Cesar Australia

Assessing habitat suitability for platypuses in upper Campaspe River and Campbells Creek.

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Contents

Abbreviations

Abbreviation	Description
HQI	habitat quality index
WRP	water reclamation plant
CTF	cease-to-flow
CVw	coefficient of weekly flow variation

Executive summary

The upper Campaspe River and Campbells Creek receive inputs of treated water discharge. This project investigated the current status of platypuses in these waterways and assessed general habitat suitability to understand any potential impacts of treated water discharge on the resident platypus populations in these waterways.

The current occurrence of platypuses in these waterways was investigated using environmental DNA (eDNA) at 13 sites throughout the upper Campaspe River and 9 sites along Campbells Creek and compared to available historical data from online databases. An experienced platypus ecologist assessed habitat quality at the same sites using a qualitative index that incorporates riparian and in-stream metrics. In addition, key flow metrics were calculated from long-term flow gauge data, water quality parameters assessed, and compared to other known platypus populations.

Platypus populations appear relatively sparse throughout both waterways. During the current eDNA surveys, platypuses were only detected at several sites immediately downstream of the treated water discharge point on Campbells Creek. Habitat quality was generally considered poor in both waterways, due to a lack of riparian vegetation, low in-stream complexity, and poor flow regimes, particularly regular cease-to-flow events.

There was no evidence of negative impacts from the treated water discharges on platypus populations, despite some degraded water quality parameters. Macroinvertebrate communities are relatively depauperate both up- and downstream of discharge points and the only occurrence of platypuses were detected directly downstream of the water treatment plant in Castlemaine. Several other waterways in Victoria that receive treated water discharges appear to support healthy platypus populations.

Rather, sparse platypus populations in the upper Campaspe River and Campbells Creek are likely a reflection of overall poor habitat conditions from heavily modified catchment areas and seasonal cease-to-flow events.

Background

The iconic platypus (*Ornithorhynchus anatinus*) inhabits a variety of freshwater systems throughout eastern Australia (Grant 1992). The species was recently listed as Near Threatened by the International Union for the Conservation of Nature (IUCN) (Woinarski *et al.* 2014; Woinarski and Burbidge 2016), and Vulnerable in Victoria, due to mounting evidence of overall population decline and localised extinctions. As an apex predator in Australian aquatic ecosystems, platypuses are potentially vulnerable to a number of threatening processes that may degrade their aquatic habitats or reduce the availability of macroinvertebrate food resources. Such threats may include a reduction of available surface water through drought, water extraction or diversion, changes to flow regimes, clearing riparian and broader catchment vegetation, poor water quality, barriers to dispersal, entanglement in litter or fishing equipment, and predation (Grant and Temple-Smith 2003, 1998; Bino *et al.* 2020). Of concern, is the lack of contemporary data on the status of many platypus populations across their range, and quantitative assessments on the relative impacts of various threatening processes are scarce.

Aquatic ecosystems of northern Victoria have been significantly modified as a result of altered flow regimes due to water extraction, the construction of reservoirs and other flow-altering structures, and reduced run-off, widespread land clearing for agriculture, the presence of invasive species (e.g. willows, carp), and reduced water quality. All of these factors are expected to have significant impacts on aquatic-dependent fauna, such as platypuses, although the extent of declines are often unknown due to a lack of contemporary data for many species.

Coliban Water seeks to understand the suitability of the upper Campaspe River (between Carlsruhe and Redesdale) and Campbells Creek (between Castlemaine and the Loddon River) to support platypus populations, particularly with respect to an understanding of any potential impacts of treated water discharges into these waterways, as well as the current occurrence of platypuses in each waterway.

Methods

Status of platypus populations

A desktop search of available platypus data was undertaken, including online wildlife databases (platypusSPOT, Atlas of Living Australia, Victorian Biodiversity Atlas), published studies, and unpublished technical reports.

Current occurrence of platypuses in these waterways was investigated using environmental DNA (eDNA). As the name suggests, eDNA refers to the genetic material that an organism leaves behind in its environment.

Quantitative comparisons with traditional sampling methods indicate that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs et al. 2015; Lugg et al. 2018; Smart et al. 2015; Thomsen et al. 2012; Valentini et al. 2016), enabling effective detection of species at low densities.

Water samples for eDNA analyses were collected from 13 sites in the upper Campaspe River and 9 sites along Campbells Creek using a Smith-Root eDNA Sampler Backpack (Smith-Root Inc, Vancouver WA, US). Two replicate samples were collected at each site by passing water through a 5.0 μm Smith-Root self-preserving filter (Thomas *et al.* 2019) on site in order to reduce DNA degradation (Yamanaka *et al.* 2016). Clean sampling protocols were employed to minimise contamination, including new sampling equipment at each site, not entering the waterway to collect the samples, and taking care not to transfer soil, water or vegetation between sites. Filters were then stored out of sunlight before being transported to EnviroDNA's laboratory for processing.

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit). Real-time quantitative Polymerase Chain Reaction (qPCR) assays were used to amplify the target DNA, using a species-specific probe that targets a small region of DNA of the species of interest. Assays were performed in triplicate for each sample. Positive and negative controls were included for all assays, as well as an Internal Positive Control (IPC) to detect inhibition (Goldberg *et al.* 2016). Here, we considered samples as positive if at least two PCRs (out of three for each sample) returned positive detections for the target species. To minimise false positives, sites were considered equivocal if only 1 PCR returned a positive result, indicating very low levels of target DNA. While trace amounts of DNA may indicate the target species is actually present in low abundance, it may also arise from sample contamination during either the sampling or laboratory screening process, facilitated movement of DNA between waterbodies (i.e. water birds, recreational anglers, water transfers, predator scats), or dispersal from further upstream. If greater confidence is required, further sampling is recommended at equivocal sites to confirm the presence or absence of the target species. Repeat sampling is also recommended to help determine the tenure of the species at a site (i.e. resident or transient). This threshold may be considered conservative, and results from all samples at each site should be considered collectively to ascertain the likely presence of the target species at a site.

Habitat Assessment

An experienced platypus ecologist from Cesar Australia carried out on-site assessments of the habitat quality along the upper Campaspe River and Campbells Creek on 14 and 15 September 2021. Habitat quality is one the key factors influencing species' distribution and abundance, but can be difficult to quantitatively assess. We used an index of habitat quality (HQI) approach (Grant and Gill 2011; Grant 2014) that takes into account both riparian and in-stream variables known to be associated with platypus presence or foraging (Ellem *et al.* 1998; Gardner and Serena 1995; Grant 1983, 2004; Gust and Handasyde 1995; Rohweder and Baverstock 1999; M. Serena *et al.* 1998; Serena *et al.* 2001), or favourable for their invertebrate prey (Boulton and Brock 1999; Young 2001) (Table 1). The HQI has previously been validated using long-term data on platypus populations from the Melbourne Water Urban Platypus Program and has been revealed to be positively correlated with platypus abundance (indicated by captures-per-unit-effort) and reproductive output (Griffiths *et al.* 2014).

Table 1. Bank and in-stream variables assessed to generate the habitat quality index (HQI).

Habitat variable	**Known or potential benefit to platypuses**
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.

Habitat variable	Known or potential benefit to platypuses
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
Coarse organic matter (CPOM)	Primary productivity, habitat and food for benthic invertebrate prey
Large woody debris (LWD, >10cm diameter)	Habitat and food for benthic invertebrate prey
Complex benthic substrate (cobbled, gravel)	Favourable habitat for benthic invertebrate prey

The HQI was assessed at 13 sites distributed throughout the upper Campaspe River and 9 sites along Campbells Creek (Appendix 1) by visually estimating the percentage of bank or in-stream area exhibiting the relevant habitat variables along approximately a 50 m stretch of waterway, and each variable assigned a score between 0 and 4 (Table 1). The total from all variables provided a habitat score for the site as a proportion of the maximum possible score, yielding a HQI of 0 (extremely poor) to 1 (ideal habitat). An overall HQI for each waterway was calculated by averaging the scores from each site.

To maintain consistency with the previous HQI assessment in Greater Melbourne (where scores ranged from 0.467 to 0.735), we classified locations as Very Poor (0.35-0.45), Poor (0.45-0.55), Moderate (0.55-0.65), Good (0.65-0.75), or Very Good (0.65-0.75) (Griffiths *et al.* 2014, 2016).

Hydrology and Water Quality

The HQI does not incorporate several important parameters that are also likely to influence overall habitat quality for platypuses – stream hydrology and water quality. These factors are typically highly variable and cannot be easily assessed in the field. Therefore, we examined available historical flow and water quality data, both upstream and downstream of treated water discharge points, and compared the data to published thresholds of tolerance for platypuses (e.g. Serena and Pettigrove

2005) or ranges from waterways known to support healthy platypus populations (e.g. Griffiths *et al.* 2019).

Historical daily flow data (mean ML/day) was obtained from 2 flow gauges in the upper Campaspe River. No raw long term flow data was available for Campbells Creek, although some recent flow modelling had been undertaken (GHD 2019). Without long term quantitative data on platypus populations, it is impossible to correlate changes in flow regimes to any trends in the local platypus population. However, we can compare key metrics of the current flow regime, with those observed in waterways known to support healthy platypus populations. A recent analysis of flow regimes across a range of waterways in Greater Melbourne identified long-term cease to flow patterns (avCTF10 – average duration of longest annual cease to flow event over 10 years) and flow variability (CVw10 – mean weekly coefficient of flow variation) as key metrics attributed to declining platypus populations (Griffiths, Maino, *et al.* 2019). Waterways that support "healthy" platypus populations, as defined by relatively high captures per unit effort and other metrics (Griffiths and Weeks 2018), are characterised by low to no cease-to-low events, and low to moderate variability (Appendix 2).

Raw water quality data was provided by Coliban Water from a number of monitoring sites along both Campbells Creek and the upper Campaspe River. Monitoring was undertaken bimonthly between 2018-21 at sites upstream and downstream of the WRP discharge points. Average values were calculated across the sampling period, and compared between sites upstream and downstream of the discharge points.

Results & Discussion

Status of platypus populations

Common to many subcatchment areas across their range, there is a lack of any systematic survey data on platypuses in the upper Campaspe River and Campbells Creek. Therefore, no quantitative data exists, nor is there any information on population trajectories over time. Historical anecdotal records from wildlife databases are scarce, with just 9 records in the upper Campaspe River and 9 in Campbells Creek (Figure 1). Previous records for both waterways span approximately 20 years, with very few recent records (<5 yrs).

Systematic eDNA surveys in the upper Campaspe River and tributaries in September 2017 (*n* = 23), February 2019 (*n* = 8), and September 2019 (*n* = 24), yielded just one positive detection of platypus DNA at a site in Kyneton (Griffiths and Licul 2020). During the current surveys, no platypus DNA was detected from 13 sites distributed from Tylden-Woodend Rd to Redesdale (Figure 1). The current evidence suggests platypuses may still persist in the upper Campaspe River upstream of Lake Eppalock, but they are in very low abundance. It is difficult to assess population trends due to the absence of any historical systematic or quantitative data, but the very low number of reported sightings suggest numbers of platypuses may have been low for some time (i.e. 20 years or more).

No previous systematic surveys data exists for Campbells Creek, but platypuses have been recorded along its length, from the Loddon River to at least the Castlemaine Botanical Gardens. The current eDNA surveys spanned 9 sites from Castlemaine to almost the Loddon River. Platypus DNA was detected at 3 sites in the middle of the survey area, downstream of the treated water discharge point in Castlemaine (Figure 1).

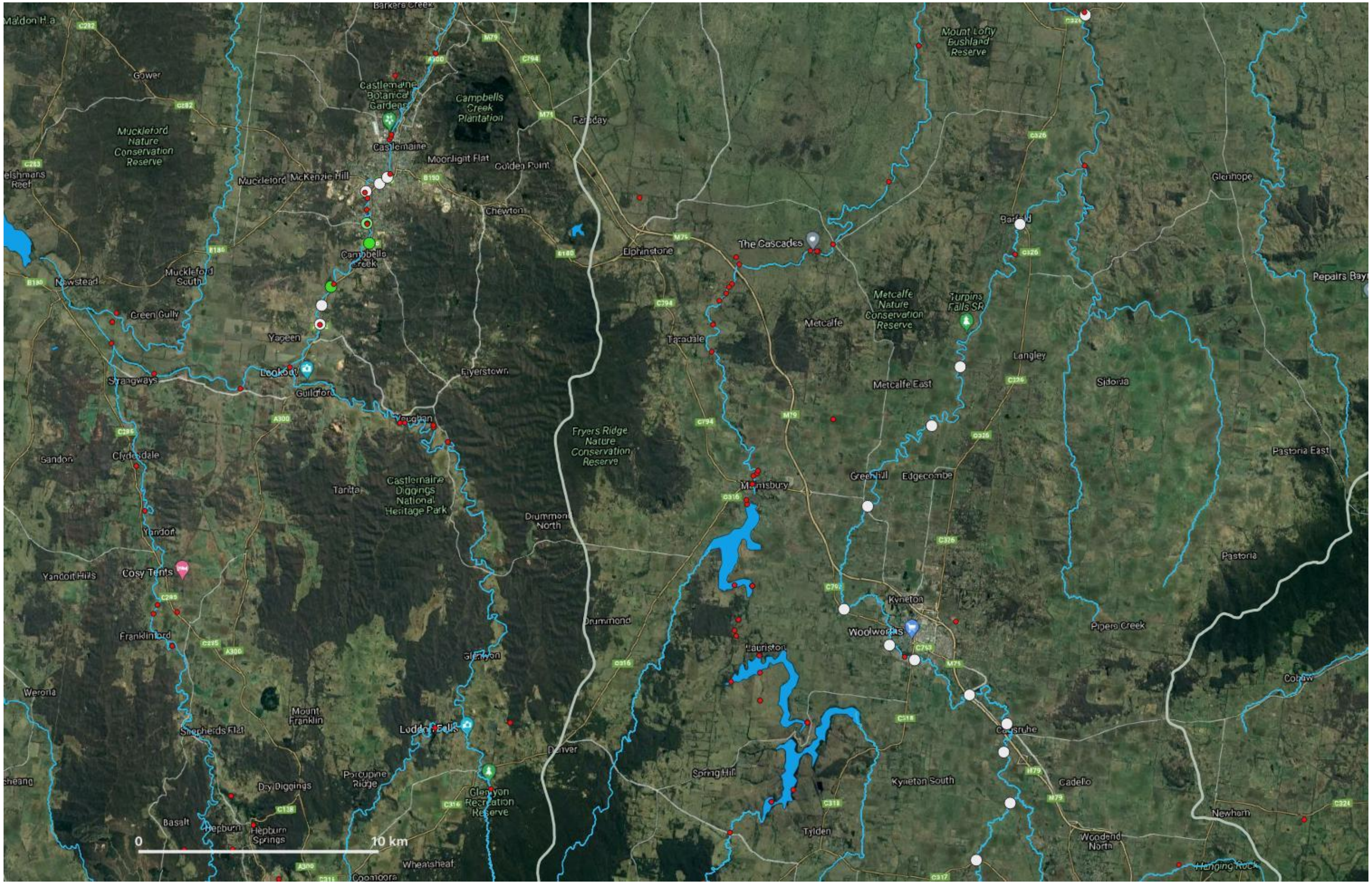

Figure 1. Results of 2021 eDNA platypus surveys along upper Campaspe River and Campbells Creek (green = positive, pale green = equivocal, grey = negative) as well as historical platypus records from online databases (small red circles).

Status of platypus populations

Platypuses are adaptable to a range of environmental conditions, as demonstrated by the wide range of aquatic habitats and climatic zones they inhabit across their range (Grant 1992; Grant and Temple-Smith 1998) However, in broad terms, there are three key components that are required for their presence:

- reliable water availability,
- abundant macroinvertebrates,
- and stable earthen banks to construct burrows.

Critically, platypuses require adequate surface water and flow regimes to support a reliable supply of macroinvertebrate prey. Platypuses are adapted to feed exclusively in water, where they forage for a range of benthic macroinvertebrates, with adults consuming about 15-30% of their bodyweight daily, increasing up to 80% bodyweight for females during lactation (Holland and Jackson 2002; Krueger *et al.* 1992). Therefore, many habitat variables associated with platypus presence and abundance are those favourable for macroinvertebrates. Platypuses are known to preferentially forage in areas of coarse benthic substrates (i.e. cobbles, rocks, pebbles) and large woody debris (Grant 2004; Serena *et al.* 2001). Other variables known to be important for platypuses include large riparian trees, overhanging vegetation, pools that are 1-3 m deep, and near vertical or undercut banks at least 0.5 m above the water (Bethge *et al.* 2003; Ellem *et al.* 1998; Grant 2004; Serena *et al.* 2001, 2000; M. Serena *et al.* 1998; M Serena *et al.* 1998). Conversely, platypuses are known to avoid areas of fine substrates (silt or clay) and dense willows (Serena *et al.* 2001).

The HQI along the upper Campaspe River ranged from 0.386 to 0.636, with scores generally improving downstream (Appendix 1). Habitat quality along the length of the surveyed waterway was generally considered Poor (46% of sites) to Moderate (38%), with two sites rated Very Poor (15%). The main variables contributing to the low habitat scores were poor riparian vegetation, leading to unconsolidated, eroded banks, the absence of large woody debris, and the extensive occurrence of sandy or silty substrate.

It should be noted that several sites are in the middle of rehabilitation works (e.g. Site 11), including woody weed removal (likely willows, poplars, and/or box thorn), stock exclusion, and native revegetation. The replanted species are still young, and have limited capacity to provide shade, organic matter, LWD, and bank stabilisation. However, the riparian scores are expected to improve in coming years, improving habitat quality for platypuses and other aquatic species.

The HQI along Campbells Creek ranged from 0.341 to 0.591 (Appendix 1). Habitat quality was poorest in the lower reaches, with the two most downstream sites rated Very Poor. While in-stream complexity was relatively poor throughout the creek, almost no riparian vegetation remained in the lower reaches (e.g. Figure 2), leading to badly eroded banks (exacerbated by stock access). Habitat quality was highest in the middle section of the creek, which corresponds to the area where platypuses were detected in the eDNA surveys.

Figure 2. An example of poor habitat quality for platypuses in lower Campbells Creek.

It's worth noting data from platypus populations in other waterways that receive treated water discharges.

Lower Jacksons Creek, downstream of Sunbury, receives discharge from the Sunbury Recycled Water Plant. When assessed in 2014, the HQI was considered Moderate (0.610; Griffiths *et al.* 2014). The local platypus population is considered healthy from long-term monitoring surveys undertaken from 1996 to 2016 (Serena and Williams 2008; Griffiths *et al.* 2016) and currently has one of the highest capture rates (CPUE) of any surveyed location throughout Greater Melbourne (Appendix 2). Indeed, it has been suggested that consistent treated water discharges helped to maintain baseflows in Jacksons Creek and sustain this population during the Millennium Drought.

The Leigh River in the Corangamite Catchment receives discharge from a water treatment plant via the Yarrowee River. Recent eDNA sampling at 6 sites along the Leigh River returned positive detections for platypuses at all sites (EnviroDNA unpublished data). While this is only a single survey with limited sites, these preliminary results suggest a relatively healthy platypus population in the Leigh River. Although not directly comparable, site occupancy of platypuses from broader eDNA surveys in the adjacent upper Barwon and upper Moorabool subcatchments has been approximately 30-50% (Griffiths *et al.* 2001; Griffiths, Song, *et al.* 2019).

Although no detailed investigations of water quality or hydrology have been undertaken in these waterways, these data suggest healthy platypus populations can persist and thrive in waterways that receive treated wastewater provided other habitat variables are suitable.

Hydrology

As an aquatic-dependent species, platypuses are vulnerable to natural and anthropogenic changes to flow regimes (Griffiths, Maino, *et al.* 2019; Jacobs *et al.* 2016; Serena *et al.* 2014; Martin *et al.* 2013). In Victoria, the alteration of river flows arises from a variety of human activities, such as surface and groundwater extraction, construction of reservoirs and other instream structures, land clearing, and the modification of catchments for agriculture and urban development. As a result, the ecological integrity of many freshwater ecosystems has been significantly and permanently altered due to reduced baseflows, increased duration and frequency of cease-to-flow events, and high variability, altered seasonality, and higher magnitude and frequency of peak flows, although the changes to flow regimes will vary in nature and scale across landscapes. Climate change and human population growth are already increasing stress (and are predicted to increase further) on Australia's river systems and aquatic fauna due to a reduction in overall rainfall, leading to reduced stream flows and increased extraction (Hope *et al.* 2017).

The long-term flow data for the upper Campaspe River at two gauges at Ashbourne (upper reach - gauge 406208; 1933-2021) and Redesdale (lower reach - gauge 406213; 1975-2021) shows an ephemeral stream with seasonal cease-to-flow events over summer that appear to be increasing in frequency and duration (Appendix 2). The Millennium Drought had an obvious severe impact from 2000-2010, but extensive cease-to-flow events have continued in the decade since.

The average annual CTF in the upper Campaspe River is 121 and 94 days in the upper and lower reaches, respectively, over the last 30 years (Figure 3). For comparison, average annual CTF of waterways that support healthy platypus populations ranges from 0 to 17 days (Griffiths, Maino, *et al.* 2019; Appendix 3). Therefore, extensive cease-to-flow events is an obvious factor inhibiting the capacity of the upper Campaspe River to support a resident platypus population. This is reflected in the current very low abundance of platypuses in the waterway.

Flow variability in the upper Campaspe River was also quite high, particularly in the upper reaches (Figure 3). The coefficient of weekly flow variability (CVw) ranged from 0.38 to 0.81 in the upper reach, and 0.28 to 0.71 in the lower reach. For comparison, CVw of waterways that support healthy platypus populations ranges from 0.22 to 0.50 (Griffiths, Maino, *et al.* 2019; Appendix 3). High hydrological variability is recognised as one of the most important factors degrading waterways, particularly smaller urban streams (Walsh et al. 2012, 2005). Rapid changes in flow rates and water levels, increased shear stress, and high frequency of disturbance events can lead to depauperate macroinvertebrate populations and prevent the establishment of aquatic macrophytes. Frequent high flow events are also likely to impact platypus foraging behaviour (Griffiths et al. 2014; Gust and Handasyde 1995).

Other flow metrics, such as number of high spells (nHS), and seasonality, were comparable to values found for waterways that support platypus populations, but these metrics have minimal impacts (Griffiths, Maino, *et al.* 2019).

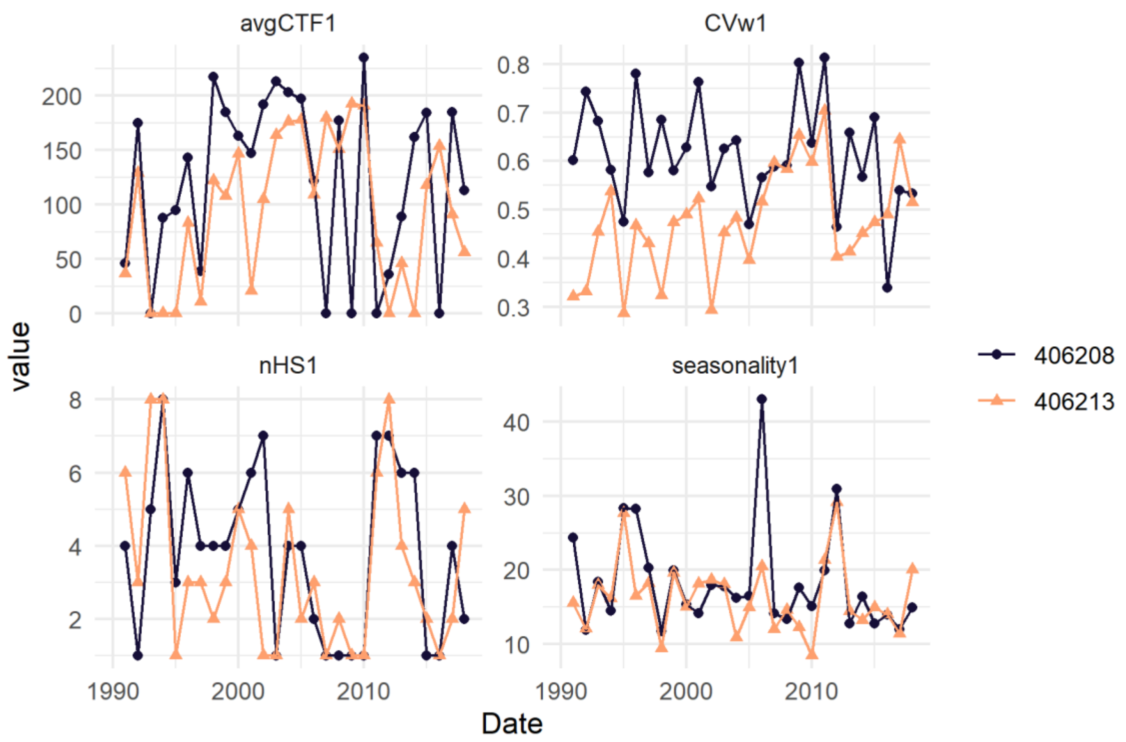

Figure 3. Key flow metrics in the upper Campaspe River calculated from daily flow data from gauges in the upper (406208) and lower (406213) reaches.

No long-term flow data is available for Campbells Creek. However, a recent study modelled stream flow in Campbells Creek, as well as the contribution of the treated water discharge from Castlemaine WRP (GHD 2019).

During the summer and autumn months, the contribution from the WRP is often the only available flow in the creek, suggesting Campbells Creek is a naturally ephemeral stream. Indeed, although there was water flow throughout the survey area during the current field visit, which occurred during a relatively wet period, the size and depth of the channel and presence of extensive instream reed beds suggests the upper reaches are regularly dry. It appears that the upper reaches of Campbells Creek experience regular and extended seasonal CTF events, while a minimal summer baseflow is generally maintained in the lower reaches due to the input from the WRP. Therefore, habitat suitability in the lower reaches is higher due to the availability of reliable surface water, which corresponds to the area where platypuses were detected during the current eDNA surveys.

As platypuses are completely dependent on aquatic ecosystems, cease-to-flow (CTF) events inherently reduce habitat availability for platypuses and macroinvertebrates due to a reduction in the total wetted area. Intuitively, this will lower food availability and the carrying capacity of aquatic systems, as well as inhibit platypus movements and dispersal (Griffiths and Weeks 2015), as riffle areas dry up. Although platypuses are known to undertake some terrestrial dispersal, it does expose them to predation and hyperthermia. Some systems will cope with CTF events better than others, due to the presence of deeper pools (number, size, and distribution) that provide refuge areas until conditions improve. Other impacts from CTF events include increased water temperature, and lower dissolved oxygen, which can further decrease macroinvertebrate abundance (Connolly et al. 2005; Chessman 2009). While platypuses can persist in waterways with CTF events, particularly with suitable refuge pools, longer and more frequent CTF events will severely limit the suitability of a waterway to support enough platypuses to maintain a viable population in the long term. Although it has not been directly quantified, it is likely that CTF events will have a more significant negative impact on platypuses than riparian or in-stream habitat quality, as measured by the HQI. It appears that a lack of permanent reliable surface water is the biggest issue in these two waterways.

Water Quality

The relationship between various water quality parameters and platypus populations (occurrence, abundance, recruitment) is poorly understood. The platypus's widespread distribution across a variety of waterway and habitat types illustrates the species' tolerance to a range of water quality parameters. As expected, treated water discharges from WRP result in downstream changes to several key water quality parameters, particularly total nitrogen (TN) and total phosphorous (TP), although discharges largely meet licensing criteria (GHD 2019; Biosis 2021). Higher abundance of platypuses in Melbourne streams has previously been associated with lower levels of TP, TN and suspended solids, although the analysis did not explore correlations or interactions between explanatory variables and sample sizes were small (Serena and Pettigrove 2005). Average values for TP and TN in the upper Campaspe River and Campbells Creek are above the median values found for waterways described as having medium (total P 0.048mg/L; total N 0.55 mg/L) or low (total P 0.099 mg/L; total N 0.67 mg/L) platypus density.

Few water quality metrics are likely to impact platypuses directly, but rather act indirectly through a reduction in their macroinvertebrate prey. Despite this, there is no evidence of impacts on downstream macroinvertebrate communities with taxa richness generally poor throughout the catchment, but with no observable difference between sites upstream and downstream of the treated water discharge points (Biosis 2021).

Conclusions

This study assessed the suitability of habitat for platypuses in the upper Campaspe River and Campbells Creek using several different metrics – a qualitative field assessment of riparian and in-stream metrics (HQI), calculating key flow metrics, and investigating several water quality parameters. Habitat was considered relatively poor in both waterways, with low HQI and poor flow regimes. Poor habitat quality is generally reflected in depauperate macroinvertebrate communities and sparse platypus populations. Extended (and increasing) seasonal cease-to-flow events appear to be the most significant issue, which would be exacerbated in the absence of treated water discharges from WRPs. Riparian vegetation and in-stream complexity were also considered poor throughout the study areas, although moderate local patches were evident. THE WRP inputs result in reduced water quality downstream of the discharge points, particularly with respect to elevated Total Nitrogen and Total Phosphorous. However, these are within the known tolerance ranges of platypuses, and while this may lead to reduced macroinvertebrate diversity, the generalist and opportunistic diet of platypuses suggest total macroinvertebrate abundance, rather than the presence of specific taxa is more important.

There was no evidence of higher platypus abundance or occurrence upstream of WRP discharge points, compared to downstream areas, suggesting the WRP discharges are not having significant detrimental impacts on platypus populations in either waterway. Indeed, the only occurrence of platypuses from the current eDNA surveys were recorded at several sites immediately downstream of the discharge point in Campbells Creek.

Platypuses are known to occur, and in some instances thrive, downstream of discharge points of other WRP in Victoria. Given the ephemeral nature of these two waterways, it is likely that the increased water availability provides greater net benefit to platypuses than any potential negative influence of reduced food availability. Nevertheless, platypus populations in both waterways appear to be relatively sparse, which is likely a reflection of overall poor habitat conditions from heavily modified catchment areas and regular cease-to-flow events.

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References

Bethge P., Munks S., Otley H. & Nicol S. (2003) Diving behaviour, dive cycles and aerobic dive limit in the platypus *Ornithorhynchus anatinus*. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **136** , 799–809.

Biggs J., Ewald N., Valentini A. *et al.* (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.029. [online].

Bino G., Kingsford R. T. & Wintle B. A. (2020) A stitch in time – Synergistic impacts to platypus metapopulation extinction risk. *Biol. Conserv.* **242** , 108399. [online].

Biosis (2021) *Coliban Water: 2020 Biological Stream Health Monitoring*. Coliban Water.

Boulton A. J. & Brock M. A. (1999) *Australian Freshwater Ecology: processes and management*. Gleneagles Publishing, Adelaide.

Ellem B. A., Bryant A. & O'Connor A. (1998) Statistical modelling of platypus, *Ornithorhynchus anatinus*, habitat preferences using generalized linear models. *Aust. Mammal.*

Gardner J. L. & Serena M. (1995) Spatial-Organization and movement patterns of adult male platypus, *Ornithorhynchus anatinus* (monotremata: Ornithorhynchidae). *Aust. J. Zool.* **43** , 91–103.

GHD (2019) *EPA Castlemaine WRP PAN Support Discharge Risk Assessment*. Coliban Region Water Corporation.

Goldberg C. S., Turner C. R., Deiner K. *et al.* (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.* **7** , 1299–1307.

Grant T. (2004) Depth and substrate selection by platypuses, *Ornithorhynchus anatinus*, in the lower Hastings River, New South Wales. *Proc. Linn. Soc. New South Wales*.

Grant T. (2014) The platypus and the environmental impact assessment process: more cogitations of a consultant. *Consult. Ecol.*

Grant T. & Gill G. (2011) *Manning River Platypus Monitoring; summer 2011 (Report to Mid Coast Water)*. Cronulla, NSW.

Grant T. R. (1983) Body temperatures of free-ranging platypuses, ornithorhynchus anatinus (Monotremata), with observations on their use of burrows. *Aust. J. Zool.* **31** , 117–122.

Grant T. R. (1992) Historical and current distribution of the platypus, *Ornithorhynchus anatinus*, in Australia. In: *Platypus and Echidnas* (ed M. L. Augee) pp. 232–254 Royal Zoological Society of New South Wales.

Grant T. R. & Temple-Smith P. D. (1998) Field biology of the platypus (*Ornithorhynchus anatinus*): historical and current perspectives. *Philos. Trans. R. Soc. London Ser. B-Biological Sci.* **353** , 1081–1091.

Grant T. R. & Temple-Smith P. D. (2003) Conservation of the platypus, *Ornithorhynchus anatinus*: Threats and challenges. *Aquat. Ecosyst. Heal. Manag.* **6** , 5–18.

Griffiths J., Kelly T., van Rooyen A. & Weeks A. (2014) *Distribution and relative abundance of platypuses in the greater Melbourne area: survey results 2013/14. (Report to Melbourne Water)*. cesar, Parkville.

Griffiths J. & Licul S. (2020) *Mapping the distribution of platypuses and blackfish in the upper Campaspe region using citizen scientists and environmental DNA. Prepared for: Upper Campaspe Landcare Network*. EnviroDNA, Parkville, VIC.

Griffiths J., Licul S. & Weeks A. (2001) *Determining the distribution of platypuses & short-finned eels in the Moorabool River subcatchment. Report for Moorabool Catchment Landcare Group.* EnviroDNA, Parkville, VIC.

Griffiths J., Maino J. & Weeks A. (2019) *Identifying key flow variables and quantifying their impact on platypus populations (Report to Melbourne Water)*. **cesar**, Parkville, VIC.

Griffiths J., van Rooyen A. & Weeks A. (2016) *Distribution and relative abundance of platypuses in the greater Melbourne area: survey results 2015/16. (Report to Melbourne Water)*. **cesar**, Parkville.

Griffiths J., Song S. & Weeks A. (2019) *Determining the distribution of platypuses in the upper Barwon region using environmental DNA. (Prepared for Upper Barwon Landcare Network)*. EnviroDNA, Parkville, VIC.

Griffiths J. & Weeks A. (2018) *Platypus Strategic Management Plan for Melbourne ' s Catchments (Report to Melbourne Water).* **cesar**, Parkville, VIC.

Gust N. & Handasyde K. (1995) Seasonal variation in the ranging behaviour of the platypus (*Ornithorynchus anatinus*) on the Goulburn River, Victoria. *Aust. J. Zool.* **43** , 193–208.

Holland N. & Jackson S. M. (2002) Reproductive behaviour and food consumption associated with the captive breeding of platypus (*Ornithorhynchus anatinus*). *J. Zool.*

Hope P., Timbal B., Hendon H., Ekström M. & Potter N. (2017) *A synthesis of findings from the Victorian Climate Initiative (VicCI)*. Australia.

Jacobs, APC & **cesar** (2016) *Understanding the environmental water requirements of platypus. Report by Jacobs, Australian Platypus Conservancy and* ***cesar*** *for Melbourne Water.*

Krueger B., Hunter S. & Serena M. (1992) Husbandry, diet and behaviour of platypus *Ornithorhynchus anatinus* at Healesville Sanctuary. *Int. Zoo Yearb.*

Lugg W. H., Griffiths J., van Rooyen A. R., Weeks A. R. & Tingley R. (2018) Optimal survey designs for environmental DNA sampling. *Methods Ecol. Evol.* **9** , 1049–1059.

Martin E. H., Walsh C. J., Serena M. & Webb J. A. (2013) Urban stormwater runoff limits distribution of platypus. *Austral Ecol.* doi: DOI: 10.1111/aec.12083.

Rohweder D. A. & Baverstock P. R. (1999) Distribution of platypus *Ornithorhynchus anatinus* in the Richmond river catchment, northern New South Wales. *Aust. Zool.* **31** , 30–37.

Serena M. & Pettigrove V. (2005) Relationship of sediment toxicants and water quality to the distribution of platypus populations in urban streams. *J. North Am. Benthol. Soc.* **24** , 679–689. [online].

Serena M., Swinnerton M., Worley M. & Williams G. A. (2000) *Status and habitat relationships of platypus in the Diamond and Arthurs Creek catchments: I. Results of surveys and habitat studies along Running Creek, summer 2000. (Report to Melbourne Water)*. Australian Platypus Conservancy, Whittlesea.

Serena M, Thomas J. L. & Williams G. A. (1998) *Status and habitat relationships of platypus in the Dandenong Creek Catchment: II. Results of surveys and radio-tracking studies, September 1997 - March 1998. (Report to Melbourne Water)*. Australian Platypus Conservancy, Whittlesea.

Serena M., Thomas J. L., Williams G. A. & Officer R. C. E. (1998) Use of stream and river habitats by the platypus, *Ornithorhynchus anatinus*, in an urban fringe environment. *Aust. J. Zool.* **46** , 267–282.

Serena M. & Williams G. A. (2008) *Distribution and management of platypus in the greater Melbourne region. (Report to Melbourne Water)*. Australian Platypus Conservancy, Wiseleigh.

Serena M., Williams G. A., Weeks A. R. & Griffiths J. (2014) Variation in platypus (Ornithorhynchus anatinus) life-history attributes and population trajectories in urban streams. *Aust. J. Zool.* **62** , 223–234.

Serena M., Worley M., Swinnerton M. & Williams G. A. (2001) Effect of food availability and habitat on the distribution of platypus (*Ornithorhynchus anatinus*) foraging activity. *Aust. J. Zool.* **49** , 263–277.

Smart A. S., Tingley R., Weeks A. R., Van Rooyen A. R. & McCarthy M. A. (2015) Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol. Appl.* **25** , 1944–1952.

Thomas A. C., Nguyen P. L., Howard J. & Goldberg C. S. (2019) A self-preserving, partially biodegradable eDNA filter. *Methods Ecol. Evol.* **10** , 1136–1141.

Thomsen P. F., Kielgast J., Iversen L. L. *et al.* (2012) Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.* **21** , 2565–2573.

Valentini A., Taberlet P., Miaud C. *et al.* (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* **25** , 929–942.

Woinarski J. & Burbidge A. A. (2016) *Ornithorhynchus anatinus*. The IUCN Red List of Threatened Species.

Woinarski J. C. Z., Burbidge A. A. & Harrison P. L. (2014) *The action plan for Australian mammals 2012*. CSIRO Publishing, Collingwood.

Yamanaka H., Motozawa H., Tsuji S., Miyazawa R. C., Takahara T. & Minamoto T. (2016) On-site filtration of water samples for environmental DNA analysis to avoid DNA degradation during transportation. *Ecol. Res.* **31** , 963–967.

Young W. J. (2001) Rivers as Ecological Systems: The Murray-Darling Basin.

Appendix 1: Habitat quality index scores for all sites along Campbells Creek and upper Campaspe River.

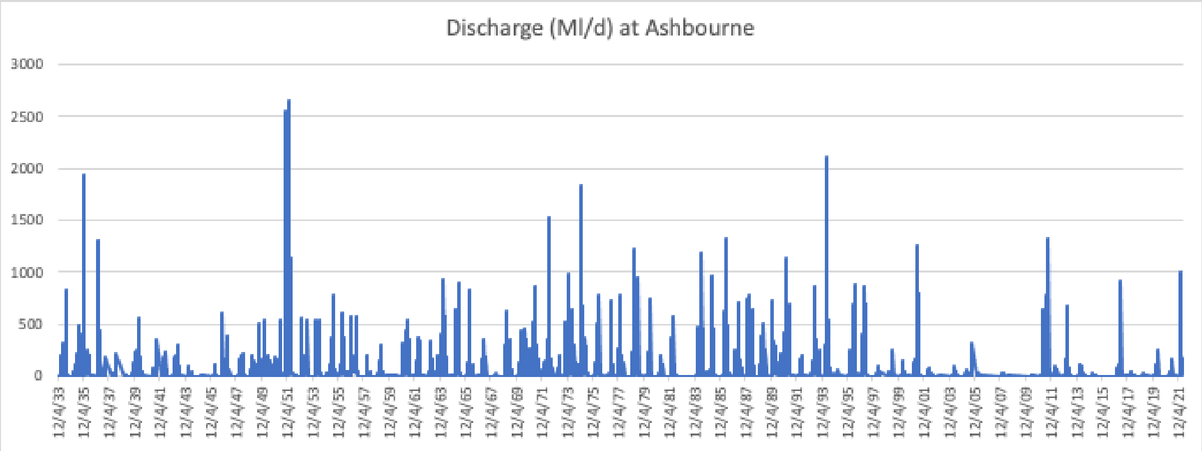
Table A1.1: Campbells Creek

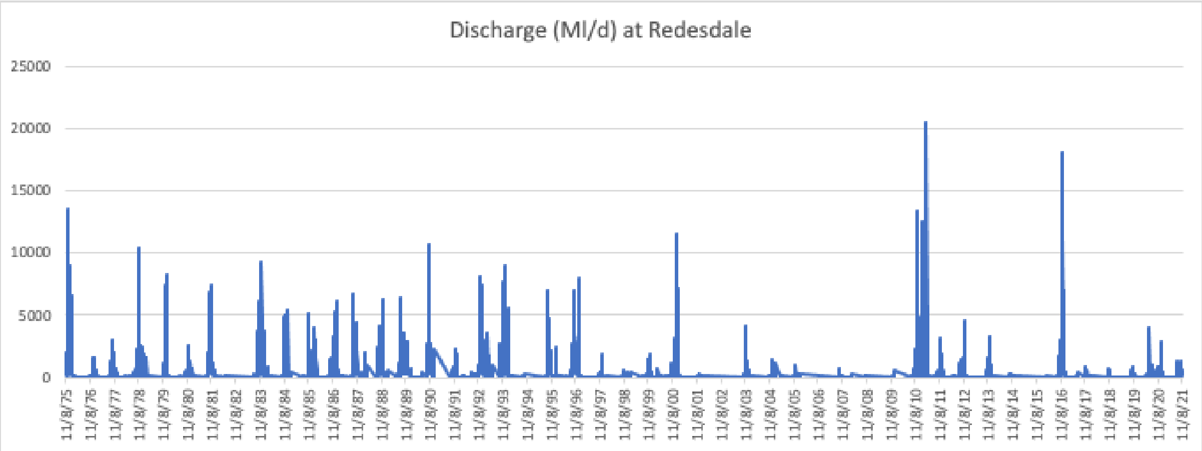
Site Code	CC01	CC02	CC03	CC04	CC05	CC06	CC07	CC08	CC09
Consolidated banks	1	0	2	2	2	2	2	3	4
Trees on banks	1	1	1	2	2	3	1	2	3
Overhanging veg.	1	0	1	1	3	1	1	1	3
Earthen banks	4	4	4	4	4	4	4	3	4
Bank height >1m	2	3	2	2	2	3	1	3	1
Bank profile	2	2	2	2	2	3	1	2	0
Absence of erosion	1	0	2	1	2	2	2	3	3
Pool depth (>1m, <5m)	1	1	1	2	2	3	2	3	0
Coarse organic matter	1	1	1	2	2	2	3	1	1
Large woody debris	2	2	2	2	2	2	3	2	3
Benthic substrate	1	1	1	1	1	1	1	1	1
HQI score	0.386	0.341	0.432	0.477	0.545	0.591	0.477	0.545	0.523

Table A1.2: Upper Campaspe River

Site Code	6	7	9	CR60	8	11	Kyneton	12	62	15	61	39	14
Consolidated banks	3	3	3	3	1	2	1	1	3	2	3	1	2
Trees on banks	2	2	2	2	1	1	2	1	2	1	1	1	1
Overhanging veg.	3	2	2	2	1	2	1	1	2	1	2	1	2
Earthen banks	4	3	3	4	4	4	4	4	4	4	4	4	4
Bank height >1m	3	3	2	2	1	3	3	1	2	3	2	2	3
Bank profile	2	2	2	2	1	2	2	2	2	2	2	1	2
Absence of erosion	3	3	3	3	2	2	1	1	3	2	2	1	1
Pool depth (>1m, <5m)	2	3	1	1	1	3	4	2	3	3	1	1	1
Coarse organic matter	2	2	2	2	3	0	1	1	0	0	1	1	1
Large woody debris	2	2	3	2	3	2	2	2	2	2	2	3	3
Benthic substrate	2	2	3	3	2	1	1	2	2	1	2	1	1
HQI score	0.636	0.614	0.591	0.591	0.455	0.500	0.500	0.409	0.568	0.477	0.500	0.386	0.477

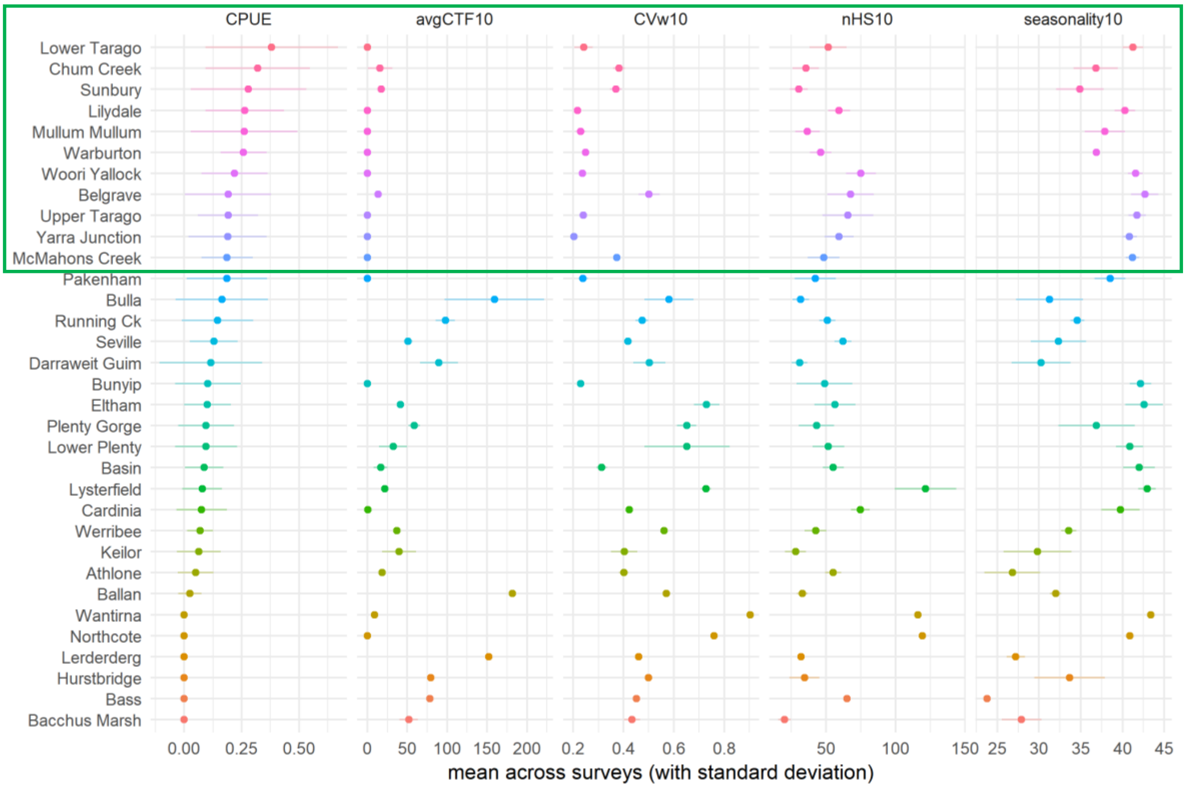
Appendix 2. Long-term daily flow data in the upper Campaspe River





*Note different time and flow scales between graphs.

Appendix 3. Overview of platypus populations and key flow metrics for survey locations across greater Melbourne.



* Green box indicate "healthy' platypus populations (from Griffiths *et al.* 2019).