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Morphological and life-history plastic responses to predators and competitors in two brown frogs, *Rana dalmatina* and *R. Latastei*

Sergio Castellano[1] · Daniele Seglie[2] · Olivier Friard[1]

Abstract

Predation and competition are two major factors that drive natural selection. When they vary unpredictably, selection has promoted the evolution of plastic responses in behavioral, morphological and life-history traits. These responses are not independent of each other and often represent a trade-off between conflicting interests. We conducted a common-garden experiment to study the effects of predation and competition on the morphology and life history of *R. dalmatina* and *R. latastei* tadpoles. The experiment used a randomized-block design, where tadpoles were raised either with or without siblings and either with or without predators. Regardless of the treatments, *R. dalmatina* grew faster, developed proportionally larger tails, proportionally smaller bodies, and completed metamorphosis earlier than *R. latastei*. Both species developed relatively larger bodies with competitors, and relatively larger tails with predators. While the relative increase in body size with competitors was similar in the two species, the increase in tail size with predators was greater in *R. dalmatina*, suggesting that this species invested more in defense than *R latastei*. Competitors delayed metamorphosis in both species and in *R. latastei* they negatively affected froglet body size and shape. Conversely, predators delayed the metamorphosis only in *R. dalmatina*. This delay was the long-term cost paid by *R. dalmatina* for the short-term benefits of developing more effective behavioral and morphological defensive traits.

Significance statement

Tadpoles develop plastic morphological and life-history traits in responses to predators and competitors. This plasticity is costly in terms of both performance and function. Our study shows that two closely related brown frogs have evolved different trade-offs in their plastic responses to predators and competitors. *Rana dalmatina*, which grew and developed faster than *R. latastei*, was more sensitive to predators and developed more effective morphological defenses. Both species delayed metamorphosis at high density, but *R. dalmatina* did it more than *R. latastei*. Predators did not affect the duration of larval development in *R. latastei*, whereas in *R. dalmatina*, they further delayed metamorphosis. This delay was the long-term cost of the *R. dalmatina*'s larger investment in short-term defensive traits.

Introduction

Phenotypic plasticity is the phenomenon in which a single genotype develops different phenotypes, depending on the environment it experiences (West-Eberhard 2003). Plasticity is a property of the genotype and it might be adaptive, non-adaptive, or neutral, depending on its effects on fitness (Ghalambor et al. 2007). Neutral and non-adaptive phenotypic plasticity is often a passive response (Smith-Gill 1983) to stressful conditions, such as resource limitation, and it can be fully explained in terms of the proximate

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✉ Sergio Castellano
  sergio.castellano@unito.it

1   Department of Life Science and Systems Biology, University
    of Turin, Via Accademia Albertina 13, Torino 10123, Italy

2   ELEADE Soc. Coop, C.Le Montresco 1, Turin,
    Chiaverano 10010, Italy

mechanisms regulating growth and metabolism (Parsons et al. 2020). In contrast, adaptive plasticity is an active process (Smith-Gill 1983), in that it allows individuals to adjust their phenotypes to the environment, usually, with positive fitness effects. Since adaptive plasticity has evolved by natural selection, it is an "evolutionary character" (Wagner 2001), whose variation must be understood in terms of both proximate mechanisms and ultimate causes.

One of the strongest evidence for adaptive plasticity comes from studies of how organisms respond to predators and/or competitors. In the presence of predators, organisms modify their phenotype in a way that decreases the risk of being attacked or increases the chances of survival after being attacked. In the presence of competitors, they develop traits that increase the ability to collect and store resources from the environment. These plastic changes are often multivariate in that they involve different types of phenotypic characters, such as behavioral, morphological and life-history traits (Beckerman et al. 2010). For example, water fleas (*Daphnia* sp.) increase helmet sharpness in the presence of predator fish and develop longer spines in the presence of predator midge (Dodson 1988), but reduce the size of these traits when exposed to high intra- or inter-specific competition (Burns 2000). When exposed to fish, which selectively feed on larger-than-average individuals, water fleas tend to reproduce earlier at a smaller size, whereas, when exposed to midges, which preferentially select for smaller-than-average individuals, they tend to reproduce later at a larger size (Beckerman et al. 2010).

The traits involved in multivariate plastic responses are not fully independent of each other. If they are constrained to covary in a direction opposite to what natural selection favors, then natural selection is likely to promote an optimal trade-off in their expression. Trade-offs can be of two different types, sometimes called "performance" and "functional" trade-offs (Arnold 1992; Ghalambor et al. 2004; Berberi and Careau 2019). A "performance" trade-off arises when the variation in a trait has conflicting effects on different components of fitness. For example, when exposed to predators, prey become elusive and spend proportionally more time hiding and less time feeding, but this makes them less effective in dealing with competitors. A "functional" trade-off arises when limiting resources are allocated to two traits so that an increase in the first trait results in a decrease in the other. As mentioned above, when predators select for smaller-than-average individuals, water fleas tend to invest more in development and less in growth. As a consequence, prey reproduce earlier but at a smaller size than when predators preferentially feed on larger-than-average water fleas.

Similar multivariate plastic responses are known to occur also in species with complex life cycles, such as anurans. In these species, the biotic interactions experienced by tadpoles in the aquatic environment are usually more intense and variable than those experienced during the post-metamorphic terrestrial stages. Natural selection on tadpoles has thus favored the evolution of adaptive plastic responses in behavioral, morphological and life-history traits (Benard 2004). In the presence of competitors, tadpoles increase activity and develop larger bodies with smaller tails, which might improve feeding efficiency under limiting resources (Relyea 2002). These changes in morphology are expected to mitigate the negative effects of intra-specific competition on growth and development, which however remain evident, because at high density tadpoles tend to metamorphose later and at a smaller size. As observed in water fleas, in the presence of ambush predators (i.e. dragonfly larvae), tadpoles decrease activity and develop a small body and a proportionally larger tail (Innes-Gold et al. 2019). In these species, since metamorphosis represents an opportunity to escape from aquatic predators, many theoretical models predict that, independent of the type of predators, when the risk is high, tadpoles should metamorphose earlier at a smaller size (Benard 2004). Empirical studies have provided convincing evidence for plastic development in tadpoles, but only a few studies have shown support to the theoretical predictions. Indeed, only few studies showed predator cues to cause a smaller size and/or a shorter time at metamorphosis, whereas most studies showed that, with predator cues, tadpoles emerged either at the same time or later and with the same or larger size (Benard 2004; Relyea 2007). The trade-off hypothesis of adaptive plastic traits explains the apparent contrast between theoretical predictions and empirical evidence as the consequence of the multivariate nature of the anti-predator plastic response. Tadpoles that reduce activity and feed less in the presence of predators might not have enough resources to grow and develop fast enough to metamorphose earlier. As for the competitor-induced variation in growth and development, also the predator-induced variation in growth and development cannot be viewed as adaptive per se, but as the costs imposed by the adaptive plastic changes in behavioral and morphological traits. As a corollary of this hypothesis, we may predict that if two species differ in the strength of the plastic response to predators, then the species with the strongest adaptive response should show also the strongest non-adaptive response to predators. For example, if, in the presence of predators, the tadpoles of the first species develop proportionally larger tails than those of the second species, then the tadpoles of the first species are expected to pay proportionally higher costs for their stronger response and, as a consequence, to grow and to develop more slowly than tadpoles of the second species.

In the present study, we test the trade-off hypothesis of adaptive plastic traits by comparing the morphological and life-history plastic responses to competitors and predators of two European brown frogs, *Rana dalmatina* and *R. latastei*. These are sister species (Veith et al. 2003; Yuan et al. 2016) with a similar ecology. They live in the deciduous forests of lowland plans. They are predominantly terrestrial and move to the aquatic environments only during the short breeding season, in late winter or early spring (Dalpasso et al. 2022). Both species survived the Pleistocene glaciations in single refugia in different regions of southern Europe (Ficetola et al. 2007; Vences et al. 2013), but while *R. dalmatina* has expanded its range to much of the territories of central Europe, *R. latastei* has remained endemic to northern Italy and to neighboring territories (Sillero et al. 2014). In sympatry, the two species often share the same breeding site, although *R. latastei* lays eggs in deeper water, further from the shoreline (Romagnoli et al. 2020). In previous studies (Castellano et al. 2022, 2023), we showed that tadpoles of the two species differed in their behavior, both quantitatively (the amount of swimming) and qualitatively (speed and duration of swimming), with *R. dalmatina* moving more and faster than *R. latastei*. Both species adaptively change their behavior in the presence of both predators (dragonfly larvae) and competitors. Although these changes were qualitatively similar in the two species, they differed quantitatively. In particular, the most active species, *R. dalmatina*, was less sensitive than *R. latastei* to competitors (Castellano et al. 2022), but more sensitive to predators (Castellano et al. 2023). Here, we show results of a common garden experiment, where tadpoles of the two species were raised to metamorphosis either with or without predator cues and in conditions of either low or high density. The tadpoles were from two breeding populations situated a few kilometers apart at the westernmost edge of the species' ranges. In this region, the species coexist in sympatry and frequently engage in syntopic breeding. The experiment was designed to measure the plastic responses to predators and competitors in both morphological (tail and body shape) and life-history traits (body size and age at metamorphosis). As predicted by the trade-off hypothesis of plastic traits, we expect that, with predators, the most active and behaviorally plastic species, *R. dalmatina*, will develop proportionally larger tails than *R. latastei*, in order to compensate for the higher risk of being detected by predators with a higher probability of escaping from their attacks. We also expect that *R. dalmatina* will pay higher costs for its higher behavioral plasticity to predators and that these costs might be associated with non-adaptive changes in larval growth and development.

Methods

Animals were initially collected as eggs from two breeding ponds located in Special Areas of Conservation of the Po-river basin, in Piedmont, North-western Italy. Four clutches of *Rana dalmatina* were collected on 16 March 2022 from the site "Po morto di Carignano" (IT1110025 SAC), whereas four clutches of *R. latastei* were collected on 16 March ($n = 3$) and 22 March ($n = 1$) 2022 from the site "Confluenza Po-Varaita" (IT1160013 SAC). The clutches were transported to our field research station and placed outdoors, in separate 60-l tanks of well water until hatching.

At Gosner (1960) stage 23–24, a haphazard sample of hatchlings from each clutch was collected and individuals were photographed with a Leica EZ4W stereo zoom microscope. From the pictures, we measured hatchling total length (starting size). Since at this stage, larvae rely on maternal yolk reserves rather than actively feeding, we assumed that variation in starting size, both between and within species, was unaffected by the environment and depended only on genetic and epigenetic (i.e. maternal effect) factors. These hatchlings were then returned to their tank and allowed to grow with their siblings for 20 days, when the experiment began. In this phase and during the experiment, tadpoles were fed fish vegetable flakes *ad libitum*.

We started the experiment when tadpoles were 20 days old. From each clutch, with a dip net, we blindly collected 48 tadpoles at Gosner stage 25–27, which were photographed (see below) and then randomly assigned to experimental treatments. The experiment used a randomized block design, with four blocks and two replicates per block and with a factorial combination of density and predator treatments within each block (Fig. 1). Specifically, a block consisted of four fiberglass troughs ($217 \times 40 \times 15$cm) (Lamar, Udine s.r.l.), two with predators and two without. In each trough, we placed four small containers ($33.5 \times 19 \times 12$cm) with one tadpole in about 5 l of water and two larger containers ($40 \times 34 \times 17$cm) with 10 tadpoles in about 10 l of water. In a trough, three containers (two small and one large) hosted tadpoles from one clutch of *R. latastei*, the other three containers hosted tadpoles from one clutch of *R. dalmatina*. Since there was one clutch per species within each block, the experiment did not allow to disentangle the effects of block from those of clutch. To facilitate homogeneous water flow through the containers within a trough, we cut two windows into the large sides of the containers (window size in large containers: 25×10cm; window size in small containers: 16×6cm) and sealed them with 1-mm plastic mesh. In the troughs with predators, three dragonfly larvae (gen. *Aeshna*) were individually kept into perforated plastic cages (base diameter $= 15$ cm), placed outside the containers but in close contact with one of the two windows,

Fig. 1 A schematic representation of an experimental block. Each block included four troughs, two with caged predators (dragonfly larvae, gray "D" circles) and two control troughs without predators. Within each trough, there were four small containers (each housing one tadpole) and two larger containers (each housing 10 tadpoles). These containers were coded based on species (using the first letter, L or D), clutch (corresponding to the block number), ontogenetic treatment (D for dragonfly; C for control), and replicate number. The entire experiment comprised four blocks
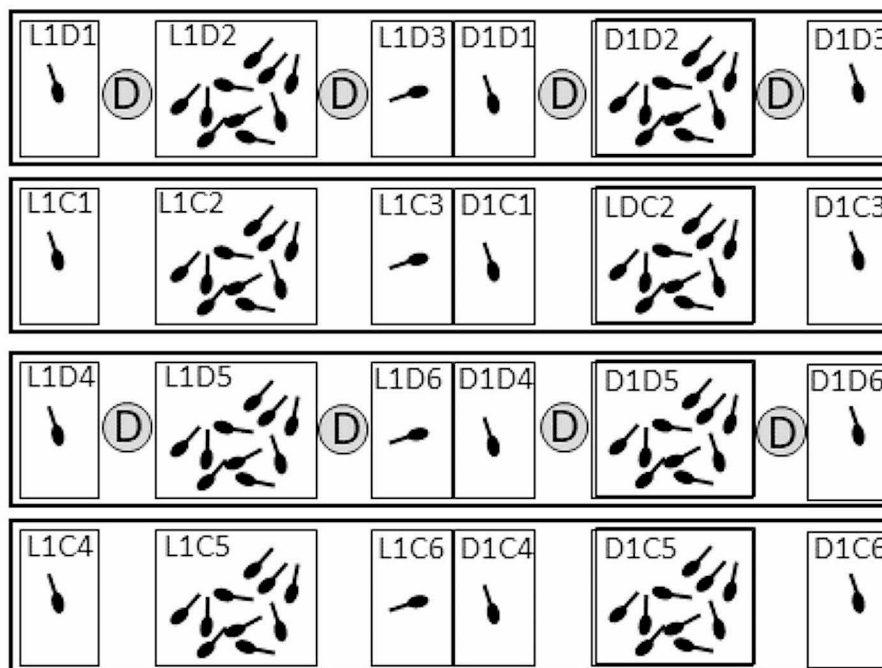
so that tadpoles could perceive the predator presence both visually and chemically (Hettyey et al. 2012). Dragonfly larvae were fed twice a week with small tadpoles, to produce digestion released alarm cues (Hettyey et al. 2015). All troughs were on a lawn under a shelter of 50% knitted shade cloth material, to avoid full sunshine. When tadpoles approached metamorphosis (i.e. the emergence of forelimbs, Gosner stage 42), they were transferred from the troughs to new containers, closed with a perforated cover to prevent escape, and with a small amount of water to prevent drowning. They were kept there for a few days until the tail was completely reabsorbed.

During the experiment, tadpoles were photographed three times, when they were 20 days old, when they were 40 days old and when they completed metamorphosis. Pictures were taken with a Raspberry Pi v2.1 8 MP camera on a Raspberry Pi model 3B+. Both tadpoles and froglets were placed on a Petri dish, lined with graph paper. Tadpoles were photographed from their lateral view and, with a custom-designed program written in Python3, we measured three morphometric traits: total length (the distance between the tip of the head and the tip of the tail), tail area and body area. Froglets were anaesthetized in a 0.1% MS222 solution and placed ventrally with femurs and humerus perpendicular to the animal sagittal plane. From these pictures, we measured the snout-vent length (SVL), the elbow-to-elbow distance (fore-limb size) and the knee-to-knee distance (hind-limb size) (see Fig. S1). Besides these morphometric traits, we measured the age at metamorphosis, defined as the number

of days from hatching to completion of larval development. To minimize observer bias, blinded methods were used when all data were analyzed.

After being recovered from the anesthesia, froglets were released close to their pond of origin.
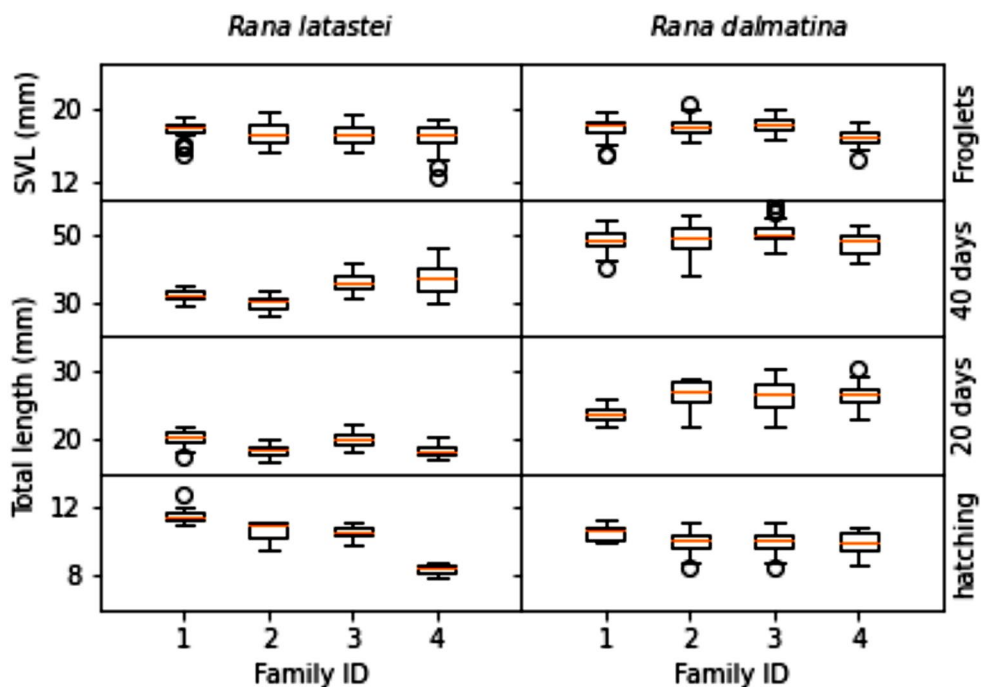
Statistical analyses

To analyze between-species differences in both morphometric and life-history traits, we carried out univariate general mixed-effects models, with two random factors, clutch (nested within species) and trough (nested within block). The analyses were conducted on four datasets, separately. The first and the second datasets included the measurements of tadpole size (log-transformed total length) either before (at hatching) or at the beginning of the experiment (day 20). The analyses on these datasets, which included the species as the only fixed factor, allowed us to test for differences between species and, for each species, to compute the portion of among-clutch variation, also known as the intra-class correlation coefficient r_I (Sokal and Rohlf 1995) or repeatability (Nakagawa and Schielzeth 2010).

The third and four datasets were collected either during (when tadpoles were 40 days old) or at the end of the experiment (when tadpoles had completed metamorphosis). The mixed models, on these datasets, included as fixed factors the species and the two ontogenetic treatments (density and predator). For tadpoles, when the response variables were the log-transformed tail or body sizes, the models used the

log-transformed total length as a covariate. Likewise, for froglets, when the response variables were either the log-transformed hind- or fore-limb lengths, the models included the log-transformed SVL as a covariate. For all analyses, the models included the two- and three-way interactions (i.e. species*density*predator). However, since the three-way interactions were not statistically significant, we pooled them with the error term to improve the statistical power of the tests.

All mixed-effect models were fitted using the brms package (Burkner 2017, 2018) in R v. 4.1.2 (R Development Core team 2021), which adopts a Bayesian inference based on STAN. In all models, we used the default non-informative priors and results were obtained by running 4 chains of 8000 iterations each, with warmups of 4000 iterations. We checked visually traces and distributions of all models to detect autocorrelation and sampling stationary (Faraway 2016) and used Rhat values to check for chain convergence (Burkner 2017). From the predictors' posterior distributions, we evaluated their posterior means, with their 95% credible intervals (CI). Predictors, whose 95% CI did not include zero, were assumed to have a statistically significant effect on the response variable. The brms package allowed us to compute the posterior distributions of the within- and among-clutch variances. We used these posteriors to compute the intra-class correlation coefficient r_I of the response variables and to test for significant variation among clutches within species ($H_0 : r_{I,species} > 0$) and for significant differences in r_I between species ($H_0 : r_{I,latastei} - r_{I,dalmatina} \neq 0$). In the latter case, for each iteration, we computed the between-species difference in r_I and, then, we estimated the posterior mean of the differences and its 95% CI.

Results

Hatching and pre-treatment growth

At hatching, tadpoles of the two species did not differ in total length (*R. latastei* effect = -0.07, 95% CI: -0.37, 0.22), but they did differ in the relative amount of among-clutch size variation. As shown in Fig. 2, at hatching, the among-clutch intra-class correlation coefficient of *R. latastei* ($r = 0.91$, 95% CI: 0.74, 0.91) was higher than that of *R. dalmatina* ($r = 0.24$, CI: 0.00, 0.85) and the difference was statistically significant (95% CI of the difference between posterior values: 0.08, 0.96).

At age 20 days, when the experiment began, *R. dalmatina* tadpoles had already grown larger than *R. latastei* tadpoles (*R. latastei* effect = -6.86, 95% CI: -7.22, -6.49). In *R. latastei*, the among-clutch intra-class correlation coefficient was lower than that observed at hatching ($r = 0.658$, 95% CI: 0.31, 0.94), whereas in *R. dalmatina* it was higher ($r = 0.502$, 95% CI: 0.19, 0.86) and the between-species difference was no longer statistically significant (95% CI of the difference between posterior values: -0.34, 0.62).

Treatment effects on tadpoles.

At age 40 days, *Rana dalmatina* tadpoles were still larger with proportionally larger tails than *R. latastei* tadpoles (Table 1). The size-adjusted body area of *R. dalmatina* tadpoles were smaller than those of *R. latastei*, but differences were not statistically significant (Table 1).

Density had a weak negative, marginally significant effect on size, but a stronger effect on both tail and body area (Fig. 3). The effect on tail area differed between species and

Fig. 2 Box plot of the distribution of tadpole size at different stages of their development, separated by species and clutch. Boxes indicate the inter quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR. Outliers are shown as individual points beyond the whiskers

Table 1 Summary of the fixed and random effects on morphological traits of 40-days old tadpoles, estimated from the posterior distribution of Bayesian GLMMs. Statistically significant effects are in bold (CI, credibility interval)

	Ln(total length)		Ln(tail areas)		Ln(Body areas)	
	coefficient	95% CI	coefficient	95% CI	coefficient	95% CI
Rana latastei tadpoles raised without predators at low density	**3.54**	(3.44, 3.64)	**-2.37**	(-2.68, -2.07)	**-1.58**	(-1.99, -1.07)
The effect of *Rana dalmatina* (RD)	**0.38**	(0.24, 0.50)	**0.10**	(0.01, 0.18)	-0.11	(-0.28, 0.06)
The effect of high density	-0.02	(-0.05, 0.00)	-0.01	(-0.03, 0.01)	**0.05**	(0.02, 0.08)
The effect of predators	-0.01	(-0.08, 0.05)	**0.04**	(0.02, 0.06)	0.00	(-0.03, 0.03)
The effect of high density on RD tadpoles	-0.02	(-0.04, 0.01)	**-0.04**	(-0.08, -0.01)	0.03	(-0.02, 0.07)
The effect of predators on RD tadpoles	**0.06**	(0.03, 0.09)	**0.03**	(0.01, 0.06)	0.02	(-0.01, 0.06)
The allometric effect of tadpole size (total length)			**1.99**	(1.90, 2.08)	**1.61**	(1.48, 1.73)
Random effects						
Clutch (σ)	0.1	(0.03, 0.18)	0.04	(0.02, 0.09)	0.11	(0.06, 0.22)
Trough (σ)	0.6	(0.04, 0.10)	0.01	(0.00, 0.03)	0.01	(0.00, 0.04)
Residual (σ)	0.06	(0.06, 0.07)	0.06	(0.05, 0.06)	0.07	(0.07, 0.08)
IC correlation coefficient (RD)	0.29	(0.02, 0.93)	0.18	(0.00, 0.78)	0.53	(0.14, 0.96)
IC correlation coefficient (RL)	0.73	(0.36, 0.98)	0.69	(0.31, 0.98)	0.78	(0.45, 0.99)

was statistically significant only in *R. dalmatina* tadpoles, which developed a proportionally smaller tail at high density (Fig. 3b). The effect on body area was similar in the two species: at high density tadpoles developed a proportionally larger body than their siblings raised alone (Fig. 3c; Table 1).

The presence of predators had no effect on tadpole body area, a weak effect on their total length and a stronger effect on their tail area (Table 1). In *R. dalmatina*, total length tended to increase with predators, whereas in *R. latastei* it slightly decreased. In both species, predators caused tadpoles to develop wider tails and their effects were significantly stronger in *R. dalmatina* than in *R. latastei*. Density and predator effects were mainly additive and we found no evidence for statistically significant interactions between them for all three morphometric traits (High Density * Predators coefficient (95% CI): Ln(Total length) = 0.06 (-0.02, 0.14); Ln(tail area) = 0.03 (-0.02, 0.08); Ln(Body area) = -0.01 (-0.08, 0.06), not shown in Table 1).

In both species, the random factor "Clutch" explained a significant amount of variation in all three morphometric traits (Table 1, the lower values of 95% CI of posterior distributions were greater than zero). By comparing the posterior values of the intra-class correlation coefficients of the two species, we always observe higher mean values in *R.*

latastei than in *R. dalmatina*, although differences were not statistically significant (i.e. the 95% CI of the posterior distributions included always zero, results not shown).

Treatment effects on metamorphosis

R. dalmatina completed metamorphosis earlier than *R. latastei* (Table 2). In both species, the age at metamorphosis increased at high density, and the increase was larger in *R. dalmatina* than in *R. latastei* (Fig. 4a). Predators did not affect the age at metamorphosis of *R. latastei*, but they did that of *R. dalmatina*. When exposed to predators, *R. dalmatina* tadpoles metamorphosed significantly later than their siblings raised without predators (Fig. 4a).

At metamorphosis, *R. dalmatina* and *R. latastei* did not differ in SVL, and in relative fore-limb size, but they did differ in the relative hind-limb size, which was proportionally smaller in *R. dalmatina* (Table 2; Fig. 4b-d). Froglet morphology was not affected significantly by the presence of predators, whereas it was affected by the presence of competitors and the effect was statistically significant in *R. latastei*, but weak or absent in *R. dalmatina*. Specifically, at high density *R. latastei* metamorphosed at a smaller SVL and with proportionally shorter hind limbs (Fig. 4b, c).
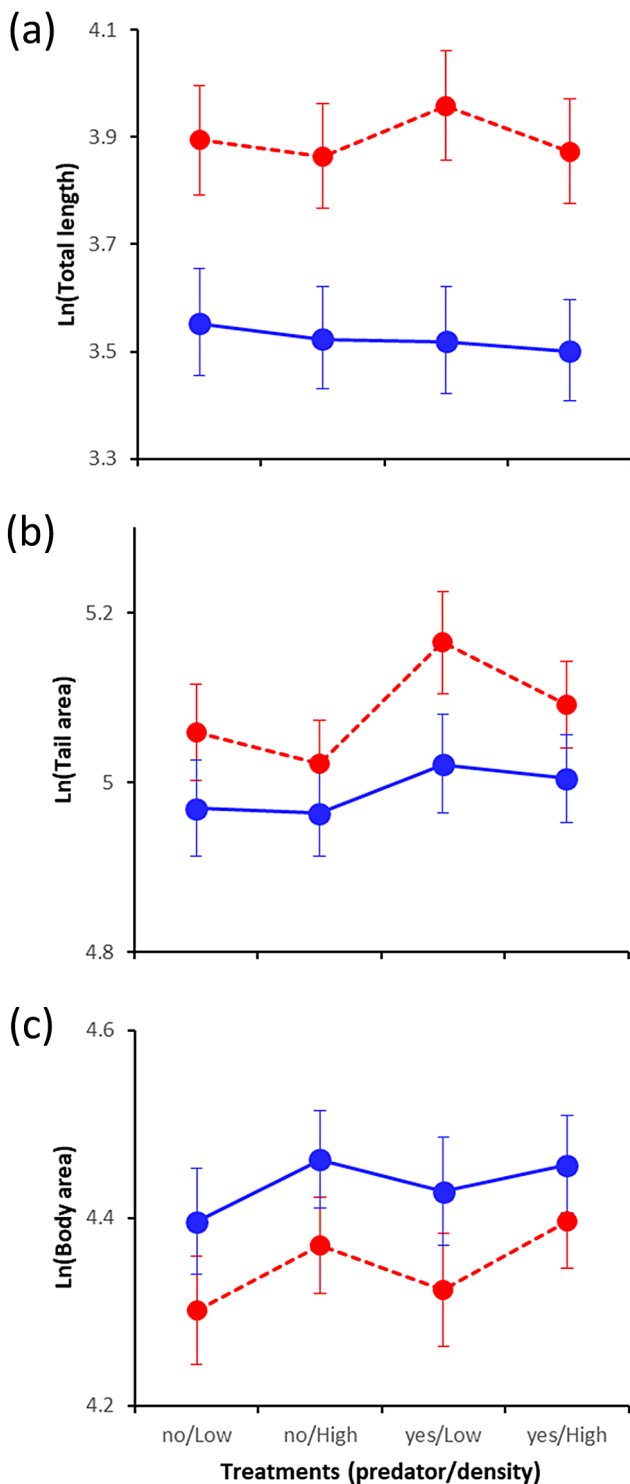
Fig. 3 Effects of the four experimental treatments on tadpole morphometric traits of *R. dalmatina* (red) and *R. latastei* (blue). Estimated mean marginal effects are shown with 95% credibility intervals represented by bars

The random factor "Clutch" explained a significant portion of trait variation in both the highly plastic (age at metamorphosis) and the less plastic traits (morphological traits). The IC correlation coefficient of the age at metamorphosis was larger in *R. latastei*, the less plastic species, than in *R. dalmatina*. The reverse was true for the morphological traits, which were more plastic in *R. latastei* than in *R. dalmatina*.

Discussion

R. dalmatina and *R. latastei* share a similar ecology and often breed in sympatry. However, previous studies showed that tadpoles of *R. latastei* were less active, spent more time resting at the bottom, and made shorter movements than tadpoles of *R. dalmatina* (Castellano et al. 2022, 2023). Possibly because of these differences, *R. latastei* was found to be more sensitive to the presence of competitors and less sensitive to the presence of predators than *R. dalmatina*. In this study, we analyzed the effects of competitors and predators on the morphology and the larval life-history of *R. latastei* and *R. dalmatina*. Because our study did not include multiple populations within species, the results may not represent general differences between species. Nevertheless, as the two populations coexist in the same habitat, we reasonably assume that the observed differences reflect distinct responses to similar environmental conditions. From this assumption and from the trade-off hypothesis of adaptive plasticity, we derived two predictions.

First, given *R. dalmatina*'s greater behavioral plasticity in response to predators, we predicted that it would exhibit a stronger morphological response to predators than to competitors. Conversely, given *R. latastei*'s greater behavioral plasticity to competitors, we predicted that it would show a stronger morphological response to competitors. Results were consistent with this prediction: in both species, tail area increased in the presence of predators, but the increase was larger in *R. dalmatina* than in *R. latastei*. In contrast, the plastic response to competitors was inconsistent with the prediction. In fact, body area increased with competitors, but the increase was similar in the two species.

The second prediction was that *R. dalmatina* would incur higher costs due to its stronger plastic responses to predators compared to *R. latastei*, and that these costs might be associated with non-adaptive changes in larval growth and development. Results were consistent with this second prediction. Tadpoles of *R. dalmatina* that were raised with predators metamorphosed later, at a similar size, but with proportionally smaller fore-limb size than their siblings raised without predators. In contrast, the presence of predators did not affect the age and the size at metamorphosis of *R. latastei* tadpoles, but the presence of competitors did.

Table 2 Summary of the fixed and random effects on morphological and life-history traits of newly metamorphosed froglets, estimated from the posterior distribution of Bayesian GLMMs. Statistically significant effects are in bold (CI, credibility interval)

	Age		SVL		Hindlimb size		Forelimb size	
	coefficient	95% CI	coefficient	95% CI	coefficient	95% CI	coefficient	95% CI
Rana latastei froglets raised without predators at low density	**81.16**	(78.35, 83.96)	**2.9**	(1.74, 4.83)	**-0.66**	(-1.93, -0.38)	**-0.36**	(-0.63, -0.09)
The effect of *Rana dalmatina* (RD)	**-7.85**	(-11.89,-3.87)	0.04	(-0.01, 0.09)	**-0.05**	(-0.09, -0.02)	-0.01	(-0.05, 0.02)
The effect of high density	**2.05**	(0.64, 3.49)	**-0.06**	(-0.08, -0.04)	**-0.03**	(-0.06, -0.01)	**-0.02**	(-0.04, -0.00)
The effect of predators	-0.42	(-1.61, 0.80)	0.01	(-0.01, 0.04)	0.00	(-0.01, 0.02)	0.00	(-0.02, 0.02)
The effect of high density on RD froglets	**2.42**	(0.44, 4.34)	**0.06**	(0.03, 0.10)	**0.03**	(0.00, 0.06)	0.01	(-0.02, 0.03)
The effect of predators on RD froglets	**3.57**	(2.01, 5.17)	0.01	(-0.01, 0.04)	0.01	(-0.02, 0.03)	0.02	(-0.01, 0.04)
The allometric effect of SVL					**1.22**	(1.13, 1.32)	**1.01**	(0.92, 1.10)
Random effects								
Clutch (σ)	2.36	(1.21, 4.55)	0.03	(0.02, 0.07)	0.04	(0.02, 0.09)	0.03	(0.01, 0.07)
Trough (σ)	1.39	(0.02, 6.48)	0.60	(0.00, 3.36)	0.58	(0.00, 3.03)	0.51	(0.00, 3.31)
Residual (σ)	3.32	(3.05, 3.61)	0.06	(0.05, 0.06)	0.09	(0.08, 1.00)	0.08	(0.07, 0.08)
IC correlation coefficient (RD)	0.16	(0.01, 0.57)	0.24	(0.02, 0.93)	0.44	(0.09, 0.94)	0.38	(0.06, 0.93)
IC correlation coefficient (RL)	0.47	(0.15, 0.84)	0.20	(0.02, 0.76)	0.07	(0.00, 0.48)	0.07	(0.00, 0.50)

At high density, *R. latastei* tadpoles metamorphosed slightly older and at a smaller size, with proportionally smaller hind and fore limbs.

The adaptive plastic response to predators

Although, at hatching, tadpoles of the two species showed similar size, *R. dalmatina* grew faster and metamorphosed earlier than *R. latastei*. However, once metamorphosis was completed, froglets of the two species no longer differed in size. These differences in larval growth rate and age at metamorphosis are consistent with the results of previous studies that showed a general higher activity in *R. dalmatina* than in *R. latastei* (Castellano et al. 2022, 2023) and support the hypothesis that the two species occupy different positions along a fast-slow continuum of larval development (Réale et al. 2010), with *R. dalmatina* showing "faster" traits than *R. latastei*. In tadpoles, growth and development are affected by metabolic rate, which in turn might be related to activity and feeding efficiency (Beck and Congdon 2000). As observed in other frogs (Altwegg and Reyer 2003), a fast larval development might have long term beneficial effects on post-metamorphic growth and survival, but it also imposes short term costs, because more active tadpoles would need a larger amount of resources and would inevitably suffer a higher predation risk.

Tadpoles have evolved several plastic adaptations to cope with predators (Benard 2004; Relyea 2007). The most common response is behavioral: tadpoles decrease activity in the presence of predators. In a previous work (Castellano et al. 2023), we showed that tadpoles of both species moved not only less, but also faster when exposed to, and that the plastic response was stronger in *R. dalmatina* than in the less active *R. latastei*. These results were consistent with the hypothesis that *R. dalmatina*, because of its higher activity, suffered a higher predation risk and, consequently, decreased activity more than *R. latastei*. Another widespread plastic response to predators is morphological: when exposed to, tadpoles develop deep tails, which improve the probability of surviving to predators' attacks (Blair and Wassersug 2000). In the present work, we show that tails were larger in *R. dalmatina* and, in both species, tail area increased in the presence of predators, but the increase was larger in the most active species, *R. dalmatina*. These results provide evidence that the same selective pressures responsible for interspecific differences in behavioral plasticity might have promoted consistent differences in morphological plasticity.

Our results resemble those obtained by Smith and Van Buskirk (1995) in a transplant experiment on two North-American treefrogs, *Pseudacris triseriata* and *P. crucifer*. The two species showed consistent differences in the morphological and behavioral plastic responses to predators. *P.*
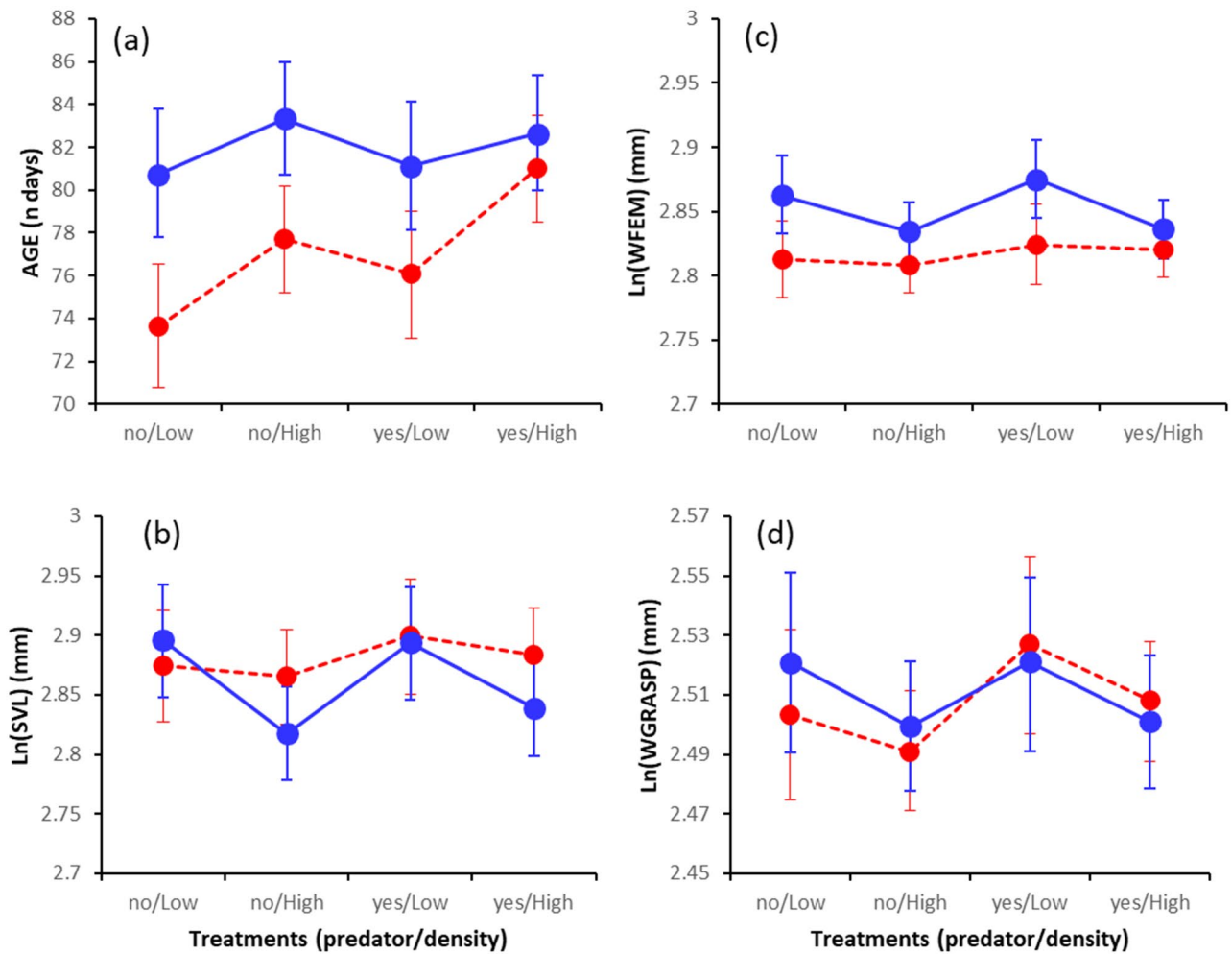
Fig. 4 Effects of the four experimental treatments on Age, Body (SVL), and Limb size (WGRASP: elbow-to-elbow distance; WFEM: kneel-to-kneel distance) of newly-metamorphosed frogs of *R. dalma-* *tina* (red) and *R. latastei* (blue). Estimated mean marginal effects are shown with 95% credibility intervals represented by bars

triseriata tadpoles had smaller tail muscles and tail fin and grew faster and metamorphosed earlier than *P. crucifer* tadpoles. In the presence of predators, *P. triseriata* decreased activity and increased tail size more than *P. crucifer*. The authors explained the pattern as the effect of the different ecology of the two species. In *P. crucifer*, which inhabits ponds with high predator risk but low intra-specific competition, natural selection has favored adaptations that prioritize predator defense over resource acquisition. The opposite is true for *P. triseriata*, which lives in ponds where predator risk is generally low but competition high. This species has evolved less effective defenses against predators, but the ability to develop them, when needed. Unlike *Pseudacris* species, *R. dalmatina* and *R. latastei* did not show clear ecological differences and, where their distribution ranges overlap, they often breed in syntopy. For this reason, establishing whether the differences in the plastic

response to predators between species were the causes or the consequences of different predator-selection regimes is challenging. If *R. latastei* experienced stronger predator selection than *R. dalmatina*, then the direct effect of selection was the evolution of risk-averse behaviors, while the slower larval developmental strategy and the reduced plasticity were indirect consequences. In contrast, if the "slow" life-history traits of *R. latastei* were directly selected, then the low sensitivity to predators was an indirect effect of this selective regime.

The adaptive plastic response to competitors and its interaction with predators

Tadpoles have evolved behavioral and morphological plastic responses to cope not only with predators, but also with competitors (Relyea 2002, 2004). When the presence of

other tadpoles benefits the individual, natural selection may have favored the ability to recognize and aggregate with their kin (Blaustein and Waldman 1992). This cooperative behavior allows the facilitative effects of group living to be shared among relatives, ultimately increasing individual inclusive fitness (Halverson et al. 2006).

However, in most cases, the presence of other tadpoles incurs costs that outweigh the benefits. When tadpole density is high, food availability per capita decreases. Consequently, tadpoles are forced to allocate more time and energy in resource acquisition. Relyea (2002) observed that in *R. sylvatica*, tadpoles increased activity mainly when exposed to intraspecific competitors, while maintaining constant activity when exposed to interspecific competitors. In a previous study (Castellano et al. 2022), we found that *R. latastei* tadpoles increased activity in the presence of conspecifics and even more in the presence of heterospecifics, whereas *R. dalmatina* did not show significant changes in both conditions.

In many species, tadpoles often exhibit also plastic morphological responses to competitors. In *R. sylvatica*, for examples, tadpoles developed proportionally shallower tails and larger bodies when raised at high density. These plastic changes in body and tail shape are in the opposite direction of those induced by predators and have been suggested to be adaptive because of their positive effects on growth rate (Relyea 2002).

In the present study, tadpoles raised with siblings were smaller in both species and they had a proportionally larger body than tadpoles raised alone. However, the effect of density on tail shape differed between the two species. In *R. latastei*, density had no effect on tail shape, whereas in *R. dalmatina*, it had the opposite effect of that of predators, making the tail proportionally smaller. These results suggest that, in *R. dalmatina*, there exists a functional trade-of on tail development. Specifically, tadpoles need to invest resources to develop a large tail. In the presence of predators, a larger amount of resources is invested in tail development. However, when competition for resources increases, tadpoles have less resources to invest in defensive traits and they develop a much smaller tail than when resources are abundant. In contrast, *R. latastei* does not show any evidence for a trade-off on tail development, possibly because of its lower investment in developing defensive traits.

The costs of adaptive plasticity

In species with a complex life-cycle, such as frogs, the growth-development trade-off predicts that an increase in predation risk would force tadpoles to develop faster, to metamorphose earlier and at a smaller size (Werner 1986; Abrams and Rowe 1996). However, these predictions have been rarely confirmed (Benard 2004; Relyea 2007) and our results are in line with this trend. Tadpoles raised with predators metamorphosed at the same age (*R. latastei*) or later (*R. dalmatina*) than their siblings raised without predators. These results suggest that natural selection favored an adaptive trade-off not between life-history traits, but between morphological, behavioral and life-history traits. In fact, in the presence of predators, tadpoles invested more in defense and less in resource acquisition and feeding efficiency. They reduced the time spent feeding, developed relatively small bodies and large tails, and, consequently, grew and developed slowly. Moreover, since in our experiment tadpoles were fed *ad libitum*, food was not a limiting resource per se. This suggests that predators might have not only decreased the rate of resource acquisition, but they might have also forced tadpoles to invest more in maintenance and less in growth and development, as observed in the Atlantic salmon, *Salmo salar* (Millidine et al. 2006).

As the effects of predators on larval behavior and morphology varied between species, the effects on metamorphosis also differed between the two species. In *R. latastei*, predators had weak effects on age and size at metamorphosis and no effects on froglet limb size. In contrast, in *R. dalmatina*, tadpoles raised with predators tended to metamorphose later than those raised without predators, as observed in many other frogs (Laurila et al. 1998; Laurila and Kujasalo 1999; Nicieza 2000). In temperate anurans, a delay in metamorphosis can be costly in terms of fitness because it reduces the time available for feeding and growth before hibernation. Frogs that hibernate at a smaller body size might be less able to withstand the harsh winter temperatures (Alvarez and Nicieza 2002). The trade-off hypothesis of adaptive plasticity suggests that tadpoles payed a post-metamorphic cost, in the form of delayed metamorphosis, to improve their larval survival in response to predators. In *R. dalmatina*, the post-metamorphic costs were higher than in *R. latastei* because tadpoles of this species exhibited a stronger pre-metamorphic adaptive response to predators.

Unlike the plastic behavioral and morphological responses to predators, the plastic responses to competitors are supposed to increase resource acquisition in order to mitigate the negative effects of competition on tadpole growth and/or development (Relyea and Hoverman 2003). As discussed above, *R. latastei* exhibited a stronger behavioral plastic response to competitors than *R. dalmatina*, while the morphological plastic response was similar in both species. Although the effect of competitors on the age at metamorphosis was stronger in *R. dalmatina* than in *R. latastei*, the effects on body, hind-limb and fore-limb size were stronger on *R. latastei*. This suggests that *R. latastei* suffered more from competition than *R. dalmatina*, despite exhibiting a stronger behavioral response to competitors.

In summary, both *R. dalmatina* and *R. latastei* have evolved the ability to plastically modify their behavior, morphology, and larval growth in response to specific predation and competition conditions. These plastic responses are interconnected and reflect a life-history strategy that flexibly allocates resources among defense, growth, and development functions. While the qualitative aspects of their strategies are similar in the two species, there are quantitative differences between them. *R. dalmatina* exhibits higher activity levels and a faster growth and development, compared to *R. latastei*, suggesting that *R. dalmatina* had higher rates of resource acquisition and processing than *R. latastei*. In *R. dalmatina*, competition has a marginal impact on growth and development, whereas predation plays a more significant role. Conversely, in *R. latastei*, the situation is reversed. We hypothesize that these differences arose from the unique selection histories experienced by the two species. *R. dalmatina* may have adapted to more temporary breeding ponds, where competition for food and space is often high, but predation risk is low, because ephemeral habitats are unsuitable for predators that require a stable aquatic environment to complete their development. In contrast, *R. latastei* may have adapted to more permanent breeding ponds, where conditions are consistent and predictable. Since the risk of desiccation is low and nutrient availability highly predictable in these sites, tadpoles may have evolved slow growth and development rates, which exposed them to a lower predation risk. Future comparative studies on a much larger number of species will allow to investigate the association between breeding habitat, tadpole behavior and larval life history.

Declarations

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