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The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia

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Abstract The introduction of fish has decimated many amphibian populations through increased predation, primarily on their larvae. Some amphibian species now occupy marginal habitats as a response to the presence of introduced fish predators. Such habitats may include ephemeral water bodies where fish do not usually occur, although breeding in these suboptimal environments may incur some cost to a species if its larvae are not adapted to develop under these conditions. We investigated this scenario of amphibian decline using the endangered green and golden bell frog (*Litoria aurea*) and the introduced mosquitofish (*Gambusia holbrooki*) in a factorial experiment to determine the responses of tadpoles to declining water levels and the introduced predator. Tadpoles metamorphosed asynchronously but did not accelerate development in declining water or when housed with mosquitofish. Mass at metamorphosis was 30% less in declining water. Mass increased with time to metamorphosis in constant water-level treatments, but decreased in declining water. Tadpoles did not respond to mosquitofish and were therefore assumed to be naive to this predator. These results suggest that ephemeral habitats may be suboptimal for breeding, and tadpoles appear better suited to develop in permanent water bodies free of introduced fish. Intra-clutch variability in larval development (i.e. bet-hedging) may allow *L. aurea* to cope with unpredictable pond duration, whereby even permanent water bodies may occasionally dry out. The responses observed in *L. aurea* suggest that introduced fish may have reduced the suitability of permanent water bodies as breeding sites for other pond-breeding amphibian species. The use of less favourable ephemeral habitats as breeding sites may be responsible for some of the declines reported in amphibians since the 1970s.

Keywords Amphibian declines · Environmental uncertainty · Fish predation · Habitat · Tadpole life-history

Introduction

A major threat to animal populations worldwide is the introduction and spread of exotic species. Invasive species may outcompete native ones (Kupferberg 1997) or decimate local populations through predation (Dickman 1996). In fact, biological invasions have the potential to eliminate entire species from the landscape, thereby allowing exotic species to dominate (Barel et al. 1985; Fritts and Rodda 1998; Hobbs and Mooney 1998). A challenge for conservation biologists is to determine the conditions under which a native and introduced species can co-exist, given that eradication of exotics can be extremely difficult, if not impossible.

Many amphibian populations have been severely affected by introduced species. The widespread introduction of fish unintentionally, for recreational purposes or as agents of biological control, has been implicated in the decline of a number of species. The mountain yellow-legged frog (*Rana muscosa*) and red-legged frog (*R. aurora draytonii*), for example, are now restricted in their distribution in California largely as a result of introduced species of fish, which eat their larvae (Bradford et al. 1993; Fisher and Shaffer 1996; Knapp and Matthews 2000). Introduced fish have been shown to limit the distribution of *Hyla arborea* in Sweden (Brönmark and Edenhamn 1994) and reduce amphibian species richness in Ontario, Canada (Hecnar and M'Closkey 1997). They have also been implicated in the decline of the spotted frog (*Litoria spenceri*) in south-eastern Australia (Gillespie 2001). Introduced fish have therefore been proposed as one of the causes of the general decline observed in amphibian populations around the world.

Owing to the presence of introduced fish, some amphibian species may now occupy less favourable habi-

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tats. Tadpoles adapted to life in fish-free, but permanent, water bodies may not exhibit the appropriate behaviour to evade predation, such as reduced activity or spatial avoidance (Petranka et al. 1987; Lawler 1989; Stauffer and Semlitsch 1993; Relyea 2001a, b). Consequently, they may have reduced larval survival in the presence of fish (Kats et al. 1988; Adams 2000). These amphibian species may now occur predominantly in ephemeral water bodies because they lay their eggs in permanent and in ephemeral waters, but only those in ephemeral water bodies survive, since these usually do not contain fish because they dry out periodically. Alternatively, the amphibian adults may only lay eggs in fish-free water bodies, which tend to be ephemeral sites.

Many amphibian species which breed in relatively permanent ponds have tadpoles with long and asynchronous development periods (bet-hedging species), whereas those in ephemeral water bodies have shorter or more synchronous development periods (Wilbur and Collins 1973). The latter (e.g. *Scaphiopus* sp.) often exhibit adaptive plasticity (i.e. phenotypic plasticity) which enables them to complete metamorphosis in a rapidly drying aquatic environment by accelerating the larval period (Newman 1989, 1992). However, these individuals usually metamorphose at a smaller size (Crump 1989; Newman 1989; Laurila and Kujasalo 1999), which may affect subsequent survival and reproductive success (Smith 1987; Berven 1990; Morey and Reznick 2001).

Tadpoles that exploit the bet-hedging strategy may suffer increased mortality in more permanent water bodies as a result of exposure to introduced fish or, in fish-free ephemeral waters, from desiccation when the pond dries. Therefore, while ephemeral ponds may favour increased tadpole survival because they generally lack fish, this advantage may be offset by the uncertainty in pond duration. We explored these aspects of tadpole life-history using the introduced mosquitofish (*Gambusia holbrooki*) and the green and golden bell frog (*Litoria aurea*) as our model species.

Litoria aurea originally inhabited permanent ponds, swamps, lagoons and farm dams on the coast and adjacent ranges of New South Wales and Victoria in south-eastern Australia (Courtice and Grigg 1975; Cogger 2000). Although the species was once common throughout its range in New South Wales, it underwent a massive range contraction in the mid-1970s, and many populations have disappeared (White and Pyke 1996). The species is now listed as endangered in New South Wales in the Threatened Species Conservation Act 1995 and nationally as vulnerable in the Environmental Protection and Biodiversity Conservation Act 2000. However, there is no evidence of a decline in populations further south in the East Gippsland region of Victoria (Gillespie 1996). Several hypotheses have been presented in an attempt to explain the range contraction, such as habitat destruction and modification, increased susceptibility to UV-B exposure and increased predation from introduced fish, the mosquitofish in particular (van de Mortel and Buttemer 1996; White and Pyke 1996).

The mosquitofish has been introduced into many wetlands worldwide to control mosquito larvae, but is also known to eat the eggs and tadpoles of several species of frogs (e.g. Grubb 1972; Webb and Joss 1997; Goodsell and Kats 1999; Lawler et al. 1999; Komak and Crossland 2000). The fish was introduced to Australia from North America in 1925 as an exercise in biological control and is now widespread and common in many waterways in south-eastern Australia, except in East Gippsland where it is absent (McDowall 1980; Gillespie 1996). The eggs and tadpoles of *L. aurea* are also vulnerable to predation by mosquitofish (Morgan and Buttemer 1996; Pyke and White 2000). Consequently, predation by mosquitofish is listed as a key threatening process in the New South Wales Threatened Species Conservation Act 1995.

Recent field observations in New South Wales suggest that *L. aurea* now apparently requires ephemeral water bodies for breeding because mosquitofish are generally absent from these (Pyke and White 1996). It is assumed therefore that ephemerality is an optimum condition, even a prerequisite, for successful reproduction. However, prior to the range contraction, breeding habitat throughout the species' range was reported to be permanent ponds (Courtice and Grigg 1975), and populations in Victoria presently breed in permanent and ephemeral ponds where mosquitofish are absent (Gillespie 1996). Furthermore, breeding may have always occurred both in permanent and ephemeral water bodies, but was not documented in the latter because they were not included in the field surveys conducted by Courtice and Grigg. Therefore, in the populations that remain in New South Wales, *L. aurea* may use permanent and ephemeral water bodies as ovipository sites, but tadpoles from ephemeral water bodies predominantly survive to metamorphosis because of the spread of mosquitofish in permanent water bodies. It has not been determined whether the life-history of the tadpole of *L. aurea* is characteristic of an ephemeral or permanent pond-breeding species. If it is suited to develop in permanent water bodies, then ephemeral ponds may not be the ideal habitat.

Despite the argument that fish-free ephemeral water bodies are the optimal habitat and a prerequisite for successful breeding (Pyke and White 1996), it remains unknown whether *L. aurea* possesses the mechanisms that may enable it to co-exist with mosquitofish. Furthermore, it has not been determined whether its larvae can adapt to declining water levels. We therefore investigated the role of declining water levels and mosquitofish on *L. aurea* in a 2×2 factorial experiment. We also made behavioural observations to determine whether tadpoles reduce their activity in the presence of mosquitofish and whether they spatially avoid this introduced fish.

Materials and methods

Experimental design

Tadpoles from two spawn masses collected from captive *L. aurea* were combined for the experiment. The male parents of both clutches were second generation (F2) captive progeny, while the

Table 1 Mean total body length (mm \pm SE, $n=6$) of *Litoria aurea* tadpoles at the start of the experiment. Each replicate consisted of 15 tadpoles. There was no significant interaction between predator and hydroperiod

Predator absent		Predator present		F ($df=1,20$)	P
Constant volume	Declining volume	Constant volume	Declining volume		
39.1 \pm 1.13	40.0 \pm 0.82	42.4 \pm 0.26	39.9 \pm 0.88	Predator:	3.54
				Hydroperiod:	1.05
					0.07
					0.32

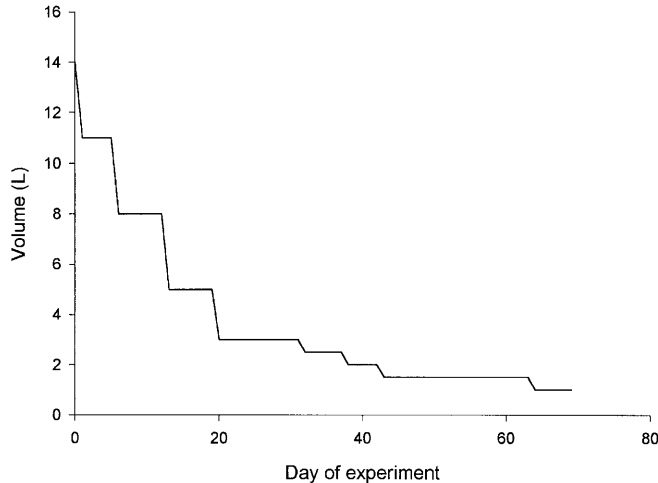


Fig. 1 Removal of water in the declining water level treatments

females were first generation (F1) and therefore had wild parents. The wild-caught parents were from a pond that fluctuated widely in its water level, usually between 0 and 1 m in depth. The tadpoles were housed in 14 l of aged water in white plastic containers (41 \times 28 \times 16 cm deep) until the majority had reached development stage 25 (Gosner 1960).

The experiment was conducted in a laboratory at a temperature of 25 \pm 3 $^{\circ}$ C, a humidity of 50 \pm 30%, and a 12 h light:12 h dark photoperiod. The experimental design was a two-way analysis of variance (ANOVA), with the factors being hydroperiod (either constant or declining water volume) and predator (either present or absent). Twenty-four containers (41 \times 28 \times 16 cm deep) were arranged so that each factor combination occurred once in each of six blocks. A randomised-block arrangement was adopted to minimise any bias that might have arisen from container position, for example, from variation in temperature across the room.

The experiment commenced on 7 November 2000 (day 0). Each replicate consisted of 15 tadpoles (stage 25; Gosner 1960) selected at random and placed in each of the 24 experimental containers (24 \times 15=360 tadpoles). The water volume in the constant-water-level treatments was maintained at 14 l, and the volume in the declining treatments was reduced from 14 l to 11 l on day 0, after the addition of the tadpoles, and randomly thereafter to a minimum of 1 l on day 64 (Fig. 1). This volume was maintained until all individuals had metamorphosed. To compensate for evaporation during the experiment, water was added to the containers as required to maintain the desired volume.

Exposure to the predator (mosquitofish) was organised so that no direct physical contact was possible between the fish and tadpoles, because mosquitofish would quickly kill and eat the tadpoles. However, the experiment required that tadpoles could detect the presence of this fish, either visually or chemically, or both. We therefore housed the fish in clear plastic tubs with fitted lids (10 \times 15 \times 7 cm deep). Small holes were drilled into the sides and a rectangular section of the lid cut away to facilitate water flow between the tub and container. A net (mesh size 1 mm) was placed under the lid and held secure with elastic bands to prevent the fish from escaping. One tub was immersed and positioned at a ran-

domly selected end of each of the 24 replicates. They were placed in each of the 12 "predator absent" treatments to control for any reaction the tadpoles might have to the presence of the tub itself. One mosquitofish (3–5 cm), selected at random from a separate container, was added to a tub in each of the 12 "predator present" treatments. Fish were collected locally from a small pond. Tadpoles and fish were introduced together on day 0 of the experiment.

Tadpole length (tip of snout to tail end) was measured at the start of the experiment to the nearest 0.5 mm. The mass of individual tadpoles was not obtained, owing to concern over possible harm to them because of their small size. For each replicate, the lengths of the 15 tadpoles were averaged to give a mean. A two-way ANOVA revealed no significant differences between the treatments (Table 1). The containers were examined every 24 h until all tadpoles had metamorphosed. Any dead tadpoles were removed and were not replaced. Metamorphosis was defined by the emergence of the first forelimb (stage 42; Gosner 1960). Tadpoles were fed boiled lettuce ad libitum throughout the experiment, and mosquitofish were fed Friskies fish flakes. The water in each container was changed every 3rd day and replaced with aged tap water treated with Stress Coat (Aquarium Pharmaceuticals Inc., Chalfont, Pa., USA) to remove chlorine and neutralise chloramines.

Response variables

We measured three response variables: (1) duration of larval period, (2) mass at metamorphosis and (3) tadpole behaviour. We used ANOVA to investigate how the two treatments affected these variables. The null hypotheses were that predator presence and hydroperiod had no significant effect on the response variables, and no interaction was evident. Proportional data were arcsine square-root transformed, though back-transformed means are presented in the Figures.

At metamorphosis, individuals were removed from the containers, gently blotted on absorbent paper and weighed to the nearest 0.01 g using an electronic balance. The length of the larval period was measured as the number of days elapsed from the start of the experiment.

To investigate whether tadpoles respond behaviourally to the presence of mosquitofish, we recorded their behaviour using instantaneous sampling once a week over three consecutive weeks (days 11, 16 and 23). The responses scored were the proportion of tadpoles (1) occupying the predator half of the container, (2) active (i.e. swimming), and (3) feeding. The latter was recorded by starving tadpoles for 12 h then placing a 10 \times 10-cm piece of boiled lettuce in the centre of the container and recording the number feeding at 1 min.

Results

Metamorphosis

The first tadpole metamorphosed on day 29 of the experiment. Thereafter, new individuals transformed almost every day until the final tadpole metamorphosed on day 69 (Fig. 2). The mean time to metamorphosis was

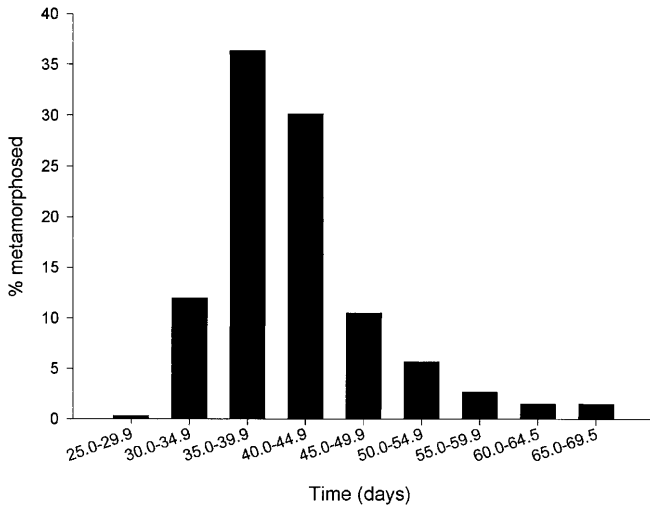


Fig. 2 Frequency distribution of the time to metamorphosis of *Litoria aurea* ($n=337$ individuals)

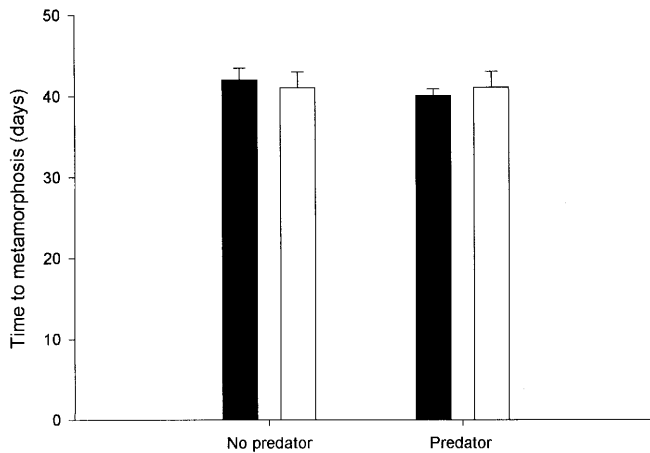


Fig. 3 Mean time (+1 SE, $n=6$) to metamorphosis of *Litoria aurea*. There were no significant differences within or between the two treatments (predator: $F=0.30$, $df=1,20$, $P=0.59$; hydroperiod: $F=0.00$, $df=1,20$, $P=0.96$; interaction: $F=0.26$, $df=1,20$, $P=0.62$). Closed bars represent the constant water level treatments; open bars represent the declining water level treatments

41 days, although nearly half (48.2%) of the cohort metamorphosed in the 10 days after the first tadpole metamorphosed. Of the 360 tadpoles at the start of the experiment, 23 died. However, survivorship was not significantly different between the treatments (predator: $F=0.25$, $df=1,20$, $P=0.63$; hydroperiod: $F=1.33$, $df=1,20$, $P=0.26$; interaction: $F=0.01$, $df=1,20$, $P=0.95$).

The duration of the larval period was unaffected by either predator presence or hydroperiod (Fig. 3). The presence of mosquitofish had no effect on the mass at metamorphosis (Fig. 4). However, the mean mass for metamorphs was 30% lower in declining water, and there was a significant interaction between predator and hydroperiod (Fig. 4).

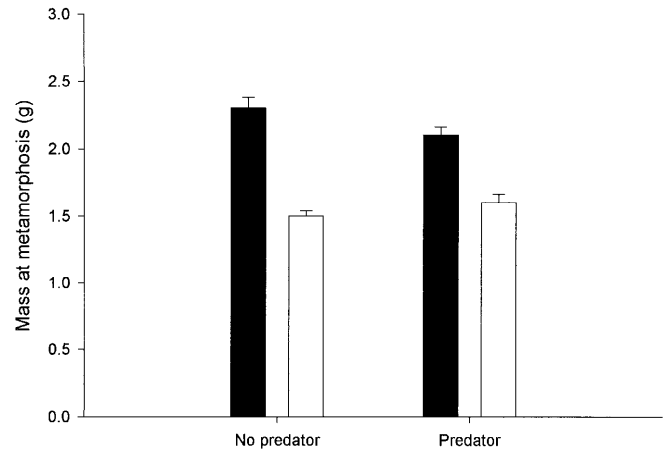


Fig. 4 Mean mass (+1 SE, $n=6$) at metamorphosis of *Litoria aurea*. There were significant differences between the constant and declining water treatments (predator: $F=0.58$, $df=1,20$, $P=0.46$; hydroperiod: $F=99.50$, $df=1,20$, $P<0.001$; interaction: $F=5.07$, $df=1,20$, $P=0.04$). Closed bars represent the constant water level treatments; open bars represent the declining water level treatments

Owing to the significant result obtained for mass at metamorphosis and hydroperiod, we determined whether there was a relationship between mass and time to metamorphosis in the constant versus the declining water level treatments. We did so by plotting the time to metamorphosis versus the mass at metamorphosis for each tadpole, with blocks pooled into the four factor combinations. That is, no mosquitofish/constant water level, no mosquitofish/declining water level, mosquitofish/constant water level, and mosquitofish/declining water level. In the constant water level treatments, there was a significant increase in tadpole mass with time (Fig. 5). However, in the declining water level treatments, mass decreased with time.

Behaviour

Tadpoles did not avoid mosquitofish in the constant water level treatments by retreating to the “no predator” half of the container (Fig. 6). However, the proportion of tadpoles occupying the “no predator” half in the declining treatments almost reached statistical significance ($P=0.06$). The number of tadpoles that were active (i.e. swimming) did not differ between the predator and hydroperiod treatments (predator: $F=0.17$, $df=1,20$, $P=0.69$; hydroperiod: $F=0.02$, $df=1,20$, $P=0.89$; interaction: $F=0.04$, $df=1,20$, $P=0.84$). Mosquitofish did not reduce tadpole feeding, although more tadpoles grazed in a declining than in a constant water volume (Fig. 7). There was no significant interaction between predator and hydroperiod in the feeding observations (Fig. 7). Owing to the lack of developmental and behavioural responses, it appeared that *L. aurea* was naive to the presence of mosquitofish.

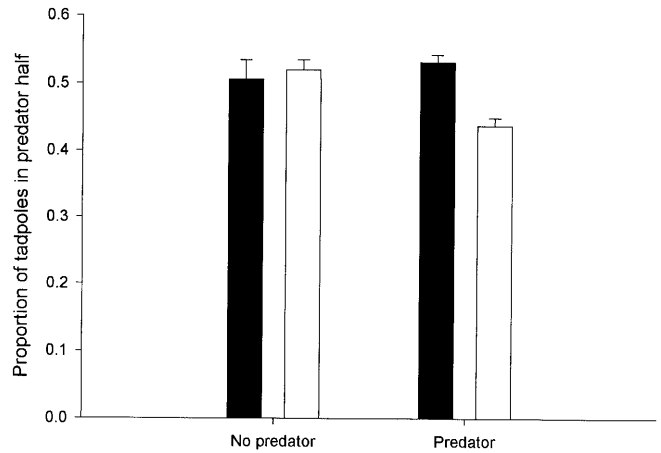
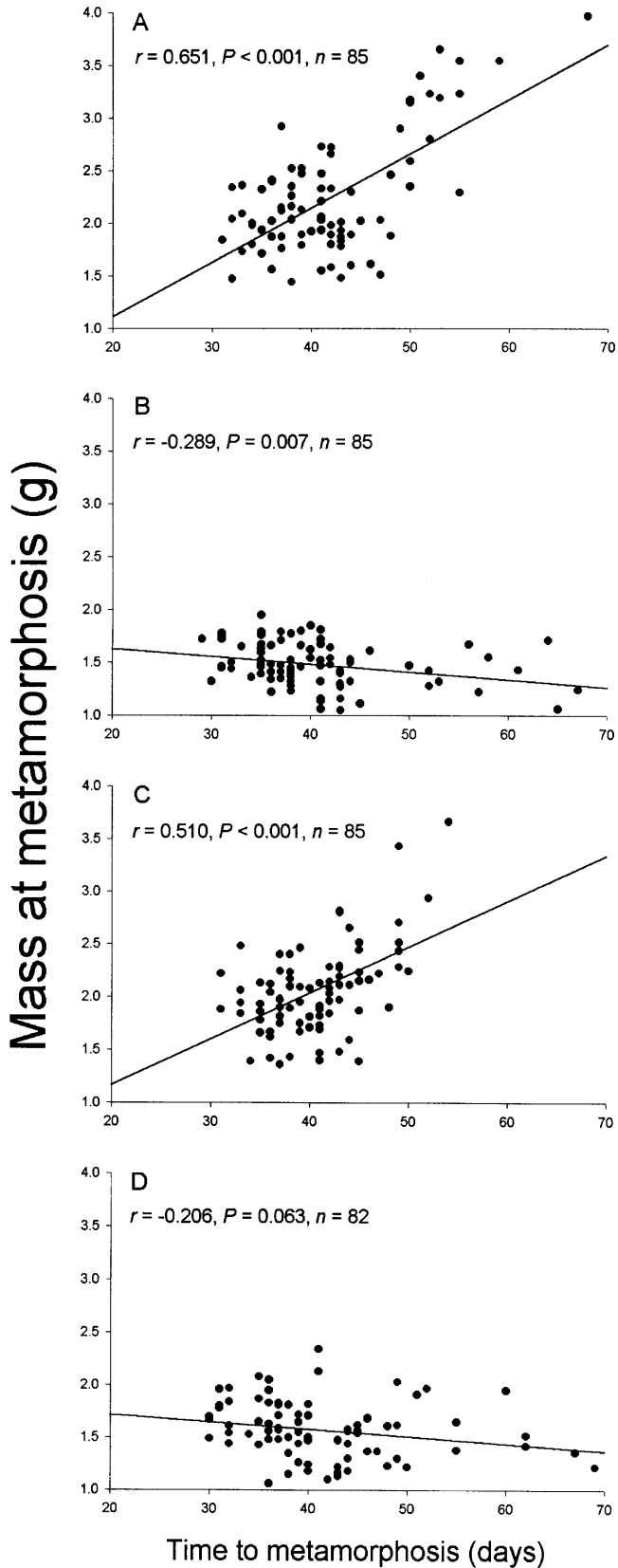


Fig. 6 Proportion of *Litoria aurea* tadpoles (+1 SE, $n=6$) occupying the side of the container which housed one mosquitofish (i.e. “predator half”). There was no statistically significant difference in the distribution of tadpoles (predator: $F=2.48$, $df=1,20$, $P=0.15$; hydroperiod: $F=4.93$, $df=1,20$, $P=0.06$; interaction: $F=8.94$, $df=1,20$, $P=0.02$), although fewer tadpoles occupied the predator half in the declining water. *Closed bars* represent the constant water level treatments; *open bars* represent the declining water level treatments

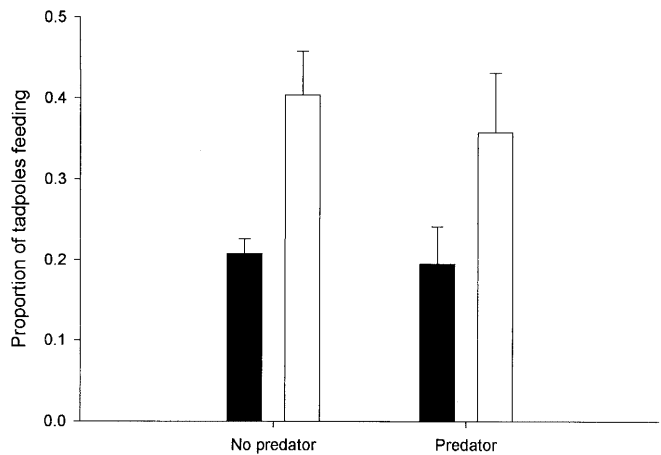


Fig. 7 Proportion of *Litoria aurea* tadpoles (+1 SE, $n=6$) feeding. Significantly more tadpoles were feeding under the declining water treatment than under the constant water level treatment (predator: $F=0.39$, $df=1,20$, $P=0.55$; hydroperiod: $F=12.25$, $df=1,20$, $P=0.01$; interaction: $F=0.07$, $df=1,20$, $P=0.79$). *Closed bars* represent the constant water level treatments; *open bars* represent the declining water level treatments

Discussion

The introduced mosquitofish has been implicated in the decline of *Litoria aurea* because it is a voracious predator of its eggs and tadpoles. Although once reported to be a permanent pond-breeder (Courtice and Grigg 1975), *L. aurea* now apparently requires ephemeral water bodies for breeding because they generally lack mosquitofish (Pyke and White 1996). We tested the responses of *L. aurea* tadpoles to the presence of mosquitofish and to a declining water volume. The experimental

cohort reached metamorphosis asynchronously over 41 days. Tadpoles exhibited no behavioural or developmental responses to the presence of mosquitofish. Individuals metamorphosed at a smaller mass in a declining water volume, but there was no reduction in mean time to metamorphosis. In constant water level treatments, mass at metamorphosis increased in individuals with a prolonged larval period. These results suggest that *L. aurea* tadpoles are adapted to develop in mosquito-free water bodies at the more permanent end of the hydroperiod gradient. Individual variability in the duration of the larval phase implies that a bet-hedging strategy exists within a cohort, probably to cope with environmental uncertainty.

The cohort raised in our experiment metamorphosed asynchronously between day 29 and day 69. This intra-clutch variability in duration of larval period has several benefits for species living in environments where rainfall is unpredictable and where even permanent water bodies may dry occasionally, especially during drought periods induced by El Niño. For instance, individuals that metamorphose first escape desiccation, though at the cost of a smaller size at metamorphosis.

However, there are also benefits for tadpoles that are slower to develop. These individuals, whilst being vulnerable to pond desiccation, have more time to gather resources in order to metamorphose at a larger size. This was observed in our study in which mass at metamorphosis, in the constant water level treatments, increased in individuals with longer development times. These benefits may carry on into the terrestrial life phase, where larger metamorphs have greater locomotory capacity (Goater et al. 1993; Beck and Congdon 2000) and higher tolerance to dehydration (Newman and Dunham 1994), both of which may increase survival to maturity (Smith 1987). It appears that a bet-hedging strategy would maximise the chance of at least part of a cohort surviving to metamorphosis in water bodies of unpredictable duration. Because the larvae of *L. aurea* have no ability to adapt to a declining water volume, this would best be achieved in water bodies which are at the more permanent end of the hydroperiod spectrum.

Bet-hedging within a cohort may be achieved genetically, whereby individuals are programmed to develop at a fixed interval (Newman 1988). This would predispose individuals to vary in their abilities to cope with an uncertain aquatic environment. The large clutch size of *L. aurea* (4,000–6,000 eggs; van de Mortel and Buttemer 1996), which would include a wide spectrum of development times, may insure against losing an entire cohort if pond drying occurs.

That tadpoles metamorphosed at a smaller size in declining water, but that there was no acceleration in development, suggests that factors other than water volume were responsible. For instance, competition between tadpoles in small water volumes can reduce mass at metamorphosis (Wilbur and Collins 1973; Semlitsch and Caldwell 1982; Tejedo and Reques 1994). However, tadpoles in our experiment were fed ad libitum, thereby

eliminating the potentially confounding effects of competition. An alternative explanation may be that crowding in the declining water treatments resulted in the production of chemicals (or an alga) by tadpoles that were inhibitory to growth but not to development (Rose 1960; Petranka 1989). Although water in the constant and in the declining water treatments was changed frequently (every 3 days) to remove accumulated waste products, this may have been offset by the high larval density in the declining water. Prior to emergence of the first metamorph, for instance, the constant water volume housed one tadpole per litre, whereas density in the declining water at day 27 was three tadpoles per litre. Moreover, the smaller metamorphic size may have been caused by stress from interference because of a reduced swimming volume. Therefore, the smaller mass at metamorphosis observed in our experiment may be a result of a deterioration in the aquatic environment and not of competition. Furthermore, prolonged exposure of tadpoles to these adverse conditions resulted in a smaller mass at metamorphosis.

A number of experimental caveats may explain the responses observed in the behaviour of tadpoles. Although not statistically significant, there were fewer tadpoles occupying the no-predator half in the declining water level treatments. This may be due to the reduction in space available to the tadpoles in this half as a consequence of the water volume displaced by the container housing the mosquitofish. We do not consider it to be biologically significant, since there was no evidence of predator avoidance in the constant water treatments and, if tadpoles were able to respond to the presence of mosquitofish, it would be expected both in constant and declining water level treatments. Encounter rates may be responsible for the greater numbers of tadpoles observed feeding in the declining water, because the probability of a tadpole finding the food would increase in smaller water volumes.

Although tadpoles in more permanent water bodies may benefit from a prolonged developmental period, there are risks from predation if mosquitofish are present. This fish is more likely to persist in these water bodies, since they are eliminated from more ephemeral ones when they dry. While some species may respond to predation risk through a number of strategies (Lawler 1989; Relyea 2001a, b), *L. aurea* was observed to possess none of these and appeared to be naive to the presence of mosquitofish. Hence, individual cohorts would be expected to incur significant losses if they co-occur with mosquitofish.

In contrast, the common and widespread Australian frog *Limnodynastes tasmaniensis* has been shown to avoid mosquitofish under experimental conditions (Lane and Mahony, unpublished data). This species has not been reported to be in decline, even though it co-exists with mosquitofish in many water bodies (Hamer 1998). This may be possible because of its ability to respond to the presence of mosquitofish, which would reduce losses through predation.

Differential sensitivity to predation by mosquitofish may occur in Australian frogs, and their ability to co-exist may depend on previous exposure to native fish predators. It appears that *Litoria aurea* has no history of co-existence with fish, despite its larval life-history being suited to more permanent water bodies where fish are more likely to be present. However, such water bodies may still dry occasionally, thus eliminating fish, which reinvade only when flooding occurs. Moreover, densities of native fish known to eat the tadpoles of *L. aurea* (e.g. gudgeons; Pyke and White 2000) probably never reach those of mosquitofish, which may become very high to the point at which it is regarded as a pest in some areas (Merrick and Schmida 1984). Therefore, densities of native fish in these water bodies, if they occur at all, are likely to be low, which would result in few encounters between fish and tadpoles. Finally, native gudgeons have been shown to have lower levels of predation on the tadpoles of *L. aurea* than mosquitofish (Pyke and White 2000).

Historical descriptions of the habitat of *L. aurea* include permanent ponds (e.g. Courtice and Grigg 1975), and there is no mention of its use of ephemeral water bodies, although this may be because they were not included in previous surveys. This conflicts with current descriptions. For instance, Pyke and White (1996) analysed the habitat of 74 locations where *L. aurea* has been recorded and found that the majority of breeding sites during the study were ephemeral water bodies which generally lacked fish. They also found that *L. aurea* had disappeared from many permanent water bodies where mosquitofish were present. However, the tadpoles of *L. aurea* have been observed in permanent water bodies in the Gippsland region of Victoria, which is free of mosquitofish (Gillespie 1996). Therefore, it is likely that *L. aurea* uses permanent and ephemeral water bodies as ovipository sites throughout its present range, but recruitment to the terrestrial stage occurs predominantly in ephemeral water bodies in areas where mosquitofish occur, such as New South Wales. Although there have been observations of tadpoles co-existing with mosquitofish in permanent water bodies (e.g. van de Mortel and Goldingay 1998; Hamer et al. 2002), it is likely that the reproductive output at these sites was reduced because of fish predation.

The breeding habitat of *Rana muscosa* in California's Sierra Nevada, originally deep lakes, is now predominantly shallower ones, because of the presence of introduced trout which can only persist in deeper water bodies (Knapp and Matthews 2000). However, the long development time of *R. muscosa* tadpoles (2–4 years) renders them especially vulnerable to desiccation in shallow lakes during summer. Therefore, the deep lakes that *R. muscosa* once inhabited are now unsuitable, since they are generally occupied by trout and the species is now restricted to marginal habitats where it is becoming extinct. This seems to be a similar scenario to that of populations of *L. aurea* in New South Wales and may be one that is limiting anuran reproduction elsewhere to less than ideal habitats.

Although mosquitofish are eliminated from ephemeral water bodies when they dry out, their removal from permanent ones is often difficult and may be impossible. However, several methods may be employed to improve larval survival at these sites. For instance, fish poisons such as rotenone have been successfully used in a number of situations to remove fish when tadpoles were not present (e.g. Brönmark and Edenhamn 1994; Hamer 1998). Alternatively, increasing the structural complexity of water bodies by providing dense aquatic vegetation may reduce fish/tadpole encounters, and consequently decrease the number of tadpoles killed. This may be a more appropriate means of managing sites for *L. aurea*, because mosquitofish often reinfest water bodies from the surrounding catchment when flooding occurs (Hamer 1998). Furthermore, recruitment has been observed in densely vegetated permanent water bodies that contain mosquitofish (van de Mortel and Goldingay 1998; Hamer 1998), indicating that the two can co-exist under these conditions.

The fitness of populations of *L. aurea* may be compromised if individuals metamorphose from ephemeral ponds at a smaller size; that is, if they metamorphose at all in rapidly drying habitats. This would be especially so in those water bodies with a higher frequency of drying out. The result could be reduced recruitment to the adult population (Morey and Reznick 2001), lowered fecundity and reproductive success (Berven 1982), which would affect subsequent generations, or the complete loss of a cohort if the pond dries. Moreover, if *L. aurea* breeds in permanent water bodies containing mosquitofish and little aquatic vegetation, there are likely to be few tadpoles surviving to metamorphosis.

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