Platypus mucormycosis and its conservation implications

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Abstract

Since 1982 some Tasmanian platypuses have been affected by a fungal disease called mucormycosis. This disease causes skin lesions, morbidity and mortality in these iconic Australian animals. Here we review the contemporary understanding of the epidemiology of mucormycosis and its potential impacts on the conservation status of platypuses. *Mucor amphibiorum,* the fungal pathogen responsible for mucormycosis, may have been transported to Tasmania with infected frogs from mainland Australia. Although there are no confirmed cases of mucormycosis from platypuses in mainland Australia, mucormycosis appears to have spread widely in Tasmania since 1982. In the 25 years to 2007, ulcerated platypuses were captured across 11 river catchments that cover 24% of the land area of Tasmania. The potential disease spread is much larger, with unconfirmed public observations over the same period reporting ulcerated platypuses in 11 additional catchments spanning another 31% of the state. Though a number of mechanisms are suggested, it is currently unclear what the route of individual infection is, or how mucormycosis is spread within or between catchments in Tasmania. There is currently insufficient fundamental information on the distribution and impacts of the disease to rigorously assess the threat. For instance, it is unclear whether mucormycosis has persisted in platypus populations that were historically affected, whether the proportion of infected animals has changed within these areas, or whether platypuses still persist and reproduce successfully in formerly infected areas. Difficulties in reliably quantifying platypus abundance compromise researchers' ability to determine impacts on populations.

Key words: platypus (*Ornithorynchus anatinus*), mucormycosis, *Mucor amphibiorum*, Tasmania, conservation.

Introduction

The platypus (*Ornithorhynchus anatinus*) is an iconic semi-aquatic monotreme whose oddities have intrigued people worldwide since the first specimens reached Europe at the end of the 18th century. For almost two centuries since its anatomy was first described (Home 1802), this globally recognised egg-laying mammal (Fig. 1) has elicited excitement and controversy in the zoological world. Recently the species' genome has been documented (Warren *et al.* 2008), which provides clues on the function and evolution of mammalian genomes, and has renewed international interest in the animal's unique amalgam of avian, reptilian, mammalian and derived characteristics.

The platypus is the only living representative of a significant lineage of platypus-like animals with a 100 million year fossil history that are relatively unchanged since the time of the dinosaurs (Long *et al.* 2002). Platypuses have remarkable morphological features including highly sophisticated neuroanatomy and electro-receptors, a specialized venom delivery system that is unique among mammals, and a cloaca, which forms a common opening to the reproductive, urinary and gastrointestinal systems. Platypuses are long lived for small mammals, surviving up to 21 years of age in the wild (Grant 2004).

Platypus conservation status

Platypuses are an integral part of the biodiversity of many eastern Australian freshwater ecosystems and their conservation is widely regarded as important because of their unique features, status, identity and ecological niche. Platypuses are protected by legislation in all States in which they occur (Grant & Temple-Smith 2003), and since they rarely breed in captivity, their conservation in the wild is of particular importance (Warren *et al.* 2008). Platypuses are widely distributed across eastern mainland Australia and are found widely throughout Tasmania (Grant & Temple-Smith 1998). Tasmania has the largest platypuses in Australia (Temple-Smith *et al.* unpublished data), and a myriad of suitable streams, rivers, lakes and farm dams contribute to a large platypus metapopulation. However, whether Tasmania represents optimum habitats for platypuses in Australia is arguable, since robust population estimates for this species are not available, particularly over large spatial scales. Across Australia the geographic range of platypuses is markedly discontinuous, but spans 3500 km from cool temperate environments to the tropics (Grant 2007). The current nationwide distribution of platypuses, with individuals distributed across 27 degrees of latitude, appears to have changed little from pre-European times (Grant & Temple-Smith 2003). Platypuses occupy diverse fresh-water habitats from sea level to altitudes of around 1000 m, where they feed predominantly on benthic invertebrates (Grant & Temple-Smith 1998). Platypuses are currently considered 'common' and widespread throughout much of their geographical range, although they are potentially vulnerable to disease, introduced predators, and a number of human-mediated changes to their environment (Grant & Temple-Smith 2003).

Platypuses are difficult animals to study, and despite their wide distribution many Australians have never seen one in the wild. Historically many platypuses did not survive for prolonged periods, or breed in captivity (Whittington 1991). Although platypuses have been kept in captivity since the 1800's, they have only bred in captivity on six occasions (Booth & Connolly 2008). Despite improved captive husbandry techniques in the last decade, there is still a lot to learn about platypus reproduction. Investigations of platypus ecology and life history in the wild are confounded by their secretive, nocturnal behaviour, aquatic life style and burrow use.

Their behaviour hampers understanding of their population dynamics, and many fundamental aspects of their mating behaviour, reproductive cycle and venom function remain obscure. Furthermore the current and future conservation status of platypuses is not easily predicted since their abundance is not readily measured (Grant & Temple-Smith 2003). The International Union for the Conservation of Nature (IUCN) classifies the platypus as a 'species of least concern'. However, some researchers feel they should be reclassified as 'potentially vulnerable' because of their reliance on aquatic environments under stress from drought, climate change and degradation by human activities e.g. (Grant & Temple-Smith 2003; Grant 2007).

Platypuses are dependent on fresh-water habitats, and have a very limited capacity for dispersing long distances across much of arid Australia. Catastrophic events in isolated water bodies may therefore further fragment their distribution and potentially reduce their geographic range (Carrick 1991). On smaller spatial scales, several studies have also reported fragmentation of platypus distribution within individual river systems (reviewed by Grant & Temple Smith 2003). This was attributed to poor land management practices resulting in stream bank erosion, loss of riparian vegetation and channel sedimentation. There is also evidence for adverse effects of river regulation and impoundments, introduced species, poor water quality, fisheries by-catch mortality and disease on platypus populations, although none of these threats has been well studied (Grant & Temple-Smith 2003). Since their abundance is difficult to determine, population trends are generally unknown and the specific impacts of disease or environmental perturbations have been suggested and implicated, but rarely rigorously demonstrated.

Platypus diseases

Platypuses generally suffer from few significant diseases in the wild (Munday *et al.* 1998), although studies around Australia have reported twenty-six causative agents of potential disease or parasitism (Whittington 1992). The causative agents include viral, fungal, bacterial, protozoan and metazoan pathogens, most of which result in sub-clinical infections, are asymptomatic and/or have no known effects on platypuses (Whittington 1992; Booth & Connolly 2008). The fungus *Mucor amphibiorum* (Fig. 2) is the only pathogen thought to cause significant morbidity and mortality in wild platypuses (Whittington 1992). *M. amphibiorum* causes an ulcerative skin disease in Tasmanian platypuses variously referred to as ulcerative dermatitis (Munday & Peel 1983), mycotic granulomatous dermatitis (Connolly *et al.* 1999), mucormycosis (Connolly *et al.* 2000), and ulcerative mycotic dermatitis (Whittington *et al.* 2002). We refer to the disease as mucormycosis throughout this review.

Clinical signs of platypus mucormycosis

Platypuses infected with *M. amphibiorum* can develop severe granulomatous and often ulcerative dermatitis on their legs, tails and backs (Fig. 3). The gross appearance of skin lesions in affected platypuses ranges from raised red nodules or plaques, which sometimes exude purulent material, to ulcerated lesions with central cavitation, red exuding centres and raised epidermal margins (Fig. 3) (Connolly *et al.* 2000). Older lesions may be covered either partly or fully by thickened and irregular epidermis, with histological examination of skin biopsies from infected animals revealing discrete, poorly encapsulated granulomas, or more commonly a diffuse granulomatous or pyogranulomatous inflammation (Connolly *et al.* 2000). The fungal infection has also been detected in the lungs and musculature of infected animals submitted for necropsy (Munday *et al.* 1998). Diseased individuals may have single or multiple cutaneous lesions, typically from 5 to 50 mm in diameter (Munday & Peel 1983; Connolly *et al.* 2000). The largest recorded lesions are around 70 mm in diameter (occupying roughly 4% of their body's surface area), and can invade the musculature to a depth of 10 mm below the skin (Stewart & Munday 2005). When the fungus invades tissue, it does not grow in the hyphal form associated with other *Mucor* infections, instead if forms circular bodies known as sphaerules that replicate and spread when the parent sphaerule ruptures (Stewart & Munday 2005). Sphaerules refer to the bodies found in vivo, while sphaerule-like bodies refers to the bodies grown in vitro (Stewart 1998). Miliary lesions up to 1mm in diameter can also occur in the lungs of infected platypuses (Munday & Peel 1983), although whether this occurs in all infected individuals is unclear. Active ulcers cause animals' obvious discomfort and they spend considerable time scratching and rubbing against objects, with severely ulcerated animals becoming debilitated and often flyblown (Munday *et al.* 1998). The ugly skin lesions and ulcers caused by mucormycosis engender public sympathy and support for platypus research and conservation efforts.

The fungal pathogen *Mucor amphibiorum*

M. amphibiorum is probably an endemic Australian fungal species, although it could potentially have been introduced to Australia from Hawaii with cane toads (*Bufo marinus*) released in coastal north Queensland in 1935 (Speare *et al.* 1994). On mainland Australia, *M. amphibiorum* has been found in soil from tropical Queensland (Speare *et al.* 1994; Stewart & Munday 2005), and has been reported from captive frogs held in Queensland, New South Wales, Victoria and Western Australia (Creeper *et al.* 1998; Stewart & Munday 2005). *M. amphibiorum* can infect and kill frogs and toads in the wild (Frank 1975; Speare *et al.* 1994). There are documented cases of amphibians transferring *M. amphibiorum* overseas (Frank *et al.* 1974), and between Australian states (Slocombe *et al.* 1995). The majority of amphibian mortalities have been observed in captive populations, with initial interest in the fungus arising from it infecting and rapidly killing a variety of frog species housed together in a European collection (Frank 1975). The fungus is widespread in cane toads in northern mainland Australia, although mean prevalence of infection is only around 1% of animals at the infected sites (Speare *et al.* 1994). It is currently unclear how significant mucormycosis is to native Australian frog

populations in the wild, although research to date has been limited (Speare *et al.* 1994).

In tropical North Queensland *M. amphibiorum* grows well, forms spores rapidly, and potentially persists in soil for many months (Speare *et al.* 1994). It is currently unclear whether the same is true for either the soil or water of cool-temperate Tasmania. It has been suggested that *M. amphibiorum* infects platypuses, and not other mammals, because the animal's relatively low body temperature of 32°C is closer to the optimum growth temperature of 30–34°C for *M. amphibiorum* than other mammals with body temperatures exceeding 36°C (Munday *et al.* 1998). Like many *Mucor* species, *M. amphibiorum* has positive and negative mating types and forms zygospores when the mating types are crossed, which provides an unambiguous method of identifying both the species and its mating type. Recent research indicates the positive mating strain of this dimorphic fungus (exclusively present in Tasmanian platypuses tested to date) is particularly virulent to cane toads and influences the severity of their infection (Stewart & Munday 2005). These researchers suggested that platypus mucormycosis may be attributable to either an endemic strain of *M. amphibiorum* that has mutated and become pathogenic to platypuses, or that a pathogenic strain of *M. amphibiorum* may have been introduced into Tasmania, where it is infecting a naïve platypus population. Tasmanian platypuses have been isolated from mainland Australia by sea level rises on a number of occasions, with the most recent isolation occurring over approximately the last 12,000 years Galloway & Kemp 1981). Differences in susceptibility to *M. amphibiorum* may result from genetic differences between Tasmanian and mainland platypuses since it is thought that the pathogen has only recently been introduced to Tasmania, whereas mainland populations may have evolved with the organism over a longer period of time (Booth & Connolly 2008).

Introduction of *Mucor amphibiorum* to Tasmania

It is possible that *M. amphibiorum* was introduced to Tasmania via infected frogs transported from mainland Australia. Green tree frogs (*Litoria caerulea*) have been specifically identified as a likely potential vector since they can be infected with mucormycosis in the wild (Berger *et al.* 1997), have previously been implicated in spreading the fungus amongst amphibians (Frank 1974), and are thought to be regularly transported around Australia with Queensland bananas (Munday *et al.* 1998). It is also possible that *M. amphibiorum* arrived via the illegal introduction of frogs as pets into the state or via infected cane toads (*Bufo marinus*) in fruit shipments from northern Australia. Neither of these possibilities has previously been suggested in the scientific literature, despite being viable (though less likely) alternative explanations for introduction. The first evidence for the fungus being present in Tasmania was obtained in 1982 when four sick, ulcerated individuals were found in a short section of the Elizabeth River (Munday & Peel 1983). Initial investigations struggled to find any apparently viable organisms that may have been responsible for the disease, although an unknown fungal species from the *Mucor* genus was isolated and grown on Sabouraud's agar (Munday & Peel 1983). The causative agent was subsequently identified as *M. amphibiorum* from ulcerated platypuses a decade later (Obendorf *et al.* 1993).

Fig. 1 A healthy Tasmanian platypus in the wild. Photo: Nick Gust.

Mucormycosis spread in Tasmania

The initial discovery of ulcerated platypuses in 1982 was in the Elizabeth River near Campbell Town in central-eastern Tasmania (Fig. 4). Over the next 15 years until 1997, mucormycosis was known only from tributaries of the wider Tamar River catchment area, a relatively discrete geographic zone with physical barriers on all sides except the northwest (Munday & Peel 1983; Obendorf *et al.* 1993; Connolly *et al.* 1996). Mucormycosis was hypothesised to have spread from the Elizabeth River, to the tributaries of the Tamar River (Munday *et al.* 1998). These authors assumed that subsequent westward spread may have eventually occurred via infected platypuses travelling overland between catchments. They predicted that if infected platypuses or other potential vectors can move from one catchment to another, then mucormycosis is likely to continue to spread, with more isolated, steeper sided catchments eventually becoming affected. Without some form of control, they felt mucormycosis may eventually spread to most areas in Tasmania with possible severe consequences for relatively isolated platypus populations. Since 1982 mucormycosis appears to have spread across much of northern Tasmania, although increased awareness and reporting of diseased animals may account for some of the apparent pattern of spread. In the mid 1990's, trapping surveys and opportunistic post mortems revealed infected platypuses in sites throughout the north-central part of the state. Diseased animals were confirmed from the Supply, Meander, Liffey, South Esk and Elizabeth Rivers and Brumby's Creek (Obendorf *et al.* 1993; Connolly *et al.* 1996;

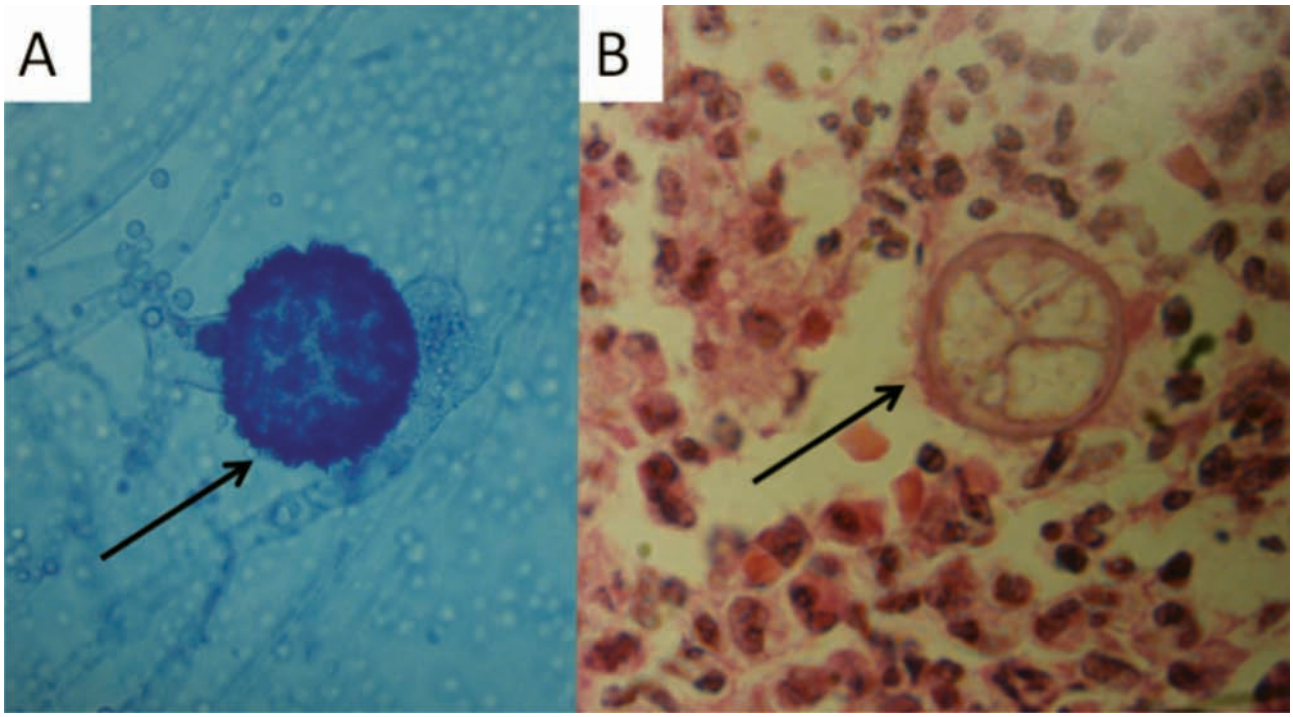

Fig. 2 *Mucor amphibiorum*. **A.** *In vitro* with a zygospore indicated from crossing positive and negative mating strains. **B.** *In vivo* and a sphaerule-like body indicated growing in an ulcerated platypus. Photos: Niall Stewart.

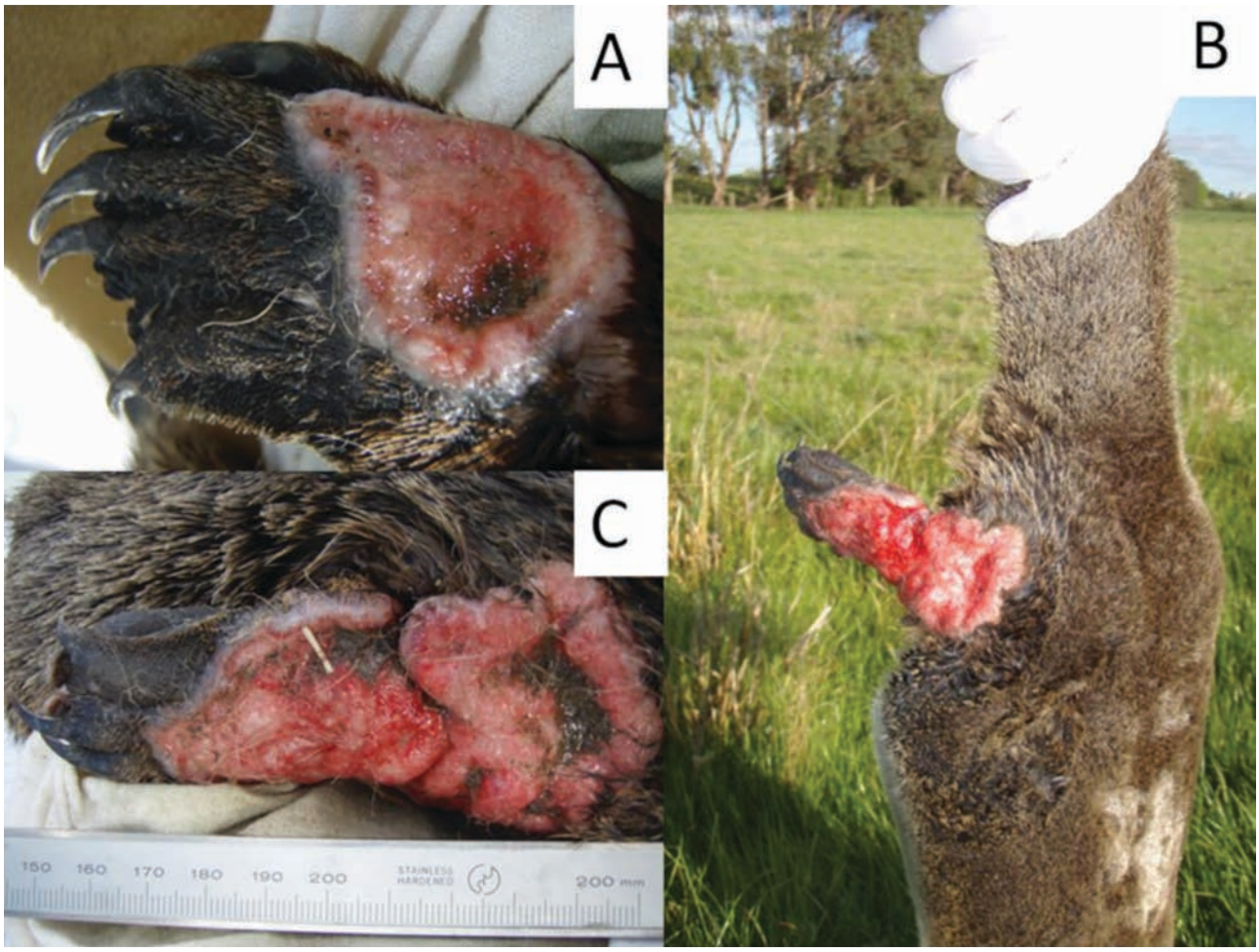

Fig. 3 A Tasmanian platypus with mucormycosis, showing ulcers on **A** dorsal surface of front foot, **B** and **C** dorsal surface of hind leg. Photos: Nick Gust.

Connolly *et al.* 1998; Munday *et al.* 1998). In the last decade additional ulcerated animals were captured, and ulcerated platypuses sighted in catchments outside known infected areas, which suggest mucormycosis is still spreading. In the 25 years from 1982 to 2007 at least one ulcerated animal was captured in each of 11 separate

Table 1 Tasmanian river catchments with evidence of platypus mucormycosis from 1982 to 2007. The most reliable evidence for disease is the confirmed presence of ulcerated platypuses[1], with public sightings[2] suggesting a much larger potentially infected area.

Evidence of infection	Total number of cases	Number of catchments affected	Total area of catchments[3] potentially affected (km^2)	Combined catchment areas as a % of mainland Tasmania
Ulcerated platypuses	55	11	15,742	24
Ulcerated platypuses and public sightings	132	22	36,220	55

[1] Live or dead platypuses examined by researchers and ulcers/lesions typical of mucormycosis present. Data collected from peer-reviewed scientific papers including (Munday & Peel 1983, Obendorf *et al.* 1993, Connolly *et al.* 1998, Munday *et al.* 1998, Stewart & Munday 2005), and unpublished pathology reports.

[2] An apparently ulcerated animal sighted and reported by the public. Animal was not captured and thus disease status is currently unverified.

[3] Catchment boundaries and areas defined by Information and Land Services Division, DPIW. The land area of Tasmania was calculated from all catchments, excluding the offshore King and Furneaux Islands groups.

river catchments (Fig. 4). Together these catchments contribute approximately 24% of the land area of Tasmania (Table 1). Public observations roughly double the suspected spatial extent of the disease. If reported public sightings are shown to be correct, then mucormycosis may now have spread across 22 catchments and 55% of Tasmania (Table 1, Fig. 4). Platypuses in Tasmania's Wilderness World Heritage Area in the South-West of the state may be impacted by mucormycosis, however, the region has not been surveyed for platypus mucormycosis and the current distribution of the disease is poorly resolved both within and between catchments.

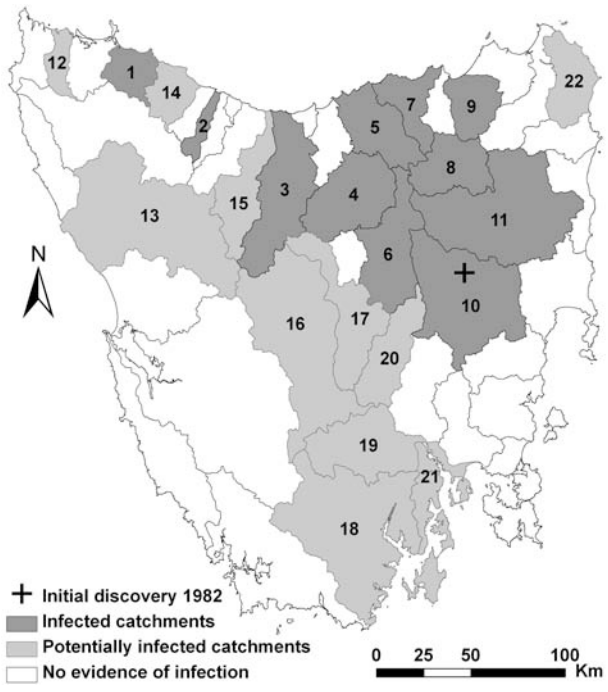

Fig. 4 Cumulative evidence for ulcerated platypuses in Tasmanian river catchments from 1982 to 2007. The cross indicates where the first ulcerated platypuses were discovered in 1982 on the Elizabeth River near Campbell Town. "Infected catchments" have had one or more ulcerated platypuses confirmed since 1982, and include (from west to east): 1. Black-Detention, 2. Emu, 3. Mersey, 4. Meander, 5 Tamar, 6. Brumby's-Lake, 7. Pipers, 8. North Esk, 9. Great Forester-Brid, 10. Macquarie, and 11. South Esk. "Potentially infected catchments" contain unconfirmed public sightings of ulcerated animals and include: 12. Montagu, 13. Pieman, 14. Inglis, 15. Forth-Wilmot, 16. Upper Derwent, 17. Ouse, 18. Huon, 19. Lower Derwent, 20. Clyde, 21. Derwent Estuary-Bruny, and 22. Musselroe-Ansons. "No evidence of infection" indicates catchments where public observations have only reported healthy platypuses, and there are no records of ulceration in either captured or dead animals.

Routes of individual mucormycosis infection

It is currently unclear what routes of infection enable *M. amphibiorum* to affect individual platypus. Contamination of skin wounds has been suggested to provide the initial route of infection (Obendorf *et al.* 1993), although the consistent occurrence of lung lesions led to postulated infection via inhalation into the respiratory tract, with subsequent dissemination to the dermal layer (Munday *et al.* 1998). Four potential routes of entry for *M. amphibiorum* are indicated below.

i. Skin Wounds

If *M. amphibiorum* is present in the environment (in water or soil), it could infect platypuses via contaminating skin abrasions, spur wounds or cuts. The cutaneous distribution of mucormycosis lesions is consistent with the causative agent gaining entry via skin wounds (Obendorf *et al.* 1993; Connolly *et al.* 2000). Platypuses regularly have spur puncture wounds from other platypuses, and other abrasions or wounds from sharp objects or interactions with various organisms including crustaceans, eels (*Anguilla australis*), water rats (*Hydromys chryogaster*), or the previous attachment sites of ticks or mites (Connolly *et al.* 1998).

ii. Inhalation

The consistent occurrence of lung lesions also raises the possibility that the initial infection is respiratory (Munday & Peel 1983; Obendorf *et al.* 1993; Connolly *et al.* 1998), with propagules potentially inhaled and subsequently disseminated by the blood to single or multiple cutaneous sites.

iii. Ectoparasites

Most wild platypuses in Tasmania are parasitised by the tick *Ixodes ornithoryhynchi*, which are unique to platypuses and thought to be transferred among individuals from shared burrow use. Platypus ticks can transmit protozoan parasites (Whittington 1992) that infect red blood cells (Munday & Whittington 1998), and could potentially also spread *M. amphibiorum*. A platypus tick found adjacent to an ulcer has also previously been found to contain sphaerule-like bodies (Connolly *et al.* 1998).

iv. Ingestion

If dietary items are infected with *M. amphibiorum* platypuses may be infected by ingesting them. Frogs are a potentially infected food source, and are occasionally consumed by platypuses (Faragher *et al.* 1979).

However, frogs are rarely reported from dietary studies of platypuses, and to date no frogs with mucormycosis have been detected in Tasmania. This route of infection is speculative and perhaps more likely if either commonly consumed benthic invertebrates are infected by *M. amphibiorum,* or the fungus is ingested incidentally whilst platypuses forage amongst benthic substrates.

Mucormycosis transmission within and between catchments

With an unknown route of individual infection, it is also currently unclear how mucormycosis is spread between platypuses within a catchment, or amongst catchments in Tasmania. The mechanism for transmission of mucormycosis within and between platypus populations is subject to considerable speculation, and is important to determine in order to develop appropriate management. Within a catchment mucormycosis spread amongst individuals may be facilitated by shared use of contaminated burrows (Munday *et al.* 1998). Adult platypuses use multiple short resting burrows within their home ranges, with individual burrows often used by different animals over time, and sometimes simultaneously (Grant *et al.* 1992; Gust & Handasyde 1995; Serena *et al.* 1998; Otley *et al.* 2000). Longer nesting burrow complexes are also shared for months each year by females and their dependant young during lactation (Grant 2007). Sharing of burrows could facilitate the transfer of mucormycosis between individuals if the burrow soil or air is infected, through transfer of infected ticks, or if physical contact with infected individuals facilitates transfer via skin wounds.

Inadvertent anthropogenic spread of *M. amphibiorum* may help explain the spread of the disease particularly between catchments. It could occur for instance through movement of infected soil or water, or if salmon or trout fingerlings at hatcheries become infected and are transported around the state. Infected platypuses or frogs that disperse naturally or are transported by humans from one catchment to another could also help to spread the disease. In this scenario spread would be limited by the capacity of these species to move over land between catchments, and accelerated by human transport of infected individuals around the state.

With poor understanding of the environmental niche of *M. amphibiorum*, its environmental distribution and tolerances, we can only speculate on how it is being spread. The persistence of *M. amphibiorum* in the Tasmanian environment is suggested by a 27-year history of platypus disease. Preliminary attempts to isolate *M. amphibiorum* from Tasmanian soil samples were unsuccessful even in areas where platypus mucormycosis was common (Connolly *et al.* 1998). In a sampled tropical Australian environment, *M. amphibiorum* appears to grow well and persist in soil for up to a year (Speare *et al.* 1994). Since cane toads (*Bufo marinus*) commonly ingest soil when they feed, the fungus could be ingested with soil contaminating prey items (Speare *et al.* 1994). *M. amphibiorum* has been isolated from the faeces of toads both with internal lesions and no pathological changes (Frank 1975; Speare *et al.* 1994). Therefore, *B. marinus* has the potential to disseminate *M. amphibiorum* on the mainland. Similar mechanisms of amphibian based spread are possible in Tasmania via some of the 11 species of native frogs described in the state by (Litteljohn 2003). Attempts to culture the fungus from Tasmanian frogs have previously been unsuccessful (Connolly *et al.* 1998), although sample sizes were small and the possibility remains that Tasmanian frogs are involved in spreading the disease.

The spread of mucormycosis within and between catchments may also reflect differences in the general health of platypuses and their susceptibility to infection. As most fungal infections are opportunistic, it is possible that Tasmanian platypuses are in some way immuno-compromised (Stewart *et al.* 2008). Platypuses exposed to poor water quality, pesticides or other toxic compounds may become immuno-suppressed and more susceptible to mucormycosis. In this scenario the more polluted catchments are predicted to have more susceptible and/or infected individuals. However to date there is little evidence that mucormycosis distribution or prevalence is associated with measures of poor water quality (Connolly *et al.* 1998). A study of persistent organochlorine pesticides found these pollutants were present in the tail fat of platypuses from different geographic regions of Tasmania, with higher concentrations related to areas of high intensity agricultural activity, industrial and hydroelectric developments (Munday *et al.* 2002). These authors found no significant difference in the concentrations of organochlorines in *Mucor*-infected and healthy platypuses. As such it appears these common environmental pollutants do not play a critical role in the epidemiology of mucormycosis (Booth & Connolly 2008).

Disease prevalence

Disease prevalence refers to the proportion of infected animals in a population. In the affected areas of Northern Tasmania studied in the mid 1990s, approximately one third (35%) of captured platypuses were ulcerated and infected with mucormycosis as determined by mycological culture of infected tissues on Sabouraud's dextrose agar (Connolly *et al.* 1998; Stewart 1998). Brumby's Creek in Tasmania's northern midlands was the best studied location with 36 platypuses captured in 1994, 12 of which were infected with mucormycosis (Connolly *et al.* 1998). Although a number of other rivers were also investigated by these researchers, sample sizes were generally small (<5 individuals) in each location, which compromises accurate estimates of disease prevalence. An enzyme-linked immunosorbent assay (ELISA) was subsequently developed to assess the serological responses of platypuses to *M. amphibiorum* (Whittington *et al.* 2002). Platypuses with clinical mucormycosis had elevated anti-*M. amphibiorum* antibody levels compared to unaffected animals. The ELISA results suggested that platypuses in affected rivers may have been exposed to *M. amphibiorum* at a higher frequency than the occurrence of clinical disease indicated, although the specificity of the antigen used in the ELISA was unclear (Whittington *et al.* 2002). A

positive *Mucor* ELISA result could indicate exposure to either *M. amphibiorum, or M. ciricilloides,* a closely related species commonly found in the Tasmanian environment (Stewart pers. comm.). As such it does not necessarily indicate current or previous mucormycosis infection (Connolly pers. comm.). The prevalence of platypus mucormycosis has not been measured for the past 14 years in any catchments around Tasmania and requires investigation.

Potential impacts of mucormycosis

The impact of mucormycosis on Tasmanian platypus populations can broadly be considered a function of its per-capita impacts, geographic spread, prevalence within local populations, and the disease's persistence in populations through time. Per-capita impacts refer to the mortality and reproductive consequences of mucormycosis infection for individuals. Per-capita impacts reflect the disease induced mortality rates or potential for recovery, the duration of illness and changes to affected individual's reproductive success. The impacts of mucormycosis on platypuses are currently poorly understood at both the individual and population level. For instance it is unclear what proportion of infected animals die from mucormycosis, how long infections persist, and how disease susceptibility varies amongst individuals. As noted by Whittington 1992, the role of disease in regulating wildlife populations often goes uninvestigated even in diurnal species that are easily studied.

Platypus mortalities have often been attributed to mucormycosis (e.g. Connelly *et al.* 1998; Munday *et al.* 1998; Munday & Peel 2005), and it is generally believed that severely ulcerated individuals perish (Booth & Connolly 2008). Death is often attributed to secondary bacterial infections or impaired ability to control body temperature and forage efficiently (Munday & Peel 1983; Obendorf *et al.* 1993; Connolly *et al.* 1998). The proportion of infected animals that die from mucormycosis, and the time from infection to mortality are both currently unknown. There are suggestions that some platypus can potentially recover from mucormycosis infection. Tasmanian platypuses with healed or apparently healing lesions were captured and seen in the endemic area in the early 1990's (Munday *et al.* 1998). Completely healed lesions, consisting of bulbous scars 3 mm deep and 15 mm in diameter have been found in animals captured in the endemic area, and when captured six months later, one of these animals had no detectable, residual lesions (Munday *et al.* 1998). These authors noted that healed lesions consisted mainly of fibrous tissue with a limited inflammatory component and scant organisms.

Historically mucormycosis was thought to be responsible for high morbidity and, presumably, mortality rates in platypuses in the areas where it occurs (Stewart & Munday 2005), although the cryptic, nocturnal nature of the animal made accurate mortality rates difficult to determine and most animals that die are not recovered for autopsy. Of 25 dead platypuses obtained for autopsy in 1994, only 2 (8%) of deaths were attributed to mucormycosis, while 10 (40%) were killed by dogs and 7 (28%) were hit by vehicles when crossing roads (Connolly *et al.* 1998). However, apportioning relative mortality rates on the basis of these autopsies may be biased and potentially underestimates the relative importance of mucormycosis mortality. Members of the public are probably more likely to find a platypus carcass if it is killed on a road, or by a dog, than one that has succumbed to mucormycosis in its burrow or somewhere along a waterway with limited human access.

The progressive and fatal nature of mucormycosis and the infection of multiple animals in different populations suggest the disease may be exerting an effect on the population dynamics of Tasmanian platypuses (Whittington 1992). However, there are currently major difficulties in assessing platypus abundance, such that reliably determining population declines due to mucormycosis are compromised. In the years since platypus mucormycosis surveys were conducted at Brumby's Creek in 1994 (Connolly *et al.* 1998), little additional ecological information has been collected in Tasmania to help assess the impacts of mucormycosis, or its conservation significance for platypuses. There is currently insufficient fundamental information on the distribution of mucormycosis, or its impacts to rigorously assess the threat. For instance, it is unclear whether mucormycosis has persisted in platypus populations that were historically affected or whether the proportion of infected animals or demographics has changed within these areas. Indeed whether platypuses still persist and reproduce successfully in these formerly affected areas is currently unknown. Field surveys in Tasmania are required to address these fundamental gaps in knowledge to help assess the potential ecological impacts of mucormycosis on this iconic Australian animal.

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