# INVESTIGATION INTO INDIVIDUAL HEALTH AND EXPOSURE TO INFECTIOUS AGENTS OF PLATYPUSES (*ORNITHORHYNCHUS ANATINUS*) IN TWO RIVER CATCHMENTS IN NORTHWEST TASMANIA

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ABSTRACT: Changes in the health of individuals within wildlife populations can be a cause or effect of population declines in wildlife species. Aspects of individual platypus (*Ornithorhynchus anatinus*) health have been reported. However, holistic studies investigating potential synergistic effects of both pathogens and environmental factors are needed to expand understanding of platypus individual health. We collected baseline data on the health of platypuses in two Tasmanian river catchments (including evidence of the potentially fatal fungal disease mucormycosis) and on individual, demographic, and geographic patterns associated with health data results. We examined 130 wild platypuses from the Inglis River Catchment and 24 platypuses from the Seabrook Creek Catchment in northwest Tasmania between 29 August 2011 and 31 August 2013. More than 90% of captured platypuses were infected with ticks, *Theileria* spp., and trypanosomes. Evidence of exposure to other infections, including *Salmonella* spp., *Leptospira* spp., and intestinal parasites, was low (<10%). Three platypuses had single fungal granulomas in the webbing of a forefoot, but no evidence of mucormycosis was found in any of the study animals. Possible subclinical hepatopathies or cholangiohepatopathies were found in six platypuses. Exposure to infectious agents did not cluster geographically, demographically, or in individuals, and there was minimal evidence of morbidity resulting from infection. This study has provided important baseline data for monitoring the effects of threatening processes, including mucormycosis, on the health of infected populations.

*Key words:* Aquatic animal, individual health, mucormycosis, *Ornithorhynchus anatinus*, platypus.

## INTRODUCTION

The health of individuals within wildlife populations can have important effects on reproductive success and longevity and, therefore, population health. Infectious diseases have been involved to varying extents in species extinctions (Cunningham and Daszak 1998; Daszak and Cunningham 1999; Schloegel et al. 2006), and the health of individuals can be an indicator of species decline (Munson and Karesh 2002). However, in many situations there has been little baseline information on individual health before these species were observed to be in decline.

The platypus (*Ornithorhynchus anatinus*) is a semiaquatic mammal found only in eastern Australia, with its distribution ranging from Cooktown in Queensland to Tasmania. It is one of only five extant species of the order Monotremata (subclass Prototheria), the only egg-laying mammals, and the only extant species of the family Ornithorhynchidae (Flannery and Groves 1998; Booth 2003). A range of issues relating to individual platypus health has been addressed by previous studies. Platypus body mass, body size, and

body condition have commonly been reported (Grant and Temple-Smith 1983; Serena and Williams 1997; Connolly and Obendorf 1998). Reference ranges for hematology and biochemistry parameters have been produced (Whittington and Grant 1984; Booth and Connolly 2008; Geraghty et al. 2011). Infection with, or exposure to, a range of infectious agents has also been reported (Table 1). The most notable of these is the fungus *Mucor amphibiorum*, the causative agent of mucormycosis in platypuses. Mucormycosis has been reported in certain river catchments in Tasmania but, despite the presence of *M. amphibiorum* in amphibians and the environment, the disease has never been reported in mainland states (Spear et al. 1994; Stewart and Munday 2005). Clinical signs of mucormycosis vary from nonulcerated, hairless nodules (<10 mm diameter) or plaques (10–54 mm diameter) (Connolly and Obendorf 1998; Connolly et al. 2001) to cutaneous ulcers (5–100 mm diameter) (Munday et al. 1998; Connolly et al. 2001). These ulcers can progress to involve underlying muscle up to 10 mm below the skin, and lesions are sometimes found in the internal organs, particularly the lungs (Munday et al. 1998; Connolly et al. 2001; Stewart and Munday 2005). The ulcers are assumed to lead to impaired thermoregulation (due to loss of fur) and impaired mobility (Connolly and Obendorf 1998) and are likely to predispose affected animals to secondary infections and flystrike (Connolly and Obendorf 1998; Munday et al. 1998). Mucormycosis can be fatal and is considered a conservation threat (Munday et al. 1998).

Despite the variety of investigations into individual platypus health, no study has used the full range of available health assessment techniques in the same population to give a holistic analysis of individual health. We gathered baseline data on a broad range of individual health parameters for platypuses in the Inglis River Catchment (from which a preliminary study of 26 platypuses had reported morphometric data and an absence of evidence of mucormycosis; Macgregor 2008; Macgregor et al. 2010) and in the Seabrook Creek Catchment in northwest Tasmania. We looked for individual, demographic, and geographic patterns associated with health data results. We investigated, in detail, any cases that showed clinical similarities to mucormycosis.

## MATERIALS AND METHODS

### Study animals

We performed fieldwork in the Inglis Catchment (41°3′S, 145°38′E) in northwest Tasmania 29 August 2011 to 31 August 2013 (Fig. 1, left). The Inglis Catchment is an area of land defined for management and administration purposes (CFEV 2005) and consists of one large river basin (the Inglis River Catchment; further divided into subcatchments for the purposes of this project) and several smaller hydrologically distinct areas. In addition to nine subcatchments in the Inglis River Catchment, fieldwork was performed in the hydrologically distinct Seabrook Creek Catchment (named for this project) to the east of the study area (Fig. 1, left).

We performed health examinations on 130 wild platypuses (53 adult females, two juvenile females, 65 adult males, six subadult males, and four juvenile males) from the Inglis River Catchment and 24 platypuses (10 adult females, one juvenile female, 11 adult males, and two juvenile males) from the Seabrook Creek Catchment (Fig. 1, left). All individuals were captured using fyke nets (Macgregor et al. 2010). Repeat examinations were performed on 10 individuals (two adult females recaptured once, six adult males recaptured once, and two adult males recaptured twice). The fieldwork schedule in part aimed for captures to be distributed evenly between seasons. During the 24-mo study, there were 45 platypus captures in spring (September–November), 42 in summer (December–February), 44 in autumn (March–May), and 35 in winter (June–August).

### Individual health examinations and sampling

Before anesthesia, each platypus was examined for external abnormalities and given a visual assessment for alertness (spontaneous movement or responsiveness to visual, auditory, and tactile stimuli during handling and examination [or both]). Body mass was measured (±10 g using digital scales; Rapala VMC Corporation, Vääksy, Asikkala, Finland) by weighing the platypus in its holding sack before anesthesia and subtracting the weight of the sack.

A range of morphometric examinations were performed under anesthesia. Sex and age were

TABLE 1.    Infections reported in platypuses (*Ornithorhynchus anatinus*) and their effects.

| Infectious agent | Disease in platypus | Source |
|---|---|---|
| **Viruses** | | |
| Adenomavirus-like agent | Minor lesions in kidneys | Whittington et al. 1990 |
| Papilloma virus (possible diagnosis on basis of clinical signs) | Papules on webbing of feet | Munday et al. 1998 |
| **Bacteria** | | |
| *Leptospira* spp. | Unknown, but seroconversion has been observed and spiral bacteria have been demonstrated in the renal cortex of a platypus that drowned in a fishing net | Munday et al. 1998; Loewenstein et al. 2008 |
| *Salmonella* spp. | Diarrhea in one case, systemic disease in two cases | Munday et al. 1998 |
| *Corynebacterium ulcerans* | Cutaneous ulcer | Macgregor et al. 2010 |
| *Dermatophilus congolensis* | Scabs on skin | Lunn et al. 2016 |
| Widespread bacteria | Possibly secondary infections or contaminants | Whittington and McColl 1983; Munday et al. 1998 |
| **Fungi** | | |
| *Mucor amphibiorum* | Mucormycosis—lesions in skin and sometimes internal organs; may result in death | Munday and Peel 1983; Obendorf et al. 1993; Connolly and Obendorf 1998; Munday et al. 1998; Stewart 2001 |
| *Trichophyton mentagrophytes* var *mentagrophytes* | Alopecia of the tail | Whittington 1992 cited by Munday et al. 1998 |
| Unknown fungal organism | Cutaneous granuloma | Macgregor et al. 2010 |
| **Protozoa** | | |
| *Theileria ornithorhynchi* | Usually no effect; hemolytic anemia has been observed in two heavily infected animals | Collins et al. 1986; Kessel et al. 2014 |
| *Trypanosoma binneyi* | None observed | Munday et al. 1998; Paparini et al. 2014 |
| *Toxoplasma gondii* | None observed | McColl 1983 |
| Coccidia | None observed | Munday et al. 1998 |
| **Trematodes** | | |
| *Mehlisia ornithorhyni* | None observed | McColl 1983; Whittington and Spratt 1989 |
| *Maritrema ornithorhynchi* | None observed | Munday et al. 1998 |
| *Moreauia mirabilis* | None observed | Munday et al. 1998 |
| **Cestodes** | | |
| *Spirametra erinacei* | Focal pneumonia | Whittington et al. 1992 |
| **Nematodes** | | |
| Rhabditoid & filarioid spp. | Mild tissue changes in skin | Spratt and Whittington 1989; Whittington and Spratt 1989 |
| **Arthropods** | | |
| *Pygiopsylla hopli* | Skin irritation | Munday et al. 1998 |
| *Pygiopsylla zethi* | Skin irritation | Munday et al. 1998 |
| Trombiculid mites (2 species) | Skin irritation | Munday et al. 1998 |
| *Ixodes ornithorhynchi* | Mild, chronic dermatitis | McColl 1983; Whittington and Spratt 1989; Munday et al. 1998 |
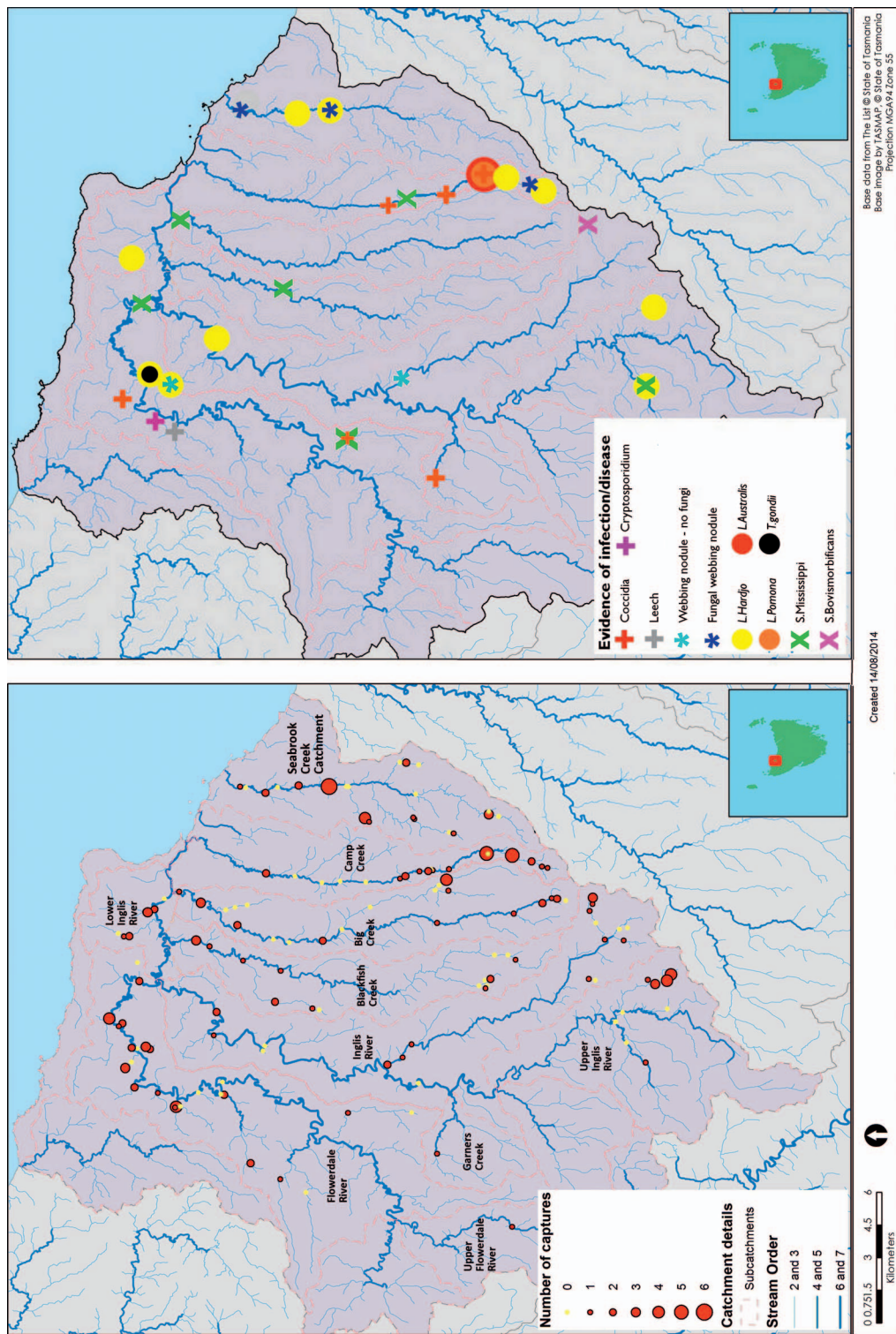
FIGURE 1.   The Inglis Catchment in Tasmania, Australia. (Left) The Seabrook Creek Catchment and subcatchments (named for this project) of the Inglis River Catchment where platypus (*Ornithorhynchus anatinus*) live capture-release fieldwork was performed. Stream orders are as described by Strahler (1957). (Right) Locations of capture sites of platypuses with evidence of disease or exposure to infectious agents.

determined by presence and morphology of spur and month of capture (Temple-Smith 1973; Grant and Llewellyn 1991; Williams et al. 2013; Macgregor 2015). Tail volume index, as a measure of body condition (1=very good, 5=very poor), was assessed as described by Grant and Carrick (1978). All measurements taken were in millimeters: Bill width at its widest point was measured using Vernier calipers; total body length (TBL; tip of bill to tip of tail, measured over dorsum) was measured using a tape measure; tail length (distance from the tip of the tail, not including length of hair cover, to the caudal muscles of the body) was measured using a tape measure; and mid-tail fat depth (fat depth adjacent to the bone and musculature of tail, at mid-point along the length of the tail) was measured using ultrasonography.

Relative tail fat depth$_{TBL}$ (RFD$_{TBL}$) and body condition index$_{TBL}$ (BCI$_{TBL}$) (Macgregor 2015) were calculated as additional body condition indices as follows:

$$RFD_{TBL}1 = 10^9 \times \text{mid-tail fat depth}^{1.7}/TBL^3$$

$$BCI_{TBL}1 = 10^9 \times \text{body mass}/TBL^3$$

Biologic samples were collected under anesthesia for laboratory analysis. Ticks were removed using forceps and placed in 70% ethanol for identification. Excreta (up to 3 mL) were collected by pipette via the cloaca for parasitology, culture, and sensitivity. A cloacal swab was taken for microscopy, culture, and sensitivity. Blood (up to 2 mL) was collected using a 3-mL syringe and a 23-ga needle from the bill sinus for biochemistry, hematology, microscopy, and *Leptospira* spp.-*Toxoplasma gondii* serology. Surface swabs were taken from skin lesions for bacterial and fungal culture. Punch biopsies were collected from skin nodules for histology, fungal and bacterial culture, and fungal PCR.

### Laboratory testing

Panfungal PCR was performed on a fungal culture isolate by SA Pathology, Women's and Children's Hospital, North Adelaide, South Australia. Panfungal PCR was also performed, at Westmead Hospital Mycology Laboratory, Westmead, New South Wales, on shavings from paraffin-embedded histology samples from two platypuses. *Salmonella* typing was performed by the Salmonella Reference Laboratory, Microbiological Diagnostic Unit, Melbourne. Ticks were identified by microscopic examination by Andrea Paparini, School of Veterinary and Life Sciences, Murdoch University. All other laboratory testing, described shortly, was performed at the Department of Primary Industries, Parks, Water and Environment Tasmania Animal Health Laboratory, Mount Pleasant laboratories, Prospect, Tasmania. Packed cell volumes were determined manually and other hematology parameters were determined using a Sysmex KX21N automated hematology analyzer (Sysmex Corporation, Kobe, Hyogo, Japan). Blood smear slides were reviewed for blood parasites and consistency with automated analyzer results. A Konelab 20XTi (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) analyzer was used to analyze sera for biochemical analytes. Modified agglutination tests were performed to detect antibodies to *Toxoplasma gondii*. Microscopic agglutination tests were performed to detect antibodies to *Leptospira interogans* serovars Hardjo, Pomona, Icterohaemorrhagiae, Tarassovi, Australis, and Canicola. A flotation test (using saturated magnesium sulfate) was conducted for parasitology on excreta where there was >1 g of sample. A wet preparation was used when there was insufficient sample for the flotation test.

### Hematology and biochemistry

Hematology and biochemistry results were examined for each individual. Outliers were considered to have possible significance in relation to health if they were considerably outside the relevant reference interval, as determined by Macgregor (2015), or if we thought more than one outlier in the same individual might be attributable to the same pathologic process.

### Statistical analysis

For observed prevalences of exposure to infectious agents which were >0, confidence intervals were calculated using the formula:

$$95\% \text{ confidence limits} = \text{obs} \pm \{\text{obs}/(1-\text{obs})/n\}^{\frac{1}{2}},$$

where obs=observed prevalence and $n$=number of individuals sampled. When obs=0, the upper confidence intervals limit was calculated using the following formula (Hanley and Lippman-Hand 1983):

$$95\% \text{ confidence interval upper limit} = 300/n$$

The significance of differences between adult males and adult females in the distributions of body lengths and body weights were tested using the Mann-Whitney $U$-test using Statistica 8.0 (Stat Soft Inc., Tulsa, Oklahoma, USA). To assess whether the indicators of exposure to infectious agents were more or less common in particular groups of platypus, two sets of tests were performed. Firstly, for each age-sex category, a two-tailed Fisher's exact test was performed for each indicator using a 2×2 contingency table

TABLE 2.  Hematology and biochemistry results of possible significance in relation to health from platypuses (*Ornithorhynchus anatinus*) captured in the Inglis Catchment, Tasmania between 29 August 2011 and 31 August 2013.

| Parameter[a] | Units[b] | 95% Reference interval (Macgregor 2015) | Platypus no. 43 | 122 | 94 | 57 | 78 | 84 | 19 | 125 |
|---|---|---|---|---|---|---|---|---|---|---|
| AST | IU | 429–1134 | 1,759 | 3,017 | 1,444 | | | | | |
| ALT | IU | 135–498 | 860 | 1,124 | 542 | | | | | |
| GLDH | IU | 38–258 | 542 | 1,374 | 356 | | | | | |
| GGT | IU | 0–8 | | | | 59 | 54 | 57 | | |
| Eosinophils | $\times 10^9$/L | 0–2.18 | | | | | | | 3.62 | 3.68 |

[a] AST = aspartate aminotransferase; ALT = alanine aminotransferase; GLDH = glutamate dehydrogenase; GGT = gamma-glutamyl transferase.

[b] IU = international units.

containing entries for each of the positive and negative results in the relevant age-sex category and for positive and negative results in the other age-sex categories combined. Secondly, for each subcatchment, a two-tailed Fisher's exact test was performed for each indicator using a 2×2 contingency table containing entries for each of the positive and negative results in the relevant subcatchment and for positive and negative results in the other subcatchments combined. To investigate possible associations between morphometrics and evidence of exposure to infectious agents, for adult males and adult females and for each indicator of exposure to infection Mann-Whitney *U*-tests were performed comparing the distributions of results for measures of body size and body condition between positive and negative platypuses. Consistent with the views of Perneger (1998), there was no correction for multiple testing, and the conventional $P<0.05$ significance level was used. Conclusions were drawn based on cautious consideration of the statistical results with emphasis on whether the findings were biologically plausible (Perneger 1998).

## RESULTS

### Morphometrics

The (mean, median, range) body masses (kg) of adult males (2.06, 2.02, 1.54–2.93) were significantly greater than those of adult females (1.32, 1.32, 0.97–1.64) ($U=20$, $P<0.001$). Similarly, the TBLs in centimeters (mean, median, range) of adult males (55.1, 54.5, 51–63.5) were significantly greater than those of adult females (47.4, 48, 43.5–52) ($U=10$, $P<0.001$). The mean, SD, and range of values for morphometric data from each

study catchment and subcatchment are listed in the Supplementary Table.

### Hematology and biochemistry

Three platypuses had aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutamate dehydrogenase (GLDH) that were all above the reference intervals, three had gamma-glutamyl transferase (GGT) well above the reference intervals, and two had eosinophilia (Table 2).

### Exposure to infectious agents

Results of investigations into exposure to infectious agents are summarized in Table 3, and the geographic location of the positive findings for infections-titers with prevalences of <90% are illustrated in Figure 1, right. Ticks from 28 platypuses were examined and all were identified as *Ixodes ornithorhynchi* (Paparini et al. 2014). The Fisher's exact tests showed no significant variation in prevalences of infectious agent exposure between age-sex classes or between subcatchments. Of the 50 Mann-Whitney *U*-tests performed, comparing the distributions of results for measures of body size and body condition between platypuses that were positive and negative for exposure to infectious agents, three significant differences were found: a higher median and mean TBL for trypanosome-positive adult female platypuses; a higher median and mean body mass for adult female platypuses with

TABLE 3.   Evidence of exposure to infectious diseases in platypuses (*Ornithorhynchus anatinus*) in the Inglis Catchment, Tasmania between 29 August 2011 and 31 August 2013. Numbers in parentheses indicate numbers tested in each age-sex category.

| Infectious agent or condition | Testing method | No. tested | No. adult female positives | No. adult male positives | No. adult male positives | No. juvenile female positives | No. adult male positives | Total no. positives | Observed prevalence (%) | Confidence interval (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Salmonella* spp. | Bacterial culture | 151 | 2 (62) | 5 (75) | 0 (6) | 0 (3) | 0 (5) | 7 | 4.6 | 2.9–6.3 |
| *Leptospira* spp. | Serology[a] | 115 | 4 (49) | 7 (57) | 0 (4) | 0 (2) | 0 (3) | 11 | 9.6 | 6.9–12.3 |
| Fungal granuloma in foot webbing | Clinical exam, histology, culture, PCR | 154 | 3 (63) | 0 (76) | 0 (6) | 0 (3) | 0 (6) | 3 | 1.9 | 0.8–3.0 |
| Other granuloma in foot webbing | Clinical exam, histology, culture | 154 | 1 (63) | 1 (76) | 0 (6) | 0 (3) | 0 (6) | 2 | 1.3 | 0.4–2.2 |
| Mucormycosis | Clinical exam, histology, culture, PCR | 154 | 0 (63) | 0 (76) | 0 (6) | 0 (3) | 0 (6) | 0 | 0 | 0–2 |
| *Theileria* spp. | Microscopy on blood film | 142 | 53 (60) | 67 (71) | 5 (5) | 3 (3) | 3 (3) | 131 | 92.3 | 90.1–94.5 |
| Trypanosomes | Microscopy on blood film | 143 | 52 (60) | 68 (71) | 4 (5) | 3 (3) | 4 (4) | 131 | 91.6 | 89.3–93.9 |
| *Toxoplasma gondii* | Serology[b] | 110 | 1 (48) | 0 (53) | 0 (4) | 0 (2) | 0 (3) | 1 | 0.9 | 0.0–1.8 |
| *Cryptosporidia* spp. | Excreta microscopy | 112 | 0 (52) | 1 (54) | 0 (4) | 0 (1) | 0 (1) | 1 | 0.9 | 0.0–1.8 |
| Coccidial oocyst | Excreta microscopy | 112 | 1 (52) | 5 (54) | 0 (4) | 0 (1) | 0 (1) | 6 | 5.4 | 3.3–7.5 |
| Ticks present | Clinical examination | 153 | 58 (63) | 74 (75) | 6 (6) | 3 (3) | 6 (6) | 147 | 96.1 | 94.5–97.7 |
| Leeches present | Clinical examination | 154 | 0 (63) | 2 (76) | 0 (6) | 0 (3) | 0 (6) | 2 | 1.2 | 0.3–2.1 |

[a] Microscopic agglutination test.
[b] Modified agglutination test.

TABLE 4. Statistically significant results from Mann-Whitney $U$-tests comparing the distributions of morphometrics between platypuses (*Ornithorhynchus anatinus*), captured in the Inglis Catchment, Tasmania between 29 August 2011 and 31 August 2013, which were positive and those that were negative for exposure to infectious agents.

| Grouping variable (positive vs. negative) | Sex | Dependent variable | $U$-value | $P$-value | Median (positive) | Median (negative) |
|---|---|---|---|---|---|---|
| Trypanosome infection | Female | Total body length | 107 | 0.03 | 48 cm | 45.8 cm |
| *Leptospira* spp. serologic titer | Female | $RFD_{TBL}$[a] (no units) | 5 | 0.04 | 6.4 | 12.9 |
| Fungal granuloma | Female | Body mass | 27 | 0.04 | 1.42 kg | 1.32 kg |

[a] $RFD_{TBL}$ = relative tail fat depth total body length.

fungal granulomas; and a lower median and mean $RFD_{TBL}$ for adult female platypuses that were antibody-positive for *Leptospira* spp. (Table 4).

Single nodules were observed in the webbing of the front feet of five adult female platypuses and biopsy specimens were collected. The nodules were firm, roughly spherical in shape, and ranged in size from ~3–7 mm in diameter with a slightly reddened surface appearance but no ulceration. The smallest nodule was a mild, suppurative dermatitis, containing some foreign material on histology, and was considered to be a foreign-body reaction (Platypus 148). Another nodule was a moderate, chronic dermatitis with mixed inflammatory cells, no evidence of fungal infection, but with some spirochetes present (Platypus 99; Fig. 2a). Three webbing nodules contained fungal elements on histology. One was a dermatitis containing mixed inflammatory cells and fungal hyphae only (Platypus 48; Fig. 2b), the other two were dermal granulomatous-pyogranulomatous lesions containing fungal hyphae and spherules (Platypuses 95 and 125; Fig. 2c–e). Panfungal PCR performed on paraffin-embedded shavings did not resolve the identity of the fungal organisms in one nodule containing fungal hyphae (Platypus 48) or in one containing fungal hyphae and spherules (Platypus 95) due to the presence of multiple fungal species. However, the results were not consistent with *Mucor* spp. infection. *Phomopsis/Diaporthe* complex DNA was detected in the paraffin-embedded shavings from the other nodule containing fungal hyphae and spherules (Platypus 125; Fig. 2c–e). One fungal colony was cultured from a fresh specimen from this nodule. This isolate was identified as a *Phomopsis/Diaporthe* sp. by DNA sequencing. Fungal culture was negative for the sample from Platypus 48 but was not performed on samples from Platypuses 95, 99, and 148 due to small biopsy size.

**Patterns within health data**

The platypus with a nodule from which the *Phomopsis/Diaporthe* sp. was isolated also had a high eosinophil count ($3.68 \times 10^9$/L). No other platypuses with notable hematology-biochemistry outliers had evidence of exposure to infectious agents or clinical disease. Infection with both *Theileria* spp. and Trypanosomes was found in 85% of platypuses. The only individual to have a serologic titer to *Toxoplasma gondii* also had a low titer (1:50) to *L. interrogans* serovar Hardjo. One individual that was shedding coccidian-like oocysts also had *Salmonella* serovar Mississippi in its excreta. Another individual from which *Salmonella* Mississippi was cultured also had a low serologic titer to *L. interogans* serovar Hardjo. The single serologic titers to *L. interogans* serovar Pomona and to *L. interogans* serovar Australis were found in the same individual.

**DISCUSSION**

We provide baseline data on individual platypus health and important reference data for a wide range of specific health parameters. There were no individual, geographic, or age-
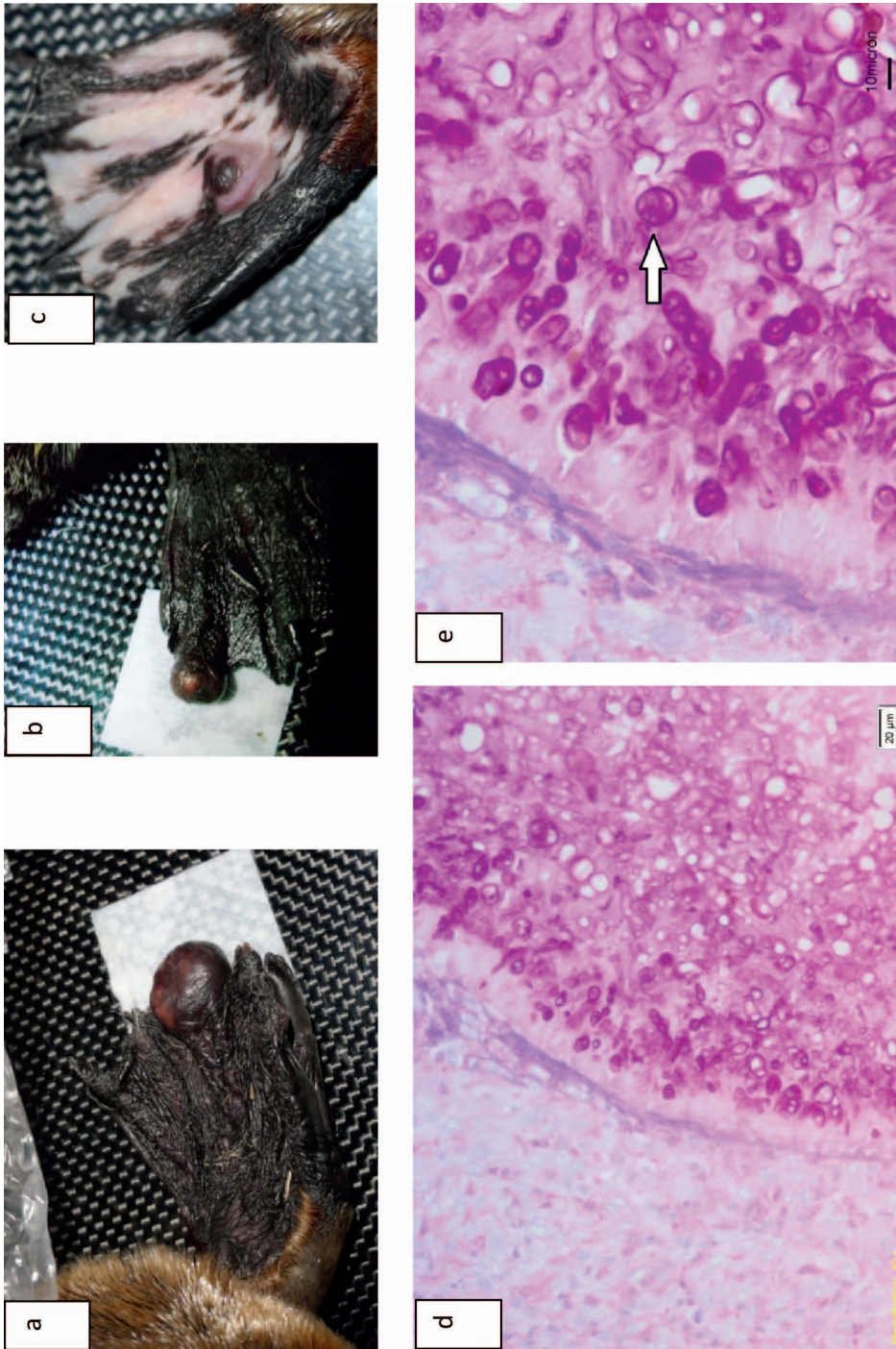
FIGURE 2.   Nodules in foot webbing; (a) left forefoot of Platypus 99, gross appearance; (b) left forefoot of Platypus 48, gross appearance; (c) right forefoot of Platypus 125, gross appearance (note the patchy pigmentation which can be normal); (d) and (e) periodic acid-Schiff stain of histologic slides from Platypus 125 (arrow=spherule). (d) Bar=20 μm. (e) Bar=10 μm. Photos: (a) David McArtor, (b) David Maleca (c) Helen Robertson, (d) and (e) Graeme Knowles.

sex class patterns in the exposure to infectious agents within the study population, and only minimal evidence was found of morbidity associated with exposure to infectious agents. We found no evidence of mucormycosis in the study animals but found a new differential diagnosis for this disease.

Platypuses are sexually dimorphic in body length and body mass and are generally larger in Tasmania (Grant and Temple-Smith 1983; Connolly and Obendorf 1998; Munks et al. 1998; Bethge 2002; Koch et al. 2006; Gust and Griffiths 2011). Our findings are consistent with these observations. Studies of Tasmanian platypuses have shown mean mass in different river systems in the ranges 0.91–1.65 kg and 1.47–2.5 kg for females and males, respectively (Connolly and Obendorf 1998; Stewart 2001; Bethge 2002; Koch et al. 2006; Gust and Griffiths 2011). Mean body masses in our study were near the middle of these ranges and were very close to the mean values of 1.3 kg for females and 2.1 kg for males reported by Macgregor (2008) in the Inglis River Catchment. Gust and Griffiths (2011) reported mean body length values for platypuses from different river systems as 42–48 cm for females and 48–56 cm for males. Our results were close to the upper ends of these ranges and were higher than the values of 43 cm and 49 cm, respectively, reported by Macgregor (2008), also in the Inglis River Catchment. This finding may be a result of the prevention of hunching or lateral curving of the spine, associated with our use of anesthesia, which might have reduced the measured body length in previous studies.

The absence of differences in the rates of exposure to infectious agents between sub-catchments and age-sex categories of platypuses indicates that there are no particular foci of infectious challenge to platypuses in the Inglis River Catchment. The results of the Mann-Whitney $U$-tests for the distributions of morphometric results between exposure-positive and -negative animals provide minimal evidence of morbidity from infectious disease. The higher median and mean TBL for trypanosome-positive adult female platypuses, and the higher median and mean body mass

for adult females with fungal granulomas, do not suggest a negative disease impact. The lower median and mean $RFD_{TBL}$ for adult female platypuses positive for *Leptospira* spp. antibody might suggest a disease impact. However, the absence of a similar result for the other two measures of body condition in female platypuses, or for any of the measures of body condition in males, suggests this result may be a statistical anomaly.

The isolation of *Salmonella* spp. from wildlife is not uncommon, and prevalences similar to or higher than the $4.6\pm1.7\%$ we observed have been reported for a range of avian, reptilian, and mammalian species (Quessy and Messier 1992; Handeland et al. 2002; Renter et al. 2006; Phalen et al. 2010; Scheelings et al. 2011). Six of the seven isolates in this study were identified as *Salmonella* Mississippi (Edwards et al. 1943). Approximately 80% of the human infections with this serovar in Australia occur in Tasmania and are thought to be associated with exposure to native animals and the drinking of untreated water (Ball 1992; Obendorf 1993; Ashbolt and Kirk 2006). This serovar has not previously been reported in platypuses. However, given the reported associations with wildlife species and water, it is not a surprising finding. *Salmonella* Bovismorbificans, the other serovar isolated in this project, is relatively common in Australia in animals (particularly cattle) and humans (Liesegang et al. 2002; Stafford et al. 2002; Animal Health Australia 2012, 2013, 2014; Iveson et al. 2013). Outbreaks of human gastrointestinal disease due to *Salmonella* Bovismorbificans have been associated with raw vegetables in Europe and Australia (Liesegang et al. 2002; Stafford et al. 2002), and this has led to speculation that the bacteria could survive in soil and watery habitats, leading to infection via vegetables. In Australia, the association of this serovar with cows, and the possibility that it survives well in aquatic environments, would appear to make platypuses in the two study populations (which inhabit an area where pasture for grazing cattle is a common land use) at risk of infection. *Salmonella* spp. are common in wild

animals and infections are usually subclinical (Uhart et al. 2011). Consistent with this, there were no indications of clinical disease in the *Salmonella*-positive platypuses in this study. However, there is potential for disease in infected animals if they become stressed by other factors (Uhart et al. 2011). In addition, from a fieldworker-safety viewpoint, the 4.6% *Salmonella* spp. prevalence we observed should be considered and incorporated into hazard reduction plans for research projects that involve handling platypuses.

The prevalence of detectable antibody to *Leptospira* (9.6%) was low compared to previous studies in platypuses, despite *Leptospira* Pomona or *Leptospira* Hardjo being widespread in the Tasmanian cattle population (G. Knowles pers. comm.). Prevalences of antibody to *Leptospira* Hardjo of 47%, approximately 50%, and 66% have been reported in three studies of platypuses in mainland populations (McColl and Whittington 1985; Munday et al. 1998; Loewenstein et al. 2008). The two possible explanations for the low prevalence in our study are a low exposure to infection or a high mortality rate. Although McColl (1983) observed mild chronic interstitial nephritis, which could be consistent with *Leptospira* infection, during five of 20 platypus necropsies, *Leptospira* spp. are not known to cause clinical disease in platypuses (Munday et al. 1998).

There were no obvious signs that the webbing nodules observed in five platypuses significantly affected these individuals, and these cases are probably more significant in their similarity to the disease mucormycosis than they are as a cause of poor individual health. Histologically, mucormycosis lesions have been described as granulomas or lesions with granulomatous-pyogranulomatous inflammation containing spherules characteristic of *M. amphibiorum* infection (Connolly et al. 2000). Daughter spherules (single, 11.3±2.5 µm in diameter) and mother spherules (18.0±5.8 µm and containing a mean of 4.7±3.2 daughter spherules) have been reported as a characteristic finding (Connolly et al. 2000). Organisms in the *Phomopsis/Diaporthe* complex can lead to disease in a wide range of plant hosts. They have also been reported as the cause of subcutaneous infections in the fingers of two immunosuppressed people, most likely via accidental inoculation of the organism into subcutaneous tissues by pricking with plant material (Sutton et al. 1999; Garcia-Reyne et al. 2011). The ability of certain fungi to change between unicellular (spherules) and multicellular filamentous (hyphae) forms in response to environmental changes is known as fungal dimorphism (Nadal et al. 2008). It has been identified to commonly occur in a range of species including certain plant pathogens, six human pathogens, and *Mucor* spp. (Connolly et al. 2000; Stewart and Munday 2005; Klein and Tebbets 2007; Nadal et al. 2008). Understanding is incomplete of the triggers for this process and the species capable of undergoing it (Nadal et al. 2008; Garcia-Reyne et al. 2011). However, our findings suggest that *Phomopsis* spp. should be added to the list of dimorphic fungi. In addition, the absence of *Mucor* spp. in the lesions from Platypuses 125 and 95 has implications for the diagnosis of mucormycosis. Connolly (2009) stated that suggestive lesions and a culture of *M. amphibiorum* are required for a diagnosis of mucormycosis and that further support for this diagnosis can be provided by the presence of spherules on wet or histologic sections or by detection of antibodies to *M. amphibiorum* by enzyme-linked immunosorbent assay. Our findings support this, indicating that spherules in fine needle aspirates or impression smears may lead to false positives if not confirmed by histology (in which the finding of fungal hyphae would not be characteristic of mucormycosis), fungal culture, or PCR.

The introduction of foreign material, fungal organisms, and possibly spiral bacteria during foraging seems to be a likely cause of the webbing nodules we observed. Further research is required to determine whether Platypuses 125 and 95, who were both captured in the Seabrook Creek Catchment and whose lesions both contained fungal spherules and hyphae, reflect one-off cases or a low but consistent prevalence of infection with a particular *Phomopsis/Diaporthe* species.

The high prevalence of *Theileria*, trypanosomes, and ticks is consistent with findings of previous studies and the suggestion that, except for two reports of juveniles with *Theileria*, these organisms do not usually lead to significant disease in platypuses (Munday et al. 1998; Booth and Connolly 2008; Kessell et al. 2014). The prevalence of coccidia, cryptosporidium, antibodies to *T. gondii*, and leeches were low and are unlikely to be having an effect at a population level in our study area.

Although one individual (Platypus 125) had an eosinophilia as well as a fungal dermatitis, the other individual (Platypus 19) with an elevated eosinophil count was clinically normal and had no other biochemical or hematologic findings to explain the raised eosinophil count. The three platypuses with AST, ALT, and GLDH above reference intervals and the three with GGT above reference intervals appeared clinically normal, suggesting subclinical hepatopathies or cholangiohepatopathies (G. Knowles pers. comm.).

Overall, this study does not raise concerns about platypus health in the Inglis Catchment. The observed absence of mucormycosis is particularly encouraging for the two populations, as mucormycosis is considered a conservation threat (Munday et al. 1998). Data from this project could make a suitable comparison for a future study using similar methods to investigate the effects of mucormycosis or other threats on individual health in affected populations.

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## SUPPLEMENTARY MATERIAL

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