Patterns of natural selection on size at metamorphosis in water frogs

Altwegg, Res; Reyer, Heinz-Ulrich

Patterns of natural selection on size at metamorphosis in water frogs

Abstract

Strategies for optimal metamorphosis are key adaptations in organisms with complex life cycles, and the components of the larval growth environment causing variation in this trait are well studied empirically and theoretically. However, when relating these findings to a broader evolutionary or ecological context, usually the following assumptions are made: (1) size at metamorphosis positively relates to future fitness, and (2) the larval growth environment affects fitness mainly through its effect on timing of and size at metamorphosis. These assumptions remain poorly tested, because data on postmetamorphic fitness components are still rare. We created variation in timing of and size at metamorphosis by manipulating larval competition, nonlethal presence of predators, pond drying, and onset of larval development, and measured the consequences for subsequent terrestrial survival and growth in 1564 individually marked water frogs (Rana lessonae and R. esculenta), raised in enclosures in their natural environment. Individuals metamorphosing at a large size had an increased chance of survival during the following terrestrial stage (mean linear selection gradient: 0.09), grew faster and were larger at maturity than individuals metamorphosing at smaller sizes. Late metamorphosing individuals had a lower survival rate (mean linear selection gradient: -0.03) and grew more slowly than early metamorphosing ones. We found these patterns to be consistent over the three years of the study and the two species, and the results did not depend on the nature of the larval growth manipulation. Furthermore, individuals did not compensate for a small size at metamorphosis by enhancing their postmetamorphic growth. Thus, we found simple relationships between larval growth and postmetamorphic fitness components, and support for this frequently made assumption. Our results suggest postmetamorphic selection for fast larval growth and provide a quantitative estimate for the water frog example.

PATTERNS OF NATURAL SELECTION ON SIZE AT METAMORPHOSIS IN WATER FROGS

Res Altwegg and Heinz-Ulrich Reyer

Institute of Zoology, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland.

Author for correspondence:

Res Altwegg

Email: altweggr@zool.unizh.ch

Phone: ++41 1 635 49 82

Fax: ++41 1 635 68 21

Abstract.—Strategies for optimal metamorphosis are key adaptations in organisms with complex life cycles, and the components of the larval growth environment causing variation in this trait are well studied empirically and theoretically. However, when relating these findings to a broader evolutionary or ecological context, usually the following assumptions are made: 1) size at metamorphosis positively relates to future fitness, and 2) the larval growth environment affects fitness mainly through its effect on timing of and size at metamorphosis. These assumptions remain poorly tested, because data on post-metamorphic fitness components are still rare. We created variation in timing of and size at metamorphosis by manipulating larval competition, non-lethal presence of predators, pond drying, and onset of larval development, and measured the consequences for subsequent terrestrial survival and growth in 1564 individually marked water frogs (*Rana lessonae* and *R. esculenta*), raised in their natural environment. Individuals metamorphosing at a large size had an increased chance of survival during the following terrestrial stage (mean linear selection gradient: 0.09), grew faster and were larger at maturity than individuals metamorphosing at smaller sizes. Late metamorphosing individuals had a lower survival rate (mean linear selection gradient: -0.03) and grew more slowly than early metamorphosing ones. We found these patterns to be consistent over the three years of the study and the two species, and the results did not depend on the nature of the larval growth manipulation. Furthermore, individuals did not compensate for a small size at metamorphosis by enhancing their post-metamorphic growth. We thus found simple relationships between larval growth and post-metamorphic fitness components, and thus support for this frequently made assumption. Our results suggest post-metamorphic selection for fast larval growth and provide a quantitative estimate for the water frog example.

Key words.—AICc, capture mark recapture, complex life cycles, life history, manipulated phenotype, metamorphosis, survival

INTRODUCTION

Organisms with complex life cycles occupy different niches during their ontogeny. The shifts between subsequent niches are often accompanied by dramatic morphological transformations, termed metamorphosis (Wilbur 1980; Cohen 1985). As growth opportunities and predation risk vary between the habitats associated with the niches, the ability to time metamorphosis in a way that will optimize fitness is a key adaptation of any species with a complex life cycle.

Several models have been proposed that explain how the timing of metamorphosis is optimized (Wilbur and Collins 1973; Smith-Gill and Berven 1979; Travis 1984; Werner 1986; Rowe and Ludwig 1991, recently reviewed in Harris 1999). Critical elements of these models are the patterns of postmetamorphic survival and growth, which the models either include as explicit terms or implicit assumptions. Empirical studies applying these models to predict metamorphosis in a number of different organisms have almost exclusively focused on the pre-metamorphic stage (Alford and Harris 1988; Skelly and Werner 1990; Hensley 1993; Twombly 1996; Flanagin et al. 2000), assuming simple relationships between pre-metamorphic traits and post-metamorphic fitness components.. However, empirical data to test this assumption are still rare. In order to improve an understanding of how evolution acts in organisms with complex life cycles, empirical answers the following questions are urgently needed.

1) How much does a certain change in size at metamorphosis affect subsequent survival and growth under natural conditions, and is there selection on timing of metamorphosis? Theory assumes a positive relationship between size at metamorphosis and fitness, and empirical studies on several groups of organisms now demonstrate that a large size at metamorphosis enhances survival, fecundity and/or growth in later life stages (insects: Taylor et al. 1998; fish: Ward and Slaney 1988; Ryding and Skalski 1999; amphibians: Smith

1987; Berven 1990; Scott 1994; Goater 1994; Morey and Reznick 2001). A larger size may enhance fitness through improved jumping ability and stamina, or lower risk of starvation and desiccation (John-Adler and Morin 1990). While these studies convincingly demonstrate the presence of the assumed pattern, quantitative estimates based on the fate of marked individuals under natural conditions, and estimates of selection strength on metamorphic characters are still lacking.

2) How much does the relationship between size at metamorphosis and subsequent survival/growth depend on the time of the season? In seasonal environments, particular combinations of timing of and size at metamorphosis may be favored or disfavored by natural selection. For example, a large size at metamorphosis may be particularly important late in the season when terrestrial growth conditions deteriorate.

3) Do timing of and size at metamorphosis alone determine subsequent survival and growth? This is assumed by theory and most empirical studies, but there may be carry-over effects of the larval growth environment on these fitness components In this case, age and size at metamorphosis may not be good measures for fitness.

4) Does size at metamorphosis determine size at maturity, or can individuals compensate for a small size at metamorphosis by enhancing postmetamorphic growth? If compensatory growth is common, individual differences in size at metamorphosis may reflect different growth strategies rather than differences in quality.

Our experimental study on water frogs (*Rana lessonae* and *R. esculenta*) was designed to address the questions raised in the previous paragraphs. Over a period of three years, we monitored postmetamorphic growth and survival of 1564 individually marked animals raised under natural conditions. As part of a larger study, we manipulated timing of and size at metamorphosis by raising the larvae under different densities, in the presence or absence of caged predatory dragonfly larvae, under different drying regimes. We chose these larval manipulations to reflect variation in natural conditions that potentially affect metamorphosis.

High larval density, presence of predators, and long hydroperiods delays metamorphosis, while high larval density and short hydroperiods decreases size at metamorphosis (Altwegg 2002a; Altwegg 2002b). We did not consider chemicals, such as agricultural toxins, which well may affect post-metamorphic fitness components.

Here, we examined post-metamorphic survival and growth in relation to timing of and size at metamorphosis. Timing of metamorphosis here means the day of the season on which an individual metamorphosed. However, timing of metamorphosis is equivalent to age at metamorphosis in our experiment, because larval development started at similar times in the season for all individuals. We considered three periods in the postmetamorphic lifewhich reflect slightly different aspects of a frog's fitness: (a) the first weeks in the terrestrial habitat, which show the immediate consequences of metamorphic decisions; (b) from metamorphosis to first spring, which appears to be a period of high mortality in amphibians (Clarke 1974; Clarke 1977; Pechmann et al. 1991); and (c) one additional year in which the surviving individuals reach sexual maturity. Survival until and size at maturity are fitness components with a strong effect on population dynamics (Taylor and Scott 1997; Biek et al. 2002). We estimated the strength of natural selection on timing of and size at metamorphosis using survival between metamorphosis and the following spring as a fitness measure. As timing of and size at metamorphosis are related through the larval growth rate, with the consequence that a change in any of the three traits neceessarily affects one or both of the other traits (Abrams and Rowe 1996), we also report correlational selection on age and size. Finally, we assess the repeatability of the qualitative and quantitative results over the three years of the study.

METHODS

The study species

The western palearctic water frogs form a species group that is widely distributed across Europe and includes many types of mixed populations containing two or three species at one location (Graf and Polls Pelaz 1989). *Rana esculenta* is originally an interspecific hybrid between *R. lessonae* and *R. ridibunda* (Berger 1964) with a reproductive system known as hybridogenesis (Schultz 1969; Berger 1988; Graf and Polls Pelaz 1989). Som et al. (2000) present a recent description of the water frog system present in Switzerland. Around Zürich, the area of our study, mixed populations of *Rana lessonae* and *R. esculenta* occur, with species composition varying between sites (Blankenhorn et al. 1973; Holenweg Peter et al. 2002). Within and between ponds, there is considerable variation in size at and timing of metamorphosis, the latter being inflated by the extended breeding season of the adults (Abt Tietje and Reyer, *unpublished manuscript*). Seasonality imposes time constraints on the larval period because tadpoles cannot survive the winter.

Manipulation of larval growth environment

Larvae of *Rana lessonae* and *R. esculenta* were raised in 1998, 1999, and 2000 using 1100 L fiberglass tanks ($1.4m^3$)., We created variation in time to and size at metamorphosis through (a) different densities (30, 60 or 90 tadpoles/tank in 1998; 25 or 75/tank in 2000), (b) raising tadpoles in the presence or absence of caged (thus non-lethal) predacious dragonfly larvae (*Anax imperator*) (1998, 1999) or (c) by imposing time constraints on the development by manipulating the start of development and by simulating pond drying (1999). Further details of the experimental treatments and their effects on metamorphosis are described elsewhere (Altwegg 2002a; Altwegg 2002b; *unpublished manuscript*).

In all three years, the tadpoles metamorphosed between 20 July and 1 October, and 90-94% of the individuals had completed metamorphosis when we ended the experiments. When

the froglets had resorbed their tail (stage 46, Gosner 1960), we estimated their body size by measuring head width, snout-vent length, and tibia length to the nearest 0.01mm using a slide caliper. The first component of a principal component analysis on these three measures explained between 96% and 98% of their variance and was therefore a very good estimate of the overall body size. We calculated separate principal component analyses for each year, and, for examining variation in survival between the years, we calculated a single principal component analysis using all data.

Monitoring survival and growth in the terrestrial habitat

When the freshly metamorphosed froglets had completed tail resorption, we marked individuals with a unique combination of toe clips. We never clipped more than one toe per foot, and we did not clip the first toe of the front feet, which plays an important role in male mating behavior. During the few days between metamorphosis and their release into field enclosures (<7 days for 91% of the individuals), the froglets were kept at low densities (up to ten individuals) in five-liter plastic tubs, and fed crickets ad libitum. The individuals were grouped randomly with respect to their larval history and body size. The number of froglets studied in the different years are shown in Table 1.

Starting on 6 August in 1998, 3 August 1999, and 28 July 2000, we assigned marked individuals evenly to open-top field enclosures and released them at least once every two weeks, until maximal densities of 4.5 (in 1998), 6 (in 1999), and 2.8 or 8.3 (in 2000) individuals per m^2 were eventually reached. Frog densities differed between years because different numbers of frogs were available in each year. Even though densities were confounded with the year effect, we were able to examine the effect of terrestrial density by comapring high and low densitty treatments within the year 2000. These enclosures were situated in a forest near Zürich, Switzerland, where water frogs are known to spend the winter (Holenweg and Reyer 2000). They measured 18 m^2 in 1998 and 1999 and 9 m^2 in 2000 and

were designed to keep the froglets in conditions that were as natural as possible. We randomly assigned individuals to four enclosures in 1998, to six enclosures in 1999, and to twelve enclosures in 2000. All enclosures were located in the same forest clearing. Fences, constructed of fine-meshed steel wiring and strengthened in 1999 and 2000 with a plastic shade cloth, extended approximately 40cm into the soil. While a 15cm wide overhanging plastic strip prevented the frogs from climbing out, potential predators or small invertebrates could enter the enclosure unhindered. Each enclosure had a shallow-bottomed plastic bowl with a diameter of 65cm that served as a small permanent pool.

First weeks data.- Every two weeks until mid October of the years 1998 and 1999, but not in 2000, we recaptured the surviving froglets by carefully searching every enclosure for 30 minutes. The captured frogs were identified, weighed and measured in the same way as after metamorphosis, and released back into the enclosures on the same day.

Over winter data.- In all three years after release in spring, we recaptured and removed every surviving individual by repeating recapture efforts until no more individuals were found. We are confident that we caught all survivors at this stage because the frogs emerge in spring to spend their summer in ponds.

Second year data.- We monitored survival and growth during the second terrestrial year in 41 of the frogs that had gone through their larval stage in 1998 and survived their first winter in the terrestrial habitat (Table 1). The frogs were transferred to two fenced artificial ponds near Zürich, Switzerland. The ponds were 60cm deep, with a surface area of $40m^2$, and were surrounded by fine-meshed steel fences similar to those used in the terrestrial experiment (for details see Thurnheer and Reyer 2001). On 15 May and 22 June 2000, we recaptured the surviving frogs, identified, weighed and measured them. Males with vocal sacs and thumb pads, and females larger than 44mm (*R. lessonae*) or 55mm (*R. esculenta*), were considered sexually mature (Bättig 1998).

Statistical analyses

The main goals of this study were, first, to quantify the relationship between size at metamorphosis and survival / growth during three periods of postmetamorphic life; second, to examine whether this relationship depended on the time of the season; third, to find potential effects of the larval growth conditions on post-metamorphic life not accounted for by variation in date of and size at metamorphosis; and, fourth to examine whether individuals can compensate for a small size at metamorphosis by increasing their post-metamorphic growth.

To achieve these goals, we studied post-metamorphic survival and growth in relation to size at and date of metamorphosis. Our measure of size was the first principal component extracted from body length, tibia length and head width, which we considered to be less affected by short term fluctuations than, for example, body mass. In those individuals for which we measured both mass and size, these two measures were highly correlated (r=0.95, n=638). Date of metamorphosis is equivalent to age at metamorphosis within the years 1998 and 2000 because all tadpoles started their development simultaneously. In 1999, and between years, date of and age at metamorphosis were highly correlated (r=0.76, n=1564). The qualitative results are the same for date of and age at metamorphosis and we therefore treat these two variables as being equivalent in this study.

Throughout this study, we made use of two different statistical concepts, significance testing and model selection. In the former case, we tested the data against the null-hypothesis that the statistical relationship between two variables equals zero. This was done, when the experimental design allowed us to consider one single model (e.g. a regression). However, in some cases we felt that several statistical models (e.g. two regression models including a different set of explanatory variables each) could plausibly explain the data. In this case, each model represented a different biological hypothesis and support for each hypothesis was evaluated by assessing the parsimony of the corresponding model (Burnham and Anderson 2002). Parsimony is the ability of a model to represent the structure in the data with a minimal

number of parameters. Rather than relying on an arbitrary cutoff value (e.g. 5%), model selection ranks the models according to their parsimony and allows assessing the strength of evidence for any given model compared to the other models in the set.

We conducted separate analyses for each year, and used individuals as replicates in all analyses. The goal was to explore the sources of variation in performance between individuals, and we used multiple enclosures to reduce the effect of potential experimental accidents rather than for applying any treatment. Nevertheless, there may be some degree of non-independence between individuals living in the same enclosure. A preliminary analysis however showed that survival and growth never differed significantly between enclosures (all $P>0.1$), and thus we did not further include this factor in our analyses. Albeit replicated, the larval treatments present a further potential source of non-independence between individuals as several individuals shared the same larval environment. Our analysis is biased towards finding effects of the larval treatments on terrestrial performance that are in fact spurious (Hurlbert 1984). This is a conservative bias because we expected not to (and did not) find such effects.

Survival.—During the fall periods in 1998 and 1999, we conducted bi-weekly recaptures in the field enclosures. We analyzed survival rates during this time with capture-mark-recapture (CMR) methods, implemented in program MARK (Lebreton et al. 1992; White and Burnham 1999). CMR methods allow to model the recapture rate (probability of an individual to be caught at time *t*, given that it is alive at that time) independently of the survival rate which is confounded by different detection probabilities, for instance for small and large individuals. Our most general model allowed the recapture rate to be affected by body size and to vary between capture occasions. We chose ten survival models (Table 2), which allowed us to examine the effect of the following variables on survival: individual variation in date of and size at metamorphosis, general variation in survival rates between the recapture intervals throughout the fall (henceforth referred to as time variation), and terrestrial

age (the first two weeks since introduction into the enclosures vs. later terrestrial stage).

Therefore, the global (most parameterized) model included time dependence in two age

classes, and the individual covariates date and size at metamorphosis.

We divided the *first weeks data* into three sets, one containing the data for the fall of

1998, and the other two for the fall of 1999, split into two sets of enclosures. This latter

division was necessary because the recapture events were not synchronized in all enclosures,

which lead to different recapture rate patterns. We evaluated the models following basic

capture-mark-recapture methodology (Lebreton et al. 1992), and based model selection on the

small-sample-size adjusted Akaike's information criterion (AICc)(Burnham and Anderson

2002). We started with the global model, simplified the recapture model, and then fitted the a

priori selected survival models using the most parsimonious structure of the recapture model

(Lebreton et al. 1992).

We analyzed survival during the second terrestrial year (*second year data*) using the

same CMR methodology. The global model considered effects of date and size at

metamorphosis and size after the first year on survival during the second yearas well as

differences between the two study ponds in frog survival and recapture probabilities. The

other models assumed some of these effects to be absent.

The capture-mark-recapture approach assumes equal survival and catchability among

individuals within a group at time *t*, which needs to be tested with a goodness-of-fit (GOF)

test. Unfortunately, there is no GOF test for models in which individual covariates are

present. We expected size at metamorphosis to have a strong effect on survival, and we

therefore conducted the GOF test for a model with four size classes and time dependence,

using Tests 2 + 3 in program RELEASE (Burnham et al. 1987). This model provided an

acceptable fit to the data (1998: χ^2=37.7, df=36, *P*=0.4; 1999, enclosure-set 1: χ^2=35.6,

df=42, *P*=0.8, enclosure set 2 χ^2=58.7, df=44, *P*=0.07). Based on AICc, the global models for

all data sets fitted the data better than the models used for the GOF (ΔAICc > 27). The global

models were therefore good starting points for model selection. We used the GOF test provided by the bootstrap option in program MARK (White and Burnham 1999) to assess the validity of our modeling approach for the data of the second terrestrial year. The test showed that the model taking into account variation in time and variation between the two ponds provided an adequate fit for the data ($P = 0.25$, based on 1000 bootstrap replicates).

The effects of date of and size at metamorphosis, as well as larval treatments on survival until the next spring (*over winter data*) were analyzed as logistic regressions using proc GENMOD in SAS (SAS Institute Inc. 1996). We considered four candidate models which we compared by model selection using AICc (Burnham and Anderson 2002). The first model included factors that we had manipulated, such as larval treatments, number of toes clipped, and species identity. The second and the third models considered linear and quadratic effects of date of and size at metamorphosis, respectively, and the fourth model included manipulated factors in addition to date of and size at metamorphosis. If date of and size at metamorphosis alone were important for post-metamorphic survival, we expected models one and four to have low support from the data. In 2000, we considered an additional model corresponding to the hypothesis that survival and survival selection on date of and size at metamorphosis depended on density in the terrestrial life stage. This model consists of the main effects of terrestrial density, date of and size at metamorphosis, and the interactions between terrestrial density and the two metamorphic traits.

Selection.—We quantified survival selection on date of and size at metamorphosis using standardized selection differentials and standardized selection gradients. We first standardized both traits to a mean of zero and a standard deviation of one. Differentials were then calculated as the difference in trait value before and after selection (Brodie et al. 1995). Following Janzen and Stern (1998), we transformed the partial regression coefficients of a logistic regression to obtain linear and quadratic selection gradients (Lande and Arnold 1983).

Correlational selection on date of and size at metamorphosis was calculated as the linear

selection gradient on the product of the two traits (Brodie 1992).

*Growth.—*We analyzed the effect of size at metamorphosis on growth during the first

weeks in the terrestrial habitat, and over the winter, by regressing size at the end of the time

period on size at metamorphosis. Only individuals that survived the period, and, for the fall,

only those that were recaptured on a particular occasion, entered the analysis. If there was no

effect of size on growth, the slope of the regression is expected to be close to one. Then, we

analyzed the effect of date of metamorphosis and larval treatments on growth by conducting

an ANCOVA on the residuals of the regression analysis (Ranta et al. 1994). Due to sparse

data, we used a simple correlation analysis to assess the relationship between date and size at

metamorphosis and size in the second summer of the terrestrial stage.

RESULTS

Survival

Individuals that metamorphosed at a large size and early in the season were more

likely to survive during the first weeks of the terrestrial phase than smaller or later

metamorphosing ones (Table 2). Models 8 to 10, which assume absence of one or both of

these individual characteristics, had essentially no support in either year (Table 2). The AICc

selected best models (model 7 in 1998 and 1999 / set 1, and model 2 in 1999 / set 2) included

the factor terrestrial age, and suggested that survival was generally lower during the first two

weeks after metamorphosis (age 1) than during the later terrestrial stage (age 2, Fig. 1).

Survival varied over the time of the season, both in the freshly metamorphosed froglets and in

the older ones (Fig. 1). Models assuming that either one or both age classes had constant

survival (models 3 to 5) had low support in both years. The effect of size at metamorphosis on

survival differed between the release batches (Fig. 2), as the strong interaction between size and time dependent survival during age class 1 shows (comparing models 6 and 7).

In 1998, 86 of 326 (26%) individuals, in 1999, 69 of 638 (11%) individuals, and in 2000, 171 of 600 (28%) individuals survived from metamorphosis until the next spring. In all years, the variation in survival between individuals was best explained by date of and size at metamorphosis (Table 3). Survival was positively related to size at metamorphosis (Fig. 3, 1998: slope on the logit scale 2.0, se=0.3, χ^2_1= 47.4, $P<0.001$; 1999: 1.0, se=0.2, χ^2_1= 22.2, $P<0.001$; 2000: 0.48, se=0.14, χ^2_1= 12.2, $P<0.001$) and negatively or not significantly to date of metamorphosis (1998: slope on the logit scale -0.03, se=0.01, χ^2_1=8.1, $P=0.005$; 1999: -0.01, se=0.01, χ^2_1=2.58, $P=0.1$; 2000: 0.003, se=0.01, χ^2_1= 0.06, $P=0.8$). In all years, the models including either the manipulated factors only or all factors (models 1 and 4, Table 3) had low support, suggesting that the manipulated factors did not affect survival beyond what was explained by date of and size at metamorphosis. In 2000, different terrestrial densities did not affect survival or survival selection on date of and size at metamorphosis to a detectable degree. Model 5, corresponding to the hypothesis of such an effect was poorly supported by the data. Survival varied significantly between years ($\chi^2_{2,1555}$=11.5, $P=0.003$), and the effect of size at but not date of metamorphosis on survival differed significantly between years (size: $\chi^2_{2,1555}$=26.6, $P<0.001$; date: $\chi^2_{2,1555}$=3.9, $P=0.14$).

Survival during the second year of the terrestrial stage was not affected by date of and size at metamorphosis, or by size after the first year. The model with constant survival rate and pond specific recapture rates (model 5, Table 4) was clearly the AICc selected best model, with more than six times (ratio of the Akaike Weights: 0.632/0.104) better support than the next best model. Model selection also suggested that survival rates were equal in the two experimental ponds, averaging 0.52 (se=0.12).

Selection strength

Model selection favored the hypothesis that selection on date of and size at metamorphosis were non-linear in all three years of the study (model 3, Table 3). In 1998 and 1999, selection favored early metamorphosis, and in all three years, a larger size at metamorphosis was selected for (Table 5). Quadratic selection on size at metamorphosis was negative in 1998 and 1999, indicating that this trait was under stronger selection among relatively small individuals than among larger ones. The other quadratic selection gradients and all linear selection gradients on the products of the two metamorphic traits included zero in their confidence interval (Table 5). We thus found no evidence for correlational selection. Selection differentials, i.e. differences in trait means before and after selection, were always larger in their absolute values than the corresponding selection gradients. This indicates that part of the selection on date of and size at metamorphosis acted through correlated traits not measured in this study.

Growth

Larger individuals increased more rapidly in size than small individuals (Fig. 4), as the regression analyses of size after the first two weeks in the terrestrial habitat and size after the winter on size at metamorphosis showed. The slopes of these regression analyses were significantly steeper than unity in the fall of 1999 and winter of 2000 (for statistical details see figure legend). Non-random mortality with respect to size may have affected this result. The true effects were probably stronger, since the slow-growing small individuals may be less likely to survive than faster-growing ones. An ANCOVA on the residuals of these regression analyses showed that individuals metamorphosing later had lower growth during both fall and winter in all years (coefficients ranging from -0.01 to -0.06, all $P<0.001$). The characteristics of the larval growth environment, predators and larval density, on the other hand, did not significantly affect growth, neither during the fall nor over the winter (all $P>0.3$). There was

also no detectable effect of terrestrial density on growth in 2000 ($F_{1,176}$, $P=0.41$). After accounting for date of and size at metamorphosis, *Rana esculenta* tended to grow faster than *R. lessonae* in 1998 (fall: $F_{1,71}=2.9$, $P=0.09$; winter: $F_{1,83}=4.01$, $P=0.05$).

At the beginning of their second terrestrial summer, all individuals except for one female *R. esculenta* had reached sexual maturity. The second terrestrial year was also the period when the frogs gained most of their size at maturity. The average size in the second summer was 62.7mm (sd=4.0) snout-vent length, 29.7mm (sd=3.4) tibia length and 20.2mm (sd=1.6) head width in *R. lessonae*, and 67.5mm (sd=8.3) snout-vent length, 34.3mm (sd=4.4) tibia length and 23.1mm (sd=3.0) head width in *R. esculenta*. On average, individuals increased their mass on average by 32g (sd=22.7), from 1.5g at metamorphosis to 33.5g in the second year. A positive correlation between the PC1 of these measures and size at metamorphosis indicated that individuals metamorphosing at large size maintained this headstart until adulthood (N=18, r=0.48, $P=0.046$). But adult size was not significantly related to date of metamorphosis (N=18, r=0.37, $P=0.13$).

DISCUSSION

This study examined the patterns of post-metamorphic survival and growth in relation to size at and timing of metamorphosis in water frogs (*Rana lessonae* and *R. esculenta*). A large size at metamorphosis always led to increased survival, and early metamorphosis always improved subsequent growth. The strength of these relationships varied over the years of the study, with terrestrial age of the individuals, and over the time of the season. Overall, survival was lowest during the first weeks in the terrestrial habitat, then slightly higher - but variable - during the fall and winter, and still higher during the second year. Growth declined towards the end of the season, and most of the size gain until maturity was achieved during the second terrestrial year. We found no evidence for a direct effect of larval growth conditions on post-

metamorphic survival or growth, once date and size at metamorphosis were accounted for. And there was no evidence for compensatory post-metamorphic growth. In contrary, in some years growth was positively related to size at metamorphosis, which still positively correlated with size at maturity. Below, we discuss the four questions posed in the Introduction one after the other.

1) How much does a certain change in size at metamorphosis affect subsequent survival and growth, and is there selection on timing of metamorphosis?

We found strong survival selection for larger size at metamorphosis in all three years, with linear selection gradients ranging from 0.052 to 0.158 and quadratic selection gradients ranging from -0.018 to -0.056. We further found direct selection for earlier metamorphosis in 1998 with a linear selection gradient was -0.060. These figures are in the range of earlier estimates of selection strength on life-history traits under natural conditions (Kingsolver et al. 2001). A positive relationship between size at metamorphosis and future fitness components has been found in different taxa (insects: Taylor et al. 1998; fish: Ward and Slaney 1988; Ryding and Skalski 1999; amphibians: Smith 1987; Berven 1990; Scott 1994; Goater 1994; Morey and Reznick 2001). Our study goes beyond earlier ones by quantifying this relationship on the basis of a large number of observed individuals, and by obtaining estimates for replicate years. When postmetamorphic survival is used as a measure of fitness, this results in the estimated fitness surface for date of and size at metamorphosis shown in Fig. 3. The effect of variation in metamorphosis on subsequent growth, not included in this graph, would make the surface steeper, but hardly change its shape, since growth declined with later metamorphosis and smaller size at metamorphosis. Slow growing tadpoles also run an increasing risk of getting trapped in cold water during late fall, and not being able to metamorphose at all. Including this consideration would lower the fitness surface at the end of the season. This imaginative fitness surface corresponds well with the assumed fitness

function of the models for optimal metamorphosis (Wilbur and Collins 1973; Werner 1986; Rowe and Ludwig 1991). All models assume a positive association between size at metamorphosis and fitness. We here provide quantitative empirical evidence for refinement of these models.

2) How much does the relationship between size at metamorphosis and subsequent survival/growth depend on the time of the season?

Our data also show that there was considerable variation in the strength of survival selection on size at metamorphosis, both within a given season and between the three years of the study (Fig. 2, Table 5). However, we could not find a clear pattern in this variation, for example a gradual change in selection strength over the season. Consequently, we also found no evidence for correlational selection on date of and size at metamorphosis. Metamorphic responses are likely to be adaptive only in the long run, since tadpoles are unable to predict the terrestrial survival conditions they will encounter in a given season. Variation in selection strength will often lead to seemingly suboptimal metamorphic responses if the current conditions deviate from the long term average. While the optimal strategy for metamorphosis must rely on patterns of terrestrial growth that are consistent across years, individuals can only react to changes in the larval growth potential (Wilbur and Collins 1973). On the other hand, we found that realized growth rates steadily declined throughout the season in all years. This result suggests a deterioration of the growth potential in the terrestrial habitat. The terrestrial habitat may have deteriorated due to falling temperatures with associated lower activities of the froglets and their arthropod prey, and due to increasing competition as more individuals entered the terrestrial life stage. However, the absence of an effect of terrestrial density in 2000, and additional data (R. Altwegg, *unpublished manuscript*) suggest that the effect of terrestrial density on post-metamorphic growth is small.

3) Are there carry-over effects of the larval growth environment on post-metamorphic fitness components?

After controlling for date of and size at metamorphosis, we found no further effects of the larval environment on post-metamorphic survival and growth. For the range of larval environments that we considered, a change in size at and date of metamorphosis always led to an equal change in postmetamorphic performance, regardless of which of the larval treatments (predators, density, pond drying or delayed development) caused the change. This result is fortunate for research programs in larval ecology and life history, because it suggests that metamorphic responses are in fact important fitness correlates which can easily be measured when only the larval stage is studied. This has always been assumed, but the assumption and the available evidence have been based on mostly observational studies relating metamorphic traits to adult performance in natural populations (e.g. Smith 1987; Semlitsch et al. 1988; Berven 1990). Due to difficulties of marking many animals in the field, these studies often examined differences between groups rather than individuals, and the causes of the variation in metamorphic traits were largely unknown (but see Scott 1994; Morey and Reznick 2001). By combining an experimental approach with a field study, our experiment shows that earlier metamorphosis at a larger size, and thus faster larval growth, indeed leads to higher survival and faster growth after metamorphosis, and therefore probably to higher fitness.

4) Can individuals compensate for a small size at metamorphosis by enhancing their post-metamorphic growth?

We found that variation in size at metamorphosis was maintained or even increased during the subsequent growth periods. Thus, there is no evidence for compensatory growth. In contrast to our study, compensatory growth was found in post-metamorphic spadefoot toads (Morey and Reznick 2001), and appears to be widespread among organisms with indetermined growth (reviewed in Metcalfe and Monaghan 2001). A possible explanation for

the absence of compensatory growth in our study is that individuals were maximizing their growth rate during the life stages observed here. This would imply that costs of rapid growth are small relative to its benefits during these life stages (Arendt 1997).

In conclusion, this study has shown that in water frogs, metamorphic responses are tightly linked to important fitness components during later life, and it examined the shape of this relationship and its variation, which determine selection on important life-history responses. such knowledge of the causes and consequences of variation in life histories is important in order to understand the mechanisms that drive population dynamics and species interactions in a community (McPeek and Peckarsky 1998), including coexistence of waterfrog species (Hellriegel and Reyer 2000).

ACKNOWLEDGMENTS

LITERATURE CITED

Abrams, P. A., and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution 50: 1052-1061.

Alford, R. A., and R. N. Harris. 1988. Effects of larval growth history on anuran metamorphosis. Am. Nat. 131: 91-106.

Altwegg, R. 2002a. Predator-induced life-history plasticity under time constraints in pool frogs. Ecology 83: 2542-2551.

Altwegg, R. 2002b. Trait-mediated indirect effects and complex life cycles in two European frogs. Evol. Ecol. Res. 4: 519-536.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q. Rev. Biol. 72: 149-177.

Bättig, I. 1998. Fortpflanzungswahrscheinlichkeit bei weiblichen *Rana lessonae, Rana esculenta* und *Rana ridibunda*. MSc thesis. University of Zürich, Switzerland.

Berger, L. 1964. Is *Rana esculenta lessonae* Camerano a distinct species? Ann. Zool. (Warsaw) 22: 45-61.

Berger, L. 1988. On the origin of genetic systems in European waterfrog hybrids. Zool. Poloniae 35: 5-32.

Berven, K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71: 1599-1608.

Biek, R., W. C. Funk, B. A. Maxell, and L. S. Mills. 2002. What is missing in amphibian decline research: insights from ecological sensitivity analysis. Cons. Biol. 16: 728-734.

Blankenhorn, H. J., H. Heusser, and P. Notter. 1973. Zur Verbreitung von *Rana esculenta* Linnaeus und *Rana lessonae* Camerano im Zürcher Oberland. Rev. Suisse Zool. 80: 662-666.

Brodie, E. D. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. Evolution 46: 1284-1298.

Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. Trends Ecol. Evol. 10: 313-318.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

Burnham, K. P., D. R. Anderson, G. C. White, C. Brownie, and K. H. Pollock. 1987. Design and analysis methods for fish survival experiments based on release-recapture. American Fishing Society, Monograph 5. Bethesda, MD.

Clarke, R. D. 1974. Postmetamorphic growth rates in a natural population of Fowler's toad, *Bufo woodhousei fowleri*. Can. J. Zool. 52.

Clarke, R. D. 1977. Postmetamorphic survivorship of fowler's toad, *Bufo woodhousei fowleri*. Copeia 1977: 594-597.

Cohen, J. 1985. Metamorphosis: introduction, usages, and evolution. Pp. 1-19 *in* M. Balls, and M. Bownes, eds. Metamorphosis. Oxford University Press, Oxford.

Flanagin, V. L., S. P. Haase, and S. A. Juliano. 2000. Effects of growth rates on development to metamorphosis in the lubber grasshopper, *Romalea microptera*. Oecologia 125: 162-169.

Goater, C. P. 1994. Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. Ecology 75: 2264-2274.

Gosner, N. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

Graf, J.-D., and M. Polls Pelaz. 1989. Evolutionary genetics of the *Rana esculenta* complex. Pp. 289-302 *in* R. M. Dawley, and J. P. Bogart, eds. Evolution and ecology of unisexual vertebrates. New York State Museum, Albany, New York.

Harris, R. N. 1999. The anuran tadpole: evolution and maintenance. Pp. 279-294 *in* R. W. McDiarmid, and R. Altig, eds. Tadpoles: the biology of anuran larvae. The University of Chicago Press, Chicago.

Hellriegel, B., and H.-U. Reyer 2000. Factors influencing the composition of mixed populations of a hemiclonal hybrid and its sexual host. J. Evol. Biol. 13: 906-918.

Hensley, F. R. 1993. Ontogenetic loss of phenotypic plasticity of age at metamorphosis in tadpoles. Ecology 74: 2405-2412.

Holenweg, A.-K., and H.-U. Reyer. 2000. Hibernation behavior of *Rana lessonae* and *R. esculenta* in their natural habitat. Oecologia 123: 41-47.

Holenweg Peter, A.-K., H.-U. Reyer, and G. Abt Tietje. 2002. Ecological factors affecting genotype and sex ratios in mixed populations of *Rana lessonae, R. ridibunda,* and their hybridogenetic associate *R. esculenta*. Ecoscience 9: 1-11.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52: 1564-1571.

John-Adler, H. B., and P. J. Morin. 1990. Effects of larval density on jumping ability and stamina in newly metamorphosed *Bufo woodhousii fowleri*. Copeia 1990: 856-860.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A.

Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157: 245-261.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on related characters. Evolution 37: 1210-1226.

Lebreton, J. D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecol. Monogr. 62: 67-118.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol. Evol. 16: 254-260.

Morey, S., and D. Reznick. 2001. Effects of larval density on postmetamorphic spadefoot toads (*Spea hammondii*). Ecology 82: 510-522.

Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253: 892-895.

Ranta, E., A. Laurila, and J. Elmberg. 1994. Reinventing the wheel: analysis of sexual dimorphism in body size. Oikos 70: 313-321.

Rowe, L., and D. Ludwig. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology 72: 413-427.

Ryding, K. E., and J. R. Skalski. 1999. Multivariate regression relationships between ocean conditions and early marine survival of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 56: 2374-2384.

SAS Institute Inc. 1996. SAS / STAT Software: changes and enhancements through release

6.11. SAS Institute Inc., Cary, North Carolina.

Schultz, R. J. 1969. Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Nat. 103: 605-619.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75: 1383-1396.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69: 184-192.

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. Ecology 71: 2313-2322.

Smith, D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68: 344-350.

Smith-Gill, S. J., and K. A. Berven. 1979. Predicting amphibian metamorphosis. Am. Nat. 113: 563-585.

Som, C., B. R. Anholt, and H.-U. Reyer. 2000. The effect of assortative mating on the coexistence of a hybridogenetic waterfrog and its sexual host. Am. Nat. 156: 34-46.

Taylor, B. E., and D. E. Scott. 1997. Effects of larval density dependence on population dynamics of *Ambystoma opacum*. Herpetologica 53: 132-145.

Taylor, B. W., C. R. Anderson, and B. L. Peckarsky. 1998. Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. Oecologia 114: 494-502.

Thurnheer, S., and H.-U. Reyer. 2001. Spatial distribution and survival rate of waterfrog tadpoles in relation to biotic and abiotic factors: a field experiment. Amphibia-Reptilia 22: 21-32.

Travis, J. 1984. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology 65: 1155-1160.

Twombly, S. 1996. Timing of metamorphosis in a freshwater crustacean: comparison with anuran models. Ecology 77: 1855-1866.

Ward, B. R., and P. A. Slaney. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Can. J. Fish. Aquat. Sci. 45: 1110-1122.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and optimal size at transformation. Am. Nat. 128: 319-341.

White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46: S120-139.

Wilbur, H. M. 1980. Complex life cycles. Ann. Rev. Ecol. Syst. 11: 67-93.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182: 1305-1314.

Table 1: Numbers of froglets studied. The froglets originated from the cohorts 1998, 1999, and 2000, and were studied during three phases of their postmetamorphic life: the first weeks, over the first winter, and during the second terrestrial year.

cohort	species	first weeks	over winter	second year
1998	*Rana lessonae*	157	157	20
	Rana esculenta	169	169	21
1999	*Rana lessonae*	638	638	
2000	*Rana lessonae*		600	
Total		964	1564	41

Table 2: Model selection for survival of freshly metamorphosed froglets during the fall 1998 (Table 2a) and 1999 (split into set1, Table 2b, and set2, Table 2c). The capture-mark-recapture models were run in program MARK (White and Burnham 1999). The table lists the ten candidate survival models (model selection for the recapture model not shown), which contained a subset or combinations of the following factors (see Methods for more detail): terrestrial age(age, i.e. the first two weeks after metamorphosis vs. the later terrestrial stage), time variation (t), size at metamorphosis (size), and date of metamorphosis (date). A short description of each model is given in Table 2a. Deviance is the difference in log (likelihood) between the current model and the saturated model, i.e. the one in which the number of parameters equals the sample size. K is the number of parameters, Akaike's information criterion (AICc) assesses the parsimony of a model, and Delta AICc is the difference in AICc of a particular model to the best one (in bold). The Akaike weight gives the relative support by the data that a certain model has, in relation to the other models.

Factors in model	Description of model	Deviance	K	AICc	Delta AICc	Akaike weight
a) 1998, recapture model contained time and size effect						
1 t+size+date	no age effect	1149.70	14	1178.51	7.01	0.028
2 age-t/t+size+date	parallel time variation in both age classes	1147.90	15	1178.82	7.32	0.024
3 age-t/c+size+date	no time variation in second age class	1152.07	14	1180.87	9.37	0.009
4 age-c/t+size+date	not time variation in first age class	1159.91	14	1188.71	17.21	0.000
5 age-c/c+size+date	no time variation in either age class	1166.33	11	1188.83	17.34	0.000
6 age-t*t+size+date	age classes vary independently over time	1147.29	18	1184.61	13.11	0.001
7 age-t*t+size+date+t.size	all effects included, interaction between time and size	**1132.03**	**19**	**1171.50**	**0.00**	**0.937**
8 age-t/t+date	no size effect	1226.33	14	1255.14	83.64	0.000
9 age-t/t+size	no date effect	1165.59	14	1194.40	22.90	0.000
10 age-t/t	neither size nor date effects	1253.74	12	1278.33	106.83	0.000

$\Sigma=1$

Factors in model	Deviance	K	AICc	Delta AICc	Akaike weight
b) 1999 / set 1, recapture model contained time effect					
1 t+size+date	1629.45	13	1655.94	4.47	0.088
2 age-t/t+size+date	1629.41	14	1657.98	6.50	0.032
3 age-t/c+size+date	1629.36	14	1657.94	6.46	0.033
4 age-c/t+size+date	1633.44	14	1662.01	10.53	0.004
5 age-c/c+size+date	1651.51	11	1673.87	22.39	0.000
6 age-t*t+size+date	1623.34	18	1660.27	8.80	0.010
7 age-t*t+size+date+t.size	**1612.43**	**19**	**1651.48**	**0.00**	**0.826**
8 age-t/t+date	1645.17	13	1671.66	20.19	0.000
9 age-t/t+size	1634.83	13	1661.32	9.85	0.006
10 age-t/t	1651.22	12	1675.65	24.17	0.000
					$\Sigma=1$
c) 1999 / set 2, recapture model contained time effect					
1 t+size+date	1485.15	13	1511.62	6.11	0.024
2 age-t/t+size+date	**1476.97**	**14**	**1505.51**	**0.00**	**0.505**
3 age-t/c+size+date	1479.80	14	1508.34	2.83	0.123
4 age-c/t+size+date	1484.37	14	1512.91	7.40	0.012
5 age-c/c+size+date	1487.32	11	1509.66	4.14	0.064
6 age-t*t+size+date	1474.82	18	1511.70	6.18	0.023
7 age-t*t+size+date+t.size	1468.72	19	1507.70	2.19	0.169
8 age-t/t+date	1505.30	13	1531.76	26.25	0.000
9 age-t/t+size	1482.71	13	1509.18	3.66	0.081
10 age-t/t	1507.38	12	1531.77	26.26	0.000
					$\Sigma=1$

Table 3: Logistic regression models of characteristics of the larval growth environment and date of and size at metamorphosis on survival over the first winter. The models were run in proc GENMOD in SAS (SAS Institute Inc. 1996). The table shows the factors included in a particular model: date of metamorphosis (date, date2 includes quadratic term), size at metamorphosis (size, size2 includes quadratic term), larval density (dens), presence of caged predators during the larval stage (pred), pond drying (dry), start of larval development (start), species, enclosure, and number of toes clipped (toes). In 2000, we included an additional model to examine terrestrial density dependence (see Methods). For every model, the table shows its maximum log Likelihood, the number of parameters (K), the small sample size adjusted Akaike's Information Criterion (AICc), the difference in AICc to the best model (in bold) (Delta AICc), and the Akaike weight, which indicates the relative support for the particular model.

1998: Model survival = function of	Log Likelihood	K	AICc	Delta AICc	Akaike weight
1) dens, pred, species, toes	-161.014	6	334.291	32.065	0.000
2) date, size	-149.379	4	306.882	4.655	0.089
3) date^2, size^2, date x size	**-143.937**	**7**	**302.227**	**0.000**	**0.908**
4) dens, pred, species, toes, date, size	-148.567	8	313.589	11.362	0.003
					$\sum=1$

1999: Model survival = function of					
1) pred, dry, start, toes	-209.979	11	442.380	22.642	0.000
2) date, size	-206.857	4	421.777	2.039	0.265
3) date^2, size^2, date x size	**-202.780**	**7**	**419.738**	**0.000**	**0.733**
4) pred, dry, start, toes, date, size	-202.434	13	431.452	11.714	0.002
					$\sum=1$

2000: Model survival = function of					
1) dens, toes	-352.714	5	715.528	3.701	0.071
2) date, size	-352.275	4	712.617	0.790	0.303
3) date^2, size^2, date x size	**-348.819**	**7**	**711.827**	**0.000**	**0.449**
4) dens, toes, date, size	-351.101	7	716.390	4.563	0.046
5) effect of terrestrial density	-350.046	7	714.281	2.454	0.132
					$\sum=1$

Table 4: Summary of model selection for survival during the second terrestrial year. The models were run in program MARK (White and Burnham 1999). The effect of size at metamorphosis (msize), size in the next spring, i.e. at the beginning of the time period considered here (ssize), date of metamorphosis (mdate) and differences between the two experimental ponds (pond) on survival were examined. Models 1 to 5 account for differences in recapture rates between the ponds. See legend of Table 2 for additional information.

	Factors in model: survival	recapture	Deviance	K	AICc	Delta AICc	Akaike Weight
1	ssize, msize, mdate	pond	78.25	9	100.64	9.24	0.006
2	msize, mdate	pond	79.18	8	98.60	7.21	0.017
3	pond	pond	78.87	7	95.48	4.08	0.082
4	ssize	pond	78.41	7	95.01	3.61	0.104
5	**constant**	**pond**	**80.07**	**5**	**91.40**	**0**	**0.632**
6	pond	constant	84.66	5	95.99	4.6	0.063
7	constant	constant	88.68	3	95.19	3.79	0.095
							$\Sigma=1$

Table 5: Standardized selection differentials (S), and standardized linear (β) and quadratic (γ) selection gradients on date of and size at metamorphosis in water frogs during three subsequent years. Correlational selection on the two metamorphic traits is given as the standardized linear selection coefficient on their interaction. Linear selection gradients were obtained from model 2, Table 3, whereas quadratic and correlational selection gradients were obtained from model 3, Table 3. Confidence intervals (95%) are given in parentheses.

		S	β	γ
1998	date	-0.383	-0.060 (-0.103/-0.018)	0.034 (-0.012/0.080)
	size	0.765	0.158 (0.111/0.205)	-0.046 (-0.080/-0.012)
	date x size		0.008 (-0.047/0.064)	
1999	date	-0.137	-0.020 (-0.046/0.005)	-0.022 (-0.051/0.007)
	size	0.540	0.052 (0.030/0.074)	-0.018 (-0.035/-0.001)
	date x size		0.010 (-0.020/0.040)	
2000	date	-0.075	0.005 (-0.035/0.045)	0.008 (-0.048/0.033)
	size	0.228	0.067 (0.026/0.106)	-0.056 (-0.112/0.001)
	date x size		0.018 (-0.031/0.068)	

Fig. 1: Survival over a two-week period during the fall and winter for freshly metamorphosed froglets (age 1), and for froglets that survived their first two weeks in the terrestrial stage (age 2, solid line). The values shown are the expected values (± SE) for an individual of average phenotype, taken from the best fitting model (see Table 2). The estimate for the winter is calculated for the whole winter but adjusted to a two-week interval. a) fall / winter 1998/99 (n=326), b) fall / winter 1999/2000, enclosure set 1 (n=341), c) fall / winter 1999/2000, enclosure set 2 (n=297).

Fig. 2: Relationship between survival and body size at metamorphosis for the different release batches (groups of individuals released at the same time) during the fall 1998, and fall 1999 (two sets of enclosures). The curves were estimated using the parameter values provided by model 7, Table 2.

Fig. 3: Survival over the winter as a function of size at and date of metamorphosis (n=1564). This figure pools data from three cohorts. Contours show lines of equal survival, averaged over the three years, given by the logistic regression equation: logit (survival)= -1.366+0.87*size-0.021*time.

Fig. 4: Growth during the first two weeks in fall (circles and dotted line) and over the first winter (triangles and dashed line) in the terrestrial habitat. The solid line, with slope=1, marks the line of no growth. The fitted regression lines are shown for both periods (fall 1998: 1.09, se=0.07, deviation from unity: $t=1.3$, $P=0.2$; winter 1998: 1.09, se=0.16, deviation from unity: $t_{1,83}=0.6$, $P=0.5$; fall 1999: slope=1.22, se=0.04, deviation from unity: $t=4.9$, $P<0.001$; winter 1999: slope=1.02, se=0.24 deviation from unity: $t_{1,68}=0.07$, $P>0.5$; winter 2000: slope=1.39, se=0.06 deviation from unity: $t_{1,170}=6.33$, $P<0.001$). Open symbols represent *R. lessonae*, the black symbols in the uppermost figure show *R. esculenta.*
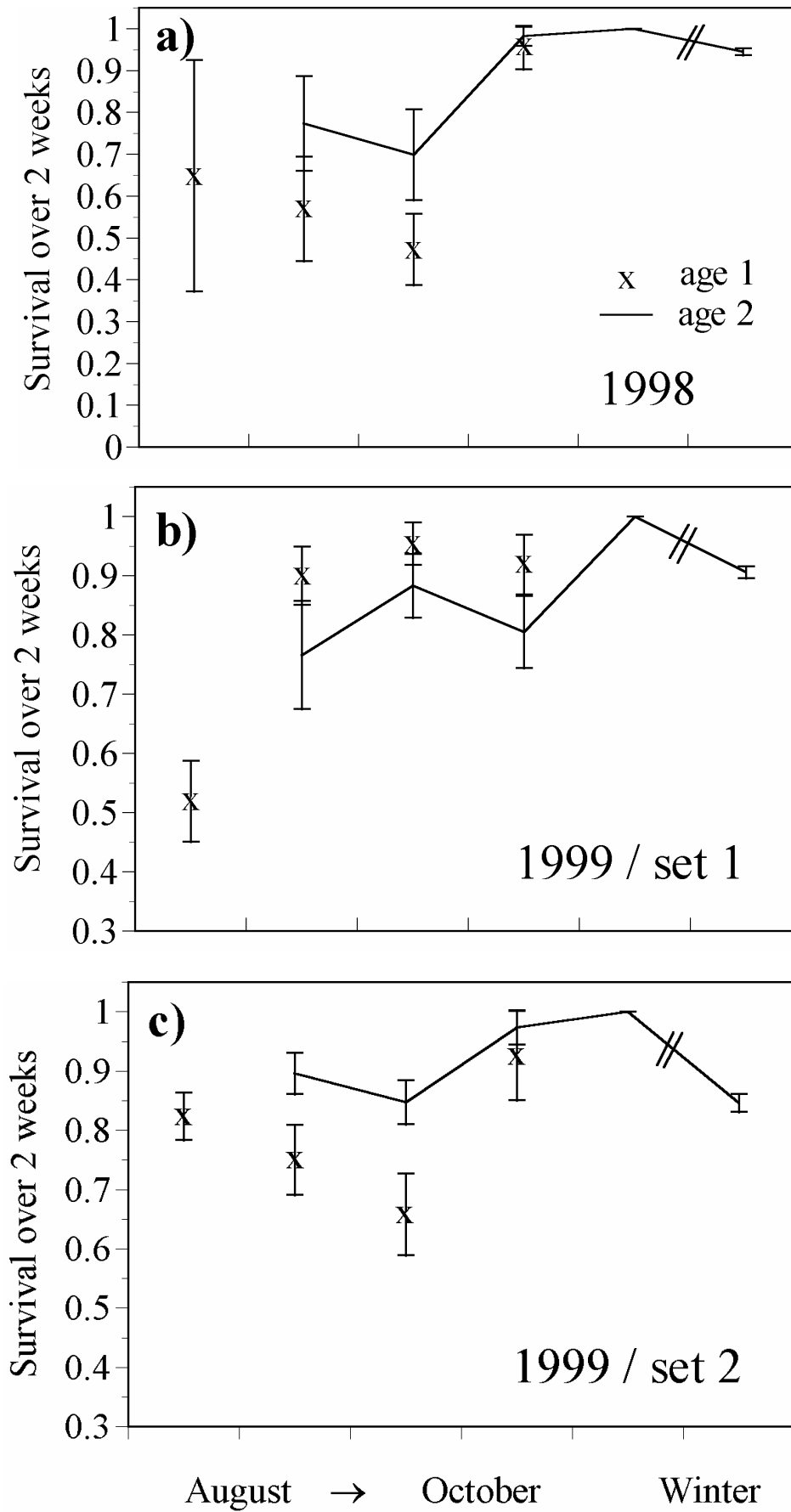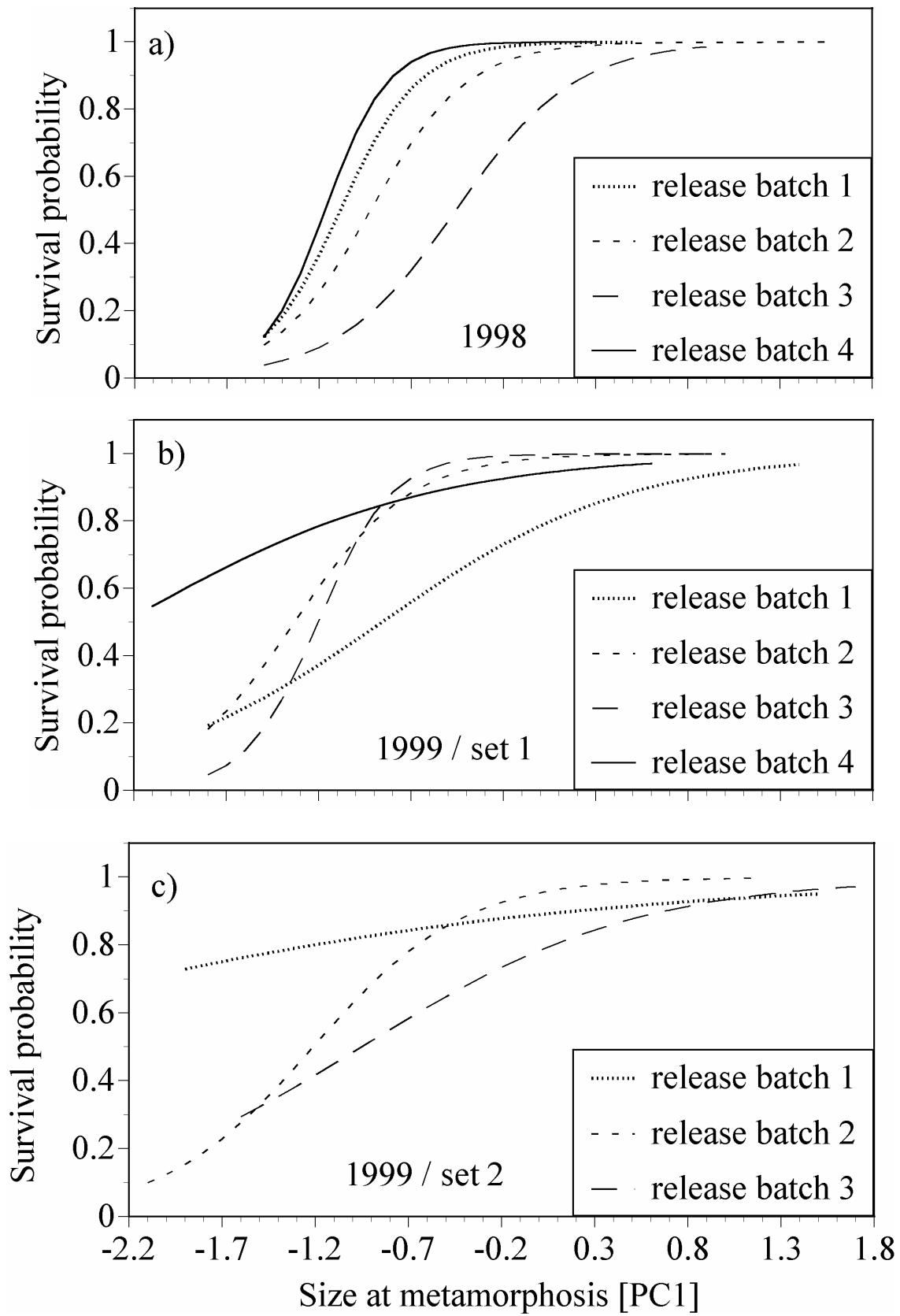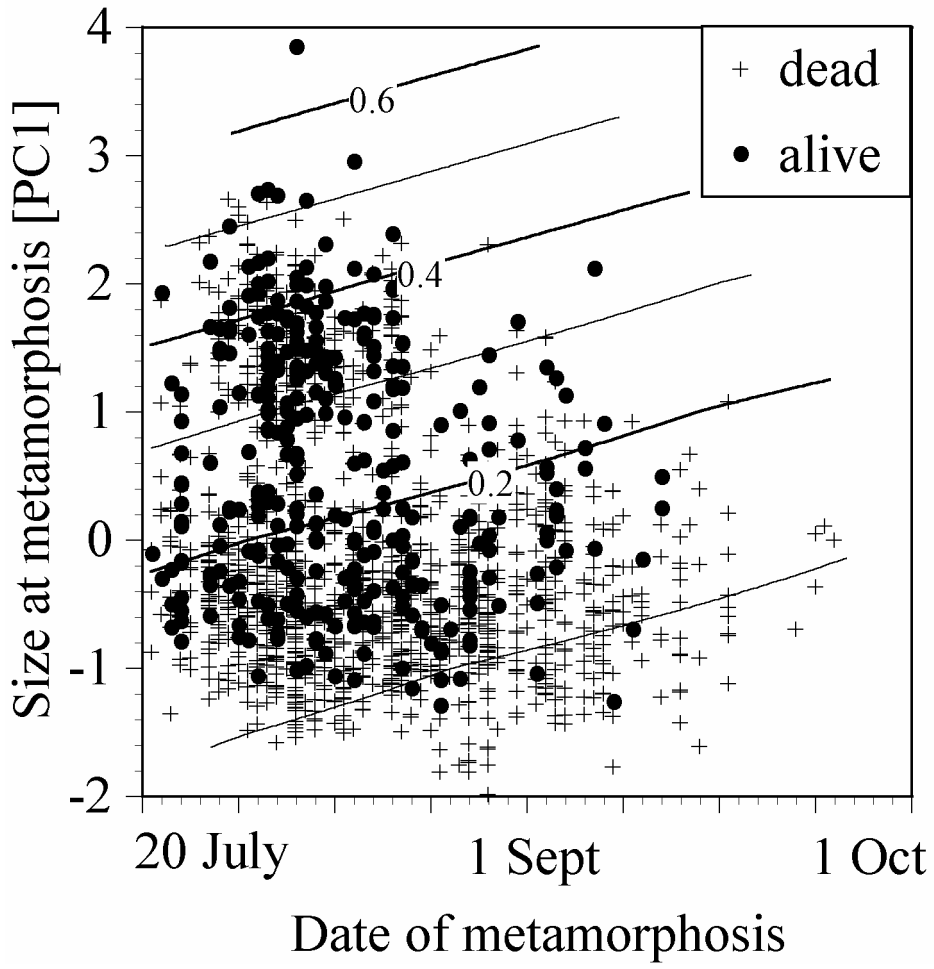
Fig. 1:

Fig. 2:

Fig. 3:

Fig. 4: