UPPSALA
UNIVERSITET

Local adaptation of larval life history in the moor frog *Rana arvalis* across a landscape mosaic

Monique Lustenhouwer

Degree project in biology, Master of science (2 years), 2012
Examensarbete i biologi 30 hp till masterexamen, 2012
Biology Education Centre and Department of Population Biology and Conservation Biology, Uppsala University
Supervisor: Anssi Laurila
External opponent: Karoline Fritzsche

Contents

Abstract

Growth rate is an important life history trait, which impacts fitness indirectly through its effect on the age and size at maturity, as well as directly through costs associated with accelerated growth such as increased predation risk. Genetic variation and plasticity in growth are widespread in nature, and local adaptation of growth rate may evolve due to divergent selection in different environments, for example related to predation risk, temperature or time constraints. I studied local adaptation of larval life history in the moor frog *Rana arvalis,* in a local network of ponds close to Uppsala. Local adaptation of growth rate and survival was studied in a reciprocal transplant experiment between ponds with different habitat characteristics. Meanwhile, differences among the populations in intrinsic growth, activity and response to predation were studied in a common garden experiment in the laboratory, where tadpoles were raised in the presence or absence of a predator and tested in direct predation trials. In the field, differences in growth among populations were found, independent of which pond the tadpoles were raised in, indicating that the ponds were similar growth environments. Survival differences among the populations depended on the pond, but local populations did not do better than foreign ones. In the laboratory, similar patterns in growth rate were found. All populations were highly plastic in their response to predation, having lower growth and activity in the predator-induced treatment and decreased mortality in the predation trials. Tadpole size was an important factor in escaping predation. One population clearly grew faster than the others in the field and in the lab, which could be explained in terms of its habitat of origin but was most likely related to the relatively late hatching of this population. Future studies are necessary concerning the possible costs of this accelerated growth and the importance of breeding phenology. Apart from the one differential population, I did not find evidence of local adaptation in the field or in the laboratory. The influence of habitat characteristics on tadpole life history was difficult to study, due to the limited number of ponds and many environmental differences among them. However, this thesis was a valuable pilot study concerning the design of experiments to study factors promoting and constraining local adaptation in landscape mosaics. An understanding of local adaptation at the scale at which gene flow occurs is important for the conservation of populations in fragmented landscapes as well as for the study of ecological speciation.

Introduction

Body size has long been recognized as a key animal trait, because of allometric relationships in life history, physiology and behavior (Peters 1986). Body size is generally considered to be positively correlated with fitness through positive effects on for example fecundity, mating success, offspring quality and life span (Dmitriew 2011). Because adult body size is constrained by development time (Kingsolver & Pfennig 2004), growth rate plays an important role in the determination of the age and size at maturity (Dmitriew 2011). Growth rates are however not always maximized, and both growth rate plasticity (Abrams & Rowe 1996) and genetic variation in growth rate among populations of the same species (*e.g.,* Riska *et al.* 1984; Billerbeck *et al.* 2000; Dmitriew *et al.* 2010) are widespread in nature (Dmitriew 2011). Local adaptation of growth rate may evolve due to divergent selection in different environments (Kawecki & Ebert 2004). In addition to the effect of resource availability (Arendt & Wilson 1997; Morey & Reznick 2000), several other abiotic and biotic factors are known to be associated with growth rate variation, and trade-offs between these factors may play an important role in the determination of optimal life history strategies (Dmitriew 2011).

One classic constraint on high growth rates is the trade-off between growth and predation risk (Werner & Gilliam 1984). Predation risk generally selects against high growth because it requires high foraging activity, which increases exposure to predators (Ali *et al.* 2003; Dmitriew 2011). A reduction of growth under predation risk can potentially have a negative impact on fitness through a decrease in development time or adult body size (Dmitriew 2011). Common garden studies have found indications of an increased risk of predation in fast-growing amphibian larvae and fish (Munch & Conover 2003; Laurila *et al.* 2008; Dmitriew 2011). On the other hand, a high growth rate can also be an advantage in the case of gape-limited predation, because the total exposure time to predation risk is reduced when a size refuge with low predation risk is reached quickly (Urban 2007a). Studies on larvae of the spotted salamander, *Ambystoma maculatum*, suggest that an induced increase in growth rate to reach a size refuge may be an adaptive response to size-selective predation risk (Urban 2008).

Time constraints also play an important role in the evolution of growth rate (Dmitriew 2011). Short growing seasons at high latitudes promote higher growth rates and decreased development time (Blanckenhorn & Demont 2004). Time constraints can vary from year to year, especially in ectotherms where temperature plays an important role in breeding phenology. Environmental constraints which are experienced in early development can lead to compensatory growth, which has been shown in anurans after delayed hatching under low temperature (Orizaola *et al.* 2010). The ability of strong compensatory growth suggests there are costs of accelerated growth rates, which can for example be related to predation avoidance; differential anti-predator strategies in relation to breeding phenology have been found in anuran larvae (Orizaola *et al.* 2012; Dahl *et al.* 2012). Another type of time constraint which affects many organisms with an aquatic larval stage is the hydroperiod of their habitat. Populations from temporary wetlands run a high risk of desiccation before metamorphosis is

reached (Newman 1992), in contrast to populations from permanent ponds. High growth and development rates are therefore adaptive in temporary wetlands. Local adaptation to a short pond hydroperiod by increased growth and development rates has for example been found in the Natterjack toad *Epidalea calamita* (Rogell *et al.* 2009). Moreover, predation densities are expected to vary more in space and time in temporary ponds, which promotes the evolution of plasticity in growth rate and morphology in response to predators (Van Buskirk & Relyea 1998; Lardner 2000).

Temperature is another important environmental factor concerning growth rates, as it impacts both the metabolism and activity of ectotherms (Bullock 1955). Environmental effects on phenotypic variation in growth rate should therefore be taken into account in the study of local adaptation, in addition to genetic effects. When environmental effects (plasticity) act in the opposite direction of adaptive genetic change along an environmental gradient, countergradient variation occurs (Conover *et al.* 2009). Genetic adaptations can for example compensate for the negative effects of low temperatures on metabolic rates and mask phenotypic variation along the gradient. The opposite case is cogradient variation, where plasticity and genetic effects act in the same direction and enhance phenotypic variation (Conover *et al.* 2009). Evidence of countergradient variation has been found in systems where selection pressures on growth rate act in the opposite direction of the effect of temperature (Conover & Schultz 1995), for example along altitudinal gradients in frogs (Berven *et al.* 1979) and lizards (Smith *et al.* 1994), and along latitudinal gradients in fish (Conover & Present 1990), frogs (Riha & Berven 1991) and gastropod larvae (Dehnel 1955). On a smaller geographic scale, countergradient variation in growth rate has been found in populations of the common frog, *Rana temporaria*, coming from open and closed-canopy ponds that differ in temperature and predator density. In the laboratory, *R. temporaria* tadpoles from cooler ponds with low predator densities grow faster than those coming from warmer ponds with high predator densities, under high and low temperatures (Richter-Boix *et al.* 2010). In these examples, populations from warmer environments show submaximal growth, which suggests there are short or long-term costs of high growth rates related to other traits (Dmitriew 2011).

In this thesis, I studied local adaptation of larval life history in the moor frog *Rana arvalis*, using populations from a local network of wetlands with different habitat characteristics close to Uppsala. These populations show differences in growth and development rates when raised in a common environment in the lab, which are associated to differentiation in a thyroid hormone receptor gene which is correlated with larval phenotypes (Richter-Boix *et al.* 2011, *submitted manuscript*). However, the genetic differences among populations in neutral molecular markers are very weak, suggesting that there is ample gene flow among local populations (Richter-Boix *et al.* 2011). Understanding local adaptation at the spatial scale at which gene flow occurs is important for conservation of local populations in landscape mosaics as well as for the study of ecological speciation (Rundle & Nosil 2005; Richter-Boix *et al.* 2011). Anuran tadpoles are a good system to study local adaption of larval

life history, because they are known to respond to many different environmental factors. The main aim of this thesis was to find out whether the *Rana arvalis* populations were adapted to their local pond conditions, and how plastic their responses were when they were transferred to (a) another pond or (b) common laboratory conditions. I considered adaptation of (a) growth rate and (b) defense against predators. The project consisted of two complementary parts:

1) A reciprocal transplant experiment in the field between ponds with different canopy closure (temperature), hydroperiod and predator densities, to test whether populations were locally adapted in terms of growth rate and survival;
2) A laboratory rearing experiment in the presence and absence of predators to study differences in behavior and growth among the populations in a common garden, combined with direct predation trials to test differences among the populations in their survival in the presence of a free-ranging predator.

Common garden and reciprocal transplant experiments are two classic designs in the study of local adaption (Kawecki & Ebert 2004). Common garden experiments focus on possible genetic differences between populations, which may cause them to respond differently to the same controlled environmental conditions. By exposing populations to an environmental gradient, differences in phenotypic plasticity can be studied as well. A downside of common garden experiments is that important environmental factors may be overlooked in the design, and that certain genotypes may just be well-adapted to the laboratory conditions by chance (Kawecki & Ebert 2004). Reciprocal transplant experiments have the advantage to capture more complex natural conditions, which comes with the disadvantage that it is then more difficult to determine which specific environmental factors affected the response of the different genotypes (Kawecki & Ebert 2004). In my study, the reciprocal transplant experiment was designed to study the effect of differences in the complex pond environments on the growth rate of the populations, while the aim of the common garden experiment was to study differences in intrinsic growth rate among populations and their response to predators, which could not be tested in the field.

I expected that an interplay between the effects of pond canopy cover, pond hydroperiod and predator density would influence growth rate and defense against predators in my study populations. Considering the possibility of countergradient variation in growth associated with temperature and the costs of high growth rates for predator avoidance, I hypothesized that populations from cool closed-canopy ponds should have higher growth rates than those from open-canopy ponds in both types of environments, but worse defense against predators. Furthermore, I predicted that populations from temporary ponds should show more plasticity in their response to predators than those from permanent ponds, because of the unpredictable fluctuation of predator densities in temporary habitats. However, the temporary ponds which dry out fastest may have constant very low predator densities, in which case plasticity would not be beneficial (Van Buskirk & Relyea 1998; Lardner 2000).

Methods

Rana arvalis is a small brown frog with a geographic range covering Europe from northern France up to Sweden and Finland, extending eastwards across Europe and Asia to Siberia. It is found in both open and forested freshwater habitats, including ponds, marshes and temporarily flooded fields (Arnold *et al.* 2000). Spawning occurs in a brief time window in early spring shortly after hibernation ends, and egg clutches are laid in shallow, often temporary waters. Natural predators of *R. arvalis* tadpoles include aeshnid and libellulid dragonfly larvae, newts, diving beetles and their larvae and notonectid bugs (Laurila *et al.* 2008). In my study I used aeshnid dragonfly larvae as predators, which are known to catch tadpoles easily and eagerly under laboratory conditions.

From 13 to 25 April 2012, I collected freshly laid egg clutches from six *R. arvalis* breeding sites which were located in a 40 x 40 km area in the Uppsala and Enköping municipalities in central Sweden (Fig. 1). This is a flat area consisting of a mixture of farms, villages and forests, which are mainly made up of *Picea abies* and *Pinus sylvestris* stands mixed with several species of deciduous trees (Richter-Boix *et al.* 2011). The six study sites (named pond A-F) included both permanent ponds and temporarily flooded areas, ranging from a completely open site to a forest marsh (Table 1). The study sites were selected from the 17 ponds used by Richter-Boix et al. (2011), based on pond characteristics, the availability of clutches and the expected hydroperiod of the ponds. Richter-Boix et al. (2011) conducted a PCA analysis of the 17 ponds and classified them into two habitat types: forest marshes with low temperatures and predator densities and much emergent vegetation, and open ponds with higher temperature and predator densities and less emergent vegetation. A similar analysis could not be done with my small sample of these 17 ponds. Instead I based my expectations on the general patterns found by Richter-Boix et al. (2011), and conducted additional measurements of the conditions in my study ponds to assess year-to-year variability (see below).

From each of the six study sites, I sampled 8-10 clutches and transported half of each clutch, containing approximately 400 eggs, to the laboratory in Uppsala. There the clutches were split once more and kept in 0.9L vials, with the water being changed twice a week. I will hereafter refer to the tadpoles coming from pond A as population A, etc. Because the sampling of clutches spanned two weeks due to differences among the sites in spawning date, we kept the clutches at different temperatures (12, 16 and 19 °C) in the lab in order to synchronize their hatching (Table 2). Ultimately all clutches were moved to a laboratory room with a controlled temperature of 19 °C and a photoperiod of 18 hours light and 6 hours darkness.

After hatching, the tadpoles were raised in 3L buckets and water was changed twice a week. I used reconstituted soft water (RSW; APHA 1985) for all experiments. When the tadpoles reached Gosner developmental stage 25, the absorption of external gills (Gosner 1960), I started feeding them chopped spinach *ad libitum*. Most tadpoles from populations A-C, E and F reached stage 25 within the same time period of 3-4 days, and therefore all further experiments with them were conducted

synchronously. The tadpoles from population D, which had been sampled last, were 6 days behind the other populations in development and thus all experiments with the D tadpoles were conducted 6 days after the respective experiments with the other populations (Table 2). From the 8-10 clutches we collected from each population, we selected 7 clutches to use in the experiments (excluding clutches with abnormal or asynchronous development and then selecting randomly). I fed the tadpoles for three to six days after they reached stage 25 before starting the field and laboratory experiments.

Figure 1. Location of the study area in Sweden. Adapted from Richter-Boix et al. (2011).

Table 1. Habitat characteristics of the ponds. Deme, GPS, Type, Canopy, Pred08 and T08 are data from Richter-Boix et al. (2011). Deme is the number of the corresponding pond in Richter-Boix et al. (2011), which are there named demes; type indicates the pond hydroperiod (permanent (P) or temporary (T)), canopy the percentage of forest canopy cover, and Pred08 the predator density according to Richter-Boix et al. (2011). Pred12 are predator density observations from the present study (number of predators/sweep). Pred type is the type of predators that has been caught in this study (DA: aeshnid dragonfly larva, DL: libellulid dragonfly larva, N: newt, BA: diving beetle adult, BL: diving beetle larva, L: leech and NB: notonectid bug). T08 the mean water temperature measured in 2008 and T12 the mean water surface temperature measured in 2012. Note that in pond D canopy cover has decreased since 2008 due to logging.

Pond	Deme	GPS	Type	Canopy	Pred08	Pred12	Pred type	T08	T12
A	4	59°45'17.34"N 17°2'6.72"E	P	40	1.54	0.25	DL, N, BA	12.24	13.04
B	14	59°50'29.46"N 17°21'48.18"E	T	80	0.86	1.00	DA, BA	9.49	13.54
C	5	59°51'10.17"N 17°28'21.31"E	P	0	2.38	1.25	DA, DL, N, BA	12.86	14.39
D	12	59°46'44.88"N 17°1'24.90"E	T	40	0.44	0.40	BA, BL, L	11.89	13.47
E	1	59°43'54.12"N 16°59'9.24"E	T	60	0.88	0.75	DL, N, BA, BL, NB	11.15	-
F	3	59°44'28.92"N 16°50'22.32"E	P	0	3.12	0.25	DL, BL	13.94	-

Table 2. Specific methods and development of populations A-F. Dates of egg collection (eggs), hatching (hatched), Gosner stage 25 (stage 25), time in the lab and field experiment (lab and field), and the temperature conditions under which the eggs were developing (temperature conditions).

Site	Eggs	Temperature conditions	Hatched	Stage 25	Lab	Field
A	4/12	12° 8 days, 16° 2 days, 19° from 4/23	4/23	4/27-28	5/4 – 5/22	5/5 – 5/31
B	4/18	16° 2 days, 19° from 4/20	4/25	4/30-5/1	5/4 – 5/22	5/5 – 5/31
C	4/19	16° 1 days, 19° from 4/20	4/25	4/30-5/1	5/4 – 5/22	5/5 – 5/31
D	4/25	19° from 4/25	4/29-30	5/5-6	5/10 – 5/28	5/11 – 6/6
E	4/13	12° 8 days, 16° 2 days, 19° from 4/23	4/23-24	4/28-29	5/4 – 5/22	-
F	4/18	16° 2 days, 19° from 4/20	4/23-25	4/30-5/1	5/4 – 5/22	-

Field experiment

Sites A-D were used for the field experiment. Sites E and F were excluded for logistic reasons, and because the risk of desiccation of the pond before the end of the experiment was too high at these temporarily flooded sites. I conducted a reciprocal transplant experiment in which we raised tadpoles from each population in cages in their home pond and in the three foreign ponds. In each pond, the cages were replicated over four spatial blocks, each containing one cage per population. This made a total of 4 populations x 4 ponds x 4 blocks = 64 cages. Each cage contained seven tadpoles which had been randomly selected, one from each of the seven experimental clutches belonging to their respective population. The tadpoles were selected and photographed on the day prior to release into the cages. Dorsal photographs were taken at the cage level while the tadpoles were swimming in a petri dish filled with water.

The cages were made from 42 x 25 x 24 cm plastic containers with strong plastic net (mesh size 1 mm) glued over holes in the bottom (36 x 22 cm) and sides (39 x 24 cm). The mesh-covered holes allowed water exchange through the containers. The lid of the cage was made of soft mosquito net (mesh size 2 mm), sown into an elastic band which fitted exactly over the top of the cage. The cages were positioned in shallow water so that they were submerged for about three quarters. However, the cages could float and they would not get completely submerged if the water level of the pond rose. To each cage I added approximately 5g of rabbit pellets and 7g of dried aspen leaves, to provide shelter, food and a substrate for other organisms to grow on. The cages were deposited into the ponds 16-17 days before the start of the experiment, so that additional resources (*e.g.* algae, bacteria) could accumulate on them.

The field experiment started on May 5th for populations A-C and on May 11th for population D. The cages were checked weekly, and moved to deeper parts of the pond if necessary. After 26 days, the tadpoles were collected and stored directly in 95% alcohol. Dorsal and lateral pictures of the collected tadpoles were taken at the cage level, after the stored tadpoles had shortly been soaked in water. In eight cages, no tadpoles were left. Likely causes are a hole in the cage resulting in escape or predation, and mortality of all the tadpoles due to a lack of resources, low water quality or other causes.

The photographs of the tadpoles were analyzed using imageJ software version 1.45s (Rasband 1997) with the ObjectJ plugin (Vischer & Nastase 2012). The body length (in mm) of the tadpoles was

measured from the dorsal angle, along a straight line between the eyes from the tip of the nose to the start of the tail. Mean body size before and after the experiment was calculated per cage, and the growth rate (mm/day) per cage was defined as the mean body size after minus the mean body size before the experiment, divided by 26 days.

My response variables were growth rate and the number of remaining tadpoles per cage at the end of the field experiment. All statistical analyses were done in R version 2.15.1 (R Core Team 2012). The growth rate data were analyzed with linear mixed-effects models (LME), using the lme function from the nlme package in R (Pinheiro *et al.* 2012). Population and pond were entered in the model as fixed effects and block as a random effect nested within pond. I chose to always retain the random effect block in the model while evaluating the fixed effects, because the blocks were part of the experimental design as a measure to capture environmental variation within the ponds, rather than an explicit focus of the study. Testing whether the blocks were significantly different from each other was irrelevant in this case (Hurlbert 1984). I started with a model including both the additive and interaction terms of population and pond, and compared this model with an additive model using a likelihood ratio test. This procedure required the models to be fit using maximum likelihood (ML), but the final model was fit using the preferred restricted maximum likelihood (REML) method.

The data on the number of surviving tadpoles were analyzed using generalized linear mixed models (GLMMs) with a binomial distribution. The models were fit using the Gauss-Hermite quadrature technique (Pinheiro & Chao 2006), which allows for likelihood-based inference, such as the Akaike information criterion (AIC) and hypothesis testing, of the estimates (Bolker *et al.* 2009). Like for the growth rate data, I started with a model including the additive and interaction terms of population and pond as fixed effects, and block as a random effect nested in pond. This model was compared with an additive model based on AIC; the model with the lowest AIC was considered to be the best model. The main effects of this model were then tested using type II Wald chisquare tests, which are preferred over likelihood ratio tests for testing fixed effects in GLMMs (Bolker *et al.* 2009). The data were analyzed using the glmer function from the lme4 package in R (Bates *et al.* 2012 p. 4) and the Anova function from the car package (Fox & Weisberg 2011).

Laboratory experiment

All six populations were used in the laboratory experiments. The tadpoles were reared in a walk-in climate-controlled room at 19°C, in presence or absence of a caged predator, to study differences in growth rate among the populations and their response in growth rate and behavior to the presence of the predator. Subsequently, I conducted direct predation trials with a free-ranging predator to study population and predator treatment effects on tadpole survival.

I reared the tadpoles in plastic tanks (38 x 28 x 13 cm) filled with 10 L of RSW water, which were positioned on four possible shelf heights (blocks 1-4, from the top to the bottom shelf). I used a total

of 6 populations x 2 treatments x 8 replicates[1] = 96 tanks. Fourteen tadpoles were released into each tank, two from each experimental clutch belonging to their population. The tanks were positioned according to a randomized block design with two replicates form each treatment combination in each of the four blocks. In each tank, a transparent cylindrical predator cage (diameter 11 cm, height 21 cm) was hung 2 cm above the bottom of the tank. The bottom of the cage consisted of a double net (mesh size 1.5 mm), so the tadpoles would receive both visual and chemical cues of predator presence. Aeshnid dragonfly larvae, which had been caught from experimental pond C, were released into half of the cages. The predators were fed approximately 2 tadpoles every other day, and the tadpoles were fed chopped spinach ad libitum. Water was changed once a week.

The laboratory experiment started on May 4th for populations A,B,C,E and F and on May 10th for population D (day 0). Prior to release into the tanks, photographs were taken of all tadpoles per tank together in the same way as for the field experiment. On days 11 and 17, I recorded the behavior of the tadpoles. Each tank was approached carefully and the tadpoles were observed for 30 seconds, and the number of tadpoles that had moved and the number of tadpoles that had been seen were noted. All observations were done by the same person, and the tanks had random numbers as labels to reduce the risk of bias. The observations were repeated three times on each of the two days.

The growth experiment lasted for 18 days, after which the remaining tadpoles in each tank (there was a little tadpole mortality during the experiment) were split into two groups of six and photographed per group while swimming in a petri dish. The two groups were kept in 0.9 L vials with some spinach and used in two runs of direct predation trials in the two following days. The predation trials were conducted in the same tanks as where the tadpoles had been raised in. Before the start of the trials, all tanks were emptied, rinsed with tap water and filled again with 10L of RSW water and a layer of aspen leaves for shelter. The predators consisted of a mix of predators that had been kept in the cages before and newly caught predators; these were distributed evenly over the populations and treatments whenever possible. The predators had been starved for two days prior to the experiment. On the day of the trial, a group of six tadpoles was released into their original tank and allowed to acclimatize for one hour, after which one predator was added to the tank. After 21.5 hours (including six hours of darkness), the predators were removed and the number of surviving tadpoles and the number of tadpoles that survived but sustained damage, were counted. The tanks were then emptied, rinsed and refilled with water and aspen leaves, after which another predation trial was conducted with the second group of six tadpoles from each tank. The same procedure was followed for the tadpoles from population D, with the exception that some of the predators that had been used in the first predation trials had to be used again for logistic reasons. These were distributed evenly across treatments. After all predation trials were finished, the body length of the predators was measured.

[1] An exception was population E, were accidentally only 6 replicates received a predator and 10 did not.

The analysis of the laboratory experiment consisted of three parts, (i) the growth data, (ii) the behavior data and (iii) the survival data. Considering tadpole growth, the body length of the tadpoles was measured from the photographs in the same way as for the field experiment. Means per tank were calculated from all tadpoles measured on the single picture taken at the start, and the two pictures taken of the two groups of six at the end of the experiment. Growth rate per tank (mm/day) was defined as the difference between these two means, divided by 18. The growth data were analyzed with an ANOVA with growth rate as the response variable and population, treatment (predator or no predator) and block all as fixed factors. Block entered the model as a fixed rather than as a random factor because the blocks represented four specific shelf heights in my design which had known differences in temperature and light, in contrast to the blocks in the field experiment which were a random sample of possible environmental variation in the ponds. I started the analysis with a model containing all two-way interactions and progressively discarded interactions which were not significant.

The behavior data from day 11 and day 17 were analyzed separately. First, the fraction of moving tadpoles was calculated per tank for each of the three runs of measurements on the day, by dividing the number of moving tadpoles by the number of seen tadpoles. The mean of those three fractions was taken as the response variable. After an arcsine transformation, the data were analyzed using an ANOVA with population, treatment and block as fixed factors, following the same procedure as for the growth data.

The data from the predation trials were analyzed using GLMMs with a binomial distribution, estimated by the Gauss-Hermite quadrature technique, with the number of surviving tadpoles per tank as the response variable. Population, treatment, block and run (trial 1 or 2) were entered in the model as fixed factors, tank was considered a random factor and predator length and mean tadpole size were continuous covariates. The initial model included the population:treatment, run:treatment, block:treatment and tadpole size:predator length interactions, which were considered the most meaningful. The model was then simplified by comparing models using AIC, progressively removing interactions and choosing the models with the lower AICs. The main effects of the final model were then tested using type II Wald chisquare tests. Next, the analysis was performed a second time, this time excluding the tanks where all tadpoles survived and none of them were damaged (the "zeros"), which could indicate the incapability or unwillingness of the predator to catch tadpoles. This could for example be the case if the predator was too small or if the predator was about to mold.

Pond characteristics

Water surface temperature data were collected at 15-minute intervals in ponds A-D for the duration of the field experiment (May 6th to June 6th) using two data loggers (HOBO Water Temp v2 Data Logger) per pond. The data loggers were attached to a cage and were mostly floating on the water surface. Data from periods where there was evidence that air temperature had accidentally been

recorded were removed from the analysis. One of the loggers from pond A was removed from the analysis completely because the data showed obvious errors. Mean daily temperatures were calculated per pond and from those means the average water surface temperature over the entire period was calculated for each pond.

In mid-June, after the experiments were finished, I sampled the predator communities in all ponds. Twenty dipnet sweeps of 1.5 – 2 m were conducted per pond (net diameter 32 cm, mesh size 8 mm), spread over various locations, and a photograph was taken of all aeshnid and libellulid dragonfly larvae, newts, diving beetles and their larvae, notonectid bugs and leeches that were caught. All predators were counted and the number of predators per dipnet sweep was calculated as a relative measure of predator density. It should be noted that pond F was difficult to sample because it had almost completely dried out, and a higher predator density may have been found earlier in the season.

Results

Field experiment

The mean growth rate of all the field tadpoles was 0.23 mm/day (Fig. 2). There was no significant interaction effect between population and pond on growth rate (LR = 10.83, p = 0.29), so I used an additive model instead. Overall, population had a significant effect on growth rate (F=9.93, p < 0.0001; Table 3), but pond did not (F= 2.17, p = 0.14). Population D generally grew faster than the other populations, while the lowest growth rates were found for population A. Though not statistically significant, the lowest growth rates among the ponds were found in pond D (Fig. 4).

On average, 4.7 out of 7 tadpoles remained per cage by the end of the field experiment. Assuming that no predation or escape had occurred, the number of remaining tadpoles was seen as an indication of the survival of the tadpoles under the local pond conditions. In contrast to the growth data, the model containing the interaction term between population and pond better explained the data than an additive model (AIC = 132.0 with df = 17 and AIC = 136.0 with df = 8, respectively). Wald chisquare tests of the interaction model showed that the interaction effect was significant (χ^2 = 18.612, df = 9, p = 0.013; Table 4), indicating that there were differences among the populations in survival in response to the local pond conditions. However, it should be noted that the standard errors of the means of the response of the populations in the different ponds were very large (Fig. 3). Only population C survived best in their home pond; otherwise population D always survived the best (particularly in pond B). Tadpoles from population A showed the highest survival in pond C, while population B showed similar responses in all ponds (Fig. 3).

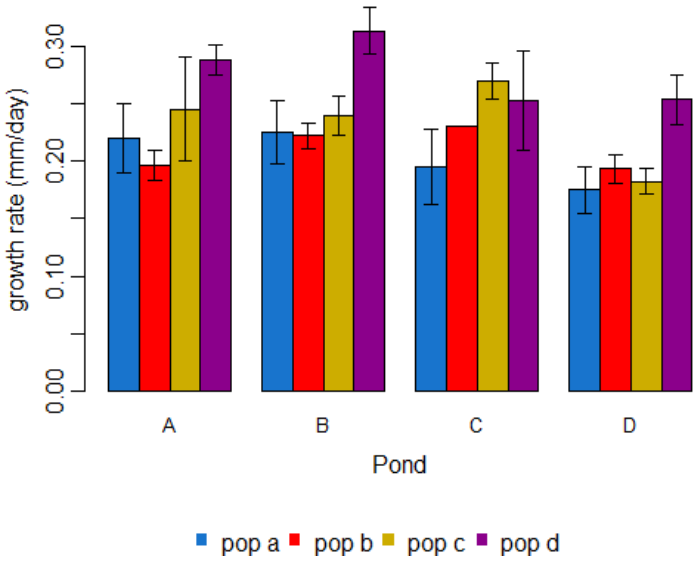

Figure 2. Growth of the field tadpoles. Mean growth rate (mm/day) per pond and population +/- SE. N = 55.

Table 3. Growth of the field tadpoles, linear mixed-effects model fit by REML. Fixed effects: pop and pond, random effect: block nested in pond. Additive model (pond:pop interaction was not significant and discarded). Number of observations: 55, number of groups: 16. Significance levels indicate *($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Fixed effects	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	36	910.8423	<.0001***
pop	3	36	9.9295	0.0001***
pond	3	12	2.1681	0.1448
Random effects	**Intercept**	**Residuals**		
Std. Dev.	0.021	0.039		

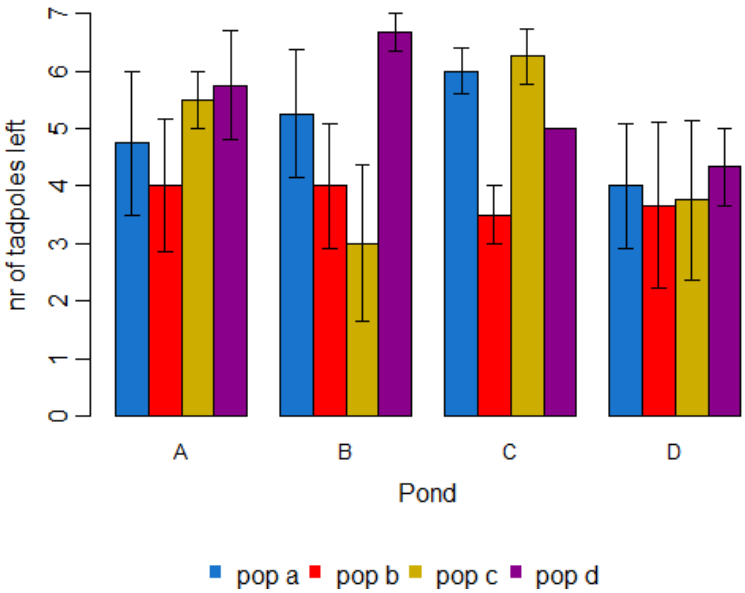

Figure 3. Survival of the field tadpoles. Mean number of remaining tadpoles per pond and population +/- SE. N = 55.

Table 4. Survival of the field tadpoles, generalized linear mixed model with a binomial distribution and Gauss-Hermite quadrature approximation. Analysis of deviance table, type II Wald chisquare tests. Fixed effects: population and pond, random effect: block nested in pond.

Fixed effects	**Chisq**	**Df**	**Pr(>Chisq)**
pop	10.815	3	0.013*
pond	2.588	3	0.460
pop:pond	18.612	9	0.029*

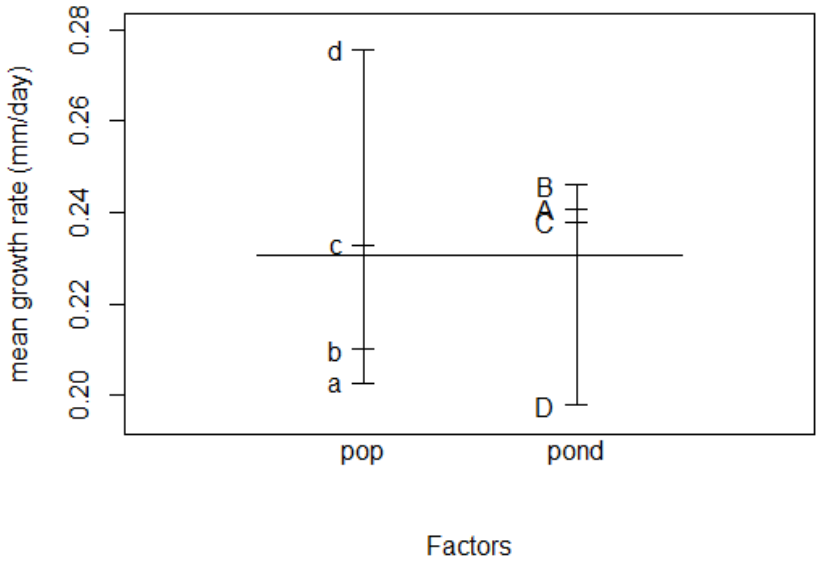

Figure 4. Differences in growth rates among the populations and ponds. Mean growth rate (mm/day) per population and pond.

Laboratory experiment

The tadpoles in the laboratory had an average growth rate of 0.34 mm/day, which is considerably faster than the field tadpoles (Fig. 5). The population:treatment, population:block and treatment:block interactions all were non-significant, so an additive model was chosen for further analysis. Population ($F_{5,96}$ = 9.72, $p < 0.0001$), treatment ($F_{1,96}$ = 7.86, $p = 0.0062$) and block ($F_{3,96}$ = 5.55, $p = 0.0016$) all had a significant effect on growth rate (Table 5). Tadpoles had a reduced growth rate in presence of a predator (Fig. 5). Pairwise comparisons of populations using a Tukey HSD test revealed that population D had a higher growth rate than all other populations, while none of the other populations significantly differed from one another (Fig. 6). Tadpoles from block 1had significantly higher growth rates than those from block 3 and 4 (Fig. 7).

Considering the behavior of the tadpoles, an average of 28% of the tadpoles was observed moving on day 11 (Fig. 8, left). All two-way interactions were non-significant, but an additive model showed significant effects of population ($F_{5,96}$ = 3.54, $p = 0.0058$) and treatment ($F_{1,96}$ = 110.0, $p < 0.0001$) on tadpole activity (Table 6). Tadpoles moved much less in the presence of a predator (Fig. 8). Population D was significantly more active than populations A, C and F, and close to significantly more active than populations B and E (Tukey HSD; Fig. 9). The other populations did not significantly differ from one another. Block had no significant effect on tadpole behavior ($F_{3,96}$ = 0.69, $p = 0.56$). On day 17, tadpole activity levels had increased to an overall 34 % (Fig. 8, right). There were still no significant interactions, and tadpoles still moved significantly less in the presence of a predator ($F_{1,96}$ = 90.3, $p < 0.0001$; Table 7). However, there was no longer an effect of population ($F_{5,96}$ = 0.962, $p = 0.45$), while the overall block effect was now almost significant ($F_{3,96}$ = 2.61, $p = 0.057$).

An average of 4.5 out of 6 tadpoles survived the predation trials (Fig. 10, left), and 4 out of 6 came through without any damage. The final model contained only one interaction, namely the interaction between the two covariates, tadpole size and predator length (Chisq = 5.53, df = 1, p = 0.019; Table 8). These were thus very important covariates to take into account considering tadpole survival. A possible explanation for the interaction effect could be that small predators didn't catch many large tadpoles - because they were too hard to catch, or the predators couldn't eat many - while large predators discriminated less among tadpoles of different size. There was a significant effect of treatment, as the tadpoles that were raised in the presence of a predator had a higher survival in the predation trials (Chisq = 4.82, df = 1, p = 0.028). This was particularly visible for populations D-F (Fig. 10, left). However, population did not have a significant effect on tadpole survival (Chisq = 2.98, df = 5, p = 0.70), nor did block (Chisq = 4.46, df = 3, p = 0.22) or run (Chisq = 2.00, df = 1, p = 0.16).

The results of the subsequent analysis, where the tanks where no tadpoles had been eaten or damaged had been excluded, were slightly different (Fig. 10, right). Mainly, there was no longer a significant interaction between the covariates tadpole size and predator length; instead the final model was the additive model containing all the variables of interest (Table 8). An explanation could be that small predators that did not catch any tadpoles were now removed from the analysis, cancelling the interaction effect. Tadpole size had a significant positive effect (Chisq. = 9.65, df = 1, p = 0.0019) and predator length had a significant negative effect (Chisq. = 9.18, df = 1, p = 0.0024) on tadpole survival. The effect of the predator treatment on tadpole survival was stronger this time (Chisq. = 8.19, df = 1, p = 0.0042). Population (Chisq. = 0.90, df = 5, p = 0.97), block (Chisq = 5.87, df = 3, p = 0.12) and run (Chisq. = 0.14, df = 1, p = 0.70) still had no significant effect on tadpole survival.

Finally, I repeated the analysis excluding tadpole size from the model, to evaluate to what extent size differences among the populations affected differences in their survival. This extra step was important because the tadpoles from population D were on average much larger at the time of the predation trials (Fig. 11). Excluding tadpole size, there was no longer a significant effect of treatment (Chisq. = 1.84, df = 1, p = 0.17; Table 9). A likely explanation is that the tadpoles which had been raised in the presence of a predator were smaller on average. The positive effect of tadpole size on survival decreased the difference between the treatment groups when tadpole size was not taken into account in the model. Interestingly, the population effect was much stronger when tadpole size was excluded, and almost became significant (Chisq. = 10.93, df = 5, p = 0.053). The differences in size among the populations were thus contributing to differences in survival, as the larger tadpoles – which mainly belonged to population D – had a higher chance to escape predation due to their size. The analysis without zeros showed a similar pattern, though the treatment effect was now significant (Chisq. = 4.62, df = 1, p = 0.032). Thus both with and without tadpole size in the model, the treatment effect was greater if the zeros were removed.

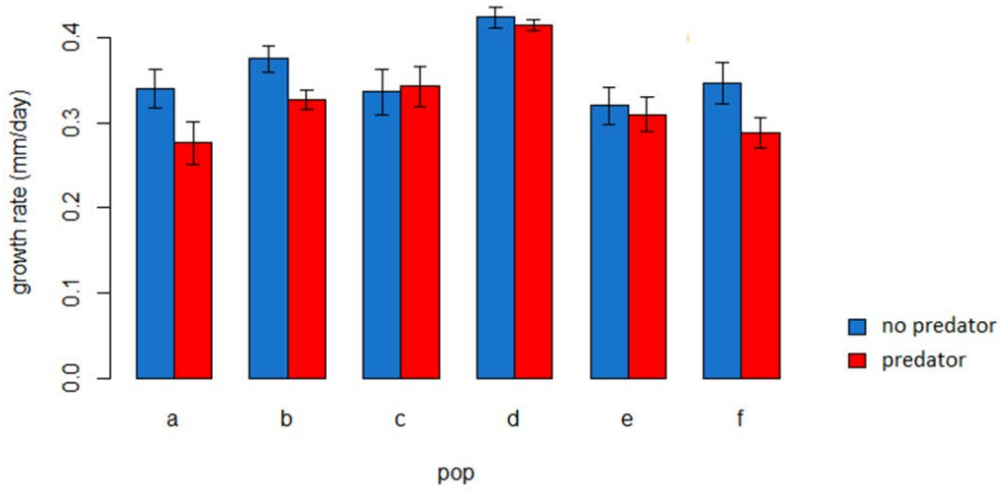

Figure 5. Growth of the tadpoles in the laboratory. Mean growth rate (mm/day) per population an d treatment, +/- SE. N = 96.

Table 5. Growth of the tadpoles in the laboratory, factorial ANOVA with three fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pop	5	0.136	0.027	9.722	<0.0001***
Treatment	1	0.022	0.022	7.865	0.0062**
Block	3	0.047	0.016	5.553	0.0016**
Residuals	86	0.240	0.003		

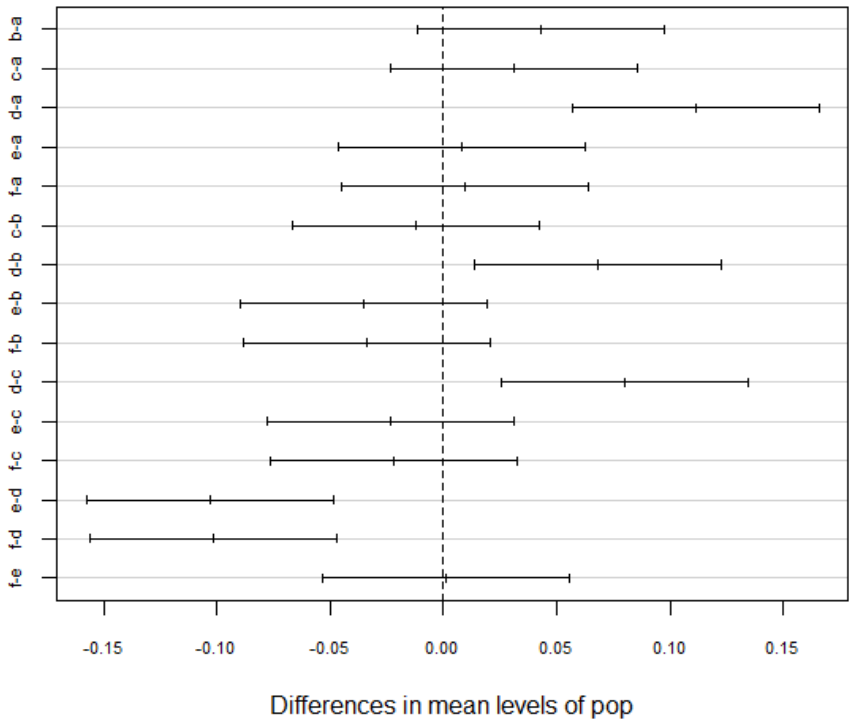

Figure 6. Growth rate of the tadpoles in the laboratory, pairwise comparisons of populations (Tukey HSD, 95% family-wise confidence level).

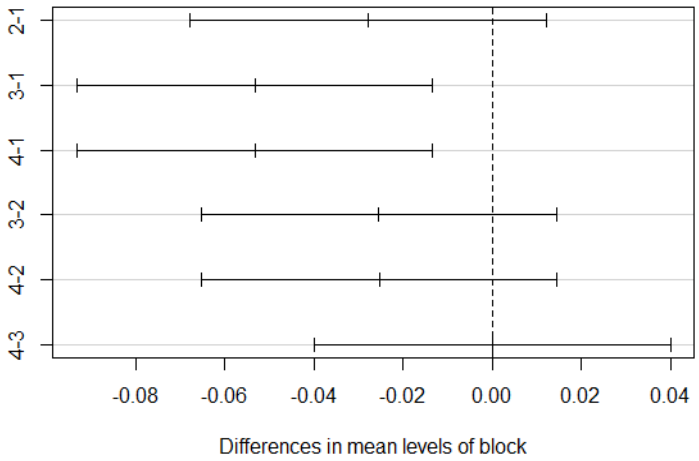

Figure 7. Growth rate of the tadpoles in the laboratory, pairwise comparisons of blocks (Tukey HSD, 95% family-wise confidence level).

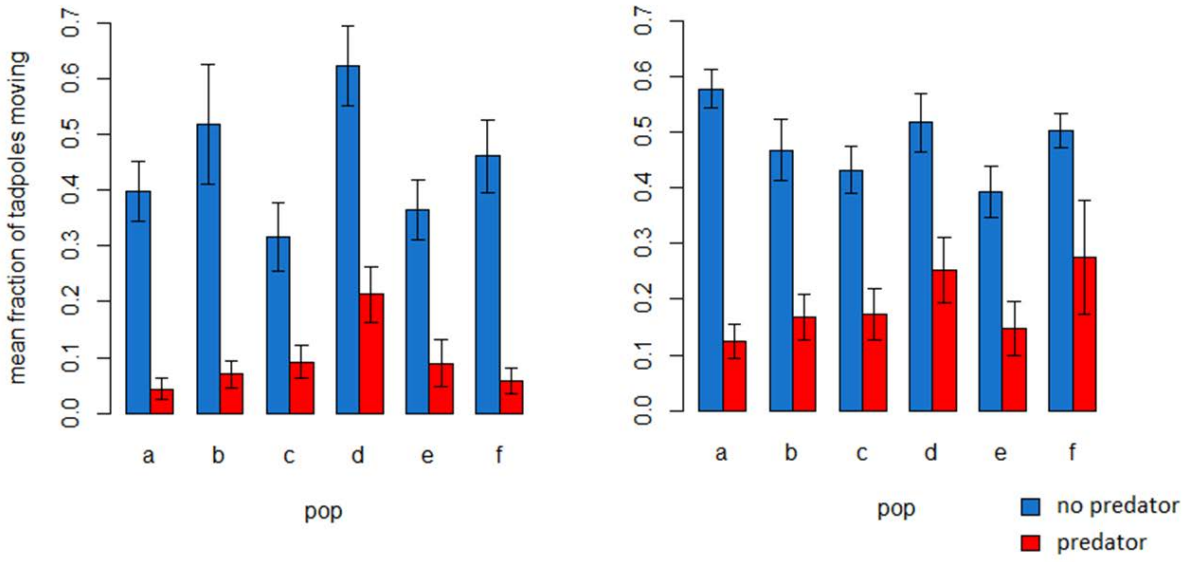

Figure 8. Tadpole activity on day 11 (left) and day 17 (right). Mean fraction of moving tadpoles over 3 behavior trials on a day +/- SE, with and without a predator present.

Table 6. ANOVA table for behavior data, day 11.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
population	5	0.8407	0.1681	3.5442	0.00582	**
treatment	1	5.2199	5.2199	110.0274	<0.0001	***
block	3	0.0982	0.0327	0.6901	0.56051	
residuals	86	4.08	0.0474			

Table 7. ANOVA table for behavior data, day 17.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
population	5	0.16053	0.03211	0.9615	0.44604	
treatment	1	3.01525	3.01525	90.3029	< 0.0001	***
block	3	0.26153	0.08718	2.6108	0.05656	.
Residuals	86	2.87157	0.03339			

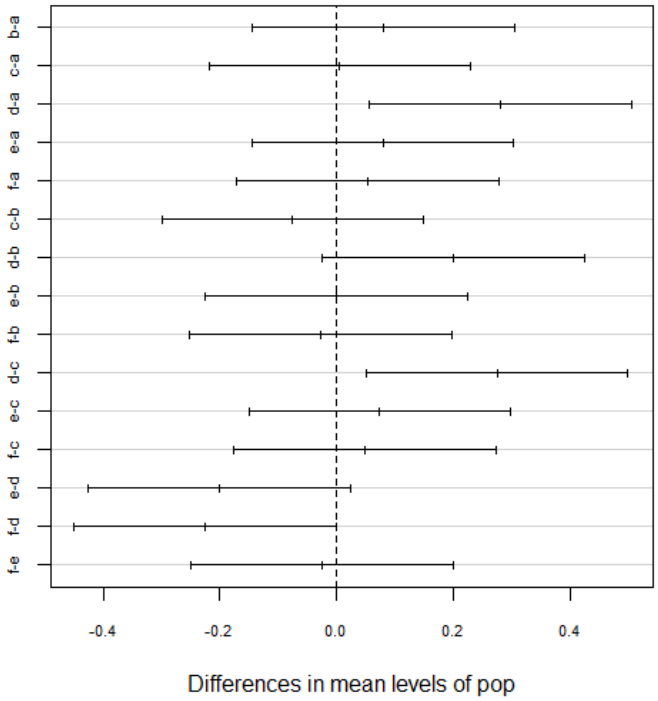

Figure 9. Pairwise comparisons of populations in tadpole behavior on day 11 (Tukey HSD, 95 % family-wise confidence level).

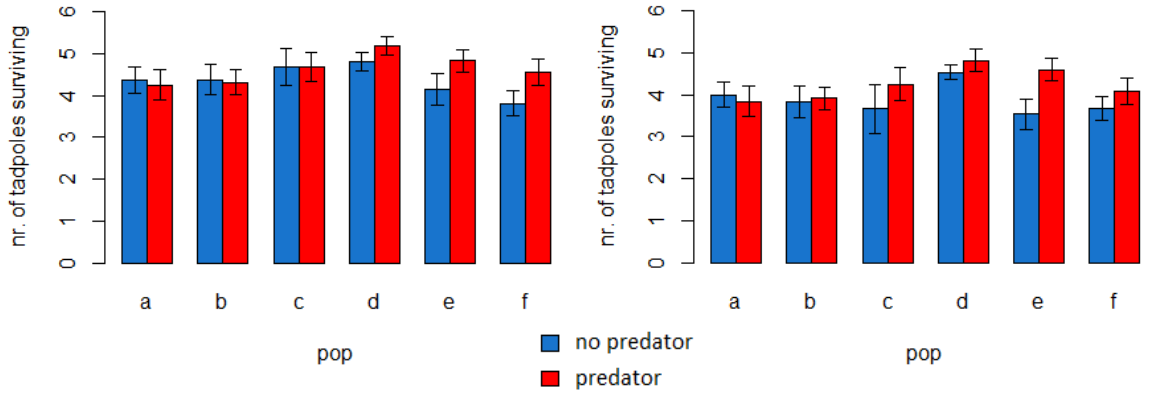

Figure 10. Survival of the different populations during the predation trials, with all observations included (left, n=184) and after the removal of the "zeros" removed (n=141).

Table 8. Survival of the lab tadpoles; analysis of deviance table, type II Wald chisquare tests.

	Including zeros			*Excluding zeros*		
	Chisq	**Df**	**Pr(>Chisq)**	**Chisq**	**Df**	**Pr(>Chisq)**
tadpole size	13.86	1	0.0002***	9.65	1	0.0019**
predator length	19.33	1	< 0.0001***	9.18	1	0.0024**
treatment	4.82	1	0.0282*	8.19	1	0.0042**
population	2.98	5	0.7028	0.90	5	0.9704
block	4.46	3	0.2160	5.87	3	0.1182
run	2.00	1	0.1574	0.14	1	0.7041
tadpole size:predator length	5.53	1	0.0187*		n.s.	

Table 9. Survival of the lab tadpoles, excluding tadpole size from the model; type II Wald chisquare tests.

	Including zeros			*Excluding zeros*		
	Chisq	**Df**	**Pr(>Chisq)**	**Chisq**	**Df**	**Pr(>Chisq)**
predator length	17.85	1	< 0.0001***	8.60	1	0.0034**
treatment	1.84	1	0.1747	4.62	1	0.0315*
population	10.93	5	0.0527 .	9.94	5	0.0769 .
block	5.14	3	0.1615	4.19	3	0.2412
run	2.22	1	0.1359	0.10	1	0.7550

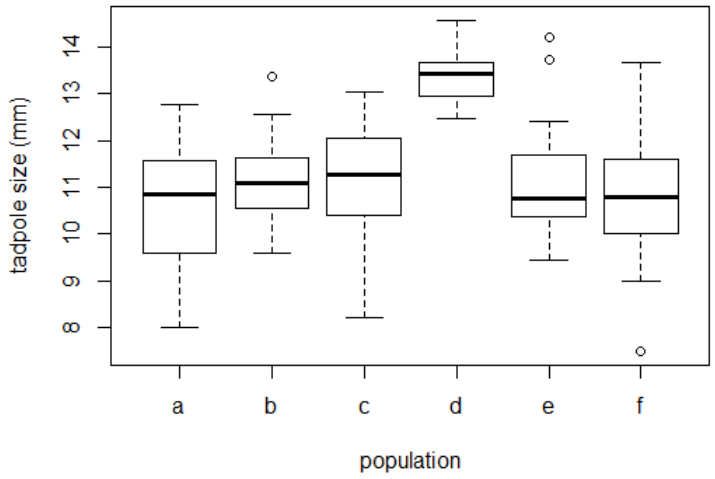

Figure 11. Body size (mm) of the laboratory tadpoles at the start of the predation trials.

Pond characteristics

Water surface temperature was clearly highest in pond C (14.39 °C on average), followed by pond B (13.54 °C), D (13.47 °C) and A (13.04 °C). The water temperature showed similar fluctuations over time in all four of the ponds (Fig. 12). Pond C was the most open pond and clearly the warmest.

There were profound differences among the ponds in both predator density and composition (Table 1, Fig. 13). In pond A I caught many differed kinds of predators, including aeshnid and libellulid dragonfly larvae, newts, notonectid bugs and leeches, but the density of predators was low, 0.25 predators/sweep. Pond B contained many large aeshnid dragonfly larvae and overall predator density was high (1 predator/sweep). This is in contrast with the general pattern that forest marshes have low predator densities (Richter-Boix *et al.* 2011). Pond C contained a high density of all kinds of predators (1.25 predators/sweep), similar in composition to population A with the addition of aeshnid dragonfly larvae. Predator densities in pond D were low (0.4 predators/sweep); I mainly caught some libellulid dragonfly larvae and leeches, and a few beetle larvae. Pond E contained many different kinds of predators in medium density (0.75 predators/sweep), though no aeshnid dragonfly larvae were caught. The predator community of pond F was hard to evaluate, because the pond had almost completely dried out already. However I did catch some beetle larvae and libellulid dragonfly larvae (0.25 predators/sweep). Pond F is an open pond located next to a farm, and I suspect that this is normally a permanent pond which this year has dried out due to something that happened in the surroundings, such as sudden high water drainage.

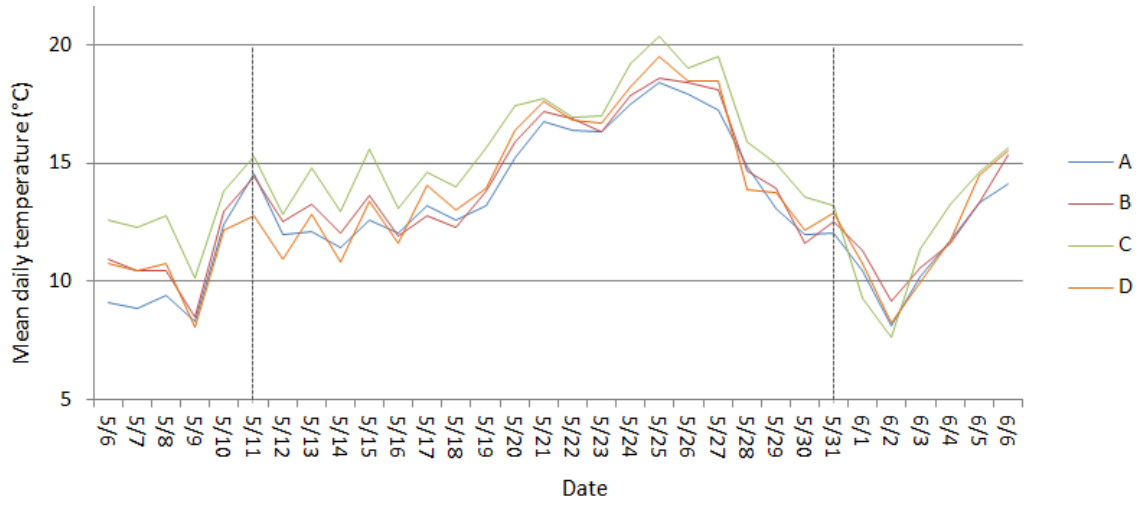

Figure 12. Water surface temperature (°C) in ponds A-D during the field experiment. The first dotted line indicates when population D entered the experiment, the second dotted line indicates when populations A-C were removed from the experiment.

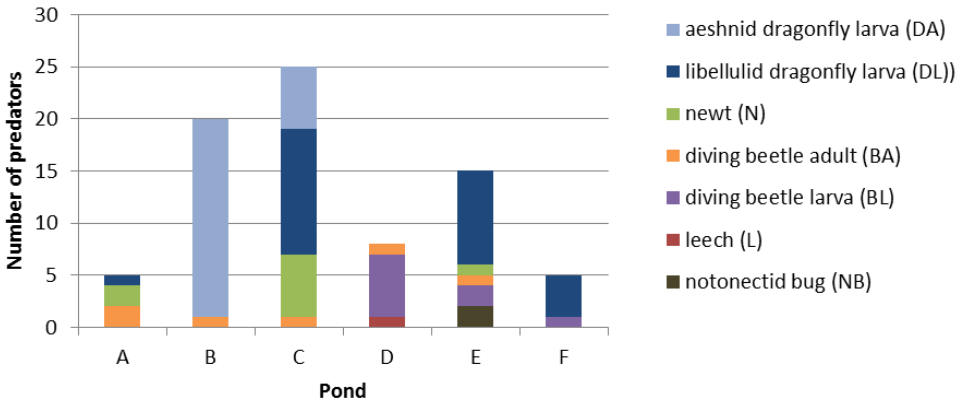

Figure 13. Predator composition of the ponds.

Discussion

In a reciprocal transplant experiment designed to study local adaptation of tadpole life history, I found that tadpoles coming from ponds with different canopy cover, predator density and hydroperiod had significantly different growth rates, independent of which pond they were raised in (Fig. 2, Table 3). This can be concluded from the fact that there was a significant effect of population, but not of pond. An interaction effect between pond and population would have been an indication of local adaptation (Kawecki & Ebert 2004), but was not found. Tadpoles had similar growth rates in all four pond environments, suggesting that the ponds did not differ much from each other as a growth environment. However, another explanation could be that the tadpoles experienced similar growth conditions due to the design of the cages, if the environment within the cages did not reflect the local pond conditions well enough. Nevertheless, the tadpoles from population D had the highest growth rate in all ponds except pond C (where they grew only slightly less fast than population C), while the growth rates in their pond of origin where the lowest overall (Fig. 4). I suggest that this contrast could be an indication of countergradient adaptation; population D could have compensated in growth for the generally more unfavorable conditions in pond D, and could therefore have been able to grow faster in the other ponds.

Considering the survival of the tadpoles in the field experiment, an interaction effect between pond and population was found. This was however not conclusive evidence of local adaptation, because the tadpoles that were raised in their home pond in most cases did not do better than the foreign tadpoles in their pond (Fig. 3). Interestingly, there were clear differences among the populations in the plasticity of their response to the different environments; the survival of population C differed very much among the ponds, while population B had a constant, low survival rate (Fig. 3). Except for these stronger differences among the ponds, the survival data roughly followed the same pattern as the growth data, suggesting that the two response variables were affected by the same processes acting in the ponds. An explanation for the survival differences among the ponds could for example be variation in resource availability or water quality.

There are some examples in the literature of reciprocal transplant experiments to study local adaptation of growth rate. Niewiarowski & Roosenburg (1993) studied the relative importance of genetic and environmental effects on growth rate in a reciprocal transplant experiment with two populations of the fence lizard, *Sceloporus undulatus* from different thermal biophysical environments. They found an interaction between population and environment, which was an indication that differences between populations were genetically based. In another lizard species from the same genus, *Sceloporus jarrovi*, Smith *et al.* (1994) found indications of genetic differences in growth rates in a transplant experiment of populations from different altitudes. However, genetic differences among populations are not always found. Morrison and Hero (2003) conducted a reciprocal transplant experiment with tadpoles from two tropical frog species, *Litoria chloris* and

Litoria pearsoniana, between populations from streams at different altitudes. As in my study, the tadpoles were hatched in the laboratory and then moved to field cages in the streams, with the difference that the tadpoles were raised individually. Growth rate seemed to be primarily affected by environmental variation rather than genetic effects, the most important of which was variation in water temperature. Tadpole survival in the field cages was not affected by either tadpole origin or altitude. It remains an open question what are the main factors influencing tadpole survival in this type of field enclosures.

The growth rates of the different populations were also studied in a common garden experiment in the laboratory, to evaluate possible differences in intrinsic growth rates among the populations. Interestingly, the tadpoles from population D also grew fastest in the laboratory (Fig. 5, 6), which adds support to the hypothesis that countergradient variation in growth explains the higher growth in population D. The other populations did not significantly differ from one another, which suggests there were no differences in intrinsic growth rates or in compensatory growth responses among those populations. Common garden experiments studying differences in growth rate are much more common in the literature than reciprocal transplant experiments. Similar experiments with frog species have found indications of local adaptation of growth rate to differences in forest canopy cover (Rana sylvatica: Skelly 2004; Rana temporaria: Richter-Boix *et al.* 2010), though this is not the only environmental factor for which there are indications of local adaptation. In *Rana arvalis* populations coming from the same 17 ponds used by Richter-Boix *et al.* (2011), where my study ponds were also selected from, a relation has been found between growth rate and a distinct set of habitat characteristics. Growth rates were higher in populations coming from forest marshes with a high canopy cover, few predators and low water temperatures. Combined with a relation between phenotypic variation and a candidate gene, and evidence for weak population structure for neutral markers, there was a strong case for divergent selection shaping population differences in life history (Richter-Boix *et al.*, *submitted manuscript*).

All populations showed a plastic response to predation cues in the laboratory, having a lower growth rate in presence of a predator. This could for example have been due to reduced foraging activity of the tadpoles, as predicted by theory (Werner & Anholt 1993). There was no interaction between treatment and population, suggesting that all populations showed the same degree of growth rate plasticity. Lardner (1998) also found an overall reduction in growth rate of *Rana arvalis* tadpoles in response to predation cues and no differences in growth rate among populations related to predation risk in the source ponds. However, tadpoles from source ponds with a high predation pressure did have a higher development rate, which reduces the total exposure time to predation risk. Other studies have found reduced growth rates across populations in the presence of predators in common garden experiments with *Rana temporaria* (Van Buskirk & Arioli 2005; Laurila *et al.* 2008).

Tadpoles also greatly reduced their activity levels in the presence of a predator. This is a common observation in anurans (Van Buskirk & Arioli 2005; Laurila *et al.* 2006, 2008) and is in accordance with theoretical predictions (Werner & Anholt 1993). Empirical evidence for the relation between activity level and predation risk has been found in many taxa (epibenthic prey: Ware 1973; zooplankton: Wright & O'Brien 1982; damselfies: McPeek 1990). Though predator-induced tadpoles reduced activity levels in my study, the difference between the two predator treatments decreased between day 11 and day 17, because the predator-induced tadpoles became more active over time (Fig. 8). This could have been related to the increased size of the tadpoles (Nunes *et al.* 2012). An exception to this pattern was population D, which showed higher activity levels than all other populations on day 11 (Fig. 9), but did not differ significantly from them anymore on day 17. A possible explanation could be that the total biomass of tadpoles within each tank increased as the tadpoles were growing, especially in the case of population D, increasing competition. Teplitsky and Laurila (2007) showed a shift from morphological and behavioral defense in response to predation cues to morphological defense only at higher tadpole densities. The pattern in my data could have been caused by a similar mechanism.

Concerning tadpole survival in the presence of free-ranging predators, I initially did not find differences among the populations when including the important covariates of tadpole size and predator length. There was an overall plastic response to the predator treatment, with predator-induced tadpoles having higher survival rates in the direct predation trials (Fig. 10). A previous study of *Rana temporaria* populations across a 1500-km latitudinal gradient did not find a significant effect of a predator induction treatment on tadpole survival in direct predation trials. However, there was an effect of population, as populations from northern latitudes where predator densities are lower had higher mortality rates. This was hypothesized to be related to the higher activity levels of these populations, which were in turn likely to be connected to the need for high growth rates during the short growing season in the north (Laurila *et al.* 2008). A similar pattern might have been found in my study, if differences between open- and closed-canopy ponds had more closely mirrored latitudinal gradients in temperature and predator density.

A mechanistic explanation for the higher survival rates of the predator-induced tadpoles in my study could be adaptive differences in morphology. Tadpoles are highly plastic in their morphology, and known induced responses to predation are the development of a smaller body size and deeper tail (Relyea 2001). Van Buskirk and Relyea (1998) found that predator-induced *Rana sylvatica* tadpoles had deeper tail-fins, lower growth rates and lower mortality in direct predation trials. Morphological data of the laboratory tadpoles would have been necessary to evaluate whether this pattern occurred in my study as well, but could not be collected from the type of photographs that were taken. The amount of data that can be extracted from the photographs could have been greatly increased by photographing the tadpoles from the side while they were alive. However, this would have exposed the tadpoles to an extra stress factor, which I chose not to risk right before the start of the predation trials.

At first it seemed surprising that population D, which had been growing faster than the other populations in both the field and the laboratory, did not pay a cost of this high growth rate in terms of a lower survival in the predation trials. However, a subsequent analysis of the data excluding tadpole size as a covariate from the model revealed that population D probably had a relatively high survival rate because the tadpoles were about 2 mm bigger than the tadpoles from the other populations at the time of the predation trials (Fig. 11). Because the survival rate of the tadpoles increased with tadpole size, population D did not significantly differ from the other populations in the original model, while the survival rate of population D may have been lower than that of the other populations, had the tadpoles been tested at the same size. The high growth rate of population D can thus be seen as an adaptive way to escape predation by quickly reaching a size refuge (Urban 2007a; b; Urban *et al.* 2008).

Overall, apart from the differential results for population D, I did not find convincing evidence of local adaptation in the field or in the laboratory. There were generally no interactions between population and pond or treatment, and for the only variable where this was the case (survival in the field experiment), local populations did not do better than foreign ones. This seems surprising at first, especially considering that strong indications of divergent selection related to habitat types have been found using the larger network of breeding ponds that my study areas were part of (Richter-Boix *et al.* 2011, *submitted manuscript*). However the number of study areas was small and there were many environmental factors that could potentially counteract each other and that were hard to disentangle. The ponds also did not match the general patterns of correlations of habitat characteristics found in previous studies (Richter-Boix *et al.* 2011). Moreover, some of the pond environments had changed since previous studies due to logging and other habitat modifications, and predator densities vary greatly from year to year. Finally, I was limited in my selection of the ponds due to the dry early spring, which caused many temporarily flooded areas with known past *Rana arvalis* populations to be too dry for spawning this year.

On the other hand, all populations were highly plastic in their response to predation, decreasing growth and activity in the predator-induced treatment and decreasing mortality in the predation trials. The overall plastic response to predation cues can be considered as highly adaptive, especially in environments with fluctuating predator densities such as temporary wetlands (Lardner 2000). Though not statistically significant, populations D, E and F from the more temporary ponds seemed to show a greater difference in survival among the predator treatments than the other populations (Fig. 10, left).

The one population that really differed from all the others was population D. The question is whether this difference can be attributed to habitat differences between the ponds, or is due to the fact that population D had a late breeding time compared to the other populations. Considering habitat characteristics, pond D had a short hydroperiod, medium water temperature, low canopy cover (though this was of recent origin because of logging) and low predator densities (Table 1). High growth rates

can be adaptive in such an environment, because the risk of pond desiccation is high, the cost of high activity levels is low due to the limited predation risk, and colder temperatures promote countergradient variation in growth (Richter-Boix et al., *submitted manuscript*). Arguably, pond F shared many of these habitat conditions with pond D, though Richter-Boix *et al.* (2011) found very high predator densities there. However, because population D was the only population that differed much from the other populations, it seems likely that breeding time was of great importance in determining growth rate, and that population D showed compensatory growth after late hatching. In addition to in anurans (Orizaola *et al.* 2010), this phenomenon is also known in damselflies (De Block & Stoks 2004).

The potential importance of the effect of breeding time on growth rate raises the issue that breeding time could not be studied explicitly in my experimental design, because the hatching of the populations was synchronized at the start. Originally it was my aim to focus on the effect of differences in habitat characteristics on growth rate and behavior, rather than on the effect of breeding phenology. As synchronization of hatching ensured that the populations would experience similar environmental conditions in the field, this was the best compromise for the purposes of my study. The risk of this method lies in the potential of compensatory growth in artificially delayed populations (Orizaola *et al.* 2010). However, the artificially delayed populations grew more slowly rather than faster than the undelayed population D. Instead it is more likely that population D showed compensatory growth due to its naturally delayed hatching. If the hatching of the five other populations had not been synchronized, greater differences in growth rate among these populations may have been found as well, if the natural timing of hatching was important.

Ideally, the egg clutches I used should have come from lines that had been in a common environment in the lab for several generations, to rule out maternal effects (Kawecki & Ebert 2004). However, this was not practically possible for this project. Another suggestion for improvement is that the predators that were used in the predation trials should be caught earlier, to give them time to acclimatize to the laboratory conditions. In my study many of the freshly caught predators responded to their new, warmer environment by molding, which sometimes occurred during the predation trials and affected the willingness of the predators to hunt for prey. Early capture of the predators would also ensure that enough predators are caught so that no predators have to be used twice in the predation trials.

More analyses can still be done with my data; it would be interesting to try to estimate the development stage from the pictures of the field tadpoles, which may show more variation among populations than just growth rate. Additionally, morphological measurements may be possible from these pictures. It would be of great interest to quantify differences in tail length and depth and see whether the populations are differentiated in morphology, and whether these differences can be attributed to differences in habitat characteristics and/or breeding time. Because the design of the field

cages turned out to work quite well, my main suggestion for future studies would be a transplant experiment in the field on a much larger scale, comparable with the 17 sites used by Richter-Boix *et al.* (2011). This would greatly increase the potential to evaluate the effects of habitat differences among the ponds, especially in combination with more detailed measurements of pond canopy cover, predator density and hydroperiod as well as water temperature and productivity (as a measure of the general growing conditions of the pond).The effect of differences in breeding time among the ponds on growth rate should be a major focus of future studies. The hatching of the clutches should therefore not be synchronized artificially and I would recommend performing the experiments asynchronously in accordance with the natural breeding time of the populations.

This thesis was a valuable pilot study. I found a method to conduct a reciprocal transplant experiment with these populations which was robust against predation and stochastic events. Furthermore, breeding phenology was identified as an important factor to take into account in local adaptation of these populations, and it became clear that more precise methods are necessary to assess the impact of habitat differences among the ponds.

There were indications of local adaptation in population D, as its high growth rate in both the field and the lab matched expectations from previous studies concerning the short hydroperiod, low temperature and low predation risk of pond D (Dmitriew 2011). Furthermore, the high growth rate could be explained as compensatory growth following late hatching (Orizaola *et al.* 2010). Molecular methods would be necessary to determine whether the differences between population D and the other populations were genetic or plastic, such as a comparison between neutral genetic variation among the populations and variation for a candidate gene related to tadpole life history (Richter-Boix *et al.* 2011). It would also be informative to repeat the study over several years and see whether the differences among the populations are stable, or can be related to environmental fluctuations.

Another question that remains is whether, and how, population D would experience a cost of its high growth. It could be that the costs lie early in development, when the tadpoles from population D had higher activity levels than all other populations and could theoretically be exposed to higher predation risk (Werner & Anholt 1993). Alternatively there may be costs of high growth later in life, in the form of reduced reproduction (Auer *et al.* 2010), increased oxidative stress or worse immune function (De Block & Stoks 2008). A greater understanding of the costs and benefits of high growth rates on a small geographic scale will be necessary to gather a complete image of the factors promoting and constraining local adaptation of tadpole life history in landscape mosaics.

Acknowledgements

I would like to thank Anssi Laurila for giving me the opportunity to work in his lab and for his helpful and enthusiastic supervision, Alex Richter-Boix for his great advice and all his help with the field work and Nora Kolter for her invaluable help in the lab and in the field. I would also like to thank Germán Orizaola for his useful advice, Charlie Lindqvist for helping me out with sewing all those lids and during the field and lab work, Andrea Murillo for her help and company in the lab, and Glib Mazepa, Marios Thoma and Olja Toljagic for helping me in the field.

References

Abrams PA, Rowe L (1996) The Effects of Predation on the Age and Size of Maturity of Prey. *Evolution*, **50**, 1052.

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries*, **4**, 147–190.

APHA (1985) *Standard methods for the examination of water and waste water*. American Public Health Association, Washington, D.C., USA.

Arendt JD, Wilson DS (1997) Optimistic Growth: Competition and an Ontogenetic Niche-Shift Select for Rapid Growth in Pumpkinseed Sunfish (Lepomis gibbosus). *Evolution*, **51**, 1946.

Arnold EN, Burton JA, Ovenden DW (2000) Field Guide to the Reptiles and Amphibians of Britain & Europe. In: *Reptiles and Amphibians of Britain & Europe* , p. 80. Harpercollins Pub Ltd.

Auer SK, Arendt JD, Chandramouli R, Reznick DN (2010) Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (Poecilia reticulata). *Ecology Letters*, no–no.

Bates D, Maechler M, Bolker B (2012) *lme4: Linear mixed-effects models using S4 classes*.

Berven KA, Gill DE, Smith-Gill SJ (1979) Countergradient Selection in the Green Frog, Rana clamitans. *Evolution*, **33**, 609.

Billerbeck JM, Schultz ET, Conover DO (2000) Adaptive variation in energy acquisition and allocation among latitudinal populations of the Atlantic silverside. *Oecologia*, **122**, 210–219.

Blanckenhorn WU, Demont M (2004) Bergmann and Converse Bergmann Latitudinal Clines in Arthropods: Two Ends of a Continuum? *Integrative and Comparative Biology*, **44**, 413–424.

De Block M, Stoks R (2004) Life-history variation in relation to time constraints in a damselfly. *Oecologia*, **140**, 68–75.

De Block M, Stoks R (2008) Compensatory growth and oxidative stress in a damselfly. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 781–785.

Bolker BM, Brooks ME, Clark CJ *et al.* (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, **24**, 127–135.

Bullock TH (1955) Compensation for temperature in the metabolism and activity of poikilotherms. *Biological Reviews*, **30**, 311–342.

Van Buskirk J, Arioli M (2005) Habitat specialization and adaptive phenotypic divergence of anuran populations. *Journal of Evolutionary Biology*, **18**, 596–608.

Van Buskirk J, Relyea RA (1998) Selection for phenotypic plasticity in Rana sylvatica tadpoles. *Biological Journal of the Linnean Society*, **65**, 301–328.

Conover DO, Duffy TA, Hice LA (2009) The Covariance between Genetic and Environmental Influences across Ecological Gradients. *Annals of the New York Academy of Sciences*, **1168**, 100–129.

Conover DO, Present TMC (1990) Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia*, **83**, 316–324.
Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution*, **10**, 248–252.
Dahl E, Orizaola G, Nicieza AG, Laurila A (2012) Time constraints and flexibility of growth strategies: geographic variation in catch-up growth responses in amphibian larvae (S Meiri, Ed,). *Journal of Animal Ecology*, n/a–n/a.
Dehnel PA (1955) Rates of Growth of Gastropods as a Function of Latitude. *Physiological Zoology*, **28**, 115–144.
Dmitriew CM (2011) The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, **86**, 97–116.
Dmitriew C, Blows MW, Rowe L (2010) Ontogenetic Change in Genetic Variance in Size Depends on Growth Environment. *The American Naturalist*, **175**, 640–649.
Fox J, Weisberg S (2011) *An R Companion to Applied Regression*. Thousand Oaks, CA: Sage.
Gosner KL (1960) A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica*, **16**, 183–190.
Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, **54**, 187–211.
Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology letters*, **7**, 1225–1241.
Kingsolver JG, Pfennig DW (2004) Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution*, **58**, 1608.
Lardner B (1998) Plasticity or fixed adaptive traits? Strategies for predation avoidance in Rana arvalis tadpoles. *Oecologia*, **117**, 119–126.
Lardner B (2000) Morphological and life history responses to predators in larvae of seven anurans. *Oikos*, **88**, 169–180.
Laurila A, Lindgren B, Laugen AT (2008) Antipredator defenses along a latitudinal gradient in rana temporaria. *Ecology*, **89**, 1399–1413.
Laurila A, Pakkasmaa S, Merilä J (2006) Population divergence in growth rate and antipredator defences in Rana arvalis. *Oecologia*, **147**, 585–595.
McPeek MA (1990) Behavioral Differences between Enallagma Species (Odonata) Influencing Differential Vulnerability to Predators. *Ecology*, **71**, 1714.
Morey S, Reznick D (2000) A comparative analysis of plasticity in larval development in three species of spadefoot toads. *Ecology*, **81**, 1736–1749.
Morrison C, Hero J-M (2003) Altitudinal Variation in Growth and Development Rates of Tadpoles of Litoria chloris and Litoria pearsoniana in Southeast Queensland, Australia. *Journal of Herpetology*, **37**, 59–64.
Munch SB, Conover DO (2003) Rapid growth results in increased susceptibility to predation in Menidia menidia. *Evolution*, **57**, 2119.
Newman R (1992) Adaptive Plasticity in Amphibian Metamorphosis. *Bioscience*, **42**, 671–678.
Niewiarowski PH, Roosenburg W (1993) Reciprocal Transplant Reveals Sources of Variation in Growth Rates of the Lizard Sceloporus Undulatus. *Ecology*, **74**, 1992.
Nunes AL, Richter-Boix A, Laurila A, Rebelo R (2012) Do anuran larvae respond behaviourally to chemical cues from an invasive crayfish predator? A community-wide study. *Oecologia*.
Orizaola G, Dahl E, Laurila A (2010) Compensating for delayed hatching across consecutive life-history stages in an amphibian. *Oikos*, **119**, 980–987.
Orizaola G, Dahl E, Nicieza AG, Laurila A (2012) Amphibian life history and anti-predator strategies are affected by time constraints driven by breeding phenology. *Oecologia*, **in press**.
Peters RH (1986) *The Ecological Implications of Body Size*. Cambridge University Press.

Pinheiro JC, Bates D, DebRoy S, Sarkar D, R Core Team (2012) *nlme: Linear and Nonlinear Mixed Effects Models*.

Pinheiro JC, Chao EC (2006) Efficient Laplacian and Adaptive Gaussian Quadrature Algorithms for Multilevel Generalized Linear Mixed Models. *Journal of Computational and Graphical Statistics*, **15**, 58–81.

R Core Team (2012) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Australia.

Rasband WS (1997) *ImageJ*. U.S. National Institutes of Health, Bethesda, MD, USA.

Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, **82**, 523–540.

Richter-Boix A, Quintela M, Laurila A Fine-grained adaptive divergence in an amphibian: selection and non-random gene flow restrict effective migration among demes. ***Submitted manuscript.***

Richter-Boix A, Quintela M, Segelbacher G, Laurila A (2011) Genetic analysis of differentiation among breeding ponds reveals a candidate gene for local adaptation in Rana arvalis. *Molecular Ecology*, **20**, 1582–1600.

Richter-Boix A, Teplitsky C, Rogell B, Laurila A (2010) Local selection modifies phenotypic divergence among Rana temporaria populations in the presence of gene flow. *Molecular Ecology*, **19**, 716–731.

Riha VF, Berven KA (1991) An analysis of latitudinal variation in the larval development of the wood frog (Rana sylvatica). *Copeia*, 209–221.

Riska B, Atchley WR, Rutledge JJ (1984) A Genetic Analysis of Targeted Growth in Mice. *Genetics*, **107**, 79–101.

Rogell B, Hofman M, Eklund M, Laurila A, Höglund J (2009) The interaction of multiple environmental stressors affects adaptation to a novel habitat in the natterjack toad Bufo calamita. *Journal of Evolutionary Biology*, **22**, 2267–2277.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecology letters*, **8**, 336–352.

Skelly DK (2004) Microgeographic countergradient variation in the wood frog, Rana sylvatica. *Evolution*, **58**, 160–165.

Smith GR, Ballinger RE, Nietfeldt JW (1994) Elevational Variation of Growth Rates in Neonate Sceloporus jarrovi: An Experimental Evaluation. *Functional Ecology*, **8**, 215.

Teplitsky C, Laurila A (2007) Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. *Ecology*, **88**, 1641–1646.

Urban MC (2007a) The growth-predation risk trade-off under a growing gape-limited predation threat. *Ecology*, **88**, 2587–2597.

Urban MC (2007b) Predator size and phenology shape prey survival in temporary ponds. *Oecologia*, **154**, 571–580.

Urban MC (2008) Salamander evolution across a latitudinal cline in gape-limited predation risk. *Oikos*, **117**, 1037–1049.

Urban M, Leibold M, Amarasekare P *et al.* (2008) The evolutionary ecology of metacommunities. *Trends in Ecology & Evolution*, **23**, 311–317.

Vischer N, Nastase S (2012) *ObjectJ*. University of Amsterdam.

Ware DM (1973) Risk of Epibenthic Prey to Predation by Rainbow Trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, **30**, 787–797.

Werner EE, Anholt BR (1993) Ecological Consequences of the Trade-Off between Growth and Mortality Rates Mediated by Foraging Activity. *The American Naturalist*, **142**, 242.

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. *Annual review of ecology and systematics*, **15**, 393–425.

Wright DI, O'Brien WJ (1982) Differential Location of Chaoborus Larvae and Daphnia by Fish: The Importance of Motion and Visible Size. *American Midland Naturalist*, **108**, 68.