Distribution and Potential Spread of Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* in the Tasmanian Wilderness World Heritage Area

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Abstract

Chytridiomycosis is an emerging infectious disease caused by the pathogen *Batrachochytrium dendrobatidis* and has been linked to the decline and extinction of amphibian species throughout the world. *B. dendrobatidis* was first detected in Tasmania in 2004 and subsequently found in a range of urban and rural habitats in eastern and northern parts of the State. We report on a survey of the distribution of *B. dendrobatidis* within and around the Tasmanian Wilderness World Heritage Area (TWWHA). The TWWHA is a 1.38 million hectare area of significant fauna conservation value, which provides the majority of habitat for Tasmania's three endemic frog species (*Litoria burrowsae*, *Bryobatrachus nimbus* and *Crinia tasmaniensis*). We surveyed 62 sites within and around the TWWHA including 33 sites within the boundaries of the TWWHA. *B. dendrobatidis* was found to cause oral chytridiomycosis in tadpoles of all four frog species surveyed (*Litoria burrowsae, L. ewingii, Crinia signifera*, and *C. tasmaniensis). B. dendrobatidis* was detected at 16 (26%) of the 62 sites surveyed, including 15 (52%) of the 29 sites surrounding the TWWHA and one of the 33 (3%) sites surveyed inside the TWWHA. The relatively low incidence of the disease within the TWWHA suggests that the majority of the TWWHA is currently free of the pathogen despite the region providing what appears to be optimal conditions for the persistence of *B. dendrobatidis*. For all survey sites within and around the TWWHA, the presence of *B. dendrobatidis* was strongly associated with the presence of gravel roads. The wide distribution of *B. dendrobatidis* in areas of Tasmania with high levels of human disturbance

and its very limited occurrence in remote wilderness areas suggests that anthropogenic activities may facilitate the dissemination of the pathogen on a landscape scale. Because the majority of the TWWHA is not readily accessible and is largely free of *B. dendrobatidis* and that Tasmanian frogs reproduce in ponds rather than streams, it may be feasible to control the spread of the disease in the TWWHA. Potential high-risk activities and associated management prescriptions are discussed.

Introduction

Chytridiomycosis is caused by the fungal pathogen *Batrachochytrium dendrobatidis* and is recognised as an emerging infectious disease (EID) of amphibians (Daszak et al. 2003). It has been implicated as the causative agent responsible for numerous amphibian mortality events (eg. Bell et al., 2004; Diaz et al., 2007; Berger, 1998; Green and Kagarise Sherman, 2001; La Marca et al., 2005; Lips et al., 2003a), and in the rapid decline and extinction of amphibian species throughout the tropical and temperate zones of Australasia (eg. Bell et al., 2004; Schoegel, et al. 2005; Berger et al., 1998; Laurance et al., 1996; Skerratt et al., 2007), the Americas (eg. Berger et al., 1998; Bradley et al., 2002; Green et al., 2002; La Marca et al., 2005; Lips et al., 2006; Muths et al., 2003; Ron and Merino, 2000), Africa (Lane et al., 2003; Weldon, 2002; Weldon et al., 2004) and Europe (eg. Bosch et al., 2001; Garner et al., 2005).

Within Australia *B. dendrobatidis* has a scattered distribution throughout most of the eastern seaboard from northern Queensland to Victoria, extending south to Tasmania, west to South Australia and the southwestern region of Western Australia and north to the Kimberley region of Western Australia (Berger et al., 2004; DEH, 2005). The threat posed by the pathogen has been recognised by the classification of chytridiomycosis as a "*key threatening process*" pursuant to the Commonwealth *Environmental Protection and Biodiversity Conservation Act*

1999 and by the development of the *Infection of amphibians with chytrid fungus resulting in chytridiomycosis, Threat Abatement Plan 2006* (DEH, 2005).

The role of anthropogenic-mediated environmental change in the emergence of chytridiomycosis is complex and poorly understood. Current evidence suggests that the emergence of chytridiomycosis is likely to be the result of both direct human influence, through the transportation of infected frogs into naive populations, and the indirect impact of global climate change (Carey, 2003; Williams et al., 2002). Growing evidence supports the link between global climatic change and a shift towards optimal temperature regimes for chytridiomycosis in the landscape (Pounds et al., 2006; Laurance 2008). However few studies have investigated the localised impact of direct human disturbance on the distribution and spread of *B. dendrobatidis*.

Environmental conditions across most of Tasmania are suitable for the establishment and persistence of *B. dendrobatidis* and the habitat and life history traits of the Tasmanian endemic anuran species are similar to those of anuran species occurring elsewhere whose decline has been linked to chytridiomycosis (Drew et al., 2006). *B. dendrobatidis* was first detected in Tasmanian in 2004 and was subsequently found in five anuran species in urban and rural areas of the south east and the central north (Obendorf 2005). This wide distribution of the disease suggests it was introduced to the State sometime before 2004; possibly by inadvertent translocation of infected frogs in imported fresh produce from mainland Australia or the illegal importation of amphibians for pets. The vast majority of western Tasmania has not been surveyed for *B. dendrobatidis*, particularly the Tasmanian Wilderness World Heritage Area (TWWHA) which plays a significant role in the conservation of Tasmania's vertebrate fauna including anurans (Driessen and Mallick 2003). In particular the distributions of *B. nimbus* and *L. burrowsae* are primarily restricted to the TWWHA. The other Tasmanian

endemic anuran, *C. tasmaniensis,* is common and widely distributed in the TWWHA but also occurs in other parts of Tasmania.

The aim of the present study was to determine the presence and distribution of *B. dendrobatidis* within and immediately around the TWWHA, and to identify factors associated with the presence of the disease in order to direct appropriate management actions.

Methods

Study Area

The study was focused on the south and western portion of Tasmania; an area characterised by high annual rainfall (1200 –3200 mm) and low annual temperature (12-21 °C mean maximum temperature). The TWWHA is one of the largest temperate wilderness areas in the southern hemisphere, encompassing over 1.38 millions hectares or 20% of the Tasmanian land mass. The area is characterised by a mountainous landscape, low soil fertility and limited disturbance associated with European settlement. Dominant vegetation types are buttongrass moorland, rainforest, wet sclerophyll forest, wet scrub and alpine treeless communities. The remote location of, and limited vehicular access to, much of the TWWHA has resulted in minimal human impact in the region. Today, only a handful of minor commercial industries occur in the region including beekeeping, ecotourism, hydro-electric power generation and commercial fishing (Driessen and Mallick 2003).

Field sampling

Surveys for *B. dendrobatidis* were conducted between October 2005 and June 2007. Historical location records of *L. burrowsae* (stored on the Tasmanian Natural Values Atlas, a computerised database maintained by the Department of Primary Industries and Water) were used to provide a base set of sampling sites. The distribution of *L. burrowsae* was surveyed in

conjunction with the chytrid survey and will be reported elsewhere. Additional sampling sites were added opportunistically to the base-set to provide an overall spatial coverage of the southwest, with a specific focus on the TWWHA.

At each sampling site the following environmental characteristics were recorded: altitude, aquatic pH, vegetation type (forest or non-forest) and land tenure. Aquatic pH was determined using a Hach™ pH test kit. Anthropogenic disturbance factors associated with sampling sites were defined by delineating a 100 m radius assessment area around each sampling site using MapInfo Professional 8.0 GIS (Mapinfo Corporation). The factors recorded were presence of gravel roads, bitumen roads, walking tracks, fire trails and human infrastructure.

Chytridiomycosis assessment

Tadpoles were used for chytridiomycosis assessment due to the ease with which sufficient numbers could be captured at each sampling site. Infection status at each sampling site was determined using a combination of visual assessment of chytridiomycosis-induced mouth-part abnormalities (Obendorf and Dalton, 2006) and real-time Taqman Polymerase Chain Reaction (PCR) assay (Boyle et al., 2004). By grading mouthpart abnormalities in the field we were able to identify a subset of tadpoles that exhibited clinical signs of chytridiomycosis that could be used for follow-up Taqman PCR analysis. Sixty tadpoles were collected from each site and visually assessed for mouthpart depigmentation. A subset of 12 tadpoles, including any with mouthpart depigmentation or else a random sample, was swabbed individually by placing a fine-tip swab (MW100, Medical Wire and Equipment) on the mouthparts until the oral disc closed on the swab (Obendorf and Dalton, 2006). All tadpoles were released unharmed at the point of capture. Adult anurans captured opportunistically at sampling sites were swabbed twice over their ventral, dorsal and lateral surfaces. Care was taken not to collect debris or dirt on the swab during the sampling of both tadpoles and frogs

and to avoid cross contamination between swab samples. Swabs taken from tadpoles were pooled according to species with each sampling site represented by a minimum of three samples comprising of four swabs each (Hyatt et al., 2007). Pooling of swabs provided a cost-effect method for PCR analyses that permitted additional sites to be assessed. Swabs taken from frogs were not pooled and were analysed individually. Pooled swabs were stored at -10°C before transportation to James Cook University where they were analysed in triplicate and a positive result for *B. dendrobatidis* presence was recorded if all three replicates reacted for a single sample.

All field operations were conducted in accordance with the hygiene protocols for disease control (Speare, 2001). Equipment used in the field was washed down and sterilised with 95% ethanol between successive sampling sites. An additional wash down and equipment drying was undertaken between sampling periods.

Data analysis

We used logistic regression to determine the degree of association between site factors and the presence of *B. dendrobatidis,* however the unbalanced distribution and small number of sampling sites limited the power of the test and interpretation of results. Analysis was undertaken in SPSS version 15.0 with binary logistic models fitted to individual risks factors. Student t tests were used to assess differences in aquatic pH between infected and uninfected sites.

Results

A total of 62 wetland sites were sampled for the presence of *B. dendrobatidis* within and around the TWWHA, with the pathogen detected at 26% (n=16) of sites (Fig. 1, Table 1). Thirty three sites were sampled within the TWWHA and one (3%) was infected; this site was

adjacent to an access track to hydro-electric pylons and is within 1.5 km of the TWWHA boundary. The land tenures of sites surveyed within the TWWHA were national park and conservation area (Table 1). Twenty nine sites were sampled outside the boundary of the TWWHA and 15 (52%) were infected. State Forest (16 sites) was the most common land tenure sampled outside the TWWHA with the remaining sites occurring on various tenures (Table 1). *B. dendrobatidis* was detected at 69% of the State Forest sites and 39% of the pooled remaining sites (13) occurring outside the TWWHA.

Chytridiomycosis was detected in the tadpole stage of all four frog species sampled (*Litoria burrowsae, L. ewingii, Crinia tasmaniensis and C. signifera);* however, it was not detected in any of the adult frogs sampled (*L. burrowsae,* n = 6; *L. ewingii,* n = 1; and *Crinia spp*., n=12). There was little evidence to suggest that any of the frog species sampled were more or less likely to occur in areas where *B. dendrobatidis* was present (Table 2).

The presence of *B. dendrobatidis* appeared to be strongly associated with sampling sites that had gravel roads and forest vegetation present (Table 3). There was also a weak association with sites that were situated below 1000 m elevation. No association was found between the presence of *B. dendrobatidis* and roads sealed with bitumen. Although aquatic pH was slightly higher at infected sites (mean ± standard error: 6.0 ± 0.24, n = 16) than uninfected sites (5.6 ± 0.24, n = 46) the difference was not statistically significant ($t = 1.72$, $df = 60$, $P = 0.08$). The high pH at infected sites reflected an easterly bias in the sampling of infected sites where pH is generally higher. *B. dendrobatidis* was detected in highly acidic pools with the lowest pH recorded equalling 5.1. Walking tracks were weakly associated with the absence of *B. dendrobatidis* suggesting an association with remote locations.

Discussion

Understanding the distributional patterns of *B. dendrobatidis* and the identification of *B. dendrobatidis* free areas is essential for effective management and control of the pathogen (Skerratt et al., 2007). The results of the present study suggest that most of the TWWHA and southwest Tasmania remains free of *B. dendrobatidis* and that there is an opportunity to control the further spread of the disease into this high conservation area. Understanding the processes involved in the dissemination of *B. dendrobatidis* will be critical to controlling the spread of the disease into the TWWHA as well as other disease free areas of Tasmania.

Several pieces of evidence indicate that anthropogenic processes rather than interspecific anuran transmission may be primarily responsible for facilitating the spread of the pathogen on a landscape scale in Tasmania. Firstly, the high incidence of *B. dendrobatidis* in landscapes associated with human disturbance and activity outside the borders of the TWWHA; secondly, the absence of *B. dendrobatidis* throughout the remoter, undisturbed regions of the TWWHA; and thirdly, Tasmanian frogs species typically do not breed in stream habitats limiting the distribution of the pathogen by natural movement of water and anurans. However, despite several disturbance and environmental factors being strongly associated with the presence of *B. dendrobatidis*, the precise mechanisms of human-facilitated spread cannot be defined from the present study.

Roads

In the present study the presence of *B. dendrobatidis* was strongly associated with the presence of gravel roads. Roads can influence disease distribution, prevalence and pathogenicity by facilitating the translocation of pathogens or altering conditions to favour disease emergence (Daszak et al., 2000; Patz, 2004; Urban, 2006) and road construction provides access to previously undisturbed locations. No association was found between the

presence of *B. dendrobatidis* and roads sealed with bitumen suggesting that the presence of the disease relates to some factor that specifically relates to gravel road construction, maintenance and/or where they occur. In Tasmania, regular road maintenance procedures for high-use gravel roads involve the transportation and relocation of soils and water along the road. Water collected from nearby wetlands is used to reduce dust formation during maintenance procedures, while moist soils are transported long distances to repair the road structure. Water and soil run-off from the road surfaces into nearby wetlands during such procedures may facilitate the spread of water-borne *B. dendrobatidis* zoospores and infected tadpoles into new areas. *B. dendrobatidis* can persist for extended periods in water, moist soils and non-amphibian keratin based tissues without a host substrate (Johnson and Speare, 2003; 2005). Water collected from wild sources for use in dust suppression on gravel roads is suggested by Urban (2006) to facilitate the rapid dispersal of parasitic trematodes in Northern Alaska. Similarly soil and mud attached to heavy machinery and vehicles has been demonstrated to spread the plant pathogen *phytophthora* into undisturbed forest communities in Australia and America (Jules, 2002; Wills, 1993). It is possible that the spread of *B. dendrobatidis* is also occurring through similar mechanisms in Tasmania, with the translocation of *B. dendrobatidis* and infected tadpoles in moist soils and mud on heavy machinery used in road maintenance operations.

Risk to Tasmanian anurans

B. dendrobatidis was detected in *Litoria ewingii, L. burrowsae, Crinia tasmaniensis* and *C. signifera* in pool habitats along the eastern and northern borders of the TWWHA. The potential impact of *B. dendrobatidis* on Tasmania's anurans fauna is of significant concern, with two of Tasmania's three endemic anuran species restricted in range and habitat. Although population declines in these species have not been recorded, similarities in their life history

traits and habitat preferences with declining anuran species throughout the world indicate that *B. dendrobatidis* poses a significant threat to these species.

Host-specific habitat traits are thought to influence the persistence and impact of *B. dendrobatidis* within a population or species. However, patterns of anuran response in regions of increased *B. dendrobatidis* prevalence and pathogenicity remain disparate, with differing species and populations not being impacted equally (Lips et al., 2003b). Chytridiomycosis may have a greater impact on selected populations that reside in aquatic and riparian habitats, while sympatric species may be resistant to development of chytridiomycosis and act as reservoirs of *B. dendrobatidis* (Daszak et al., 2003; Lips et al., 2003b; Woodhams and Alford, 2005). Obendorf (2005) hypothesised that widespread and abundant species including *L. ewingii* and *C. signifera* may, in some cases, resist the development of chytridiomycosis and act as reservoirs for *B. dendrobatidis* in Tasmania. The findings of Ricardo (2006) support this hypothesis, with captive *L. ewingii* metamorphs able to survive with high intensity infections of *B. dendrobatidis* for at least 31 days post metamorphosis. However, further work on the susceptibility and survivorship of wild infected anurans in Tasmania is required.

Management

The results of the present study indicate that the TWWHA is predominantly free of *B. dendrobatidis* and that human facilitated movement of the disease is likely to be the main cause of spread rather than interspecific anuran transmission. Given that the majority of the TWWHA is not readily accessible by people, particularly by road, it may be feasible to control the spread of the disease in the TWWHA. Although further investigations into the precise mechanisms underlying human facilitated movement are required to fine-tune management and control actions, a precautionary management approach should be adopted in relation to movement of water, soil and amphibians by people. This will involve identifying activities in which people currently transport water and soil into the TWWHA, assessing the

risk of introducing the disease from these activities and adopting alternative measures where the risk is unacceptable. The transportation of amphibian into reserved land is currently illegal under the *National Parks and Reserves Management Act 2002*. The public, land management agencies and researchers need to be informed about the disease, the consequences of spreading the disease and how they can avoid spreading the disease particularly when visiting remote areas of the TWWHA. Monitoring sites need to be established at key locations within the TWWHA to detect further spread of the disease. Wherever possible management of the disease should be linked with management of other fungal diseases such *Mucor amphibiorum* and *Phytohpthora cinnamomi*.

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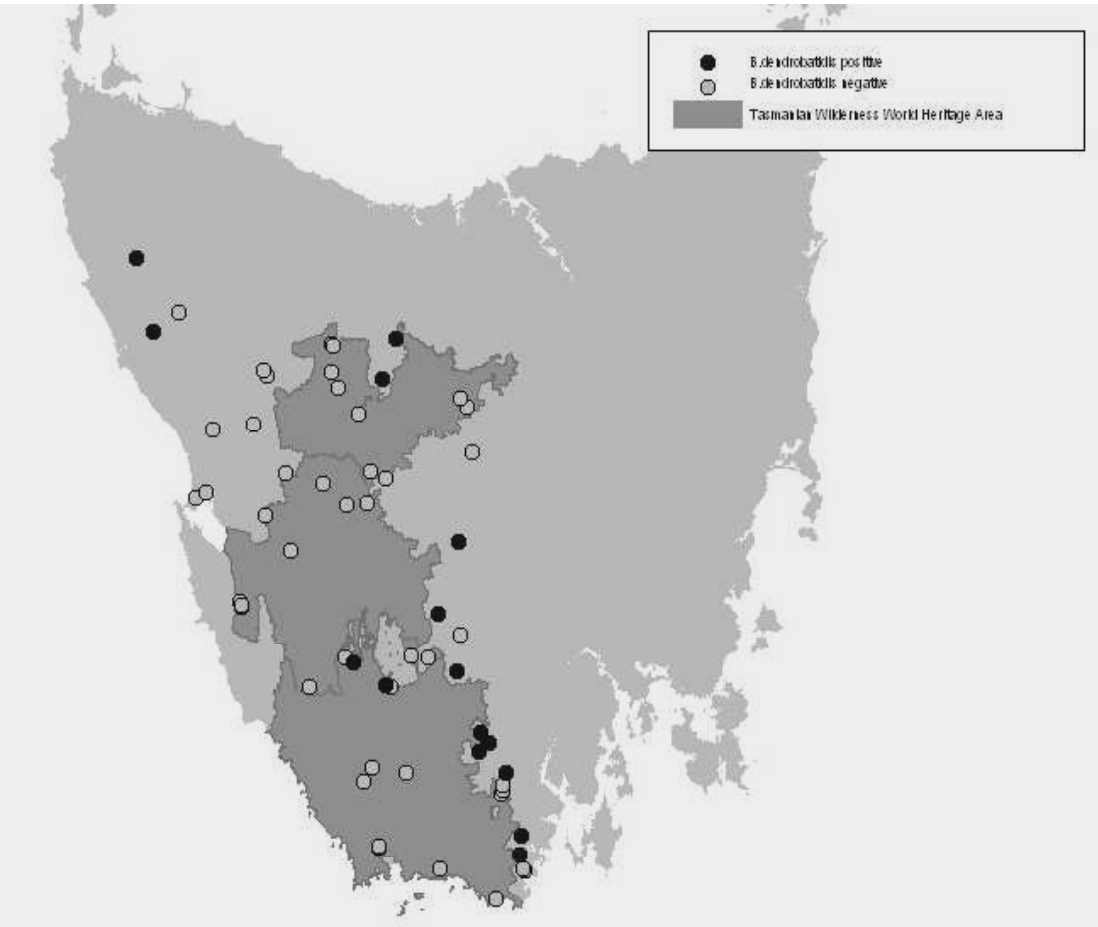

Figure 1. Distribution of *B. dendrobatidis* within and around the Tasmanian Wilderness World Heritage Area (darker shading). Dark circles = *B. dendrobatidis* positive, Light circles = *B. dendrobatidis* negative.

Table 1. Number of Sites where *B. dendrobatidis* was detected by land tenure.

Land Tenure	***B. dendrobatidis* absent**		***B. dendrobatidis* present**	
	within TWWHA	**outside TWWHA**	**within TWWHA**	**outside TWWHA**
National Parks[1]	27	1	1	0
Conservation Area[1]	5	1	0	1
Nature Reserve[1]	0	0	0	1
Regional Reserve[1]	0	4	0	0
Private Reserve[2]	0	0	0	1
State Forest	0	5	0	11
Hydro Tasmania	0	0	0	1
Unallocated Crown Land	0	3	0	0
Total	32	14	1	15

[1]Land reserved under the *Nature Conservation Act 2002*

[2]Local Council Reserve

TWWHA = Tasmanian Wilderness World Heritage Area

Table 2. Occurrence of frog species at sites where *B. dendrobatidis* was present or absent. N = number of sites, % = number of sites with frog species present as a percentage of total infected sites (16) and uninfected sites (46).

Species	***D. dendrobatidis* absent**		***D. dendrobatidis* present**	
	n	***%***	***n***	***%***
Crinia signifiera	20	43	10	63
Crinia tasmaniensis	33	71	9	56
Litoria burrowsae	12	26	3	18
Litoria ewingii	38	83	14	88

Table 3. Number of sites with *D. dendrobatidis* present in relation to disturbance and environmental factors. Probability values from binary logistic model for each individual factor is shown.

Factor present or absent	**No. of sites with *D. dendrobatidis* absent**	**No. of sites with *D. dendrobatidis* present**	**% diseased**	**Probability**
Gravel road				
No	37	0	0%	<0.001
Yes	9	16	64%	
Bitumen road				
No	36	15	29%	0.12
Yes	10	1	9%	
Walking track				
No	25	14	36%	0.012
Yes	21	2	9%	
Elevation				
≥1000m	8	0	0%	0.013
<1000m	38	16	30%	
Vegetation				
Forest	14	11	44%	0.007
Non-forest	32	5	14%	