Environmental DNA (eDNA) Survey for Platypus Across Ipswich

2022

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Review Table

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Contents

Summary

The Wildlife Preservation Society of Queensland (Wildlife Queensland) is a not-for-profit, non-Government wildlife advocacy organisation. Wildlife Queensland manage the PlatypusWatch Network, a focus group for community engagement, conservation, education and research on the platypus in Queensland.

To better understand platypus distribution in the Ipswich region, Wildlife Queensland has partnered with the Ipswich City Council who have provided funding for an environmental DNA (eDNA) project. This project is in its seventh year, collating important distribution data on local platypus populations.

Since the 2021 eDNA sampling period South East Queensland has experienced substantial flooding events (November 2021 and February/March 2022), which impact platypus distribution and habitat. Therefore, this year's sampling was important to determine any impact these events may have caused on populations.

This year (2022), 22 sites were sampled across 10 waterways, one site was included within the Brisbane LGA. Platypus DNA was not identified in any samples collected within the Ipswich region. There were three equivocal sites, meaning there were traces of DNA found but not enough to confidently confirm presence of platypuses. Two of these three waterways have had positive DNA in previous years of sampling. One site was sampled in a lower section of Sandy Creek in the Brisbane City Council LGA, and was positive.

Habitat quality index varied across the sample locations and all sites were visibly impacted by flood waters. Erosion was prominent and vegetation disturbed, some sites would favour priority rehabilitation. There is also pipeline infrastructure extending along Opossum Creek which may impact the surrounding riparian zone and disturb the platypus population in the creek.

This year's results present some concern with regards to the density and viability of the platypus populations inhabiting waterways in Ipswich. This may be due to potential flood displacement of animals or the water conditions the samples were collected from. A second round of sampling later during this year's (2022) breeding season should be considered and continuing annual eDNA surveys will remain essential for monitoring this cryptic species to help determine persistence and changes in populations across the Ipswich region.

The on-going implementation of habitat protection and rehabilitation around the catchments in eastern Ipswich will aid the long-term viability of the local platypus population. Platypus preferred habitat features should be protected or rehabilitated in these catchments to better conserve platypus populations.

Introduction

Platypus are listed as 'near threatened' on the International Union for Conservation of Nature Red List (IUCN) (Woinarski and Burbidge 2016). Continued monitoring of platypus populations across their range is vital to understand their future conservation requirements. The species is found in freshwater habitats of varying quality. However, they require key habitat requirements including deep pools, high consolidated sloping banks and cobbled stony substrates to carry out their life history events (Grant 2007).

The platypus' elusive behaviour, making species distribution and abundance difficult to confirm through observation surveys; they may not be observed in an area but may still be present (Grant 2012). Environmental DNA (eDNA) analysis is a powerful tool to detect platypus DNA within waterways without the time and labour constraints of using traditional techniques. This method of detecting a species is cost effective, accurate and has an absolute minimal environmental disturbance (Goldberg et al. 2011). The Wildlife Preservation Society of Queensland in partnership with the Melbourne based consultancy EnviroDNA have developed this sampling program over the last seven years for the purpose of defining the current distribution of platypus across the south east Queensland region.

In 2016 an environmental DNA project targeting platypus was developed within the Ipswich City Council region. This survey has occurred once annually for seven years, providing Wildlife Queensland with a clear indication on the distribution of platypus across the region.

In Ipswich, 50 sites across 12 waterways have been sampled (Appendix 2) over seven consecutive years. This year, 22 sites across ten waterways were sampled for platypus eDNA with three new sites being included in the survey. This year only one positive was detected in the lower section of Sandy Creek in Wacol, Brisbane City Council LGA.

This year's eDNA sampling is of great importance due to the occurrence of two major flood events within south-east Queensland. Floods impact platypus populations by destroying habitat and impacting food resources. It can also directly impact the animal by inundating burrows and flushing individuals downstream, away from their territories. However, with the increased amount of water entering the catchment it can also help with connectivity of smaller tributaries, especially during breeding season.

This project important is for identifying platypus population persistence and distribution. The ongoing monitoring of platypuses within the Ipswich region may help facilitate the detection of any decline in current populations and potential displacement due to the flooding events. The project also helps to identify key habitat characteristics associated with platypus inhabitation and detect any deterioration of that habitat over time of after major weather events, such as this year's flooding. These results will enable a better

understanding of platypus habitat and may help drive future conservation strategies to protect platypuses and their habitat.

Aim

1. To accurately identify platypus presence in selected waterways within the Ipswich City Council local government area (LGA) using the eDNA method;
2. Establish and contribute to a longitudinal platypus survey in key locations across Ipswich;
3. Ability to identify platypus distribution changes over time; and
4. Determine habitat quality in association with platypus presence.

Methods

Observation data

Queensland State Government Wildnet database, Atlas of Living Australia and Platypus Watch Network records were used to source recent platypus sightings records within Queensland and specifically the Ipswich LGA.

Site selection for environmental DNA sample collection sites

Wildlife Queensland in conjunction with Jack McCann (Waterway Health Officer, Ipswich City Council) selected sites to be sampled based on their history of platypus sightings data. The sample location details are provided in Table 1 and Figure 1. Three new samples sites were added, Bundamba Creek (BUND10), upon a recoded sighting in May 2022, Ironpot Creek (IRPOT01) and Purga Creek (PURG02).

Table 1: Water sample locations in eight waterways.

Site Code	Location	Latitude	Longitude
BREM01	Bremer River	-27.6179	152.7413
BREM03	Bremer River	-27.6367	152.7461
BREM07	Bremer River	-27.6355	152.7316
BUND08	Bundamba Creek	-27.6359	152.7908
BUND04	Bundamba Creek	-27.6186	152.8077
BUND10	Bundamba Creek	-27.6942	152.8043
GOOD02	Goodna Creek	-27.6074	152.872
IP01	Iron Pot Creek	-27.6015	152.7326
OPOSS01	Opossum Creek	-27.6562	152.9002
OPOSS02	Opossum Creek	-27.6453	152.8942
PUR02	Purga Creek	-27.6828	152.7284
SAND01	Sandy Creek	-27.6253	152.9209
SAND02	Sandy Creek ***BCC***	-27.6061	152.9279
SAND04	Sandy Creek	-27.6347	152.9264
SIXM03	Six Mile Creek	-27.6067	152.8588
SIXM04	Six Mile Creek	-27.6558	152.8401
SIXM06	Six Mile Creek	-27.6394	152.8463
WARR01	Warrill Creek	-27.6575	152.699
WOOG02	Woogaroo Creek	-27.618	152.9065
WOOG03	Woogaroo Creek	-27.6322	152.9038
WOOG04	Woogaroo Creek	-27.6473	152.8881
WOOG01	Woogaroo Creek	-27.6152	152.9087

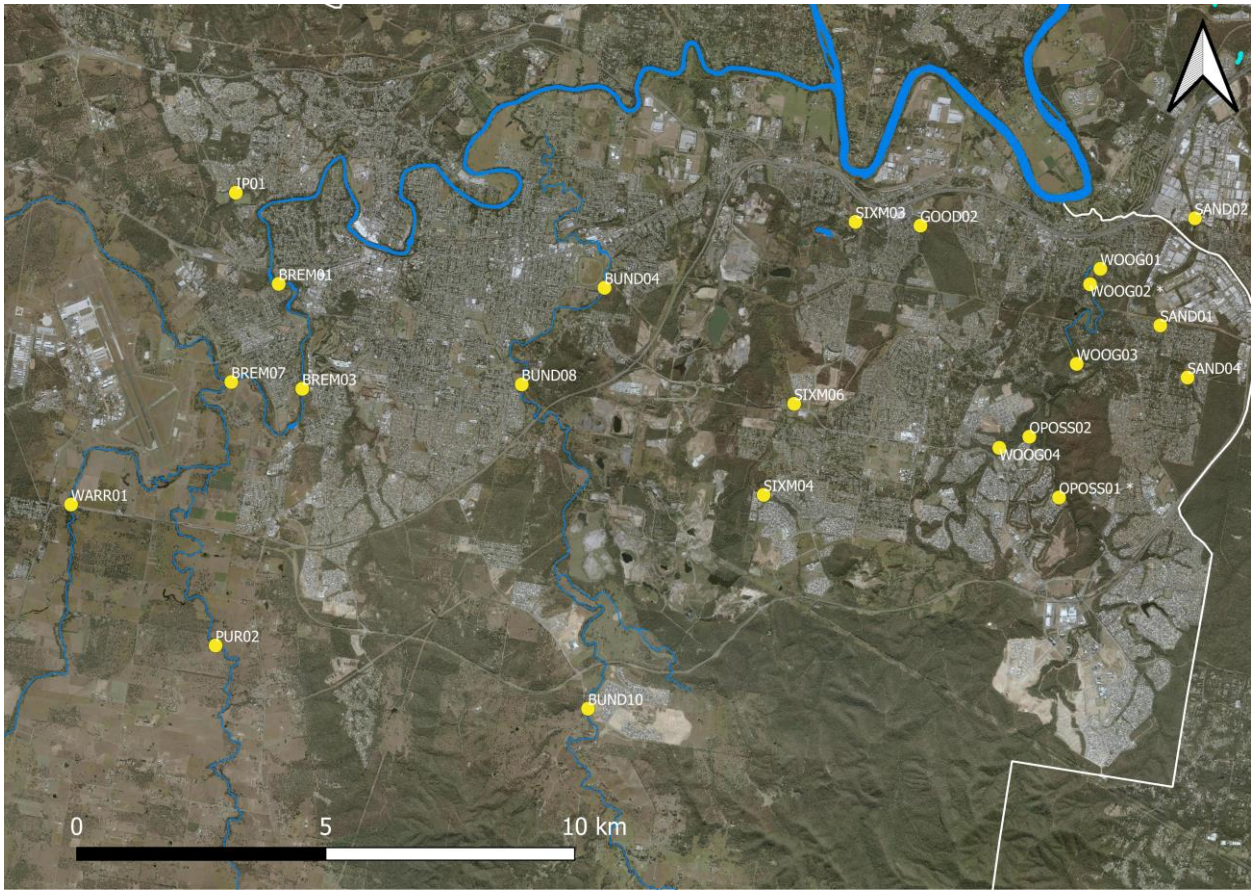

Figure 1: Locations of chosen waterways within Ipswich City Council Region, Bremer River, Bundamba Creek, Iron Pot Creek, Goodna Creek, Purga Creek, Opossum Creek, Sandy Creek, Six Mile Creek, Warrill Creek and Woogaroo Creek.

Environmental DNA Sampling to determine platypus distribution

Environmental DNA method as per EnviroDNA method (Griffiths et al. 2015). Water samples were collected in duplicate at one or more independent sites in each identified waterway (i.e., upper, mid and lower sections). Water sample volumes between 100 ml and 500 ml were collected and filtered through a 1.2µm filter (Sterivex) using a 50 ml sterile syringe. Filters containing collected filtrate material were preserved using 100% ethanol and transported overnight to the EnviroDNA laboratory (Melbourne) for analysis. Care was taken to avoid contamination between sites following prescribed methods.

DNA was extracted from the filters using DNA extraction kit (Qiagen DNeasy Blood and Tissue kit). Species-specific primers were developed by EnviroDNA to target a small section (57 bp) of the platypus mitochondrial gene *cytochrome b* (CytB) (Life Technologies). Amplification of the target DNA will be completed through the use of real time polymerase chain reaction (PCR) assays. Each sample is prepared in triplicate and all assays contained positive and negative controls.

Water samples were collected 2nd and 3rd of June 2022. The sample period was chosen to take advantage of an expected increase in platypus activity associated with platypus breeding behaviours.

Habitat Quality Index

A selection of habitat characteristics known to be associated and beneficial for platypus requirements was collated into a habitat quality index (Grant 2014), allowing for a quick assessment of the habitat quality of each sample site. Each Ipswich eDNA sample location was assessed using the habitat quality index (Table 2).

Table 2: Habitat Quality Index table with known or potential benefit to platypuses (Adapted from Grant 2014).

Habitat variable	**Known or potential benefit to platypuses**	**SCORE**
Bank variables (Score 0 = none, 1 = <25%, 2 = 25-49%, 3 = 50-74%, 4 = >75%)		
Consolidated banks	Maintenance of burrows, reduced in-stream sedimentation	
Large-medium sized trees on banks	Consolidation of banks, organic input to aquatic ecosystem	
Overhanging vegetation <2m above water	Consolidation of banks, organic input to aquatic ecosystem, lower predation risk due to shelter while foraging and entering/leaving burrows	
Earthen banks	Allows construction and maintenance of burrows	
Bank height >1m	Preferred bank morphology for burrows construction and maintenance	
Concave or near vertical banks	Secure access to burrow, hide entrance, lower predation risk	
Absence of erosion	Maintenance of burrows, maintenance of riparian vegetation, reduced in-stream sedimentation	
In-stream variables (Score 0 = none, 1 = <25%, 2 = 25-49%, 3 = 50-74%, 4 = >75%)		
Pool depth (>1m but<5m)	Preferred foraging depth for platypuses, lower risk of predation	
Large woody debris (LWD, >10cm diameter)	Habitat and food for benthic invertebrate prey	
Complex benthic substrate (cobbled, gravel)	Favourable habitat for benthic invertebrate prey	
Coarse organic matter – if visible	Favourable habitat for benthic invertebrate prey	
Total		\44

Results

Environmental DNA (platypus)

Ten waterways (21 sites) were sampled in the Ipswich LGA during the 2022 eDNA survey (2/3 June 2022). No platypus DNA was able to be strongly detected at any survey sites this year.

One site was sampled outside the ICC region at SAND02, Wacol Brisbane and platypus DNA was detected, downstream of the Ipswich site SAND01 (Figure 2, Appendix 1).
Equivocal results were recorded at WOOG04, GOOD02 and SAND01.

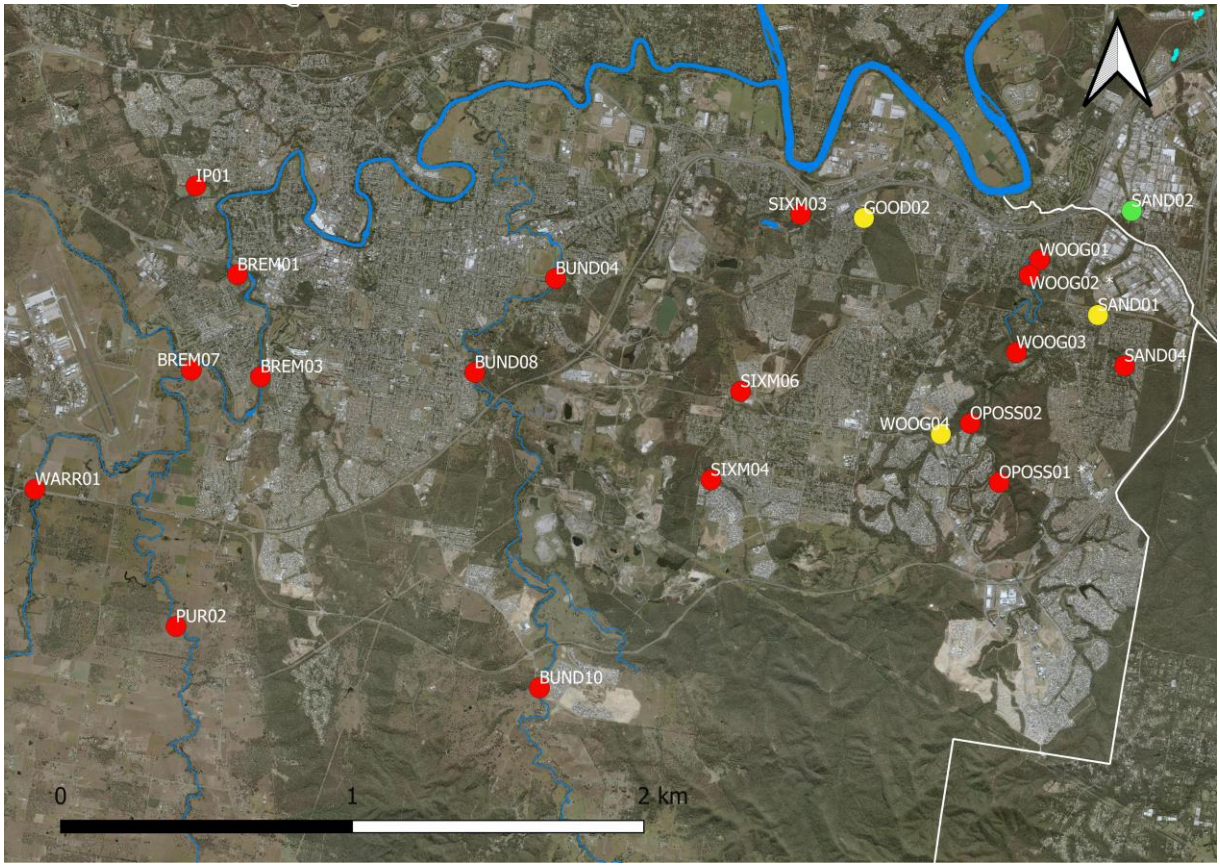

Figure 2: Environmental DNA results for Ipswich City Council sites 2022. Positive samples (green circles), negative samples (red circles), equivocal samples (yellow circles).

Habitat Quality Index

The habitat quality scores (Table 3) ranged from 39% to 86%. Opossum Creek site 3 scored low (39%) due to a lack of deep pools, minimal high stable banks, vegetated by invasive species, including in-stream and had a muddy/sandy substrate. Woogaroo Creek site 2 recorded the highest habitat score quality (86%) and had dense vegetation cover, stable consolidated banks and complex gravel and cobbled substrate. All sites had noticeable levels of flood damage, some had more sever levels of damage than others.

Table 3: Habitat quality index score and percent for each sample location.

Waterway	Site	HQI score	Percentage (%) (HQI/44)
Bremer River	BREM01	35	80
Bremer River	BREM03	34	77
Bremer River	BREM07	36	81
Bundamba Creek	BUND04	30	68
Bundamba Creek	BUND08	25	57
Bundamba Creek	BUND10	27	61
Iron Pot Creek	IRPOT01	21	48
Opossum Creek	OPOSS01	26	59
Opossum Creek	OPOSS02	20	45
Purga Creek	PURGA02	27	61
Sandy Creek	SAND01	22	50
Sandy Creek	SAND02	23	52
Sandy Creek	SAND04	32	72
Six Mile Creek	SIXM03	33	75
Six Mile Creek	SIXM04	21	48
Six Mile Creek	SIXM06	24	55
Warrill Creek	WARR01	29	65
Woogaroo Creek	WOOG02	38	86
Woogaroo Creek	WOOG03	26	59
Woogaroo Creek	WOOG04	34	55

Discussion and Recommendations

The presence of platypus DNA at sample sites within the Ipswich region has been found to be intermittent over the past seven consecutive years of eDNA sampling (Appendix 2).
These data suggests that the eastern catchments of the Ipswich Region sustain a small platypus population. However, this year the lack of strong sample detections for platypus DNA is a concern.

Habitat quality across the region was noted to be variable between all sites (45 - 86%), and there was visible damage due to flooding. Sedimentation continues to be an issue in Woogaroo and Opossum Creek. This is a major concern for the longevity of platypuses inhabiting the Woogaroo catchment. Sedimentation will cause deep pools to become shallow as a result of a build-up of silt, in conjunction with the smothering of their food source as silt settles out of the water column (Boulton et al. 2014). Platypuses rely on pools between one and five meters deep to forage effectively and successfully mate (Grant 2007). They forage for up to 12 hours a night/day ingesting 30% of their body weight (Grant 2007), therefore need areas of high food abundance and diversity.

Platypuses rely on waterways for migration (Furlan et al. 2013) consequently, decreased habitat quality and connectivity between catchments will contribute to isolate populations (Furlan et al. 2013; Griffths et al. 2014; Weeks 2014). They are also better protected in deeper water from terrestrial predators (moving through shallower waters risks predation (Grant 2007)). Therefore, water quality and the connectivity between waterways is highly important within the region and may be conserved by maintaining and rehabilitating preferred habitat features in which platypuses depend upon and to reduce further degradation to water quality and riparian habitat.

A number of sample sites were noted to have significant damage resulting from for the two flood events (Table 4). These include sites where platypuses have and have not been detected. Where feasible, Ipswich Council could prioritise restoration activities that target improving platypus habitat.

Table 4. Sites for rehabilitation assessment, photos Appendix 3.

Platypus detected site	**Feature**	**Assess**
BUND04 & BUND08	Banks – sandy Overhanging vegetation	Erosion
Opossum Creek all sites OPOSS01 – Creekside Park	Banks and connectivity under causeway	Erosion and disturbance due to pipeline upgrade
WOOD01 & WOOG02	Banks - sandy	Erosion
WOOG03	Eugene Street reserve pool	Pool depth
Platypus not detected		
BREM01	Banks and native vegetation	Erosion
PURGA02	Banks – sandy Overhanging vegetation	Erosion

A particular site in Woogaroo Creek, at Eugene St Reserve contains a pool of water that has shown to be important for platypuses' inhabitation in the catchment. Wildlife Queensland recommends further inspection of this pool to determine what impact the two floods have had on the size and depth of the pool. Possible restoration actions include bank stabilisation and revegetation and dredging/excavation of the poo if necessary (note, such an activity would require guidance by subject matter professionals). Mapping, measuring and monitoring other pools/waterholes in the catchments would also benefit the knowledge base around refuge pools for platypus populations in the region.

The seven years of platypus occurrence data collected during this project indicate that Woogaroo, Opossum and Sandy Creeks require increased protection and rehabilitation to protect the platypus population. The other waterways should still be considered for rehabilitation including the identification of barriers to platypus dispersal/movement within catchments. Water volume and flow is an important contributor to platypus movement and should be considered for mitigation where activities that reduce water volume/flow occur (excessive irrigation allocation, barriers and stormwater management - especially during the wet season where rapid water flow impacts juvenile platypus when they emerge from the den).

The results obtained during the 2022 eDNA survey represent the least number of positive samples in seven years. There is the evidence to suggest that platypuses are persisting in the Ipswich region, however, these individuals and populations are highly vulnerable. The outcome may relate to possible impacts on eDNA detection resulting from unusually high water volume, flow rate and turbidity; there a reasonable case for a second eDNA survey to resample later in the 2022 breeding season (once the catchments have settled down after the rainfall events). It is also recommend continuing the annual eDNA sampling (May/June) within the Ipswich LGA to continue to monitor extant populations (Woogaroo Creek and Opossum Creek) but also track transient animals moving in and out of systems, as possibly seen in Bundamba and Six Mile Creek. The longitudinal data collected by this project is important not only to track platypus populations over time but to develop and support

catchment management programs to rehabilitate areas. It is also recommended that detailed habitat assessments including aquatic macroinvertebrate surveys be implemented, to identify in-stream pool areas, barriers that may inhibit platypus movement within the connected waterways and associated food abundance and diversity.

Preservation of platypuses will be a conservation benefit within the Ipswich region, as they encourage habitat protection and increase the overall quality of the waterways in which they live.

Acknowledgements

We respectfully acknowledge the Traditional Owners upon the lands in which this research was conducted and deeply respect their connection to Country. We pay our respects to their Elders past and present.

The Wildlife Preservation Society of Queensland would like to thank senior ecologist Josh Griffiths from EnviroDNA Australia who has generously given us his time to guide and advise on implementing the eDNA project. We are also grateful to the Ipswich City Council officers, Jack McCann, Tim Shields and Phil Smith for helping develop the project for the Ipswich region over the last seven years.

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Appendix 1. Environmental DNA results 2022

				Volume	**eDNAresult**						
Site	Waterway	Latitude	Longitude	***default set to 1 if na**	**LabID**	**Cycle number**	**Result (copies/L) for sample**	**Scoring (out of 3 technical reps)**	**Conclusion (for SITE)**	**Total score (for SITE)**	**copies DNA per litre for SITE (averaged)**
BUND10	Bundamba Creek	-27.6942	152.8043	500	12.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	12.2	NA	0.00E+00	0			
IP01	Iron Pot Creek	-27.6015	152.7326	500	13.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	13.2	NA	0.00E+00	0			
GOOD02	Goodna Creek	-27.6074	152.872	490	14.1	37.69	4.90E+05	1	Equivocal	1	2.45E+05
				410	14.2	NA	0.00E+00	0			
SAND01	Sandy Creek	-27.6253	152.9209	500	15.1	NA	0.00E+00	0	Equivocal	1	1.92E+05
				500	15.2	38.10	3.84E+05	1			
SAND04	Sandy Creek	-27.6347	152.9264	500	16.1	NA	0.00E+00	0	Negative	0	0.00E+00
				430	16.2	NA	0.00E+00	0			
WOOG03	Woogaroo Creek	-27.6322	152.9038	500	17.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	17.2	NA	0.00E+00	0			
OPOSS02	Opossum Creek	-27.6453	152.8942	100	18.1	NA	0.00E+00	0	Negative	0	0.00E+00
				150	18.2	NA	0.00E+00	0			
WARR01	Warrill Creek	-27.6575	152.699	270	19.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	19.2	NA	0.00E+00	0			
BUND04	Bundamba Creek	-27.6186	152.8077	450	20.1	NA	0.00E+00	0	Negative	0	0.00E+00
				150	20.2	NA	0.00E+00	0			
BREM01	Bremer River	-27.6179	152.7413	200	21.1	NA	0.00E+00	0	Negative	0	0.00E+00
				225	21.2	NA	0.00E+00	0			
SIXM04	Six Mile Creek	-27.6558	152.8401	450	22.1	NA	0.00E+00	0	Negative	0	0.00E+00

				300	22.2	NA	0.00E+00	0			
SIXM03	Six Mile Creek	-27.6067	152.8588	500	23.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	23.2	NA	0.00E+00	0			
SAND02	Sandy Creek	-27.6061	152.9279	500	24.1	37.49	5.51E+05	2	Positive	4	4.23E+05
				500	24.2	38.66	2.95E+05	2			
PUR02	Purga Creek	-27.6828	152.7284	500	25.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	25.2	NA	0.00E+00	0			
OPOSS01	Opossum Creek	-27.6562	152.9002	290	26.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	26.2	NA	0.00E+00	0			
WOOG04	Woogaroo Creek	-27.6473	152.8881	500	27.1	37.69	4.80E+05	1	Equivocal	1	2.40E+05
				500	27.2	NA	0.00E+00	0			
BREM07	Bremer River	-27.6355	152.7316	150	28.1	NA	0.00E+00	0	Negative	0	0.00E+00
				250	28.2	NA	0.00E+00	0			
SIXM06	Six Mile Creek	-27.6394	152.8463	300	29.1	NA	0.00E+00	0	Negative	0	0.00E+00
				300	29.2	NA	0.00E+00	0			
BUND08	Bundamba Creek	-27.6359	152.7908	500	30.1	NA	0.00E+00	0	Negative	0	0.00E+00
				500	30.2	NA	0.00E+00	0			
BREM03	Bremer River	-27.6367	152.7461	500	31.1	NA	0.00E+00	0	Negative	0	0.00E+00
				425	31.2	NA	0.00E+00	0			
WOOG01	Woogaroo Creek	-27.6152	152.9087	250	32.1	NA	0.00E+00	0	Negative	0	0.00E+00
				250	32.2	NA	0.00E+00	0			
WOOG02	Woogaroo Creek	-27.618	152.9065	500	33.1	NA	0.00E+00	0	Negative	0	0.00E+00
				230	33.2	NA	0.00E+00	0			

Appendix 2. Ipswich region eDNA site results 2016 - 2022.

Waterway	Site	Latitude	Longitude	2016	2017	2018	2019	2020	2021	2022
Bremer River	BREM01	-27.61788	152.7413	Negative	Negative	Negative				Negative
	BREM02	-27.62259	152.7429	Negative	Negative	Negative				
	BREM03	-27.63672	152.7461	Negative	Negative	Negative				Negative
	BREM04	-27.64281	152.7437	Negative	Negative	Negative				
	BREM05	-27.6488	152.6354	Negative	Negative	Negative				
	BREM06	-27.60257	152.7562						Negative	
	BREM07	-27.63428	152.734609						Negative	Negative
	BREM08	-27.62708	152.66868						Negative	
	BREM09	-27.59151	152.78093						Negative	
	BREM10	-27.60263	152.7443						Negative	
Bundamba Creek	BUND01	-27.62493	152.7971	Negative	Negative	Positive	Negative	Negative		
	BUND02	-27.60978	152.8	Negative	Negative	Negative	Negative			
	BUND03	-27.60458	152.8011	Negative	Negative	Negative	Negative			
	BUND04	-27.61858	152.8077				Positive	Negative		Negative
	BUND05	-27.63623	152.79086				Negative			
	BUND06	-27.69946	152.8036				Negative			
	BUND07	-27.591295	152.795659						Negative	
	BUND08	-27.635791	152.790683						Negative	Negative
	BUND09	-27.600108	152.798394						Negative	
	BUND10	-27.69416	152.8043							Negative
Goodna Creek	GOOD01	-27.6008	152.8804				Negative			
	GOOD02	-27.60741	152.872				Negative			Equivocal
	GOOD03	-27.61475	152.8692				Negative			
Iron Pot Creek	IP01	-27.6015	152.73256							Negative
Opossum Creek	OPOSS01	-27.6564	152.8997		Negative	Positive	Negative	Positive	Equivocal	Negative

	OPOSS02	-27.6453	152.894				Positive		Positive	Negative
	OPOSS03	-27.664777	152.907268						Negative	
	OPOSS04	-27.67459	152.9083				Negative			
Purga Creek	PURGA01	-27.71238	152.7333	Negative		Negative				
	PURGA02	-27.68282	152.7284							Negative
Sandy Creek	SAND01	-27.62534	152.9209		Negative	Positive	Negative		Positive	Equivocal
	SAND02	-27.6061	152.9279			Positive		Positive	Positive	Positive
	SAND03	-27.5706	152.93			Negative		Positive		
	SAND04	-27.6347	152.9264			Negative	Negative			Negative
	SAND05	-27.63868	152.9278				Negative			
Six Mile Creek	SIXM01	-27.64161	152.8449	Negative	Negative	Negative	Negative			
	SIXM02	-27.6177	152.847			Positive	Negative			
	SIXM03	-27.6068	152.8592			Positive	Negative		Negative	Negative
	SIXM04	-27.65569	152.84				Positive		Negative	Negative
	SIXM05	-27.63737	152.8454				Negative			
	SIXM06	-27.639423	152.846278						Equivocal	Negative
Warrill Creek	WARR01	-27.6575	152.699		Negative	Negative				Negative
	WARR02	-27.7494	152.6858		Negative					
Woogaroo Creek	WOOG01	-27.61517	152.9087	Negative	Equivocal	Positive	Negative			Negative
	WOOG02	-27.61783	152.9065	Positive	Negative	Equivocal	Negative		Negative	Negative
	WOOG03	-27.63219	152.9038	Positive	Negative	Equivocal	Positive	Negative	Positive	
	WOOG04	-27.64733	152.8881				Negative			Equivocal
	WOOG05	-27.65879	152.8808				Negative			
	WOOG06	-27.60948	152.906017						Equivocal	
Black Snake	BSC01	-27.552270	152.597829						NA	
Deebing Creek	DEEB01	-27.62507	152.75136						Negative	
	DEEB02	-27.63432	152.75426						Negative	
Franklin Vale Creek	FV01	-27.73232	152.4718						Negative	
	FV02	-27.72107	152.47568						Negative	

Appendix 3: Site assessments

Platypus detected site	**Feature**	**Assess**		
BUND04 & BUND08	Banks – sandy Overhanging vegetation	Erosion		
Opossum Creek all sites OPOSS01 – Creekside Park	Banks and connectivity under causeway	Erosion and disturbance due to pipeline upgrade		

WOOG01 & WOOG02	Banks - sandy	Erosion		
WOOG03	Eugene Street reserve pool	Pool depth		
Platypus not detected				
BREM01	Banks	Erosion		

PURGA02	Banks – sandy Overhanging vegetation	Erosion		

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Appendix 4: Urban impacts to platypuses.

Threat	**Details**	**Causing**	**Impact to platypus**	**Reference**
Habitat destruction from land use	Removal of native vegetation	Erosion of banks	Fewer burrowing sites	Grant (2007)
		Sedimentation	Pool depth become shallower	Grant and Temple-Smith (2003); Grant (2007)
			Smother's food source	Boulton et al. (2014)
	Water pollution – chemicals and littering	Stormwater runoff from roads	Pollutants impact food sources Pharmaceuticals ingested from food source	Serena and Pettigrove (2005); Richmond et al. (2018)
		Rubbish	Entanglement	Serena and Williams (2021)
	Modification of waterways	Weirs, dams, irrigation	Unfavourable water levels and flow Altered follow regimes	Grant and Temple-Smith (2003); Kolomyjec (2010); Hawke (2020)
		Stormwater runoff from roads	Change in habitat productivity and female reproductive success	Australian Platypus Conservancy (n.a.); Martin et al. (2014)
			Channel morphology – erosion	Walsh et al. (2005); Martin et al. (2014)
			Diving energetics – hard and fast flows reduce foraging efficiency	Griffiths et al. (2014b)