Northern Australia Environmental Resources Hub

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Tilapia eDNA survey along the Walsh, Mitchell and Wild river catchments

Report

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Cover photographs

Front cover: eDNA sampling at Leafgold Weir (photo Brendan Ebner).

Back cover: Tinaroo irrigation channel adjacent to the Mareeba Wetlands (photo Cecilia Villacorta Rath).

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Acronyms and abbreviations

eDNA ..... environmental DNA

LOD..... limit of detection

NESP ..... National Environmental Science Program

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

TropWATER. Centre for Tropical Water and Aquatic Ecosystem Research

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Executive summary

Tilapia is an invasive fish species that has rapidly spread across river catchments in Queensland. We conducted eDNA sampling at three river catchments in north Queensland: the Walsh and Mitchell rivers in the Mitchell River catchment and the Wild River in the Herbert River catchment, as well as at the Tinaroo Dam and an associated irrigation channel. Within each catchment, we sampled between five and 14 sites and at each site, we collected five replicate water samples of 30 mL volume. Environmental DNA was extracted, purified and screened for presence of two tilapia species using one species-specific quantitative PCR (qPCR) assay: Mozambique tilapia (*Oreochromis mossambicus*) and spotted tilapia (*Tilapia mariae*). We found presence of tilapia eDNA at most sites of known occurrence, except Mutchilba. We also found presence of tilapia eDNA at Mareeba Wetlands, constituting the first record of species presence there. Based on the results, we provide recommendations for the use of eDNA surveys to map the distribution of the species in north Queensland catchments as well as an early warning tool at sites that are currently at risk of invasion.

1. Introduction

Mozambique tilapia (*Oreochromis mossambicus*) and the spotted tilapia (*Tilapia mariae*) are exotic pest species in Australia and declared as noxious fish in Queensland by the Biosecurity Act 2014. These fish are efficient colonisers that can easily adapt to a wide range of environments and exhibit reproductive strategies that ensure the survival of recruits (Russell, Thuesen, & Thomson, 2012a, 2012b). This, added to their aggressive and competitive behaviour, can result in a significant alteration of native fish communities in newly invaded systems (Canonico, Arthington, Mccrary, & Thieme, 2005).

Mozambique tilapia was imported into Australia during the 1970s through the aquarium trade industry and since then, it has spread and become established in south-east Queensland, north Queensland, New South Wales and the Pilbara drainage of Western Australia (Thuesen et al., 2011). The first report of this species in north Queensland was at ornamental ponds in the Cairns region. During the late 1980s to early 1990s, most of the nearby Barron River catchment and adjacent creeks, as well as Tinaroo Dam (on the Barron River), had established populations of Mozambique tilapia (Russell et al., 2012a). Later, in the early 2000s, the species was detected in the Wild River weirs near Herberton (Hogan & Vallance, 2004), the Burdekin River catchment (Veitch, Burrows, & Webb, 2006), Eureka Creek on the Walsh River catchment (Pearce, 2009; Pearce, Perna, & Hedge, 2009) and the Endeavour River in Cooktown (Webb, 2007). Of those locations, an eradication plan was put in place at Eureka Creek, due to the potential for that introduction to spread throughout the Gulf of Carpentaria rivers. Following application of rotenone (a chemical lethal to fish) to a 5km length of Eureka Creek in 2009, the species was considered possibly eradicated from Eureka Creek (Russell et al., 2012a).

The spotted tilapia was originally found in north Queensland (Cairns and Barron catchment) and Victoria in the 1990s, although the exact source and time of the introduction is unknown (Greiner & Gregg, 2008). This species was reported from the Walsh River catchment in 2017. The same year, in response to that report, an eDNA survey at Eureka Creek detected the presence of spotted tilapia at the same public campground that had been the centre of the rotenone control treatment in 2009 (Edmunds, Cooper, Huerlimann, Robson, & Burrows, 2019). The spread of both tilapia species has mostly been human-mediated; however, dispersal through the Tinaroo Dam irrigation channel into downstream locations could have been possible. A fish screen was installed along the irrigation channel in 2008 to prevent fish movement through the irrigation channel system, although tilapia are sometimes found in the irrigation channels downstream of this screen, possibly introduced directly into the channels.

The extensive river systems of the Gulf of Carpentaria have for many years been at high risk of tilapia invasion (Russell et al., 2012a), given the abundance of both tilapia species in the adjacent Barron River catchment and the irrigation channels that connect Tinaroo Dam and the Barron River catchment, with the Walsh River and Eureka Creek (both part of the Mitchell catchment in the Gulf of Carpentaria). The 2009 rotenone treatment (Russell et al., 2012a) appeared to eradicte the then recently established tilapia population (mostly Mozambique tilapia although a single spotted tilapia as also caught) but in more recent years, there have been reported and now confirmed sightings of tilapia in the same location.

Extensive tilapia infestations require removing fish via different methods as well as targeted education programs to stop human-mediated translocation (Russell et al., 2012a). It is known

that invasive fish eradication is often difficult to achieve, especially in open systems (Russell et al., 2012a). Prevention of tilapia spread into new systems would be more efficient in order to preserve the aquatic biodiversity (Canonico et al., 2005). Although frequent monitoring in areas at high risk of new tilapia invasions is needed, such programs are often too costly and impossible to persist through time, especially in remote areas of northern Australia. Environmental DNA (eDNA), the DNA released into the environment via mucous, faeces, skin cells, etc., and that can be isolated from water samples and screened for species of interest (Jerde, Mahon, Chadderton, & Lodge, 2011) is an excellent additional survey method. The eDNA-based approach has been used successfully for the detection of invasive tilapia (Edmunds et al., 2019; Robson et al., 2016; Villacorta-Rath & Burrows, 2020; Villacorta-Rath, Edmunds, & Burrows, 2019) in northern Australia.

In the present study, we surveyed several sites along three catchments through eDNA analysis: the Walsh, Mitchell and Wild rivers. We also included Tinaroo Dam as a positive control system known to harbour a large tilapia population, and sampled multiple sites along the Tinaroo irrigation channel in order to determine whether the water coming into the Walsh and Mitchell rivers contained tilapia eDNA transported from the dam. Mapping the tilapia distribution along these three northern Australia river catchments would help inform which tilapia-free areas are at higher risk of invasion.

2. Methodology

2.1 eDNA sampling

Water samples for eDNA analysis were directly collected and preserved from 34 sites along the Walsh, Mitchell and Wild river catcments, as well as in Tinaroo Dam and its associated irrigation channels before they dropped into the Walsh River and Mareeba Wetlands, which are considered important downstream ecological receiving environments. Sampling occurred during July and October 2019 (Table 2.1, Figure 2.1). At each site, five replicate 30 mL samples were collected using a clean falcon tube of 50 mL capacity and decanting into another falcon tube containing 10 mL of Longmire's preservative solution. At every site, a field blank was also taken to ensure that the process of sample collection did not introduce contamination. The field blank consisted of decanting 30 mL of laboratory-grade water into a falcon tube containing 10 mL of preservative solution.

2.2 Environmental DNA extractions

eDNA extractions were carried out at the dedicated eDNA laboratory at the Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville. Prior to eDNA extraction, bench top surfaces and the floor were decontaminated with 10% bleach and subsequently wiped with water and ethanol. Falcon tubes and tube lids containing the field samples were wiped using the same procedure to avoid cross-contamination during tube handling.

We followed a glycogen-aided isopropanol precipitation protocol developed at TropWATER, as described by (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. 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).

Table 2.1. Field sites along the Walsh, Mitchell and Wild river catchments as well as Tinaroo Dam and the Tinaroo irrigation channels sampled for tilapia eDNA detection. The numbers in brackets next to the site names correspond to numbers on Figure 2.1.

River catchment	Site name and number	Latitude	Longitude	Collection date
Tinaroo Dam	Tinaroo Dam (1)	-17.1716815	145.5523058	10/07/2019
	Tinaroo spillway (2)	-17.1638480	145.5440094	10/07/2019
Irrigation Channel	Fish Screen (3)	17.1531	145.43152	28/10/2019
	Channel-Granite (4)	-17.132829	145.385271	10/07/2019
	Channel-Walsh (5)	-17.173889	145.307472	28/10/2019
	North Walsh Chanel (6)	-17.044949	145.308714	10/07/2019
	Channel-Mareeba Wetlands (7)	-16.940833	145.322500	29/10/2019
Walsh	Cattle Creek (8)	-17.054674	145.285499	10/07/2019
	Mareeba-Dimbulah (9)	-17.120900	145.269634	28/10/2019
	Collins Weir (10)	-17.257690	145.293158	28/10/20196
	Watsonville d/s (11)	-17.35048	145.3248	29/10/2019
	Watsonville u/s (12)	-17.351750	145.333639	29/10/2019
	Mutchilba (13)	-17.135730	145.207608	09/07/2019
	Bruce Weir (14)	-17.111094	145.115385	09/07/2019
	Wolfram Road (15)	-17.143694	145.095929	09/07/2019
	Leafgold Weir (16)	-17.149202	145.052950	09/07/2019
	Eureka Creek campground (17)	-17.187230	145.041199	09/07/2019
	Eureka Creek u/s (18)	-17.212996	145.063420	09/07/2019
	Emu Creek (19)	-17.379041	144.952621	09/07/2019
	Chillagoe Weir (20)	-17.144196	144.524755	09/07/2019
	Rookwood (21)	-16.9918008	144.300031	09/07/2019

Table 2.1 continued.

River catchment	Site name and number	Latitude	Longitude	Collection date
Mitchell	Cetinich Road (22)	-16.938289	145.328514	11/07/2019
	Two-mile Creek (23)	-16.916194	145.384778	11/07/2019
	Lake Mitchell (24)	-16.794438	145.358047	11/07/2019
	Rifle Creek campground (25)	-16.664806	145.327464	11/07/2019
	Rifle Creek u/s (26)	-16.65277	145.33292	29/10/2019
	Mitchel River (27)	-16.66904	145.19406	30/10/2019
	McLeod River (28)	-16.497592	145.002323	11/07/2019
	Hurricane Station (29)	-16.561417	144.888528	29/10/2019
Wild	Big Weir (30)	-17.366861	145.431000	10/07/2019
	Small Weir (31)	-17.3640103	145.4258336	10/07/2019
	D/s weirs (32)	-17.3611482	145.4091385	10/07/2019
	Herberton (33)	-17.380389	145.387417	10/07/2019
	Herberton d/s (34)	-17.429778	145.384583	10/07/2019

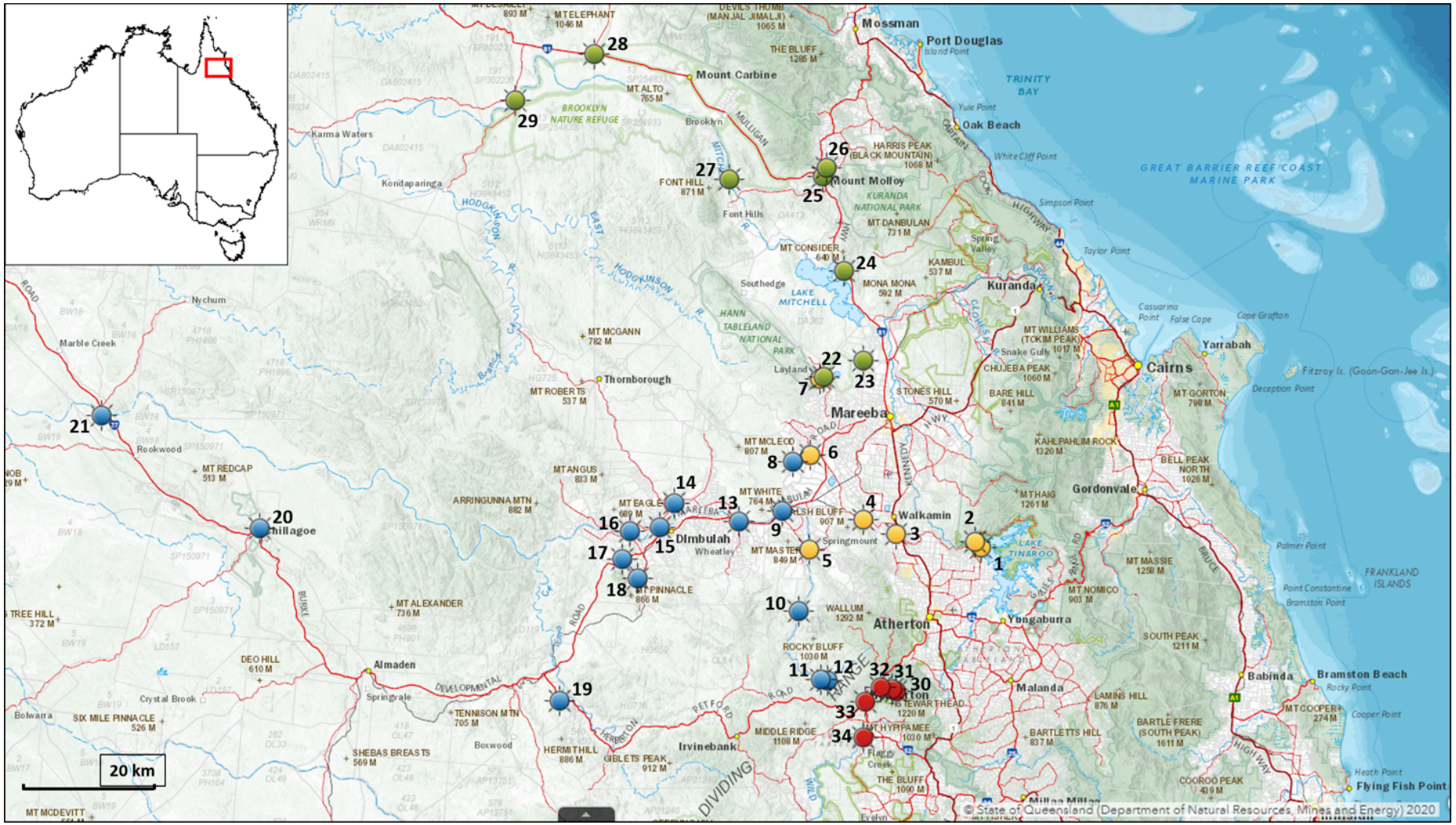

Figure 2.1. Field sites sampled for tilapia eDNA detection in the Tinaroo Dam and Tinaroo Irrigation Channel (yellow, numbers 1–7), Walsh River catchment (blue, numbers 8–21), Mitchell River catchment (green, numbers 22–29) and Wild River catchment (red, numbers 30–34).

2.3 Detection of species-specific DNA by quantitative polymerase chain reaction (qPCR)

Detection of tilapia was performed using a primer pair developed at TropWATER that targets the *16S* mitochondrial gene of *Oreochromis mossambicus* and *Tilapia mariae* (Edmunds & Burrows, 2019). The limit of detection (LOD) of each assay was determined through 8 serial dilutions and set to 2.65 x 10^{-6} ng DNA per µL for *O. mossambicus* and 2.81 x 10^{-6} ng DNA per µL for *T. mariae*.

qPCR assays were run on a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd) in a total of three white 384-well plates sealed with optical films (Thermo Fisher Scientific Australia Pty Ltd). We ran 20 µL reactions and 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 were as follows: initial denaturation and activation at 95°C for 2 min, then 60 cycles of 95°C for 15 secs and 60°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. Eight technical replicates of each sample were used for all qPCR analyses, representing 48% of the total available DNA elution volume. Additionally, we ran five standards in each plate ranging from 2.95 x 10^{4} to 2.95 copies of DNA per µL. Finally, four no template controls (NTC) were used. The NTC samples did not contain the target species DNA and their lack of amplification indicated that no contamination was introduced during plate handling.

2.4 Data analysis

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) before export and subsequent analyses in Microsoft Excel. Samples were considered putative positive detections if: (1) the amplification curve crossed the common fluorescence threshold within 55 cycles; (2) the efficiency of the plate was above 95%; and (3) the melt curve analysis showed a dissociation temperature peak at 82.76°C (± 0.69 – 99.7% confidence interval) for *O. mossambicus*, and 83.07°C (± 0.52 – 99.7% confidence interval) for *T. mariae*. Amplicons from putative positive detections were sequenced at Australian Genome Research Facility to determine if they were true detections. A nucleotide BLAST was performed and amplicon sequences from the samples considered putative positive detections were considered as true detections if there was ≥ 89% pairwise identity with the *16S* gene of each species and the amount of eDNA was above the LOD. Samples that had an eDNA concentration lower than the LOD were still considered putative detections if they aligned to the tilapia *16S* mitochondrial gene.

3. Results

We detected tilapia eDNA at sites of confirmed presence of spotted and Mozambique tilapia as well as sites with no prior knowledge of tilapia occurrence. As there are established populations of both tilapia species in Tinaroo Dam, we sampled a series of sites in the dam and the irrigation channels emanating from the dam to determine whether tilapia eDNA was present and transported downstream to the downstream ecologically important environments of the Walsh River and the Mareeba Wetlands. While both species were detected at both the dam and the fish screen of the irrigation channel, no tilapia eDNA was detectable at the junction of the channel and the Mareeba Wetlands (Table 3.1). However, one technical replicate showed positive amplification of spotted tilapia at the junction of the irrigation channel and the Walsh River (Table 3.1). In the Walsh River, both species were detected around the Dimbulah area, including Bruce Weir and Leafgold Weir, as well as Eureka Creek, a tributary of the Walsh River (Table 3.1). Within the Mitchell River catchment, both species' eDNA was also detected near the Mareeba Wetlands and at Rifle Creek, a tributary of the Mitchell River (Table 3.1). Finally, two weirs on the Wild River catchment showed presence of Mozambique tilapia eDNA only (Table 3.1).

Amplicon sequences from Mozambique tilapia detections matched the *O. niloticus 16S* ribosomal RNA gene (accession number MH699843) between positions 319 – 372 bp with 96% pairwise identity, the *O. upembae 16S* ribosomal RNA gene (accession number MK788837.1) between positions 351 – 382 bp with 100% pairwise identity, or the *O. urolepis 16S* ribosomal RNA gene (accession number MK788839.1) between positions 343 – 382 bp with ≥ 98% pairwise identity. Additionally, amplicons sequences from spotted tilapia detections matched the *T. mariae 16S* ribosomal RNA gene (accession number GQ168026 or GQ168026.1) between positions 344 – 401 bp with ≥ 89% pairwise identity. All field and extraction control samples were verified to be devoid of the target species eDNA by qPCR.

3.1 Tinaroo Dam and irrigation channel

Spotted tilapia eDNA was detected in 100% of the field replicates collected within the dam, as well as at the dam spillway (Table 3.1), indicating that the water going into the irrigation channel contained a large amount of spotted tilapia eDNA. Those two sites also contained Mozambique tilapia eDNA; however, it was less abundant, with only only 20% of the field replicates and 2.5% of the qPCR technical replicates showing positive amplification (Table 3.1).

The irrigation channel showed presence of spotted tilapia eDNA in 100% of the field replicates and 45% of the qPCR technical replicates collected at the fish screen, approximatey 17 km downstream from the spillway (Table 3.1). Environmental DNA of Mozambique tilapia was also present at the screen, at a similar percentage of field and qPCR technical replicates as the spillway (Table 3.1). Moreover, we detected the presence of spotted tilapia eDNA 6 km downstream from the screen (approximately 21 km downstream from the dam spillway), but no Mozambique tilapia (Table 3.1).

Finally, we collected water at the junction of the irrigation channel and the Walsh River, as well as the Mitchell River (Mareeba Wetlands) to determine whether tilapia eDNA had been transported from Tinaroo Dam. No positive detections of either species were found at the intersection of the channel and Mareeba wetlands (Table 3.1) and therefore, any detections

within the Mitchell River catchment would indicate tilapia are present locally. There was one positive detection of spotted tilapia; however, the amount of eDNA was below the accepted LOD. The LOD is the minimum DNA concentration that can be reliably detected by an assay (Bustin et al., 2009). Since concentrations lower than the LOD cannot be assigned as positive detections with certainty, we have therefore termed them 'inconclusive' from here on.

3.2 Walsh River catchment

We sampled 14 sites along the Walsh River catchment, including four weirs (Bruce, Leafgold, Chillagoe and Collins Weirs) and three tributaries (Cattle, Eureka and Emu Creeks) (Table 2.1, Figure 2.1). Spotted and Mozambique tilapia eDNA were detected at Bruce Weir, with a higher percentage of positive detections of spotted tilapia (Table 3.1). Spotted tilapia eDNA was also detected at Wolfram Road (60% field replicates, 10% of qPCR technical replicates), Leafgold Weir (20% field replicates, 2.5% of qPCR technical replicates) and Eureka Creek campground (80% field replicates, 57.5% of qPCR technical replicates) (Table 3.1). One qPCR technical replicate from Eureka Creek upstream, located approximately 5 km from Eureka Creek campground, and one qPCR tehnical replicate from Rookwood showed positive amplification of spotted tilapia (Table 3.1). However, the eDNA concentrations were below the assay LOD. Finally, the northeastern sites of the Walsh River (Cattle Creek, Mareeba-Dimbulah Road and Mutchilba), southeastern sites (Watsonville upstream, Watsonville downstream and Chillagoe Weir) as well as Emu Creek and did not show presence of spotted and Mozambique tilapia eDNA (Table 3.1).

3.3 Mitchell River catchment

A total of eight sites were screened for presence of spotted and Mozambique tilapia eDNA (Table 2.1, Figure 2.1). We detected both tilapia species eDNA at two sites adjacent to the Mareeba Wetlands. In Two-Mile Creek, Mozambique tilapia eDNA was detected in 20% of the field replicates and 2.5% of the qPCR technical replicates (Table 3.1). Additionally, one qPCR technical replicate from Cetinich Road showed positive amplification of spotted tilapia and Mozambique tilapia eDNA; however, the eDNA concentrations were below the assay's LOD (Table 3.1). Spotted tilapia eDNA was detected at the campground on Rifle Creek, a tributary of the Mitchell River, as well as approximately 1.8 km upstream from the campground (Table 3.1). We also collected water samples from the Mitchell River, before the junction with Rifle Creek, but no tilapia eDNA was detected at this site (Table 3.1). Similarly, no tilapia eDNA was detected in the northern Mitchell River tributary (McLeod River) or on the western area of the Mitchell River (Hurricane Station) (Table 3.1). Finally, we also collected water samples from Lake Mitchell and did not detect presence of tilapia eDNA (Table 3.1), however, we only smapled one site adjacent to the highway and we cannot conclude that tilapia are not present at that site based on that sample size.

3.4 Wild River catchment

Mozambique tilapia eDNA was detected in two weirs on the Wild River catchment as well as at a site approximately 2 km downstream from the weirs. All field replicates from the most upstream weir (Big Weir) and 20% of qPCR technical replicates exhibited positive Mozambique tilapia amplification (Table 3.1). Adiditionally, 60% of field replicates and 15% of qPCR technical replicates from the Small Weir, <1 km downstream from Big Weir, and only

20% of field replicates and 2.5% of qPCR technical replicates from the dowsntream site exhibited positive Mozambique tilapia amplification (Table 3.1).

Table 3.1. Summary of tilapia percentage of positive detections using eDNA analysis at 35 field sites in northern Australia using the 16S mitochondrial gene. (*) indicates putative positive detections. 'd/s' = downstream, 'u/s' = upstream.

River catchment	Site name	Species	Field site			qPCR analysis		
			No. field replicates	No. positive fied replicates	% positive detections	No. technical replicates	No. positive technical replicates	% positive detections
Tinaroo Dam	Tinaroo Dam	*T. mariae*	5	5	100	40	30	75
		O. mossambicus		1	20		1	2.5
	Tinaroo spillway	*T. mariae*	5	5	100	40	32	80
		O. mossambicus		1	20		1	2.5
Irrigation Channel	Screen	*T. mariae*	5	5	100	40	18	45
		O. mossambicus		2	40		2	5
	Channel-Granite	*T. mariae*	5	4	80	40	7	17.5
	Channel-Walsh	*T. mariae**	5	1	20	40	1	2.5
	Channel-Wetlands	-	5	0	0	40	0	0
	North Walsh Chanel	-	5	0	0	40	0	0
Walsh	Cattle Creek	-	5	0	0	40	0	0
	Mareeba-Dimbulah	-	5	0	0	40	0	0
	Collins Weir	-	5	0	0	40	0	0
	Mutchilba	-	5	0	0	40	0	0

Table 3.1 continued.

River catchment	Site name	Species	Field site			qPCR analysis		
			No. field replicates	No. positive fied replicates	% positive detections	No. technical replicates	No. positive technical replicates	% positive detections
Walsh	Watsonville u/s	-	5	0	0	40	0	0
	Watsonville d/s	-	5	0	0	40	0	0
	Bruce Weir	*T. mariae*	5	3	60	40	11	27.5
		O. mossambicus		1	20		1	2.5
	Wolfram Road	*T. mariae*	5	3	60	40	4	10
	Leafgold Weir	*T. mariae*	5	1	20	40	1	2.5
	Eureka Creek campground	*T. mariae*	5	4	80	40	23	57.5
	Eureka Creek u/s	*T. mariae**	5	1	20	40	1	2.5
	Emu Creek	-	5	0	0	40	0	0
	Chillagoe Weir	-	5	0	0	40	0	0
	Rookwood	*T. mariae**	5	1	20	40	1	2.5
Mitchell	Two-mile Creek	*O. mossambicus*	5	1	20	40	2	2.5
	Cetinich Road	*T. mariae**	5	1	20	40	1	2.5
		*O. mossambicus**		1	20		1	2.5

Table 3.1 continued.

River catchment	Site name	Species	Field site			qPCR analysis		
			No. field replicates	No. positive fied replicates	% positive detections	No. technical replicates	No. positive technical replicates	% positive detections
Mitchell	Lake Mitchell	-	5	0	0	40	0	0
	Rifle Creek campground	*T. mariae*	5	1	20	40	2	5
	Rifle Creek u/s	*T. mariae*	5	1	20	40	1	2.5
	Mitchel River **(dry)**	-	5	0	0	40	0	0
	McLeod River	-	5	0	0	40	0	0
	Hurricane Station	-	5	0	0	40	0	0
Wild	Big Weir	*O. mossambicus*	5	5	100	40	8	20
	Small Weir	*O. mossambicus*	5	3	60	40	6	15
	D/s weirs	*O. mossambicus*	5	1	2	40	1	2.5
	Herberton	-	5	0	0	40	0	0
	Herberton d/s	-	5	0	0	40	0	0

4. Discussion

The present study conducted eDNA analysis targeting spotted and Mozambique tilapia along three river catchments in north Queensland with historical and unknown tilapia records. We conducted a first sampling round during July 2019 and, based on the tilapia eDNA detections, a second sampling round was carried out in October 2019 to fill gaps in knowledge. On the Walsh River catchment, we targeted the two main weirs (Bruce and Collins), two smaller weirs (Leafgold and Chillagoe), as well as a series of sites along the river and tributary creeks. Given that human-mediated dispersal of tilapia is the most likely source of introduction into new areas (Russell et al., 2012a), we sampled sites located around towns, such as upstream and downstream Watsonville, Dimbulah and Mutchilba. On the Mitchell River catchment, we surveyed the Mareeba Wetlands, Lake Mitchell, two major tributaries of the river (Rifle Creek and McLeod River) and a remote location along the river on Hurricane Station. Water from Tinaroo Dam in the Barron Ruver catchment flows via irrigation channels into both the Walsh and Mitchell rivers. The Walsh River is a tributary of the Mitchell River so tilapia can spread from one to the other. There are multiple points of entry for tilapia across the connected system. Therefore, monitoring for tilapia should be widespread to provide early detection of new incursions. This study demonstrates that eDNA sampling is a viable method of providing regular, widespread monitoring for tilapia that can be applied by non-specialists (e.g. government agency staff, community members) who collect water samples. We also sampled four sites along the nearby Wild River, which drains to the Herbert River which is largely free of tilapia except for a nascent population in the headwaters of the Wild River.

By collecting 150 mL of water at a single point, we detected tilapia eDNA at sites of known presence of the species as well as at sites where tilapia had never been reported before. We provide recommendations on how eDNA surveys can be incorporated in tilapia monitoring programs to stop the spread of the species.

4.1 Tinaroo Dam and irrigation channel

Mozambique tilapia was first detected in the Tinaroo Dam during the late 1990s, and spotted tilapia was detected a few years later. Currently, there are established populations of both species at the dam (Russell et al., 2012b). Since eDNA can be displaced downstream from a source, especially if the species is abundant, it was important in the present study to determine the extent of eDNA transport along the Tinaroo Irrigation Channel. We were able to detect presence of both tilapia species at the dam, at the fish screen (located approximately 17 km downstream from the spillway) and near Granite Creek, located 21 km downstream from the spillway. While there were no tilapia eDNA detections at the intersection of the irrigation channel with the Mareeba Wetlands, an inconclusive spotted tilapia detection at the intersection of the channel and Walsh River could mean that tilapia eDNA can be transported > 21 km from the dam. The high amount of tilapia eDNA present in the water from Tinaroo Dam possibly results in long distance transport. Similar long distance eDNA detection has been observed along rivers outflowing from a lake containing large numbers of fish and invertebrates (Deiner & Altermatt, 2014; Pont et al., 2018). It is also possible that the tilapia eDNA detected along the irrigation channel is sourced from a local tilapia population residing within the channel system. Regardless of the source of tilapia eDNA along the irrigation channel, the potential transport of tilapia eDNA to the Walsh River

could not have been a confounding factor for tilapia detection in the present study. This is because the next site that we sampled, Mareeba-Dimbulah, was located 9 km downstream from the channel junction, and based on previous literature assessing eDNA transport, it is highly unlikely that the small concentration of eDNA detected in the channel would be carried more than 1 km downstream (Jane et al., 2015; Schumer et al., 2019; Wood, Erdman, York, Trial, & Kinnison, 2020).

4.2 Walsh River catchment

We surveyed a site on the Walsh River (Collins Weir) that is well upstream of where the Tinaroo irrigation channel drops into the Walsh River and did not detect presence of tilapia eDNA there. A previous eDNA survey conducted during 2018 also failed to detect tilapia eDNA in Collins Weir (Edmunds et al., 2019). There is no anecdotal evidence that tilapia inhabits this weir, although its geographical location downstream of Watsonville constitutes a risk of invasion, if tilapia was present near that town. We collected water samples at two sites around Watsonville and did not detect presence of tilapia eDNA. Similarly, no tilapia eDNA was detected in the northern tributaries of the Walsh River, Cattle Creek and the North Walsh channel. Further monitoring at these sites should be carried out to maintain the tilapia-free status of the areas.

On the other hand, we detected both tilapia species at the other major weir on the Walsh River, Bruce Weir. The Queensland Department of Agriculture and Fisheries had previously detected spotted tilapia near Bruce Weir in 2017. During early 2019, Biosecurity Queensland conducted an electrofishing assessment of several water bodies, including Bruce Weir. The electrofishing work revealed presence of both tilapia species (Bonnie Holmes, *pers. comm.*) and validates our findings. During the same electrofishing survey, Biosecurity Queensland detected tilapia presence at Mutchilba. We conducted sampling at an access area near the town and did not detect tilapia eDNA. Given that the electrofishing survey found tilapia at this site, our result could represent a false negative. However, it is also possible that we sampled at a site upstream from the tilapia population, thus providing a negative result.

We detected spotted tilapia eDNA at Wolfram Road and Leafgold Weirs, around Dimbulah, as well as at Eureka Creek. As mentioned earlier, previous incursions of Mozambique tilapia had been recorded at Eureka Creek. Although the species was thought to be eradicated (Russell et al., 2012a), spotted tilapia was detected through an eDNA survey during 2017 (Edmunds et al., 2019). An inconclusive spotted tilapia detection upstream from the Eureka Creek campground could indicate that the species is established along a larger stretch of the creek. Another tributary of the Walsh River that we sampled in the present study is Emu Creek, a seasonal watercourse. No tilapia detections were found here and there were no other records of tilapia there either. This water body is a seasonal creek, which limits the natural movement of the tilapia into it.

The last tributary of the Walsh River that we surveyed in the present study was the weir on Chillagoe Creek. No tilapia eDNA was detected at this site. This was an expected result, since the weir is a spring-fed system and it is also an intermittent waterbody, therefore tilapia is not expected to freely swim upstream from the Walsh River. Finally, we surveyed Rookwood, a remote site on the Walsh River 30 km northwest from Chillagoe and had an inconclusive spotted tilapia detection at this site. Biosecurity Queensland reports presence of spotted tilapia around this area and the inconclusive detection could suggest that the

population is very low and that increasing the sampling effort (number of sites and water volume) could lead to positive results.

The Walsh River is a major tributary of the Mitchell River and the junction of both occurs near Gamboola, approximately 100 km from Rookwood. Tilapia transport during wet season could further spread the species into the Gulf of Carpentaria. The spread of this invasive fish into the Gulf Rivers systems could potentially lead to the spread of the species into the Northern Territory.

From the above data, it appears that tilapia are present in the reaches of the Walsh River within the irrigation area (including Bruce Weirm Leafgold Weir and Eurake Creek) but not in the Walsh River upstream of the irrigation area (Collins Weir and above), or tributaries such as Cattle Creek, Emu Creek and Chillagoe Creek.

4.3 Mitchell River catchment

We demonstrated that the water carried by the Tinaroo Irrigation Channel into the Mareeba Wetlands did not contain tilapia eDNA; therefore, we can conclude that all detections within the Mitchell River catchment arised from local presence of tilapia.

We detected Mozambique tilapia eDNA at Two-mile Creek, a creek that runs through the Mareeba wetlands, constituting the first report of Mozambique tilapia presence in the wetlands. There was also an inconclusive detection of spotted tilapia at this site. Similarly, Cetinich Road, adjacent to the wetlands had inconclusive detection of spotted and Mozambique tilapia. While we cannot claim with certainty that there is tilapia presence at Cetinich Road, the fact that we detected Mozambique tilapia at Two-Mile Creek indicates that this species inhabits the wetlands. The wetlands receive excess seasonal water from the Mareeba Dimbulah Irrigation Area, which comprises a potential source of natural tilapia movement from the Walsh River. At the same time, presence of tilapia in the wetlands represents a very high risk of movement of tilapia into the Mitchell River catchment (Barron/Mitchell Tilapia Management Group, 2004).

Following Two-mile Creek downstream, we sampled Lake Mitchell, which is mainly fed by the water from Two-mile Creek and the Mareeba Wetlands. Although we did not detect tilapia eDNA at Lake Mitchell, the fact that at one of the species was detected at the wetlands, could suggest that tilapia are present at the lake undetected or that they may soon invade from upstream sources. The false negative detection at the Lake may also be due to the fact that because of lack of site access, we only sampled one site from this large waterbody (approximitely 40 km perimeter) and therefore it is not possible to make conclusive inferences based on such a limited sampling effort. Environmental DNA is not dispersed randomnly in water – its density is highest in the target species' immediate surroundings and decreases exponentially with distance (Goldberg, Strickler, & Fremier, 2018). Therefore the probability of detection of the target species is related to the survey protocol (number of replicates and volume of water collected) as well as the level of eDNA dipersion (Furlan, Gleeson, Hardy, & Duncan, 2016). Survey sensitivity needs to be taken into account before designing field sampling schemes, especially for detection of low-abudance species, such as new incursions or post-eradication of invasive species (Furlan, Gleeson, Wisniewski, Yick, & Duncan, 2019). For larger waterbodies, such as Lake Mitchell, increasing field replication would improve precision and avoid false negative detections (Pilliod, Goldberg, Arkle, & Waits, 2013; Turner et al., 2014).

On the other hand, spotted tilapia was detected at Rifle Creek campground, a popular tourist destination in Mount Molloy. Given that spotted tilapia eDNA was detected during the first sampling event (July 2019), we conducted further sampling at a site approximately 1.8 km upstream from this site during October 2019. Similar percentage of positive tilapia eDNA detections were observed at both sites on this creek; therefore, this data indicates that spotted tilapia is now established in this waterway. Therefore, conducting eDNA sampling around the Julatten-Rifle Creek area is recommended to be able to accurately determine the extent of the species distribution in this Mitchell River tributary.

Additionally, after detecting spotted tilapia at Rifle Creek during the July sampling event, we decided to sample one site on Mitchell River located before the junction with Rifle Creek. The selected site was set far enough from Lake Mitchell (approximately 23 km downstream) to avoid false positive detections due to potential tilapia eDNA transport from the lake. During the time of the year when the sampling was carried out, this stretch of the Mitchell River was not flowing and water was collected from a large pool of water. This reduced the chances of tilapia eDNA detection even if the species was present in the area. Not surprisingly, we did not detect tilapia eDNA at this site. However, if the species was present in Lake Mitchell, tilapia could swim downstream into the Mitchell River during the wet season and even spread into north-western areas of the catchment.

Finally, McLeod River, a tributary of the Mitchell River, as well as the Mitchell River at Hurricane Station did not have presence of tilapia eDNA. There is no anecdotal evidence of the species presence at these downstream sites, and therefore we conclude that the lack of detections is due to absence of the species. Further eDNA monitoring in the tilapia-free areas, such as McLeod River and Hurricane Station, can help detecting early signal of the species presence (Villacorta-Rath et al., 2020).

In the Mitchell River catchment, eDNA analyses presented here indicate that tilapia are present in the Mareeba Wetlands, Two-mile Creek, Lake Mitchell and Rifle Creek, but they have not yet spread along most of the upper Mitchell River itself or other tributaries.

4.4 Wild River catchment

While there are historical records of Mozambique tilapia at the two weirs on the upper reaches of the Wild River that supply potable water to the Herberton township (Russell et al., 2012b), no tilapia have been recorded downstream from the weirs (Thuesen et al., 2011). In the present study, we conducted eDNA sampling at the weirs, immediately downstream from them, as well as at two sites near Herberton to confirm that the area remains tilapia-free. While tilapia eDNA was transported 1 km downstream from the weirs, no tilapia eDNA was detectable at the Herberton township (approximately 6 km downstream from the small weir). This eDNA transport distance differs from the tilapia eDNA detection obtained downstream from the Tinaroo Dam, where we detected eDNA approximately 21 km downstream from the spillway. This difference could be due to the abundance of the target species at the source and the water discharge. During the year 2011, tilapia were removed via electrofishing from both Herberton Weirs and the population was considered to have being reduced considerably (Thuesen et al., 2011). Although this removal took place almost 10 years before the present eDNA survey, it is likely that the tilapia population at the Tinaroo Dam is currently much larger than at the Herberton Weirs, producing a higher amount of eDNA that can be transported downstream. Also, the higher water discharge from Tinaroo Dam would promote

eDNA transport over longer distances. Previous studies have shown that mean eDNA transport distance and retention in a system are highly influenced by water discharge (Jane et al., 2015; Jerde et al., 2016; Shogren et al., 2017, 2018). High flow results in fast particle transport and resuspension (Wipfli, Richardson, & Naiman, 2007), potentially transporting eDNA further downstream (Fremier, Strickler, Parzych, Powers, & Goldberg, 2019; Webster et al., 1987). Therefore, it is important to measure eDNA downstream transport at each system separately.

5. Conclusions and recommendations

Environmental DNA analysis is a sensitive tool to detect presence of tilapia in still and flowing waters. Here, tilapia eDNA was detected at sites with historical records of the species, demonstrating that replicate sample collection at a single site along a creek can provide reliable species detection. However, when targetting larger water bodies (i.e. dams and weirs), sampling more than one site is recommended to avoid false negative results (i.e. concluding the species is absent when it is not).

Using eDNA analysis, we provided evidence of tilapia presence at sites where the species had not been recorded yet. Validation of our results via conventional sampling methods that require sighting the species at the Mareeba Wetlands would be valuable to conclude that tilapia has spread further into new areas.

Environmental DNA sampling is a time-efficient method that does not require specialised equipment or staff, which is particularly relevant in remote areas of north Queensland. Therefore, eDNA analysis could be used to delimit the distribution of tilapia to inform management and make decisions about eradication measures for the species. Also, implementing regular eDNA sampling at sites at the edge of an invasion could constitute an early-warning system that trigger early eradication efforts. The ease-of-use of the eDNA field collection protocol allows for community involvement in sample collection, resulting in negligible field costs and increased awareness of invasive species in the community.

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