

A review of the Green and Golden Bell Frog *Litoria aurea* breeding program at Taronga Zoo

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ABSTRACT

The Green and Golden Bell Frog *Litoria aurea* is a threatened species, having declined greatly in abundance throughout its range in recent decades. In 1994, Taronga Zoo, Sydney, established a captive population of bell frogs, after obtaining a small number of frogs from a suburban site planned for development. Our aim was to maintain and breed bell frogs in captivity and to provide progeny for release at suitable sites in order to establish new and self-sustaining populations. The program later expanded to include bell frogs from two other locations in New South Wales.

Successful breeding has been regularly achieved and fourth generation captive-bred individuals have been released. Approximately 20,000 tadpoles and metamorph frogs were provided for release at five sites between 1996 and 2004, although no new populations of bell frogs were established. Education has been another key component of this program at Taronga Zoo, with school and community groups actively involved in releases, monitoring and habitat restoration. One outcome of this component was the creation of an interactive CD-ROM, to provide an educational resource on frog conservation, that was distributed to schools and various community groups Australia-wide.

Key words: *Litoria aurea*, Green and Golden Bell Frog, zoo, reintroduction, captive breeding, conservation education

Introduction

A major focus of modern zoos is to promote and contribute to the conservation of threatened species. Zoos are often well-positioned to participate in *ex-situ* conservation programs by educating the general public on conservation issues, exhibiting animals where they will be seen by large numbers of people and having the facilities and expertise to maintain and breed a diversity of animals. Frogs provide an almost ideal candidate for *ex-situ* conservation programs due to their small size, minimal resource needs and the high numbers of progeny that can be produced by some species. Additionally, they are a high priority, with many amphibian species dramatically declining or disappearing in the past three decades (Berger *et al.* 1998; Stuart *et al.* 2004). The threatened Green and Golden Bell Frog *Litoria aurea* is a species that has received a great deal of public and scientific attention in recent years (Pyke and White 2001; DEC 2005). Captive bell frogs are now held for insurance or educational purposes at a number of institutions, including Taronga Zoo, the Australian Reptile Park, Newcastle University and also by a small number of private keepers.

Since 1994, Taronga Zoo has had a considerable involvement in the conservation of the Green and Golden Bell Frog (hereafter bell frog) by establishing captive breeding programs. In this paper, we provide an overview of the many activities that have taken place at the zoo involving this species. In particular, we focus on summarising information on the husbandry, reproduction and life history of the species in captivity, the provision of animals for release and research purposes, and the setting

up of educational programs to benefit the conservation of the species. We have not detailed the fieldwork involved with release attempts, because with the exception of one site at Botany, Taronga Zoo's involvement in releases has been restricted to simply providing tadpoles to the appropriate researchers conducting the releases. For two of these reintroduction attempts, this information has been published elsewhere (White 2006; Pyke *et al.* 2008)

Source Populations

The bell frog breeding program was established at Taronga Zoo in 1994 after a disused urban site, marked for development, in the Sydney suburb of Rosebery was found to contain a small population of bell frogs. The New South Wales (NSW) National Parks and Wildlife Service authorised the collection of frogs from this population following investigations and recommendations by environmental consultants (Cogger 1993; Fanning and White 1994). In October 1994, 10 adult frogs were collected from this site and taken to Taronga Zoo to establish a captive breeding colony. The aims of this program were to successfully maintain and breed this species in captivity and produce captive-bred progeny that could be used for releases into the wild. Individuals from the Rosebery provenance have gone on to be the main focus of Taronga Zoo's bell frog breeding and release program over the last 10 years, with releases occurring in the Sydney suburbs of Botany, Long Reef (Pyke *et al.* 2008) and Marrickville (White 2006).

In 1998, the program expanded when a population of bell frogs at Arncliffe was identified as requiring captive breeding as a condition of consent for a development proposal. An assessment found that the construction of the M5 East Motorway might impact on a population of bell frogs that inhabited a degraded freshwater wetland at the site. As a result, a Plan of Management (White 1998) was prepared that identified the need to create new breeding pools near the inhabited wetland and establish a captive breeding colony as an insurance population. In February 1998, six bell frogs were collected at Arncliffe and taken to Taronga Zoo.

In 2002, a third population of bell frogs was established at the zoo as a consequence of a development proposal in the suburb of Woonona, near Wollongong, on the south coast of New South Wales. In a similar situation to the Arncliffe population, the developer was required to create new breeding pools and have a captive breeding population established before consent could be granted. Populations from these three provenances currently exist at Taronga Zoo and progeny from each of these populations have been released.

Husbandry

A number of different enclosures and techniques have been used in the 10 years of maintaining and breeding bell frogs at Taronga Zoo. Individuals have been housed successfully in both indoor and outdoor enclosures throughout the year. For over three years in the initial stages of the program, bell frogs were maintained and bred in an 8 m by 8 m outdoor wire aviary with a substrate of river sand and grass, containing four ephemeral and four deep permanent ponds (Hobcroft 1997a). As Sydney is within the distribution of this species, outdoor housing allows the animals to experience natural temperature and photoperiod cycles. However, it does reduce the ability to closely monitor individuals and may expose them to quarantine risks. With the threat of diseases, such as chytrid fungus, which has been detected in wild Striped Marsh Frogs (*Limnodynastes peroni*) at Taronga Zoo, all bell frogs are now only housed indoors.

Bell frogs at the zoo are currently housed in a variety of display and holding enclosures. One method that has worked conveniently at the zoo for holding large numbers of bell frogs is glass aquaria (measuring 47 cm x 50 cm x 20 cm) with a waterproofed wooden hood (measuring 47 cm x 50 cm x 40 cm). The aquaria are connected in rows of three, with removable wooden dividers separating each aquarium (Fig. 1). A door is situated at the front of the hood above each aquarium to allow keeper access. Fine-gauge steel mesh panels in both the door and roof of the hood provide ventilation. A drain is located in the base of each aquarium to facilitate cleaning.

Adult bell frogs are kept in these aquaria in single-sex groups of two to three animals. Within each enclosure, frogs have access to both water and land. Artificial plants and sections of PVC pipes cut in half longitudinally are provided for shelter. Artificial furnishings are preferred in order to prevent accidental introduction of pathogens



Figure 1. Enclosures typical of that used to house bell frogs at Taronga Zoo. Source: Taronga Zoo.

and to allow for easy sterilisation. All adult bell frogs at the zoo are fed three times a week, whilst metamorphs and juveniles are fed daily. The diet consists mostly of crickets, but occasionally cockroaches and houseflies. Invertebrates are dusted with Rep-cal calcium powder or Herptivite multivitamin powder on every other feed. Between feeding days, the enclosures are drained and rinsed to remove waste.

Most recently, tadpoles have been raised in large 250 litre white plastic tubs. They are usually kept at a density of one spawn, or up to one thousands tadpoles, per tub. However, spawn may be divided between two tubs when there are larger numbers of tadpoles (i.e. greater than 1000 tadpoles). The water is filtered with biological and mechanical filtration, in which the water passes through a series of layers, including filter wool, bioballs and biological filter matting. A 10% water change, using aged water, is performed daily to prevent a build up of ammonia and other waste material. All tap water has been aged prior to use by allowing it to settle in a storage container for a number of days so that chlorine can be removed. Tadpoles are fed daily on a diet consisting mostly of frozen endive and lettuce, supplemented with commercial algal fish flakes and frozen bloodworms.

Reproduction

When attempting to breed bell frogs at the zoo, an adult pair from separate known lineages within a population is selected and set up either in their holding aquarium (described above) or a 68 litre white plastic tub (35 cm x 55 cm x 40 cm). Identifying unrelated adults is possible as each adult bell frog is implanted with a Passive Integrated Transponder (PIT). Aged water is filled to a depth of 10-15 cm to allow the frogs to fully submerge. Floating polystyrene blocks and submerged artificial plants are present to ensure the frogs can rest out of the water. To stimulate breeding when pairs have been introduced, a recording of calling male bell frogs is played on a 3-minute continuous tape from approximately 1800 h at night to 0600 h the following morning. Utilising this tape often spontaneously initiates calling behaviour in

captive males (McFadden pers. obs.). Calling behaviour has also been observed to increase largely before the onset of rain, despite being kept indoors at a relatively constant temperature (McFadden pers. obs.). Males have been observed calling at any time of the day and night and show little desire to feed at this time. This seems not to occur in females, because some individuals will continue to feed, even whilst in amplexus.

Amplexus has been recorded both during the day and night, and typically has not continued for more than 24 hours. However, Hobcroft (1997b) observed amplexus to continue for five days in one pair at Taronga Zoo. Although amplexus is a precursor to spawning, successful spawns do not always result from amplexus with many pairs separating early (McFadden pers. obs.). Only 67 of 135 (50%) pairings during three consecutive breeding seasons (2000/1, 2001/2 and 2002/3) produced spawn. Of these, 16 pairs (12%) produced significant quantities of spawn that were fertile or viable, with many producing infertile or very small amounts of spawn. Spawning has mostly occurred at Taronga Zoo between the months of September and February, although successful spawns have been recorded as late as May. This is largely consistent with wild bell frogs, which have been recorded spawning mostly between October and February (Pyke and White 2001). Spawning typically occurs during the night in the absence of staff, between 1700 h and 0600 h, although it has been recorded as late as 0730 h on occasions.

Although spawn masses as large as 11682 eggs have been recorded for this species (van de Mortel and Goldingay 1998), the average wild spawn size is significantly less than 3773 eggs (Pyke and White 2001). From seven spawns counted at Taronga Zoo during 2004 and 2005, we found the average spawn from captive animals to be very similar with 3885 eggs (range 2241-5840). This is much less than the average of 6047 eggs previously documented using captive individuals from Kurnell and Taronga Zoo (White and Pyke 2002). Fertility rates in these seven spawns also varied widely, ranging from 10-88% with an average of 58%. This is within the range of the 46-77% hatching rate previously found for this species (Pyke and White 2001), but significantly less than the 96% fertility rate documented in one population of wild bell frogs (White and Pyke 2002). Spawning by individually housed females has been noted on at least three occasions, whilst calling males were present in other enclosures within the room. This indicates that amplexus is not necessary for a female to release spawn.

Life History

In over a decade of keeping bell frogs at Taronga Zoo, up to fourth generation offspring have been produced and provided for release into the field. To maintain the number of individuals and the genetic diversity within the collection, up to six frogs have often been retained from selected clutches. Frogs retained are usually selected from those quickest to reach metamorphosis. A total of 101 captive bred bell frogs have been raised to maturity at Taronga Zoo (52 males and 49 females).

Under natural conditions, male bell frogs are able to breed as early as 6-9 months of age, whilst females do not become sexually mature until approximately 18 months (Pyke and White 2001). A similar age to sexual maturity has been found in the captive population at Taronga Zoo. The earliest that a male has sired fertile spawn is at 8 months of age. Although the earliest that females typically produce young is approximately one year later at 20 months of age; one female produced a viable spawn of approximately 2000 eggs at 10.5 months of age. Reproduction at such an early age in captivity is probably due to a reliable source of food and warmer winter temperatures. Young male bell frogs at Taronga Zoo have been observed in amplexus at 5 months and 2 weeks of age. The oldest observed breeding age has been 7 years and 11 months for a male and 8 years for a female.

The average longevity of bell frogs at Taronga Zoo is 6.2 years, with a small number of individuals reaching 10 years. The average longevity in healthy captive frogs may be higher, but a mycobacterial infection afflicted the main Rosebery breeding population causing earlier mortality in a number of frogs, thus reducing the average longevity.

Releases

One of the major aims of the Green and Golden Bell Frog breeding program at Taronga Zoo has been to provide captive-bred progeny for release into suitable sites in order to establish self-sustaining populations or supplement existing populations if required. In over nine years of breeding, approximately 20 000 tadpoles and recently metamorphosed frogs have been released at five locations (Table 1). This has included the release of up to fourth-generation captive bred animals. Sites at Botany, Long Reef and Marrickville received animals of Rosebery provenance, whilst releases at Arncliffe and Woonona involved progeny from their respective locations.

Prior to each release, a screening of tadpoles for diseases takes place on two occasions, spaced at least two weeks apart. On each occasion, 15 tadpoles from each batch are selected to be euthanased and autopsied. This involved sectioning of the animals to screen for chytrid fungus or bacterial and viral infections. This process is necessary to ensure that the release of diseased or parasitised tadpoles does not take place.

The sites at Sir Joseph Banks Reserve, Botany, and Long Reef Golf Course both involved attempts to reintroduce populations of bell frogs (Pyke *et al.* 2008). Both sites also required a large amount of restoration work to provide suitable habitat. At Botany, large ponds were created (up to 15 m²), suitable vegetation was planted both within and surrounding the ponds, rocks and wooden boards were provided for shelter and a frog-proof fence was built to surround the new habitat. Similar levels of involvement were also needed to prepare the Long Reef site (Pyke *et al.* 2008).

Table 1. Total releases of captive-bred *Litoria aurea* progeny from Taronga Zoo at five release locations, spanning nine breeding seasons. All releases are of tadpoles unless otherwise indicated.

Year	Botany	Long Reef	Marrickville	Arncliffe	Woonona
95/96	2055	-	-	-	-
96/97	1000	-	-	-	-
97/98	-	1468	22	-	-
98/99	691	628	100	42	-
99/00	993	1543 ¹	-	996	-
00/01	350	2000	-	30	-
01/02	743	1424	-	17	-
02/03	-	62	-	-	-
03/04	-	3753 ²	-	504	1557
Total	5832	10878	122	1589	1557

¹ Release of 5 adult frogs included in the total.

² Release of 352 metamorphs included in the total.

Although the Botany and Long Reef sites experienced large releases of progeny over a 7-year period, neither site has been successful in establishing a self-sustaining population of bell frogs. The precise reasons for these failures are unknown but a number of factors are suspected. At Botany, human disturbance, the introduction of the Plague Minnow *Gambusia holbrooki* into a release pool and predation by waterbirds have been suggested (Hobcroft 1997a). During surveys, small numbers of metamorphosed frogs were found in late Summer and early Autumn but none were located after Winter. Disturbance by humans and predators was suspected to be a primary reason for these animals not to survive throughout winter (Hobcroft 1997a). However, it is possible that the amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, may have been at least partly responsible for these disappearances over winter. Chytridiomycosis, caused by *B. dendrobatidis*, is thought to be responsible for the decline and disappearance of a number of Australian frog species (Berger et al. 1998, 1999). This pathogen is more virulent at cooler temperatures, resulting in greater die-offs during winter (Berger et al. 2004). However, this pathogen was not described until after early releases at the Botany site. As a result, frogs at this site were not tested for chytridiomycosis so its impact on this population cannot be confirmed.

Sites at Arncliffe and Woonona differed from the Botany and Long Reef locations in that remnant populations of bell frogs were still present. At these locations, new habitat was created to offset the risks of habitat loss resulting from the development. It was anticipated that frogs at these sites would naturally migrate to and reproduce in these created wetlands. Hence, the primary purpose of holding these frogs in captivity was to act as an insurance population should natural colonisation fail. This explains the lower number of progeny released at these two sites (Table 1).

Research

Because large numbers of bell frog progeny can be produced in captivity, it provides an ideal source of specimens for research purposes. By using captive-bred offspring for various studies reduces any interference

with wild populations by minimising the collection of animals or spawn. Progeny of bell frogs produced at Taronga Zoo have been used for a wide range of research purposes, including published studies determining the impact of introduced Plague Minnow on tadpoles (Morgan and Buttemer 1996), the impact of water parameters on tadpoles (Penman 1998), genetic studies (Burns 2004) and the fecundity of captive animals (White and Pyke 2002).

Education

With the release of tadpoles at the Sir Joseph Banks Reserve site in Botany, Taronga Zoo staff developed a program to educate the local community on the importance of frog conservation, sustainability and biodiversity, in addition to collecting data and improving habitat. This program was established in 1998 as Frog Focus Botany and was the first time in Australia that school students were involved with the release and management of an endangered frog species. Frog Focus Botany was a partnership between the NSW Department of Education and Training and the Zoological Parks Board of NSW. It was also supported financially by Parks & Leisure Australia through its NSW Region. The program was delivered by Taronga Zoo Herpetofauna and Education staff, Botany Bay City Council and involved students from two local schools (JJ Cahill High School and Botany Public School) and a Botany Community Group.

The program was in operation for five years. A variety of activities were completed including regular visits to Taronga Zoo and the frog breeding unit, and weekly visits for the students and community members to the Botany release site. At the release site the students and community members participated in a variety of activities including the sighting, capturing, weighing and measuring of frogs (Fig. 2) and tadpoles (Fig. 3), site cleaning, tree planting and watering, and construction of a frog-proof fence. Over the five years of the program, the students and community group helped with the release of hundreds of bell frog tadpoles into the prepared site.



Figure 2. School children participating in the monitoring of bell frogs at Botany. Source: Taronga Zoo.



Figure 3. School children conducting a survey for tadpoles at the bell frog release site in Botany. Source: Taronga Zoo.

A total of 10 'Frog Focus Botany' community information sessions was held over the course of the program and a dedicated community group was established. The community group consisted of interested parents of the school students, residents who lived near the site and members of Botany Rotary Club and Maroubra Lions Club. A total of 90 active community members were involved and received regular community flyers updating them on the progress of the program. In 1999, the school students created a Frog Focus Botany page for their school's website where students continually wrote and updated information on the progress of the frogs for viewing on the Internet.

To support the vision of Frog Focus Botany and the development of community frog conservation programs, the Australian Stock Exchange financially sponsored the next phase of this project - 'ASX Frog Focus'. In November 2000, the ASX Frog Focus website was launched and went on to win three major awards, including 'The Australian Awards for Excellence in Educational Publishing'. The national launch of an interactive CD-ROM occurred in September 2001. The CD-ROM met all environmental education

school curriculum components and focused on themes ranging from frog conservation to habitat restoration. The resource provided information on environmentally friendly practices from constructing a frog pond, to how to get involved in recovery programs for frog species.

Around 12000 copies of the ASX Frog Focus CD-ROM were delivered free to every school in Australia giving teachers, students and their communities a resource that aimed to motivate and engage them in the study and conservation of Australian frogs. Over 5000 copies were also given free to councils, community groups, environmental educators, scientists, frog interest groups, home schoolers and international educators. In 2000, ASX Frog Focus also became the 'education' component of the National Zoo Amphibian Project (NZAP), which was coordinated and delivered through the Australasian Regional Association of Zoological Parks and Aquaria (ARAZPA).

Frog Focus Botany and ASX Frog Focus were successful in providing conservation education programs even though the release of the Green and Golden Bell Frogs failed to lead to an established population. At the end of the programs, the Botany community and students from all over Australia had a resource to develop a greater understanding of frogs, their local environment and the importance of protecting them. Both Frog Focus programs demonstrated that the key to conservation is an increase in knowledge, positive changes in our behaviour and local community support.

Conclusions

When the Green and Golden Bell Frog program was first established at Taronga Zoo, the early aims included successfully maintaining this species in captivity, breeding from second-generation animals and providing animals for release in reintroduction trials. These aims were all achieved in the early parts of the project. The breeding program went on to involve the breeding and release of fourth-generation animals in addition to providing offspring for a range of research projects. Through this work, we were able to establish captive breeding protocols that would enable the successful maintenance and reproduction of this relatively hardy species in captivity. The educational programs that stemmed from this project were also a great achievement, incorporating *in-situ* conservation work with the local community.

Although Taronga Zoo has been actively involved in the captive component of the recovery of this species since 1994, no releases of tadpoles have taken place since March 2004. The zoo continues to maintain bell frogs from the Arncliffe and Woonona sites as insurance populations, but there is no requirement to release animals at either of these sites. The captive Rosebery population of frogs at Taronga Zoo, contracted a mycobacterial infection, resulting in the decline of this population. As this disease cannot be treated, the final 10 individuals carrying heavy mycobacterial loads were euthanased in May 2005. The only progeny remaining from this population exist in an educational section of the zoo.

The attempted reintroductions using zoo-bred progeny have failed for reasons that are not known, but may possibly include the presence of chytrid fungus or the lack of suitable habitat. Despite this, we believe that this species would make a good candidate for future releases due to the continuing decline of the species and the ability to produce large quantities of offspring in captivity. Although the zoo is not currently involved in actively producing animals for release, it is still possible for future involvement should a suitable release location be

established with sufficient external resources to monitor reintroduction attempts. Future releases may benefit from the screening of frogs at release sites to determine the status of chytrid fungus in these areas and assess the impact that this has on reintroduction attempts. Additionally, should resources be available, consideration should be given to releasing bell frogs at various life stages, including eggs, tadpoles, metamorphosed frogs and 1-year olds within an experimental framework to determine the most suitable life stage at which to release this species.

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