The disappearance of the Stuttering Frog *Mixophyes balbus* at Macquarie Pass National Park, New South Wales

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ABSTRACT

We monitored a population of Stuttering Frog *Mixophyes balbus* tadpoles in Macquarie Pass National Park near Albion Park on the south coast of New South Wales. The species was initially detected by the presence of tadpoles at one site in 2000. Surveys thereafter were conducted at this site and the broader catchment of Macquarie Rivulet in an attempt to detect adults and or other tadpoles. Fifteen 250 m transects located along perennial creeks were surveyed at night for 30 min each and diurnal searches for tadpoles were conducted along approximately five km of creekline. About 200-300 tadpoles were recorded at this same site in January 2005; this cohort declined to about 15 tadpoles by September 2005. A total of 20 small tadpoles were taken into captivity in January 2005 in an attempt to maintain a captive population. Swab samples taken from free-living tadpoles indicated the presence of the frog chytrid fungus, *Bactrachochytrium dendrobatidis*. *Mixophyes balbus* may no longer persist at this site and managers now have to decide if the last cohort should be allowed to breed in captivity or an attempt made to outbreed this contracted genetic base. The captive population raises the dilemma, what should we do with captive populations that represents the last genetic vestige of an isolated population that may not persist in the wild?

Key words: *Mixophyes balbus,* monitoring population, chytrid, Macquarie Pass National Park New South Wales.

Introduction

The Stuttering Frog *Mixophyes balbus* (Straughan 1968) is listed in New South Wales (NSW) as an endangered species under Schedule 1 of the *Threatened Species Conservation (TSC) Act, 1995* and nationally as vulnerable on the schedules of the *Environment Protection and Biodiversity Conservation (EPBC) Act 1999*. This species has declined substantially in distribution and abundance on the south coast of New South Wales since the 1980's. Although extensive targeted surveys for *M. balbus* have been conducted in the southern portion of its NSW range there have been only a few animals found over the last twelve years (Hunter 2001; Lemckert *et al.* 1997; Daly *et al.* 2002; DECC 2006). The Atlas of Victorian Wildlife database has only three records of the species (Hunter and Gillespie 2006) from the East Gippsland area. Intensive surveys specifically targeting this species between 1998-2006 have failed to detect any individuals (Hunter and Gillespie 2006).

Within the Sydney basin *M. balbus* persists in scattered small populations in the Blue Mountains National Park and the Watagan Mountains, north of Sydney (White *et al.* 2009; G. Daly unpub. data). Populations previously recorded in the 1990's from Garrawarra Nature Reserve and Budgong near Nowra no longer appear to persist (G. Daly unpub. data). Hence it seems likely that this species is now extinct south of the Sydney basin, although further survey work is required to determine this with certainty.

A number of studies have implicated the disease chytridiomycosis in the decline of frog species in Australia and other parts of the world (Berger *et al.* 1998; Hyatt *et al.* 2007). The disease has been listed on the NSW *TSC Act,* 1995 and the Commonwealth *EPBC Act,* 1999 as a 'key threatening process'. Sick and dead *M. balbus* infected with the chytrid fungus have been detected in the Blue Mountains National Park (DECC 2006) and this disease is highly implicated in the decline of this species (Daly *et al.* 2002).

In view of the status of *M. balbus* in the southern portion of its range we monitored breeding, as evidenced by the presence of tadpoles in Macquarie Pass National Park from 2001 to 2006. Diurnal surveys were conducted annually (up to November 2009) at this site for tadpoles and targeted surveys for adults and tadpoles were conducted within the broader catchment. Samples taken from *M. balbus* tadpoles were tested for chytrid. This paper presents findings of monitoring and systematic surveys *M. balbus* in Macquarie Pass National Park and Tongarra Nature Reserve.

We also describe the actions that have been taken to date to conserve the genetic unit that the Macquarie Pass animals represent. This includes the capture and transportation of tadpoles to various institutions in order to maintain an ex-situ population. Finally we outline some of the problems and challenges facing the conservation of this genetic unit and consider some options for managers.

Methods

Study area

Macquarie Pass National Park (NP) (1064 ha) and Macquarie Pass State Conservation Area (166 ha), are located some 15 km west of Albion Park, on the south coast of NSW (Fig. 1). Although the conservation areas surveyed are given separate titles they form a contiguous block of bushland along the Illawarra escarpment.

Site Selection

Fifteen sites were surveyed within the reserves (Fig.1). The sites varied in altitude from 50 to 400 m Australian Height Datum (Table 1). The survey sites were chosen because previous surveys in the region (Daly *et al.* 2002) indicated that *M. balbus* was highly associated with second and third order streams that had rock pools and overhangs, riffle zones and mesic vegetation. Each site consisted of a 250 m long search area, herein termed a

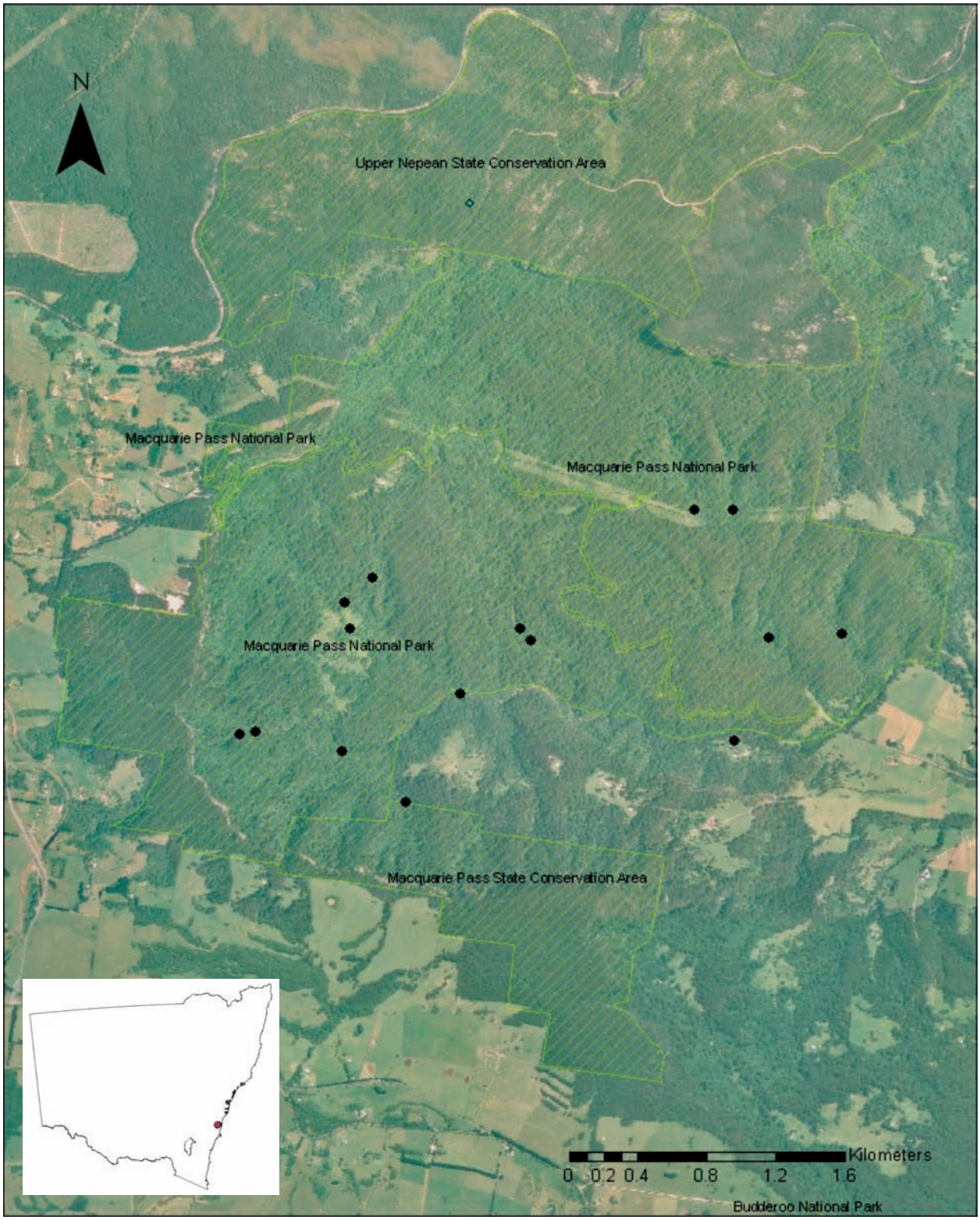

Figure 1. Sites surveyed for *Mixophyes balbus* in Macquarie Pass NP. Note: hatched area represent National Park estate

Table 1. Site locations and date and effort for nocturnal surveys.

Grid references - Eastings and northings in ADG Z56, AMG 66 datum. Altitude (Alt) in metres Australian Height Datum. Eastern Standard Time used for search periods.

Site Name	Easting	Northing	Altitude metres	Date of survey	Nocturnal Survey Period (hours)
Clover Hill 1a	284170	6172450	300	11.12.04	21.01 - 21.31
Clover Hill 1b	284840	6171910	170	11.12.04	22.22 - 22.52
Clover Hill 2a	285250	6172225	250	11.12.04	23.10 - 23.40
Clover Hill 2b	285190	6172300	250	11.12.04	23.10 - 23.40
Tongarra 1	284525	6171270	260	11.1.05	20.15 - 20.45
MacRivulet 1a	283650	6171690	400	4.1.05	22.07 - 22.37
MacRivulet 1b	283560	6171675	400	4.1.05	21.30 - 22.00
MacRivulet 2a	286430	6171635	100	13.12.04	23.50 - 24.20
North Arm 1	286630	6172245	100	13.12.04	21.30 - 22.00
North Arm 2	286420	6173000	270	13.12.04	20.56 - 21.36
North Arm 3	287050	6172265	50	13.12.04	22.53 - 23.22
North Arm 4	286200	6173000	250	13.12.04	21.36 - 21.56
Nurrewin Weir 1	284330	6172600	300	11.12.04	23.10 - 23.40
Nurrewin Weir 2	284200	6172300	400	13.12.04	19.38 - 20.08
South Arm 1	284150	6171575	270	4.1.05	20.50 - 21.20

transect. These covered a range of habitats within the reserves (Gaia Research 2005). The survey sites were visited during the day prior to nocturnal surveys in order to describe habitat characteristics such as the surrounding vegetation, existence of shallow pools and presence of fish.

Habitat

Survey sites were located along second and third order perennial creeks. The Kiama soil landscape series sheet (DLWC) indicates that the area has soils derived from the Illawarra escarpment and Cambewarra landscapes. The Cambewarra landscape is based on latites and the Illawarra escarpment group on Hawkesbury sandstone talus (Hazelton, 1993). The habitat within the immediate area of the creek typically varied from narrow races of water on exposed rounded sandstone boulders or latites to sandy pools (up to 1.5 m deep). The water turbidity was consistent and all sites had clear water.

The vegetation of most of the survey sites consisted of a canopy of Brown Barrel *Eucalyptus fastigata,* Gully Gum *E. smithii,* Yellow Stringybark *E. muellerana* and Turpentine *Syncarpia glomulifera.* This association is defined by NPWS (2002) as Moist Brown Barrel Forest. The mid-canopy was primarily closed forest composed of Lillypilly *Acmena smithii,* Coachwood *Ceratopetalum apetalum,* Cabbage Tree Palm *Livistona australis* and Sassafras *Doryphora sassafras*. Beside the creeks there was an abundance of ferns such as King Fern *Todea barbara,* Rough Tree Fern *Cyathea australis,* Spiky Tree Fern *C. leichardtiana* and Soft Tree Fern *Dicksonia australis* on their edges. The exception to this was site MR2, which had a canopy of River Oak *Casuarina cunninghamiana* and Turpentine. Macquarie Pass NP, dedicated in 1969, has been selectively logged prior to dedication. Some sites added to the park have been historically cleared and used for agriculture prior to their dedication as Park, for example old Nurrowin and Clover Hill farms.

Frog surveys

A single visit, night survey of all 15 sites was conducted between October 2004 to January 2005 (Table 1) over a period that corresponded with the breeding season of the species (Anstis, 2002). Surveys involved spotlighting along approximately 250 m of creek for 30 min. Fifty watt/12 volt Spotlights (50 W, 12 V) were used to observe frogs, tadpoles and spawn. Calls of *M. balbus* were broadcast continually from a cassette player (Optimus CTR-116) while surveying. Surveys were conducted between dusk and 23.40 Eastern Standard Time. The temperatures, during the surveys ranged between 14.6-20.5º C (air) and 13.9-18.3ºC (water). Additional diurnal searches were made along sections of creekline within the catchment during this period for *M. balbus* tadpoles. The NSW NPWS hygiene protocol for frogs (2008) was followed.

Tadpole Monitoring

Approximately 30 *Mixophyes balbus* tadpoles were detected during diurnal searches at one site in Macquarie Pass National Park (Clover Hill 1a) on 17 February 2000 (Daly *et al.* 2002). Thereafter annual inspections were conducted until 2005 when monitoring became more frequent until early 2006 when searches were conducted more infrequently (Table 2).

Recovery action – collection for ex-situ population

On 13 January 2005 twenty *M. balbus* tadpoles were collected from the wild at Clover Hill 1a. The protocols and approvals for the collection are detailed in Hunter (2004). Ten *M. balbus* tadpoles were transported to the Melbourne Zoo and ten *M. balbus* tadpoles to the Amphibian Research Centre, Werribee, Victoria by plane in individual ten litre plastic containers. All equipment

Table 2. Number and size of *Mixophyes balbus* tadpoles detected at Macquarie Pass NP

Date of survey	Survey period (EST)	No. of *M. balbus tadpoles*	Length (snout-vent)	Length (total)	Gosner stage
5.1.05	not recorded	42	circa 10	circa 25	25
11.1.05	not recorded	33	circa 10	circa 25	25
13.1.05	not recorded	200-300	10	circa 25	25
19.1.05	20 *M. balbus* tadpoles removed as an emergency recovery action				
17.3.05	18.58-19.19	5	not recorded	not recorded	not recorded
23.7.05	17.35-17.50	25	22	60	25
14.9.05	18.17-18.46	15	22	55	25
26.10.05	18.53-19.35	15	not recorded	not recorded	29
10.12.05	20.00-20.30	4	25	70	41
23.1.06	12.07-12.37	0			
8.3.06	10.28-10.44	0			
18.12.07	13.33-13.48	0			
17.11.09	10.33-10.50	0			

used to capture and transport tadpoles was either new in sterile packaging, or sterilised with 70% ethanol prior to arriving at the site. The buckets were filled with stream water and the lids fastened such that there is very little or no air space remaining in the bucket. The objective was to collect sufficient animals to form a nucleus for a potential captive breeding program, to ensure the preservation of the clearly declining south-coast population.

Swab sampling

The mouthparts of twelve *M. balbus* wild tadpoles were swab-sampled for chytrid fungus *Batrachochytrium dendrobatidis* (Bd) on 26 October 2005. The samples were sent to CSIRO Australian Animal Health Laboratory for testing. Tadpoles were removed from the pool with a scoop net and individually placed in a small plastic bucket containing water from the pool containing the *M. balbus* tadpoles. A fresh scoop net was used for each tadpole, or the net was washed in pure alcohol between captures and re-used to avoid contamination.

Eight of the *M. balbus* tadpoles taken into captivity and held at Melbourne Zoo were also tested for the Bd on on 9 November 2005 (two tadpoles having died). The samples were sent to CSIRO Australian Animal Health Laboratory for testing (G. Gillespie pers. comm.).

Results

Streamside searches

Stuttering frogs

Mixophyes balbus was detected at one site (Clover Hill 1a) by the presence of tadpoles in September 2004. A new cohort of tadpoles was detected at the same location in January 2005. The tadpoles found in 2004 were relatively advanced (Gosner stage 25) being approximately 40 mm in total length, whereas those found in January 2005 were smaller being only about 25 mm in total length. Tadpoles observed in 2004 were all in a single pool below a road culvert, whereas tadpoles found in 2005 were in pools above, within and below the culvert.

The survey effort in the summer of 2004 totalled 7.5 h of nocturnal survey time and 6 h diurnal survey time. Approximately 3.75 km of creeks were surveyed during nocturnal searches and 5 km were surveyed during diurnal searches. No adult frogs were ever seen or responded to the broadcast of call playback, and no tadpoles were detected at any other sites.

Other species of frog and fish detected

Four species of frog were detected during the diurnal surveys, mostly through the identification of tadpoles (Table 1). The encounter rates were Southern Leaf Green Tree Frog *Litoria nudidigitus* (73 % of diurnal and 80 % of nocturnal sites), Lesueur's Tree Frog *L. lesueuri* (7 % of diurnal and 20 % of nocturnal sites), the Common Eastern Froglet *Crinia signifera* (13 % of diurnal and 7 % of nocturnal sites) and Verreaux's Tree Frog *L. verreauxii* (7 % of nocturnal sites).

Fish and Yabbies were present in most of the creeks surveyed. The fish included Eel *Anguilla* sp., Climbing Galaxia *Galaxia brevipennis*, Spotted Galaxia *G. maculata*, Gudgeons *Gobiomorphus* spp. and Smelt *Retropinna semoni*. The latter three species were only observed in the lower catchment of Macquarie Rivulet.

Tadpole Monitoring

Mixophyes balbus tadpoles were observed between January and December 2005 in the pools immediately above and below a road culvert. The survey effort totalled approximately 3.5 h for diurnal and 4 h for nocturnal surveys. These *M. balbus* tadpoles took 11 months to develop to the late Gosner developmental stage (41-43) pre-metamorphling.

Table 3. Frog species detected during nocturnal surveys for *Mixophyes balbus.*

Litoria nudidigitus = Ln, *Litoria lesueuri* = Ll, *Litoria verreauxii* = Lv, *Crinia signifera* = Cs, *Mixophyes balbus* = Mb. O – observed, W – heard calling, T = tadpoles,

Suffix 1-4 and a, b, c indicates separate survey sites

Site Name	Ln	Ll	Lv	Cs	Mb	Total Sp.
Clover Hill 1a	W				T	2
Clover Hill 1b	W					1
Clover Hill 2a						0
Clover Hill 2b						0
Tongarra 1	T	O				2
MacRivulet 1a	O					1
MacRivulet 1b	O					1
MacRivulet 2a	O	O				2
North Arm 1	O					1
North Arm 2		O		W		2
North Arm 3	O					1
North Arm 4	W					1
Nurrewin Weir 1	W		W			2
Nurrewin Weir 2	W					1
South Arm 1	W					1

Figure 2. *Mixophyes balbus* tadpoles placed in separate clean containers ready for translocation.

Figure 3. The last free-living *Mixophyes balbus* tadpole observed at Macquarie Pass NP.

During development tadpoles exhibited several changes in behaviour. When they were small (< 25 mm total length) they congregated in the shallows during the day and some hid under stones. They could be flushed from their hiding places by disturbances to the stones. The tadpoles swam into deeper water and took refuge under other stones when disturbed. Fewer tadpoles were observed during night surveys as they dispersed into deeper (circa 1.0 metre) water and presumable hid under stones. When the tadpoles were approximately 60 mm in total length they were not easily observed during the day as they took refuge under the stones in the shallows. At night they could be coaxed to leave their hiding places by poking the substrate. Animals were counted as they dispersed into the deeper water or while visible on the substrate. During the latter Gosner stages of development (stages 41-43) the tadpoles were detected (at night) in a very shallow side pool. The depth of this pool was approximately 25 mm and the tadpoles hid under the dead leaves.

The tadpoles were quite sedentary. After January 2005 all tadpoles were found in the two pools immediately below the culvert. The number of tadpoles declined during the year. From the estimated 200-300 tadpoles (100 actually observed) their numbers fell to 15 or less during nine months. Only four tadpoles were found during the December 2005 survey and their late stage of development indicated that other members of this cohort had completed metamorphosis. No tadpoles were detected during the January 2006 - 2009 surveys, indicating that additional breeding had not occurred at this site.

Swab samples

Results from the Bd testing of swab samples taken from 12 free living *M. balbus* tadpoles at Macquarie Pass NP on 26 October 2006 were all positive for chytrid (D. Hunter pers. comm.). Results from the Bd testing of tadpoles held by Melbourne Zoo conducted on 9 November 2005 were 1 positive / 7 negative. The single positive tadpole was treated but subsequently died. Repeat analysis for Bd on the 7 remaining Melbourne Zoo tadpoles on 24 November 2005 were all negative.

Recovery actions to date

Captive population

Of the ten animals sent to Melbourne Zoo, seven survived, metamorphed by late December 2005 and persist (G. Gillespie pers. comm.). Of the ten tadpoles sent to the Amphibian Research Centre all animals were metamorphosing and surviving as of 7 March 2006. All remaining animals have grown and are now sexually mature.

Genetic analysis

A mitochondrial DNA study (Naomi Doak pers. comm.) of *M. balbus* recognized a significant genetic distinctiveness between populations north and south of the Taree district. However, these results are incomplete and require further genetic sampling. In view of the declining status for the southern populations of *M. balbus* and the unresolved genetic status of the species a more detailed study on the species' genetic status was prepared (Donnellan, 2008). This study found that from the samples taken from twenty locations in NSW there were two distinct lineages: one from animals north of Mt Royal and the other from Barrington Tops to Macquarie Pass.

Discussion

Decline in the population

At Macquarie Pass NP adult *M. balbus* declined to a few (possibly two) frogs that bred at one location between 2000 and 2006. No tadpoles have been detected since that time and wild frogs may no longer persist. This decline is consistent with other surveys of areas with historic records of *M. balbus* on the south coast of NSW (Lemckert *et al.* 1997, Hunter 2001, Daly *et al.* 2002).

Chytrid is strongly implicated as the major causal agent for the decline of *M. balbus* in southern NSW. Swabs taken from wild tadpoles tested positive for the presence of the pathogen in all animals. In another study, dead adults and metamorphling *M. balbus* were found at Ruby Creek in the southern Blue Mountains. Pathology conducted on these animals indicted the presence of chytrid (DEC 2006). Evidence from the current study and DEC (2006) indicate chytrid infects the animals during their aquatic phase and leads to mortality early within the terrestrial phase. The fungus compromises recruitment of frogs into the adult breeding population and may also cause death of adult frogs. The implication is that chytrid has caused the decline and local extinction of almost all populations of *M. balbus* from the Watagan Mountains NSW to Gippsland Victoria.

No *M. balbus* tadpoles were detected in late January or early March 2006 or surveys conducted in 2008 and 2009. We suggest that *M. balbus* may no longer persist in Macquarie Pass National Park.

Captive population

The tadpoles taken from Macquarie Pass NP may represent the last genetic provenance of the south coast of NSW. Managers now face a number of decisions in regard to these animals. What shall be done with them? Should we allow them to breed, outbreed with genetic material collected from south of the Hunter valley (i.e. Ruby Creek or the Watagan Mountains), release them back to Macquarie Pass NP or simply euthanase them. These questions were raised at the time that an application for ex-situ collection was made (R. Wellington pers. comm.).

If the *M. balbus* from the Macquarie Pass collection (now sexually mature) are allowed to interbreed then it is highly likely that the offspring will have reduced fitness as they are almost certainly siblings from the one spawn. As this genome has been demonstrated unable to persist in the presence of chytrid, it would be useless re-introducing offspring to the wild.

If the *M. balbus* were to be outbred, then which surviving population to choose from? The nearest extant population is at Ruby Creek in the Blue Mountains some 80 km north-west of Macquarie Pass. The Ruby Creek population persist with chytrid and any collection from the wild would have to be screened for this disease and quarantined until such time that they were considered safe to pair with the Macquarie Pass animals.

Captive *M. balbus* at Melbourne Zoo originate from the north coast of NSW and could be used to outbreed with the Macquarie Pass animals. This scenario could be justified if the two *M. balbus* lineages were found to interbreed at a site in the Barrington Tops - Mt Royal area. Some populations of this northern lineage appear to have recovered from earlier, dramatic declines, and at some sites are again common (G. Daly unpub. data). This suggests that this lineage may have developed immunity to chytrid. However, if *M. balbus* represents two species as preliminary genetic work suggests, then this would open a new debate for managers; would it be applicable to reintroduce these chytrid immune, captive 'hybrids' back to Macquarie Pass?

Conclusions

This study found that *M. balbus* has further declined on the south coast of NSW and may be locally extinct at Macquarie Pass NP. There appears to be a trend of extinction in this species from higher to lower latitudes suggesting some link with average temperatures. Chytrid is highly implicated in this species decline.

By taking *M. balbus* tadpoles from Macquarie Pass NP into captivity, genetic stock from this region was secured, but their future is uncertain. Daly *et al.* (2008) quantified some of the costs associated with the re-introduction of an endangered species of amphibian. This is an expensive exercise in terms of financial and human investments. There is a real desire to stop extinctions and the subsequent decline of biodiversity. However, if the cause for the decline is not arrested then releasing the captive frogs or breeding the siblings would be a worthless exercise. The animals could either be euthanased or outbred with compatible genetic lines that have persisted (evolved) with chytrid for reintroduction. State and federal government agencies have to provide guidance on the fate of these animals in accord with recovery plans. The situation of M. balbus at Macquarie Pass illustrates the need for clear direction from government agencies on declining populations, emergency translocations and reintroduction strategies.

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