



**Universität
Zürich** ^{UZH}

**The effect of density on the performance of larval and
juvenile toads: implications for ponds constructed for
amphibian conservation**



Master thesis

Tobias Weber, 30th January 2015

Supervised by:

Dr. Benedikt Schmidt, KARCH & University of Zurich, Switzerland

Prof. Dr. Arpat Ozgul University of Zurich, Switzerland

University of Zurich, Switzerland

Institute of Evolutionary Biology and Environmental Studies

Abstract

Anthropogenic activities have major impacts on habitats. With the loss of habitats, also their inhabitants are endangered. To protect species, which rely on specific habitats, different strategies have been acquired. One of them is to build new habitats where the species can survive. Since in conservation work, financial and personnel resources are limited, it is of great benefit if a project can be built on an evidence-based background. One aspect that contributes to the success of a project, is the knowledge about the effect of density on the inhabitants of a habitat. I looked at it, by investigating tadpoles in ponds, whose development is influenced by the density of individuals. Therefore I combined a controlled experiment with data collected in the field. As study species I used the natterjack toad (*Bufo calamita*), which is a highly endangered amphibian species in Central Europe due to anthropogenic habitat destruction. I conducted experiments with different density treatments of larvae and I reared the juveniles for an additional month. My study revealed that density significantly lowers larval fitness by negatively affecting body mass, development time, survival rate and metamorphic rate. There was also a carry-over effect of density visible from the larval stage to the juvenile stage. Juveniles that experienced high larval density had a reduced body mass after one month. The effect of density in field-collected data was not very pronounced. Only the development stage had an influence on body mass. However, due to the fact that the density in the artificial ponds was much higher than in the mesocosms, it can be assumed that there is also an influence of density in the ponds.

My study illustrates that without having an evidence-based conservation, it is hard to create effective conservation strategies. With my findings on the effect of density, I contribute to future pond building projects by providing information, which can be used to achieve a successful conservation of species.

Zusammenfassung

Anthropogene Aktivitäten haben einen bedeutenden Einfluss auf Habitate. Schweizweit sind bereits 90% der Feuchtgebiete durch Eingriffe des Menschen verloren gegangen. Mit dem Verschwinden von Feuchtgebieten sind auch deren Bewohner bedroht. Um Arten, welche auf Feuchtgebiete angewiesen sind zu schützen, gibt es verschiedene Strategien. Eine davon ist, dass man neue Habitate errichtet, in denen die Arten überleben können. Um ein neues Habitat anzulegen, werden detaillierte Informationen über die Anforderungen einer Art an ihre Umwelt benötigt. Da im Naturschutz die finanziellen und personellen Ressourcen begrenzt sind, ist es von grossem Nutzen, wenn man auf einer evidenz-basierten Grundlage ein Projekt aufbauen kann. Daher ist es wichtig, dass man ein spezifisches Wissen erarbeitet und somit ein erfolgreiches Projekt aufbaut. Ein Aspekt, welcher zu diesem Erfolg beiträgt, ist das Wissen über die Auswirkungen von Dichte auf die Bewohner eines Habitates. Ich untersuchte dies mit Hilfe von Kaulquappen, da deren Entwicklung sehr anfällig auf Veränderungen in der Individuendichte ist. Um den Einfluss von Dichte in den von Menschenhand geschaffenen Teichen zu untersuchen, kombinierte ich ein kontrolliertes Experiment mit Felddaten. Die Art, mit der ich meine Experimente durchgeführt habe, ist die Kreuzkröte (*Bufo calamita*), eine Art, die durch die anthropogene Zerstörung ihres Lebensraums in Europa vom Aussterben bedroht ist.

Ich führte meine Experimente an der Universität Zürich durch, wobei ich die Kaulquappen der Kreuzkröte unter verschiedenen Dichten aufzog und die Juvenilen dann noch für einen weiteren Monat beobachtete. Meine Studie zeigte, dass die Dichte in der die Kaulquappen aufwuchsen, einen wesentlichen Einfluss auf ihre Fitness hatte. Ein negativer Effekt wurde beim Körpergewicht, der Entwicklungszeit, der Überlebensrate und der Metamorphoserate der Kaulquappen festgestellt.

Negative Effekte, die sich auf das Wachstum von Kaulquappen auswirkten, hatten auch einen Einfluss auf die Juvenilen (Übertragungseffekt). Juvenile Kreuzkröten, welche als Kaulquappe einer erhöhten Dichte ausgesetzt waren, hatten nach einem Monat ein geringeres Körpergewicht als diejenigen, welche eine kleinere Kaulquappendichte erfuhren.

Im Gegensatz zu meinem kontrollierten Experiment konnte der negative Einfluss der Dichte auf das Wachstum der Kreuzkröte im Feld nur bedingt festgestellt werden. Nur das Entwicklungsstadium der Kaulquappen hatte einen Einfluss auf das Körpergewicht. Die Dichte, das Teichvolumen und die Temperatur hatten keinen Einfluss. Da die Kaulquappendichte in den von Menschenhand erstellten Teichen jedoch beträchtlich grösser war als in meiner experimentellen Studie, kann man davon ausgehen, dass die Dichte dennoch einen Einfluss hat.

Die Ergebnisse meiner Studie helfen dabei dem Erfolg zukünftiger Teichbauprojekten beizutragen. Mit dem Wissen über dichteabhängige Prozesse, hat man Informationen über einen Grundpfeiler, welcher bei einem Projekt eine wichtige Rolle spielt. Daher ist es wichtig ein evidenz-basiertes Grundwissen zu erarbeiten, um zum nachhaltigen Erfolg von Naturschutzprojekten beizutragen.

Acknowledgments

I thank Dr. Benedikt Schmidt and Prof. Dr. Arpat Ozgul for supervising me during my work on this Master Thesis. They were a great help by giving me advice and ideas during many discussions we had. I am also grateful to Dr. Josh Van Buskirk on letting me use his facilities during my practical work at the University and lending me his temperature loggers for performing my measurements. A special thank goes to Dr. Mollie Brooks who helped me with my struggles in the analysis of my data. I also want to thank the governments of the cantons of Zurich, Aargau and Basel-Land for giving me permission to enter the nature reserves and letting me perform my research. I am thanking my fellow students in the Masters-office for the great time we had and for taking care of my last three toadlets while I was not there. Furthermore, I want to thank all my friends and family members who supported me during this project by assisting me with fieldwork and proofreading my thesis.

1. Introduction

1.1. Conservation of pond ecosystems

The loss of habitats can have severe consequences on species. Habitat destruction leads to a reduction of population size and a decrease in species richness and diversity (Brooks et al., 2002). The majority of habitat loss is due to anthropogenic activities, it is thus in our responsibility to protect the species that are affected (Dobson et al., 1997). Therefore, it is important that conservation attempts focus on habitat protection, restoration and the establishment of new habitats. To achieve a successful conservation specific knowledge on the ecology and life history of species is essential. Most of the conservation attempts are based on common knowledge and not always based on scientific evidence (Sutherland et al., 2004). Without an evidence-based and efficient conservation management, conservation actions can lead to unintended results (Pullin et al., 2004). An important contribution to conservation is the gain and sharing of knowledge about the success of conservation strategies (Sutherland et al., 2013).

Ponds represent an ecosystem that suffered severely from anthropogenic activities. During the last century, there was a strong decline in the number of ponds due to drainages and conversion of land use (Curado et al., 2011). As a conservation attempt, new ponds have been built to compensate the anthropogenic habitat loss (Rannap et al., 2009). These ponds should replace the previously destroyed habitats, which led to the decline of species. They should provide new mating and breeding places as well as new refuges. By monitoring these ponds, evidence-based knowledge can be gained which is a great benefit for new projects where the lack of

time and resources is a problem. Recent evidence-based conservation attempts now are promising and show first signs of success in creating a stable biodiversity in ponds (Rannap et al., 2009).

By improving conservation strategies, we can protect and manage endangered species. One class of animals which contains many endangered taxa are amphibians (Stuart et al., 2004). Amphibians are endangered because they live in ponds and wetlands that are continuously being lost. With the establishment of new ponds, so called artificial ponds, conservationists hoped to reduce the decline of habitats. However, artificial ponds do not always lead to a successful result (Shulse et al., 2010). If a pond fails to provide a successful habitat it is not necessarily a failure but represents an opportunity for biologists to further understand the dynamics of the pond ecosystem. This information can then be used to improve future projects. By improving pond projects, the conditions for local species will become better, with the goal on establishing a healthy community.

Studies showed that there are three main effects which have an impact on communities in ponds: competition, predation and disturbance (Wilbur, 1987). Out of those, competition seems to have the strongest influence (Purrenhage & Boone, 2009). Competition arises when a high density of individuals share the same resources in the same habitat. If a newly built pond is not providing enough space, density rises and provokes competition. The smaller the size of a newly built pond, the higher the density of individuals living in it. The increased density can therefore have negative effects on the pond community and can decrease the fitness of individuals and the population as a whole (Tejedo & Reques, 1992). Among the organisms living in a pond, tadpoles are strongly affected by density. The development of tadpoles under density stress is drastically affected (Morin, 1983; Semlitsch & Caldwell, 1982). Therefore, artificial ponds, that have been built for the

protection of species, should be designed in a more sensitive way, to become aware of any density influenced effects (Curado et al., 2011).

1.2. Purpose of this Master thesis

With my master thesis I want to investigate whether density dependence occurs in newly built artificial ponds in Switzerland and how density influences the development of larval and juvenile amphibians. This study is of considerable interest to conservation biology, because it tests whether the success of artificial ponds is impeded by density dependence. If there is density dependence, the current conservation management should be improved. Efficient conservation of the natterjack toad is necessary because the native population declined by 60% of the total population since the mid-1980ties (Schmidt & Zumbach, 2005).

I want to test whether density dependence affects larval and juvenile performance under experimental conditions. I also measured tadpole size in artificial ponds and tested whether it was affected by density or environmental factors. Thereby, I get information about the current situation of my study species, and I will find out if the benefit of artificial ponds is affected by density.

2. Methods

This thesis consists of three parts, two of which were conducted at the University of Zurich. The third part was a field study. The two experiments conducted at the University aimed to test effects of density on larval and juvenile performance of *Bufo calamita*. Fieldwork was conducted at several artificial ponds in Northern and Northwestern Switzerland.

2.1. Study Species *Bufo calamita*

B. calamita is a species that occurs in Switzerland as well as in the rest of Middle and West Europe. There are also local populations in Great Britain and Sweden (Sinsch, 1998). Populations mostly occur in altitudes from 300-500 m.a.s.l. and become scarce in altitudes after 600 m.a.s.l. . *B. calamita* is a pioneer species and therefore arrives as one of the first species at new ponds (Meyer, Zumbach, Schmidt, & Monney, 2009).

Its natural occurrence is limited to floodplains and other areas that are temporarily flooded. In those habitats, they prefer areas with sandy or gravelly grounds and almost no vegetation. There, *B. calamita* makes use of the temporary ponds, providing newly formed water bodies (Grossenbacher, 1988). Nowadays such habitats are very rare. To have holms, a river or stream must be under natural conditions, which is in conflict with human landscape management. We used to prefer straightened rivers because they minimize the risk of floods, which can destroy the surrounding landscape. Due to those drastic changes in their environment, *B. calamita* was forced to colonize new areas, which are mainly formed by human activities. In gravel pits they found perfect conditions, which resemble those of their

natural habitat. The area is characterized by poor vegetation, the water bodies are only formed temporarily and the ground is very loose. Other areas where they find ephemeral ponds are in construction sites, military training areas or flooded farmlands (Grossenbacher, 1988; Mermod et al., 2010).

As an attempt to protect *B. calamita*, artificial ponds have been built in gravel pits, resembling their natural habitat. Those artificial ponds are without any vegetation and can be manually drained. The fact that there is no vegetation in and around those ponds benefits the species because it first of all provides more sunlight and also the open area provides sandy and gravelly grounds, so called immature soils. Nevertheless, the manual desiccation is the most important factor that influences the life of *B. calamita* (Lippuner, 2013a). The desiccation of water bodies is very important because it lowers the physical pressure of interspecific competition. It is not possible for other species, preferring the same water bodies, to live under such conditions. Additionally, it also lowers the chance of predators settling (Wilbur, 1987). The greater amount of sunlight benefits *B. calamita* in two ways. First, it helps the water bodies to become warmer, which is preferred by *B. calamita*. This helps them to go through the larval stage much quicker and therefore avoid competition by other individuals. Second, it benefits the adult individuals in their thermoregulation. In addition to the greater amount of sunlight in open areas, the immature soil is important for the adult individuals. They immerse themselves in the ground to reach specific microhabitats that are warm and moist. Therefore soft grounds are preferred (Lippuner, 2013b). Nevertheless, the increased depletion of those gravel pits is destroying their last refuges. Therefore, *B. calamita* is considered as “stark gefährdet” (highly endangered) in the red list of endangered amphibians of Switzerland.

2.2 Experiments

2.2.1. Part I: Density dependence in the larval stage of *B. calamita*

Density can strongly influence the development of tadpoles. Therefore, I conducted an experiment with *B. calamita* tadpoles to investigate the effect of larval density on their body mass, larval development time, survival rate and metamorphosis rate.

The experiment was situated at the campus of the University of Zurich. I conducted the density experiment by establishing mesocosms that varied in density (i.e., the number of larvae per mesocosm). The densities in the experiment were 5, 10, 15, 20 and 30 tadpoles per mesocosm, each density being replicated ten times (Figure 1). This led to a total of 50 mesocosms. I chose this range of densities to mimic density variation in natural ponds (Wilbur, 1987). Every mesocosm was filled with 80 liters of water and 0.2 liters of sand. The top was covered with a lid of shade cloth, whereby natural evaporation was simulated. Two days after filling the mesocosms, I added 0.4 l aged water inoculated with plankton to each of the mesocosms to induce the establishment of a freshwater phyto- and zooplankton community.



Figure 1. Setup of my mesocosms at the University of Zurich

The tadpoles were collected as spawn at two artificial ponds (Table 5, appendix). Two egg strings were collected from a pond in Rütifeld Glattfelden ZH and one egg string from a pond in Gemeindegube Stammheim ZH. The spawn was collected on 13th May 2014 and 15th May 2014, respectively. Tadpoles hatched 10 days after the spawn collection. After hatching, I haphazardly distributed randomly chosen tadpoles to the mesocosms according to the assigned density treatments. Since I was interested in their response to density treatments and not in the genetic differentiation between ponds, I did not consider the origin of tadpoles. The experiment was terminated after 85 days.

During the experiment the number of tadpoles was recorded every second day. Thereby, I was able to see when and how many of the tadpoles died. 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 tadpoles were weighed twice: Once when they reached Gosner stage 42 (Gosner, 1960) and were collected in the mesocosms and a second time when they finished their metamorphosis with full tail absorption (Gosner stage 45).

The tadpole density dependence experiment had four response variables and one explanatory variable. Mesocosm was used as a random effect for mass and development time because tadpoles from the same mesocosm were not independent. (Table 1.)

Table 1. Response and explanatory variables used in the mesocosm experiment with tadpoles

Response Variable	Explanatory Variable	Random effect
Body mass after metamorphosis	Density	Mesocosm
Development time		
Survival rate		
Metamorphosis rate		

The body mass is the mass of the tadpole after full tail absorption. The development time is the number of days from the start of the experiment to the day at which the tadpole reached metamorphosis (stage 42). The survival rate is the proportion of tadpoles that survived until the end of the experiment (85 days). The metamorphosis rate is the proportion of tadpoles that metamorphosed before the end of the experiment.

The data was analyzed in R (R Core Team, 2014). I used the LME4 package (Bolker et al., 2009) to analyze the body mass and the development time. I used a density where I excluded the tadpoles that died during the experiment. The two

models were then compared with a likelihood ratio test. To analyze the effect of density on the survival rate itself and the metamorphosis rate I used a generalized linear model (R Protocol 1, appendix).

2.2.2. Part II: Density dependence in the juvenile stage of *B. calamita*

I investigated whether the density that the newly metamorphosed tadpoles, so-called toadlets, experienced had an influence on their survival and growth. This experiment was conducted by rearing the toadlets for an additional month.

As in the first part of my experiment, I used mesocosms to rear the toadlets. Instead of mainly aquatic mesocosms they contained a mix of land and water habitat. Every mesocosm was holding 0.5 liter sand, a hiding place and some water. The mesocosms were positioned skewed on a piece of wood so the water was concentrated on one side of it. The water was replaced as soon as it evaporated. As hiding places, I used bottom plates of flowerpots, bricks and some pieces of wood (Figure 2). The mesocosms were covered with a fine mesh of shaded cloth, preventing any predators from attacking the toadlets.



Figure 2. Inner view of a mesocosm for housing toadlets of *B. calamita*.

I prepared a total of 20 mesocosms with each containing a density of five toadlets. Toadlets within the same mesocosm were from the same larval density treatment. There were four replicates for each larval density. The toadlets in the mesocosms were fed with collembolans on a daily basis *ad libitum*. The collembolans were bought at a local pet store. Every individual was counted once a week to measure the survival of the toadlets and after one month I measured the mass of the individuals that survived.

I had two response variables and two explanatory variables in my toadlet experiment. Mesocosm was chosen as a random effect, to account for common environmental effects (Table 2).

Table 2 Response and explanatory variables and random effect used in the toadlet experiment

Response Variable	Explanatory Variables	Random Effect
Body mass Survival rate	Density Initial mass	Mesocosm

The body mass is the mass of the toadlets after the duration of one month. The survival rate consists of the number of toadlets that survived one month. The density is the density the toadlets experienced as tadpoles and the initial mass is the mean mass of the toadlets when I started the experiment.

To analyze the effect of density and initial mass on the body mass I used the LME4 package. This was done with the help of a model selection where I used a stepwise variable reduction to look at the significance of these variables. The two models were then compared using an ANOVA. To analyze the effect of density and initial mass on the survival rate I used a generalized linear model (R Protocol 2, appendix)

2.2.3. Part III: Tadpole density measurements at artificial ponds

In the third part of my project I was interested if the density in the artificial ponds has an influence on the body mass of the tadpoles. Furthermore, I investigated the effect of developmental stage, temperature and pond volume on the body mass. I conducted the fieldwork at 20 artificial ponds in Northern Switzerland (Table 5, appendix). I investigated ponds in three cantons, the cantons of Zurich, Aargau and Baselland (Figure 3).

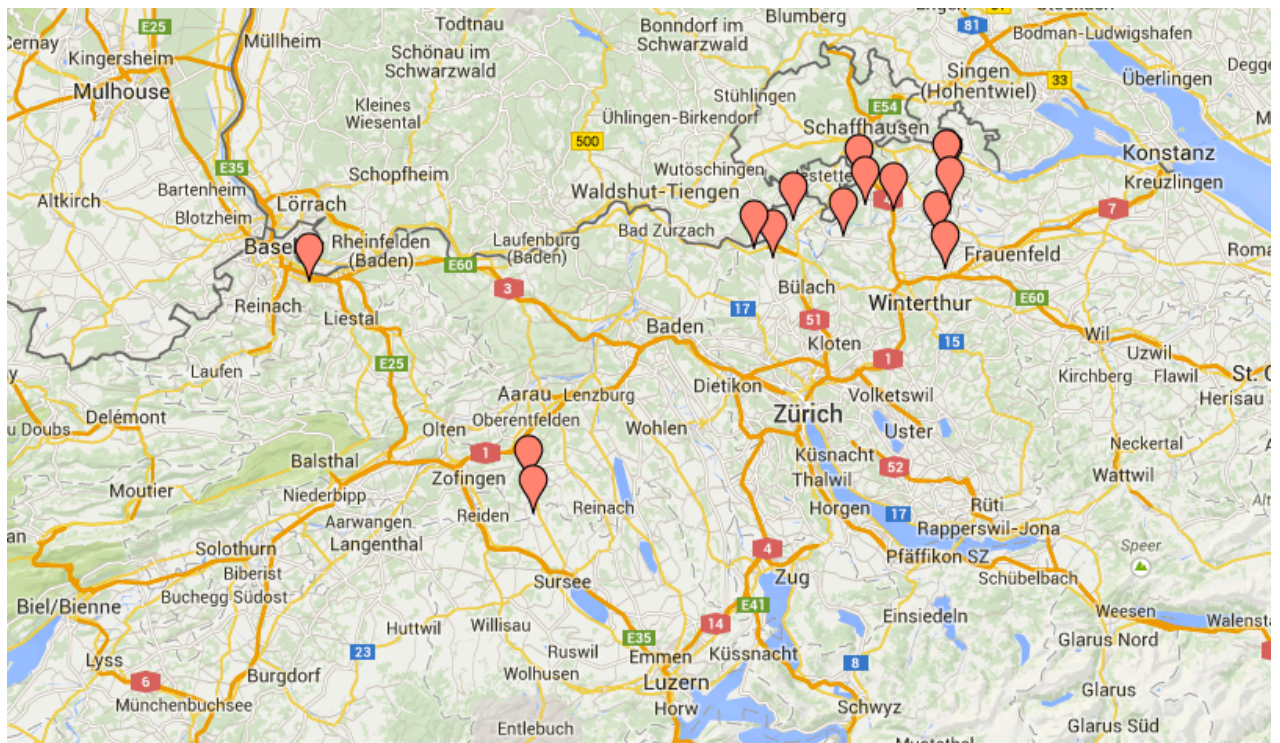


Figure 3. Locations of my study ponds in the northern part of Switzerland. They are distributed among three cantons. Some of the locations contained more than one pond, which was observed (Table 5, appendix).

Body mass of the tadpoles was used as the response variable. The explanatory variables were measured individually and I looked at their effect on body mass. Pond represents a random effect. This makes sure that the ponds did not influence the results itself (Table 3).

Table 3. Response and Explanatory Variables and Random effect used in the pond experiment

Response Variable	Explanatory Variables	Random Effect
Body mass	Density Pond Volume Temperature Development Stage	Pond

I collected the data at each of the 20 ponds. I haphazardly sampled 50 tadpoles, measured the body mass and determined their development stage. I calculated the tadpole density and pond area and I measured the water temperature. To measure the body mass I used a micro scale (max 50g, min 0.001g, Type=FC50, www.waagen4you.ch). To estimate the density, I took pictures of a frame of 0.25m² and repeated it haphazardly ten times over the whole surface of the pond. Due to the clear water and the lack of vegetation, I was able to count the tadpoles in each of the pictures. For each pond I calculated the average density per frame. The volume of the ponds was calculated individually depending on its shape (Figure 4). Most of the ponds had a round or triangular shape. The temperature was measured with temperature loggers over a period of 2 weeks after I weighed the tadpoles.



Figure 4. Example of an artificial pond from Altikon ZH

For analyzing the data collected from the ponds I used R and the LME4 package as well as the MuMin Package (Burnham et al., 2010). I investigated the effect of my explanatory variables (Table 3) and their interaction terms on body mass (R Protocol 3, appendix).

3. Results

3.1. Part I: The effect of density on tadpoles of *Bufo calamita* in mesocosms

To investigate the influence of density on tadpole fitness, I recorded the body mass (mg), larval development time (days), the proportion of surviving metamorphs and the proportion of metamorphosed tadpoles under five different density treatments.

I used a linear mixed effect regression (LMER) to investigate the effect of density on body mass and development time. Density negatively influenced body mass (df=4, estimate=-2.2322, std. error=0.1432, $p < 0.0001$; Figure 5). Tadpoles in high-density mesocosms had a significantly longer development time (df=4, Estimate=1.5295, Std. Error=0.1563, $p < 0.0001$; Figure 6).

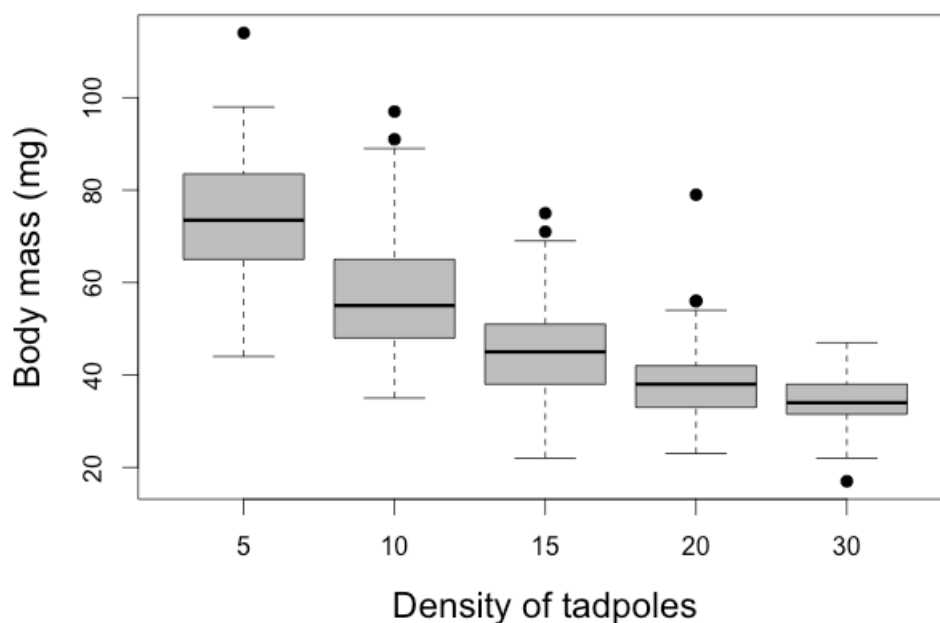


Figure 5. Body mass at metamorphosis of *Bufo calamita* tadpoles in five experimental density treatments. Points above and under the error bars are outliers. Each data point is one tadpole (n=378).

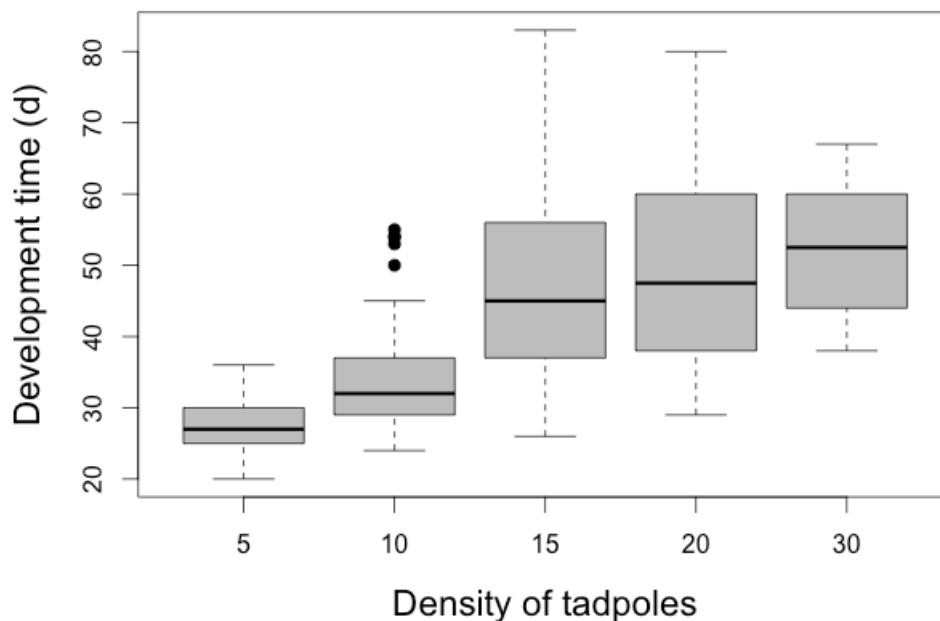


Figure 6. Development time of *Bufo calamita* tadpoles in five density treatments. Outliers are shown by points above the error bars. Each data point is one tadpole (n=378).

I used generalized linear models (GLM) to assess the effects of density on the proportion of toadlets surviving and the proportion of tadpoles that underwent metamorphosis during the experiment. Larval survivorship significantly decreases with density (df=4, Estimate=-0.15254, Std. Error=0.01826, $p < 0.0001$; Figure 7). The same pattern was observed for the number of metamorphs (Figure 8). Whereas nearly all tadpoles in low-density treatments completed metamorphosis, only few larvae under high-density treatments developed into toadlets. The effect of density was highly significant (df=4, Estimate=-0.32556, Std. Error=0.02243, $p < 0.0001$).

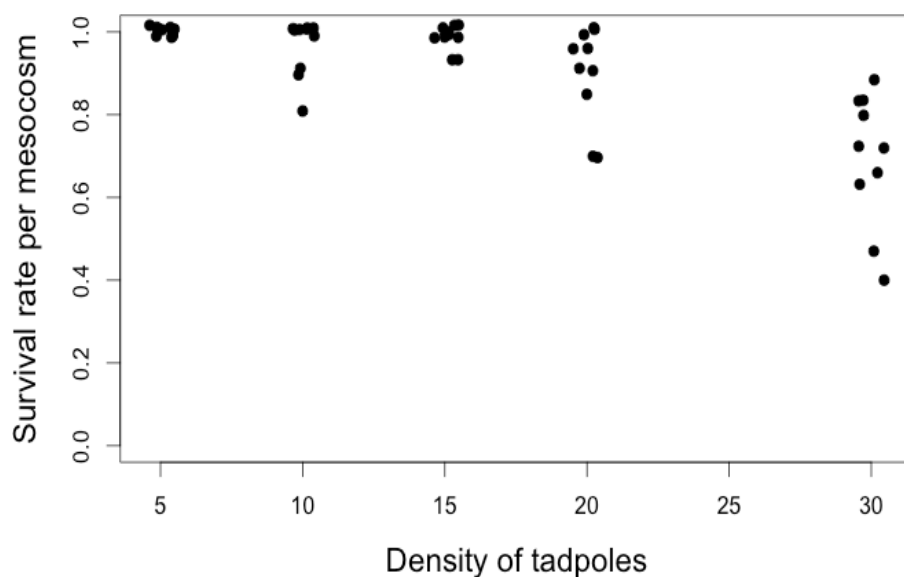


Figure 7. Survival rate of *Bufo calamita* tadpoles in five density treatments. Data points were jittered to show overlaying data points. Each data point represents one mesocosm (n=50).

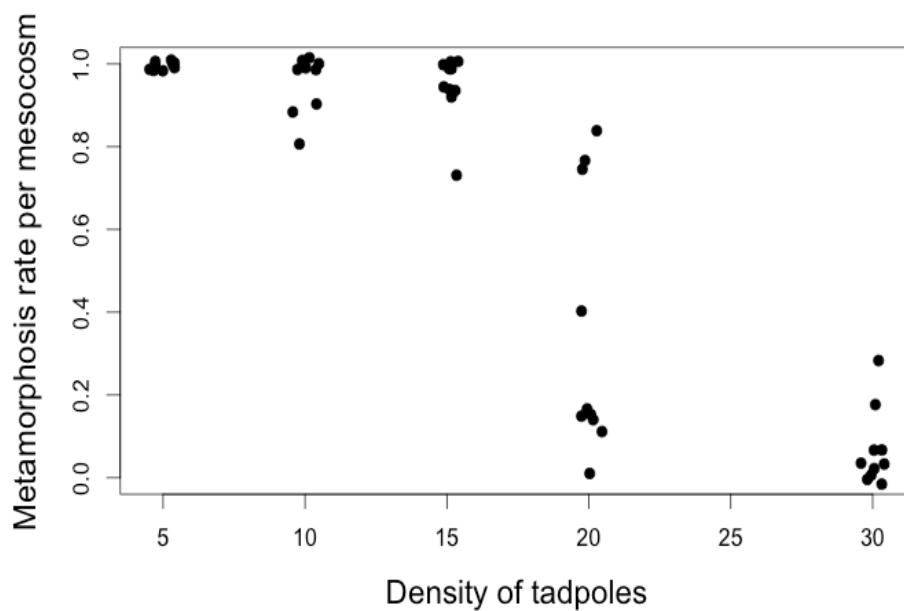


Figure 8. Proportion of metamorphs of *Bufo calamita* tadpoles in five density treatments. Every data point represents one mesocosm (n=50).

3.2. Part II: The effect of density and initial mass on the toadlets of *Bufo calamita*

I tested for the influence of density in the larval stage on the performance of toadlets, by keeping them at the University for an additional month. I also tested for the influence of the initial mass of the toadlets. The initial mass (mean per mesocosm) correlated positively with the mass after one month ($df=18$, Estimate=0.01636, Std. Error=0.005399, $p < 0.01$; Figure 9). Furthermore, the initial mass also had a significant positive influence on the survival rate of toadlets ($df=18$, Estimate=0.05900, Std. Error=0.02173, $p < 0.001$; Figure 10).

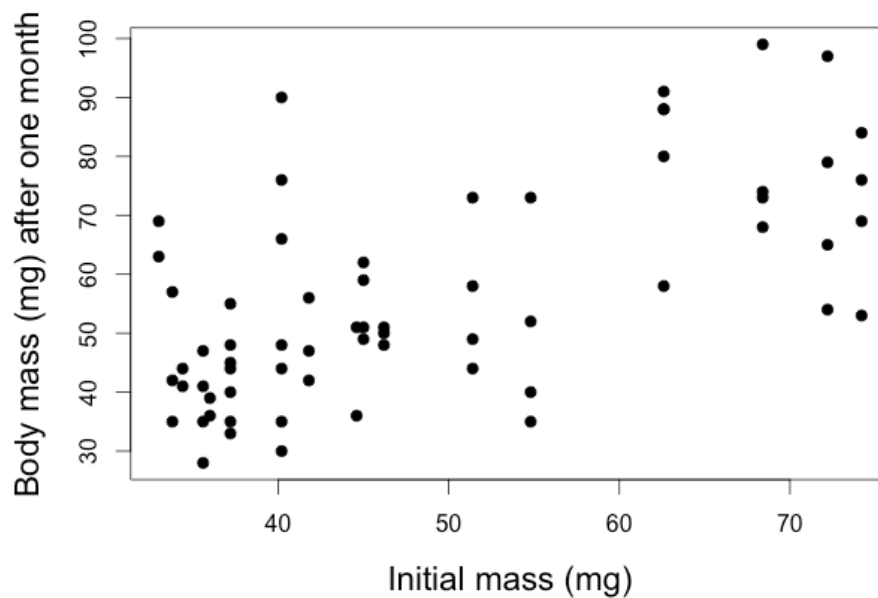


Figure 9. Body mass of *Bufo calamita* toadlets after one month plotted against their initial mass. Each data point represents one individual ($n=64$).

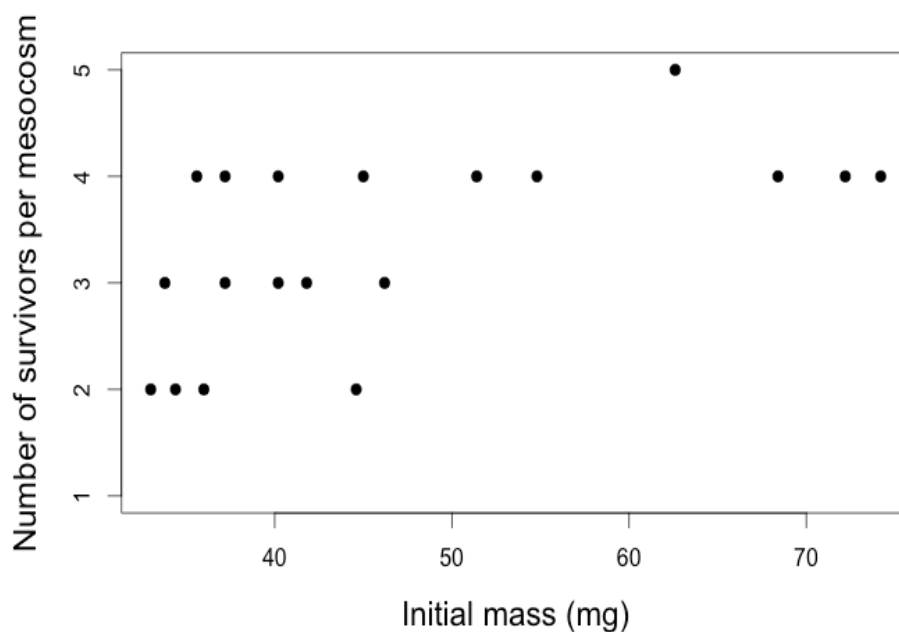


Figure 50. Effect of initial mass on the number of survivors per mesocosm. Each data point stands for one mesocosm (n=19).

The survival rate of toadlets declined significantly with increasing larval density treatment (df=4, Estimate=-0.06942, Std. Error=0.02879, $p < 0.01$, Figure 11). There was no significant effect of larval density on toadlet body mass (df=4, Estimate=0.004566, Std. Error=0.008752, $p=0.5811$, Figure 12).

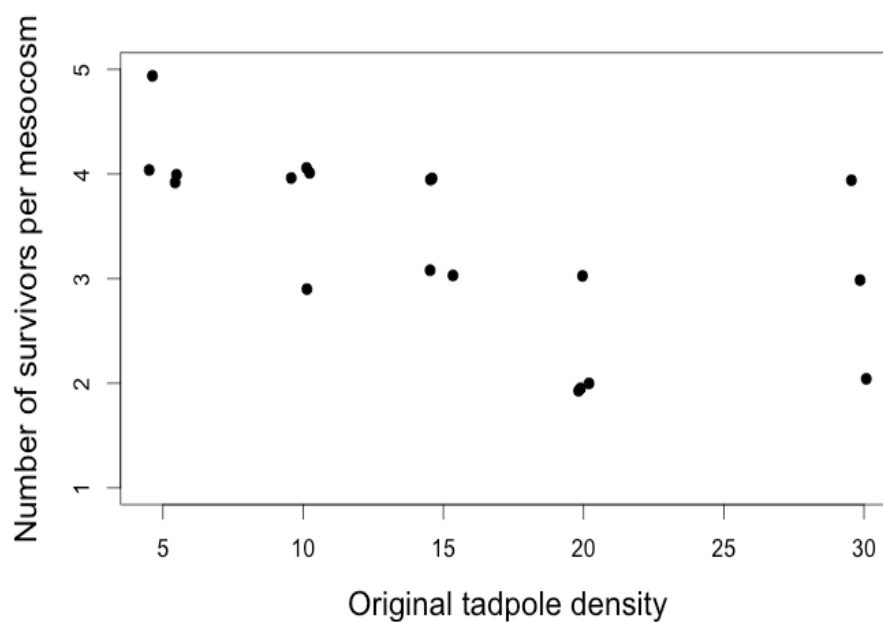


Figure 11. Number of survivors of *Bufo calamita* toadlets influenced by their density in the larval stage. Each data point represents one mesocosm (n=19). The density treatment “30” has only three mesocosms because the number of tadpoles that underwent metamorphosis was too small.

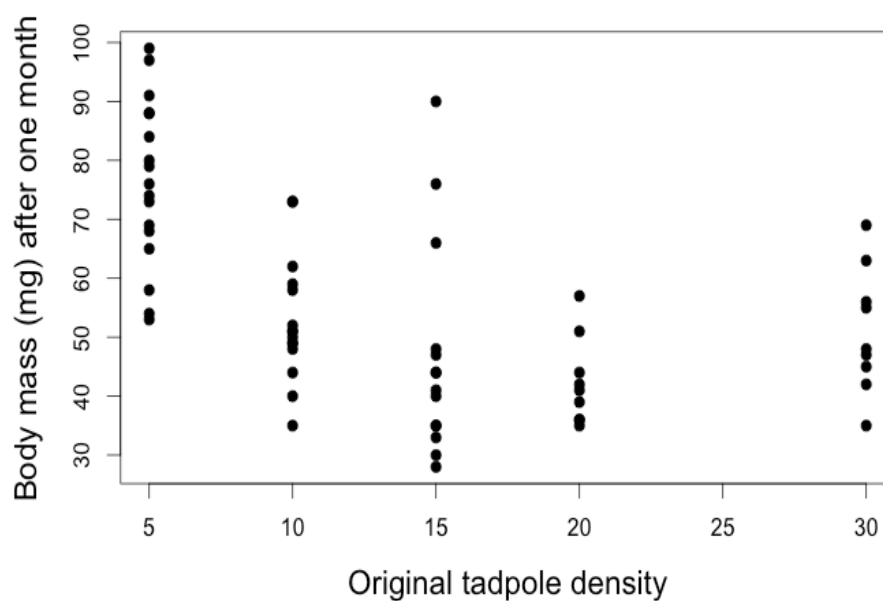


Figure 12. Body mass of *Bufo calamita* toadlets after one month plotted against their larval density treatment. Each data point represents one individual (n=64).

3.3. Part III: The investigation of the artificial ponds created for the conservation of *Bufo calamita*

During my fieldwork, I investigated tadpoles of *Bufo calamita* in twenty ponds in the north and northeastern parts of Switzerland. I estimated the effect of density, water temperature, volume and Gosner stage on tadpole body mass. The average water temperature was measured over a period of two weeks, reaching from 21.98°C to 25.95°C, the estimated pond surface ranged between 3.4m² and 175.19m² and the estimated pond volumes ranged from 0.134 m³ to 23.315 m³.

Table 4. Model-averaged parameter estimates for the effects of explanatory variables on body mass.

	Density	Gosner Stage	Temperature	Volume	Density x Gosner Stage
Average Estimate	-6.118	27.313	-1.613	6.379	-9.783
Average Std. Error	16.695	1.385	7.510	5.911	5.925

Model selection showed that no model was clearly the best one (Table 5, appendix); the most parsimonious model had an AIC (Akaike's Information Criterion) weight of 0.123. All top-ranking models included tadpole density and Gosner stage as explanatory variables for tadpole body mass. Pond volume, pond water temperature and the interaction density-by-Gosner stage were also well supported by the data. Support for all other interactions was weak. With the exception of Gosner stage, all standard errors are wide and the confidence intervals for the parameter estimates include zero (Table 4). The effect of density was not significant; no effect was detected (Figure 13). Volume did also not influence the body mass of the tadpoles; the variation among the different volumes was too big (Figure 16). Temperature does not seem to influence body mass; there is considerable variation

in body mass within and among ponds (Figure 14). As expected, body size correlates positively with the Gosner stage (Figure 15).

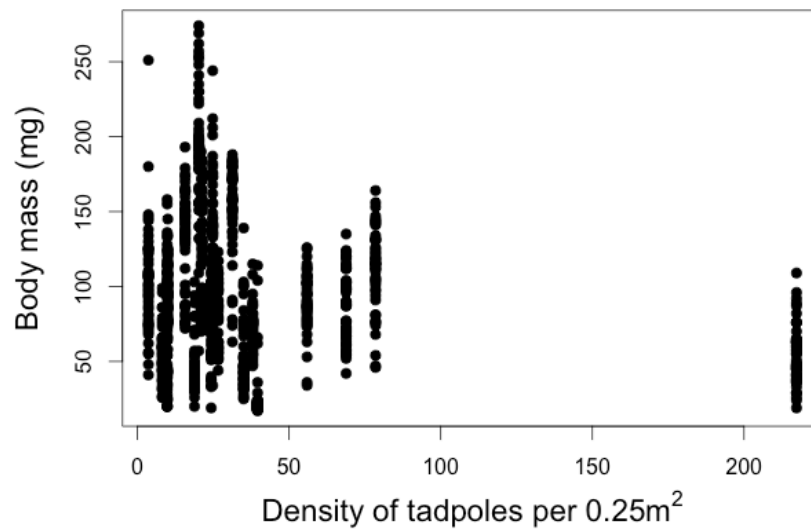


Figure 13. Body mass of *Bufo calamita* tadpoles compared to the density in a 0.25m² square. Every vertical line of data points stands for one pond (n=20) and every data point stands for one individual (n=1000). 50 individuals were measured per pond. The large number of tadpoles in the pond to the far right happened to be a pond with newly hatched tadpoles.

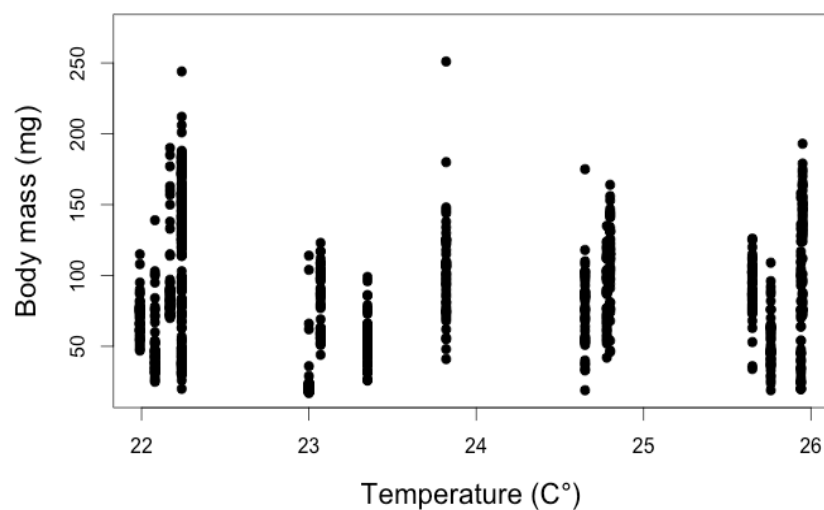


Figure 14. The effect of temperature on the body mass of *Bufo calamita* tadpoles. Every vertical line of data points stands for one pond (n=20) and every data point represents one individual (n=1000).

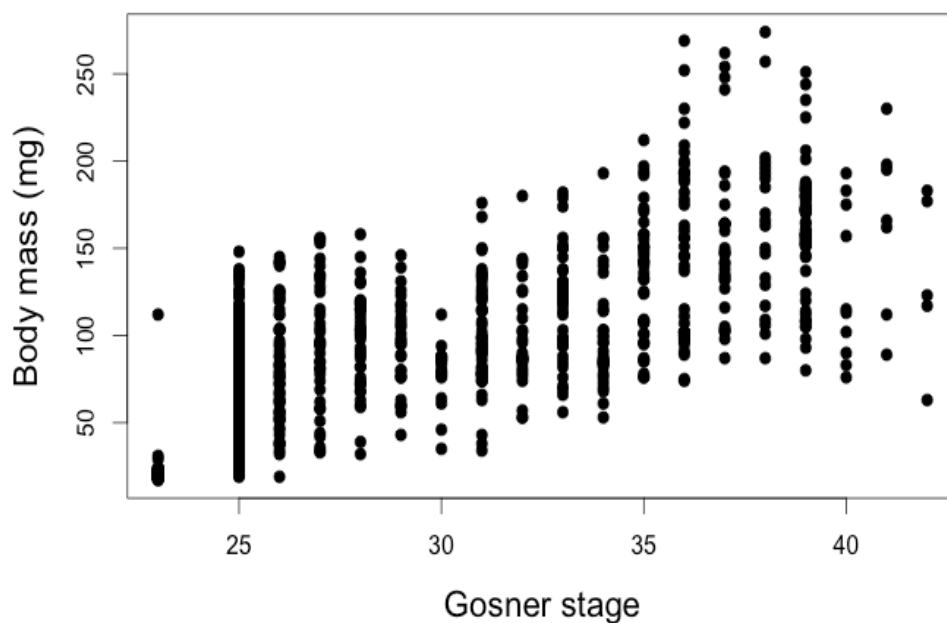


Figure 15. The effect of Gosner Stage on the body mass of *Bufo calamita* tadpoles. Every vertical line of data points stands for one pond (n=20) and every data point represents one individual (n=1000).

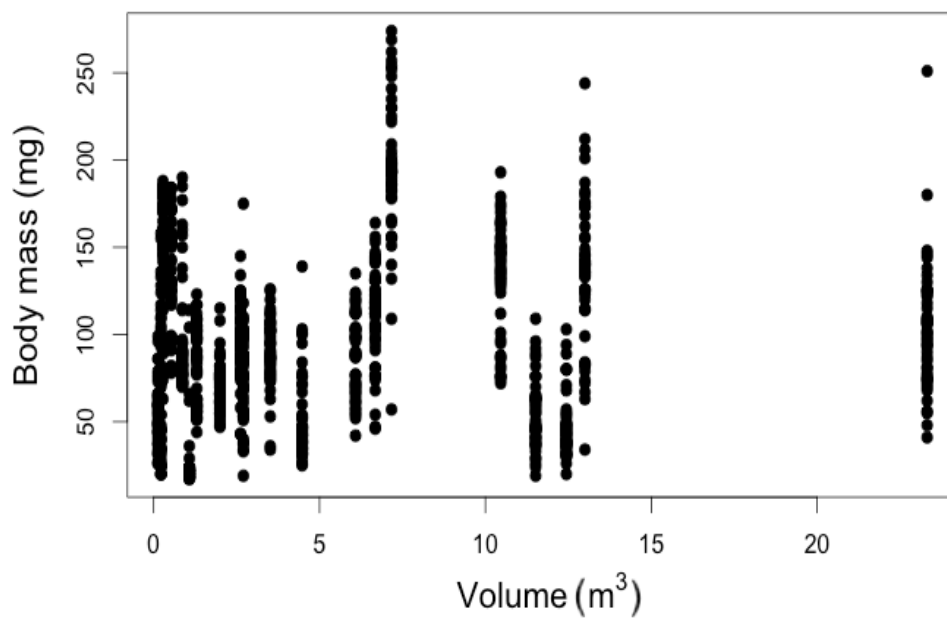


Figure 16. The effect of pond volume on the body mass of tadpoles of *B. calamita*. Every vertical line of data points stands for one pond (n=20) and every data point represents one individual (n=1000).

4. Discussion

Density dependence can have a strong influence on the development of larval amphibians through larval competition for food and space (Wilbur, 1987). Furthermore, the effect of density experienced in the larval stage can translate into fitness effects at the adult and reproductive stages (Goater, 1994; Golay & Durrer, 1996). My findings largely support these findings. Increased density reduced size at metamorphosis, increased the length of the larval period, reduced survival probability, reduced the probability to metamorphose and negatively affected post-metamorphic juvenile growth and survival. Smaller tadpoles develop into smaller toadlets, which in turn may have a reduced survival rate, hence lower fitness. Therefore, density dependence can affect the viability of the population (Altwegg, 2003).

Mass at metamorphosis and the duration of the larval period were both negatively affected by larval density in the mesocosm experiment (Figure 5 & 6). Tadpoles in the lowest density treatment (5 individuals per mesocosm) gained almost double the weight compared to individuals in the highest density treatment (30 individuals per mesocosm). This corresponds to findings of Tejedo & Reques (1992) and Golay & Durrer (1996) who demonstrated that larvae of *B. calamita* are smaller under high density treatments.

Furthermore, larval development time (Figure 6) significantly increased with density. Thus, the cumulative risk of competition during the larval stage is higher in ponds with high density; since larvae with fast development can escape the resource limited water body. A density-mediated reduction of development time has been reported in *Bufo americanus* and other species in which competition plays a major role in the initiation of metamorphosis (Semlitsch & Caldwell, 1982; Wilbur, 1987). The observation that increased density leads to a prolonged larval period could be of

interest for assessments of pond suitability. When density is low and conditions for growth favorable, tadpoles will spend a short time in the pond. Hence, for most of the time, there may be no tadpoles in the pond. In contrast, tadpoles in high density ponds will be present a much longer time. This may mislead assessments of pond suitability. Density also had a negative impact on the proportion of tadpoles that survived and the proportion of tadpoles that went into metamorphosis. In the low-density treatment, none of the individuals died during the experiment, whereas in high-density treatments, the survival rate sharply decreased (Figure 7). In my experiment, the survival rate takes into account all the individuals that were still alive at the end of the experiment. This does not differentiate between the ones that reached metamorphosis and those who did not. The negative effect of density is even stronger on the rate of successful metamorphosed individuals than on survival rate. This finding corresponds to the results of Semlitsch (1982), who tested density effects on *Scaphiopus holbrooki* (Southern Spadefoot Toads) and Wilbur (1977) on *Bufo americanus*. Wilbur (1977) reports some variation in adult body size under the high-density treatment. A similar pattern was visible in my results; however, the number of larvae that achieved a successful metamorphosis prior to the end of my experiment was very low. The experiment was terminated after 85 days, due to a slowdown of the metamorphosing of the remaining tadpoles. The continuing of the experiment would have led to the unnecessary deaths of many tadpoles, which was not intended.

Juvenile performance was also affected by larval density. Both, density and mass at metamorphosis showed an effect on the toadlets. A carry-over effect of density was detected from the larval stage to the juvenile stage (Goater, 1994). Toadlets that experienced high larval density had a lower probability to survive for an additional month after metamorphosis than toadlets from the low-density treatment

(Figure 11). This was also shown in the work of Golay & Durrer (1996) with *B. calamita*, where they state that the effects experienced as tadpoles are still visible in the juvenile stage. Due to the fact that in my experiment the tadpoles coming from the high densities did not successfully metamorph, it can be assumed that the effect would be even stronger for them. Another important point is, that toadlets do not only experience the density from the tadpole stage, they also undergo an additional density effect, when large groups of tadpoles metamorph into large groups of juveniles that then further compete for resources.

In the field, there was no significant effect of larval density, pond volume or pond water temperature on tadpole body mass. This was probably because there was almost as much variation in body size within ponds as there was variation among ponds. The density-by-Gosner stage interaction however was significant. Therefore, density can affect the body mass of tadpoles at certain Gosner stages. The other interactions showed only little effect on the tadpoles, which can be neglected. The analysis I used gave me information about how close my models are to the best model. Gosner Stage had a strong positive effect on body mass, which increased with the developmental stage. As shown in Figure 15, growth ceases at Gosner Stage 42 and the further development leads to a size decrease due to the absorption of the tail. The effect of density, temperature and volume has to be taken with caution due to the large standard errors. I expect a clearer result by an increased sample size (i.e. recording more ponds with high variation in density), and by the applying more precise parameter estimates, such as the pond volume. Nevertheless, my field data indicates that the larval density in natural environments may be much higher than the environmental treatments used in this study, as all ponds, except for one pond (Rheinau) (Table 7, appendix), exceed the laboratory density treatments. This indicates that density effects could also appear in the

artificial ponds, which were built for the conservation of *B. calamita*. By looking at Figure 13 we can see a decrease of body mass in the higher densities of tadpoles. The effect, however, was not statistically significant. The large density of more than 200 tadpoles per 0.25m² is due to newly hatched tadpoles.

By measuring the ponds with more sophisticated methods, the accessible volume for the tadpoles would be estimated more precisely. This seems to be important because the pond volume plays a relevant role in the development of tadpoles when it comes to the reduction of density dependent problems. The pond volume can also be increased by additional ponds which therefore increase the cumulative pond volume (Beebee et al., 1996).

4.1. Conclusions

This study documents the importance of larval density in the development of tadpoles and its relevance to wildlife management and conservation biology. Mesocosm experiments demonstrated that body size (and various other fitness traits) of *B. calamita* larvae is negatively affected under high larval densities. In addition, field data may support this finding because density in the field was higher than in the experiment, illustrating the potentially detrimental effect of inappropriate conservation actions. A decrease in fitness of juvenile toads likely leads to reduced adult fitness, affecting the whole population. Increasing the size of artificial ponds would temporarily reduce negative density effects. However, if the population grows it will reach the carrying capacity of larger ponds. But, a greater carrying capacity in a bigger pond is better than a smaller carrying capacity in a small pond. An alternative management strategy was investigated by Denton et al. (1997), who showed that the establishment of several additional small ponds positively influenced the population

size of *B. calamita* in Great Britain. Nevertheless, successful conservation actions must not only act on the site of the water bodies for larval development and reproduction, also key requirements of juvenile and adult individuals must be fulfilled. With the establishment of said artificial ponds, the conservationists are able to investigate the efficiency of the conservation attempts and gain an evidence-based knowledge. It will help future projects, by saving personnel and financial resources and therefore lead to a more sustainable way of conservation. Artificial ponds are therefore an important tool to prevent a species from its extinction and can be even more successful the more information is available.

5. References

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6. Appendix

Table 5. Names and locations of the observed ponds during the fieldwork.

City	Name of area	Coordinates	Number of ponds
Rheinau	Rhinauerfeld	689.271/277.376	1
Marthalen	Steinacher	689.889/274.725	1
Flaach	Buchbrunnen	687.521/270.784	2
Kleinandelfingen	Müllersbuck	694.087/273.845	1
Waltalingen	Raffolterseekiesgrube	701.558/274.992	1
Unterstammheim	Gemeindegrube	701.162/278.316	1
Unterstammheim	Bötschigrube	700.397/278.532	1
Altikon	Büelhüsli	699.957/270.260	1
Rickenbach	Ebnet	701.081/266.528	1
Wil	Kiesgrube Wil	680.911/272.133	1
Glattfelden	Rütifeld	678.028/267.598	1
Weiach	Kiesgrube Weiach	675.776/269.017	2
Staffelbach	Pelzacker	646.286/237.389	1
Reitnau	Feldmatt	646.867/233.492	2
Muttenz		617.183/263.867	3

Table 6. Results from the linear mixed regression analysis of the pond data. The table shows the different models, the parameter estimates, d.f, log-likelihood, AIC, deltaAIC and Akaike weights. Den = Density, Gos = Gosner Stage, Temp = Temperature and Vol = Volume.

Intercept	Density	Gosner Stage	Temperature	Volume	Den:Gos	Den:Temp	Den:Vol	Gos:Temp	Temp:Vol	df	Log Lik.	AIC	ΔAIC	weight
89.36	-11.78	27.33		7.739	-10.96					7	-4066.21	8146.5	0	0.123
89.67	-10.51	27.31	-1.421	8.19	-9.02			-2.572		9	-4064.24	8146.7	0.14	0.116
89.35	-10.83	27.2			-11.16					6	-4067.55	8147.2	0.64	0.090
94.04	6.582	27.32	-4.934	10.14	-9.148	-13.39		-2.655		10	-4063.61	8147.5	0.93	0.078
88.81	-12.43	27.33	-0.5805	8.721	-8.6			-2.691	6.32	10	-4063.71	8147.7	1.13	0.070
89.66	-9.982	27.15	-0.3736		-9.391			-2.417		8	-4065.84	8147.8	1.3	0.064
89.99	-5.949	27.26		4.839	-11.26		-6.373			8	-4066.02	8148.2	1.65	0.055
90.36	-4.247	27.22	-1.303	5.041	-9.337		-6.9	-2.616		10	-4063.99	8148.2	1.69	0.053
93.34	5.383	27.34	-4.214	10.8	-8.681	-14.03		-2.802	6.687	11	-4062.98	8148.3	1.71	0.052
89.33	-11.17	27.35	-1.586	7.901	-10.91					8	-4066.16	8148.5	1.94	0.047
89.34	-10.61	27.21	-0.5661		-11.14					7	-4067.54	8149.2	2.66	0.033
89.46	-6.858	27.25	-0.522	5.937	-8.907		-6.035	-2.727	5.971	11	-4063.51	8149.3	2.78	0.031
93.46	4.995	27.35	-4.917	9.742	-11.08	-12.67				9	-4065.65	8149.5	2.97	0.028
94.17	6.699	27.33	-5.112	10.58	-9.118	-14.01	0.7533	-2.654		11	-4063.61	8149.5	2.98	0.028
88.56	-12.91	27.36	-0.8541	8.352	-10.65				5.532	9	-4065.8	8149.8	3.25	0.024
90.8	-5.466	27.14	-1.23		-9.443	-3.508		-2.424		9	-4065.8	8149.8	3.26	0.024
89.95	-5.469	27.28	-1.483	5.035	-11.2		-6.274			9	-4065.98	8150.2	3.61	0.021
93.89	5.857	27.39	-4.964	12.74	-8.517	-16.8	3.302	-2.803	6.951	12	-4062.94	8150.2	3.69	0.020
90.74		29.02	-3.527	8.496				-3.499		7	-4068.26	8150.6	4.1	0.016
92.82	3.85	27.37	-4.285	10.3	-10.8	-13.21			5.85	10	-4065.22	8150.7	4.15	0.015
90.39	-6.474	27.2	-1.35		-11.19	-3.211				8	-4067.51	8151.2	4.64	0.012

Table 7. Number of tadpoles per m³ in each pond and number of tadpoles per m³ according to the densities in the mesocosms.

Pond Location	Tadpoles/m ³	Mesocosm density	Tadpoles/m ³
Rheinau	95	5	62.5
Marthalen	870	10	125
Flaach 1	12290	15	187.5
Flaach 2	2140	20	250
Kleinandelfingen	3220	30	375
Waltalingen	730		
Unterstammheim	530		
Gemeindegrube			
Unterstammheim	1220		
Bötschigrube			
Altikon	2860		
Rickenbach	510		
Wil	1090		
Glattfelden	630		
Weiach 1	1260		
Weiach 2	3030		
Staffelbach	1050		
Reitnau 1	620		
Reitnau 2	920		
Basel 1	1340		
Basel 2	1520		
Basel 3	930		

R protocol 1:

R code used for the analysis of the effect of density on the larvae.

Body mass:

```
model1<-lmer(mass~density+survivalrate+(1|mesocosm), data=dd)
```

```
model2<-lmer(mass~survivalrate+(1|mesocosm), data=dd)
```

```
anova(model1,model2)
```

Development time:

```
model1<-lmer(age~density+survivalrate+(1|mesocosm), data=dd)
```

```
model2<-lmer(age~survivalrate+(1|mesocosm), data=dd)
```

```
anova(model1,model2)
```

Survival rate:

```
model<-glm(cbind(survivors,density-survivors)~density, data=dd, family=binomial)
```

Metamorphosis rate:

```
model<-glm(cbind(metamorphs,density-metamorphs)~density, data=dd,
```

```
family=binomial)
```

R Protocol 2

R code used for the analysis of the effect of density on the juveniles.

Body mass:

```
model1<-lmer(log(mass)~initialmass+density+(1|mesocosm), data=dd)
```

```
model2<-lmer(log(mass)~density+(1|mesocosm), data=dd)
```

```
anova(model1,model2)
```

Survival rate:

```
model<-glm(cbind(survivors,density-survivors)~density+initialmass, data=dd, family=  
binomial)
```

R Protocol 3

R code used for the analysis of the data collected in the artificial ponds.

```
model<-lmer(mass~density+volume+temperature+volume:temperature+  
density:volume+density:temperature+(1|pond), data=na.omit(dd), REML=F)  
dredgemodel<-dredge(model)
```

Statement of Authorship:

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Zurich, 30.01.2015

Tobias Weber