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Environmental DNA survey of Torres Strait Islands for invasive species – 2022 sampling campaign

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Executive Summary

The Torres Strait Regional Authority (TSRA) engaged the Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) to conduct environmental DNA (eDNA) analysis of water samples collected at Saibai, Mua, Kirriri and Muralag Islands during the 2022 sampling campaign. One or two freshwater bodies (sites) were sampled on each island. At each site, five 30 mL water samples and one field blank were collected. Environmental DNA from these water samples was extracted, purified, and analysed at our TropWATER laboratory to screened for the presence of invasive species, using species-specific quantitative PCR (qPCR) assays. The species targeted on each island were: Kirriri Island and Muralag Island – cane toad (*Rhinella marina*); Mua Island – cane toad and snakehead fish (*Channa striata*); Saibai Island – cane toad, snakehead and tilapia (*Oreochromis mossambicus* and *Tilapia mariae*). No presence of eDNA from any of the invasive species assessed was detected at any of the sites and islands surveyed in the 2022 sampling campaign.

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1. INTRODUCTION

The Torres Strait Islands are an archipelago situated between the Cape York Peninsula, far north Queensland, and central-southern New Guinea. Their geographical location, in particular the northern islands of Boigu and Saibai, makes the archipelago a likely pathway of entry into Australia for invasive freshwater fish from Papua New Guinea (PNG) (Waltham, Burrows, and Schaffer 2014), (Burrows and Perna 2009). Invasive species are a threat to native freshwater fish populations and habitat (Hitchcock et al. 2012).

The habitat of Saibai and Boigu is mainly swamplands with intermittent streams, which is suitable habitat for a range of invasive pest fishes (Hitchcock et al. 2012). Snakehead fish (*Channa striata*) is a fish species exotic to the Torres Strait, that has a high potential for invading the northern Torres Strait Islands. This fish is native to southeast Asia, from India to Indonesia, and has invaded southern PNG coastal villages approximately 10 years ago (Burrows and Perna 2009). Snakeheads are large predatory fish that can survive without water for extended periods and disperse via land (Lee and Ng 1994). Furthermore, if this exotic pest fish is translocated to mainland Queensland, it may spread through the Cape York Peninsula , threatening native bird, reptile and fish species through competition and predation (Hitchcock et al. 2012).

Several tilapia species are already established in coastal PNG, and Saibai Island authorities have been concerned about the spread of these fish (Waltham, Burrows, and Schaffer 2014). In August 2018, one individual fish, reported to be tilapia, was caught on cast net in Malil Pupu, Saibai Island, a water body of approximately 250 m by 300 m in size. However, this specimen was not retained, and no photos were taken to confirm identification. Furthermore, a specimen caught on Saibai Island this year (2022), has recently been confirmed as tilapia via sequencing of retained tissue sample. Two species of tilapia are now well established in Queensland: Mozambique tilapia, *Oreochromis mossambicus*, are found in 20 of the 76 catchments in Queensland (Webb 2007; Russell, Thuesen, and Thomson 2012). The spotted tilapia, *Tilapia mariae*, have an established population in the Walsh River, Gulf of Carpentaria, from which they are expected to spread much further in coming years. Both species are steadily expanding their range, largely due to human assistance and close monitoring is required to assess their spread (Edmunds and Burrows 2019a). Tilapia are a threat to native fish due to competitive displacement, predation and habitat alteration, and pose a threat as vectors for diseases and parasites (Russell, Thuesen, and Thomson 2012). Recently, during the 2020 eDNA sampling campaign, Mozambique tilapia eDNA was detected at the northern Torres Strait Island, Saibai Island (Villacorta-Rath 2021). As such, it is important that monitoring of other northern islands, such as Boigu Island, to determine whether this species is also present there.

Conversely, the central and southern Torres Strait Islands are exposed to invasive species through their close connection with mainland Queensland. One of the main threats to native biodiversity in the southern islands is the cane toad, *Rhinella marina*. The cane toad's ability to adapt to new environments due to their wide temperature tolerance range (Zug and Zug 1979) and high toxicity, hence low predation, has enabled them

to progressively spread across northern Australia (Tingley et al., 2017). The Threat Abatement Plan (Arnhem and Kiessling 2011) identified four Torres Strait Islands with no records of cane toads, but where the native populations of reptiles and birds can be highly impacted if an invasion occurred: Badu, Boigu, Horn and Muralag islands. Currently, cane toads are found on Thursday and Horn islands, where eradication and awareness programs are currently in place to mitigate the spread of this pest (tsra.gov.au).

Given the large number of freshwater bodies on the Torres Strait Islands and the vast number of exotic species to survey, additional methods supplementing the traditional methods of netting, trapping and electrofishing should be implemented. Detection of environmental DNA (eDNA), DNA released into the environment through shedding of skin cells, mucous, faeces, etc. in water samples, is a sensitive survey method that can be used in addition to traditional survey methods to provide an extensive screen for species of interest (Jerde et al. 2011). The eDNA survey approach has been used successfully for the detection of invasive tilapia (Noble et al. 2014; Robson et al. 2016; LePort, Jerry, and Burrows 2016) and cane toads (Tingley et al. 2019; Villacorta-Rath et al. 2020). Environmental DNA monitoring can provide a sensitive early warning tool for invasive species and inform the success of eradication programs.

The objective of the 2022 eDNA-based survey campaign was to assess six islands in the Torres Strait (Boigu, Badu, Moa, Kirriri, Saibai and Muralag Islands) for presence of cane toad, snakehead and tilapia eDNA. Only four islands, however, were able to be surveyed during the 2022 sampling campaign (Moa, Kirriri, Saibai and Muralag Islands). Here we report the results from eDNA sampling carried out by the Torres Strait Regional Authority (TSRA) and analysed by TropWATER, James Cook University, Townsville.

2. METHODS

2.1 Field collection of eDNA samples

Environmental DNA sampling kits were sent to the TSRA prior to their field collection. All tubes, solutions and materials for collecting field water samples for eDNA analysis are supplied in the eDNA kit, along with a field collection protocol. Field collection was carried out by the TSRA Indigenous rangers, who followed TropWATER's eDNA collection protocol, using the eDNA kits supplied. Water samples were collected from one to three different sites on each island (Table 1). At each site, five field water samples and one field blank were collected. For each field sample, 30 mL of water was collected and transferred into a tube containing 10 mL of preservative solution, making a final volume of 40 mL per field sample. A field blank was collected by transferring 30 mL of laboratory-grade water into a tube containing 10 mL preservative solution.

Table 1 Sampling site and location for the direct water collection of samples in the northern, central, and southern Torres Strait Islands.

Location	Site	Latitude (°S)	Longitude (°E)	Collection date
Kirriri Island	Francis Well 2	10.557780	142.218869	24/04/2022
	Oval Well	10.558476	142.219695	24/04/2022
	Gela Well	10.558476	142.219695	24/04/2022
Muralag Island	Keenan Well	10.599010	142.203877	24/04/2022
Moa Island	Yardi	10.195267	142.330871	31/05/2022
	Airport Dam	10.228737	142.223086	31/05/2022
Saibai Island	Buthu May	9.381862	142.612544	31/05/2022
	Mag	9.393040	142.634922	31/05/2022

2.2 Environmental DNA extractions

Prior to laboratory extraction of eDNA, bench top surfaces and floors in a dedicated eDNA laboratory were decontaminated with 10% v/v bleach, as per standard operating procedure. We followed a DNA precipitation method protocol described in Villacorta-Rath et al. (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 (3,270 g; 90 min; 22°C), discarded the supernatant, dissolved the pellet in 600 µL lysis buffer (guanidinium hydrochloride and TritonX) and froze the samples overnight. 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. Samples were then centrifuged (20,000 g; 30 min; 22°C), the supernatant discarded, and the pellet washed twice with 70% ethanol before resuspending in 100 µL elution buffer. DNA purification was subsequently 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 Inhibition tests

We tested inhibition in water samples by spiking 80 copies of double-stranded synthetic DNA fragments (gBlocks™ Integrated DNA Technologies Pty Ltd, New South Wales, Australia) of a rainforest frog species (*Litoria dayi*) that does not occur on the Torres Strait Islands, in duplicate qPCR replicates from all field samples collected at each site. Additionally, we spiked the same number of DNA copies into three technical replicates containing only laboratory-grade water. A sample was considered inhibited if it exhibited a Delta Ct (ΔCt) of

3 (i.e. a shift in Ct values of 3 or more cycles) when comparing the spiked field samples to the spiked laboratory-grade water (Cao et al. 2012).

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

Kirriri and Muralag islands were screened for presence of cane toad eDNA only. Moa and Badu islands were screened for presence of both cane toad and snakehead fish eDNA, and Saibai and Boigu islands were screened for presence of cane toad, snakehead, spotted tilapia and Mozambique tilapia eDNA. Detection of each species-specific eDNA by quantitative real-time PCR (qPCR) consisted on using three different qPCR assays specifically designed to detect cane toads (Edmunds and Burrows 2019b), spotted and Mozambique tilapia (Edmunds and Burrows 2019a), and snakehead fish (Edmunds and Burrows 2019c).

All qPCR assays were run on a QuantStudio™ 3 or a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC) in white 96-well or 384-well plates, respectively, and sealed with optical films (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC). Quantitative real-time PCR of eDNA for each species from Saibai Island and Boigu Island was carried out using four qPCR replicates of each field sample, whereas eight qPCR replicates of each field sample for each species were carried out for all other islands. The difference in the number of qPCR replicates used between islands was due to the requirement for screening a greater number of target species at Saibai and Boigu islands. All qPCR plates contained triplicate no-template control (NTC) replicates, as well as triplicate positive control replicates consisting of double-stranded synthetic DNA fragments (qBlocks^{TM} Integrated DNA Technologies Pty Ltd, New South Wales, Australia).

For cane toad and snakehead eDNA detection, each qPCR assay contained 6 µL of template DNA and 14 µL of master mix [10 µL PowerUp SYBR Green Master Mix (Thermo Fisher Scientific Australia Pty Ltd, Scoresby VIC); 1 µL forward primer (5 µM for cane toad; 10 µM for snakehead); 1 µL reverse primer (5 µM for cane toad; 10 µM for snakehead); 2 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}.

For tilapia species eDNA 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 (10 µM); 1 µL reverse primer (10 µM); 3 µL MilliQ® water). Thermal cycling conditions for the tilapia assay 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), exported and subsequent

analysed in Microsoft Excel (version 15.41). A field site was considered positive for a species detection if at least one of the eight (or four) qPCR replicates for that 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 putative positive detections

qPCR products 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

Confirmation Sanger sequencing detected no positives for any of the target species, at any of the sites sampled in the 2022 sampling campaign (Table 2).

Additionally, no field sample was considered inhibited when analysed by qPCR. Slight inhibition was observed in some samples ($3 < \Delta Ct < 6$) (Table 3). Strong inhibition was observed in three field samples from Kirriri Island and Moa Island sites ($\Delta Ct > 10$). These field samples were diluted (1:10) prior to running the qPCR to reduce inhibition. Finally, all field and extraction control samples were verified to be devoid of the target species eDNA by qPCR. Therefore, all qPCR assays were accurate reflections of collected site-specific eDNA.

Table 2 Summary of cane toad, tilapia and snakehead eDNA detections for the 2022 sampling campaign.

Location	Site	Species	# Field samples	Field Samples # Positive detections	% Positive detections	# Technical replicates	qPCR analysis # Positive detections	% Positive detections
Kirriri Island	Francis Well2	Cane toad	5	0	0	40	0	0
	Oval Well	Cane toad	5	0	0	40	0	0
	Gela Well	Cane toad	5	0	0	40	0	0
Muralag Island	Keenan Well	Cane toad	5	0	0	40	0	0
Moa Island	Yardi	Cane toad	5	0	0	40	0	0
		Snakehead	5	0	0	40	0	0
	Airport Dam	Cane toad	5	0	0	40	0	0
		Snakehead	5	0	0	40	0	0
Saibai Island	Buthu May	Cane toad	5	0	0	20	0	0
		Snakehead	5	0	0	20	0	0
		Spotted tilapia	5	0	0	20	0	0
		Mozambique tilapia	5	0	0	20	0	0
	Mag	Cane toad	5	0	0	20	0	0
		Snakehead	5	0	0	20	0	0
		Spotted tilapia	5	0	0	20	0	0
		Mozambique tilapia	5	0	0	20	0	0

Table 3 Variation in cycle amplification (ΔCt) between spiked field samples and spiked water samples - samples in bold indicate presence of inhibition.

Location	Sample Name	Δ Ct
Kirriri	Francis Well Well2 Control	3.010
	Francis Well_Rep1	**3.798**
	Francis Well_Rep2	2.295
	Francis Well_Rep3	**3.828**
	Francis Well_Rep4	2.402
	Francis Well_Rep5	1.363
	Gela Well Well Control	1.234
	Gela Well_Rep1	**3.576**
	Gela Well_Rep2	**13.297**
	Gela Well_Rep3	**5.978**
	Gela Well_Rep4	**3.280**
	Gela Well_Rep5	**3.860**
	Oval Well_Control	0.711
	Oval Well_Rep1	2.479
	Oval Well_Rep2	1.179
	Oval Well_Rep3	1.487
	Oval Well_Rep4	**3.493**
	Oval Well_Rep5	1.979
Moa	**Airport Dam_Control**	**28.775**
	Airport Dam_Rep1	**4.991**
	Airport Dam_Rep2	2.910
	Airport Dam_Rep3	5.508
	Airport Dam_Rep4	0.000
	Airport Dam_Rep5	0.000
	Yardi_Control	**33.399**
	Yardi_Rep1	**4.125**
	Yardi_Rep2	2.254
	Yardi_Rep3	**3.490**
	Yardi_Rep4	2.653
	Yardi_Rep5	2.105
Muralag	Keenan Well_Control	0.586
	Keenan Well_Rep1	**3.420**
	Keenan Well_Rep2	2.589
	Keenan Well_Rep3	2.977
	Keenan Well_Rep4	1.511
	Keenan Well_Rep5	1.859
Saibai	Buthu May_Control	0.349288
	Buthu May_Rep1	0.71105
	Buthu May_Rep2	0.630463
	Buthu May_Rep3	0.880549
	Buthu May_Rep4	0.590367
	Buthu May_Rep5	0.508897
	Mag_Control	0.29804
	Mag_Rep1	0.410267
	Mag_Rep2	0.463079
	Mag_Rep3	0.408158
	Mag_Rep4	0.463935
	Mag_Rep4	0.463935
	Mag_Rep5	0.347914

4 DISCUSSION

Environmental DNA is a sensitive tool for monitoring species that persist at low abundances, as in early incursions of invasive species (Dejean et al. 2012; Smart et al. 2015). In the present study we screened water samples collected at Saibai Island for the presence of one invasive amphibian (cane toads), and three invasive fish species (Mozambique tilapia, spotted tilapia and snakehead). None of the target species eDNA was detected at any of the surveyed islands. This may indicate that the current mitigation programs are successful, or that the sampling sites selected were not representative of where these invasive species were present.

A study on the detection threshold of cane toad eDNA in water suggested that one toad is able to be detected after coming in contact with a waterbody for as short as five minutes (Villacorta-Rath et al. 2020). Further, cane toad eDNA degrades in water within four days after it was shed (Villacorta-Rath et al. 2020). Therefore, negative detections for cane toad eDNA may infer that no cane toads were near the water bodies sampled, withing four days prior to sampling, but not that there are no cane toads on the Island.

Mozambique tilapia eDNA was recently detected at Saibai Island, during the 2020 eDNA sampling campaign (Villacorta-Rath 2021). Furthermore, a specimen confirmed to be tilapia by James Cook University, was caught on Saibai Island in 2022 (Waltham, N. *pers. com.*). This specimen was not captured at one of the sites sampled in this current survey. Consequently, broadening the survey area and increasing the number of sites surveyed for tilapia eDNA may give a clearer picture of current incursions. It is also recommended that regular eDNA sampling surveys continue, in particular on northern Torres Strait islands, to enable early detection, and manage existing incursions of tilapia and other invasive species.

5 REFERENCES

Arnhem, C, and Ntdi Kiessling. 2011. “Threat Abatement Plan.” *Department of the Environment and Heritage*. http://cutlass.deh.gov.au/biodiversity/threatened/publications/tap/pubs/marine-debris-background-paper.rtf.

Burrows, Damien, and C. Perna. 2009. “A Survey for Exotic Freshwater Fish on Saibai Island and Thursday Island, Torres Strait.” Townsville. https://www.tropwater.com/publications/technical-reports/.

Cao, Y., J. F. Griffith, S. Dorevitch, and S. B. Weisberg. 2012. “Effectiveness of QPCR Permutations, Internal Controls and Dilution as Means for Minimizing the Impact of Inhibition While Measuring Enterococcus in Environmental Waters.” *Journal of Applied Microbiology* 113 (1): 66–75. https://doi.org/10.1111/j.1365-2672.2012.05305.x.

Dejean, Tony, Alice Valentini, Christian Miquel, Pierre Taberlet, Eva Bellemain, and Claude Miaud. 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: 953–59. https://doi.org/10.1111/j.1365-2664.2012.02171.x.

Edmunds, R. C., and Damien W. Burrows. 2019a. “Development of Revised eDNA Assay for Tilapia (*Oreochromis mossambicus* and *Tilapia mariae*): Technical Report,” no. 07/19: 32. https://www.tropwater.com/publications/technical-reports/.

Edmunds, R. C., and Damien W. Burrows. 2019b. “Development of *Rhinella marina* (Cane Toad) eDNA Assay.” Townsville. https://www.tropwater.com/publications/technical-reports/.

Edmunds, R. C, and Damien Burrows. 2019c. “Development of eDNA Assay for Invasive *Channa striata* (Snakehead Murrel).” Townsville. https://www.tropwater.com/publications/technical-reports/.

Goldberg, Caren S., Cameron R. Turner, Kristy Deiner, Katy E. Klymus, Philip Francis Thomsen, Melanie A. Murphy, Stephen F. Spear, et al. 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, Garrick, Marcus A. Finn, Damien W. Burrows, and Jeffrey W. Johnson. 2012. “Fishes from Fresh and Brackish Waters of Islands in Torres Strait, Far North Queensland.” *Memoirs of the Queensland Museum* 56 (1): 13–24.

Jerde, Christopher L., Andrew R. Mahon, W. Lindsay Chadderton, and David M. Lodge. 2011. “‘Sight-Unseen’ Detection of Rare Aquatic Species Using Environmental DNA.” *Conservation Letters* 4 (2): 150–57. https://doi.org/10.1111/j.1755-263X.2010.00158.x.

Lee, P. G., and Peter K.L. Ng. 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.

LePort, A., Dean R. Jerry, and Damien Burrows. 2016. “Environmental DNA (eDNA) Survey of Tilapia in Central Queensland: May and October 2016.” Townsville. https://www.tropwater.com/publications/technical-reports/.

Noble, Tansyn H., Heather L.A. Robson, Richard J. Saunders, and Dean R. Jerry. 2014. “The Utility of

eDNA as a Tilapia Surveillance Tool." Townsville.

Robson, Heather L.A., Tansyn H. Noble, Richard J. Saunders, Simon K. Robson, Damien W. Burrows, and Dean R. Jerry. 2016. "Fine-tuning for the Tropics: Application of eDNA Technology for Invasive Fish Detection in Tropical Freshwater Ecosystems." *Molecular Ecology Resources* 16 (4): 922–32.

Russell, D. J., P. A. Thuesen, and F. E. Thomson. 2012. "A Review of the Biology, Ecology, Distribution and Control of Mozambique Tilapia, *Oreochromis mossambicus* (Peters 1852) (Pisces: Cichlidae) with Particular Emphasis on Invasive Australian Populations." *Reviews in Fish Biology and Fisheries* 22 (3): 533–54. https://doi.org/10.1007/s11160-011-9249-z.

Smart, A. S., R. Tingley, A. R. Weeks, A. R. Van Rooyen, and M. A. McCarthy. 2015. "Environmental DNA Sampling Is More Sensitive than a Traditional Survey Technique for Detecting an Aquatic Invader." *Ecological Applications* 25 (7): 1944–52. https://doi.org/10.1890/14-1751.1.

Tingley, R., M. Greenlees, S. Oertel, A. R. van Rooyen, and A. R. Weeks. 2019. "Environmental DNA Sampling as a Surveillance Tool for Cane Toad *Rhinella marina* Introductions on Offshore Islands." *Biological Invasions* 21 (1): 1–6. https://doi.org/10.1007/s10530-018-1810-4.

Tingley, Reid, G. Ward-Fear, Lin Schwarzkopf, M. Greenlees, B. L. Phillips, G. Brown, S. Clulow, et al. 2017. "New Weapons in the Toad Toolkit: A Revie of Methods to Control and Mitigate the Biodiversity Impacts on Invasive Ane Toads (*Rhinella marina*)." *The Quarterlysa Review of Biology* 92 (2): 123–49.

Villacorta-Rath, Cecilia. 2021. "Environmental DNA Survey of the Torres Strait Islands for Four Invasive Species: *Rhinella marina* (Cane Toad), *Oreochromis mossambicus* (Mozambique Tilapia), *Tilapia mariae* (Spotted Tilapia), and *Channa striata* (Snakehead)." Townsville.

Villacorta-Rath, Cecilia, Adeshina I. Adekunle, Richard C. Edmunds, Jan M. Strugnell, Lin Schwarzkopf, and Damien Burrows. 2020. "Can Environmental DNA Be Used to Detect First Arrivals of the Cane Toad, *Rhinella marina* , into Novel Locations?" *Environmental DNA*, June, edn3.114. https://doi.org/10.1002/edn3.114.

Waltham, Nathan, Damien Burrows, and Jason Schaffer. 2014. "Freshwater Pest Fish on Boigu, Saibai, Badu and Mabuiag Islands in the Torres Straits (June 2014 Survey)." https://www.tropwater.com/publications/technical-reports/.

Webb, A. C. 2007. "Status of Non-Native Freshwater Fishes in Tropical Northern Queensland, Including Establishment Success, Rates of Spread, Range and Introduction Pathways." *Journal and Proceedings of the Royal Society of New South Wales* 140: 63–78.

Zug, George R, and Patricia B Zug. 1979. "A Natural History Resume of Native Populations." *Smithsonian Contributions Press* Smithsonia (284): 1–58.