

Larval over-wintering: plasticity in the timing of life-history events in the common frog

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Abstract

In ectothermic animals living in temperate regions, winter is a critical time. During early ontogeny, the stage or size an individual reaches at the onset of winter can have a significant effect on its survival and other life-history characteristics. Generally, temperate amphibian larvae complete metamorphosis in the summer and spend their first winter as juveniles. However, some anuran amphibians show variation in this aspect of their life history, with some individuals remaining as aquatic larvae during winter. We investigated growth and development during the larval period of *Rana temporaria* at a field where over-wintering has been recorded. In the laboratory, we investigated whether larval over-wintering could be induced at temperatures equivalent to those recorded in the field pond; this was done at both high and low food availability. Larvae within the field pond showed a bimodal distribution in developmental stage as early as July, when temperatures were still increasing, separating individuals that would metamorphose and over-winter as juveniles from those that would spend the winter as larvae. Individuals that over-wintered as larvae remained at a relatively undeveloped larval stage throughout the summer and autumn. The decision on whether to over-winter as a larva appears to be made relatively early in the season. Those individuals that adopt this slow developmental trajectory may benefit in being able to metamorphose at a larger size in Spring. In the laboratory, mean temperature and food availability affected development and growth, but did not affect the life-history stage at which individuals over-wintered. Our results suggest that there are other factors, in addition to temperature and food availability that contribute to the observed plasticity in development pattern and over-wintering strategy.

Introduction

In many animals, the timing of life-history transitions is known to vary in response to environmental conditions experienced during early development. In temperate regions, the life-history stage or size that an individual reaches by the onset of winter can have a significant effect on survival and life-history trajectory (Gotthard, 2001). This has been well studied in a number of species, including the Atlantic salmon *Salmo salar*, where parr migrate to sea either after their first year, or delay migration for one or more years, spending additional time in the larval habitat (Thorpe, 1977; Metcalfe, Huntingford & Thorpe, 1988). In salmon, the decision on whether to undergo smolting is made long before the onset of winter, and a bimodal distribution in size is evident relatively early in the growing season. This separates the two modal groups that will either undergo smolting within the same year, or delay until the next (Thorpe, 1977; Thorpe, Metcalfe & Huntingford, 1992). A similar phenomenon has also been observed in the duration of the larval phase in some amphibians, with some

tadpoles within a pond not completing metamorphosis in the year they were spawned, as is most often the case, but instead over-wintering as larvae and metamorphosing into juvenile froglets the following spring (e.g. Pintar, 2000).

Generally, temperate amphibian larvae exploit freshwater habitats during the spring and summer, grow rapidly, and complete metamorphosis before leaving the aquatic habitat before the onset of winter (Wilbur & Collins, 1973; Werner, 1986; Stebbins & Cohen, 1995). Reports of over-wintering by anuran larvae are increasing, in both public media and the scientific literature (e.g. Collins & Lewis, 1979; Archibald & Downie, 1996; Pintar, 2000; Lai *et al.*, 2002). However, there has been little detailed or experimental investigation of this variation in the duration of the tadpole phase. It is not known whether a bimodal distribution, which could appear in either developmental stage or size, arises in natural populations where over-wintering of amphibian larvae occurs. Similarly, it is not known in amphibians at what point the over-wintering stage of an individual is determined, whether (as in salmon) it occurs early in the season or later as a result of ecological factors.

However, it has been predicted (Lai *et al.*, 2002) that, in anuran amphibians that over-winter as larvae, development should be arrested early (*c.* Gosner (1960) stages 32–35) to protect the developing hind limbs from prolonged exposure during cold winter temperatures and that this reduction in development would be accompanied by a reallocation of investment into growth.

Descriptive studies have suggested an increase in the occurrence of larval over-wintering with higher latitude in *Rana catesbeiana* (Collins, 1979) and altitude in *Rana sauteri* (Lai *et al.*, 2002), both of which have been attributed to temperature. Temperature and food availability have been shown to influence the proportion of individuals over-wintering as larvae in the common frog *Rana temporaria* under laboratory conditions (Walsh, 2008). However, no attempt has been made to examine the pattern of over-wintering larvae in relation to conditions in the field.

In the present study, we investigated growth and development throughout the year in a field population of *R. temporaria* tadpoles to determine whether a bimodal distribution in size or developmental rate occurs during the growing season, which could be linked to over-wintering as larvae. Observations from the field were also used to determine the developmental stage at which tadpoles over-wintered. We also investigated growth and development in a laboratory population based on temperatures experienced at the field site where regular over-wintering occurs. Resource availability in the field population was not known, so the temperature regimes were applied at relatively high and low food availabilities in the laboratory, because the effect of temperature could vary with feeding conditions.

Methods and materials

Field site

A survey of garden and parkland ponds around Glasgow, Scotland, was conducted in the winter of 2004 to identify suitable sites to study over-wintering in *R. temporaria* larvae. Over-wintering *R. temporaria* larvae were observed in *c.* 20% of the 21 ponds surveyed. Only one of the ponds where over-wintering occurred was large enough to be a suitable study site and contained sufficient numbers of over-wintering larvae. This was Drumtian Pond, located near the village of Killearn, Scotland [NS 525855]. It was constructed in 2001, and measures 5 × 8 m. The pond has vertical sides, with exit ramps for metamorphosing froglets along the sides, and water depth is around 1 m. *Rana temporaria* spawn, from several locations in western Scotland, was added in 2001, 2002 and 2004. The pond is now an established breeding site for adult *R. temporaria*. Over-wintering tadpoles were first observed in the pond in 2002 (P. Monaghan, unpubl. data).

The field site, in addition to having adult and larval *R. temporaria*, has other species present, including several that are predators of tadpoles (e.g. newts *Lissotriton* (= *Triturus*) spp, dragonfly larvae and beetles from the family Dytiscidae). There are no fish present. The pond

bottom is covered in a layer of fine organic matter and dead leaves. There is little aquatic vegetation, but high levels of micro-algae. It was not possible to accurately assess tadpole density at the field site, because distribution within the pond was very patchy and clumps of tadpoles remained concealed in the bottom sediment making it difficult to determine our capture efficiency.

To quantify the thermal environment, temperature data were collected continuously for 1 year beginning in March 2005 using waterproof TinyTalk data loggers. Two loggers were used: one to establish temperature just below (1 cm) the pond's surface, the second to record temperature at the pond bottom. Data loggers recorded the temperature every 11 min and the data were downloaded every 2 weeks.

Tadpole growth and development were monitored on a monthly basis starting in mid-May, until the study concluded in mid-January 2006. Up to 80 individuals were randomly collected from the pond once per month using a large pond net. As the season progressed, it became more difficult to capture sufficient numbers, so a standardized capture effort of 30 min was used. Individuals were taken to the laboratory for measurement and returned to the pond within 24 h. Mass (± 0.1 mg) was measured using an electronic balance. Snout–vent length (SVL) (± 0.1 mm) was measured with dial callipers. Development was measured by staging individuals, at $\times 10$ magnification, according to Gosner's (1960) staging table. Individuals that had not commenced metamorphosis by November following their spawning were considered to be over-wintering.

Laboratory experiments

Drumtian Pond was originally stocked with tadpoles taken from a number of sites in the Glasgow/Loch Lomond area. Although this was done relatively recently, it is possible that conditions in the pond have favoured a particular genotype. Therefore, in order to test whether a thermal regime similar to that recorded in Drumtian Pond would itself induce over-wintering in tadpoles collected from other populations, several clumps of *R. temporaria* spawn were collected in mid-March 2005 from Robroyston Marsh and Queens' Park in Glasgow, Scotland. It was not possible to also have samples of the Drumtian tadpoles in the laboratory for similar study, because this would have caused significant depletion of tadpole numbers, and potentially altered resource availability, in Drumtian Pond. The egg clumps taken from Glasgow to the laboratory were maintained together in a 40 L tank at 5.5 °C until after hatching and the tadpoles were large enough to handle (*c.* Gosner stage 25), at which point the tadpoles were transferred to experimental tanks.

Experimental tanks were of 12 L capacity measuring 30 × 20 × 20 cm, and filled with 11 L of de-chlorinated, copper-free, aerated water. From April, water temperatures in the tanks were matched to the temperatures recorded at the bottom of Drumtian Pond, where tadpoles in the field were observed to spend the majority of their time. Tank temperatures were re-set on a fortnightly basis, based on the

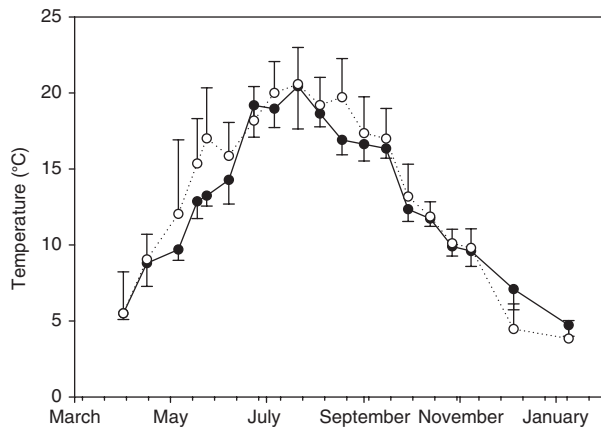


Figure 1 Water temperature throughout the season, calculated as the fortnightly mean temperatures (with SD) at the surface (○) and the bottom (●) of Drumtian Pond. The temperatures recorded at the bottom of Drumtian Pond were used for the laboratory experiments as described in the text.

mean temperature recorded in Drumtian Pond over the 2-week period (Fig. 1).

In order to examine whether resource availability influenced larval over-wintering probability, the tadpoles in the laboratory were fed on a 3:1 rabbit pellet:fish flake mixture (c. 10% protein), given three times a week. Two food availabilities were established: high food availability constituted a 50% increase over the level recommended by Relyea (2001) that is 9% of total tadpole biomass per tank; low food availability was set at 3% of the total tadpole biomass per tank. Total tadpole biomass per tank was calculated as the average mass of all tadpoles measured in the laboratory in both food level treatments multiplied by the number of individuals remaining in the tank. This was done to standardize the food provided between the treatment groups, because tadpole masses diverged between treatments. Photoperiod was set to reflect ambient conditions and ranged from 7 L:17 D in December to 17 L:7 D in July; it was changed monthly to reflect the monthly average photoperiod.

In total, eight experimental tanks were established, each containing 75 randomly allocated *R. temporaria* tadpoles from both collection locations (density: 6.82 tadpoles L⁻¹), with four tanks for each food availability treatment. Tanks were cleaned bi-weekly and checked weekly for any mortality; dead individuals were removed from the tanks. Tanks were topped-up as required with de-chlorinated, copper-free water to maintain a constant water level. All tadpoles not used in laboratory experiments were deposited at the field site.

Ten randomly selected individuals from each tank were measured monthly using the same methods as for field tadpoles. Tanks were also checked weekly for individuals entering metamorphosis. Individuals that had commenced metamorphosis (Gosner stage 42; fore-limbs emerged) were transferred to individual tubs with a small amount of water.

On completion of metamorphosis, individuals were released near the sites from where the spawn was collected.

Data analysis

All values are given as mean \pm SE, unless otherwise stated. All analysis was performed using SPSS v15. The occurrence of bimodality in the distribution of developmental stage was assessed by visual examination of frequency distributions for each month data were available. Mass and SVL measurements, which did not display a bimodal distribution, were assessed for normality. Comparisons of the mass and SVL among the field and two laboratory populations in mid-July were performed using generalized linear mixed models, with tank as a random factor. Differences in developmental stage among the field and laboratory populations was analysed using a non-parametric Kruskal-Wallis test.

Results

Over-wintering in the field

A bimodal distribution of developmental stage among the tadpoles collected from Drumtian Pond became clearly visible from July, and persisted until the onset of winter in November (Fig. 2). Both mass and SVL, with the exception of the months shortly after hatching, were normally distributed (Table 1). Metamorphosing individuals, spawned in the current breeding season (2005), were observed in samples from the pond on several occasions during the summer of 2005.

Over-wintering tadpoles were recorded in the field in 2005/2006, with a sample of 34 individuals captured as tadpoles on the 8th of November. At the onset of winter in November, remaining tadpoles had reached a mean Gosner stage of 32.7 ± 0.7 . Stages 32–33 are characterized by small hind-limb buds that are only beginning to differentiate into toes. Tadpoles were recorded continuously throughout the winter, with samples of tadpoles being captured in December (mean Gosner stage = 34.1 ± 0.6 , $n = 26$) and January (mean Gosner stage = 35.8 ± 0.4 , $n = 19$). Tadpoles spawned in the spring of 2005 were still present at the time of the 2006 spring spawning in March, and were then at advanced developmental stages (c. Gosner stage 39–40).

Effect of food availability on tadpole over-wintering

Survival through to metamorphosis in the laboratory was relatively low ($39.67 \pm 8.12\%$ survival). There was no difference in mortality between the two treatments ($t = 0.457$, d.f. = 6, $P = 0.664$), and thus the change in tadpole density was consistent across the food availability treatments. Over-wintering was not observed in either the high or low food treatment. Individuals from both food treatments were first observed to commence metamorphosis in the beginning of July. In the high food treatment, all surviving individuals

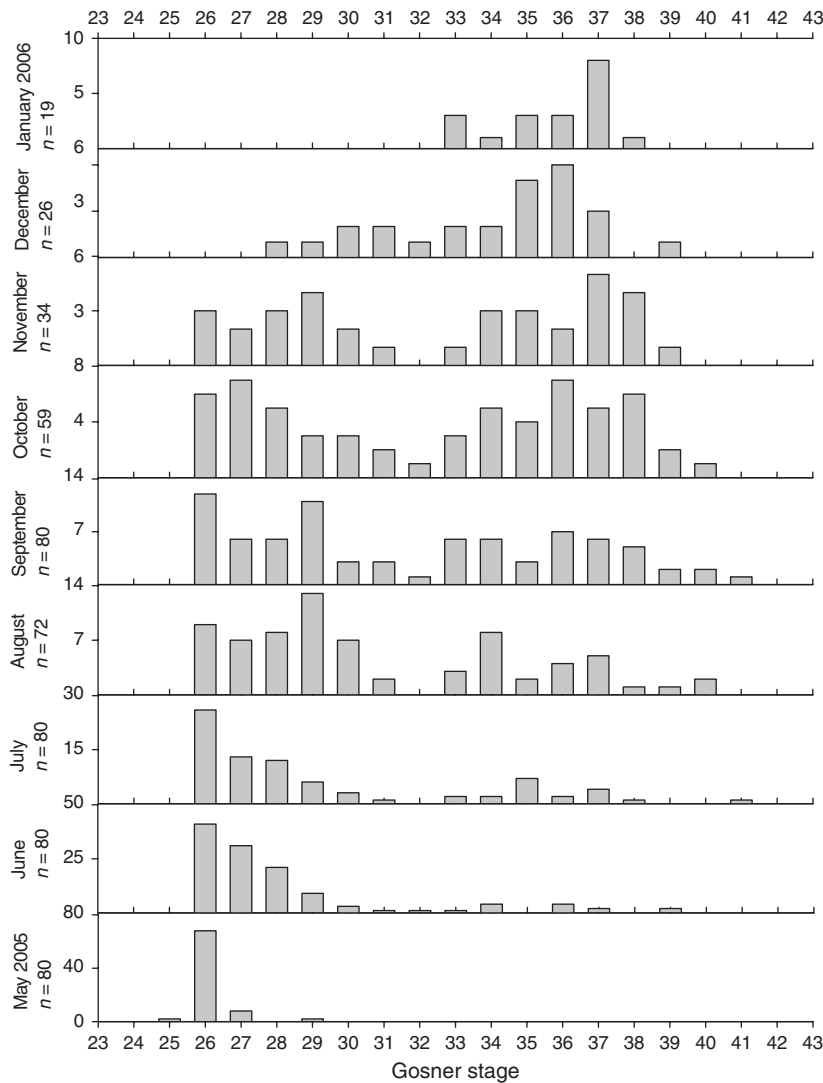


Figure 2 Monthly developmental stage frequency distributions of tadpoles found in the field at Drumtian Pond.

Table 1 Monthly mean (\pm SE) mass (mg) and snout-vent length (SVL) (mm) of individuals from Drumtian Pond and the high and low food treatments in the laboratory

	Low food treatment		High food treatment		Drumtian Pond	
	Mass	SVL	Mass	SVL	Mass	SVL
April 2005	26.2 \pm 1.6	4.9 \pm 0.1	23.0 \pm 1.3	4.7 \pm 0.1	–	–
May	98.5 \pm 47.8	7.5 \pm 0.1	101.4 \pm 4.2	7.6 \pm 0.1	71.2 \pm 4.5*	6.8 \pm 0.1*
June	304.8 \pm 16.5	11.8 \pm 0.2	439.3 \pm 15.1	13.4 \pm 0.2	172.7 \pm 10.0*	9.4 \pm 0.2
July	293.9 \pm 12.9	12.0 \pm 0.2	402.0 \pm 14.8	12.9 \pm 0.2	233.6 \pm 12.7	10.6 \pm 0.2
August	233.5 \pm 42.8	11.0 \pm 0.6	–	–	320.0 \pm 13.8	11.9 \pm 0.2
September	–	–	–	–	371.3 \pm 13.8	12.3 \pm 0.2
October	–	–	–	–	392.6 \pm 13.3	12.6 \pm 0.1
November	–	–	–	–	360.9 \pm 23.2	12.4 \pm 0.2
December	–	–	–	–	374.3 \pm 20.9	12.7 \pm 0.2
January 2006	–	–	–	–	371.5 \pm 21.7	12.8 \pm 0.3

Monthly size measurements that deviated from normality ($P < 0.01$) are marked with an asterisk.

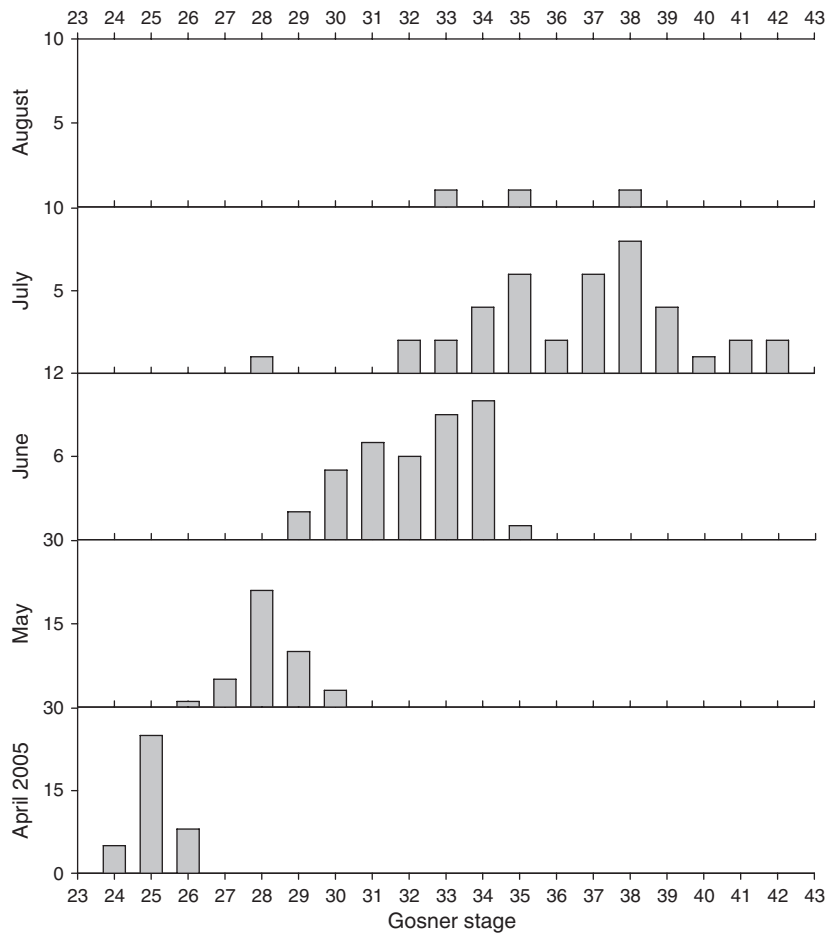


Figure 3 Monthly developmental stage frequency distributions of tadpoles reared in the laboratory under low food availability. In each month $n=40$, except August ($n=3$).

had commenced metamorphosis by the end of July. All but one individual in the low food treatment had commenced metamorphosis by the end of August; the remaining tadpole died in early September.

In both food availability treatments in the laboratory, a single size distribution was maintained throughout development, with mass and SVL normally distributed in every month (Table 1). A bimodal distribution in developmental stage was not established in either the low (Fig. 3) or high food treatment (Fig. 4). The distribution of developmental stage did develop a 'tail' in the low food treatment in July, with some individuals trailing behind the majority in development rate (Fig. 3).

Pattern of development

Since all surviving individuals from the laboratory treatments completed metamorphosis by the end of August, it was not possible to compare laboratory and field over-wintering individuals. Therefore, comparisons were made among the rates of development and growth at peak season (July) in the laboratory treatments and Drumtian Pond.

The dataset comprised three groups: the field population, laboratory high food treatment (Lab High) and laboratory low food treatment (Lab Low). In July, field tadpoles had

the lowest mass (Table 1) of the three groups ($F_{2,9} = 19.63$, $P = 0.001$). However, the difference between the mass of the field and Lab Low individuals was only marginally significant ($P = 0.043$). SVL followed the same pattern with field individuals being shortest (Table 1), followed by Lab Low and then Lab High individuals ($F_{2,9} = 20.87$, $P < 0.001$, Table 1). The developmental stage reached by July was also significantly different in the three groups ($\chi^2 = 69.62$, $P < 0.001$), with the field tadpoles only reaching stage 29.0 ± 0.6 , while Lab Low tadpoles had developed to stage 36.6 ± 0.5 and Lab High tadpoles to stage 39.6 ± 0.2 . Tank was not found to have a significant effect in any analyses.

Although the field tadpoles on average were lower in mass than laboratory tadpoles in July, for a given stage throughout development field individuals were heavier than those in either food treatment in the laboratory ($F_{28,1525} = 9.36$, $P < 0.001$; Fig. 5). As would be expected, Lab High individuals had a greater increase in mass through development than was found in Lab Low individuals ($t = 11.70$, $P < 0.0005$).

Discussion

Over-wintering of *R. temporaria* tadpoles was confirmed in the field in Scotland, with some tadpoles completing

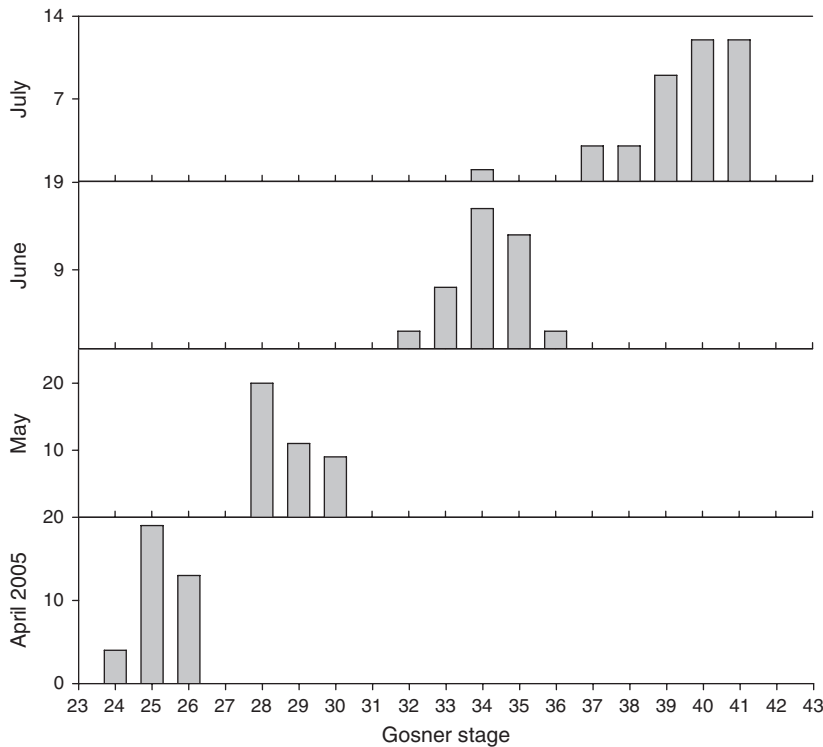


Figure 4 Monthly developmental stage frequency distributions of tadpoles reared in the laboratory under high food availability. $n=40$ in each month.

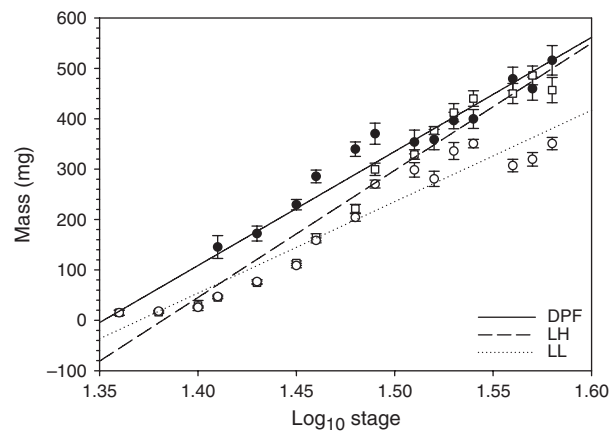


Figure 5 Mass at a given stage for Drumtian Pond (DP), Lab High (LH) and (Lab Low) LL, with SE bars. Regression lines show the increase in mass through developmental stages. ●, DP; □, LH; ○, LL.

metamorphosis in the summer and autumn while others remained in the pond as tadpoles until the following spring. In March 2006, several large, over-wintered larvae were observed in the pond at stages just before the onset of metamorphosis. Over the course of spring 2006 their numbers decreased; while mortality may have been responsible, it seems most likely that such well-developed individuals metamorphosed, as was observed by Pintar (2000). This represents a clear example of plasticity in the timing of life-history transitions. At this study site, the formation of two distinct developmental groups was evident from shortly

after the larvae became free-swimming. In contrast to the bimodality in developmental trajectories, tadpoles in the field pond showed a normal distribution in mass and SVL throughout the year. The mean mass and SVL of field tadpoles increased during the summer until November, when they levelled off, indicating that both groups were increasing in body size at a similar rate.

The formation of the two developmental groups suggests that after May, a proportion of individuals arrested their development but continued to grow, while the second group comprised successive waves of individuals progressing through intermediate developmental stages before commencing metamorphosis. The formation of the first group, which arrested development early in the tadpole phase, suggests that the individuals destined to over-winter were determined early in development and at a time when temperatures were still increasing and growth in body size was still progressing. Therefore, the occurrence of over-wintering in anuran larvae in the field may be based on a decision early in development similar to that observed in the Atlantic salmon (Thorpe, 1977; Metcalfe *et al.*, 1988). The mechanisms that determine this difference in life-history pattern are not clear. The modal group of an individual could be determined genetically, with some predetermined to over-winter as larvae regardless of local conditions. This seems unlikely since many of the adults breeding at the study pond are likely to be the result of spawn introduced from other sites where larval over-wintering has not been recorded. Additionally, over-wintering tadpoles are not universally present in nearby ponds. The formation of the two distinct groups is likely to be determined entirely by variable

responses of individuals to local environmental conditions, or differences in their competitive ability. This could come about because gene by environment interactions are likely, with certain local environmental cues triggering a particular developmental pathway in a genetic subset of the population. Alternatively, variable exposure to factors in early life could play a role.

Earlier descriptive work suggests that individuals should over-winter at stages before the development of vulnerable structures important to the adult phase (e.g. the hind limbs) (Lai *et al.*, 2002). Our field results accord with this suggestion, with the mean stage of individuals in the field being between 32 and 33 in November and below 36 in January. Before stage 36 the hind limb buds are small and held close to the body reducing their exposure to low temperatures and predator attack. This suggests that over-wintering at this developmental stage might represent an adaptive strategy. Tadpoles over-wintering might also be expected to reallocate investment away from development and into growth to reach larger sizes. This appears to be supported since field tadpoles were significantly heavier for a given stage than both laboratory treatments, but, in July, individuals from the field were significantly less well developed. The larger size of tadpoles might be an adaptive response to environmental conditions and could provide a survival advantage for individuals that will remain as larvae during the winter (Partridge & Coyne, 1997; Sogard, 1997; Renault *et al.*, 2003). Presumably, such individuals would have reduced fitness if they over-wintered as froglets than as large tadpoles.

Low temperature during the larval growth season has often been predicted (Berven, Gill & Smith-Gill, 1979; Collins, 1979; Lai *et al.*, 2002) to be a factor influencing the occurrence of over-wintering larvae in some amphibian species. In the current study, larval over-wintering was not observed in the laboratory when tadpoles, taken from another location in the same geographical area from which the field pond was originally stocked, were reared at the temperatures experienced by the larval over-wintering population in the field. While not taking spawn from the field pond for the laboratory experiments potentially introduces confounding genetic variables into the interpretation of the results, our results are not complicated by a lack in any genetic potential to over-winter as larvae. In a separate study at lower temperatures, tadpoles from the same collection sites, as used in the experiments reported here, were found to over-winter (Walsh, 2008). That this did not happen at temperatures similar to those in the field pond, where substantial numbers of tadpoles over-wintered, suggests that temperature by itself does not determine larval over-wintering as has been predicted (Berven *et al.*, 1979; Collins, 1979; Lai *et al.*, 2002). However, there are other possible reasons for our not having observed over-wintering in the laboratory. Firstly, it is possible that there has been rapid selection at this field site making over-wintering more prominent even at temperatures that would not normally induce this phenomenon. Secondly, the mean fortnightly temperature may not be the best indicator of the thermal conditions experienced by individuals in the field. Due to

diurnal fluctuations, mean temperature may not be the most commonly experienced temperature in the field. However, over the 2-week periods that the temperature was recorded continuously, the standard deviations in temperatures at the bottom of Drumtitan Pond were very low (generally < 1.5 °C; CoV: 9.8%, Fig. 1).

Predation risk, not investigated in this study but present in Drumtitan Pond and absent from the laboratory treatments, could contribute to larval over-wintering. Increased predation risk is predicted to result in shorter larval durations (Wilbur, 1980), but this is rarely found in empirical studies (Relyea, 2007), due to behavioural or induced phenotypic defences that extend the duration of larval development. Tadpoles from the field site, in this study, displayed predator-induced phenotypic traits (short, deep bodies and deep tails: P. T. Walsh, pers. obs.) common to aquatic prey species (e.g. Bronmark & Miner, 1992; Relyea, 2004). Finally, the food levels used in the laboratory treatments may have been greater in quantity or quality relative to the actual conditions in the field. The occurrence of tadpoles feeding on deceased conspecifics, which was observed in the field, has been reported to be induced by low food availabilities (Wildy *et al.*, 2001). Additionally, tadpoles in the laboratory were fed a relatively protein-rich diet, which can increase growth and development rate (Steinwascher & Travis, 1983; Kupferberg, 1997).

There was an obvious effect of food availability on growth and development, with the high food availability treatment growing and developing faster than the low food treatment. However, at temperatures similar to Drumtitan Pond, food availability in the laboratory did not influence the over-wintering strategy, with all surviving individuals completing metamorphosis before the onset of winter in both treatments.

In conclusion, over-wintering of *R. temporaria* larvae in the field appears to be determined early in development, with individuals, that will continue to remain in the pond over winter arresting development at an early stage. This provides some evidence that the developmental arrest and subsequent larval over-wintering may be adaptive. However, the mechanisms that determine the arrest in development are not clear. Our results suggest that temperature is not the main factor in determining whether individuals over-winter as larvae.

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