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## RESEARCH PAPER

# Density-dependent performance of larval and juvenile toads: Implications for amphibian conservation

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## ABSTRACT

Density dependent carry-over effects from one life history stage to another can affect the dynamics of populations. Here we study such carry-over effects from the tadpole to the postmetamorphic juvenile stage in an endangered amphibian, the natterjack toad (*Epidalea calamita*). We raised tadpoles in outdoor aquatic mesocosms at four densities and assessed juvenile performance after metamorphosis in terrestrial mesocosms. High larval density reduced mass at metamorphosis by 50 % and doubled the length of the larval period. Survival was reduced at the high densities. Larger metamorphs had higher survival in terrestrial mesocosms and remained larger than cohort members at the end of the 30-day experiment. Because juvenile survival drives amphibian population dynamics, density-dependent carry-over effects to the juvenile stage are likely to affect population viability. We discuss the implications of the results for amphibian conservation practice, both pond construction programs and surveys of amphibian populations.

## Introduction

Density dependence is thought to be one of the main drivers of animal population dynamics because population density can affect individual survival, growth and reproduction. Ultimately, the effects of density on individuals can affect the growth rate of populations and thereby regulate population dynamics (Wilbur & Collins, 1973; Turchin, 1999; Lebreton & Gimenez, 2013). Regulation (or density dependence) occurs when the negative relationship between the density of individuals and population growth rate leads to a return point around which density fluctuates (Sinclair, 1989; Dennis & Taper, 1994; Bjørnstad & Grenfell, 2001). In many systems, density dependence is caused by scramble competition for resources (e.g., food). Density dependence has been of interest for ecologists for decades but it also matters for conservation biology. Density dependence can buffer populations against external stressors (Vonesh & De la Cruz, 2002; Forbes & Calow, 2002; Henle et al., 2004).

Density-dependent performance in one life history stage can lead to carry-over effects to the next stage. Carry-over effects were defined by O'Connor et al. (2014) as “in an ecological context, carry-over effects occur in any situation in which an individual’s previous history and experience explains their current performance in a given situation.” In

many species with complex life cycles, conditions experienced during larval life induce carry-over effects by altering the growth and development of individuals, leading to phenotypic variation in metamorphic traits of individuals that translate into differences in adult performance and fitness. In mayflies, for example, size at emergence translates directly into adult fecundity (McPeck & Peckarsky, 1998). In salamanders, individuals which are larger at metamorphosis are also larger at maturity (and therefore probably more fecund; Semlitsch et al., 1988). In frogs, individuals which are larger at metamorphosis have higher survival to maturity and are larger at maturity; individuals which metamorphose late have reduced survival (Altwegg & Reyer, 2003). Carry-over effects are a widespread phenomenon in the animal kingdom and have the potential to affect the dynamics of populations (Taylor & Scott, 1997; Pechenik, 2006; Ratikainen et al., 2008; Harrison et al., 2011) and may contribute to extinction processes (Lundsgaard et al., 2023).

Density dependence is well documented in amphibians and there is ample evidence for carry-over effects. Density often reduces food availability and leads to scramble competition. It can affect growth and survival of all stages in the complex life cycle. Density-dependent larval growth and survival can be induced in experiments (Wilbur, 1980), but was also reported from natural ponds (Smith, 1983; Loman, 2004). This

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is also true for density dependence in the postmetamorphic terrestrial stages, for which there is evidence from both experiments (Goater, 1994; Altwegg, 2003) and field studies (Berven, 2009; Kissel et al., 2022). Density is also known to modulate dispersal and the expression of polyphenisms (Semlitsch, 1987; Cayuela et al., 2019). Last but not least, population growth rate can also depend on density (Cayuela et al., 2019; Meyer et al., 1998; Salvidio, 2007).

In amphibians, density in the larval stage leads to carry-over effects to later stages in the complex life cycle because larval density dependence modulates metamorphic phenotypes. Typically, at high densities, the size at metamorphosis is reduced and the length of the larval period is increased (Smith, 1983; Scott, 1990; Tejado & Reques, 1994; Ousterhout & Semlitsch, 2016). The length of the larval period and size at metamorphosis affect juvenile growth and survival (Smith, 1987; Goater, 1994; Altwegg & Reyer, 2003) but fitness benefits of large size at or an early date of metamorphosis may not be universal, as they may depend on environmental conditions such as food availability (Morey & Reznick, 2001; Schmidt et al. 2012; Earl & Semlitsch, 2013; Tarvin et al., 2015). Amphibians that metamorphose early and at a large size may have higher fitness because they can have higher lipid levels, greater endurance, better jumping ability, greater resistance to dry conditions, lower susceptibility to pathogens and parasites and a greater ability to find food and frost-free hibernacula (Goater & Ward, 1992; Goater et al., 1993; Newman & Dunham, 1994; Tejado et al., 2000; Scott et al., 2007; Tobler & Schmidt, 2010; Cabrera-Guzman et al., 2013; Sinsch & Schäfer, 2016; Sinsch et al., 2020). Such effects of larval density dependence on juvenile performance may have strong effects on amphibian population dynamics because population growth rates are highly sensitive to juvenile survival (Petrovan & Schmidt, 2019).

Here, we study the effects of larval density dependence on larval and juvenile performance in an endangered toad, the natterjack toad (*Epidalea calamita*, previously known as *Bufo calamita*). This species has undergone strong population declines and is Red Listed in many European countries (Denton et al., 1997; Dufresnes & Perrin, 2015; Cruickshank et al., 2016). It is a species for which density-dependent larval growth and carry-over effects into the terrestrial environment have been documented (Tejado & Reques, 1994; Golay, 1996; Reques & Tejado, 1997, for additional studies see Sinsch (2008)). Population models for this species suggest that population viability strongly depends on juvenile survival (Stephan et al., 2001; Stevens & Baguette, 2008; Di Minin & Griffiths, 2011). We hypothesized that higher density would lead to lower mass at metamorphosis and to a longer larval period. In addition, we hypothesized that mass at metamorphosis would lead to higher growth and survival in terrestrial juveniles. We will discuss the importance of these results for amphibian conservation.

## Materials and methods

We used an experimental approach to study the effects of density dependence on tadpoles and juveniles of the natterjack toad (*Epidalea calamita*, Fig. S1) and complemented the experiment with a field study. The experiments were conducted at the Irchel campus of the University of Zurich, Switzerland and followed standard procedures.

The natterjack toad prefers early successional temporary ponds as breeding ponds. Breeding occurs in spring and summer after heavy rains have filled temporary ponds. While an individual female produces one clutch of eggs per year, there may be a number of breeding events in a population. Clutch size ranges from 2'000 to 10'000 eggs. Sexual maturity is reached after two or three years. Longevity is seven to nine years, but most individuals have a shorter lifespan (Van Buskirk, 2003; Sinsch, 2009). While there is evidence for density dependence in the larval stage in experimental settings in this species (e.g., Reques & Tejado, 1997), the conclusion from field studies was that larval density dependence played no major role for population dynamics (e.g., Beebee et al., 1996). Time series analyses of population dynamics did not test for density dependence (e.g., Buckley et al., 2014) but population models

have assumed density dependence. For example, Stevens and Baguette (2008) assumed density dependence in their population model. They used data from Sinsch (1996) to explore the strength of density dependence from time series data but did not report the results. We repeated the analysis and show the results in the Supplement (Fig. S10, S11, S12).

## Quantifying tadpole density, mass and developmental stage in the field

Using a type of quadrat sampling, we estimated tadpole densities in 20 ponds in northern Switzerland in the cantons Basel-Landschaft, Aargau and Zürich. To do so, we used a stick which was 0.5 m long. We allowed the stick to float on the water surface and took a picture from above. From the picture, we counted the number of tadpoles in a frame of 0.25 m<sup>2</sup>. We took ten pictures at ten haphazardly chosen localities within the pond. From the ten countings, we calculated the mean as an estimate of tadpole density. We took care not to disturb tadpole priors to taking the photos. The ponds were shallow, had clear water and almost no vegetation, so the tadpoles were easy to count (see photographs in Figs. S2 and S3).

After taking the photos, we haphazardly captured 50 tadpoles, blotted them dry on a paper towel and weighed them using a balance (type FC50, precision 0.001 g). We also determined the developmental stage (Gosner, 1960).

## Experiment 1: density dependence in the larval stage

We conducted the density experiment by establishing outdoor mesocosms (Fig. S4) that varied in density (i.e., the number of tadpoles per mesocosm). We set up mesocosms with different tadpoles densities because we wanted to induce scramble competition for food (i.e., tadpoles filter phytoplankton and they graze on periphyton which grows mostly on the sides of the mesocosm). The amount of food affects growth of natterjack toad tadpoles (Griffiths et al., 1993; Tejado & Reques, 1994). While we did not quantify food availability, we assume that food was limited, especially at high densities. The densities of tadpoles in the experiment were 5, 10, 15, 20 and 30 tadpoles per mesocosm, each density being replicated ten times. This led to a total of 50 mesocosms. Density treatments were assigned randomly to mesocosms. Every mesocosm was filled with 80 liters of water and 0.2 liters of sand. Two days after filling the mesocosms, we added 0.4 l water inoculated with plankton from a nearby pond to each of the mesocosms to induce the establishment of a freshwater phyto- and zooplankton community (Wilbur, 1987). This set up mimicked the characteristics of the early successional temporary ponds used by the natterjack toad (Fig. S2). The mesocosms were covered with a lid to prevent colonization by predatory insects. Temperatures in the mesocosms were similar to temperatures measured in natural ponds (J. Meléndez Cal y Mayor, unpublished data).

Spawn strings were collected at two man-made ponds. Two egg strings were collected from a pond in Rütifeld, near Glattfelden, Switzerland, and one egg string from a pond in Gemeindegrotte, near Stammheim, Switzerland. The spawn strings were collected on 13th and 15th May 2014, respectively, and were taken to the University of Zurich. Eggs were allowed to develop in small aquaria in a shed on the experimental field on the university campus. Tadpoles hatched 10 days after the spawn was collected. Once tadpoles had reached the free-swimming Gosner (1960) stage 25, we released randomly chosen tadpoles to the mesocosms according to the assigned density treatments (ten days after filling the mesocosms). Tadpoles from the different spawns strings were mixed. The experiment was terminated after 85 days for animal welfare reasons because we observed increasing mortality among the remaining tadpoles (Zemanova, 2017; the Swiss Animal Welfare Act requires that animal suffering be minimised). The reasons for the mortality are unknown.

During the experiment the number of tadpoles was recorded every second day. After the first individual reached metamorphosis (stage 42, Gosner, 1960) they were checked on a daily basis. Every tadpole that

initiated metamorphosis was collected and kept in an individual container until metamorphosis was finished. This was done to prevent the metamorphs from drowning since the mesocosms did not provide any land area. The metamorphs were weighed twice: once when they reached Gosner stage 42 and were collected in the mesocosms and a second time when they had finished their metamorphosis with full tail absorption (Gosner stage 45).

There were four response variables. Mass at metamorphosis is the mass of the metamorphic toad after full tail absorption (Gosner stage 45). The development time is the number of days from when the experiment started until the tadpole reached metamorphosis (Gosner stage 42). The survival rate is the proportion of tadpoles that survived until the end of the experiment. The metamorphosis rate is the proportion of tadpoles that metamorphosed before the end of the experiment (given survival).

### Experiment 2: juvenile performance

We investigated whether the density that the metamorphic toadlets experienced during the larval stage had an influence on their survival and growth. This experiment was conducted by rearing the toadlets for an additional month in terrestrial outdoor mesocosms (Fig. S5). For the experiment, we used the same type of mesocosm as in the previous experiment. To mimic the terrestrial habitat, the mesocosms contained a mix of land and water habitat. Every mesocosm was holding 0.5 liters of sand and fine-grained gravel, a hiding place and some water. The mesocosms were placed at an angle so that the water collected on one side and formed a shallow puddle. Water was replaced as soon as it evaporated. Flowerpot plates, bricks, and wood pieces were used as hiding places.

We prepared a total of 20 mesocosms and added five toadlets to each mesocosm. There were four replicates for each larval density. Toadlets within the same terrestrial mesocosm were from the same larval density treatment but from different aquatic mesocosms. The groups of five toadlets were composed whenever a sufficient number had completed metamorphosis (defined as Gosner stage 46). We assigned toadlets haphazardly to terrestrial mesocosms. Toadlets were weighed before placing them in the terrestrial mesocosms. The toadlets in the mesocosms were fed with collembolans on a daily basis ad libitum. The collembolans were bought at a local pet store. Individuals were counted once a week to determine the survival of the toadlets. After one month, the experiment was halted and we counted the number of survivors and weighed all survivors. One month was enough time to detect carry-over effects both under laboratory conditions and in the field (Goater, 1994; Chelgren et al., 2006).

There were two response variables: mass of individual toadlets and the proportion of toadlets surviving to the end of the experiment.

### Statistical analysis

We used R for all analyses (R Core Team, 2020). We tested for additive effects of density (Ousterhout & Semlitsch, 2016). For the linear models, we used the R functions `lmer()` and `glm()` (Korner-Nievergelt et al., 2022). We used a general linear mixed model with normal errors to analyse the effect of density on size at metamorphosis. Density was a continuous variable in all analyses. Because mass at metamorphosis is known to depend on density in larval amphibians (Wilbur & Collins, 1973) and realized density varied among mesocosms due to mortality, we used proportion surviving in a mesocosm as a covariate. In this analysis, every individual served as an independent data point. However, because individuals within mesocosms may influence each other (Wilbur, 1987), we added “mesocosm” as a random effect to the analysis. The effects of density of the length of the larval period (i.e., days to metamorphosis) was analysed using a linear mixed model where mesocosm was the random effect. We used general linear models with binomial errors for the analysis of the proportion of tadpoles surviving in

the experiment and for the proportion of tadpoles undergoing metamorphosis (given survival to the end of the experiment). In these analyses, the mesocosm was the independent unit.

Given the small size of the toadlets at metamorphosis, it is not possible to mark or recognise them individually. Therefore, we used a general linear model with binomial errors to analyse the proportion of juveniles which survived in a mesocosm. We fit two models and used AIC to compare them (Burnham & Anderson, 2002). In the first and second model, the explanatory variables were larval density and mean mass at the start of the experiment on juvenile performance, respectively. We used mean mass at the beginning of the experiment because toadlets are not individually recognizable (i.e., we could not say which surviving toadlet had which mass at the start). A similar approach was used to analyse mass of toadlets at the end of the experiment. The difference was that we used mass of individuals toadlets as the response variable. To account for the non-independence of toadlets in the same mesocosm, we added “mesocosm” as a random effect to the analysis (i.e., a linear mixed model with normal errors).

## Results

### Quantifying tadpole density, mass and developmental stage in the field

Across ponds, mean tadpole density was 147.5 tadpoles per 0.25 m<sup>2</sup>. It ranged from 7.6 to 983.20 tadpoles per 0.25 m<sup>2</sup>. Mean tadpole mass varied from 26 to 195 mg and mean Gosner stages varied from 23 to 36 across ponds (and 25 to 42 across individuals). There was no effect of tadpole density on mass or Gosner stage (see Supplement, Fig. S8 and S9).

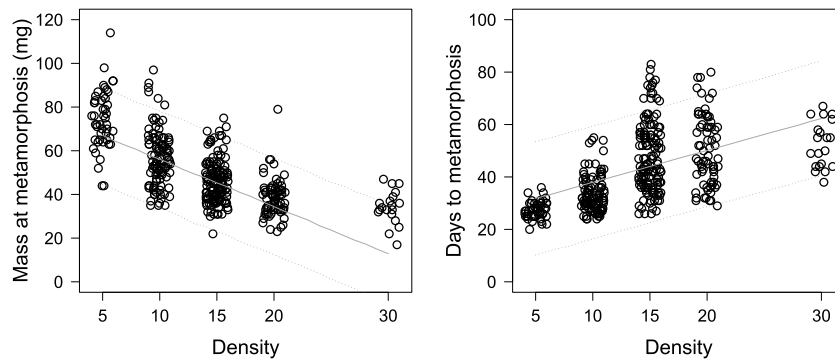
### Experiment 1: density dependence in the larval stage

Mean mass at metamorphosis was 50.5 mg (SD 15.8 mg; range across individuals: 17 to 114 mg). Both density and larval survival rate had negative effects on size at metamorphosis ( $\beta_{\text{density}} = -2.155$  (SE = 0.128),  $\beta_{\text{survival rate}} = -47.1786$  (SE = 8.7673), Fig. 1). The mean number of days to metamorphosis was 42.1 days (SD 13.9 days; range across individuals: 20 to 83 days). Higher density led to longer larval periods ( $\beta_{\text{days}} = 1.219$  (SE 0.131), Fig. 1). More details on the linear models are provided in the supplement (Table S1). The bivariate relationship (sensu Blouin, 1992) between mass between mass at metamorphosis, days to metamorphosis and density is shown in Fig. S6.

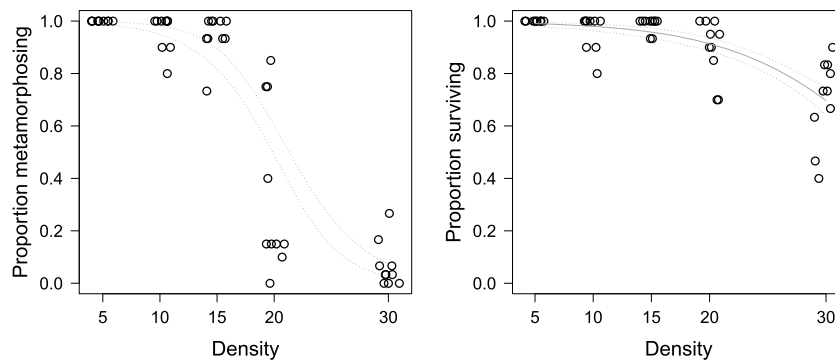
The mean proportion of individuals undergoing metamorphosis before the experiment was halted was 0.685 (SD 0.412, range across mesocosms: 0 to 1 (median = 1.0)). Larval density reduced larval survival ( $\beta_{\text{density}} = 0.152$  (SE = 0.018) (on the logit scale), Fig. 2) and the proportion of survivors undergoing metamorphosis (given survival to the end of the experiment;  $\beta_{\text{density}} = -0.326$  (SE 0.024) (on the logit scale), Fig. 2). More details on the linear models is provided in the supplement (Table S1).

### Experiment 2: juvenile performance

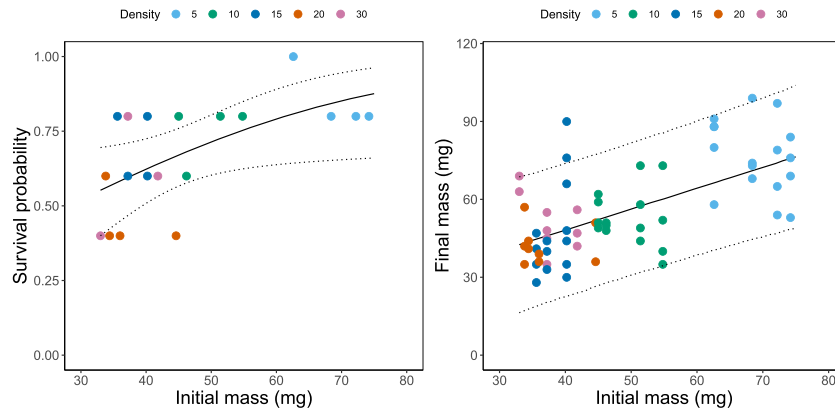
Toadlets with a mean initial mass of 40 mg had a mass of 48.1 mg (95 % CI: 22.5, 76.1 mg) after one month while those with an initial mass of 70 mg had a mass of 73.7 mg (95 % CI: 45.6, 98.9 mg). Toadlets with a mean initial mass of 40 mg had a survival probability of 0.62 (95 % CI: 0.50, 0.72) after one month while those with an initial mass of 70 mg had a survival probability of 0.85 (95 % CI, 0.65, 0.94). Juvenile survival was negatively affected by larval density and positively by mean mass at the start of the experiment ( $\beta_{\text{larval density}} = -0.058$  (SE 0.027),  $\beta_{\text{mass}} = 0.041$  (SE 0.019, Fig. 3) but the model with mass was a slightly better description of the data than the model with larval density (AIC<sub>larval density</sub> 48.8, AIC<sub>mass</sub> 48.5). Juvenile mass was also better predicted by mean mass at the start of the experiment than by larval density (AIC<sub>larval density</sub> 527.3, AIC<sub>mass</sub> 519.2). As with juvenile survival, larval density exerted a



**Fig. 1.** The effect of density on mass at metamorphosis and on days to metamorphosis. Each individual is represented by a dot. Points were jittered horizontally for better readability. The solid line shows the relationship between density and the life history traits predicted by the linear mixed models (dotted lines are the limits of the 95 % confidence interval; the statistical analysis accounted for the non-independence of individuals from the same mesocosm).



**Fig. 2.** The effect of density on the proportions metamorphosing and surviving. Each mesocosm is represented by a dot. The solid line shows the relationship between density and the life history traits predicted by the general linear models (dotted lines are the limits of the 95 % confidence interval).



**Fig. 3.** The effect of mass at metamorphosis (“initial mass”) and larval density on juvenile survival and juvenile mass after 30 days in the terrestrial mesocosms. For survival, each mesocosm is represented by a dot. For final mass, each individual is represented by a dot. The solid line shows the relationship between density and the life history traits predicted by the general linear models (dotted lines are the limits of the 95 % confidence interval; the statistical analysis for final mass accounted for the non-independence of individuals from the same mesocosm).

negative effect ( $\beta_{\text{larval density}} = -0.922$  (SE 0.376)) and mass at the start of the experiment had a positive effect ( $\beta_{\text{mass}} = 0.806$  (SE 0.185), Fig. 3). More details on the linear models is provided in the supplement (Table S1).

**Discussion**

Larval density affected the length of the larval period, size at metamorphosis, survival and the proportion of tadpoles undergoing metamorphosis. Size at metamorphosis and lengths of the larval period in the

experiment were similar to phenotypes measured in natural ponds (Tejedo & Reques, 1994). The effect of density on metamorphic phenotypes led to carry-over effects into the juvenile stage because larger mass at metamorphosis increased juvenile growth and survival, a phenomenon reported in several amphibian species (Smith, 1987; Goater, 1994; Altwegg & Reyer, 2003, Schmidt et al. 2012). While various aspects of density dependence relevant to conservation have already been investigated (e.g., Vonesh & De la Cruz, 2002; Greenberg & Green, 2013; Kross & Willson, 2022), the effects of larval density dependence and the carry-over effects into the juvenile stage and their implications for



conservation are rarely discussed. We argue that density dependence in the larval stage and carry-over have multiple effects which are relevant for conservation practice.

In the natterjack toad, a species which breeds in temporary ponds which may dry before tadpoles can complete metamorphosis, both empirical and modelling studies suggest that population size and population viability are strongly associated with the frequency of early pond drying (Beebee et al., 1996; Stevens & Baguette, 2008; Di Minin & Griffiths, 2011; Buckley et al., 2014). Furthermore, population models suggest that juvenile survival is also important. We believe that larval density dependence therefore strongly affects population dynamics through multiple pathways. Whether pond drying leads to catastrophic larval mortality depends on both meteorological conditions and larval density. While the former is stochastic and hard to predict, larval density dependence has clear effects. High larval densities increase the length of the larval period and therefore increase the risk that pond drying will lead to catastrophic larval mortality, both in frequency and magnitude (i.e., how often it occurs and how many tadpoles are affected; Loman, 2002; Newman, 1989; Smith, 1983). If tadpoles metamorphose successfully, they will have a small body size if they emerge from high density ponds. This means that survival in the juvenile stage will be low and therefore predicted population viability will be low (Stevens & Baguette, 2008; Di Minin & Griffiths, 2011).

If larval density dependence affects population viability through multiple mechanisms, then the obvious conservation solution is to reduce larval density dependence. In many places in Europe, natterjack toads use man-made ponds, often specifically designed and built for the species (e.g., Lippuner, 2013). Recent research has shown that natterjack toads are more likely to occur in patches which have a large water surface or many temporary ponds (Moor et al., 2022; Siffert et al., 2022). Thus, either increasing the number of ponds or building larger ponds suitable for the species is an obvious solution. If populations grow because there are more or larger ponds, then larval density dependence will occur again but at a larger carrying capacity. A higher carrying capacity implies that population size will increase. Larger populations are more likely to persist, at least in the short term (Schmidt & Pellet, 2005; Stevens & Baguette, 2008). The second major determinant of population viability, the frequency of early pond drying, can be minimized by building ponds where hydroperiod can be manipulated as in aquaculture ponds. By building such ponds, stochastic tadpole mortality resulting from premature pond drying can be avoided (Lippuner, 2013).

The results have implications for monitoring amphibians for conservation. Most monitoring focuses on adults, but Cruickshank et al. (2021) recently suggested that monitoring larval stages may give important additional insights into the state of populations. Cruickshank et al. (2021) showed that eggs or larvae were found only in about half of the populations, implying that populations where no eggs or larvae are found have lower viability. The experiment showed that tadpoles reached metamorphosis in about twenty days under good conditions while high density prolonged the larval stage and may have led to mortality if the temporary ponds (which are preferred by this species) run dry. Thus, in a monitoring programme one would have been more likely to detect tadpoles under high density conditions. If tadpole densities are low, then there is only a short time window to detect them. One might then erroneously infer that sites with high densities of tadpoles would harbor more viable populations than populations where tadpole density was low.

Density dependence is a fundamental process in ecology (Sinclair, 1989; Dennis & Taper, 1994; Bjørnstad & Grenfell, 2001). Here we showed that larval density leads to carry-over effects into the terrestrial juvenile stage and thereby add to the growing body of evidence for carry-over effects (Pechenik, 2006; Harrison et al., 2011; Lundsgaard et al., 2023). We argue that the carry-over effects are likely to have profound effects on population viability through multiple ecological processes. Thus, density dependence and the induced carry-over effects matters for conservation practice. Fortunately, the tools to mitigate

these effects are known.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The “Veterinäramt des Kantons Zürich” issued the animal experimentation permit for this study (ZH 66/2014). The nature conservation agency of canton Zürich (“Fachstelle Naturschutz”) allowed us to do field work in nature reserves and issued permits to collect eggs for the experiment. We thank Josh Van Buskirk for allowing us to use the mesocosm facility, Mollie Brooks for help with the statistical analysis and two anonymous reviewers for their comments on the manuscript. We received no funding for this study.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2024.02.001.

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