PLATYPUS POPULATION HEALTH ASSESSMENT IN THE INGLIS AND SEABROOK CATCHMENTS IN NORTHWEST TASMANIA

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Introduction and Aims

This paper will present a holistic approach to monitoring platypus populations that would also be applicable to other wildlife species. The project focused on platypus conservation and the aim was to develop a method, and gather baseline data, to assess the health of wild platypus populations in terms of their ability to maintain their size in the long term. A range of threats to platypuses have been described, including habitat degradation and the fungal disease mucormycosis (Grant and Temple-Smith, 2003; Gust and Griffiths, 2010; Serena and Williams, 2010). However, many of the methods used to assess population size or density in other species (e.g. remote monitoring, capture rates or mark-recapture studies) are very difficult to interpret when used to assess platypus populations (Gust and Griffiths, 2010; Grant, 2012). This complicates the assessment processes for IUCN, federal and state threatened species lists and

makes it difficult to assess the impacts of the threats they face and leads to the possibility that population declines go unnoticed until they are extreme or complete (Grant, 2012). Our approach arose partly because of these difficulties of knowing how many platypuses there are in a particular area and partly because wildlife conservation is influenced by factors in a variety of fields of study. The project relies in part on assessment of individual platypus health and is consistent with the increasing recognition of disease as an indicator and a cause of population declines in wildlife species (Munson and Karesh, 2002). We have also incorporated a wide range of more traditional ecological factors such as habitat characteristics, population distribution, short- and medium-term movements, reproduction, survivorship and measures of population density. Lastly we have assessed genetic diversity as a measure of a population's likely ability to respond to changes, and also as a guide to long-term movements within and between populations (Kolomyjec et al., 2009). It should be noted that the distribution of platypuses has changed little since European settlement and the species is not listed on any threatened species list, except in South Australia, where they only ever had a limited distribution (Grant, 2012). However, as Possingham et al. (Possingham et al., 2002) point out, the best time to perform research to ensure the survival of a species is when there is still a reasonable sized population.

Methods

Study populations

Although platypuses are known to move over land to avoid obstacles in rivers, to get to burrows and to move between water bodies (Munday et al., 1998; Mooney and Spencer, 2000), they usually move in water. Consequently, we defined a population to be all the platypuses in a connected system of water bodies. In this project we studied two neighbouring populations of platypuses near Wynyard, northwest Tasmanian: those in rivers, streams and dams draining into the Inglis River and those in water bodies draining into Seabrook Creek. The Inglis River catchment is much larger than the Seabrook creek catchment and can be divided into 11 sub catchments (CFEV database, 2005).

Fieldwork sites

We selected a total of 138 sites in our two study catchments, with sites in nine of the sub catchments in the Inglis River catchment. Sites were selected 1) to provide a broad geographical coverage of

the study area, 2) to represent the major land-uses in the catchment, 3) only if they were suitable to the capture of platypuses with fyke nets, and 4) where there was reasonable vehicular access. Habitat features for each site were collated including, sub catchment, river class, altitude (m), and area of forest cover (native and plantation combined) within a 500m radius; as well as largest river order, largest dam, and surface area of water within 500m of connected waterway.

Capture and examination

Over two years starting in August 2011 we undertook capture, mark and release of platypuses in the Inglis River and Seabrook Creek catchments. During fieldwork sessions fyke nets were set in pairs in rivers and streams with the intention of capturing platypuses moving in either direction along the waterway (Macgregor et al., 2010). Nets were generally set mid to late afternoon and removed from the water around 11pm to midnight that night. Nets were checked every hour for captured platypuses. When a platypus was found it was removed from the net immediately and placed in a holding sack to await examination.

To minimise stress on the platypuses, examination was performed in the field close to the site of capture and under anaesthesia, in a tent or farm shed (where available). Anaesthesia was induced and maintained with isoflurane delivered in oxygen via face mask. We had to take particular care to keep the platypus' body temperature within the normal range. Hyperthermia has been an issue during platypus anaesthesia on the mainland (McKee, unpublished), but during field anaesthesia in the cool Tasmanian climate platypuses appeared more likely to lose heat. So when a platypus was anaesthetised we monitored its temperature constantly and used a thermostatically-controlled heat pad and bubble wrap blanket, as necessary, to maintain their body temperature.

We recorded the platypuses weights, body condition (Tail volume index, TVI; (Grant and Carrick, 1978) and morphometrics. We also examined for signs of the ulcerative disease mucormycosis and other skin lesions, counted external parasites and collected samples for subsequent testing of blood, excreta, ticks and a skin clip. Platypuses were individually identified with a microchip inserted under the skin between the shoulder blades (scapulae) (Grant, 2004) and the internal reproductive organs were examined using ultrasonography.

Remote monitoring

We developed the use of instream microchip readers, similar to systems that have been used for monitoring a variety of other species (Kerry et al., 1993; Zydlewski et al., 2006; O'Donnell et al., 2011), for monitoring microchipped platypuses. We used two antenna types: 1) the Trovan® ANT 612 flat panel that was placed on the floor of a creek for platypuses to move over, and 2) Trovan® ANT C600, a circular hoop placed in a creek so that water flowed through the middle, and animals could also move through it. The antennas were connected to Trovan® LID650 dataloggers on the bank which recorded the microchip number, time and date of any microchipped platypus that was detected. The whole system was powered by a solar panel. *We used the antennas at 18 locations in the Inglis catchment and also at one location in the Forth Wilmot catchment where platypuses were microchipped between 1996 and 2002 by researchers involved with different studies (Otley et al., 2000; Bethge, 2002).*

Genetic analysis

Genetic analysis was performed using an existing MHC typing protocol (Lillie et al., 2012) at the Menzies Research Institute in Hobart. DNA was extracted from skin samples from platypuses in the Seabrook catchment and the MHC II DZB gene was amplified using PCR. Products were sub-cloned into pGEM-T easy and ten plasmids per product were sent to AGRF Melbourne for sequencing.

Laboratory testing

Samples of platypus blood and excreta were sent for testing (biochemistry, haematology, parasitology and microbiology) to the Animal Health Laboratory, Prospect, Launceston.

Results and Discussion

We performed 117 nights of fieldwork and captured a total of 154 individual platypuses (63 adult females, three juvenile females, 76 adult males, six subadult males, and six juvenile males), with 12 recaptures. The total capture effort for the project was 1551.42 net hours. This consisted of 416.17 net hours before sunset and 1135.25 net hours after sunset. Only three of the 166 platypus captures were before sunset. We had a similar number of captures in all four seasons (Table 1). We spent more time working in some subcatchments, but our overall capture rates were similar between

the subcatchments. Detailed analysis of the results of this project is ongoing and the data presented are preliminary results.

Table1. **Number of individuals captured in each subcatchment in each season. *** One individual captured twice in this season but only counted once in this table.

Subcatchment	Number of individuals captured			
	Spring	Summ	Autum	Winter
Inglis River catchment				
Lower Inglis River	3	2	6	0
Flowerdale River	10	10	10	3
Inglis River	4	2	6	3
Blackfish Creek	4	2	4	3
Big Creek	8*	6	4*	3
Camp Creek	6	7	3	11
Upper Inglis	5	3	3	2
Garners Creek	0	0	1	0
Upper Flowerdale	0	0	1	0
Seabrook Creek	4	9*	5	10
Total	44	41	43	35

Table 2. **Capture effort (measured in net hours) after sunset by catchment/subcatchment and season.**

	Spring	Summ	Autu	Winte	Total
Inglis River catchment					
Lower Inglis River	14.23	7.03	38.47	0.00	59.73
Flowerdale River	57.55	39.95	76.52	40.35	214.37
Inglis River	22.10	12.47	81.00	17.95	133.52
Blackfish Creek	23.03	18.85	38.37	48.02	128.27
Big Creek	41.52	33.90	50.77	52.48	178.67
Camp Creek	16.82	42.02	32.18	58.58	149.60

Upper Inglis	17.28	19.42	37.00	20.92	94.62
Garners Creek	0.00	3.98	5.47	0.00	9.45
Upper Flowerdale	0.00	3.65	5.00	0.00	8.65
Seabrook Creek	3.72	64.07	15.97	74.63	158.38
Total	196.25	245.33	380.73	312.93	1135.25

Table 3. **Capture rates (platypuses/net pair hour after sunset) by catchment/subcatchment and season.**

	Spring	Summ	Autum	Winte	Total
Inglis River catchment					
Lower Inglis River	0.21	0.28	0.16	N/A	0.18
Flowerdale River	0.17	0.25	0.13	0.07	0.15
Inglis River	0.18	0.16	0.07	0.17	0.11
Blackfish Creek	0.17	0.11	0.10	0.06	0.10
Big Creek	0.22	0.18	0.10	0.06	0.13
Camp Creek	0.36	0.17	0.09	0.19	0.18
Upper Inglis	0.29	0.15	0.08	0.10	0.14
Garners Creek	N/A	0.00	0.18	N/A	0.11
Upper Flowerdale	N/A	0.00	0.20	N/A	0.12
Seabrook Creek	1.08	0.16	0.31	0.13	0.18
Total	0.23	0.17	0.12	0.11	0.15

The number of platypuses captured at each site correlated positively with the surface area of water within 500m of connected waterway and negatively with the amount of forest cover within a 500m radius. The relationship with surface area of water is likely due to availability of foraging habitat. The negative relationship to bush cover may relate to one or both of two factors: 1) land clearing for agriculture has occurred on the most productive land and the water bodies in such areas are known to support a relatively high density of benthic macroinvertebrate communities, and 2) run-off of artificial

nutrients spread on agricultural land may change in the composition of benthic macroinvertebrate communities, and an increase in the proportion of more digestible species such as worms. This finding does not necessarily imply that agriculture has an overall positive effect on platypus populations and caution needs to be exercised in interpreting this finding. Even if the platypus population size is currently higher in agricultural areas than in less altered habitats, enhanced disease transmission and altered population structures may have adverse long-term conservation impacts.

Morphometrics

Body mass, TVI and body length of platypuses were consistent with values previously recorded for Tasmanian platypuses (Connolly and Obendorf, 1998; Otley et al., 2000; Bethge, 2002; Gust and Griffiths, 2011).

Table 4. **Morphometrics of adult female platypuses**

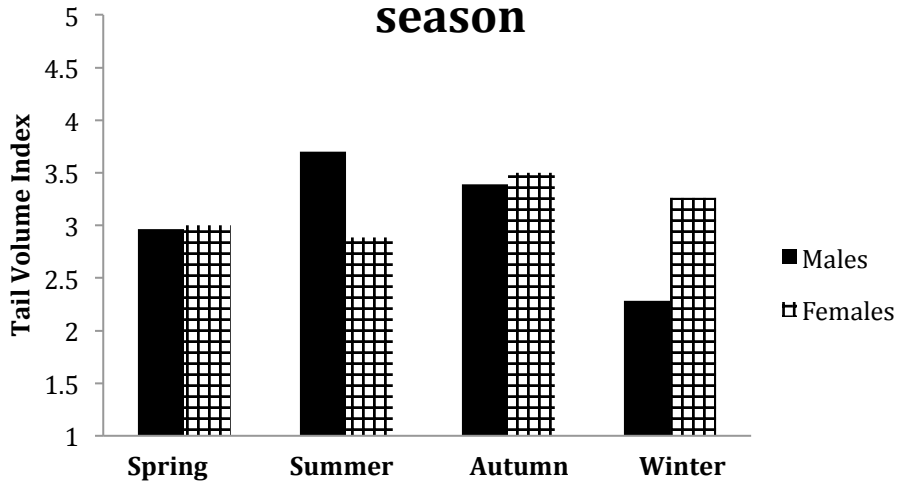
Females	n	Body mass	TVI	Body length
Inglis River catchment				
Lower Inglis River	6	1.43	3.2	48.4
Flowerdale River	14	1.32	3.1	47.7
Inglis River	7	1.27	3.4	46.4
Blackfish Creek	6	1.34	3.2	47.3
Big Creek	5	1.25	3.4	46.8
Camp Creek	8	1.27	3.5	46.7
Upper Inglis	6	1.32	2.7	48.8
Garners Creek	0	N/A	N/A	N/A
Upper Flowerdale	1	1.12	4.0	45.0
Overall Inglis	53	1.29	3.3	47.1
Seabrook Creek	10	1.35	2.9	47.5

Overall	63	1.32	3.2	47.4

Table 4. **Morphometrics of adult male platypuses**

Males	n	Body mass	TVI	Body length
Inglis River catchment				
Lower Inglis River	3	2.45	3.0	58.2
Flowerdale River	17	2.01	3.1	54.7
Inglis River	6	2.20	3.2	56.2
Blackfish Creek	6	1.85	3.7	53.8
Big Creek	14	2.08	3.1	55.5
Camp Creek	12	2.14	3.1	56.0
Upper Inglis	6	1.96	2.8	53.9
Garners Creek	1	1.68	4.0	52.0
Upper Flowerdale	0	N/A	N/A	N/A
Overall Inglis	65	2.05	3.3	55.0
Seabrook Creek	11	2.07	3.3	54.6
Overall	76	2.06	3.2	55.1

Figure 1. Tail Volume Index by season



Only seven individuals in our study (three adult females, three adult males and one juvenile male) out of 166 captures had a TVI in the lowest category of 5.

Infectious disease

We recorded a low exposure to infectious diseases. We found no ulcers consistent with mucormycosis. Four platypuses had nodules in the webbing of their feet, of which two showed histological features similar to mucormycosis (Connolly et al., 2000). However, fungal culture and PCR on samples from these platypuses did not indicate that Mucor amphibiorum was present and it is likely that these nodules were caused by opportunistic organisms that entered through skin wounds acquired during foraging. Ticks were common but internal parasites were rarely found. The prevalence of antibody titres to Leptospira spp. was low. Although Leptospira infections are not known to cause disease in platypuses, previous studies in other locations have found up to 50% of platypuses to have been exposed to the infection (Loewenstein et al., 2008). Seven platypuses (five adult males, two adult females), from six different subcatchments in the Inglis River catchment, were found to be infected with Salmonella spp; none of these individuals appeared to be in poor health. The detection of Salmonella was distributed throughout the year. Salmonella infection is not an uncommon subclinical finding in wildlife species. Six of the seven Salmonella isolates were Salmonella mississippi which is common in Tasmanian wildlife and aquatic environments (Ashbolt and Kirk, 2006). The other isolate was Salmonella bovismorbificans which is generally a cow-associated

strain in Tasmania (G. Knowles, Animal Health Laboratory, Launceston, personal communication).

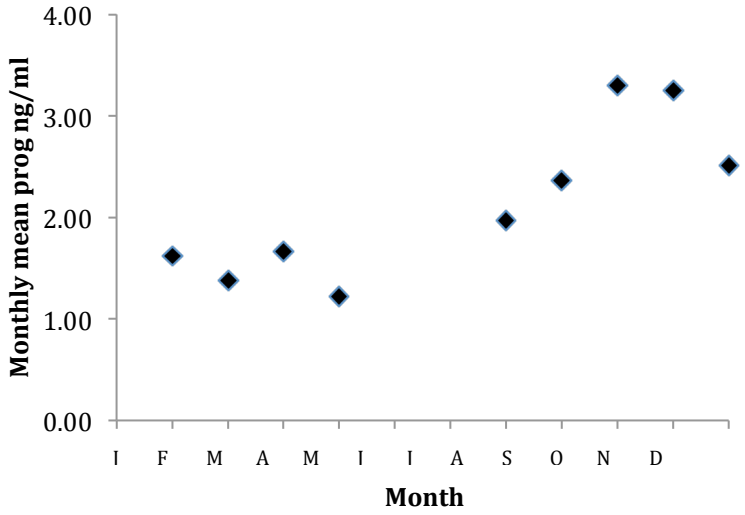
Table 5 **Evidence of exposure to infectious diseases in platypuses.**

Infectious agent	Testing method	n	n positive
Salmonella	Bacterial culture	151	7
Leptospira	Serology	115	10
Unidentified spiral bacteria	Microscopy of cloacal swab	120	7
Fungal granuloma in foot webbing	Clinical exam, histology, culture, PCR	154	3
Other granuloma in foot webbing	Clinical exam, histology, culture	154	1
Mucormycosis	Clinical exam, histology, culture, PCR	154	0
Theileria	Microscopy on blood film	142	131
Trypanosomes	Microscopy on blood film	143	131
Toxoplasma gondii	Serology	113	1
Cryptosporidia	Excreta microscopy	111	1
Coccidia	Excreta microscopy	111	5
Cestodes	Excreta microscopy	111	1
Ticks	Clinical examination	154	147
Leeches	Clinical examination	154	2

Reproductive activity

We were able to find well-developed ovarian follicles in two platypuses – one at the end of November and one in mid-December. Figure 2 shows that mean serum progesterone in females, during the second year of fieldwork, was highest from September to December.

Figure 2. Monthly serum progesterone means (Sept 2012-Aug 2013)



This pattern of serum progesterone level is consistent with previous findings (Jakubowski et al., 1998) . However, the peak levels occurred two months later than in platypuses in New South Wales (Jakubowski et al., 1998). This and the finding of well-developed ovarian follicles in November and December is consistent with previous reports that juvenile platypuses emerge from their nesting burrows about two months later in Tasmania than on the mainland (Connolly and Obendorf, 1998; Munks et al., 1999).

Remote monitoring

Results from the first 18 platypuses monitored using in-stream microchip readers indicates that platypuses investigate the antennas ~8% of the times they encounter them and that they may turn around ~1% of the times they encounter them. However, the antennas generally appear to be well tolerated by platypuses. Direction of movement studies with two antennas close together in the same creek indicated that 7% of platypus passes identified by one antenna were not detected by the second antenna. Some individuals were detected only once, some displayed a regular daily pattern of observations mostly over periods of approximately three weeks and others showed intermediate patterns of observations (Macgregor et al., In press). The frequency of observations varied between individuals which may be a result of the position of the antenna in a

particular platypus's home range (as platypuses have been shown to use different parts of their home range at different frequencies), as well as the size of the platypus's home range. Remote monitoring indicated that most platypuses continued to be present in the stretch of creek where they were captured within the time frame of this study.

One antenna has been in place at a site for over 12 months, with the four platypuses that had been captured there being regularly detected over that time. Six platypuses microchipped 4-5 years previously were detected in the Inglis River catchment and three individuals at the site in the Forth Wilmot catchment were found to still be present 14-15 years after they had been captured and microchipped. This finding indicates that this will be a useful method of assessing survivorship and hence the impacts of disease and environmental change on individuals within a population.

Immunogenetics

Of the 18 platypuses tested from the Seabrook Creek catchment, immunogenetic analysis found 12 alleles of the MHC class II DZB gene (Table 6). Of these alleles, ten have been previously described, including eight which have been previously found in Tasmanian platypuses (Lillie et al., 2012).

Table7 MHC **class II DZB alleles in platypuses from the Seabrook Creek catchment. *Alleles not previously reported from Tasmanian platypuses**

Platypus number	MHC class II DZB Allele 1 - GenBank accession numbers except for new alleles	Allele 2 (unless homozygous for allele 1) - GenBank accession numbers except for new alleles.
10	GQ165535	
12	GQ165567	
13	GQ165565*	GQ165580*
58	GQ165550	GQ165590
59	GQ165550	New allele 1
94	GQ165567	
95	GQ165535	New allele 1
97	GQ165550	GQ165590
124	GQ165542	
125	GQ165567	GQ165585
127	GQ165542	
146	GQ165546	GQ165550
152	GQ165535	
153	GQ165535	New allele 2
154	GQ165535	
155	New allele 2	
156	GQ165535	GQ165550
158	GQ165561	GQ165567

Further analysis is needed of these results, but initial indications are that this gene has a reasonable genetic diversity within this population. As genetic diversity can be a measure of a population's ability to respond to changes in its environment, and in the case of this gene that is likely to relate to infectious challenge, this population certainly compares favourably as compared to the King Island population in which all the platypuses appear to be identical at

this gene (Lillie et al., 2012). In addition, there was no evidence that certain parts of the subcatchment are isolated, with the various alleles being spread through the catchment.

Conclusion

We found no evidence that the platypus populations in the Inglis River catchment and Seabrook Creek catchment are in poor health. However, this study provides only initial baseline information. Further understanding of platypus population health would be acquired by repeating the methods in this study in areas with different characteristics such as: greater or less habitat degradation, historical evidence of the disease mucormycosis, greater isolation such as on King Island or Kangaroo Island, or evidence of population decline.

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He has a Master of Veterinary Studies degree in Conservation Medicine from Murdoch University. During this degree he performed a nine month field study investigating the prevalence of mucormycosis in platypuses in northwest Tasmania

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While still doing a small amount of work in vet practice, he is currently close to completing a PhD at the Conservation Medicine Program at Murdoch University Vet School, investigating platypus population health more broad