

Captive management and breeding of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*) (Moore 1953) at Taronga and Melbourne Zoos

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Abstract.—The Southern Corroboree Frog *Pseudophryne corroboree* is a small myobatrachid frog from south-eastern Australia that has rapidly declined in recent decades largely due to disease, caused by infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. As a key recovery effort to prevent the imminent extinction of this species, an *ex situ* captive breeding program has been established in a collaborative partnership between Australian zoological institutions and a state wildlife department. Despite initial difficulties, successful captive breeding protocols have been established. Key factors in achieving breeding in this species include providing an adequate pre-breeding cooling period for adult frogs, separation of sexes during the non-breeding period, allowing female mate-choice via the provision of numerous males per enclosure and permitting the females to attain significant mass prior to breeding. Difficulties were experienced with egg and larval mortality in early years, though these issues have since been largely resolved. To date, the success of captive breeding from 2010–2012 has permitted the reintroduction of 1,060 captive-produced eggs and an increasing captive population. size that will support conservation research and provide insurance against further declines.

Keywords. *Pseudophryne corroboree*, captive breeding, husbandry, conservation, zoo, Anura, frog, Australia

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Introduction

Over the past five decades amphibians have been declining at a rate exceeding that of other terrestrial vertebrates (Stuart et al. 2004). A large proportion of these declines are due to the spread of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which causes the disease chytridiomycosis (Berger et al. 1998; Skerratt et al. 2007). There is currently no adequate management response that can reduce the population level impacts of this pathogen on susceptible species that continue to decline (Woodhams et al. 2011; McCallum 2012), and as such, the only way to prevent their complete extinction is to secure captive assurance colonies in quarantine facilities (Gagliardo et al. 2008). The large number of frog species in this situation necessitates a large scale response, and there has been a coordinated effort globally to increase the knowledge and resources required to achieve this (Zipfel et al. 2011). Within Australia, 26 amphibian species have been identified as requiring *ex situ* intervention by the IUCN Global Amphibian Assessment, and State or Federal recovery plans (Gillespie et al. 2007). Of these species, the Southern Corroboree Frog (*Pseudophryne corroboree*) was considered the highest priority owing to its extremely precarious status in the wild (Gillespie et al. 2007). The Southern Corroboree Frog has suffered a rapid and catastrophic population decline since the mid-1980s (Osborne 1989; Osborne et al. 1999; Hunter et al. 2009b), with all the evidence implicating chytridiomycosis as the primary causal factor (Hunter et al. 2009c). It is now one of Australia's most threatened vertebrate species, with potentially fewer than 50 individuals remaining in the wild (Hunter et al. 2007), and no reproduction occurring in remnant wild populations in 2013. The species is listed as Critically Endangered by the IUCN (Hero et al. 2004). It is also listed as Critically Endangered nationally under the Environment Protection and Biodiversity Act 1999 and as Endangered under Schedule 1 of the NSW Threatened Species Conservation Act 1995.

The critically low abundance and continued decline of *P. corroboree* suggests that this species will become extinct in the wild in the very near future without immediate human intervention. Thus, persistence of the

species in the wild will depend on the success of a captive breeding program combined with the targeted *in situ* release of captive-bred progeny, and ideally mitigation of the amphibian chytrid fungus. To enable this, a collaborative *ex situ* program has been established in partnership between NSW Office of Environment and Heritage (OEH) and four captive institutions. The primary aims of this captive program are to establish an insurance population and supply captive-bred progeny for reintroduction and conservation research.

Materials and Methods

Study Species

Pseudophryne corroboree is a small, robust terrestrial myobatrachid frog that is easily recognized by its unique and striking colouration. (Fig.1) The dorsal surface is boldly marked with black and yellow longitudinal stripes, while the ventral surface consists of black, yellow and white blotches. Adults reach a maximum length of between 25 and 30 mm (Cogger 2000). The species is restricted to Kosciuszko National Park in New South Wales (NSW), Australia, where it was historically known to occur across an area of 400 km² at altitudes of 1300–1760 metres (Osborne 1989). Within this range, its breeding habitat is largely associated with ephemeral pools within sphagnum bogs or wet tussock grasslands along watercourses (Hunter et al. 2009a).

Pseudophryne corroboree breeds annually from mid to late summer, with males creating small, terrestrial nest chambers. The females typically lay 16–38 large eggs, which measure eight mm in diameter when hydrated (Hunter et al. 2007), within the nest chamber. The male remains with the nest throughout the breeding period, often attracting clutches from multiple females within a single chamber. The eggs develop in these terrestrial nests through to hatching stage, at which point they enter diapause and await autumn rains to flood the nest. Flooding stimulates the eggs to hatch and the tadpoles to move into the main pool, where they become free swimming and feeding larvae. The tadpoles remain in the pool over the winter period and reach metamorphosis in late spring to early summer.



Fig. 1. Adult Southern Corroboree Frog.

Ex Situ Captive Management

The captive *P. corroboree* population is divided between four institutions: Taronga Zoo (TZ), Melbourne Zoo (MZ), Healesville Sanctuary (HS), and the Amphibian Research Centre (ARC). The captive program was initiated at the ARC in 1997, extending to MZ in 2001, TZ in 2006, and HS in 2007. Housing the frogs at a small number of dedicated institutions has dispersed the required resources and reduced the potential threat from disease, accident or natural disaster, yet still ensures tight control of biosecurity. The source of founders for the captive population has been from eggs collected in the wild between 1997 and 2012. This paper will focus on husbandry and breeding at TZ and MZ, which held 420 and 121 frogs respectively as of 1 November 2012. Many of the frogs contributing to the captive breeding outlined in this paper were initially reared to the juvenile or adult stage at the ARC before being transferred to TZ and MZ.

At both zoos, the *P. corroboree* populations are maintained in dedicated, isolated facilities equipped with refrigeration. (Fig. 2, Fig. 3) The refrigeration system is programmed to replicate the seasonal changes in the sub-alpine climate where this species occurs. The tempera-

ture control software is programmed with temperature alarms that also disable power to the facilities should the temperature become excessively high or low. Internal lighting within the facilities is controlled by light-sensitive switches set to simulate the local photoperiod. All water entering the facilities is filtered. To date, tadpoles have been successfully reared at TZ in water that has been filtered through a reverse osmosis (RO) unit alone; RO water reconstituted with trace elements and Sydney tap water that has been passed through a filtration system that constantly circulates water through five micron paper-pleated mechanical filters and activated carbon filters. Since 2010, the water at MZ is the municipal water supply that is recirculated through a sediment filter, a carbon filter, and a UV sterilizer. It then passes through an RO unit before entry into the facility.

High levels of biosecurity that comply with current recommendations (Pessier and Mendelson 2010) are maintained at both institutions. Facilities are serviced daily prior to contact with any other animal species, dedicated footwear is located within the facilities and must be worn upon entry and protective lab coats are worn. Disposable powder-free vinyl or latex gloves are kept within the facilities and are worn when handling any animal, enclosure or equipment.



Fig. 2. Endangered amphibian complex at Melbourne Zoo.



Fig. 3. Corroboree frog breeding enclosure at Taronga Zoo.

Captive Husbandry

The husbandry protocols described below apply at both institutions unless otherwise stated.

Housing – juveniles and adults in non-breeding season

Non-breeding adult frogs were housed in clear Hagen Pal Pen terraria of two sizes (27 × 17 × 20 cm and 33 × 19 × 24 cm). Each terrarium holds 4–6 frogs. The terrarium substrate is ~two cm of washed white aquarium gravel (particle size ~4 mm) that has been heat-sterilized at 200 °C for one hour. Three mm diameter holes were drilled in the base of the terrarium for drainage. Half of the floor area was either planted with live sphagnum or had a ~three cm layer of commercially-

purchased dead, rehydrated sphagnum. At TZ, the moss was heated to 40 °C for 16 hours prior to use to ensure that any chytrid fungus zoospores were killed. At MZ, the moss was heated at 70 °C for 30 minutes, followed by 30 minutes at 40 °C. Ultraviolet light (UVB) was provided with Zoomed Repti-sun 10.0 fluorescent tubes situated 33 cm above the terrarium substrate. This typically provides UVB at between 20–30 μW / cm² at the enclosure floor, as measured on a Solarmeter 6.2.

Diet

Frogs were reared primarily on a diet of 1–10 day old hatchling crickets (*Acheta domestica*). At TZ, they were fed twice per week from early December to late April (enclosure temperature 20–22 °C), once per week throughout November and from May to late August (14–18 °C) and not at all during September and October (5–10 °C). At MZ, frogs were fed 2–3 times per week from December to May (enclosure temperature at 25 °C and 15 °C, day and night respectively). Adult frogs were not fed during the cooler period which extends from June to November, when the temperature was below 10 °C. During each feed, the frogs were offered approximately 15–20 hatchling crickets each. The crickets were dusted with either Rep-Cal calcium or Herptivite multivitamin supplement, alternating between feeds. At MZ, frogs were also occasionally fed vestigial-winged fruit flies. Enclosure substrates were sprayed with water on the day after each feed to break down and wash away faecal waste and dead crickets.

Breeding Enclosures

At TZ, eight glass breeding tanks measured 135 × 55 × 55 cm high (including a 25 cm high fly-mesh hood with access doors). In 2010 and 2012, an additional glass tank measuring 120 × 70 × 65 cm (including a 35 cm high fly-mesh lid) was used. Each of the tanks had a base substrate of washed, heat-sterilised, 5–8 mm diameter white aquarium gravel. The tanks were planted with banks of live sphagnum moss slightly sunk into the gravel substrate. All moss was collected from within the direct breeding habitat of the species. In 2010, rather than live moss, one tank had commercially-pur-

chased sphagnum moss installed around the outside of the tank to replicate the edge of a sphagnum pool.

At MZ, two different styles of enclosure have been used. A single tank was used in the 2006 and 2007 seasons. Two tanks were used in 2009 and 2010 seasons. These tanks mimicked a stream cross section with glass embankments on both sides. To replicate an alpine breeding environment, the tanks had a base of washed and heat-treated aquarium gravel, and substrate of commercially-purchased sphagnum moss (heat-treated and sterilized). These glass tanks measured $180 \times 45.5 \times 75$ cm high (including fly-mesh hoods). The second tank had the same measurements except it had a lower height of 49 cm.

In mid-2010, the Endangered Amphibian Complex (EAC) at MZ was completed and commenced operation. This is a purpose-designed facility to simulate the temperatures found in the alpine areas of Australia. This room has two separate compartments with individual temperature controls. All of the *P. corroboree* were moved into the EAC in October 2010, just prior to the onset of the breeding season. There were four glass breeding enclosures; two measured $100 \times 58 \times 70$ cm high (including 40 cm high fly-mesh hoods with access doors). The other two breeding tanks were smaller, measuring $65 \times 58 \times 70$ cm high (including the same access door). Each tank had a base substrate of white aquarium gravel which had been washed and sterilized, and commercially-purchased sphagnum moss that had been heat-treated. The moss was placed into these breeding tanks to mimic the surrounding edges of an alpine bog and water was filled into the middle area of the pool.

Temperature Cycling

At TZ, immediately after the breeding season ends in early April, the adult frogs were placed in their non-breeding enclosures in single sex groups and maintained at 15 °C. In early September, the facility was cooled to 5 °C to replicate winter temperatures. The temperature was increased to 8–10 °C in mid-October, to 15 °C (with a 12 °C night setting) in early November and to 20 °C (with a 17 °C night setting) in mid-November. Once temperatures exceeded 15 °C, feeding of frogs resumed.

At MZ, the cooling regime has varied over the years due to a lack of facilities dedicated for ensuring these animals undergo a proper winter. During 2007, adult frogs were removed from their breeding enclosure and placed into plastic Pal Pen terraria for 64 days between November and January. These were cooled to 7–9 °C in a refrigerator during this period and the frogs were not offered food. These containers were watered very lightly to help simulate overwintering in drier habitats. After this period in the refrigerator they were then placed into breeding enclosures where the temperatures varied from 16–23 °C. Prior to the 2008–09 breeding season, 18 (3.5.10) adult frogs were placed into the refrigerator where temperatures ranged between 6–8 °C for seven weeks, and then moved into breeding tanks.

Prior to the onset of the 2010 breeding season, 18 adult frogs (same individuals as previous season) were placed into the fridge for 31 days at 6–8 °C. In 2011, all adult frogs were placed into the EAC rear compartment at 5–7 °C from 29 October to 04 December (males) and 20 December (females). Moving the frogs into the new facility at MZ has allowed the frogs to undergo a full year of temperature variation, similar to those maintained at TZ.

Tadpole Management

At TZ, tadpoles were generally maintained in 145 litre glass aquaria ($122 \times 70 \times 17$ cm deep), with between 20 and 120 tadpoles per aquarium. Up to 10 tadpoles have also been reared in 11 litre plastic aquaria ($33 \times 18 \times 18$ cm). At MZ the tadpole tanks have varied over the seasons, including within the breeding tanks and in 35 L of water in glass aquaria ($75 \times 29 \times 30$ cm). The current tadpole rearing tanks in the EAC ($64 \times 58 \times 20$ cm) have removable aluminium-framed fly-mesh divides in the centre, allowing two tanks to become four if required. These tanks hold approximately 50 litres.

Daily water changes of approximately 10% were conducted using an automated irrigation timer and spray system. Weekly water quality tests were undertaken to ensure water parameters are maintained within appropriate limits (ammonia – 0 ppm, nitrates – 0 ppm, pH 6.0–7.0, conductivity <15 µS/m).

Aquarium substrate was ~1 cm of pond silt collected

from the bottom of natural pools within the species' habitat. Prior to use, the silt was heated to 40 °C for 24 hours to kill chytrid fungus zoospores (Johnson et al. 2003), a process which still allows algae to survive and grow. As well as feeding on algae, tadpoles were offered a diet of frozen endive twice per week and a 75:25 mixture of finely-powdered Sera Flora and Sera Sans fish flakes, three to four times per week. This tadpole diet has been utilized at TZ since 2007, with the heat-treated natural silt first added to tadpole rearing tanks at MZ during the 2009 breeding season. Prior to that, only endive was offered. In 2012, MZ also added finely crushed spirulina wafers.



Fig. 4. Floating hatching tray on a tadpole rearing tank.

Results

Captive Breeding at Taronga Zoo

2010

Five males were placed in each of four breeding tanks from 28–31 December 2009, to allow them time to establish nests. Six female frogs were added to each tank on 26 January 2010. Five females in each tank were six years old, while one was four years old. The male frogs began calling on 23 January. One or two males

were heard calling daily from each tank, with four frogs often heard calling from one of the tanks. Frogs often called in response to any sound (e.g., keeper entry into the facility), and could be stimulated to call at any time with a shout. In order to further stimulate calling activity, a cassette player with a 30-second continuous loop tape of a male calling was installed in both facilities on 31 January. The tape was set to come on for the first 15 minutes of each hour from 1800 to 2200 hours inclusive. The volume approximated a typical male calling in the facility, to be audible to the frogs in all tanks but not so loud as to dominate over the calling males. The calling frequency began to decrease from mid-March, ceasing on 26 March.

In late March, all tanks were searched, nests were located and the eggs removed. Six successful male nest sites were located, with two nests in each of the three tanks with live sphagnum moss. No nests were located in the tank with commercially-purchased sphagnum, despite the presence of calling males. To induce egg-laying, the three largest females from this tank were moved to another breeding tank on 28 March; two laid eggs in the following two weeks.

All nests were typically located between the sphagnum moss and the aquarium gravel. Only one nest was located inside a sphagnum clump. All nest sites were moist, but not saturated. The positioning of the eggs upon the gravel allowed for excellent drainage in the nest, but the moist sphagnum kept nest humidity at around 100%.

In total, 479 eggs were laid from a possible 24 mature females in 2010, suggesting that well over half of the females had laid eggs (Table 1). The numbers of eggs per nest varied from 36 to 130, indicating 1–4 clutches laid in each nest. Unfortunately, there was significant egg mortality, both while in the nest and following retrieval. Only 38% of eggs appeared live when removed from the nests, and 28% of the total survived eight weeks until Stage 27 (Gosner 1960), after which hatching can occur once eggs are inundated. Almost all mortality before and after removal from the nest occurred prior to Stage 14 (Gosner 1960). Eggs were kept at temperatures of 13.5–15 °C within the nest and while packed in live, moist sphagnum moss after removal, and all appeared to be well within the range of normal

moisture levels observed in wild nests. It is important to note that infertile eggs could not be distinguished from embryos that died in early developmental stages, though the majority of the 72% failed eggs did appear fertile. A total of 134 embryos reached Stage 27 (hatching), with 47 of these released to Kosciuszko NP and the remainder retained for rearing.

2011

From 12–15 January, five males were placed in each of seven breeding tanks. On 22 February, five or six female frogs were added to each of six breeding tanks, with only one female added to the remaining tank. Calling activity was recorded from 30 January to 6 April. Between one and four frogs were recorded calling from each of the tanks. Calling was more consistent from the seven year old males, with at least one male strongly calling each day. Two of the four tanks with five year olds had weak or no calling on most days. To further stimulate calling behavior, call playback was again used from 22 February.

On 25 March, a total of 422 eggs were removed from six nests in the seven tanks (Table 1). Total number of eggs varied from 16 to 135 per nest, indicating clutches from one to five females in each nest. No eggs were laid in the tank containing only one female. There was a marked difference in productivity between the five and seven year olds, with older frogs laying more eggs. Based on the number of eggs laid, it appeared that over half of the seven year old females produced eggs. Additionally, embryo survival was 83%. The five year old females produced only two clutches of eggs ($n=56$) laid in nests, while three infertile clutches were scattered over the sphagnum moss. Within these two nests, embryo mortality was also higher than the seven year olds, but far less than in the previous year (Table 1). A total of 244 healthy embryos at hatching stage were released in Kosciuszko NP, while the remainder were retained at TZ.

2012

On 15 January, four to six males were added to each of eight breeding tanks. On 20 February, five or six female frogs were added to each tank. Three of the breeding

tanks housed eight year old frogs, four housed six year old frogs, and the eighth tank housed six year old males and four year old females. Calling activity was recorded from 18 January to 08 April, with one or two males calling daily from each tank for most of this period. As calling behavior was more consistent in 2012, call playback was not utilized.

On 04 April, a total of 698 eggs were removed from 13 nests in seven tanks in the main breeding facility (Table 1). An additional 25 eggs were laid in a tank of males and females of mixed age in a second facility not detailed above. Number of eggs in each nest varied from 10–90, indicating one to three clutches being laid in each nest. Unlike 2011, there was no difference in the number of eggs produced between the two older cohorts of females, aged two years apart. Overall, 78% of embryos from these cohorts survived until hatching. However, four year old females showed lower fecundity, with only two clutches produced and 62% embryo viability until hatching. In 2012, 447 eggs at hatching stage were released and a small number were retained at TZ.



Fig. 5. Captive nest containing eggs.

Table 1. Breeding results for *P. corroboree* at Taronga Zoo in the 2010, 2011, and 2012 breeding seasons. All weights were taken just prior to breeding in January or February.

| | 2010 | | 2011 | | 2012 | |
|---|------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| No. of adult frogs used (♂.♀) | 20.24 | 15.17 | 20.18 | 14.18 | 23.15 | 5.5 |
| Age (years) | 6 | 7 | 5 | 8 | 6 | ♀: 4 ♂: 6 |
| Ave. female mass (g) (range) | 2.9 (2.2–3.6) | 3.06 (2.56–3.81) | 2.85 (2.56–3.33) | 2.83 (2.24–3.36) | 2.93 (2.60–3.36) | 2.83 (2.64–2.97) |
| Ave. male mass (g) (range) | 1.8 (1.6–1.9) | 2.17 (1.90–2.38) | 1.94 (1.53–2.29) | 1.76 (1.19–2.19) | 1.91 (1.63–2.38) | 1.88 (1.76–2.08) |
| No. of nests | 6 | 4 | 2 | 6 | 6 | 1 |
| No. of eggs produced | 479 | 316 | 106 | 316 | 329 | 53 |
| No. of eggs / total females | 20.0 | 18.6 | 5.8 | 17.6 | 21.9 | 10.6 |
| % mortality of eggs | 72 | 17 | 34 | 26 | 19 | 38 |

Captive Breeding at Melbourne Zoo

2006 and 2007

Three to five adult frogs were maintained in a single breeding enclosure each year, with 42 and 46 eggs laid respectively (Table 2). Two tadpoles hatched within the enclosure's water area in the first year, with both subsequently metamorphosing within three months of hatching, but dying within 30 days. All of the eggs laid in 2007 were infertile.

2008

Ten additional four year old frogs were added to the breeding group but did not undergo a winter cooling prior to the breeding season as they arrived into the collection just prior. Upon completing quarantine protocols, these frogs were added to the group. Two males (from the new group of frogs) consistently called and attracted females. The original founder male died post-

winter leading up to this season, therefore the existing breeding group total was reduced from five to four (all were known to be female by this stage). A total of 32 eggs were produced in what was thought to be two clutches. Two changes were implemented this season to address previous inadequate temperature control. First, eggs were removed from nests as soon as they were found, as high nest temperatures may not allow gaseous exchange, potentially asphyxiating the eggs. Second, the temperature at which eggs were held after removal from nests was reduced by placing them above cold, oxygenated water at 12 °C. Nest temperatures were 22 °C.

Many eggs died due to inadequate temperature control and only seven hatched. They were placed into a tank with water at 12 °C and all metamorphosed after 60–90 days. Three of the tadpoles presented with curvature of their tails. All metamorphs died 7–34 days post-metamorphosis and exhibited abnormal front limb emergence and mouth development. Post mortem examination of two frogs found bacterial and protozoan infections.

2009

Nineteen adult frogs were used for this breeding season, with a known sex ratio of 3.5:1.1. Six males were recorded calling from within nests. All males had constructed nests sites in and around the edges of the pond within sphagnum moss. Between 11 March and 14 April, 187 eggs were laid in the breeding tanks. Most eggs were removed from the nests immediately after being found and placed into sphagnum moss-filled containers on the surface of cold water at 8 °C. One clutch of 33 eggs was left in one nest, but there was no significance difference in egg mortality between the two rearing methods.

During May, the eggs were ready to hatch and were placed onto a floating, perforated plastic tray in a rearing tank where the water temperature was 12 °C. Water temperature was reduced to 5 °C between July-August and then gradually increased to 12-15 °C from November-December, giving the tadpoles a development period of 6-9 months.

Many eggs became cloudy and died quite early in development (Table 2). Some eggs developed a brown algal-like growth on the outer jelly layer, while others stopped developing and died in the egg. The outer casing of other eggs appeared “soft” and some tadpoles were underdeveloped and fell out of the egg membrane. Only 16 tadpoles hatched from the 187 eggs (8.5%) and 12 frogs metamorphosed. Five frogs died not long after metamorphosis, but seven were successfully raised. The metamorphs that died exhibited signs of hip dysplasia and deformed limbs, but this was not confirmed. These metamorphs were almost double the size of those from the previous seasons.

2010

After the cooling period, 20 adults were divided between two breeding enclosures. Seven males were recorded calling within nests. Male calls were recorded and three call types identified, i.e., advertisement, territorial and courtship. To enhance breeding suitability and egg production, females were moved between the two breeding enclosures to increase mate selection options. The females were weighed before being moved to more closely monitor weight fluctuations and identify females that had laid.

Once eggs were located, they were put into a fridge at 12–15 °C. Total number of eggs produced was 235. Eggs were laid between 13 March and 25 April. Egg mortality was again high at 77.5% with only 51 tadpoles hatching. After an average larval duration of six months, feeding on natural pond/bog silt and frozen endive, 43 frogs metamorphosed between October 2010 and January 2011, with post-metamorphic survival rate to one year old at 67.4% (29 frogs).

2011

The male frogs were placed in the four breeding enclosures (based on wild localities) within the EAC in December, while the females were kept separately and offered food *ad lib* for a further 16 days to allow males to establish nest sites. The three animals of unknown sex were grouped in with the females for this season. Despite the extra space and correct temperatures, only four males were heard calling, in two enclosures. After a number of weeks with little to no calling, frogs were removed from the two smaller tanks and placed into larger tanks, regardless of locality. After the movements, the number of males calling increased to six.

In total 119 eggs were laid in three clutches (average 39.6 eggs/clutch). Egg mortality was still high at 70%. These eggs produced 36 tadpoles and subsequently 33 metamorphs (91.6% larval survival rate). The post-metamorphosis survivorship was 100% until one year of age.

2012

On 28 August 2011, all adult frogs, including those whose gender was unknown, were removed from two breeding tanks and placed in plastic tanks for the remainder of their overwintering period. The males were cooled until 4 December (98 days) at temperatures varying from 5–12 °C. They were then placed into the breeding enclosures, with five males in each enclosure. Females were maintained at the above temperatures until 18 December (112 days). They continued to be kept separately from the males until the latter had started to call and had constructed nest sites. Females were placed into breeding tanks on 26 February (70 days after finishing overwintering period). Male frogs were

not moved between enclosures due to nest establishment, but females were again moved to enhance mate choice options and compatibility, and likely breeding success. There were five or six female frogs in each enclosure at any time. Eggs were laid between 17 March and 17 April 2012, with a total of 556 eggs produced. These were likely to be from 17 clutches, with average female fecundity of 46.33 eggs (if laid by 12 known females) or 39.71, if the two frogs of unknown sex were also females that contributed to breeding. Three clutches of eggs were retained at MZ (total of 68 eggs) with a 28.4% egg mortality and 100% post-metamorphosis survival rate to the time of publishing, from 46 metamorphs produced. Larval hatching data were not collated this season as all eggs were hatched via assistance from keepers. All remaining 322 eggs produced this season were transferred to Kosciuszko NP for wild release.

Eggs

Once removed from the nest, eggs were packed in moist, live sphagnum moss in round plastic disposable food containers (12 × 10.5 cm high) with a lid on, air holes around the sides, and drainage holes in the base. The eggs were kept moist by lightly misting the moss with RO water every 10–14 days. Once the tadpoles reached about Stage 27 (Gosner 1960; Anstis 2002), the eggs were inundated in the tadpole rearing tank, allowing them to hatch and swim off. An alternative method used was to place the fully developed eggs on a floating, perforated plastic tray in the tadpole rearing tank, allowing the lower 1/3 of the egg to contact the surface of the water (Figure 9). This prevented eggs from desiccating, while allowing them to be easily inspected and the tadpole to hatch and swim away when fully developed. At TZ, the eggs began to hatch at five weeks if kept at 18 °C, but could take over six months if the eggs were kept between 5–10 °C. At MZ, between 2010 and 2012, eggs hatched between 74–95 days (10.5–13.5 weeks) at 13–23 °C. In the previous breeding seasons at MZ, eggs hatched quite early, at an earlier Gosner stage, resulting in high larval mortality.

Tadpoles

At TZ, the period of larval duration was usually four and a half to six months at 14–18 °C, including a seven to ten week period of over-winter cooling at 5 °C. Larval duration is as short as seven weeks at 18 °C, but the metamorphs emerged at a much smaller size. From 2007 to 2010, TZ had 372 frogs successfully metamorphose from 431 tadpoles (86% survival).

At MZ, larval duration varied from seven weeks to eight months. Prior to 2010, larval or early juvenile mortality was very high, with few surviving substantially past metamorphosis. Since 2010, with the implementation of a winter cooling during the larval period and the addition of a silt substrate, tadpole and metamorph survival increased significantly. The larval period now averages 213 days at temperatures varying seasonally from 5–23 °C throughout the six to nine month period.

Rearing Juveniles

At TZ, a subset of 17 frogs was weighed and measured at metamorphosis in 2009: length ranged from 11.3–13.8 mm (mean 12.5 mm) and weight from 0.20–0.38 g (mean 0.28 g). They were housed in identical conditions to the adult frogs, and readily accepted day old crickets. Post-metamorphic survival in captive *P. corroboree* is typically quite high with less than 5% mortality observed in their first year at TZ, from cohorts between 2007 and 2011.

At both zoos, male frogs can be heard calling at two years of age, though most males matured at three to four years. Earliest female breeding at TZ was from a single three year old frog from 19 individual females in this age group.



Fig. 6. Southern Corroboree Frog eggs.



Fig. 7. Metamorphosing Southern Corroboree Frog.



Fig. 8. Southern Corroboree Frog metamorphs.

Table 2. Breeding results for *P. corroboree* at Melbourne Zoo from 2006 to 2012. All weights were taken just prior to breeding in February or March.

| | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 |
|---|------|-------|-------|-------------------------|-------------------------|-------------------------|-------------------------|
| No. of adult frogs used (♂.♀.unknown) | 1.2 | 1.2.2 | 2.4.8 | 6.6.7 | 7.7.6 | 10.11.3 | 10.12.2 |
| Ave. female mass (g) (range) | — | — | — | 2.46 (1.85– 2.84) | 3.17 (2.79– 3.72) | 3.42 (2.74– 3.97) | 3.58 (2.92– 4.63) |
| No clutches laid | 1-2 | 3 | 2 | 11 | 12 | 3 | 17 |
| No. of nests | — | — | 2 | 6+ | 7 | 3 | 12 |
| Eggs laid | 42 | 46 | 32 | 187 | 235 | 119 | 556 |
| Average clutch size | 21 | 15.3 | 16 | 17 | 19.58 | 39.6 | 46.33 |
| % mortality of eggs | 95.3 | 100 | 78.2 | 91.5 | 77.5 | 69.8 | 27.1 |

Discussion

The *ex situ* conservation program for *P. corroboree* is an important Australian captive breeding program due to the iconic nature of the species and the critical status of wild populations. Refinement of husbandry techniques over the last seven years has led to improved breeding success and has allowed for the release of captive-bred eggs into the wild for experimental reintroductions. The likely reasons for our increased captive breeding success include provision of an adequate winter cooling period, the timing of introduction for breeding, placing multiple males in breeding tanks, and the correct age and body weight of frogs (especially females).

Reproductive Behavior

Pseudophryne corroboree is a sub-alpine species, with wild frogs brumating at temperatures below 5 °C under a layer of snow between June and August (Green and Osborne 2012). The frogs at both institutions were exposed to an overwintering period at 5 °C, though this

period was shorter and later than in the wild in order to allow the females to increase weight between breeding seasons. We assume that a winter cooling period is important for reproduction in this species, but we did not investigate the critical overwintering temperature or minimum time required to permit reproduction. In the wild, the mean daily maximum temperature in *P. corroboree* habitat is below 5 °C for three months of the year (Bureau of Meteorology 2012).

Providing females with mate-choice by establishing multiple males in each breeding tank may have also contributed to the increase in reproductive success. Within each breeding tank, not all males established nests or called and there was a marked difference between the success of individual males, suggesting that females were demonstrating mate choice. Both zoos have also had gravid females that did not lay eggs in their breeding tanks by the end of the breeding season, but laid eggs shortly after they were moved to another tank. This suggests that they may not have been satisfied with the males or nest sites within the original tank. Female mate choice is quite widespread among

anurans, with choice determined by a number of possible factors, including call frequency, male body size or male territory (Gerhardt and Huber 2002; Sullivan et al. 1995). Although mate choice is apparent in captive *P. corroboree*, it is not clear which characteristics females utilize to assess mate quality.

The separation of sexes outside the breeding season and the timing of their introduction to breeding tanks may be additional factors contributing to breeding success. The establishment of males in breeding tanks prior to the introduction of females allowed nest construction and commencement of calling activity before females were present, which would be consistent with the timing of these events in the wild. This also allowed the females to be fed more intensively in smaller terraria while their eggs were developing. Introducing the sexes once the eggs were developed, and the males were calling strongly, appeared to initiate almost immediate reproductive behavior in the captive *P. corroboree*.

Size and age at reproduction may have dictated the level of breeding success. Under wild conditions, age to first reproduction in males is typically four years, with a small proportion reaching sexual maturity at three years (Hunter 2000). It is suspected females may take four to five years. This species may live in the wild to at least nine years (Hunter 2000). Although frogs reached maturity in the zoos at a similar age, reproductive success was greatly reduced in younger frogs. At TZ, frogs at five years of age or below had limited breeding success, with significantly fewer males calling and females laying eggs. From six years of age onwards, breeding success greatly increased. Size was also important as females at TZ below 2.5 grams did not produce eggs, and successful spawning was higher in females over three grams. At MZ, females also began to mature at four years of age, with many requiring a further one to two years before reproducing (based on egg numbers and survival to hatching). Males at MZ appeared to attain maximum breeding success at seven years of age.

At MZ, it is possible that some females showed either egg-partitioning or double-clutching from the 2009 season onwards. The strongest indication of this was in 2012 when a maximum of 14 females were present (12 known females and two additional unsexed frogs) and eggs were laid in 17 whole, or partial, clutches. The

large number of eggs per female is also consistent with this possibility as there was an average 39.7 eggs per female if all 14 females laid eggs. Under natural conditions, a female typically lays 16–38 eggs (Pengilley 1973).

Although double-clutching is not likely in the wild, it could possibly occur in captivity due to the availability of resources. Double clutching has been recorded previously in a captive *Pseudophryne australis*, though this species breeds continuously throughout the year after rainfall (Thumm and Mahony 2002), rather than seasonally in *P. corroboree*. It is also possible that females demonstrated as polyandry, laying eggs in more than one nest. Sequential polyandry has been described in another frog from this genus, *P. bibroni*, with females partitioning their eggs between the nests of up to eight males (Byrne and Keogh 2009). In this scenario, the large average clutch size could be explained by the above average mass of females allowing for greater reproductive investment resulting in larger clutches (Wells 2007; Jorgensen 1992; Kaplan 1987). Breeding females at MZ were much larger than wild females, with those producing larger clutches weighing significantly more than wild frogs.

Egg/Embryo Mortality

High mortality of captive-laid eggs and embryos has been a significant problem in this program (>65 % mortality at MZ between 2006 and 2011; 72 % at TZ in 2010). The high egg mortality seems to have been mostly resolved over the last two years, though the reasons for this are not fully understood. In the wild, excluding during drought, early embryo mortality is quite low at less than 15% (Pengilley 1992; Hunter et al. 1999). Moisture and pH characteristics of nests in captivity closely resembled those in the wild, and although nest temperatures in captivity at MZ often exceeded those in the wild, this was not the case at TZ in 2010. The fact that the same TZ breeding tank assemblages in which there was high egg/embryo mortality in 2010 (72%) experienced only 17% mortality in the following season suggests that nest substrate was not the cause of earlier mortality. Temperature may have influenced embryo mortality at MZ prior to 2012, as

nest temperatures were frequently higher than those experienced in the wild. Maintaining eggs at temperatures higher than the optimum range has been demonstrated to cause embryo mortality in anurans (Goncharov et al. 1989), including other species of *Pseudophryne* (Seymour et al. 1991).

Other possibilities considered were the husbandry of embryos once removed from the nest and inadequate nutrition of females which might result in eggs with smaller yolk supplies, or other causes of inviability. It is noteworthy that during 2008 and 2009, approximately 2,600 wild-laid embryos at various stages of development were collected and reared at TZ for three months before return to the wild. Under conditions identical to those used for captive-laid embryos, mortality was only 11%, suggesting that husbandry of the eggs post-removal from the nest was not a contributing factor. Small trials were carried out at TZ in 2011 to test for the effect of diet and supplementation on embryo mortality. Due to the subsequent low egg mortality across all treatments, the results were inconclusive, and thus the factors responsible for the high egg/embryo mortality in the early years of the program remain unclear.

Larval Mortality

Tadpoles produced by the breeding program at MZ between 2006 and 2009 showed reduced vigour, high mortality, and produced smaller frogs at metamorphosis. Two factors may have contributed to this outcome. The first is that high water temperatures caused the larval period to be reduced to two to three months and there was no simulated overwinter cooling period. Current practice with inclusion of an overwintering interval has increased the larval life-span to six to nine months at MZ, or five to six months at TZ, approximating the wild larval duration. It seems likely that a larval duration of at least 140 days may be important for development of robust larvae and metamorph frogs, and high rates of metamorphosis.

The other significant factor was probably larval nutrition. From the 2010 season onwards, heat-treated silt from a Kosciuszko NP breeding site was added to the rearing tanks, and there was an immediate increase in larval viability from that year. The likely importance of both factors are supported by results at TZ from 2007 to 2011, where tadpoles have always undergone an over-

winter cooling period and have had access to natural silt, as well as endive and fish flake. This resulted in 86% survival of larvae to metamorphosis at TZ during this period and high survivorship of metamorphs.

Conclusion

In view of its continued decline toward extinction, the survival of *P. corroboree* depends on the success of *ex situ* conservation measures. The development of successful captive-breeding protocols for this species has allowed the *ex situ* program to begin to offer *in situ* support, with the return of 738 (TZ) and 322 (MZ) captive-bred embryos to the wild between 2010 and 2012 (Hunter et al. 2010). Since the bulk of the captive population is now made up of immature frogs, the rate of production of embryos can be expected to rise over the next few years, ensuring the continued viability of the captive breeding population and greater capacity to undertake reintroductions back to the wild.

The more general lesson to be drawn from this program is that the development of reliable captive-breeding programs for species whose life history is unusual and/or not well known may invariably be both slow and highly demanding of skills and resources. It needs to be recognized that appropriate husbandry skills and breeding protocols should be in place before wild populations are reduced to critically low levels. The Sharp-snouted Day Frog (*Taudactylus acutirostris*) is a prime example of this: the delayed approval from the state government agency to establish a captive colony prior to population crashes and the combination of chytrid fungus infection (not recognized before 1998) and lack of experience in the appropriate husbandry of this genus led to the failure of a last-minute attempt to establish a captive population in 1993, and the species is now presumed extinct (Banks and McCracken 2002; Schloegel et al. 2005). Gagliardo et al. (2008) and Mendelson (2011) provide discussions of comparable instances of rescue operations for Critically Endangered amphibians in Central America. Thus, the development of husbandry protocols, for taxa with unusual biology or species in early decline, should be a conservation priority for *ex situ* institutions.

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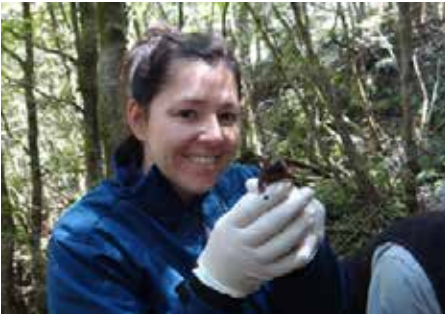
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