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Preliminary investigation into the prevalence of mucormycosis in the platypus (*Ornithorhynchus anatinus*) in three catchments in north-west Tasmania

JW Macgregor,[a,c,*] CS Holyoake,[a] SA Munks,[d,e] ID Robertson[b] and KS Warren[a]

Objective To investigate the distribution and prevalence of mucormycosis in platypus (*Ornithorhynchus anatinus*) from the Inglis, Emu and Black-Detention catchment areas in north-west Tasmania.

Procedure A field study was performed and resulted in the examination of 44 wild platypuses; in addition, one dead platypus and two live platypuses were examined after they were independently submitted to a local veterinary clinic.

Results No cases of mucormycosis were conclusively diagnosed. One platypus with signs consistent with those previously described in cases of mucormycosis was captured in the Emu River catchment. However, laboratory tests did not provide a definitive diagnosis for the lesion. Two platypuses from the Inglis catchment area had signs very similar to those previously described in cases of mucormycosis, but laboratory tests found *Corynebacterium ulcerans* to be the likely cause of the cutaneous ulcers on one of these platypuses and an unidentified fungal agent to be the cause of a cutaneous nodule in the other.

Conclusions These findings do not prove that mucormycosis is absent from the populations studied. However, they may indicate that the prevalence of disease is low. The possibility that *Mucor amphibiorum* is present in a subclinical form in platypuses, or infecting another reservoir, is not excluded. The findings also suggest that caution should be exercised when diagnosing mucormycosis based on clinical findings alone and raise the possibility that some cases may have been incorrectly diagnosed.

Keywords *Corynebacterium ulcerans*; fungal granuloma; *Mucor amphibiorum*; mucormycosis; platypus; skin lesions

Abbreviation DPIW, Department of Primary Industries, Parks, Water and Environment, Tasmania

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Mucormycosis is a disease of the platypus (*Ornithorhynchus anatinus*) that causes lesions on the skin and sometimes in the internal organs. Mucormycosis has been found in platypuses in Tasmania but not elsewhere in Australia.[1,2] It was first observed in Campbell Town in 1982 in four platypuses.[3] Obendorf et al. examined nine platypuses from northern Tasmania with similar lesions and established *Mucor amphibiorum* as the causal agent of the disease.[4] *Mucor amphibiorum* was first isolated from a captive Australian tree frog in West Germany in 1972[5] and since then it has been identified as the cause of disease in captive and wild anurans in mainland Australia, as well as being isolated from soil in Queensland.[6,7] Other than from cutaneous lesions on infected platypuses, researchers have so far been unable to isolate *M. amphibiorum* from any source in Tasmania, including soil, water, amphibians, ticks, platypus faeces and platypus nasal passages.[8–10]

Mucormycosis causes a variety of cutaneous lesions and affected platypuses may have single to multiple lesions.[1,11] In some cases, non-ulcerated, hairless nodules (<10 mm in diameter) or plaques (10–54 mm diameter), sometimes exuding purulent material, have been seen.[8,11] Other lesions have consisted of cutaneous ulcers (10–100 mm in diameter),[1,9,11] which can progress to involve underlying muscle up to a depth of 10 mm below the skin. Lesions in the internal organs are sometimes found, particularly the lungs.[1,10,11]

A definitive diagnosis of mucormycosis can be made using samples taken from cutaneous lesions and fungal culture followed by mating experiments with known *M. amphibiorum* strains.[8] Less specific diagnoses have also been made on the basis of characteristic histological findings or clinical signs.[1,8]

There are detailed reports of mucormycosis diagnosed using culture with mating experiments, clinical signs or histology, in platypuses from several waterways and water catchment areas that ultimately drain into the Tamar River in northern Tasmania.[1,3,4,8,9] There are also less detailed reports of cases of mucormycosis in the Upper Derwent River catchment, Hatfield River, Mersey River, Inglis River, Emu River catchment and Piper River catchment, none of which drain into the Tamar River Catchment.[1,8,12,13]

Three of these locations are in north-west Tasmania.[1,12,13] One case in the Inglis River[12] has been described as a public sighting, but the time when it occurred was not reported; a case from a farm dam in the Inglis catchment approximately 10 km west of Wynyard was a sighting by a member of the public (G. Hoogendorp, personal communication) in early 2007; and the third, in a tributary of the Emu River at Ridgely, has been described as a laboratory confirmed case, although the original report did not refer to it as such, and this occurred in 1996–97.[1]

Munday et al. suggested that the distribution of mucormycosis was spreading in a north-westerly direction from waterways draining into the Tamar River.[1] However, the only detailed report of the examination of animals from the north-west of mainland Tasmania involved six clinically healthy platypuses captured in the Emu River at Burnie.[8]

*Corresponding author.
[a]Conservation Medicine Program, School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia, Australia; jamesm@southcom.com.au
[b]Veterinary Epidemiology Program, School of Veterinary and Biomedical Sciences, Murdoch University, WA, Australia
[c]Wynyard Veterinary Clinic, Wynyard, Tasmania, Australia
[d]Tasmanian Forest Practices Authority, Hobart, Tasmania, Australia
[e]University of Tasmania, School of Zoology, Hobart, Tasmania, Australia

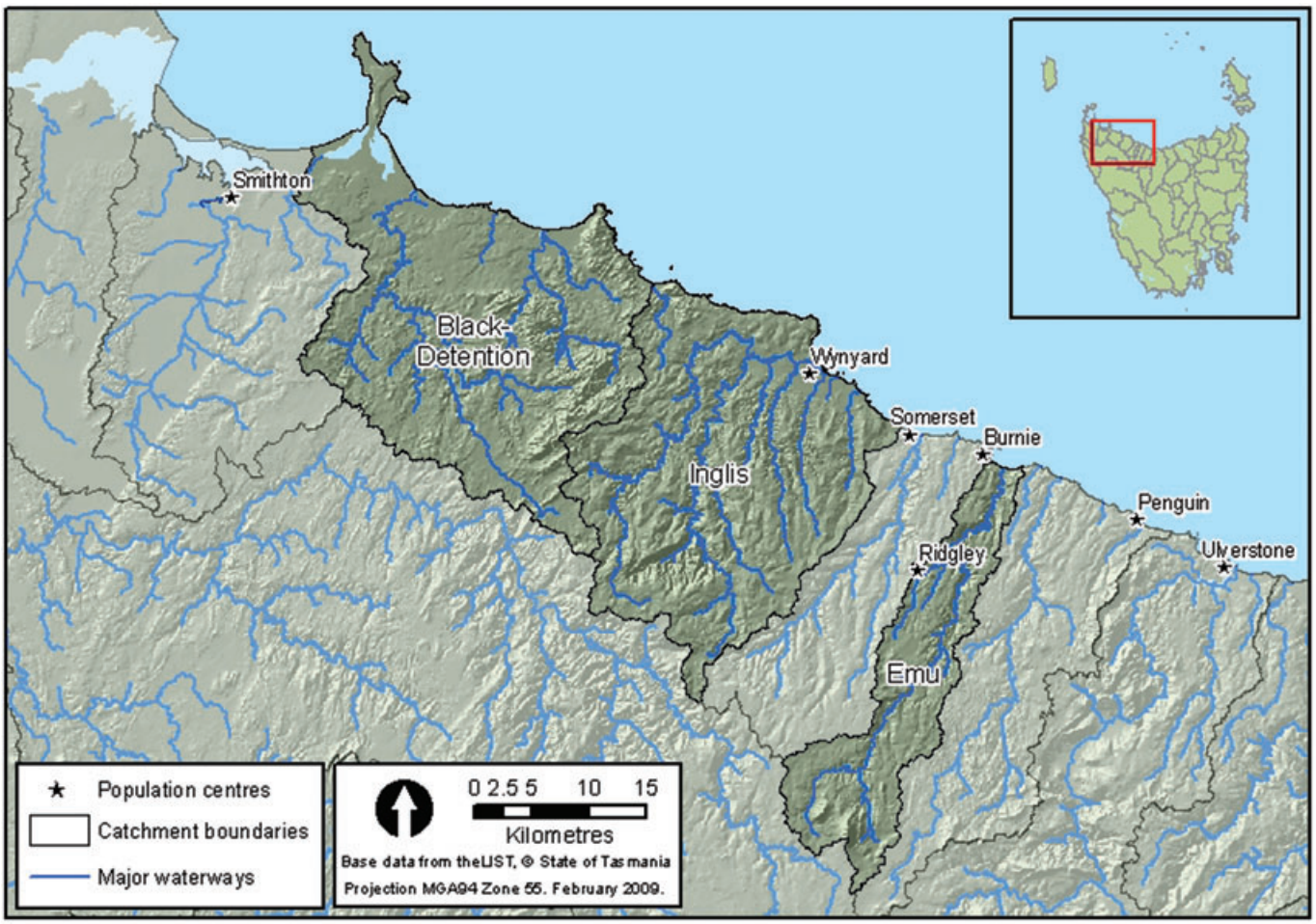

Figure 1. Location of Black-Detention, Inglis and Emu catchments in north-west Tasmania.

The aim of the present study was to address the paucity of reported research on mucormycosis in platypuses in Tasmania and, in particular, to investigate the distribution and prevalence of mucormycosis in platypuses in river catchments in the northwest of the state.

Materials and methods

Study area

This study was performed in three catchment areas in north-west Tasmania (Figure 1), within which 62 sites were selected (39 in the Inglis catchment, 19 in the Emu catchment and 4 in the Black-Detention catchment). Site selection was based on the following factors: (1) near the location of available reports of possible cases of mucormycosis, (2) suited to the capture methods being used and (3) fieldworkers considered it likely that platypuses would be captured, based on anecdotal sightings and the nature of the surrounding water bodies.

Animal capture

Platypuses were captured between December 2007 and August 2008 using either fyke nets or a gill net.[14,15] Nets were set in the late afternoon/early evening and removed from the water before 1 AM the following morning.

Fyke nets were set in pairs (one facing upstream and the other facing downstream in close proximity) with the distal end of each net secured above water level. Fyke nets were checked every 30 min. The gill net was unweighted and was set across still water (e.g. farm dams) or roughly parallel to the direction of movement of slowly moving water. The gill net was monitored constantly.

Platypuses were removed from the nets by hand as soon as they were found and placed in a hessian sack, which was tied closed and placed inside a second tied hessian sack. The double–bagged platypus was then kept in a quiet place at the site of capture with adequate ventilation and protection from adverse weather conditions. Each platypus was held in the sacks for at least 30 min before examination to allow it to relax and to allow the water on its fur to be absorbed into the hessian. The platypus was then placed in a cotton pillow case for examination, which was performed by sequentially exposing different parts of the animal's body at the opening of the pillow case. Sacks and pillow cases were used only once on each night and between uses they were washed, soaked in sodium hypochlorite, rinsed and dried in sunlight.

Captured platypuses were individually identified by implanting a microchip (Novartis Reunite®) into the subcutaneous tissues between the scapulae.[16] Platypuses were held for no longer than 4 h, the average

being 2 h. All nets were removed from the water before platypuses were released at the site of capture.

During the examination of each platypus, the following physical characteristics were measured or assessed: (1) sex and age by the presence and morphology of spurs;[17] (2) bodyweight to the nearest 10 g using digital scales (Rapala®); (3) body condition using the tail volume index;[18] (4) approximate number of ticks (0, 1–10, 10–100, 100+) and their locations; ticks were examined grossly and were classified by size as (i) adult female or (ii) larva, nymph or adult male; (5) body dimensions.

The surface of each platypus was examined and any skin abnormalities were recorded. Surface swabs, with or without fine-needle aspirates, were taken from lesions considered potentially to be mucormycosis for later laboratory investigations. Surface swabs were taken after rinsing the area with sterile water and fine-needle aspirates were taken aseptically.[8] Both types of samples could be easily collected from conscious platypuses with minimal stress to the animals, so sedation or anaesthesia was not required (N. Stewart, Menzies Research Institute, Hobart, Tasmania, personal communication).[11]

One dead and two live platypuses from the Inglis catchment were independently submitted to and examined by a veterinarian at a local veterinary clinic and information from these animals was made available to this project.

Laboratory investigations

Fungal culture, bacterial culture and histology were performed at the Animal Health Laboratory, Mt Pleasant, Department of Primary Industries, Parks, Water and Environment, Tasmania (DPIW).

DNA sequencing of a fungal isolate was performed at the Mycology Department of the Women's and Children's Hospital, Adelaide.

Results

Number of individual platypuses captured in each catchment

A total of 44 platypuses (24 females, 20 males) were captured and individually identified between December 2007 and August 2008. Of these, 23 were captured in the Inglis catchment, 20 in the Emu catchment and 1 in the Black-Detention catchment. Some individuals were captured more than once (Table 1).

Table 1. Number of times platypuses were captured at each site during the study period

No. of captures	No. of platypuses captured per catchment			
	Inglis	Emu	Black-Detention	Total
1	22	15	1	38
2	0	2	0	2
3	1	2	0	3
4	0	1	0	1
Total	23	20	1	44

Clinical findings

A variety of skin lesions were observed on 27 captured platypuses (Table 2).

The platypus listed in Table 2 as having a nodule/ulcer (platypus A) was an adult female that was examined three times within 27 days, all captures occurring in a single 200-m stretch of the Pet River in the Emu catchment. At the first capture, she was found to have a tail volume index of 3 and a bodyweight of 1.33 kg. Other body measurements were consistent with those of other adult female platypuses in good condition captured in this area (JW Macgregor, unpublished data). There was a 4-mm scar on the ventral tail and a 5-mm cutaneous nodule with a 2- to 3-mm surface scab in the right gluteal region, but otherwise the platypus appeared to be in good health. The second capture occurred 14 days later, at which time the nodule had not changed in size, but the surface scab was absent. The third capture occurred 27 days after the first. A cutaneous ulcer, 8- to 10-mm in diameter and 2- to 3-mm deep, was present where the nodule had previously been. A tick was present 15 mm medial to the ulcer. The bodyweight of the platypus had increased to 1.36 kg.

The dorsal aspect of the tails of three platypuses captured in the Inglis catchment (Figure 2) had superficial lesions that consisted of hair loss over reddened but not visibly or palpably thickened skin. One such lesion was observed to heal over the 228 days during which the platypus was captured three times. Of these three platypuses with superficial tail lesions, one also had scars of recently healed scratches on the ventral aspect of the tail and another had a laceration through the full thickness of the lateral tail margin.

One further platypus captured in the Inglis catchment also had a lesion on the dorsal aspect of the tail, which consisted of a hairless area over normal skin (classified as a scar in Table 2).

One of the two live platypuses examined at a local veterinary clinic (platypus B) had been found beside a busy road in Wynyard, in the Inglis catchment, by a member of the public who considered it to be at risk of injury. It was a male aged less than 6 months with a tail volume index of 3 and a bodyweight of 1.14 kg. Examination revealed 10 to 100 ticks behind both hindlimbs and two granulating cutaneous ulcers, approximately 7 mm in diameter, on the thoracic wall caudal to the left elbow (Figure 3).

Table 2. Numbers of various types of skin lesion observed in captured platypuses

Lesion type	No. of platypuses with lesions
Scratches on tail	1
Laceration to tail	1
Superficial lesion on tail	3
Scar on tail	8
Wound on bill	1
Scar on bill	5
Adult female tick(s)	3
Larva, nymph or adult male tick(s)	20
Nodule/ulcer	1

Figure 2. Superficial lesion on the dorsal aspect of the tail of a platypus from the Inglis catchment.

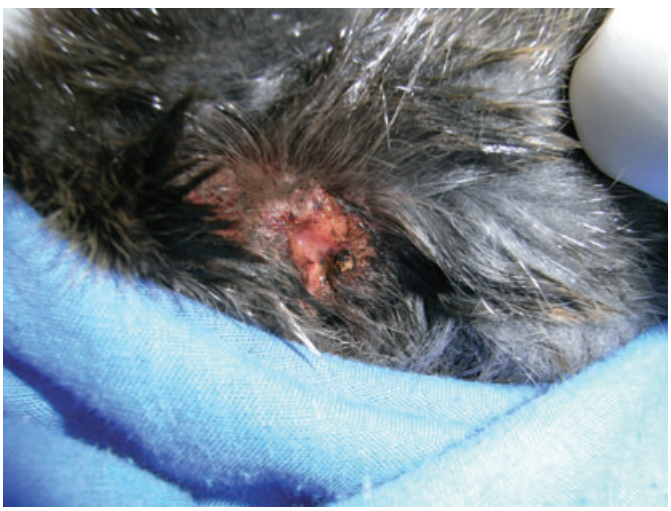

Figure 3. Skin lesions on platypus B (Inglis catchment).

An adult male road-kill platypus from the Inglis catchment (platypus C) was found to be underweight for age with a tail volume index of 5 and bodyweight of 1.7 kg (JW Macgregor, unpublished data). Examination revealed 1 to 10 juvenile ticks behind the left hindlimb and a 12-mm diameter cutaneous mass on the dorsomedial left carpus (Figure 4). Three scabs/abrasions were present on the ventral tail (Figure 5). Internal examination revealed signs of major trauma, but no suspect mucormycosis lesions were found. The intestines and urogenital system were intact, but the gastrointestinal system was severed at the stomach. The liver was severely traumatised and the diaphragm

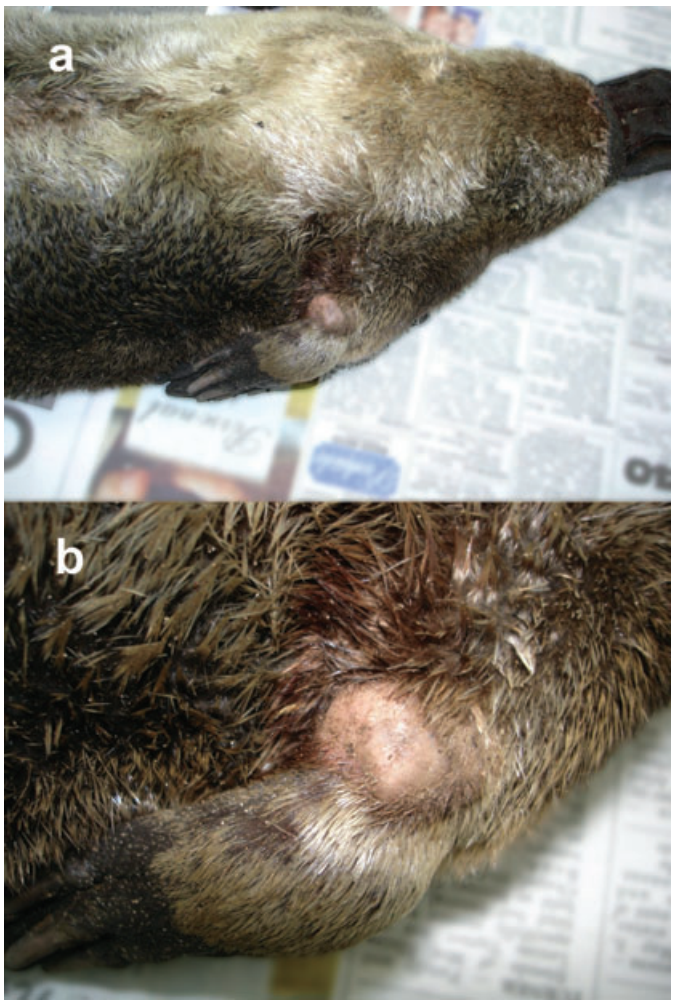

Figure 4. Platypus C (road-kill; Inglis catchment): (a) ventral aspect of chest and left forelimb, (b) cutaneous mass on carpus at higher magnification.

was ruptured. Fractured ribs were present, the lungs were severely traumatised and there was haemorrhage into the chest and heart muscle.

Histology

Histological examination was performed only on the samples from platypus C (Figure 6). The sample from the carpal mass revealed multiple, poorly encapsulated granulomas containing fungal hyphae. Section stained with gomori methenamine showed septated hyphae, branching mycelia and variably shaped conidiosphores. Spherules characteristic of *M. amphibiorum* infection were not seen and the histological appearance was not consistent with *M. amphibiorum* infection.[3,4,11] Samples from the lesions on the ventral aspect of the tail of the same platypus showed inflammation without fungal hyphae.

Mycology

Samples were taken for fungal culture from six platypuses. Surface swabs were taken from the superficial lesions on the dorsal aspects of the tails of the three platypuses in the Inglis catchment, the nodule/

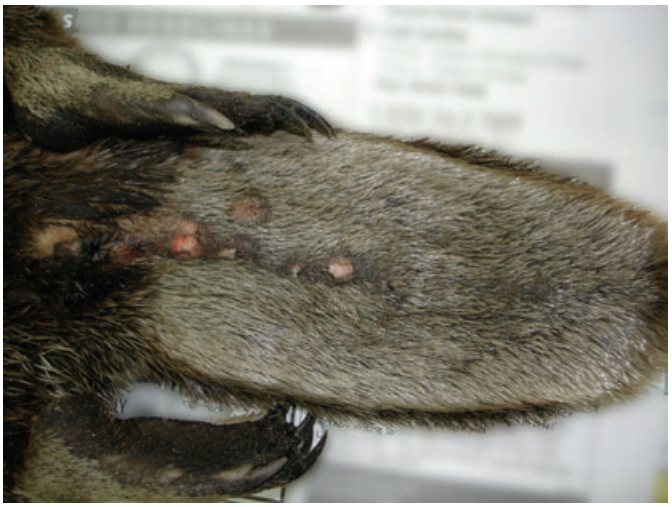

Figure 5. Lesions on ventral aspect of the tail of platypus C.

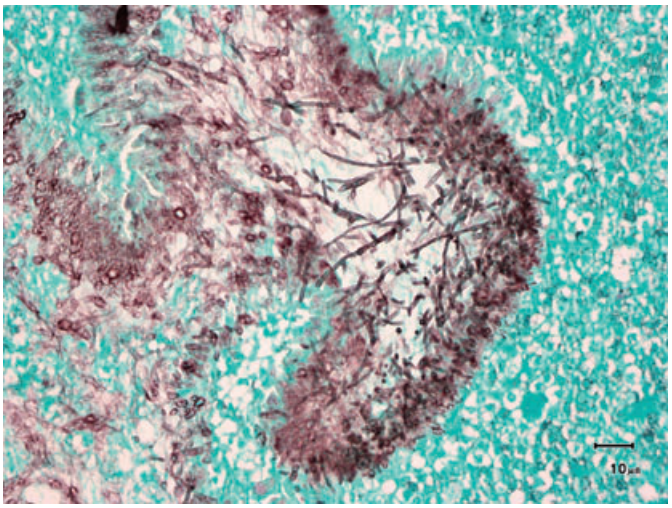

Figure 6. Histological section of the mass on the left carpus of platypus C, stained with gomori methenamine silver and showing fungal hyphae. (Photo courtesy of S. Lola, Animal Health Laboratory, Dept Primary Industries and Water.)

ulcer on platypus A and the ulcers on platypus B. Both a surface swab and a fine-needle aspirate were taken from platypus A on two occasions and a swab of the cut surface and a fresh tissue sample from the lesion on the carpus of platypus C were submitted, as well as fresh tissue samples from the lesions on the ventral aspect of the tail.

Fungal culture did not result in the growth of *M. amphibiorum* from any of these samples.

A light growth of *Epicoccum purpurascens* was cultured from the direct swab taken from the nodule of platypus A at the first capture.

Fungal culture on samples taken from lesions on the carpus and ventral tail of platypus C produced mixed growth. One isolate was similar in appearance to the fungal elements seen on the histological samples from this lesion. The form of this isolate identified it as a coelomycete, but attempts to induce sporulation to allow further identification were unsuccessful. DNA sequencing tests were also unable to identify it. In any case, it is possible that this isolate was not the same organism seen on histology, but was a contaminant.

Bacteriology

Heavy growths of *Corynebacterium ulcerans* and *Aeromonas hydrophilia* were produced on bacterial culture of the swabs from both lesions on platypus B.

Bacterial culture of a fresh sample from the mass on the carpus of platypus C produced a heavy growth of mixed bacteria, predominantly coagulase-negative *Staphylococcus*.

Discussion

The superficial lesions on the dorsal tails of the three platypuses in the Inglis catchment were considered to be consistent with skin abrasions and it was considered likely that the other skin lesions seen on the tails of two of the platypuses were caused by the same traumatic incidents that caused the abrasions. The hairless area observed on the dorsal tail of a fourth platypus in the Inglis catchment appeared consistent with a healed skin abrasion.

Connolly and Obendorf found bilateral alopecia on the tails of several female platypuses and related this to tail-holding behaviour by males during courtship.[19] In this present study, one of the affected platypuses was male and the affected females were captured at times not considered to be within the breeding season. Alopecia of the tail caused by *Trichophyton mentagrophytes* var. *mentagrophytes* has been described in the platypus by Whittington;[20] however, in the present study no dermatophytes were isolated from swabs taken from the tail lesions and future studies could investigate the cause of these lesions.

The tick burdens of platypuses in this study are consistent with previous reports in which up to 200 were found on individual animals.[1,21,22] It is likely that most, if not all, of the ticks observed in this study were *Ixodes ornithorhynchi*, which is the most commonly reported species of tick on the platypus and appears to be host-specific.[1,20,22] The pathology associated with these ticks is minor,[22] although a role for ticks in the transmission of mucormycosis has been suggested.[4,8]

In this study, only one platypus (platypus A) had a lesion consistent in appearance with mucormycosis and which could not be attributed to another cause.[1,11] Laboratory tests performed on samples taken from this platypus did not confirm a diagnosis of mucormycosis and *E. purpurascens*, which was cultured from this lesion, is an environmental saprophyte that is unlikely to be the primary pathogen in this case (J. Lentern, DPIW, personal communication).

Although the specificity is 100% for *M. amphibiorum* infection diagnosed by fungal culture followed by mating experiments, the sensitivity is likely to be less than 100%. Hence, it is possible that the laboratory tests on samples from platypus A produced a false-negative result and that the lesion observed was mucormycosis. However, despite the appearance of the lesion, it is not possible to say with confidence that mucormycosis was present in any of the populations studied. Equally, although the observed prevalence of mucormycosis

in the populations examined by the field study was 0%, it is not possible to say with confidence that mucormycosis was absent from the sampled areas. Techniques exist to assess the probability that a disease is absent in a population, but it was not possible to produce a meaningful result because of uncertainty over the expected minimum prevalence of mucormycosis in platypus populations.

Very little is known about the epidemiology of mucormycosis, including the reservoir and route of infection for platypuses. Previous studies have provided apparent prevalence values of 30% to 35% in infected populations.[1,8,9,11] However, Stewart noted that these figures are based mainly on areas where the disease has only recently been reported[9] and as such, these figures cannot be assumed to be minimum expected prevalence values. Diseases fluctuate in prevalence over time because of factors such as host immunity, host population density, environmental conditions and access to a source of the infective agent.[23] Although based on small sample sizes, Stewart postulated from his results that the prevalence of infection at Brumbys Creek, in northern Tasmania, had decreased between 1994 and 1999.[9]

If mucormycosis was present in the platypus populations surveyed in the present study, the prevalence would be at a level less than that observed in previously studied populations. It also is possible that *M. amphibiorum* was present in a subclinical form in the platypuses or infecting another reservoir.

Two species of bacteria were isolated from the cutaneous ulcers on platypus B. *Aeromonas hydrophilia* is common in aquatic environments and is likely to be a secondary pathogen (G. Knowles, DPIW, personal communication). However, the isolation of *C. ulcerans* is consistent with the clinical signs observed (G. Knowles, personal communication).

Corynebacterium ulcerans has been isolated from a variety of species, including humans, domestic animals and wild animals.[24–27] It has been isolated from a skin lesion on a Tasmanian devil (G. Knowles, personal communication). Although articles in the human literature have focussed on domestic animals as a source for *C. ulcerans* infections of people, the reservoir of disease has not been clearly demonstrated[24,26,27] and it is possible that multiple species act as reservoirs (G. Knowles, personal communication). Strains of *C. ulcerans* isolated from cutaneous ulcers are capable of producing toxins, which are likely to be involved in the development of the ulcers.[26] This is the first reported case of *C. ulcerans* infection in a platypus.

Despite uncertainties over the causal agent, the observation of fungal granulomas in the skin of platypus C is of note, because of the similarity of the lesion to those in reported cases of mucormycosis.[8] Platypus C was in poor body condition and presumably unwell before the road traffic accident. It is not possible to know if the fungal infection was the cause of the poor body condition, an effect of poor body condition or whether it was unrelated.

Cutaneous and subcutaneous fungal granulomas have been described in a variety of species of mammals, including the horse, cat, cow and humans.[28–31] It is considered that many of these infections occur by implantation of usually saprophytic organisms into the skin through local trauma.[29–31] There have also been cases in which fungal infections have disseminated to the skin and other systems after inhalation of the organism.[30]

The isolation in this study of pathogens other than *M. amphibiorum* from two lesions that had a clinical appearance similar to mucormycosis (platypuses B and C)[1,8] suggest that care should be taken when making a diagnosis solely based on clinical signs. Following on from this, it is possible that some of the published reports of mucormycosis that were based on clinical findings alone may have been incorrectly diagnosed.

Conclusion

This study has provided evidence that the prevalence of mucormycosis in populations of platypuses in the Inglis and Emu catchments was lower than would be expected in a recently infected population. Although one platypus had a lesion that appeared consistent with that of mucormycosis, laboratory tests did not provide a definitive diagnosis. The finding of two platypuses with lesions similar to mucormycosis that were attributed to infectious agents other than *M. amphibiorum* implies that caution should be exercised when diagnosing the disease on the basis of clinical findings in populations where the approximate prevalence is not known and also that some of the reports of mucormycosis in the literature have been incorrectly diagnosed.

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