Life stage specific variation in the occupancy of ponds by *Litoria aurea*, a threatened amphibian

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Abstract Breeding aggregations are a reproductive strategy to increase mate finding opportunity. However, because aggregations skew the distribution of mature animals through conspecific attraction, rather than resource availability, the distribution of breeding sites may be reduced, so that not all suitable breeding sites are used. To examine the relationship between landscape and reproductive strategies of a threatened frog, *Litoria aurea*, we studied its distribution at Sydney Olympic Park over two breeding seasons. We aimed to: (i) determine the distribution and predictors of breeding ponds; and (ii) assess the significance of dispersal in the juvenile age-class. We found that the distribution of the calling males was highly skewed and occurred in large, well-connected ponds. Despite this, breeding ponds were not aggregated; pond size was the single factor explaining the distribution of breeding ponds. Juvenile frogs dispersed from breeding ponds and were not associated with a specific pond characteristic. Less breeding occurred in the second season during which fewer ponds were used for breeding including many different ponds from the previous year. These changes suggest that breeding effort and breeding pond choice are dynamic and therefore knowledge of the factors that drive breeding events will be a powerful tool in managing species, particularly in light of changing climatic regimes.

Key words: aggregation, dispersal, distribution, frog, reproduction.

INTRODUCTION

Calling aggregations can increase mate finding opportunities when populations reach low densities (Gascoigne *et al.* 2009). However, for species to persist in low densities, aggregated sites must still allow for recruitment and dispersal into the population. Because breeding aggregations skew the distribution of mature animals through conspecific attraction, rather than resource availability (Reed & Dobson 1993) the distribution of breeding sites may be reduced, so that not all suitable breeding sites are used (Rudolf & Mark-Oliver 2005). Consequently this increases the relative importance of the selection of used sites because each site contributes to a greater proportion of the total population's recruitment. In addition, the reduced distribution of breeding habitat increases the importance of other ecological processes, such as habitat connectivity for species with dispersal during early life stages (reviewed in Amarasekare 1998).

Knowledge of the reproductive strategies of frogs is important because of the rapid declines many species have experienced. The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) causes the disease chytridiomycosis in frogs and tadpoles and has reduced many affected species to small population sizes or low densities (Skerratt *et al.* 2007). Frogs may be behaviourally buffered from Allee effects in low densities because they aggregate and chorus, a mate finding strategy that may reduce Allee effects (Stephens & Sutherland 1999). Additionally, many frog species have demographic characteristics to avoid Allee effects, such as high fecundity and rapid growth. However, Allee effects can be caused by complex interactions and their effects are difficult to predict.

The green and golden bell frog, *Litoria aurea*, is a species of frog that has declined throughout its native range and is invasive elsewhere. It rapidly declined through most of its range in a pattern consistent with exposure to the chytrid fungus (Berger *et al.* 1999; Penman *et al.* 2008). *Litoria aurea* possess life history traits of a weed species, including high fecundity and fast growth (Hamer & Mahony 2007) and these characteristics have enabled the successful establishment of invasive populations in New Zealand, New Caledonia and Vanuatu (Pyke & White 2000). During an extended breeding season mature male *L. aurea* form choruses to attract reproductive females (Christy 2000). Previous studies suggest that pond size, connectivity and vegetation affect the occupancy of *L. aurea* (Hamer *et al.* 2002a) but factors that drive choice of breeding ponds and juvenile distributions are poorly understood. To investigate reproductive

strategies of *L. aurea*, we studied its distribution during two breeding seasons. We aimed to (i) determine the pond attributes that predicted the distribution of calling males and tadpoles; and (ii) assess the significance of dispersal in the juvenile age-class.

METHODS

Study site

Sydney Olympic Park is a restored industrial wasteland in the western suburbs of Sydney, Australia. *Litoria aurea* was discovered in the grounds of the park prior to developing the site for the 2000 Sydney Olympic Games (Darcovich & O'Meara 2008). A large restoration strategy was implemented to offset habitat loss and increase the distribution of *L. aurea*, in an attempt to ensure the persistence of the population. Currently the park contains 150 ponds of varying size and hydrological regimes provide a selection of habitat for aquatic fauna. Twenty-three of these ponds occur in a drainage system invaded by the exotic fish, *Gambusia holbrooki*, which has been implicated in predation of tadpole *L. aurea* (Hamer *et al.* 2002b). Therefore, some ponds occupied by *G. holbrooki* are drained each year as a management action to temporarily rid the pond of fish (O'Meara & Darcovich 2008).

Study methods

Visual encounter surveys and calling surveys were completed in 87 ponds for two consecutive seasons at Sydney Olympic Park, by sampling each pond between 20:00 h and 04:00 h in November, December and February 2009–2011. Calling males were recorded upon arrival at each pond before and after an imitation of an *L. aurea* chorus. Frogs of all species were counted by active searching in and around each pond. Individual *L. aurea* were captured using a single use disposable plastic bag to prevent disease transmission between individuals, or recorded as being present if the catch failed. Individuals greater than 35-mm snout–vent length (SVL) were marked by injection with a passive integrated transponder (PIT) tag (Christy 1996). Males develop nuptial pads when they reach around 45 mm in length (SVL), so the presence of nuptial pads indicated a male and absence of this feature indicated a female (Christy 2000). Frogs were released at the site of capture.

Tadpole surveys were completed concurrently with visual encounter surveys and on an extra occasion in January. Minnow traps were tied to emergent vegetation and baited with a yellow glow stick between 15:00 h and 20:00 h. The number of traps set in each pond was relative to the size of the pond (between 1 and 50) and additionally a dip net was swept through the water at a relative number of sweeps at each pond. Similar catch efforts were expended in each season; 2100 frog search hours, 2623 dip net sweeps and 2003 tadpole traps were employed in 2009/2010 compared with 2490 frog search hours, 1794 dip net sweeps and 1984 tadpole traps in 2010/2011. Depth was measured up to

150 cm with pre-measured markings on the dip net in the deepest point of the pond. *Litoria aurea* spend 1.5–11 months as tadpoles (Anstis 2002; Browne *et al.* 2003); therefore, monthly trapping during the peak of the breeding season was considered adequate to sample breeding events. Rainfall was higher in the first year of sampling, 453.6 mm in 2009/2010 and 411 mm in 2010/2011 at Concord Gold Club, 2.9 km from the study site (Bureau of Meteorology).

Habitat data were sampled in each pond during April and May of 2008. Vegetation cover was quantified inside a floating quadrat made from $1\ m^2$ tubing, which followed a transect that bisected the pond. Emergent vegetation was quantified through a visual estimation of the proportion of cover of each species and summing the total. Surface area was calculated using the program MapInfo (version 8.5) by drawing a polygon around each water body at the maximum water level. Draining of ponds was completed by the Sydney Olympic Park Authority as a management tool to temporarily rid them of invasive fish (O'Meara & Darcovich 2008). Draining was attempted in each year and refilled naturally during flooding events, partial draining occurred during August 2008 and a complete drain that dried the ponds occurred during September 2009. An index for the isolation of each water body from other water bodies at the site was calculated using an adapted version of the formula developed by Hanski (1999).

$$\text{Si} = \sum_{j \neq i} \exp(-\alpha d_{ij})$$

Where S_i is the isolation of a given pond, the constant α describes how fast the numbers of migrants from another pond (termed j) decline as the distance between the two water bodies increases and d_{ji} is the distance between water bodies. The value for α was calculated by averaging the dispersal distances of *L. aurea* that were recorded in a mark–recapture study conducted in the Brickpit in January 2009 (Pickett 2009), and then taking the reciprocal of this number (Pellet *et al.* 2007). Values for d_{ji} were measured in MapInfo using an aerial photograph, and accounted for barriers to dispersal such as frog fences and underpasses, so that the shortest route around barriers rather than a straight-line route through barriers was measured where necessary. This formula for pond isolation incorporates the expected number of immigrants arriving at a given pond i from neighbouring ponds j. It is expected that the contribution of individual populations from neighbouring ponds to the pooled number of immigrants that are received by a given pond will increase as the size of that population increases and the distance between water bodies decreases (Hanski 1999). Large values of S correspond to low levels of isolation. The isolation index for a given water body was calculated by repeating the formula for all other ponds at the study site, and adding the output together to obtain a single value for each water body.

Statistical analyses

Zero-inflation models quantified the influence of predictor variables on the occupancy of ponds with calling males, juveniles and breeding events separately for each season (2009/2010 and 2010/2011). Effects included in the model were: proportion of emergent vegetation, isolation index,

Table 1. Estimates of effects with standard errors (SE) for variables in the minimum adequate model predicting the occupancy of calling male, breeding pond and juvenile *Litoria aurea* at Sydney Olympic Park during 2009–2011

| Year	Response	Effects	Estimate	SE	Liklihood ratio χ^2	P
2009/2010	Occupancy of calling males	Connectivity	0.69	0.29	9.42	0.01
		Size	0.65	0.30	5.84	0.01
		Drain	2.78	1.09	7.25	0.01
2010/2011		Connectivity	0.79	0.31	8.26	0.005
		Size	0.53	0.26	4.40	0.05
		Drain	3.08	1.16	10.68	0.001
2009/2010	Breeding ponds	Size	0.33	0.12	6.35	0.01
2010/2011		Size	1.05	0.50	3.79	0.05
2009/2010	Juveniles	Null	–	–	–	–
2010/2011	Juveniles	Null	–	–	–	–

P-values relate to likelihood ratio tests including the minimum adequate model and a model with the predictor variable excluded. Model type was Negative Binomial (NB) following likelihood ratio test in advice of Zuur *et al.* (2009).

depth, log 10 pond area, and a binary variable for draining. The maximal model was fit to all response variables and reduced to the minimum adequate model following Crawley (2007). Analyses were conducted using the pscl package in R v2.13.0 (Development Core Team 2010).

RESULTS

The distribution of mature males captured over the two seasons was highly skewed. During 2009/2010, 75 individual mature males were sampled in 35% of the available ponds and in 2008/2009, 225 individual males occupied just 38% of ponds. However, calling males were only detected in 12% and 16% of ponds respectively. Calling males were heard in one pond where mature males were not detected in 2009/2010 and two ponds in 2009/2010. In both seasons, the occupancy of calling males was positively associated with ponds that were large, drained and connected (Table 1). Depth, emergent vegetation, presence of inter-specific frogs, and season did not influence the distribution of males at either time.

During 2009/2010, 1731 tadpoles of *L. aurea* were detected in traps, 107 were caught in dip nets and 15 metamorphs were visually surveyed. The first season breeding was detected in 35% of the available ponds. The following season there was a reduction in both breeding and the distribution of breeding; we captured only 60 tadpoles in traps, 23 in dip nets and observed 16 metamorphs; these were confined to 21% of available ponds in 2010/2011. In both seasons the distribution of breeding ponds was explained solely by pond size (Table 1); larger ponds were preferred (Fig. 1). A total of 38 ponds were used for breeding but only 10 ponds were used in both years. Breeding was not limited by the number of reproductively mature females; 69 and 105 individual females greater than 65 mm were sampled in 2009/2010 and 2010/2011 respectively.
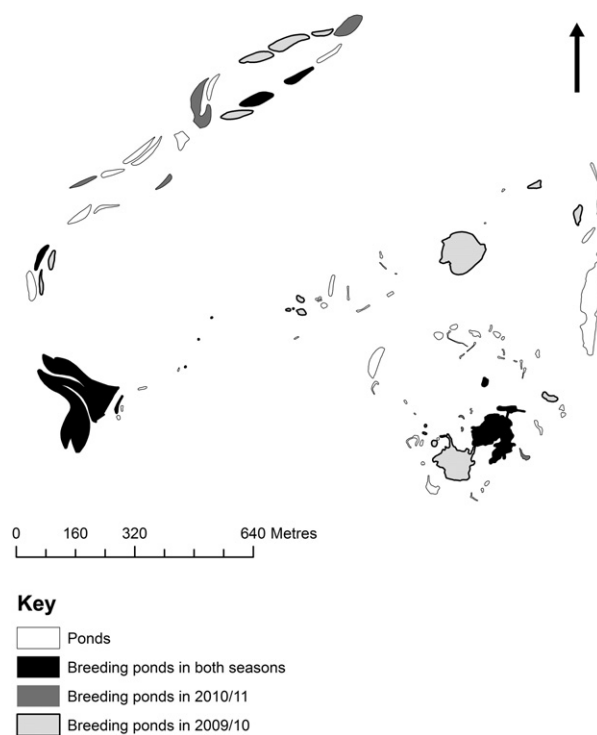
Fig. 1. Distribution of *Litoria aurea* breeding ponds in Sydney Olympic Park over two seasons. Breeding was dispersed over more ponds in 2010/2011 (light grey) when a large breeding event occurred, than in 2009/2010 (dark grey) and a subset of ponds were used in both years (black). The distributions of breeding pond were characterized by a larger size in both years.

Juveniles were more widely distributed than tadpoles and metamorphlings; we caught 232 juveniles in 38 ponds, of which 13 had been used for breeding in 2009/2010 and 416 juveniles in 26 ponds, of which seven were used for breeding in 2010/2011. Juvenile occupancy was not explained by any pond

characteristic considered (Table 1). Juveniles used 64 ponds cumulatively but only used 16 ponds during both years.

DISCUSSION

Male aggregation is a mechanism that can mitigate reproductive Allee effects because it allows females to access many males in low abundances. However, this process has the potential to reduce breeding distributions if females breed solely within aggregated ponds. While we found the distribution of calling males was restricted to closely connected ponds, we did not find such an association with the breeding distribution of *L. aurea* at Sydney Olympic Park. The distribution of breeding ponds was not negatively associated with the isolation index in either year, which suggests that the aggregated distribution of calling male frogs does not necessarily cause aggregated breeding distributions.

Fewer ponds were used for breeding in the year when less breeding occurred and some of these ponds were not used in the previous year, suggesting that both breeding levels and choice of breeding ponds is dynamic. A greater number of ponds were used for breeding in 2009/2010 when higher densities of tadpoles and metamorphlings were detected overall. Dispersal from previously oviposited sites may be a behavioural mechanism to avoid ponds where conspecific tadpoles occur. Avoidance of conspecific tadpoles occurs in other anuran species (Halloy *et al.* 2000) to avoid competition (von May *et al.* 2009) or cannibalism (Crump 1991). Pond choice was driven by only one factor for breeding; females clearly oviposited in larger ponds possibly because productivity in wetlands is positively associated with size (Vander Zanden & Fetzer 2007) and larger ponds may have more resources. It is also possible that larger ponds had more breeding simply because they cover a larger area, which increases the probability that they are used.

Breeding ponds only partially accounted for the total distribution of juvenile *L. aurea* and juvenile distribution was not explained by the same effects as breeding ponds, suggesting that juveniles will disperse away from breeding ponds. Dispersal in the juvenile stage of *L. aurea* may be beneficial because adult bell frogs are cannibalistic. If juvenile dispersal success is negatively correlated with movement distance, as with other frog species (Rittenhouse *et al.* 2009), then recruitment may be disadvantaged by breeding in well-connected ponds because juveniles will have further to travel to unused ponds. However, we did not detect an aggregated breeding distribution suggesting that even in years with little breeding, juvenile dispersal will not be restricted.

Calling male distribution was positively associated with highly connected ponds, which can provide close access to conspecifics for chorusing and mating (Table 1). Ponds greater than 40 m² were also preferred by calling male bell frogs possibly because larger ponds allow for males to chorus with less competition or because larger ponds provide more food resources (Vander Zanden & Fetzer 2007). In addition, drained ponds may be preferred because productivity can pulse when wetlands flood (Bayley 1991; Pollock *et al.* 1998), and may therefore provide more food resources. In contrast to previous studies of *L. aurea* on Kooragang Island (Newcastle, New South Wales), vegetation was not a significant predictor of occupancy (Hamer *et al.* 2002a), possibly because we solely considered calling males. In addition, the abundance of other frog species and variation in depth appear to be unimportant predictors of bell frog occupancy at Sydney Olympic Park.

Litoria aurea has life history strategies that may mitigate Allee effects such as high fecundity and fast growth. However, processes that can affect the demography of small populations are difficult to predict and following the widespread decline of many amphibians, knowledge of reproductive biology is critical to management. In the case of *L. aurea*, aggregating and chorusing to attract mates may provide a behavioural buffer but did not restrict the distribution of breeding sites. Bell frogs bred in larger ponds suggesting that resource availability is the primary driver of pond choice and the disaggregated distribution of breeding ponds is likely to provide greater opportunity for recruitment and dispersal into the population. Knowledge of factors that affect where breeding occurs is important for such highly managed species and will be useful for creation of habitat and when prioritizing existing habitats for population persistence. Factors that explain levels of breeding are not well understood in *L. aurea* and should be a priority in future research, particularly in light of the changing climate. Understanding the mechanisms that determine breeding levels will facilitate understanding of the flexibility behind choice of breeding ponds. Changes in the distributions of breeding ponds between years demonstrates that caution must be taken when considering the importance of breeding habitat in a single breeding season. In addition, the change in occupancy of ponds by *L. aurea* at different life stages highlights a need to consider ontogenetic shifts when determining the distribution of species.

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