDNA from 100 mL of water can provide high resolution for species detection in a running stream in tropical Australia (Villacorta-Rath, Hoskin, Strugnell, & Burrows, in press). By using a rainforest frog as a model species, we determined that replicate 100 mL water samples have enough power to detect the target species eDNA 22 km downstream from the lower limit of the population’s distribution (Villacorta-Rath et al., in press). The sampling sites covered in the present study were on average 2.5 km apart and extending over an approximate 12 km stretch of river, increasing the chances of encountering snakehead eDNA if the species was present within the survey area. Data on snakehead movement and seasonal patterns are limited, however

one study focusing on *C. argus* found that although most fish exhibit average home ranges of 1.2 km^2, some individuals can disperse over up to 18 km (Lapointe et al, 2013).

Therefore, we believe that the amount of water processed for the present study, the number of replicate samples collected at each site, the number of sites surveyed and the distance between them provide strong resolution to detect the target species eDNA if present at the sampling area. Additionally, the electrofishing survey failed to detect any snakeheads at the two river sections covered. Based on this, we can conclude that there is no snakehead presence at the sampling sites.

References

Barnes, M. A., Turner, C. R., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics, 17(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Blackman, R. C., Ling, K. K. S., Harper, L. R., Shum, P., Hänfling, B., & Lawson-Handley, L. (2020). Environmental DNA consistently outperforms established methods for detection of quagga mussels, Dreissena rostriformis bugensis in flowing water. BioRxiv.

Burrows, D., & Perna, C. (2009). A survey for exotic freshwater fish on Saibai Island and Thursday Island, Torres Strait. Retrieved from https://www.tropwater.com/publications/technical-reports/

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. Communications Biology, 1(1), 1–11. https://doi.org/10.1038/s42003-018-0192-6

Cristescu, M. E., & Hebert, P. D. N. (2018). Uses and misuses of environmental DNA in biodiversity science and conservation. Annual Review of Ecology, Evolution, and Systematics, 49(1), 209–230. https://doi.org/10.1146/annurev-ecolsys-110617-062306

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus. Journal of Applied Ecology, 49, 953–959. https://doi.org/10.1111/j.1365-2664.2012.02171.x

Edmunds, R. C., & Burrows, D. W. (2019). Development of revised eDNA assay for tilapia (Oreochromis mossambicus and Tilapia mariae): Technical Report. (07/19), 32. Retrieved from https://www.tropwater.com/publications/technical-reports/

Edmunds, R. C, & Burrows, D. (2019). Development of eDNA assay for invasive Channa striata (snakehead murrel). Retrieved from https://www.tropwater.com/publications/technical-reports/

Edmunds, Richard C., & Burrows, D. W. (2020). Got glycogen?: Development and multi-species validation of the novel Preserve, Precipitate, Lyse, Precipitate, Purify (PPLPP) workflow for environmental DNA extraction from Longmire's preserved water samples. Journal of Biomolecular Techniques, 31(4), 125–150. https://doi.org/10.7171/jbt.20-3104-003.

Eichmiller, J. J., Best, S. E., & Sorensen, P. W. (2016). Effects of temperature and trophic state on degradation of environmental DNA in lake water. Environmental Science and Technology, 50(4), 1859–1867. https://doi.org/10.1021/acs.est.5b05672

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. Biology Letters, 4(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Furlan, E. M., Gleeson, D., Wisniewski, C., Yick, J., & Duncan, R. P. (2019). eDNA surveys to detect species at very low densities: A case study of European carp eradication in

Tasmania, Australia. Journal of Applied Ecology, 56(11), 2505–2517. https://doi.org/10.1111/1365-2664.13485

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … TABERLET, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution, 7(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Hitchcock, G., Finn, M. A., Burrows, D. W., & Johnson, J. W. (2012). Fishes from fresh and brackish waters of islands in Torres Strait, far north Queensland. Memoirs of the Queensland Museum, 56(1), 13–24.

Hulme, P. E. (2006). Beyond control: Wider implications for the management of biological invasions. Journal of Applied Ecology, 43(5), 835–847. https://doi.org/10.1111/j.1365-2664.2006.01227.x

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters, 4(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Lapointe, N. W. R., Odenkirk, J. S., & Angermeier, P. L. (2013). Seasonal movement, dispersal, and home range of Northern Snakehead Channa argus (Actinopterygii, Perciformes) in the Potomac River catchment. Hydrobiologia, 709(1), 73–87. https://doi.org/10.1007/s10750-012-1437-x

Lee, P. G., & Ng, P. K. L. (1994). The systematics and ecology of snakeheads (Pisces: Channidae) in Peninsular Malaysia and Singapore. Hydrobiologia, 285(1–3), 59–74. https://doi.org/10.1007/BF00005654

Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). Use of "lysis buffer" in DNA isolation and its implication for museum collections. https://doi.org/10.5962/bhl.title.143318

Magnuson, J. J., Benson, B. J., & Mclain, A. S. (1994). Insights on species richness and turnover from long-term ecological research: Fishes in north temperate lakes. Integrative and Comparative Biology, 34(3), 437–451. https://doi.org/10.1093/icb/34.3.437

Nielsen, K. M., Johnsen, P. J., Bensasson, D., & Daffonchio, D. (2017). Release and persistence of extracellular DNA in the environment. Environmental Biosafety Research, 6(2007), 37–53.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. Journal of Applied Ecology, 51(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Sansom, B. J., & Sassoubre, L. M. (2017). Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. Environmental Science and Technology, 51(24), 14244–14253. https://doi.org/10.1021/acs.est.7b05199

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. Environmental Science and Technology, 50(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., … Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. Communications Biology, 1(1), 1–8. https://doi.org/10.1038/s42003-017-0005-3

Shine, R. (2012). Invasive species as drivers of evolutionary change: Cane toads in tropical Australia. Evolutionary Applications, 5(2), 107–116. https://doi.org/10.1111/j.1752-4571.2011.00201.x

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. Scientific Reports, 7(1), 1–11. https://doi.org/10.1038/s41598-017-05223-1

Shogren, A. J., Tank, J. L., Egan, S. P., Bolster, D., & Riis, T. (2019). Riverine distribution of mussel environmental DNA reflects a balance among density, transport, and removal processes. Freshwater Biology, 64(8), 1467–1479. https://doi.org/10.1111/fwb.13319

Smart, A. S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., & McCarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. Ecological Applications, 25(7), 1944–1952. https://doi.org/10.1890/14-1751.1

Smart, Adam S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., & McCarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. Ecological Applications, 25(7), 1944–1952. https://doi.org/10.1890/14-1751.1

Villacorta-Rath, C., Adekunle, A., Edmunds, R. C., Strugnell, J. M., Schwarzkopf, L., & Burrows, D. (2020). Can environmental DNA be used to detect first arrivals of the cane toad, Rhinella marina, into novel locations? Environmental DNA, 2(May), 635–646. https://doi.org/10.1002/edn3.114

Villacorta-Rath, C., Hoskin, C. J., Strugnell, J. M., & Burrows, D. W. (2021). Long distance (> 20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. PeerJ.