

Growth strategies of tadpoles along the pond permanency gradient

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Abstract The preference for particular features of water bodies for reproduction is one of the most important aspects of anuran ecology, affecting key aspects of both tadpole and adult life. The use by species of different habitats along the pond permanency gradient has been already studied, noting conflicting selective pressures from predation and desiccation risk. Here, we aim to discover physiological patterns related with this gradient. As a study system, we used the full anuran community of the NE Iberian Peninsula. We quantified growth rate, consumption rate, food assimilation and the proportion of energy allocated to growth, as well as gut length, for all species. Food consumption rate and growth allocation were the variables that defined tadpole growth, while food assimilation abilities and gut length seem to have a secondary or cryptic role in growth. More interestingly however, our data suggests a labile continuum of consumption-based versus allocation/assimilation-based growth strategies differentiating species. Differences among species follow predictions of adaptation to the pond permanency gradient selective pressures. Species from ephemeral ponds are more prone to use consumption-related growth tactics while species inhabiting ponds with longer hydroperiods are more efficient retaining and allocating energy into growth, although results seem partly shaped by strong interspecific competition. Physiological differences in growth and the usage of the assimilated energy could be an additional factor to understand how tadpoles adapt to the features of ponds they inhabit, as well as how they compete and coexist.

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Introduction

Certain features of freshwater bodies determine the biology of the entire biota that they host. Each kind of water mass is linked to a desiccation risk and is comprised of different types of predators and competitors, among other parameters, such as water quality or available food resources (Wellborn et al. 1996; Williams 2005). Accordingly, anuran species typically prefer reproducing in water bodies with a particular set of characteristics (Altig and McDiarmid 1999b). For each species, the repeated use of some kinds of water masses over evolutionary time has shaped aspects of larval biology, such as rates of development, behaviour, morphology, and phenotypic plasticity (Altig and McDiarmid 1999b; Van Buskirk 2009; Strauß et al. 2010; Richter-Boix et al. 2011). The choice of site for reproduction will have clear effects not only on larval life, but consequently on juvenile morphology and fitness (Tejedo et al. 2010; Richter-Boix et al. 2011). All these cascade effects have been demonstrated to influence the evolution of functional (Strauß et al. 2010; Van Bocxlaer et al. 2010) and morphological diversification of anurans (Gomez-Mestre and Buchholz 2006; Johansson and Richter-Boix 2013; Van Buskirk 2014).

Anuran communities from semiarid environments are a good system to study trade-offs linked to the choice of a reproduction site (e.g. da Silva Vieira et al. 2009; Dayton and Fitzgerald 2001). More precisely, anuran communities of the Mediterranean basin comprise generally well-studied species inhabiting the same area but with different preferences along the pond permanency gradient (Richter-Boix et al. 2006b, 2007a). This gradient is defined by each pond's tendency to dry out (ranging from merely a month of mean continuous water presence to permanent year-round water), but it also correlates with predation risk (Wellborn et al. 1996). Desiccation of ponds can lead to a total obliteration of a full cohort of an amphibian population, and thus desiccation risk is possibly the most important selective pressure for larvae (Semlitsch 1987; Degani and Kaplan 1999; Jakob et al. 2003; Richter-Boix et al. 2006b). Anuran species adapted to breed in ephemeral ponds, virtually free of predators, have fast developing tadpoles plus plastic abilities that enable them to accelerate development if water levels drop dramatically (Richter-Boix et al. 2006b). Desiccation risk in temporary ponds is lower, but more arthropod predators can breed in these ponds since they keep water several months. Permanent ponds, with no risk of desiccation, comprise even fish as top predator. Therefore, there is a counter-gradient between predation and desiccation risks, implying a trade-off between choosing a pond with a high number of predators or with high probabilities of drying out (Richter-Boix et al. 2006b). Although evolutionary effects of these gradients have been studied in terms of general biology and ecology in different freshwater organisms, including amphibians (e.g. De Meester et al. 2005; Serrano and Fahd 2005; Richter-Boix 2005; Gascón et al. 2008), studies trying to relate physiological traits of tadpole species with this pond permanency gradient are generally lacking (but see Richardson 2002). As a consequence, how growth-related physiological parameters (consumption rate, assimilation, proportion of energy invested in growth) of each species are related to its particular use of the pond permanency gradient is still largely unexplored. In this study, we obtained physiological data on the resource exploitation and growth physiology of the full

community of tadpole species present in the NE Iberian Mediterranean coast to explore whether physiological traits, such as consumption rate, or assimilation and growth allocation, can represent an adaptive dimension for tadpoles.

We base our predictions on the interactions among predation risk, foraging activity and physiology. As a general rule, active tadpoles can increase harvesting rate and thus develop and grow faster, becoming superior competitors (Dayton and Fitzgerald 2001; Smith et al. 2004), but increased activity can also result in increased predation risk (Morin 1983; Polocavia and Gomez-Mestre 2013). Therefore, tadpoles inhabiting ponds with high predation risk are usually found to be less active than tadpoles from ponds with low predator presence (e.g. Van Buskirk and Arioli 2005; Richter-Boix et al. 2007b). Accordingly, we predict that physiological traits enabling tadpoles to grow with low foraging rates—like high food assimilation or high energy allocation in growth—should be common in species usually inhabiting ponds with high numbers of predators, that is, long hydroperiod ponds. In these species, features positively interacting with foraging activity—like high consumption rate abilities—relate weakly to enhancing energy acquisition and therefore should have a mild link to individual fitness and positive selection. Instead, all features allowing tadpoles to grow using fewer resources are expected to be under selection in order to compensate for the lack of foraging activity (similarly as found in Thaler et al. 2012). On the other hand, species inhabiting short hydroperiod ponds that comprise fewer predators have fewer constraints on foraging rates, but must cope with desiccation risk instead. Previous experiments demonstrate that species from temporary ponds have higher activity rates both in absence and presence of predators in comparison to permanent pond breeders (Richter-Boix et al. 2007b). In the absence of activity constraints, all physiological traits that enhance growth rates could be selected for, assuming that there are no trade-offs among physiological traits. Concretely, the possibility of profiting from abundant resources (Wassersug 1975; Diaz-Paniagua 1989) through high consumption rates should be specially selected for. In summary, we expected to find that species inhabiting long hydroperiod ponds should be adapted to grow under foraging constraints, and therefore display higher assimilation and growth allocation abilities compared to species inhabiting short hydroperiod ponds. Species inhabiting short hydroperiod ponds, in turn, are usually exempt of foraging constraints and should have higher consumptive abilities compared to species from long hydroperiod ponds.

Materials and methods

Study system

Our study area is based in the NE Iberian Peninsula, which is mainly a typical Mediterranean region with hot and dry summers, mild winters and two rainy seasons in spring and autumn (Martín-Vide and Olcina 2001), but also with some areas displaying an Eurosiberian climate. The entire anuran community in this area is comprised of nine species (Pleguezuelos et al. 2002; Montori et al. 2007): *Alytes obstetricans* (Laurenti 1768), *Pelodytes punctatus* (Daudin 1802), *Pelobates cultripes* (Cuvier 1829), *Bufo calamita* (Laurenti 1768), *Bufo spinosus* Daudin 1803, *Hyla meridionalis* Boettger 1874, *Pelophylax perezi* (López Seoane 1885), *Rana temporaria* Linnaeus 1758, and the introduced *Discoglossus pictus* (Otth 1837). Mouth and gut morphology of these tadpoles are similar, fitting the traditional classification of tadpoles as filter-feeding herbivores (Altig and

McDiarmid 1999a). They feed mostly on suspended or deposited matter (mainly detritus, algae and phanerogams), and their diets are usually interpreted as simply coupled to the availability of resources (Wassersug 1975; Diaz-Paniagua 1985, 1989; Campeny 2001; Caut et al. 2012; but see Richter-Boix et al. 2007c). Some studies report negative effects of competition among species included in our study (Banks and Beebee 1987; Richter-Boix et al. 2004, 2007a), implying a role of interspecific competition in the population dynamics of the guild. However, as previously stated, physiological traits of these species potentially enhancing or interacting with competitive abilities have been largely overlooked hitherto.

In our study area, the continuous pond permanency gradient used by species can be classified into three major categories (Richter-Boix et al. 2013): (1) ephemeral pools that dry up within weeks, containing water for <2 months, with few or no predators. The anuran community of ephemeral pools is composed of *D. pictus*, *B. calamita*, and *P. punctatus*; (2) temporary ponds that flood in the spring and autumn rainfall. These ponds dry out in summer and occasionally in winter, usually containing water for more than 2 consecutive months, and comprise invertebrate predators. Species present in these ponds are *D. pictus*, *B. calamita*, *P. punctatus*, *A. obstetricans*, *P. cultripes*, *H. meridionalis*, *R. temporaria*, and *B. spinosus*; (3) permanent ponds containing water all year round, with many invertebrate predators or even fish as the top predators, comprising *P. cultripes*, *A. obstetricans*, *H. meridionalis*, *P. perezi*, and *B. spinosus*.

Experimental settings

During March to June of 2007, we conducted a set of laboratory experiments under light and temperature control (12D:12L, 22–24 °C) to study the differential exploitation of a standardized food resource by the nine tadpole species from the study area. In order to minimize the possibility of genetic particularities, we collected three egg masses from three different and separated (>3 km at least) locations for each species in the surroundings of both Garraf and Montseny Natural Parks. As *A. obstetricans* males perform parental care of their eggs, we had to directly collect tadpoles of similar sizes and Gosner Stage 25—instead of egg masses—from three different ponds within Garraf NP. All eggs—or tadpoles—of each species were collected synchronously (within 2 days) from the most typical breeding habitats in each case, excluding spawns or tadpoles from pond typologies uncommon to the species. Once collected, we transported eggs or tadpoles to the laboratory in the University of Barcelona, where we mixed and reared them in 100 × 60 × 30 cm tanks in low densities until tadpoles hatched and reached Gosner stage 25 (Gosner 1960). Then we randomly took 30 individuals from each species and raised them individually in the experimental unit containers. Experimental units consisted of two circular 1-L plastic containers (diameter 12.5 cm, depth 10 cm), one inside the other. We removed the bottom of the inner container and we added a rigid mesh (mesh size 4 mm), which was 2–3 cm away from the bottom of the other container, to prevent tadpoles from eating their own faeces. In order to familiarize tadpoles with the food used in the experiments, each tadpole was fed small pieces of Sera® Spirulina tabs placed in a small Petri dish on the mesh for 2 weeks. Despite possible differences in the ability to assimilate Spirulina among species (Altig and McDearman 1975; Steinwascher and Travis 1983), the similarities among the diets and feeding of the studied species (plus 2 weeks of familiarization) allow us to discard important biases due to the food chosen. Moreover, it was necessary to standardize the food type in order to allow interspecies comparisons. After the 2 weeks of familiarization, we starved tadpoles for 1 day to ensure that their guts were empty (Savage 1952), and we weighed them to the nearest 0.1 mg (balance: Mettler Toledo AG204 DeltaRange).

We then placed tadpoles back individually into the same containers, where they were kept for 5 days. During this period, all tadpoles were fed *Spirulina* Tabs ad libitum according to tadpole size every second day. *Spirulina* Tabs were weighed previously to incorporation into individual containers to correctly ensure the ad libitum conditions without dirtying the water unnecessarily. Every 2 days, water was changed and any uneaten food was removed carefully and placed on a small pre-weighed glass Petri dish, then replaced with a new weighed piece of *Spirulina* Tab. When tadpole faeces were detected in the Petri dish of the food, they were carefully removed by pipetting to ensure that the tadpoles only consumed *Spirulina*. After the fifth day, any uneaten food was removed and the tadpoles starved for 1 day before being weighed again. Given the reported relationships among relative gut length, growth rate, predation risk and competition (Relyea and Auld 2004) we decided to measure gut length of tadpoles. Therefore, tadpoles were then photographed and euthanized using tricaine methane sulfonate (MS-222), before their guts were extracted, unfolded and photographed. Body size and gut length were measured using SigmaScan Pro 5.0. All tadpoles used for the experiments were between Gosner stages 26 and 30. We had to reduce the number of individuals of *R. temporaria* and *P. cultripipes* prior to the experiment due to previous mortality. Survival throughout all the experimental process was high in most species (>80 %), except for *B. calamita* (50 %) and *B. spinosus* (37 %). As previous researchers have noted (Richardson 2002), *Bufo* species sometimes grow poorly when isolated, advising caution with the interpretation of these data.

Faeces (FE) and uneaten food were dried (12 h at 60 °C) and weighed to determine the dry mass of food ingested ($FI = (\text{dry mass of food given}) - (\text{dry mass of food uneaten})$). Wet body mass was converted into dry body mass using the following equation (Feder 1981): $\text{Dry mass} = 0.047^{1.06} \times \text{Wet mass}$. The increase in dry weight (IDW) was calculated by subtracting Dry Weight 1 (DW1, weight at the start of the experiment) from Dry Weight 2 (DW2, weight at the end of experiment). We then calculated daily growth rate (referred to as growth rate, $GR = IDW / [\text{days} \times DW1]$), the daily consumption rate (referred to as consumption rate, $CR = FI / [\text{days} \times DW1]$), the assimilation efficiency ($ASSM = (FI - FE) / FI$) and the growth allocation ($GA = IDW / (FI - FE)$) for each tadpole (Richardson 2002). Gut length was corrected by body length to give the relative gut length (RGL).

Statistical analyses: growth physiology

During data exploration, we detected two clearly different physiological groups of *A. obstetricans* (Figs. S1 to S6). Once these differences were statistically confirmed (see Supporting Information A, Figs. S1 to S6) through a k-means clustering analysis (Hartigan and Wong 1979), we interpreted these two groups as overwintering versus non-overwintering cohorts of *A. obstetricans* (García-París et al. 2004; Salvador and García-París 2001), and subsequently included each cohort separately into interspecific analyses. We evaluated interspecific differences among species/cohorts using one-way ANOVAs followed by TukeyHSD post hoc tests (Supporting Information A, Table S1). We also explored possible physiologic patterns related to size and possible variable correlations testing linear regressions for each combination and adjusting the α level following the Bonferroni correction (Bonferroni 1936) (Supporting information A).

After data exploration, we aimed to (1) elucidate which variables better define growth rate (GR) from an intra- and interspecific point of view, (2) elucidate if physiological variables are correlated, and (3) detect possible relationships among relative gut length (RGL) and physiological traits.

To visually explore which physiological variables could better define the growth rate of tadpoles, we first tested linear regressions for CR, GA and ASSM against GR separately for each species (Figs. S7 to S9). To statistically determine which variables better define GR for each species, we fitted a linear mixed model (LMMs) per species. These LMMs included as fixed effects all above-mentioned physiological variables, plus individual as a random effect in order to cope with overdispersion (Zuur et al. 2009). We tested each fixed term using a likelihood ratio test (LRT) between the full model and a simplified model without the variable of interest.

We also fit global LMMs (all species together) including “species” as an extra fixed effect (cohorts of *A. obstetricans* were treated separately). We then selected the best global LMM defining GR of tadpoles using AIC, AICc, BIC and LRTs. We only allowed for interactions among species and the other variables, and we included individual as a random effect again in order to cope with overdispersion. We fitted all LMMs using the function *lme* in the package *nlme* (Pinheiro et al. 2016).

To visually explore which physiological variables could be related with the amount of energy invested in growth for each species, or if physiological variables are correlated, we ran and tested linear regressions of CR, GA and ASSM against each other separately for each species (Figs. S10 to S12). We tried to detect possible relationships of all studied variables with RGL using the same methodologies (Figs. S13 to S16).

Statistical analyses: evolutionary and ecological analyses

The aim of these analyses was to: (1) detect evolutionary patterns in the mean values of physiological parameters of each species (i.e. phylogenetic signal, possible constraints), (2) relate these variables with the use of the pond permanency gradient of each species and (3) explore other interspecific patterns which may explain each species particular physiology.

For phylogenetic analyses, we worked using the ape package in R (Paradis et al. 2004) using the most comprehensive amphibian dated phylogeny to date (Gomez-Mestre et al. 2012), which includes all species in our study. In order not to distort results, we only used data from spring-born tadpoles in all phylogenetic analyses, thus excluding overwintering *Alytes obstetricans* tadpoles (Fig. S17).

First, we needed to evaluate which evolutionary model best fit the studied traits. We used “fitContinuous” in *geiger* (Harmon et al. 2008) to fit a Brownian motion (BM) and an Ornstein–Uhlenbeck (OU) model for each variable (i.e. GR, CR, GA, ASSM, RGL). We then tested the simpler model (i.e. BM) against the more complex model (i.e. OU) using likelihood ratio tests. If OU models were not significantly better than BM models, we kept the simpler model. Once we determined that BM was the best evolutionary model for all our variables (See “Results” section), we proceeded to test their phylogenetic signal.

Since the tests for phylogenetic signal are poor with a small number of species, we tested for phylogenetic signal using the four most used indices (Münkemüller et al. 2012), all suitable for BM evolutionary patterns. Thus, we estimated and tested the significance of Moran’s I (Moran 1950; Gittleman and Kot 1990) and Abouheif’s C mean (Abouheif 1999) using the function *abouheif.moran* in the package *adephylo* (Jombart et al. 2010) and using *patristic* and *oriAbouheif* methods for the proximity matrices, respectively. Pagel (1999) λ and Blomberg et al. (2003) were estimated using *phylosig* in the package *phytools* (Revell 2012), which allows the user to incorporate individual variation (following Ives et al. 2007). Randomization tests (5000 iterations) were used to determine the significance level of the latter two indices. Given that some variables gave values near to phylogenetic

relatedness and CR had a significant phylogenetic signal (see “Results” section), interspecific patterns were analyzed and discussed both regarding and disregarding phylogeny.

To detect interspecific patterns, we summarized the information in a 2D plane by conducting a principal components analysis (PCA) with GR and the variables defining it (GA and CR, see “Results” section).

In order to test if interspecific patterns are related with the pond permanency gradient and co-occurrence in ponds, we used as additional data a presence/absence data matrix (partially used in Richter-Boix et al. 2013) on 274 ponds in the studied area (Table S4). Thus, for each species we had a mean pond permanency gradient value for ponds it inhabited, and a mean value for each trait studied. We studied the relationship between the gradient and the traits of interest by means of both linear models (LM) and phylogenetic generalized least squares (PGLS) regressions. We also applied a partial mantel test (correcting for phylogenetic distance) among a matrix representing physiological dissimilarity (defined by CR, ASSM and GA using Euclidean distances) and a matrix of pond permanency gradient distance or a matrix dissimilarity of pond co-occurrence. Both matrices of ecological data were defined from the presence/absence matrix of 274 ponds (Table S4), using Bray-Curtis dissimilarity among species for co-occurrence, and linear distance among pond preference mean (1 = ephemeral, 2 = temporary, 3 = permanent) for pond permanency preference dissimilarity matrix.

All statistical analyses were performed using R (R Development Core Team 2014).

Results

Linear regressions revealed a positive relationship of both consumption rate (CR) and growth allocation (GA) with growth rate (GR) for all species. According to LMMs, these two variables had a significant role defining GR in all cases (Table S2). On the other hand, results for assimilation (ASSM) were inconsistent throughout the set of species studied (Fig. S8; Table S2). Accordingly, the best global model (all species together) explaining GR included only species (SP), CR and GA as explanatory variables (Table 1), leaving out

Table 1 Summary of the most representative linear mixed models possibly defining individual growth rate included in model selection procedures

Model	<i>df</i>	AIC	BIC	logLik	<i>p</i> > X2 versus best model
<i>gr</i> ~ <i>cr</i> + <i>ga</i>	5	−671.11	−655.06	340.55	0.0053
<i>gr</i> ~ <i>sp</i> + <i>cr</i>	12	−403.88	−365.37	213.94	<0.0001
<i>gr</i> ~ <i>sp</i> + <i>ga</i>	12	−565.56	−527.05	294.78	<0.0001
* <i>gr</i> ~ <i>sp</i> + <i>cr</i> + <i>ga</i>	13	−676.89	−635.16	351.44	(−)
<i>gr</i> ~ <i>sp</i> + <i>cr</i> + <i>ga</i> + <i>assm</i>	14	−676.09	−631.16	352.05	0.2729
<i>gr</i> ~ <i>sp</i> + <i>cr</i> + <i>ga</i> + <i>rgl</i>	14	−677.65	−632.72	352.83	0.0965
<i>gr</i> ~ <i>sp</i> + <i>cr</i> + <i>ga</i> + <i>assm</i> + <i>rgl</i>	15	−676.76	−628.62	353.38	0.1442

Models are ordered by increasing number of degrees of freedom. Log-likelihood ratio tests are done always against the best model in order to test the significance of each term. Best model is marked with an asterisk. All models include individual as a random effect (not shown in the table)

gr Growth rate, *sp*. species, *ga* growth allocation, *cr* consumption rate, *assm* assimilation efficiency, *rgl* relative gut length

ASSM. According to the criteria used and likelihood ratio tests, among the three selected explanatory variables CR and GA surpassed SP in importance.

The physiological variables CR, GA and ASSM are essentially unrelated at the intraspecific level (Figs. S10 to S12): only in 3 out of 30 regressions among these variables did we find a significant relationship. A negative relationship between GA and ASSM is seen in both bufonids (which could indicate particularities in the way the assimilated energy is invested), and a negative relationship between GA and CR occurs in overwintering tadpoles of *A. obstetricans*. The relative gut length (RGL) of tadpoles is generally unrelated to any of the physiological variables studied (Figs. S13 to S16). We found significant relationships between RGL and the other variables in only 5 out of 40 regressions tested. Interestingly, these five significant relationships were all found in only two species: *D. pictus* and *P. punctatus*. RGL of *D. pictus* related with GA, GR and CR, while RGL of *P. punctatus* related with GR and GA.

All traits were better fitted with a brownian motion model (Table S3) and among them only one trait studied, namely CR, had a significant phylogenetic signal in some tests (Table 2).

The PCA on GR, CR and GA summarized 98 % of the available information in a 2-dimensional plane. The first principal component (PC1, 64 % of variance) correlated positively with all three variables, mainly GR. Second axis (PC2, 38 % of variance) was not correlated at all with GR, instead having a clear positive relationship with CR and a negative correlation with GA. Species have significantly different values for all these individual variables, and therefore are distributed heterogeneously in this plane, defining different tactics to attain a certain GR (Fig. 1).

We did not find any significant regression or phylogenetic generalized least squares (PGLS) regression results of variables against the pond permanency gradient (Fig. 2). Species GR seems to be unrelated with its distribution across the gradient (Linear model: $F_{1,7} = 0.235$, $p = 0.630$, PGLS: $F_{1,7} = 0.144$, $p = 0.715$), but the way this growth is attained tends to be more consumption-focused in ponds with shorter hydroperiod, and more allocation- or assimilation-based in ponds with longer hydroperiod. Thus, CR is mildly higher—but far from being statistically significant—in species inhabiting ponds with short hydroperiod (Linear model: $F_{1,7} = 0.746$, $p = 0.416$, PGLS: $F_{1,7} = 1.886$, $p = 0.212$), while ASSM (Linear model: $F_{1,7} = 2.597$, $p = 0.151$, PGLS: $F_{1,7} = 3.880$, $p = 0.089$) tends to increase along with hydroperiod. On the other hand, GA (Linear model: $F_{1,7} = 0.037$, $p = 0.854$, PGLS: $F_{1,7} = 0.224$, $p = 0.650$) and RGL (Linear

Table 2 Phylogenetic signal of studied traits according to the four chosen indices

	GR	CR	ASSM	GA	RGL
Mean (Bloomberg's K)	1.0171	1.2854	0.8123	1.0278	1.0807
Mean (Pagel's L)	0.5085	0.8466	0.4568	0.4723	0.5331
p (Bloomberg's K)	0.0982	0.0300	0.2709	0.2116	0.0750
p (Pagel's L)	0.5773	0.1439	0.8221	0.7688	0.4496
p (Moran's I)	0.1115	0.0501	0.2927	0.2450	0.1651
p (Abouheif's C)	0.1823	0.0113	0.2894	0.1937	0.0646

GR growth rate, CR consumption rate, ASSM assimilation efficiency, GA growth allocation, RGL relative gut length

Significant values ($p < 0.05$) are in bold

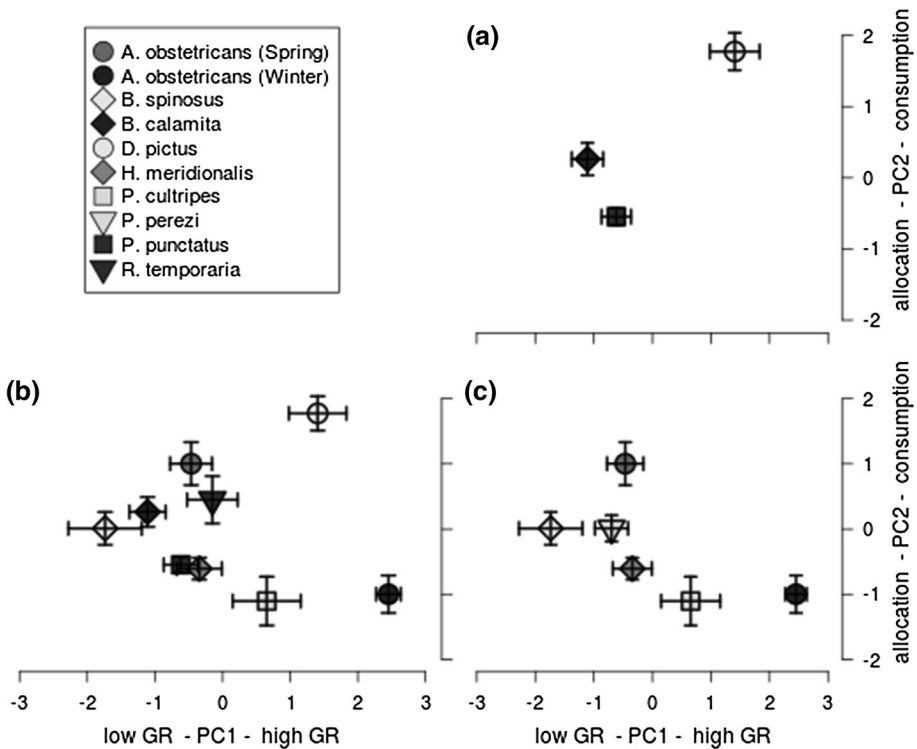


Fig. 1 Species mean \pm SE on the two first axes of a PCA using GR (= growth rate), GA (= growth allocation) and CR (= consumption rate) for all studied specimens, representing 98 % of data variability. Species are represented in all pond types where they usually breed. PC1 is positively correlated with growth rate (GR). Values in PC2 increase as tadpoles rely more on consumption and less in allocation to grow. **a** Ephemeral ponds. **b** Temporary Ponds. **c** Permanent ponds

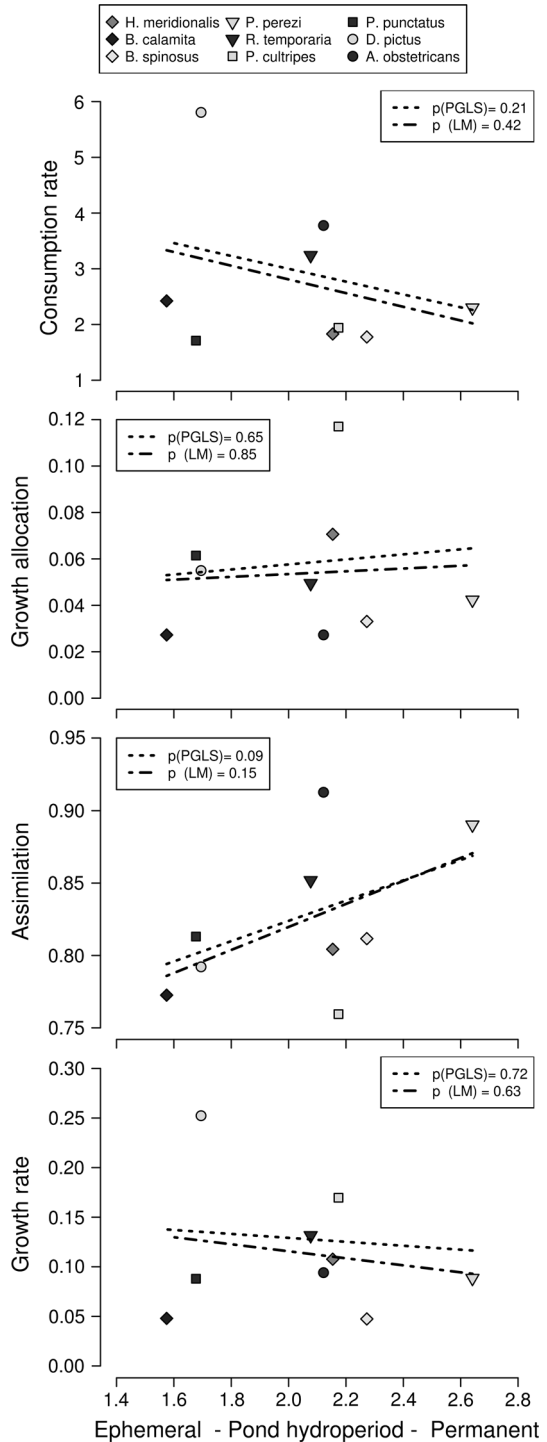
model: $F_{1,7} = 0.013$, $p = 0.913$, PGLS: $F_{1,7} = 0.385$, $p = 0.555$) showed no visible tendencies. Finally, partial Mantel tests point to a lack of a clear relationship among physiology and use of pond permanency gradient ($r = -0.00022$, $p = 0.406$) or among physiology and co-occurrence in ponds ($r = 0.00933$, $p = 0.431$).

Discussion

All physiological variables used in this study are generally uncorrelated, thus clearly representing different facets of the physiological abilities of tadpoles. But more importantly, physiological abilities represented by these variables were not randomly distributed across species. Each species has a particular physiology (Fig. 1), and significant differences among species were widespread in all features (Table S1).

Growth rates of tadpoles could depend on (1) food consumption rates, (2) the efficiency of assimilation of the ingested food and (3) energetic allocation preferences (i.e. investment of energy in growth). According to our results both at intra- and interspecific levels, only food consumption rates and the proportion of energy invested in growth visibly influence tadpole growth rates at individual level. Although they must be important for

Fig. 2 Species-level relationship among preferred pond type (horizontal axis: higher values indicate longer hydroperiods) and consumption rate, growth allocation, assimilation and growth rate. *Dots* indicate mean species value. *PGLS* phylogenetic generalized least squares, *LM* linear model



other purposes (like increasing the amount of energy available to allocate), food assimilation abilities lacked a direct relationship with growth rates. Within our array of species, the growth rate of a tadpole is defined mainly by the amount of food consumed and the capacity to allocate the energy into new somatic tissues. According to interspecific models these two factors surpass species (that is, species-specific features) in importance (Table 1).

Interestingly, the relative importance of consumption and allocation to defining growth rate is not the same for all species. Species in our study exhibit a continuum between a “consumer strategy” and an “efficient strategy”: while the growth rate of the species following the consumer strategy is based chiefly on high food consumption, species using the efficient strategy base their growth more on the ability to increase assimilation and convert the assimilated energy into growth. As prominent examples, *D. pictus* showed the highest consumer strategy, while overwintering cohorts of *A. obstetricans* and *P. cultripes* use high energetic investments in growth to produce large tadpoles.

What makes a species evolve towards one or the other strategy? At least in our array of species (with comparable results to Richardson 2002), phylogenetic relationships do not seem to be an important factor to take in consideration. We found only limited evidence of phylogenetic signal in the consumption rate of species. The value of Bloomberg’s was 1.29, higher than 1, indicating values more similar than expected phylogenetically. This could possibly reflect a constraint to evolving larger ingestion abilities without changing the general body plan of the tadpole, which is the same for all species in our study. In general terms, negative results of phylogenetic signal, in addition to the existence of discrete physiological groups within a species (*A. obstetricans*), suggest a degree of evolutionary lability in the studied traits.

From an ecological point of view, although the use of the consumer-efficient continuum seems related to the use of the pond permanency gradient by each species (Fig. 2), statistical results are not conclusive in this study. Similarly, as we hypothesized, species from ephemeral ponds tend to base growth more on consumptive abilities, while species usually inhabiting ponds with longer hydroperiods show an allocation-based tactic for growth, at the same time having higher assimilation rates (Fig. 2). These tendencies, although non-significant, coincide with the predictions of predation risk and activity levels along the pond permanency gradient. In ephemeral ponds with few predators, tadpoles should be able to base their growth in more consumptive tactics because increased activity might not be linked to a survival cost. In contrast, in permanent or long hydroperiod ponds with more abundant and complex predator communities, the need to maintain lower activity levels must be compensated for with a higher assimilation of ingested food and a higher allocation of obtained energy in growth. From another point of view, if we focus on pairs of related species or cohorts, the inhabitants of shorter hydroperiod ponds also tend to be the most consumptive. *R. temporaria* has a more flexible use of ponds and also a more consumptive physiology than the other ranid in the area (*P. perezi*), which is mainly restricted to permanent ponds. In another case, despite similarities in most biological and morphological features of the larval phase, *B. spinosus* tadpoles allocate more energy in growth than *B. calamita* (more common in ephemeral ponds). Finally, overwintering cohorts of *A. obstetricans* (mostly found in permanent ponds) also have a more allocation-based growth tactic than spring cohorts (more consumptive). Moreover, the enhanced allocation abilities of overwintering tadpoles could be useful for growth using scarcer resources during cold season (Begon et al. 1996; Bennion and Smith 2000). In this case, specific studies would be required to evaluate the ecological and evolutionary importance of these physiological differences between cohorts.

The inconclusive results for all of these tendencies could be primarily due to the relatively low number of anuran species inhabiting the area, meaning very few data for interspecific tests ($n = 9$). Moreover, only one of these species has clear preferences for permanent ponds, further impeding a powerful and reliable statistical assessment of physiological tendencies along the pond permanency gradient. On the other hand however, we cannot discard coevolutionary pressures to use different physiological tactics: efficient species may be forced to allocate even more energy to growth if they have to overcome competition from highly consumptive competitors, and vice versa. Thus, the effects of similar ecological preferences could be a dissimilarity among species instead, i.e. an overdispersion of species traits (Astor et al. 2014). Therefore, a diversification of physiological tactics among usually coexisting species cannot be disregarded. Competitively, all tadpoles present in the NW Mediterranean basin fit the traditional classification of tadpoles as filter-feeding herbivores (Altig and McDiarmid 1999a). Dietary studies yield similar results for all species, feeding on detritus, algae and phanerogams, complemented with small amounts of fungi, bacteria and small animals or their carcasses (Diaz-Paniagua 1985, 1989; Campeny 2001; Caut et al. 2012). Although a different phenology and use of the pond gradient seem to relax competition among our array of species (Jakob et al. 2003; Richter-Boix et al. 2006a, 2007a), complex larval guilds with high niche overlap between species can be easily found (Diaz-Paniagua 1985, 1989; Richter-Boix et al. 2013). Some experiments report negative fitness effects due to competitive resource depletion using species included in our study (Banks and Beebe 1987; Richter-Boix et al. 2004, 2007a). Thus, although a high availability of feeding resources seems to allow a certain niche overlap (Wassersug 1975; Diaz-Paniagua 1989), species coexisting with resource-depleting competitors like *D. pictus* (San Sebastián et al. 2015a, b) could become forced to allocate more energy in growth to overcome negative competitive effects. These or similar competitive interactions could easily blur the patterns and tendencies related to the pond permanency gradient in our data, making species that inhabit similar ponds diverge instead of converging in their physiology (Fig. 1).

Although relative gut length is a plastic feature mainly reported to change in order to enhance assimilation and growth rates (Noble 1931; Altig and Kelly 1974; Horiuchi and Koshida 1989; Relyea and Auld 2004), in this study gut length was generally unrelated to assimilation or any other variables. The only exceptions are found in *P. punctatus* and *D. pictus*, both common inhabitants of Mediterranean ephemeral ponds. Species inhabiting these ephemeral ponds are all forced to breed simultaneously when ponds fill in raining season to rapidly grow and develop before desiccation (Diaz-Paniagua 1990). The fact that significant relationships of relative gut length with growth-related features are restricted to species from these habitats (*P. punctatus* and the invasive *D. pictus*) might signal that food processing is especially important for these taxa.

Of all species, *D. pictus* showed the most extreme consumer strategy. In this particular case, this could be related with the superior ability to exploit resources described in some invasive populations (Petren and Case 1996; Kupferberg 1997; Holway 1999). Studies using native specimens of *D. pictus* would be needed to test possible physiological changes related to invasion. According to our results, although species found in ephemeral pools (*D. pictus*, *P. punctatus* and *B. calamita*) assimilate food similarly, native competitors rely less in consumption, showing a more allocative tactic. This employment of a more allocation-based strategy could be, to our knowledge, a first hypothesis explaining the apparent lack of competitive exclusion of natives *P. punctatus* and *B. calamita* by the resource-depleting invasive *D. pictus*. On the other hand however, mild physiological differences between both bufonids seem insufficient as a mechanism to avoid competitive exclusion:

studies report clear negative effects on tadpoles of *B. calamita* under competition with *B. bufo* larvae (Bardsley and Beebe 2000; Richter-Boix et al. 2007a). Further studies should disentangle if physiological factors are really important in these competitive systems.

Summing up, physiological differences among species generally follow the ecological predictions for the pond permanency gradient, but results are not clear enough to be conclusive. Despite mild interspecific physiological tendencies along the gradient (Fig. 2), once selected the list of species according to pond type, usual competitors appear rather separated (Fig. 1). Similarly as seen in Richardson (2002), phenotypic differences between species seem to persist in the same habitat, suggesting that species can display different evolutionary solutions to the same problems. The absence of convincing results may also indicate contrasting selective pressures of both competition with other tadpole species and different ecological conditions along the predation—pond desiccation risk gradient. The relative importance of competitive pressure and predation/desiccation risk shaping the physiology of tadpoles remains to be further studied.

Conclusions

Food consumption rates and the proportion of energy invested in growth (i.e. growth allocation) define tadpole growth rate, while assimilation abilities or other features like gut length seem not directly related with it. Our data suggests a labile continuum of physiological tactics from consumption-based to allocation-based growth along which species differentiate. Differences among species in this continuum generally follow predictions of adaptation to the ecological conditions along the pond permanency gradient, but also to strong competition in confined systems like ponds. The relative importance of these two selective pressures remains to be further studied. Physiological traits of each species could be an interesting field of study to understand how larval anurans adapt to the ecology of ponds, and how they compete and coexist.

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