Reintroduction of the Green and Golden Bell Frog *Litoria aurea* to Pambula on the south coast of New South Wales

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ABSTRACT

Captive bred Green and Golden Bell Frog *Litoria aurea* tadpoles were introduced to a coastal wetland near Pambula on the far south coast of New South Wales. The reintroduction involved the release of approximately 5000 captive-bred tadpoles and subsequent monitoring. Before the reintroduction could take place several requirements had to be satisfied. A re-introduction proposal was prepared for the Department of Environment and Conservation (DEC) and independently reviewed by two referees, pre-release surveys of frogs were undertaken to determine if *L. aurea* existed on the site and to ascertain if the pathogen *Bactrachochytrium dendrobatiodis* (chytrid) was present within the endemic frog population. The captive bred tadpoles were tested for the presence of chytrid prior to release. The water quality was tested at the proposed release site and potential predators were removed. The project required maintenance and breeding of the captive population, and post release surveys. The project took 4.5 years from submission of the proposal to the first release of tadpoles and to date has cost approximately $190K, of which only $37K was funded. It is anticipated that ongoing costs will be in the order of $25K per year for the next four years (2007-11). Details of the project costs and chronology of actions are given in order to assist others who wish to undertake similar projects. So far two adults have been detected 13 months post-release.

Key words: *Litoria aurea,* Green and Golden Bell Frog, Pambula, re-introduction protocols

Introduction

The Green and Golden Bell Frog *Litoria aurea* now occurs in scattered populations from East Gippsland in Victoria to Yuraygir National Park in northern New South Wales (Gillespie 1996; White and Pyke 1996; Goldingay and Newell 2005), primarily within one kilometre of the coast. This species inhabits a wide range of habitats such as natural coastal wetlands, ponds within coastal dune swale systems and quarries and dams (Pyke *et al.* 2002). *Litoria aurea* has undergone a marked decline in distribution and abundance since the 1980's and in the last decade only a few specimens have been detected in an area from Batemans Bay to Nadgee Nature Reserve, on the far south coast of New South Wales (White and Pyke 1996; Daly and Senior 2003). The species had previously been recorded at many sites within this stretch of coast and many of these historic sites have not been physically modified by humans (Daly and Senior 2003) but indirectly impacted by the introduction of an exotic fish. The Plague Minnow *Gambusia holbrooki*, a known predator of *L. aurea* eggs and tadpoles (Morgan and Buttemer 1996), currently exists in 16 areas on the far south coast of New South Wales (Daly and Senior 2003).

Litoria aurea was common in wetlands around Pambula during the early 1980's (P. Johnson, pers. obs.) and there are specimens from this location held in the Australian Museum collection (Daly and Senior 2003). However, the population crashed during the 1980's (P. Johnson, pers. obs.) and no specimens have been detected in the area since then.

There have been several attempts to reintroduce *L. aurea* into the wild from captive bred populations. The reintroductions have occurred at Long Reef Golf Course, Joseph Banks Reserve, Marrickville, Arncliffe (M5 Motorway) and Woonona (White 2007; Pyke *et al.* 2008). Most of the reintroductions have involved the release of tadpoles (some metamorphlings were released at Long Reef), but as yet no self-sustaining populations have been established (R. Wellington pers. comm.; White 2007; Pyke *et al.* 2008).

A component of the broader conservation strategy for *L. aurea* is reintroduction (DEC 2005). Trials need to be conducted at a range of sites to better understand the response of *L. aurea* to translocation. This study describes an attempt to reintroduce this species to a site near Pambula, on the New South Wales (NSW) far south coast. A re-introduction proposal was prepared for the

Department of Environment and Conservation (DEC; now known as Department of Environment and Climate Change) and independently reviewed by two referees. Adult *L. aurea* were captured from the Eden area and taken into captivity in 1988-1990. The progeny from this initial collection were released at the site near Pambula. The aim of our re-introduction was to establish a self-sustaining colony of the frogs at this site and to document the requirements to achieve this action.

Methods

Release site

Pambula (36^{O} 53' S, 149^{O} 55' E, 10 m AHD) is situated on the far south coast of NSW (Fig. 1). The town lies on a coastal plain and the substrate is mostly sandy alluvial soil. Extensive wetlands occur around Pambula and are part of the flood plain. The proposed release site was a pond within a wetland system, which runs roughly for 2 km in a north-south direction behind the dunal swales of Merimbula Beach. The tenure at the site is crown land, however, the majority of the wetland system is within Ben Boyd National Park and within land managed by Bega Valley Shire as part of their sewerage treatment system. Both agencies have been actively involved and fully endorsed the proposal to re-introduce *L. aurea* at the site.

The release pond was permanent but varied considerably in depth. During drought it has had a surface are of approximately 1000 m^2 and depth of 1.5 m but during flood the pond has covered many thousand square metres and expanded into the surrounding shallow sedgelands, which surround the pond. The pond may have been constructed in association with the nearby sewerage treatment plant, as the distal end of a pipe is exposed during drought.

Approximately 100 m from the site were two sewage exfiltration ponds (Fig. 1). These ponds were also considered useful because they were ephemeral and *L. aurea* has previously been known to use similar environments as breeding sites (Pyke and White 1996; Gaia Research 2002). In 2005 DEC issued a licence variation for the temporary discharge of treated effluent to the exfiltration ponds between the first week of the Christmas school holidays up until the last day of the Easter School holiday period, valid for 3 years up until

Figure 1. Aerial photo of the release site at Pambula, on the far south of NSW.

the end of Easter 2007 (K. McLeod, Bega Valley Council, pers. comm.). The water level of the exfiltration ponds could be artificially augmented to provide additional potential breeding sites for *L. aurea*.

The vegetation at the release site included tall sedges and rushes *Lepidosperma longitudinal*, *Juncus* spp. and Jointed Twigrush *Baumea articulata* (Figs. 2, 3). Umbrella Sedge *Cypress* sp. formed concentric beds around the sewage treatment overflow ponds. *Kunzea ericifolia* was common in the shrublayer and clumps of Sawsedge *Gahnia sieberiana* occurred in some areas around the deeper ponds in the National Park. Coastal woodland occurred within the immediate catchment of the pond and included primarily Forest Red Gum *Eucalyptus tereticornis* with small occurrences of Woollybutt *E. longifolia,* and on the better drained soils Blackbutt *E. pilularis,* Red Bloodwood *Corymbia gummifera* and Old Man Banksia *Banksia serrata*. The Pambula sewerage treatment plant (STP) lay approximately 1 km west of the proposed release site and supported areas of emergent aquatic plants (e.g. Cumbungi *Typha* spp.).

Potential threatening processes, which may act on the released animals, included predation by the Red Fox *Vulpes vulpes*. Potential native predators of *L. aurea* include the Red-bellied Black Snake *Pseudechis porphyriacus*, White-faced Heron *Ardea novaehollandiae*, Swamp Harrier *Circus approximans* and Long-necked Turtle *Chelodina longicollis.* The Princes Highway occurred 300 m west of the site and mortality of individuals may occur due to collisions with moving vehicles (see Daly 1996). The Plague Minnow *Gambusia holbrooki* did not occur at this site but eels *Anguilla* sp. were present.

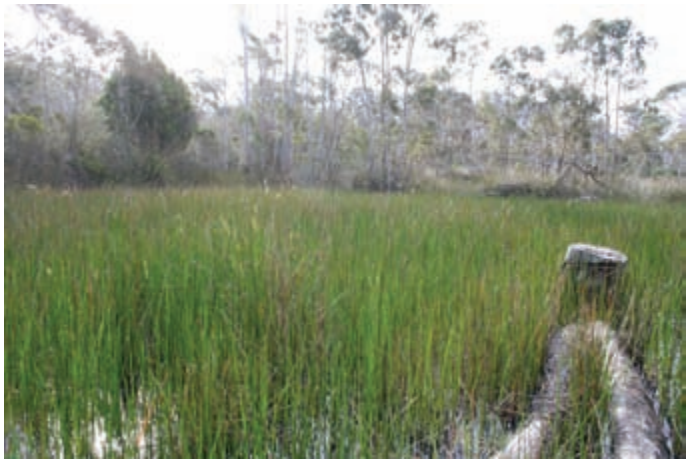

Figure 2. Release site at Pambula showing shallow portion of the pond with emergent aquatic vegetation.

Figure 3. Release site showing deep water and fringing aquatic vegetation.

Captive population

The green and golden bell frog used for the program originated from wild animals captured on the Princes Highway, south of Eden. The original captive population consisted of 45 animals (15 male and 30 females), which were collected between 1988-90.

The frogs had been held under an s120 Class 1 Keepers Licence (AK51367). This population had varied in size since 1990 due to variations in housing facilities. The adult population had varied over the last five years from 20-34 frogs (10-20 of which are female).

Captive enclosures and maintenance

The captive *L. aurea* were housed in a 4.0 x 1.5 x 2.5 m tank indoors and maintained at Coffs Harbour, New South Wales. The environment was held at temperatures with a range of 24-28ºC and the tanks received natural sunlight. Three tanks each held 4-6 frogs and were landscaped with rocks and plants. A portion of each tank had free water, which was filtered through three sections of both charcoal/sand or filter wool and bacterial balls. Frogs were fed commercially produced crickets and cockroaches.

Tanks were cleaned, disinfected with benzalkonium chloride and sun dried before use. Benzalkonium chloride is known to kill the amphibian chytrid fungus *Bactrachochytrium dendrobatiodis* (Wellington and Haering 2001). The frogs had been sprayed with a dilute (1 part per 100 volume) solution of benzalkonium chloride once per week since June 1999.

Captive breeding and transportation of tadpoles

Males initially call in September and when this occurred were removed and held in separate tanks each with a single female. The tanks were 1.0 x 0.75 x 0.5 m and filled with water to 0.1-0.2 m. Disinfected Cumbungi *Typha* sp. was provided for adults to sit above the water and Pondweed *Elodea canadensis* for the frogs to spawn on. If females double clutched within a season then different males were used as sires.

Once the adults had spawned they were removed and placed back into a communal tank and the spawning tank filled with water. A maximum of eight tanks were used for spawning. Once tadpoles were about one month old (Gosner stage 24-25) they were graded into various size groups. A total of twelve 1.0 x 0.75 x 0.5 m tanks were used to raise tadpoles. Tadpoles were fed boiled lettuce and Trout food pellets.

When tadpoles metamorphosed they were initially held in community tanks and within four weeks were graded into three size groups (2.0, 3.0 and 4.0 cm snout-vent length). Each group was maintained in a separate tank to reduce the

risk of cannibalism. Metamorphlings were fed flies. These tanks were exposed to sunlight but covered with shade cloth and landscaped with rocks and aquatic plants.

Tadpoles were transported from Coffs Harbour to Pambula within 12 hours in sealed 25 litre plastic drums. Approximately 3500 *L. aurea* tadpoles were released at Pambula in December 2005 and a further 1500 in early February 2006 and 1000 in April 2007 (Fig. 4).

Figure 4. Peter Johnson (at right) and George Malolakis (NPWS) finally release bell frog tadpoles into the wetland at Pambula after years of husbandry, testing for chytrid and other approvals.

Pathology

Bell frog tadpoles were sent to Taronga Zoo in December 2003 and 2004 for pathological screening for chytrid fungus, once they had a minimum body size of 10 mm. Thirty six tadpoles per batch (tank) were sent for screening. This number was based on sampling approximately 5% of the total spawn complement. The tadpoles utilised for pathology were euthanased in a solution of Aqui – S and preserved in 10% neutral buffered formalin. The keratinised mouthparts were sectioned and viewed for epithelial hyperplasia or hyper keratosis, and larger tadpoles were longitudinally sectioned and their livers examined for deformities such as granulomas.

Toe clip samples were also taken from frogs at the release site in 2003 and 2004, and sent to Taronga Zoo for histology. In 2003, the release was postponed because there was concern that the sample size of *L. aurea* tadpoles tested was insufficient to detect chytrid and there was some deterioration in the samples due to a delay in the testing. In October 2005, swabs were sent to CSIRO for polymerase chain reaction (PCR) testing and histology. A total of 13 *Crinia signifera*, three *Litoria verreauxii*, one *Litoria jervisiensis* and one *Limnodynastes dumerilii* were sampled from the proposed release site, and four *Litoria verreauxii* and four *Limnodynastes peronii* from a nearby wetland (Table 1).

In December 2004 (and thereafter), samples of *L. aurea* tadpoles were sent to the CSIRO laboratory in Geelong Victoria for PCR testing for chytrid. The excision, extraction and Taqman assay (Boyle *et al.* 2004) were performed in a laboratory, which had no prior exposure to chytrids, and disposable equipment was used. The samples were submitted in 3 separate pots in 70% ethanol. The pots were selected from a total of 5500 tadpoles that had been maintained in three separate tanks, 3500 in a large tank and 1000 in two smaller tanks. In 2004, 199 tadpoles were sent for analysis by Taqman real-time PCR assay. All mouthparts (oral discs) were excised from each tadpole to maximize the probability of detecting low levels of infection. They were combined in groups/lots of five for extraction using PrepMan Ultra (there was a total of 40 lots; lot number 40 comprised only 4 pooled mouthparts). Samples 1 - 12 came from pot #1, samples 13 - 24 from pot #2 and samples 25 - 40 from pot #3. The 40 samples were analysed in triplicate and at two dilutions: 1:2 and 1:10. Lot number 18 was also reanalysed at (1:10) and analysed by conventional PCR, and the reaction product sequenced to check for possible error.

Table 1. Real time PCR and immunoperoxidase results for swabs (individuals) taken at Pambula.

+ indicates positive result for *Batrachochytrium dendrobatidis*
- indicates negative result for *Batrachochytrium dendrobatidis*

Date	No. of swabs	Species	Location	Result
5/8/05	5	*Crinia signifera*	Release site	-
6/8/05	4	*Litoria verreauxii*	Pambula wetland	+
6/8/05	4	*Limnodynastes peronii*	Pambula wetland	+
4/9/05	8	*Crinia signifera*	Release site	-
4/9/05	1	*Litoria jervisiensis*	Release site	-
4/9/05	3	*Litoria verreauxii*	Release site	-
4/9/05	1	*Limnodynastes dumerilii*	Release site	-

In 2005, 77 tadpoles were sampled from the larger tank and 20 from each of the smaller tanks. Sampling numbers were based on a prevalence of 2.5% and a 95% probability of detection of chytrid. The specimens were treated in the same manner as the 2004 samples. The excision, extraction and Taqman assay were performed in a laboratory, which had no prior exposure to chytrids and disposable equipment was used. On 22 December 2006 swab samples of mouthparts were taken of 23 captive tadpoles and an additional 19 in March 2007. The samples were again analysed by Taqman real-time PCR assay in triplicate (Hyatt *et al.* 2007).

Eradication of fish and other predators

During the pre-release surveys eels were observed at the release site. Eels are potential predators of *L. aurea* tadpoles (Pyke and White 2001). To bolster the chances of tadpoles surviving, a professional freshwater fisherman was engaged to capture eels. A total of six traps were set in December 2004 and then again in December 2005 and April 2007. The traps were baited with fish and set for 2-3 consecutive days. Trapping and shooting of Red Foxes occurred in Pambula wetlands to the south of the site in October 2006 and July 2007 with 14 and three foxes killed, respectively.

Pre-release surveys for *L. aurea*

Nocturnal surveys were conducted for *L. aurea* at 25 sites from 8 km north to 28 km south of Pambula (between Bournda National Park and Towamba Swamp) from 22 November 1999 to 11 February 2000 (Table 2). Nocturnal surveys involved searches for frogs using 12-volt 50-watt spotlights and the broadcast of pre-recorded calls of *L. aurea* (call playback). Several diurnal

Table 2. Frog species detected during pre-release surveys conducted within the broad area. Playback of *L. aurea* calls was conducted at all sites except Quarry Dam Boydtown. Values are the number of individuals detected of each species.

Litoria ewingii = Le, *Litoria jervisiensis* = Lj, *Litoria nudidigita*= Ln, *Litoria peroni* = Lp, *Litoria verreauxii* = Lv, *Crinia signifera* = Cs, *Limnodynastes dumerilii* = Ld, *Limnodynastes peronii* = Lz, *Paracrinia haswelli* = *Ph, Pseudophryne bibronii* = Pb, *Uperoleia tyleri* = Ut.

Survey site	Date	Survey time	Le	Lj	Ln	Lp	Lv	Cs	Ld	Lz	Ph	Pb	Ut
Bournda Racecourse	22.11.99	20.45 -21.55	10			26				44			
Bournda Lagoon (Scotts Bay)	22.11.99	21.00-21.30							1				
Millingandi Rd - dams	22.11.99	21.45-22.10	1			1		1	1				
Merimbula Back Lake	22.11.99	20.15-20.45				2							
Tura Beach Golf Course	22.11.99	20.00-20.45	1			1		1		1			
Towamba Swamp	24.11.99	19.30-20.45				20	20				10		
dam off Nevets Rd	24.11.99	21.30-22.10			1	1	1	1			1		
Pambula (STP)	29.11.99	21.10-?	1			1	1	1		1			
Bens Swamp	29.11.99	21.15-21.45				5	10			10			
Quarry Dam Boydtown	8.12.99	nocturnal	1			1	1	1					
Tura Golf Course	8.12.99	21.00-22.00				1	1						
east of Pambula	8.12.99	not recorded	1			1		1					
Ben Boyd National Park	8.12.99	20.00-20.55	2			10	20	20		2			
Pambula Flats	16.12.99	21.00-22.30	1			1		1		1			
Tura Beach Golf Club	7.2.00	21.10-21.30						1					
Merimbula Ck (caravan park)	22.11.99	20.55-21.20				11	5	10		3			
Pambula Racecourse	6.1.00	21.00-22.00				6	4			40			
Sapphire Coast Drive	7.2.00	20.30-21.00				1							
Millingandi	7.2.00	21.55-22.30						1	5	1			
Pambula STW	7.2.00	20.30-21.00						1					
Bens Swamp	7.2.00	21.15-21.45											
Merimbula Ck (caravan park)	7.2.00	21.40-21.56						1					
Dam near Millingandi	11.2.00	not recorded				1		1		1			
Millingandi Caravan Park	7.2.00	21.50 - 21.53								1			
Release site	-.9.03	18.30 – 19.45		1		10	1				1	1	
Release site	17.9.04	18.30 – 19.45				12		3			1		2
Release site	15.12.04	not recorded		1		5	2	9		4	1		
Release site	6.8.05	18.30-20.30		10			6	7			2		
Release site	4.9.05	18.30-20.20		10				20	1	5	10		
Release site	22.12.05	20.45 – 21.45	5	1		24		2					

searches were also conducted on warm days for *L. aurea* basking on emergent aquatic vegetation.

Nocturnal searches were conducted prior to the initial release of *L. aurea* tadpoles at the proposed release site between September 2003 and December 2005, using 50-watt spotlights. Survey effort varied between 60 – 120 minutes per site. The calls of *L. aurea* were broadcast during each survey for approximately five minutes.

The NSW NPWS *Hygiene Protocol for the Control of Disease in Frogs* (2001) was followed for pre- and post-release surveys. Equipment, including footwear, was thoroughly disinfected with recommended anti-fungicide, prior to surveys. When taking swab and/or toe clip samples new gloves were used for each frog to avoid cross-contamination. Scalpel blades were washed in anti-fungicide (*Nu-Clenz*) between toe clippings.

Post-release surveys for *L. aurea*

Post release monitoring were staggered in frequency with surveys occurred five times within the first two weeks and twelve times within the first month post release of tadpoles. Thereafter monitoring was monthly until May and recommenced from late August to May the next year. In the second year monitoring was more intense in late spring and summer to coincide with the breeding season. Monitoring included diurnal and nocturnal surveys. Nocturnal surveys were mostly 60 minutes in duration. Pieces of timber were placed around the edge of the pond as refuge habitat for frogs to facilitate the detection of *L. aurea* metamorphlings.

Water quality testing

Water testing was conducted at the proposed release site using the Streamwatch faecal coliform kit and the Streamwatch smart water quality monitoring kit for dissolved oxygen, temp, pH, conductivity, turbidity and total dissolved solids. Samples were taken on 8 and 9 December 2005 and 15 March 2006.

Results

Pathology

Histopathology performed on *L. aurea* tadpoles by Taronga Zoo showed keratinised mouthparts, which appeared normal. There was no evidence of chytrid or other infectious agents within or around the mouthparts. Slides of multiple sections of bisected tadpoles showed that other organs were within normal limits of morphology.

Samples of tadpoles and toe clips sent to Taronga Zoo from the proposed release site were negative for chytrid. Several of the toe clips were too small to process into glass slides, but those that were of sufficient size (e.g. *Litoria peroni*) were examined and no chytrid fungi was evident.

One sample (Lot No. 18) of the captive bred *L. aurea* tadpoles analysed in 2004 by CSIRO returned a positive indication for chytrid. The sample was re-assayed and returned equivocal results. The reaction curves were checked in the event that the reaction kinetics or software performance were incorrect, but the curve associated with the sample appeared to be correct. Even though tadpoles from the other two tanks were not carrying chytrid, the release was postponed. Adult *L. aurea* retained indoors have never showed symptoms of chytrid.

Samples of captive bred tadpoles in 2005 were negative for chytrid. These tadpoles were utilised for re-introduction. Swab samples of tadpoles in 2006 were positive for two specimens. Samples of captive bred *L. aurea* tadpoles were negative for *B. dendrobatiodis* in 2006-7.

Swab samples taken from wild frogs during the cooler months at the release site and a nearby wetland analysed by CSIRO revealed the presence of chytrid in every sample taken at the nearby wetland but none from the proposed release site (Table 1). Chytrid was present in two common frog species 1 km from the coast but not from the release site. The infected frogs appeared to be in good health and did not show characteristics associated with chytrid.

Eradication of fish and other predators

Approximately 3 kg of small eels (less then 300 mm total length) were caught during trapping in December 2004. Approximately 25-30 kilograms of small eels were trapped in December 2005 and only five small eels were captured in April 2007. No other species of fish were found within the ponds.

Pre-release surveys

Nocturnal surveys within a radius of 20 km north and south of the release site from 22 November 1999 to 24 December 2005 detected 11 species of frog (Table 2). The species found are commonly associated with coastal wetlands and farm dams in southern NSW. The exception was *Litoria nudidigita,* which is a stream breeding species but occasionally is found in dams constructed over watercourses (Daly, pers. obs.). A small number of *Litoria aurea* (one female and two calling males) was found in association with sewerage works at Tura Beach, 5 km north of the proposed release site (Daly and Senior 2003).

Post-release surveys

Surveys at the release site were conducted on 32 separate occasions for a total of 31 h (Table 3). During that time approximately 12 *L. aurea* tadpoles were observed swimming between the weeds in the shallow areas of the pond soon after a release. Two *L. aurea* metamorphlings were detected, hiding under a piece of timber that had been positioned closed to the edge of the pond to provide additional refuge habitat and two adult frogs observed a minimum of seven months after the last release of tadpoles.

The area was in drought during a considerable portion of the post release survey period and the water level at the release site had dropped considerably over a few months. The species diversity of frogs at the release site was also considerably smaller than that detected during the pre-release surveys, indicating that the general activity of frogs was reduced. However, in October and November 2007 there were considerable rain events and the higher number of frogs detected reflected the more suitable conditions.

Table 3. Frog species detected during post-release surveys at release site. Values are the number of individuals detected of each species.

Litoria aurea = La, *Litoria ewingii* Le, *Litoria jervisiensis* = Lj, *Litoria peroni* = Lp, *Litoria verreauxii* = Lv, *Litoria peroni* = Lp, *Crinia signifera* = Cs, *Limnodynastes dumerilii insularis* = Ld *Limnodynastes peronii* = Lz, *Paracrinia haswelli* = *Ph, Pseudophryne bibronii* = Pb, *Uperoleia tyleri* = Ut.

Date	Survey period	La	Le	Lj	Lp	Lv	Cs	Ld	Lz	Ph	Ut	Comment
24/12/05	17:30 – 18.30											2,500-3,000 *Litoria aurea* tadpoles released
27/12/05	21:00 – 22.00		5		3							12 *Litoria aurea* tadpoles observed
28/12/05	16:00 – 16.30											No tadpoles observed
29/12/05	21:00 – 22.00		6		3		1					12 *Litoria aurea* tadpoles observed
5/1/06	21:00 – 22.00		6		2		1					Frogs were either juvenile or metamorphlings
9/1/06	20:30 – 21.30		12		3							12 *Litoria aurea* tadpoles observed
11/1/06	14:00 – 15.00											Water level dropping
14/1/06	21:00 – 22.00		19		2							12 *Litoria aurea* tadpoles observed
23/1/06	21:15 – 22.00		10		3							No *Litoria aurea* tadpoles observed
2/2/06	21:00 – 21.30											1500 *Litoria aurea* tadpoles released
16/2/06	21:00 – 22.00		9									No *Litoria aurea* tadpoles observed
22/2/06	20.45 - 22.15	1	12				1		2	1		Under timber circa 35 mm total length
14/3/06	21.00 - 22.00		4		1	1	5		2		1	Under timber placed beside pond as refuge
6/4/06	15.15-16.15								1			Diurnal survey – water level of pond dropping
4/5/06	21.00-22.00		4				7					About 75 mm rain three days previous to survey
29/8/06	19.00-20.00						20			6	1	
28/9/06	18.55-20.10		10		10	6	19		1	2		Many small tadpoles observed in release pond
29/9/06	14.00-15.00				2		15		1			Diurnal survey
31/10/06	20.20-21.20		2	1	20	5	10		1		5	Conservative estimate of *Litoria peroni*
5/12/06	21.10-22.10		2		12				1		3	
4/1/07	20.50-21.50	2			12		2		1		2	*Litoria aurea* detected under wood placed as refuge habitat
12/4/07												1000 *Litoria aurea* tadpoles released
27/4/07	20.00-21.00											Two *Litoria aurea* tadpoles
30/5/07	21.30-22.30								1			Water level high large areas of reedy shallows
17/9/07	20.30-21.30			10	10	10^+	10^+			4	3	Water level high large areas of reedy shallows
28/9/07	20.30-22.00	1	10	10	15	10^+	10^+	3	3	3	6	One *Litoria aurea* responded to call playback
7/10/07	19.00-20.00			4	7	4	3		2	1	6	Water level high. Eels observed in shallows
25/10/07	19.30-21.15		2		40^+	35^+	7		1	1	8	Large number of Peron's Tree Frogs
1/11/07	14.00-14.45											Diurnal survey no amphibians detected
13/11/07	21.00-22.00				25	2	4				9	Had rained the previous week
21/11/07	11.30-12.00											No frogs detected
29/11/07	20.30-21.30	2			20		4		1		10	*Litoria aurea*55-65 mm long

Water quality testing

The water at the release site had a dissolved oxygen (mg/l) content of 6.2 ppm, a pH of 5.5-6.0, turbidity (NTU) of 15-20, and total phosphates were 0.23 ppm. In general, the water was odourless and tannin stained, qualities that were consistent with other waterbodies in the area.

Discussion

The methods used for the project included maintenance and successful breeding of *L. aurea* in captivity for 17 years. Prior to release the receiving environment (six separate surveys) and 24 other sites in the broader area were surveyed for the presence of *L. aurea.* Tests were conducted at the release site (of other species of frog) and on tadpoles slated for release for the presence of chytrid. Post-release 20 surveys have been conducted and so far two adult *L. aurea* have been observed 13 months after the release.

Pre-release prerequisites

Apart from the maintenance and breeding of captive *L. aurea,* several legislative requirements had to be satisfied prior to the release being given the green light by DEC. These included the writing of a translocation proposal that was reviewed within DEC and by two external reviewers.

Also required were animal ethics committee approval, scientific licence (DEC), pathology on the health of the captive tadpoles, pathology on the frogs and water quality testing within the receiving environment. A State Fisheries (section 37) permit was obtained to fish for eels. Voucher specimens of captive *L. aurea* were sent to the Australian Museum for DNA testing. DEC (Southern and Central Directorate) had to be confident that the quarantine requirements of the captive animals were of sufficiently high standard, and that the proposed release site was chytrid free prior to giving approval.

During this project the methods used to detect chytrid have evolved. It is now accepted that as a diagnostic test histology is a relatively crude determinator of the presence of chytrid (Kriger *et al.* 2006). That is, there can be false negatives using this test alone as a screening test. PCR is the most sensitive test currently available and when used as a 'batch test', is a highly sensitive screening test (Hyatt *et al.* 2007). Given the sensitivity of PCR it was interesting that only a few tadpoles (and maybe just one) tested in 2004 were positive for chytrid. An argument could have been advanced that the existence of just a few chytrid spores did not equate to disease (Smith 2007), however, for our experiment the release of potentially infected animals would have confounded results.

Over 30 kg of eels were removed from the release site. Eels eat tadpoles and in enclosed conditions readily consume them, especially if there is little cover such as weeds (G. Daly pers. obs.). We suspected that the site harboured eels because during the pre-release surveys no tadpoles of common species such as *L. peroni* and *L. verreauxii/L/ ewingii* were observed or caught in dip nets.

If the eels had not been removed it is very unlikely that many *L. aurea* tadpoles would have survived to metamorphose. It is recommended that for any future re-introductions of frogs that, where possible, potential predators such as eels (and or *Gambusia holbrooki*) are removed or controlled from the release site. Pyke *et al.* (2008) also suggest the removal of fish from proposed *L. aurea* release sites to elevate the potential of success.

Frog species detected during pre-release and post release surveys

The surveys conducted prior to and after the release detected 11 frog species. This included some additional species not detected during previous surveys (Daly and Senior 2003). This indicates that a site has to be surveyed several times before the full assemblage of frogs can be detected, in particular, there has to be a seasonal spread of survey effort in conjunction with rain events.

All frogs (four *L. verreauxii* and four *Limnodynastes peroni*) sampled inland from the release site were positive for chytrid but did not show characteristics associated with the disease such as lethargy, anaemia or discolouration (Berger *et al.* 1999). There is an accumulating body of evidence that indicates that some species of frog live with the disease and act as a reservoir for the fungus (Hunter *et al.* 2006; Kriger and Hero 2006). This has implications for our project because *L. aurea* appears to be sensitive to the disease (DEC 2005; Penman *et al.* 2008) and may not be able to recolonise areas inland from the release site.

Criteria for success

The season when animals are released may influence the success of an introduction as background levels of chytrid infection in wild species of frog have been shown to be relatively low during summer (Kriger and Hero 2006). We released disease-free late-stage tadpoles during summer and autumn, thereby reducing the risk of chytrid infection. Semlitsch (2002) proposed several elements needed to develop recovery actions for aquatic-breeding amphibians. These included an overview of the critical local population and landscape processes required to maintain the amphibian species and threats and considerations for measuring success and long-term habitat management. Our project has addressed many of these issues. So far some tadpoles have metamorphosed and survived to the terrestrial phase and adult frogs persist. However, there are several other stages that need to be fulfilled before we can state that our strategies were successful. These include frogs breeding and the offspring developing to maturity and breeding to maintain a self-sustaining if not expanding population.

Philosophic arguments for and against reintroduction

There are a variety of views on the pros and cons of re-introducing *L. aurea*. Greer (1996) argued that translocation breaks forever the historical natural link between the organism and place, and that the process is a last resort, only to be considered to save a species or significant population from extinction.

Berger *et al.* (1999) argued that it is extremely important to demonstrate that human intervention can not only explain declining population trends in amphibians but also successfully reverse them. They recommend captive breeding and re-introducing species back into localities within their historic range.

By taking *L. aurea* into captivity in the 1980's, prior to the species decline, genetic stock from this region was secured. With the exception of Nadgee Nature Reserve, few *L. aurea* have been detected on the far south coast of NSW in the last decade (Daly and Senior 2003). The captive colony is the only genetic material from this region currently maintained in captivity. Although the original stock has now gone through approximately five generations in captivity, the animals have the most integrity to the original genetic provenance that can be used for reintroduction.

Arguments on genetic integrity aside (outbreeding v/s inbreeding depression), the question arises: do we want to intervene in an impoverished environment and attempt to restore a species in order to re-establish ecological functioning? And if the answer is yes then what lengths are we prepared to go to achieve this outcome? The current program has been time consuming, expensive and success has yet to be demonstrated.

Conclusions

Reintroductions are expensive in terms of financial and human investments (Appendix 1). Although there is a well-intentioned desire to reinstate a species into previously occupied landscapes, the factors that originally caused the decline have to be understood and mitigated prior to any reintroduction. The presence of the amphibian chytrid is suspected to be the crucial factor in the decline of *L. aurea* (DEC 2005). Evidence from this study supports this assertion because the species no longer exists in many natural areas on the far south coast of NSW. Chytrid fungus was found in healthy looking *L. verreauxii* approximately 3 km from the release site and it appears that many species of frog can persist while infected with this pathogen. Unfortunately *Litoria aurea* is highly susceptible to the disease (DEC 2005 and the extant populations appear to occur in areas where environmental factors exclude or mediate chytrid.

So far *L. aurea* has been shown to successfully colonise adjacent areas from a source population (e,g. Homebush Bay, Orica -Brick and Block site at Port Kembla and Sussex Inlet Sewage treatment ponds) but reintroduced populations have failed to establish (Pyke *et al.* 2008). Extant populations are mostly in coastal locations often with saline influence (Mahony 2001; Daly and Senior 2003,) and/or contaminated sites (White and Pyke 1996, Goldingay and Lewis 1999). With this in mind several factors were considered important as pre-requisites for an appropriate release site. The proximity of the sea and the sewerage treatment ponds were considered critical factors in the selection of the release site.

Although many precautions have been taken, our project may not be successful. Additional releases of tadpoles may be necessary before an adequate number of *L. aurea* survive to mature and breed. Monitoring will continue for the next four years (2007-2011). Ongoing costs for additional releases are expected to tally to approximately $25K per annum. The project will be considered successful when a second generation of *L. aurea* breeds and a population is sustained independent of human intervention. By documenting the process we provide other researchers with the current protocols for frog reintroduction in NSW. So far no reintroductions or translocations of *L. aurea* have satisfied the goal of self-sustaining populations. Given this, translocations as an avenue to facilitate development is not considered acceptable and has led to further population declines (e.g. Rosebery population). However, re-introduction should not be abandoned as one avenue for conservation. This is the reason why we have taken an active role to conserve this species.

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APPENDIX 1

Appendix 1. Breakdown of costs associated with the project to date.

Item	Unit costs/quantity	In kind $	Cost $
Pathology testing - PCR CSIRO and Taronga Zoo Histopathology	Various costs	4000	12,344
Materials: swabs, aqui S, spotlights, gumboots, tape recorder etc.		0	1500
Frog food		0	9698
Pre-release surveys including volunteers	$25/hr x 300	7500	0
Post release surveys (including travel time)	$45/hr x 100	4500	0
Transport cost for surveys – pre release	1500km x 52 c/km	780	0
Transport cost for surveys – post release	1125km x 52c/km	585	0
Transport of tadpoles to release site x 2 trips	4148km x 52 c/km	2157	220
Breeding facilities for frogs		7000	500
Care of captive adult population. Av. 10hrs/week x 7years	$25/hr x 3640 hrs	91,000	0
Phone hook up meetings		0	1000
Site inspection meetings	$45 x 20hr	900	0
Preparation of re-introduction proposal		0	5000
Review of proposal and supplementary		0	2800
Water quality testing kit		0	3000
Removal of eels	$30hr x 20hr	0	600
Specialist advice – Ranger time	$45/hr x 400hr	18,000	0
State Forests	$45/hr x 100hr	4500	0
Bega Shire	$45/hr x 50hr	2250	0
Specialist consultant	$100/hr x 100hr	10,000	0
Totals		**153,172**	**36,662**

Combined total of project to date $189834