

## **The effect of water quality, macroinvertebrate assemblages and habitat suitability on the distribution of Platypuses; a pilot study in Cattai Catchment, North West Sydney**

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### **Key Points**

- Platypuses are present in urban waterways. The aim of this study was to determine whether water quality, habitat quality and macroinvertebrate assemblage influences the distribution of platypuses as inferred by eDNA detection in the Cattai and Little Cattai Creek catchments, North West Sydney.
- Platypus eDNA was detected at 9 of the 18 sites and 5 out of the 9 waterways surveyed. Platypus detection was strongly associated with the quality of their habitat and instream water quality, preferring locations with wide riparian zone, and avoiding those with sand and silt accumulation, overgrowth of macrophytes, high salinity and low dissolved oxygen.
- This study indicates that the loss of riparian vegetation, an increase in aquatic weeds and increased sedimentation are important factors in developing local stream management improvements to sustain the population of platypus in the Sydney basin.

### **Abstract**

The platypus (*Ornithorhynchus anatinus*) continues to persist in urban waterways with high pollutant concentrations, altered flow regimes, channel morphology, and degraded stream substrate and riparian zones. The purpose of this study was to determine whether water quality, specific habitat features, and macroinvertebrate assemblage influences the distribution of platypus as inferred by eDNA detection in a selection of 13 sites within the Cattai catchment, in North West Sydney. Sampling took place on two separate occasions: June 2020 and December 2020. Platypus eDNA was detected in 9 of the 18 sites and 5 of the 9 waterways surveyed. Platypus activity was strongly associated with the quality of their stream habitat (riparian vegetation, absence of sand and silt accumulation, and streams lacking dense growth of macrophytes). Platypus also appeared to avoid streams with 'higher salinity', and lower dissolved oxygen. The effective conservation of this iconic species in peri-urban and urbanised catchments such as that of Sydney requires ongoing monitoring of population status and health, and prevention of further habitat damage by informed local population management strategies. This study indicates that the loss of riparian vegetation, an increase in aquatic weeds and increased sedimentation are important factors in developing local stream management improvements to sustain the population of platypus in the Sydney basin.

### **Keywords**

Platypus, habitat assessment, peri-urban, food, distribution, environmental DNA, urbanisation

### **Introduction**

The platypus (*Ornithorhynchus anatinus*) is an iconic Australian species that is distributed along the eastern coast of Australia and Tasmania, in a variety of lentic and lotic freshwater habitats (Grant 1992). The species has been impacted by habitat loss. In urban and peri-urban areas, platypus habitat has become fragmented and degraded as a result of anthropogenic factors including agricultural and metropolitan disturbances that can cause poor water quality, changes to flow regimes, and riparian and bank destruction (Grant & Temple-Smith 1998, Serena & Pettigrove 2005, Serena & Williams 2010). These factors can change the specific habitat suitability for platypus occupation. Never-the-less, platypus continue to persist in urban waterways in Melbourne, Sydney, Hobart and Brisbane with high pollutant concentrations, altered flow regimes and channel morphology, and degraded instream substrate and riparian structure (Grant 2007, Brunt et al. 2018). Records of platypus in the Sydney metropolitan area continue to decline with increasing urban expansion and subsequent severe habitat degradation (Grant 1998). Although, it is clear that platypus distribution and

abundance across the Sydney catchment has changed since European settlement, it cannot be attributed to one single human factor but rather a multiple of factors occurring simultaneous or in sequence (Grant 1998, Grant 2007). Therefore, for the effective conservation of this species in peri-urban and urbanised catchments requires an understanding of their habitat sensitivities, limitations and adaptations, ongoing monitoring of population status and health, and prevention of further habitat damage.

Current research on platypus habitat preferences have common underlying features. For example, earthy banks with consolidation of roots from native vegetation, a variety of shrubs and medium to large trees overhanging a vertical or concave bank > 1m from water level make for great habitat for building nests and shelter from predators during resting periods and lactation (Ellem et al. 1998, Grant 2014, Brunt et al. 2018). In addition, although, platypuses are considered opportunistic predators of benthic aquatic macro-invertebrates, there has been very little studies or inconclusive results on platypus habitat selectivity based on macroinvertebrates available to them (Faragher et al. 1979, Grant 1982, McLachlan-Troup et al. 2010). Never-the-less, many stream features that influence the abundance of macroinvertebrates provide habitat complexity for these prey species such as large wood debris, aquatic plants, coarse organic matter, and cobbled benthic substrate, have been positively correlated to platypus occurrence (Grant and Bishop 1998, Lunney et al 2004, Grant 2004, Grant, 2007, McLachlan- Troup et al 2010, Brunt et al. 2018). Other features correlated with platypus occurrence include productivity of the stream based on size, connective sequence of pool/riffles, and pool depth (Grant 2004). Some studies have identified habitat variables specific to urban waterways having a negative relationship with platypus occurrence including catchment imperviousness, sediment accumulation, and reduced water quality particularly increased nutrients (Phosphates (P) and Nitrates (N)) and heavy metals (Cadmium (Cd), Lead (Pb) and Zinc (Zn)) (Worley et al. 2001, Serena and Pettigrove 2005, Martin et al. 2014).

North West Sydney waterways including the Cattai Catchment have rarely, in recent years, attracted platypus interest from land managers. Apart from local sightings that are scarcely reported, active environmental group reports, and limited number of reports in BioNet data, suggest the rather small, cryptic, mobile, hard to catch, and mainly nocturnal Australian monotreme, would be considered a low density population in the catchment, at best (Grant 2014). The novel method of environmental DNA (eDNA), has the potential to ascertain the contemporary distribution of platypus on a large spatial scale to help predict current and future impacts (Griffiths et al. 2018).

The role of pollution and anthropogenic factors, habitat suitability, food resource abundance and diversity as aspects enhancing or limiting platypus occurrence in a peri-urban waterway has not been previously assessed against platypus distribution in a catchment using eDNA target species detection for presence or absence. The main purpose of this study was to determine whether these factors influence the distribution of platypus as inferred by eDNA detection in a selection of sites within the Cattai and Little Cattai Creek catchments. This study was conducted as a pilot investigation for a broader project, mapping the occurrence of platypus across the urbanised and peri-urbanised streams of the Hawkesbury-Nepean river catchment that will have implications for catchment, stream, and riparian management to improve platypus habitat and prevent further degradation in the region.

## **Methods**

### *Survey sites*

The survey was conducted within the Cattai and Little Cattai Creek catchments which enters the Hawkesbury River, north west of Sydney, New South Wales. With the assistance of BioNet data for historical distribution, the aim was to provide broad information on the presence of platypus in the Cattai and Little Cattai Catchment. Sites were selected to provide good spatial coverage throughout the catchment. The section of catchment under study traverses a range of land uses from National Park and major roads where it meets the Hawkesbury River to new urban development and agriculture in the mid-upper reaches.

## *Sampling Methodology*

### *eDNA*

The surveys were conducted on two separate occasions in June 2020 (18 sites) and December 2020 (13 sites). Water samples were collected in duplicate or triplicate at each site. Water was filtered on-site by pushing through up to 500 mL through a 0.22µm filter using a sterile 50 ml syringe. Filtered samples were stored on ice for a before overnight transport to EnviroDNA (Parkville, Victoria) for DNA extraction and processing by real-time quantitative Polymerase Chain Reaction (qPCR) (Positive > 2 qPCRs +ve; equivocal ≤ 2 qPCR +ve and negative = 0 qPCR +ve of 6-9 assays).

### *Water Quality*

Temperature, pH, and electrical conductivity were measured in five replicates at each site using onsite hand-held probe multi-meter probe (model TPS Aqua-CPA). Dissolved oxygen (DO% saturation) was similarly measured onsite with YSI digital multi-meter with four probes (model Pro DSS). Five replicate samples from each site were collected and labelled in sampling bottles for measurement of turbidity, total phosphorous and total nitrogen at Western Sydney University Hawkesbury campus laboratory. Hatch2100 Turbidimeter was used to measure the sample turbidity. Total phosphorous (P) was measured using a Hach portable spectrophotometer (Model DR/2400), following model manual method (no. 8048) PhosVer3 (Ascorbic Acid), to test phosphorous (reactive) in water, wastewater or seawater in the range 0.02 - 2.50 mg/L PO<sub>4</sub><sup>3-</sup>. Total nitrogen (N) was measured using the same spectrophotometer, following model manual method (no. 8171) Powder Pillow Cadmium reduction, to test nitrogen (nitrate) in water, wastewater or seawater in the medium range 0.1 - 10.0 mg/L NO<sub>3</sub><sup>-</sup>-N).

### *Habitat Assessment*

A rapid habitat assessment was developed to rate the habitat quality of the surveyed sites' stream, substrate, riparian zone and current urban stream effects, with regards to potential suitability for platypus presence. Variables and scoring system were identified from Grant (2007; 2014) and additional variables identified from literature. The rapid assessment scored habitat variables to the proportion of a stream exhibiting a particular variable. Scores were 0-4, where 0 represents no evidence of a variable being present, and 2 representing approx. 50% of a survey site exhibiting a variable etc. The assessment had 12 riparian variables (a maximum potential reach score 4 x 12 = 48), 5 stream and substrate variables (maximum score 4 x 5 = 20) and 3 disturbance variables (maximum score 4 x 3 = 12) for a maximum potential score for the reach of 70. A section of 50m was assessed by one assessor at each survey site. A mean site score was calculated from the scores on each side of the bank.

### *Macro-invertebrates*

The 13 sites surveyed in December 2020 were sampled for macroinvertebrates with triplicate sampling effort. A mosaic of sub-habitats swept by the net was randomised by the collector to maximise the diversity of substrates, depths and current speed. Macroinvertebrate samples were undertaken according to the Australian National River Health Program protocols (DEST et al 1994, Chessman, 1995) and at least 7 days after significant rainfall (<5mm/day). The collection methodology involved using a 30cm x 30cm kick net frame, with 250µm mesh. The net was swept through the water column for 30 seconds as sediment on the stream bed was 'kick' disturbed to dislodge rocks, sand and macroinvertebrates in the stream benthos. Net contents was transferred into a clean sample container with 70% ethanol. In the laboratory, macroinvertebrates were picked for 10 minutes in a tray then picked under a microscope for further 110 minutes or until all macroinvertebrates picked. Macroinvertebrates were identified to family level. Numbers of individuals in each family were recorded.

### *Data Analysis*

Single factor ANOVA was used to test for significant differences (for all water quality attributes, habitat assessment, and macroinvertebrate biotic indices) between sites indicating platypus presence at least once in either survey period (June or December 2020) and those sites which did not detect platypus eDNA in either

survey period. Several macroinvertebrate biotic indices were calculated for each macroinvertebrate sample at the family and order taxonomic level: EPT taxonomic richness, SIGNAL score (Chessman 1995), total insecta abundance, total gastropoda abundance and total crustacean abundance, and overall total abundance and family and order richness.

## Results

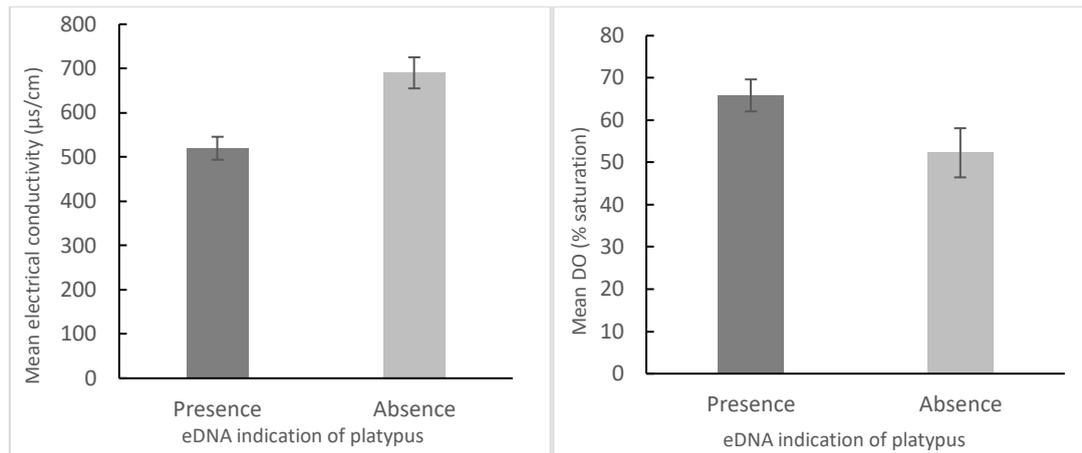
Overall platypus eDNA was detected at 9 of the 18 (50%) sites surveyed in the Cattai and Little Cattai Creek catchments over the June and December 2020 sampling period. In June all 18 sites were sampled with 8 (44%) detecting platypus eDNA and December only 13 of the 18 were surveyed (due to heavy rainfall) with 4 detecting platypus eDNA (31%; Table 1). The detection results changed for 4 sites between the June and December survey periods. Two sites in the Cattai Creek and one in Blue Gum Creek change to negative. However, Dooral Dooral returned a positive detection in the second survey period.

**Table 1. Sample sites that had positive or equivocal results for eDNA screening of water samples for platypus (*Ornithorhynchus anatinus*). Positive > 2 qPCRs +ve; equivocal ≤ 2 qPCR +ve and negative = 0 qPCR +ve of 6-9 assays.**

Creek	Suburb	Result June 2020	Result December 2020
Little Cattai Creek	Cattai	Equivocal	Not sampled
Little Cattai Creek	Cattai	Positive	Equivocal
Cattai Creek	Maraylya	Equivocal	Negative
Cattai Creek	Cattai	Equivocal	Negative
Cattai Creek	Annangrove	Equivocal	Positive
Cattai Creek	Maraylya	Positive	Equivocal
Blue Gum Creek	Annangrove	Positive	Negative
O'Haras Creek	Kenthurst	Equivocal	Not sampled
Dooral Dooral Creek	Annangrove	Negative	Positive

Little Cattai Creek had the highest detection rate (74%) across 3 sample sites. The main tributary surveyed from Hawkesbury River, Cattai Creek had a detection rate of (42%) over the 7 sites sampled. Three of the six reaches stemming from Cattai Ck; Dooral Dooral Creek, O'Haras Creek and Blue Gum Creek returned a positive detection at least once in the two survey periods. At Blue Gum Creek platypus burrows were identified.

There were no significant differences in the mean summer concentrations on total P and total N between those sites supporting platypus (P-0.13mg/L; N-1.61mg/L) and sites without platypuses (P-0.19mg/L; N-1.37mg/L) of platypus in both eDNA survey periods. However, in all surveyed sites the mean total phosphorous and nitrogen were above the ANZECC (2000) guideline trigger value. Electrical Conductivity (EC) was significantly higher for sites where platypuses were not detected than those with eDNA presence in either survey periods ( $p < 0.0005$ ); the reverse effect was true for dissolved oxygen (DO) with a significant result ( $p < 0.05$ ) (Figure 1). Turbidity was significantly higher in waterways where platypus were detected compared those in which platypus were not detected ( $p < 0.05$ ).



**Figure 1. a) Mean electrical conductivity – sites with platypus presence and absence; b) Mean dissolved oxygen – sites with platypus presence and absence**

Overall, the total mean habitat assessment score for waterways that detected platypus in either of the two eDNA sampling periods, was significantly higher than the mean score for waterways without platypus eDNA detected (Pr-78.6%, Ab-62.3%;  $p < 0.05$ ). In addition, urban stream disturbance features were significantly higher for sites with indication of platypus presence than those with eDNA absent from both survey periods ( $p < 0.02$ ), showing their avoidance of urban disturbances such as overabundance of macrophytes ( $p < 0.005$ ). The habitat variable groups assessing riparian structure, and stream and substrate scored higher in sites with platypus presence. More specifically, vegetation width  $> 3\text{m}$ , and absence of sand or silt/clay accumulation was significantly higher in sites detecting platypus than those not detecting platypus ( $P < 0.05$ ). Macroinvertebrate total family abundance, order and family richness, diversity, total crustacean abundance, and total insecta abundance were higher for waterways with indication of platypus presence than those with eDNA absent from both survey periods. All of these results were not statistically significant.

## Discussion

This pilot project used eDNA to determine the current distribution of platypus in the Cattai Catchment where no reliable recent information existed. Platypus were detected in 50% of the sites surveyed and in 5 of 9 waterways in the Cattai and Little Cattai Catchments over two sampling periods. The region has had a handful of local sightings (some unconfirmed) from community members and environmental groups which provoked this project. However, with the use of eDNA detection, the results give a reliable record of platypus occurrence, filling in gaps on platypus distribution in the catchment for local land managers, owners and environmental groups.

The study design was set-up to provide good coverage of the waterways in the catchment and target areas where local sightings had occurred. However, there were many sections not sampled that are located between positive sites. In addition, there were several sites which were only positive in one sample period or negative in both, that were downstream of positive sites or between positive sites. Although, these results show a broad distribution in the catchment from Cattai Creek entering Hawkesbury River to the headwaters in Blue Gum Creek and Dooral Dooral Creek, the slightly inconsistent pattern of presence/absence between the sampling periods could mean some locations in the catchment are used as corridors between more permanent locations within an individual's home range, some individuals detected were transient, or some residents were in their nesting burrows during the December sampling (Bino et al. 2019). However, such a pattern is also typical of low density and highly mobile species (Griffiths et al 2018).

The permanency of water, the connectivity between reaches and wetter conditions can increase movement of platypus in a catchment, particularly in the upper reaches (Grant 2007; 2014, Griffiths et al. 2018). Previous studies on platypus have shown the platypus to be opportunistic in their habitat use when faced with varying hydrological conditions including drought and flood (Gust and Handasyde 1995). Urbanised and agricultural waterways can experience hydrological strain and high variation in these conditions due to anthropogenic land

uses such as stormwater drainage, and damming. Thus, ongoing monitoring with eDNA detection across additional sites in the catchment will be an important tool in monitoring population distribution, and temporal habitat use over a longer period to develop and prioritize the appropriate conservation management strategies by land managers and owners.

Anthropogenic land uses like those observed in the Cattai catchment can change the waterways and habitat in ways that are irreversible and limit platypus distribution in the catchment (Grant 2007). This study simultaneously assessed habitat suitability at each site across three key factors important correlated with platypus occurrence; including riparian structure, stream and substrate and urban disturbance. The habitat assessment results overall support the literature from which it was developed, with platypus detected at those sites which scored higher in the habitat quality assessment. More specifically, platypus showed strong avoidance of sites that had excessive coverage of macrophytes, loss of riparian vegetation extending beyond the bank, and increased sedimentation build-up. Riparian vegetation is an important stream mechanism that provides shelter and protection for the platypus while foraging on the bank edge (Grant and Bishop et al 1998). In addition, structurally diverse, overhanging and consolidated riparian vegetation has been linked to higher productivity of the benthic organisms that the platypus feeds on, and can act to moderate stream temperature and photosynthetic productivity and provide diverse material for benthic shredder organisms (Grant and Bishop et al 1998, Giling et al 2012). Often loss of riparian vegetation coupled with altered flow regimes and channel morphology from conventional stormwater drain systems and high catchment imperviousness, increases sedimentation washed from urban and agricultural landscapes into natural waterways (Grant 2007, Martin et al 2014). The sediment accumulates in the stream and on the banks to create sand banks and sand plugs, which change physical and biological dimensions in the stream from temperature, pool size, to abundance and assemblage of macroinvertebrates (productivity) and structural integrity for nest building (Grant 2007; Grant 2014). Rain gardens, sedimentation control on building sites and regeneration of native riparian which slow water movement in heavy rainfall events and allow sediment to drop out will be key sedimentation strategies in an urbanised Cattai catchment.

Although, Grant and Bishop (1998) and Grant (2014) consider the presence of aquatic plants a feature regarded as 'good' macroinvertebrate habitat and thus likely a positive variable linked to platypus presence, this study showed that excessive aquatic plant infestations negatively relate to platypus detection. Macrophytes that choke waterways are associated with highly degraded streams and rivers suffering from eutrophication and low dissolved oxygen concentrations (Boulton et al 2014). Further investigation into this finding would be encouraged before warranting mass removal of aquatic plants to improve platypus habitat. However, nutrient levels in the catchment are concerning and could become a significant factor limiting platypus in the catchment with continued urban growth across the region.

## **Conclusions**

Determining the distribution of platypus and habitat preference across a catchment can be resourcefully and logistically limiting using traditional methods. This study employed eDNA sampling and a rapid habitat assessment to investigate platypus distribution and habitat across 9 waterways of the Cattai and Little Cattai Catchments over two sampling periods in 2020. The eDNA results provide a good baseline for current platypus distribution to enable detection of future changes. In addition, the study conducted habitat assessments, water quality and macroinvertebrate sampling simultaneously to give invaluable information on factors influencing platypus occurrence across the catchment. Platypus detection was strongly associated with the quality of their habitat and water quality, preferring locations with a wide riparian zone, and avoiding those with sand and silt accumulation, overgrowth of macrophytes, high salinity and low dissolved oxygen. In terms of future management of streams with platypus populations facing historical strain from past agricultural land clearing and nutrient loads, riparian regeneration and prevention of further bank destruction from grazing animals will be important for platypus sustainability in the region.

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