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Investigation into the Distribution of Tilapia in the Lower Pioneer Catchment

June 2015

Trent Power

REEF CATCHMENTS

Mackay REGIONAL COUNCIL

Centre for Sustainable Tropical Fisheries & Aquaculture

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Cover Figure: Lower Gooseponds, Mackay. Inset – Juvenile tilapia, similar to the specimens captured from the Gooseponds Lagoons

Contents

Background

The Lower Pioneer Catchment contains a series of tributaries in the lower reaches of the Pioneer River as well as a number of small coastal streams to the north and south of the river mouth. Located within the city limits of Mackay, the catchment encompasses ephemeral stream channels, smaller feeder creeks as well as a large number of natural and man-made offstream lagoons and dams. In 2014 there were confirmed reports of the presence of the noxious tilapia species *Oreochromus mossambicus* (referred to herein as tilapia) at a number of locations within the Gooseponds Lagoons complex on the northern side of the Pioneer River, however, the extent of the species' distribution in this area had yet to be determined.

The current investigation was part of a wider assessment being conducted by Reef Catchments Limited (RCL), Mackay Regional Council (MRC) and other project partners to determine the distribution of tilapia in the Southern Great Barrier Reef Catchments. It is hoped that once this is known, suitable control measures can be implemented to reduce the spread of the species to unaffected catchments within the region. As part of the work in the Mackay region, RCL/MRC contracted Catchment Solutions and James Cook University to conduct two rounds of sampling to provide information on the current distribution of tilapia within the Lower Pioneer Catchment. The first round was to focus on sites in close proximity to confirmed incursion locations. Based on the results of preliminary sampling the second round was to expand the site network to gauge the extent of tilapia distribution with the lower catchment. Sampling was to provide presence/absence information on tilapia at survey sites, length distribution and abundance of current tilapia populations, and species lists of fish communities at all sites sampled. Sampling was conducted using electrofishing/cast netting and environmental DNA (eDNA) methods.

Electrofishing is a non-destructive sampling method which uses a boat mounted or a backpack unit to generate an electric current, this current temporarily stuns fish which can be captured for identification and processing. Electrofishing is effective on most fish species, however, in large open sites it can be difficult to capture tilapia particularly when in low abundance (Thuesen et al 2011). Cast netting uses a circular net, of small mesh size that when thrown envelops fish within the circumference of the net. Fish are trapped in the net pocket and removed from the water for processing.

To complement electrofishing/netting, eDNA was used to test for the presence of tilapia DNA at 12 locations during preliminary surveys and 12 locations during the secondary surveys. eDNA works by using species specific genetic markers to test for the presence of tilapia DNA in water samples. This technique has proven to be a sensitive method of detection with the ability to identify the presence of tilapia where traditional methods such as electrofishing have not been successful (Power 2015, Power 2014). Water quality data was also collected to aid in the interpretation of fish community data.

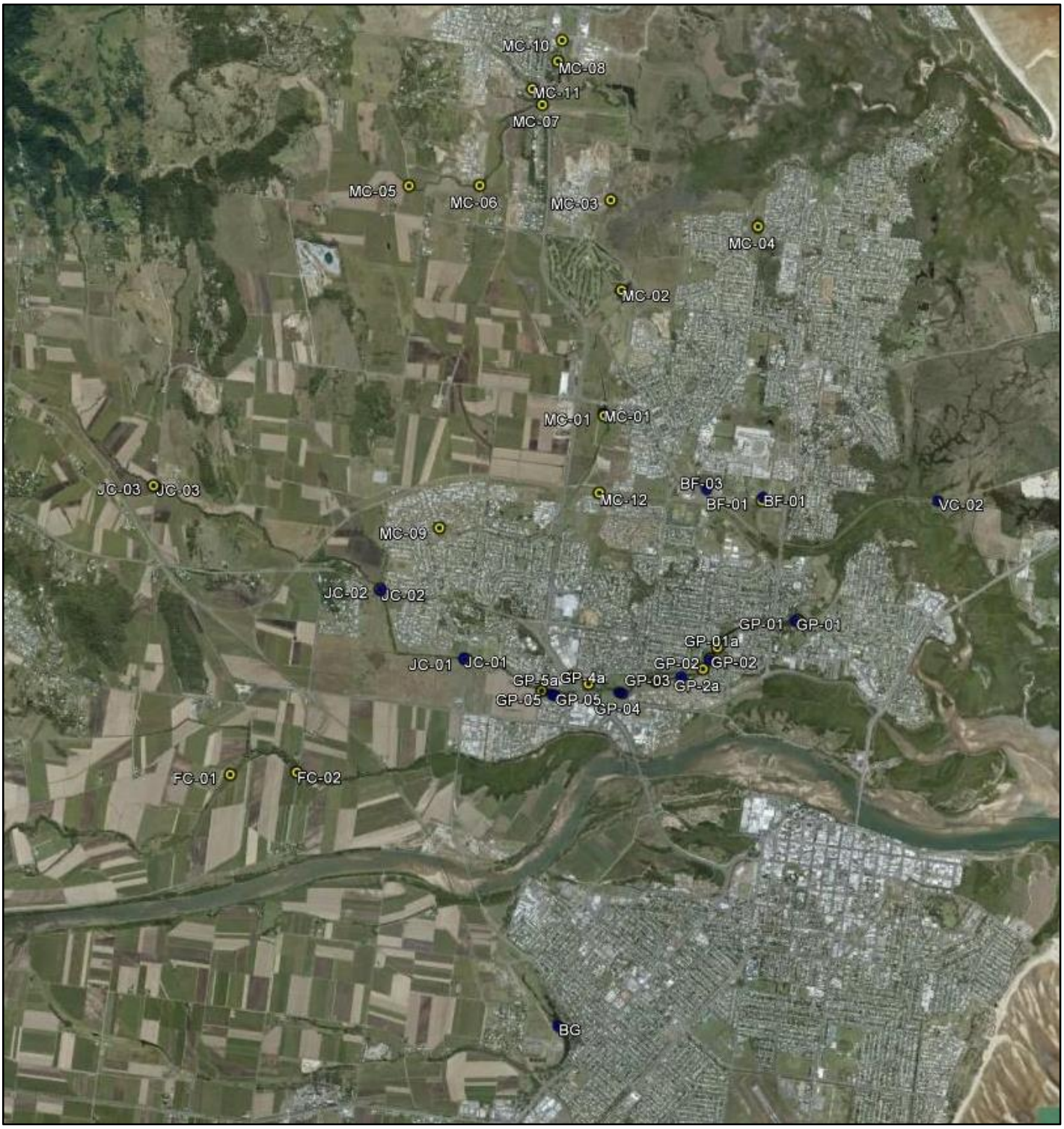

Figure 1. Location of survey sites within the Lower Pioneer Catchment. Blue dots indicate prelimiary survey sites, yellow dots indicate secondary survey sites Note - site names have been abriveated as follows: MC – McCready Creek, JC – Janes Creek, GP – Gooseponds, VC – Vines Creek, FC – Fursden Creek, BG – Botanic Gardens. Sites listed twice in this figure were sampled during both preliminary and secondary surveys. Google Earth Base Image.

Methods

Site Selection

In total 31 sites were sampled as part of the current investigation (Figure 1), 13 sites were sampled as part of the preliminary survey and 25 during the secondary survey. Several sites were sampled both rounds (Table 1). Preliminary sites included both confirmed and potential tilapia incursion locations. The location of confirmed tilapia sites was based on documented reports from that location. These sites provided the data on size distribution and abundance required for current tilapia population assessment. The location of potential tilapia sites was based on the connectivity of potential sites with confirmed tilapia sites and its location within the Lower Pioneer Catchment. Potential sites were also chosen based on likely habitat features such as waterbody type and size, riparian condition, macrophyte growth etc. to represent a range of different habitat types. Details of habitat features at sites sampled during this investigation are listed in Table 1. Criteria used for habitat descriptions are listed in Appendix 1.

A positive eDNA indication at a site in the top of McCready Creek during the preliminary survey resulted in the site network being expanded into this catchment for the secondary survey. Additional sites in Fursden creek were also included due to the proximity of this creek with the Gooseponds Lagoons complex

Table 1. Habitat description and sampling regime of sites chosen to sample for the presence of tilapia in the Lower Pioneer Catchment.

Site Name	Preliminary Survey	Secondary Survey	Water Body Type	Average Site Width	Site Length	Average Riparian Width (m)	Riparian Condition	Riparian Shade Potential	Aquatic Macrophyte
Beaconsfield 01	Efish, eDNA	Efish	Stormwater Drain	15	150	0	Very low	Very low	Moderate
Beaconsfield 03	Efish, eDNA	Not Sampled	Impondment Small	165	200	0	Very low	Very low	Low
Botanic Gardens	Efish, eDNA	Not Sampled	Instream Pool	600	50	5	Very low	Very low	Very High
Fursden Creek 01	Not Sampled	Efish, eDNA	Natural Wetland	40	450	1	Very low	Very low	Very High
Fursden Creek 02	Not Sampled	Efish, eDNA	Instream Pool	25	60	15	Moderate	High	Very low
Gooseponds 01	Efish, eDNA	Not Sampled	Instream Pool	30	1200	0	Very low	Very low	High
Gooseponds 02	Efish, eDNA	Not Sampled	Instream Pool	25	250	7	Low	Low	Moderate
Gooseponds 03	Efish, eDNA	Not Sampled	Instream Pool	15	500	5	Low	Low	High
Gooseponds 04	Efish	Not Sampled	Instream Pool	10	600	10	Low	High	Low
Gooseponds 05	Efish, eDNA	Efish	Instream Pool	60	350	2	Low	Low	Moderate
Gooseponds 1a	Not Sampled	Efish	Riffle	15	40	0	Very low	Very low	High
Gooseponds 2a	Not Sampled	Efish	Riffle	15	20	10	Low	Moderate	Moderate
Gooseponds 3a	Not Sampled	Efish	Riffle	10	30	5	Low	Low	Very High
Gooseponds 4a	Not Sampled	Efish	Stormwater Drain	10	50	0	Very low	Very low	Low
Gooseponds 5a	Not Sampled	Efish	Offstream pool	20	70	0	Very low	Very low	Very High
Gooseponds 5b	Not Sampled	Efish	Riffle	2	40	0	Very low	Very low	Moderate
Janes Creek 01	Efish, eDNA	Efish	Instream Pool	10	60	10	Low	High	Low
Janes Creek 02	Efish, eDNA	Efish	Instream Pool	10	100	10	Low	Moderate	Moderate
Janes Creek 03	Efish, eDNA	Efish	Riffle	3	100	10	Low	High	Low
McCready Creek 01	Efish, eDNA	Efish, eDNA	Instream Pool	15	100	3	Very low	Low	High
McCready Creek 03	Not Sampled	Efish, eDNA	Constructed Wetland	40	530	0	Very low	Very low	High
McCready Creek 04	Not Sampled	Efish, eDNA	Impondment Small	65	180	0	Very low	Very low	High
McCready Creek 05	Not Sampled	Efish, eDNA	Offstream pool	3	100	10	Low	High	Low
McCready Creek 06	Not Sampled	Efish, eDNA	Instream Pool	3	100	10	Low	High	Low
McCready Creek 07	Not Sampled	Efish, eDNA	Instream Pool	10	150	7	Low	High	Low
McCready Creek 08	Not Sampled	Efish, eDNA	Offstream pool	35	240	5	Low	Low	Moderate
McCready Creek 09	Not Sampled	Efish, eDNA	Constructed Wetland	30	130	0	Very low	Very low	Very High
McCready Creek 10	Not Sampled	Efish, eDNA	Stormwater Drain	5	220	0	Very low	Very low	Moderate
McCready Creek 11	Not Sampled	Efish	Riffle	3	150	1	Very low	Low	Low
McCready Creek 12	Not Sampled	Efish	Stormwater Drain	5	10	0	Very low	Very low	Very low
Vines Creek 02	Cnet, eDNA	Not Sampled	Instream Pool	5	10	2	Very low	Very low	Very low

Electrofishing/netting

General fish community and tilapia size data was collect using a variety of methods depending on site specific limitations. Electrofishing was the primary data collection which utilised large and small boat mounted units as well as a backpack unit. Sites which exceeded the conductivity limits of electrofishing were surveyed using a cast net.

Small boat configuration – 3.7 m vessel (Electrolyte) operating a Smith-Root 2.5 GPP electrofisher unit, equipped with a single boom arm, 6 dropper anode array and hull cathode. Settings were adjusted based on electrical conductivity of the water on site to maximise the effectiveness of electrofishing operations. A master and single dip-netter were employed during all sampling activities on Electrolyte.

Large boat configuration – 5.6m vessel (Discopyge II) which operated a Smith-Root 7.5 GPP electrofisher unit, two boom arms with 16 dropper anode arrays and hull cathode. Settings were adjusted based on electrical conductivity of the water on site to maximise the effectiveness of electrofishing operations. An operator and two dip-netters were employed during all sampling activities on Discopyge II

Boat electrofishing was conducted at various depths and encompassed all types of instream habitats within the waterbody. The electrofishing methodology used was a combination of power on, power off for the duration of the sampling effort. The sampling effort consisted of a series of 300 second ‘shots’ where the boat was maneuvered in and out from the shoreline as well as parallel to the shore in deeper water. The effective electric field of this unit was approximately 3 m radius (centered on the anode) to a depth of 3 m. Fish positively identified during operations but not captured were also recorded and contributed abundance and assemblage data in this report.

At sites not accessible with the boat backpack electrofishing used to collect fish community data. The backpack unit utilised was a Smith-Root Model-LR24 Backpack Electrofisher operating a 300-500 volt pulsed-DC current and a standard pulse setting (1ms). An operator and single dip-netter were employed during all backpacking operations. Sampling effort was the same used for boat mounted operations and was limited to a wading depth of 1.2 m.

Cast netting utilised a single pocket net with a mesh size of 25 mm and a drop length of 2.4 m. Sampling effort consisted of a series of ‘casts’ where the net was thrown from the bank and deployed in a circular pattern. Fish captured in the net were scooped from the water and processed. Cast netting was limited to a wading depth of 1.2 m in areas free of snags.

All tilapia captured during surveys were counted and measured to the nearest millimeter (total length). Tilapia and other non-native fish species were euthanised as per Fisheries Queensland legislation and ANZCCART procedures, and retained for gonadal development inspection or buried on site above the high water level. Native fish captured for identification purposes were processed and released within the reach or waterbody they were captured from.

Environmental eDNA (eDNA) – Extracted from supporting document *Environmental eDNA Survey of Tilapia in the Lower Pioneer Catchment (Jerry & Noblel. 2014)*.

eDNA sample consisted of five independent 2 L water samples and one negative control sample of distilled water. All samples were filtered within 24 hr of collection. Water samples (2 L) were vacuum filtered through a 20 µm nylon net filter (Merck NY2004700) and stored in the freezer until further processing.

To ensure quality control of the eDNA survey, all filtration equipment was sterilised with 10% bleach solution before use. Prior to filtration of every sample, 500 ml of deionised water was filtered through the filtration apparatus and the filter paper stored and kept for further analysis. This is called the equipment control and is used to test for equipment contamination for corresponding samples.

eDNA on the filter papers was extracted using the Bioline ISOLATE II Genomic eDNA kit (BIO-52067) following the manufacturers standard protocol. A three phase quantitative PCR (qPCR) analysis was then carried out for each sample. The qPCR protocol consists of a 40 cycle quantitation analysis followed by a melt analysis. The quantitation analysis identifies whether a sample has amplified or not and the melt analysis is used to confirm the PCR product formed is from tilapia eDNA. For each run of qPCR, positive (tilapia eDNA) and negative PCR controls were run to ensure quality control.

Phase 1 of the qPCR analysis tests each sample for PCR inhibition from contaminants in the water using an internal positive control (Qiagen RT2 qPCR Primer geDNA control, cat. no. 330011). Samples that displayed signs of PCR inhibition (e.g. failed to amplify the spiked control eDNA) were further treated using an eDNA clean up column (Bioline ISOLATE Fecal eDNA kit, BIO52082). In total, 10 samples collected from the Moranbah area required further treatment due to PCR inhibition.

Next, during phase 2, all samples were analysed for presence or absence of tilapia eDNA by conducting a qPCR using tilapine species-specific eDNA primers. Each water sample was analysed using five replicate qPCR reactions. Positive reactions were identified for tilapia eDNA when there was the presence of an amplification curve in the quantitation analysis, as well as a distinct peak of the right size compared with positive controls of known tilapia eDNA in the melt analysis. For all water samples which tested positive for tilapia eDNA the corresponding equipment control was then analysed to test for contamination (phase 3). If the equipment control was clear of contamination as well as the site negative control sample the water sample was then classified as positive for tilapia eDNA. Failure to meet any of these quality control measures resulted in the sample being classified as “status uncertain” due to eDNA contamination.

Water Quality

Water quality parameters including temperature, pH, dissolved oxygen and conductivity were measured using an Aqua Read AP-2000. The water quality sampling method involved placing the probe into the water at a depth of 0.1 m. After readings had stabilised, values were recorded for each of the water quality parameters. Secchi depths were obtained by lowering a 200 mm secchi disk into the water column until the disk could no longer be seen. The disk was then raised until the contrast between the black and white portions was discernable and the depth value recorded. To account for localised variability, water quality readings and secchi depths were averaged from readings taken at three locations within site.

Results

Preliminary Survey

Fish Communities

Pooled counts from all sites during the preliminary survey identified 28 species — 26 native and two introduced (Table 3). No tilapia were identified using electrofishing/netting methods during the preliminary surveys, however, mosquitofish (another pest species) was sampled from 8 of the 13 sites (Table 3). Tilapia DNA was detected by eDNA sampling from 4 sites, 3 sites within the Gooseponds Lagoons complex as well as McCready Creek 01 (Table 3).

The native fish assemblages present at the survey sites were typical of the communities that occur within each of the habitat types sampled. There was no discernable difference in community composition at sites with similar habitat types regardless of tilapia presence. The greatest variability in community composition appeared to be driven by variations in habitat types and condition between the sites. In general, large instream pools with abundant macrophyte growth recorded greater species diversity than small instream pools that had high riparian shade potential and low macrophyte growth. The lowest species diversity were recorded at Beaconsfield 01 and Vines Creek 02 which each recorded 3 native fish species. Beaconsfield 01 was a stormwater drain and Vines Creek 02 was a small instream pool adjacent to the estuary mangrove flats. Both sites were small in size and lacked habitat complexity.

Water quality parameters recorded during sampling were also typical of habitat, time of day and season, and were all within acceptable levels for healthy fish communities (Table 2). Vines Creek 02 recorded a very high Electrical Conductivity (EC) readings, which typical of a site which experiences occasional inundation with tidal water from the adjacent estuary.

Table2. Water quality parameters recorded during prelimiary tilapia detection sampling in the Lower Pioneer Catchment

Site	Temperature (C°)	pH	EC (µs/cm)	DO (%sat)	Secchi depth (m)
Beaconsfield 01	28.60	7.65	1322	-	0.20
Beaconsfield 03	31.00	8.08	463	102.30	0.70
Botanic Gardens	31.73	7.66	84	108.07	0.30
Gooseponds 01	31.53	7.93	272	71.87	0.58
Gooseponds 02	33.93	8.40	227	130.60	0.72
Gooseponds 03	33.50	8.44	236	112.50	1.01
Gooseponds 04	-	-	-	-	-
Gooseponds 05	30.22	7.91	390	41.57	0.59
Janes Creek 01	28.97	7.72	654	-	>0.6
Janes Creek 02	26.87	7.64	692	-	>1.2
Janes Creek 03	25.73	7.41	681	-	>0.6
McCready Creek 01	29.47	8.06	869	-	>1.2
Vines Creek 02	30.10	8.13	54300	94.00	>0.6

Table 3. Fish community assemblages recorded during secondary tilapia distribution sampling in the Lower Pioneer Catchment. ✓ denote species recorded during electrofishing operations, * denote positive eDNA indications, NS – sites not sampled for eDNA.

	Native Fish																										Pest Fish		
Common Name	*Agassizis glassfish*	*Banded scat*	*Barramundi*	*Barred grunter*	*Bony bream*	*Eastern rainbowfish*	*Empire gudgeon*	*Flyspecked hardyhead*	*Fork tailed catfish*	*Freshwater longtom*	*Hyrtl's catfish*	*Long-finned eel*	*Midgleys carp gudgeon*	*Milkfish*	*Mouth almighty*	*One-gilled eel*	*Purple-spot gudgeon*	*Sea mullet*	*Silver bidy*	*Sleepy cod*	*Snake head gudgeon*	*Sooty grunter*	*Spangled perch*	*Speckled goby*	*Tarpon*	*Yellow-fin bream*	**Total Native Species**	*Mosquito fish*	**Tilapia**	
																												Efishing/Net	**eDNA**
Beaconsfield 01						✓	✓											✓									**3**	✓		
Beaconsfield 03		✓	✓		✓	✓	✓	✓				✓		✓									✓		✓		**10**	✓		
Botanic Gardens	✓		✓		✓	✓	✓	✓				✓	✓		✓					✓				✓	✓		**12**	✓		
Gooseponds 01		✓	✓		✓	✓	✓	✓	✓	✓		✓						✓	✓	✓			✓		✓		**14**	✓		*
Gooseponds 02			✓		✓	✓	✓	✓				✓				✓		✓		✓	✓				✓		**11**			
Gooseponds 03			✓	✓	✓	✓	✓	✓				✓			✓			✓					✓		✓		**11**			*
Gooseponds 04			✓		✓	✓		✓	✓		✓	✓				✓				✓	✓						**10**			NS
Gooseponds 05			✓		✓	✓	✓	✓	✓		✓	✓								✓		✓	✓		✓		**12**			*
Janes Creek 01						✓	✓					✓													✓		**4**	✓		
Janes Creek 02						✓	✓					✓					✓										**4**	✓		
Janes Creek 03						✓	✓					✓					✓										**4**	✓		
McCready Creek 01			✓			✓	✓	✓							✓	✓		✓									**7**	✓		*
Vines Creek 02		✓																✓								✓	**3**			
Species Name	*Ambassis agassizii*	*Selenotoca multifasciata*	*Lates calcarifer*	*Amniataba percoides*	*Nematalosa erebi*	*Melanotaenia splendida splendida*	*Hypseleotris compressa*	*Craterocephalus stercusmuscarum*	*Arius graeffei*	*Strongylura kreffftii*	*Neosilurus hyrtlii*	*Anguilla reinhardtii*	*Hypseleotris species 1*	*Chanos chanos*	*Glossamia aprion*	*Ophisternon bengalense*	*Mogurnda adspersa*	*Mugil cephalus*	*Gerres filamentosus*	*Oxyeleotris lineolatus*	*Giurus margaritacea*	*Hephaestis fuliginosous*	*Leiopotherapon unicolor*	*Rediogobius bikolanus*	*Megalops cyprinoides*	*Acanthopagrus australis*	**26**	*Gambusia holbrooki*	***Oreochromis mossambicus***		

Environmental DNA

Of the 12 sites sampled, four sites resulted in positive detections for tilapia eDNA and the remaining eight sites had no detectable DNA (Table 4). The sites that resulted in positive tilapia detections were, Gooseponds 01, Gooseponds 03, Gooseponds 05 and McCready Creek 01. The number of positive samples at each site ranged from 1 – 3 and percentage of positive PCR reactions varied from 4 – 16%. All negative control samples and equipment controls of corresponding positive samples were clear of contamination.

Table 4: Results of the preliminary eDNA tilapia survey of the Lower Pioneer Catchment, November 2014. Results are presented as both an overall positive or negative assay for tilapia eDNA in each sample (total five samples per site) and positive or negative for each qPCR reaction (total 25 qPCR reactions per site).

Site	Date Sampled	Number of Samples	Number Positive	% Positive	Number of Reactions	Number Positive	% Positive
Beconsfield 01	18/11/2014	5	0	0%	25	0	0%
Beconsfield 03	17/11/2014	5	0	0%	25	0	0%
Botanic Gardens	17/11/2014	5	0	0%	25	0	0%
Gooseponds 01	18/11/2014	5	1	20%	25	1	4%
Gooseponds 02	18/11/2014	5	0	0%	25	0	0%
Gooseponds 03	18/11/2014	5	3	60%	25	4	16%
Gooseponds 05	17/11/2014	5	1	20%	25	3	12%
Janes Creek 01	17/11/2014	5	0	0%	25	0	0%
Janes Creek 02	17/11/2014	5	0	0%	25	0	0%
Janes Creek 03	17/11/2014	5	0	0%	25	0	0%
McCready Creek 01	18/11/2014	5	2	40%	25	2	8%
Vines Creek 02	18/11/2014	5	0	0%	25	0	0%

Secondary Survey

Fish Communities

Pooled counts from all sites during the secondary survey identified 20 species — 17 native and three introduced (Table 6). Tilapia were identified from two sites (Gooseponds 1a and Gooseponds 3a) using electrofishing/netting methods during the secondary survey. A total of 16 tilapia were recorded at a catch rate of 0.56 fish/min. The size of tilapia recorded were relatively small, ranging from 20 mm – 45 mm (Figure 2). Tilapia DNA was detected by eDNA sampling from a further 3 sites within the McCready Creek system (Table 6). Mosquitofish were also sampled from 21 of the 25 sites and guppy were sampled from 4 of the sample sites (Table 6).

Similar to the preliminary survey, native fish assemblages were typical of habitat type. There was no discernable difference in species composition at sites with similar habitat characteristics regardless of the presence of tilapia. Lowest species diversity was recorded from stormwater drains, followed by small instream pools with high riparian shade potential and limited macrophyte growth.

Figure 2. Average size and standard deviation of pooled tilapia captures from Gooseponds 1a and Gooseponds 3a, sampled during secondary tilapia detection surveys of the Lower Pioneer Catchment.

Water quality parameters recorded during secondary survey were generally typical of habitat, time of day and season, and within acceptable levels for healthy fish communities (Table 5). Notably, Gooseponds 5a recorded a pH reading of 10.39, discernably higher than all other sites sampled during this investigation.

Table 5. Water quality parameters recorded during secondary tilapia detection sampling in the Lower Pioneer Catchment

Site	Temperature (C°)	pH	EC (µs/cm)	DO (%sat)	Secchi depth (m)
Beaconsfield 01	23.90	8.26	245	132.70	
Fursden Creek 01	21.37	8.53	248	133.33	0.50
Fursden Creek 02	21.10	7.77	435	61.30	-
Gooseponds 01	31.97	7.12	282	106.60	0.70
Gooseponds 1a	22.90	7.35	366	71.40	-
Gooseponds 02	31.05	7.48	271	106.12	0.89
Gooseponds 2a	24.90	7.59	410	118.60	-
Gooseponds 3a	25.20	7.48	389	101.70	-
Gooseponds 04	26.20	7.19	295	21.03	1.03
Gooseponds 4a	25.90	7.48	359	119.50	-
Gooseponds 05	28.93	7.66	413	119.70	0.60
Gooseponds 5a	28.30	10.39	313	238.70	-
Gooseponds 5b	21.60	7.12	527	30.50	-
Janes Creek 01	21.00	7.20	596	68.10	-
Janes Creek 02	20.90	7.59	684	37.00	-
Janes Creek 03	21.20	7.25	691	71.40	-
McCready Creek 01	17.50	7.09	350	80.40	-
McCready Creek 02	22.60	7.94	451	84.00	0.93
McCready Creek 03	22.03	7.44	223	97.97	0.50
McCready Creek 04	22.83	6.69	94	50.97	1.03
McCready Creek 05	21.20	7.48	671	95.90	-
McCready Creek 06	19.40	7.66	696	88.70	-
McCready Creek 07	18.30	7.52	622	97.80	-
McCready Creek 08	23.40	7.57	504	100.00	1.30
McCready Creek 09	24.50	8.38	281	135.90	-
McCready Creek 10	20.10	7.77	613	107.50	-
McCready Creek 11	18.10	7.54	694	97.10	-
McCready Creek 12	25.00	8.18	133	110.70	-

Table 6. Fish communityassemblages recorded during secondary tilapia distribution sampling in the Lower Pioneer Catchment. ✓ denote species recorded during electrofishing operations, * denote positive eDNA indications, NS – sites not sampled for eDNA. Value in brackets indicates number of tilapia identified from the site.

	Native Fish																			Pest Fish		
Common Name	Agassizis glassfish	Barramundi	Bony bream	Eastern rainbowfish	Eel-tailed catfish	Empire gudgeon	Flyspecked hardyhead	Fork tailed catfish	Guppy	Long-finned eel	Midgleys carp gudgeon	One-gilled eel	Purple-spot gudgeon	Sea mullet	Sleepy cod	Sooty grunter	Spangled perch	Tarpon	**Total Native Species**	Mosquito fish	**Tilapia**	
																					Efishing/Net	**eDNA**
Beaconsfield 01				✓															1	✓		NS
Fursden Creek 01	✓			✓		✓	✓			✓								✓	6			
Fursden Creek 02						✓				✓									2	✓		
Gooseponds 01		✓													✓				2			NS
Gooseponds 1a				✓		✓	✓			✓					✓				5	✓	✓	NS
Gooseponds 02		✓													✓				2			NS
Gooseponds 2a				✓		✓	✓			✓			✓						5	✓		NS
Gooseponds 3a				✓		✓	✓			✓			✓						5	✓	✓	NS
Gooseponds 04		✓						✓							✓				3			NS
Gooseponds 4a	✓			✓		✓	✓			✓					✓				6	✓		NS
Gooseponds 05		✓	✓	✓	✓	✓	✓	✓		✓					✓	✓			10	✓		NS
Gooseponds 5a				✓		✓	✓			✓		✓			✓				6	✓		NS
Gooseponds 5b						✓			✓	✓					✓				4	✓		NS
Janes Creek 01				✓		✓				✓		✓							4	✓		NS
Janes Creek 02				✓		✓				✓							✓		4	✓		NS
Janes Creek 03				✓		✓			✓	✓		✓	✓						6	✓		NS
McCready Creek 01	✓			✓		✓	✓			✓		✓						✓	7	✓		
McCready Creek 02		✓	✓	✓		✓	✓			✓		✓						✓	8	✓		
McCready Creek 03		✓	✓	✓		✓	✓			✓	✓						✓	✓	9			
McCready Creek 04		✓	✓	✓		✓	✓			✓				✓				✓	8	✓		
McCready Creek 05				✓		✓				✓			✓						4	✓		✓
McCready Creek 06				✓		✓			✓	✓			✓						5	✓		
McCready Creek 07				✓		✓				✓									3	✓		
McCready Creek 08		✓	✓	✓		✓				✓					✓			✓	7	✓		✓
McCready Creek 09				✓		✓				✓							✓		4	✓		
McCready Creek 10	✓									✓		✓	✓						4	✓		✓
McCready Creek 11				✓		✓			✓	✓									4	✓		NS
Species Name	*Ambassis agassizii*	*Lates calcarifer*	*Nematalosa erebi*	*Melanotaenia splendida splendida*	*Tandanus tandanus*	*Hypseleotris compressa*	*Craterocephalus stercusmuscarum*	*Arius graeffei*	*Poecilia reticulata*	*Anguilla reinhardtii*	*Hypseleotris species 1*	*Ophisternon bengalense*	*Mogurnda adspersa*	*Mugil cephalus*	*Oxyeleotris lineolatus*	*Hephaestis fuliginosous*	*Leiopotherapon unicolor*	*Megalops cyprinoides*	18	*Gambusia holbrooki*	***Oreochromis mossambicus***	

Environmental DNA

Of the 12 sites sampled, three sites resulted in positive detections for tilapia eDNA and the remaining eight sites had no detectable DNA (Table 7). The sites that resulted in positive tilapia detections were, McCready Creek 05, McCready Creek 08 and McCready Creek 10. The number of positive samples at each site ranged from 1 – 3 and percentage of positive PCR reactions varied from 4 – 12%. All negative control samples and equipment controls of corresponding positive samples were clear of contamination. McCready Creek 01, which returned a positive indication for tilapia eDNA in the preliminary survey failed to return a positive indication in the secondary survey.

Table 7: Results of the preliminary eDNA tilapia survey of the Lower Pioneer Catchment, November 2014. Results are presented as both an overall positive or negative assay for tilapia eDNA in each sample (total five samples per site) and positive or negative for each qPCR reaction (total 25 qPCR reactions per site).

Site	Date Sampled	Number of Samples	Number Positive	% Positive	Number of Reactions	Number Positive	% Positive
Fursden Creek 01	26/05/2015	5	0	0%	25	0	0%
Fursden Creek 02	26/05/2015	5	0	0%	25	0	0%
McCready Creek 01	25/05/2015	5	0	0%	25	0	0%
McCready Creek 02	26/05/2015	5	0	0%	25	0	0%
McCready Creek 03	25/05/2015	5	0	0%	25	1	4%
McCready Creek 04	26/05/2015	5	0	0%	25	0	0%
McCready Creek 05	25/05/2015	5	2	40%	25	0	0%
McCready Creek 06	25/05/2015	5	0	0%	25	0	0%
McCready Creek 07	25/05/2015	5	0	0%	25	0	0%
McCready Creek 08	26/05/2015	5	3	60%	25	3	12%
McCready Creek 09	26/05/2015	5	0	0%	25	0	0%
McCready Creek 10	25/05/2015	5	1	20%	25	1	4%

Discussion

Based on the results of this investigation it is confirmed that tilapia are present at several locations in Lower Pioneer Catchment. At present the distribution appears to be limited to the Gooseponds Lagoons complex and McCready Creek with electrofishing and eDNA surveys of Fursden Creek and Botanic Gardens not identifying the presence of tilapia at either location. In addition no community reports of tilapia from other locations within the region have been received at the time of reporting.

Tilapia were captured from 2 of the 31 sites sampled as part of this investigation. Both locations were within the Goosponds Lagoons complex and had previous confirmed reports of tilapia at each site. Environmental DNA sampling detected the presence of tilapia DNA from two sites in close proximity to electrofishing captures as well as a further 5 locations where electrofishing failed to identify the presence of tilapia. Similar tilapia distribution surveys in the Fitzroy catchment that utilised both electrofishing/netting and eDNA methods documented similar results with eDNA recording higher detection rates than electrofishing/netting (Power 2014, Power 2015). The higher detection rates of eDNA sampling in the current and previous investigations suggest, as a preliminary detection method, this technique may be more effective than traditional methods such as electrofishing and netting. It must be noted however, that eDNA does not provide information on the size and abundance of tilapia, both of which are important when developing management strategies. Traditional sampling

methods also provide data on native fish assemblages, which is equally important when assessing and monitoring the impacts of tilapia and any control measures implemented.

The lack tilapia captures from eDNA positive sites in McCready Creek and the relatively low catch rates from the two sites in the Gooseponds, suggest that the abundance of tilapia in these systems is low. Although the abundance of tilapia is low, the presence of juvenile fish indicates the population has become established. In addition confirmed reports of tilapia up to 200 mm have been recorded at several locations in the Gooseponds Lagoons complex, providing further evidence of an established population. A study investigating the biology of tilapia in Wet Tropics, North Queensland reported growth rates between 50 mm to 100 mm for the first 2-3 years after which plateaus to 20 mm to 30 mm per year. With the assumption that tilapia from the Gooseponds displayed similar growth rates it was estimated that the current population has been present for 2-3 years.

Species diversity recorded during this investigation was in the most part comparable to other fish studies conducted throughout the region (Moore et al. 2007). Of concern, however, was the fish community of Fursden Creek which recorded only 6 native species, considerably less than similar habitats sampled as part of the current work. Without further investigation it is not possible to precisely identify the reason(s) for the apparent low species diversity recorded at these sites, however given the close proximity to the estuary and reduced number of catadroumous species it is likely that restrictions in connectivity would be a contributing factor. This is supported by work conducted by Moore (2015) who undertook a barrier prioritisation of the region, and identified two significant barriers (one below each of the sample locations) in the lower reaches of Fursden Creek.

The number of native fish species at several of the sites where tilapia were present was greater than tilapia free sites. Although this may be an indication that tilapia are not having as great an impact on diversity as other pressures (e.g. connectivity, habitat degradation) it is likely that competition with tilapia for resources will have some impact on local fish communities. There is evidence from controlled experiments that this species of tilapia has the potential to significantly reduce the biomass of several native macrophytes (Doupe. et al. 2010). Therefore it is possible that, in high numbers, similar reductions in macrophyte cover may occur at these sites reducing food availability for native fish.

Water quality parameters recorded at confirmed tilapia sites were typical of seasonal conditions and habitat types and did not appear to be adversely affected by current tilapia densities. The low and high DO readings from several locations can be attributed to the time sampling was conducted. DO levels in freshwater streams and lagoons with limited water circulation vary on a diurnal cycle as a result of changes in the rate of phytoplankton and macrophyte photosynthesis (Kayombo et al 2002). During the evening photosynthesis ceases while respiration of the aquatic flora and fauna continues leading to troughs in the DO cycle early morning. As photosynthesis increases throughout the day, DO levels also increase, peaking mid-afternoon. Sampling in the early morning and midafternoon corresponded to the normal troughs and peaks of the DO cycle. Similar to DO, the elevated pH readings recorded from Gooseponds 5a was most likely due to the very high level of macrophyte growth, shallow pool depth and lack of water circulation. High levels of photosynthesis reduce CO_2 levels in the water column resulting in a higher proportion of carbonate ions and spikes in pH during peak periods of photosynthesis (Tucker and D'Abramo 2008). In most circumstances increased CO_2 production in the evening reverses this allowing pH to cycle back to a lower level. The time of sampling at Gooseponds 5a corresponded to the time of the day when photosynthesis would have been at a peak with pH levels also being elevated as a result.

Recommendations

- Given the presence of breeding populations of tilapia at several locations in the Gooseponds, eradication is not feasible. A concerted effort on public awareness and education conveying the presence of tilapia and the threat this species poses on existing ecosystems will be the most effective control measure to reduce the spread of tilapia throughout the Mackay and Whitsunday region.

- Further sampling to more thoroughly determine the extent of the tilapia distribution within the Pioneer system. By determining the incursion 'front line', awareness products such as information signs can be placed at strategic sites to help raise public awareness. The use of eDNA may provide a more cost effective method for sampling a large number of sites. After the presence of tilapia is detected further sampling with other methods such as electrofishing or netting can be utilised to determine the structure of these populations and impacts to native fish.

- Investigation into the impact of tilapia on aquatic macrophytes at sites with established populations. This will aid in determining the impact tilapia have on native fish.

- There is little documented information on the impacts of tilapia in native fish in Australia. Ongoing monitoring of native fish communities will help understand the impact of tilapia within the Gooseponds and McCready Creek. Sampling annually for the next 3 years then every 3-5 years thereafter will provide suitable data to determine the impact of tilapia on existing fish assemblages.

- Habitat improvement (e.g. log hotels, riparian restoration, fishway construction etc…) of confirmed tilapia sites to increase resource availability to native fish communities and existing ecosystems and reduce competition with tilapia and other noxious species.

- Water transfer from the Gooseponds and McCready Creek should be avoided if possible. Extraction is common for road maintenance and other developments, tilapia may survive being pumped into water trucks which may then be discharged at locations close to unaffected waterways. When water extraction is not avoidable, control measures should be put in place to ensure water discharged from trucks does not run off into adjoining waterways. Such measure may include, absorption barricades (e.g. hay bales) or specific direction to operators at discharge locations.

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Supporting Document

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Appendix 1

Habitat Description Criteria

Category	Classification criteria	
Flow	Estimated rate of flow; (1) 0 m/sec, (2) <0.1 m/sec, (3) 0.1-0.5 m/sec, (4) 0.51-1.0 m/sec, (5) 1.01-3.0 m/sec, (6) >3.0 m/sec	
Water Body Type	Run	In stream water body with unbroken flowing water >0.1 m/sec
	Riffle	In stream water body with broken flowing water <0.1 m depth
	Rapid	In stream water body with broken flowing water >0.1 m depth
	Weir Pool	Water body contained within a stream channel created by an artificial structure
	In stream Pool	In stream water body contained within a stream channel with flow <0.1 m/sec
	Off stream pool	Off stream water body that connects to a stream channel during periods of elevated flow
	Impoundment Large	Water body >10 hectare that extend beyond stream channel or located off stream created by an artificial structure
	Impoundment Small	Water body <10 hectare that extend beyond stream channel or located off stream created by an artificial structure
	Natural Wetland	Naturally occurring series of interconnected water bodies that extend beyond a stream channel or located off stream
	Constructed Wetland	Artificial series of interconnected water bodies that extend beyond a stream channel or located off stream
	Stormwater Drain	Artificial drainage channels that extend beyond a stream channel or located off stream
Average Site Width	Calculated using spatial software and aerial imagery by averaging ten evenly spaced distance measurements of the water body width along the length of the site	
Site Length	Calculated using spatial software and aerial imagery by measuring the middle stream length of water body sampled, this may be portion of total water body length	
Average Riparian Width	Calculated using spatial software and aerial imagery by averaging ten evenly spaced distance measurements of riparian width along the length of the site, each measurement encompasses both banks	
Riparian Condition	Estimated condition of riparian zone; (1/Very Low) cleared to waters edge - very few trees and shrubs, (2/Low) 1-5 m width - some trees and shrubs,(3/Moderate) 5-30 m width - some trees and shrubs, 5-10 m width - many trees and shrubs (4/High) 10-30 m width - many trees and shrubs, (5/Very High) undisturbed riparian zone	
Riparian Shade Potential	Estimated potential for riparian vegetation to shade water body; (1/Very Low) <10% daylight hours, (2/Low) 10-35% of daylight hours, (3/Moderate) 35-65% daylight hours, (4/High) 75-90% daylight hours, (5/Very High) >90% daylight hours	
Aquatic Macrophyte	Estimated coverage of aquatic macrophytes at a site, includes: submerged, floating and emergent types; (1/Very Low) <10% coverage, (2/Low) 10-35% coverage, (3/Moderate) 35-65% coverage, (4/High) 65-90% coverage, (5/Very High) >90% coverage	

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