Interpopulation differences in developmental plasticity of carnivores determine the emergence of a trophic interaction

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Running head: Genetic variations in opposing plasticity

Abstract

Through its influence on trait expression, phenotypic plasticity can shape variation in ecological interaction strengths across environmental gradients. If species exhibit interpopulation differences in the plasticity of their ecological traits, their genotypes and environmental conditions may jointly determine strength of the ecological interactions. To examine this untested prediction, we experimentally investigated trophic interactions between different geographic populations of predatory and prey amphibians that vary in the plasticity of offensive and defensive morphological traits, respectively. Cannibalism-induced gigantism of *Hynobius retardatus* salamander larvae produces salamanders with wide enough gape to consume *Rana pirica* frog tadpoles—an otherwise inaccessible large prey species that, in turn, possesses an inducible morphological defense. By manipulating combinations of two geographic populations of salamanders and tadpoles and the size distribution—hence, prevalence of cannibalism—of salamander hatchlings, we revealed the interactive effect of salamander population identity and size distribution on tadpole consumption. Early life salamander size heterogeneity resulted in inter-population differences in salamander gigantism. Salamanders from the population with weaker cannibalism-induced gigantism were largely unable to consume tadpoles. However, salamanders from the population exhibiting striking gigantism predated tadpoles from both populations, though tadpole inducible defense was stronger in the geographic population with higher prevalence of salamander gigantism. Our finding suggests that geographic variation in the trait plasticity of a species determine geographic variation of the variability of interaction strength.

Key words: Genotype × Environment interaction; phenotypic plasticity; reaction norm; intraspecific variation; arms race; coevolution

Introduction

The factors determining the strength of ecological interactions are of broad relevance as such interactions determine species demography, and their effects propagate through ecological communities (Nakano et al. 1999, Persson et al. 2007, Ushio et al. 2018). Although interaction strengths depend on the densities of interacting species, they are also influenced by those species' functional traits (Sinclair et al. 2003, Schmitz et al. 2015). Functional traits are phenotypic characteristics that contribute to individual fitness and species niches including their interspecific interactions (McGill et al. 2006, Violle et al. 2007) and that drive ecological processes or characterize species responses to environmental conditions (Suding et al. 2008, Mori et al. 2013, Ross et al. 2017). Since trait expression varies among species, species-specific (i.e. mean) functional trait values have primarily been used when investigating community structure and dynamics (McGill et al. 2006, Schmitz et al. 2015, Ross et al. 2017). Yet, functional traits often vary considerably within species, and such intraspecific trait variation can alter the strength of ecological interactions (Miner et al. 2005, Miller and Rudolf 2011). Intraspecific trait variation is thus increasingly recognized as an important factor in determining community structure and dynamics (Bolnick et al. 2011, Ross et al. 2017, Des Roches et al. 2018, Raffard et al. 2018).

The effects of intraspecific trait variation on predator-prey interactions have been mainly investigated in terms of three interacting elements: genotype, phenotypic plasticity, and size. The

expression of functional traits is under genetic control (Miner et al. 2005, Pigliucci 2005, Winterhalter and Mousseau 2007). Distinct functional trait values between genetically-distinct populations will result in interpopulation differences in ecological interaction strengths (Yoshida et al. 2003, Hiltunen and Becks 2014, Bassar et al. 2017). Some individuals can change their functional traits in response to the presence or absence of predator and prey (i.e. phenotypic plasticity: changes to foraging and defensive behavior or morphology for instance), with consequences for predator-prey interaction strengths (Miner et al. 2005, Winterhalter and Mousseau 2007, Kishida et al. 2014). For example, while some prey species enhance expression of defensive traits in the presence of a specific predator (*i.e.* inducible defense), some predator species can also enhance expression of offensive traits in the presence of particular prey items (*i.e.* inducible offense, Kishida et al. 2011). Since biotic and abiotic conditions influence expression of the functional traits (Kishida et al. 2011), predator-prey interaction strengths can vary due to differential trait expression through time or space. Finally, functional traits also change through ontogeny (i.e. body growth). Because individual performance of both predator and prey generally changes as they grow larger, a species' size distribution is one factor which determines the identities and strengths of predator-prey interactions (Persson et al. 2007, Miller and Rudolf 2011, Yamaguchi et al. 2016). While body size *per se* has received much attention in the context of ecological interactions (Bolnick et al. 2011, Miller and Rudolf 2011), the consequences of developmental plasticity in body size for interaction strengths are not understood.

Numerous studies have evidenced the importance of each of the attributes above (genotype, phenotypic pasticity, and size variation) as causal mechanisms behind variation in predator-prey interaction strengths (Bolnick et al. 2011, Miller and Rudolf 2011, Des Roches et al. 2018).

These concepts have been primarily studied independently, yet are also tightly linked. For morphological traits, in particular, there is clear linkage among size variation, phenotypic plasticity, and genetic variation. Changes in body size (i.e. ordinal isometric growth) or the size of organ or body parts (i.e. allometric growth) are sometimes facultative rather than constitutive (Kishida et al. 2006, Gerber et al. 2008), and plastic responses of morphological traits differ by genotype (Miner et al. 2005, Pigliucci 2005). Hence, the ability to develop morphological traits can genetically differ among geographically distinct populations (Kishida et al. 2007, Winterhalter and Mousseau 2007). Consequently, predator-prey interaction strengths can be determined through an interaction between geographic population (i.e. genotype) and environmental conditions (Yamamichi et al. 2019). Although this integrative view is intuitive, there is little evidence of how predator-prey interaction strengths are affected in nature by genetic variation in the condition-dependent development of predator and/or prey morphological traits (Kasada et al. 2014, Yamamichi et al. 2019). Since both predator and prey jointly shape the sign and strength of their interaction (Hiltunen and Becks 2014, Bassar et al. 2017), it is therefore insufficient to address the functional traits of either party in isolation. That is, when investigating predator-prey interaction strengths, the functional traits of both predator and prey should be considered simultaneously.

Here, we investigate the potential for genetic variation in condition-dependent development of both predator and prey morphology to mechanistically shape predator-prey interaction strengths. To address this objective, we conducted an experiment using a model trophic relationship between predatory larval salamanders (*Hynobius retardatus*) and their frog tadpole prey (*Rana pirica*). This predator-prey interaction is sufficient for such a goal as both predator and prey species exhibit large phenotypic plasticity in their morphological traits which

directly affect their trophic interaction. *H. retardatus* salamander larvae can exhibit gigantism characterized as enlarged gape as an inducible offense (Michimae and Wakahara 2002), while *R. pirica* tadpoles can exhibit body enlargement by thickening epithelium tissue as an inducible defense (Kishida and Nishimura 2004). Importantly, expression of the antagonistic phenotypes also varies among the geographic populations of both species (Michimae 2006), but clear understanding of their trophic interaction across populations is still lacking. We examined whether and how population genetic differences in expression of antagonistic phenotypes of predatory salamander and prey tadpoles affects their trophic interaction by conducting a controlled multifactorial experiment manipulating the identity of both predator and prey population, as well as initial biotic conditions relevant to the emergence of offensive salamander phenotypes.

Materials and Methods

Study System

Hynobius retardatus salamanders and *Rana pirica* frogs usually spawn in small ponds in early spring in Hokkaido, Japan. Although salamander larvae are carnivores, the trophic relationship with tadpoles is not always established even if the larvae of both species cohabit the same ponds. Since tadpoles typically hatch 2–4 weeks earlier than salamanders, tadpoles are usually too large to be consumed by salamander hatchlings (Nosaka et al. 2015). A predator-prey interaction between the two species thus occurs only when salamander larvae grow rapidly. Rapid growth of salamander larvae typically results from cannibalism during their hatchling stage; individuals that successfully consume conspecifics tend to grow rapidly and become

134 ‘giants’ with much larger body and gape width (Takatsu and Kishida 2015). The ratio of
135 salamander gape to tadpole body size is greater for cannibalistic giants than for non-cannibalistic
136 salamanders. The disproportionately large gape (i.e. inducible offensive phenotype) of giant
137 salamanders can facilitate consumption of tadpoles as alternative large prey items by salamander
138 individuals with this offensive phenotype (Takatsu and Kishida 2015). Importantly, the potential
139 to become a giant salamander greatly differs among geographic populations, at least partly due to
140 genetic differences between populations (Michimae 2006, Atsumi K, unpublished data).

141 *R. pirica* tadpoles induce defense in the presence of salamander larvae, enlarging their
142 body and tail by thickening their epithelium tissue. Tadpoles with this ‘bulgy’ phenotype are
143 difficult for predatory salamander larvae to swallow (Kishida and Nishimura 2004). Notably,
144 expression of the defensive bulgy phenotype depends on predation risk; tadpoles exhibit bulgier
145 phenotypes in the presence of giant (offensive phenotype) salamanders compared to non-
146 offensive ones (Takatsu and Kishida 2015, Takatsu et al. 2017). Moreover, previous work in this
147 system demonstrated that tadpoles’ potential to express the defensive phenotype genetically
148 differs between geographic populations (Kishida et al. 2007). Thus, larvae of the two amphibian
149 species exhibit antagonistic morphological plasticity—i.e. condition-dependent allometric
150 growth in functional traits (gape width and body size of salamander larvae and body size of frog
151 tadpoles)—that characterizes both the presence and strength of their trophic interactions (Kishida
152 and Nishimura 2004, Takatsu et al. 2017), but the expression of the predator’s offensive
153 phenotype and the prey’s defensive phenotype differ between geographic populations.

154 Geographic variation in the genotypes needed to express the offensive salamander and
155 defensive tadpole phenotypes afford us an opportunity to examine whether genetic differences in
156 the expression of antagonistic phenotypes of predatory salamander and prey tadpoles influence

157 their trophic interaction. In particular, geographically distinct populations vary in their relative
158 frequency of giant salamanders (e.g., Michimae 2006). Based on our knowledge of geographic
159 variation in salamander inducible offense, we selected two localities of amphibians as
160 experimental model populations: Erimo and Chitose. Giant salamanders are more common in the
161 Erimo population (hereafter Erimo salamanders) (Michimae 2006; Atsumi and Kishida,
162 unpublished data) than in the Chitose population (hereafter Chitose salamanders). We had no
163 prior knowledge about the geographic variation in tadpole defensive phenotype expression
164 between these two geographic populations, though using other populations, Kishida *et al.* (2007)
165 revealed geographic variation in the genotypes for defensive phenotype expression of the frog
166 species. Accordingly, we expect that predator-prey interaction strengths between salamanders
167 and tadpoles are higher (more predation) when the Erimo salamanders are under heterogeneous
168 size distributions during their hatchling period, facilitating cannibalism of smaller individuals by
169 salamanders, resulting in salamander gigantism (Takatsu and Kishida 2015).
170
171 *Experimental Setting*
172 We collected eggs of both species from several ponds located in the Erimo (seven ponds around
173 42°6’ N; 143°16’E) and Chitose (three ponds around 42°48’ N; 141°35’ E) regions in mid- to
174 late-April 2018. From each region, we collected 50 salamander egg clusters and 10 tadpole egg
175 masses. We mimicked natural hatching timing so that frog tadpoles hatched 2–3 weeks earlier
176 than salamanders (Nosaka et al. 2015). We kept frog egg masses in a separate 22 L semi-
177 transparent polypropylene tank (51.3 × 37.2 × 16.6 cm high) filled with 5 L of aged tap water,
178 and the tanks were placed in an indoor facility which was maintained at 15 °C on a natural light-
179 dark (14h/10h) regime. Tadpole eggs hatched in late April (Chitose: April 23–27, Erimo: April

25–27). For each tadpole population, we mixed all tadpoles after they hatched, and reassigned 100 individuals to each 22 L tanks. We cultured tadpoles under the conditions described above for two weeks prior to the start of the experiment by putting eight pieces of rabbit chow (dry weight: 1.6 g) into each tank as food and changing the water every 2 days. Each salamander egg cluster was placed separately in a draining net which we put into 4 L semi-transparent polypropylene tanks (33.4 × 20 × 10 cm high; 5 nets per tank) filled with 3 L of aged tap water. We then placed the salamander tanks in a refrigerator and maintained at 3 °C under natural light-dark conditions.

The experiment was conducted in an experimental room in Tomakomai Experimental Forest, using semi-transparent polypropylene 22-L tanks as above. We covered the bottom of each tank with 2 cm of sand as benthic substrate, and provided two leaves of Japanese Bigleaf Magnolia (*Magnolia obovate,* dry weight: 5 g) as biotic structures. Minimum natural water (ca. 10 ml per minute), drawn from a well ~5m from a natural river, was constantly supplied into each tank using thin polypropylene hoses and water flowed out of an overflow pipe (φ = 20mm, 4cm height) inside each tank. To prevent the experimental animals leaving the system, we covered the overflow pipe with mesh net (mesh size 1mm). Overflow pipes kept water depth at 4 cm (from sandy bottom to water surface). The experimental room was maintained under natural light-dark (ca. 14h/10h) conditions and water temperature ranged from 13 to 20 ℃.

Experimental design

To examine how genetic variation in expression of antagonistic phenotypes of predatory salamander and prey tadpoles shape their trophic interactions, we conducted a three-way factorial experiment with 8 treatment combinations. We manipulated combinations of the two

geographic populations of the predators (Erimo versus Chitose salamanders) and prey (Erimo versus Chitose tadpoles) and salamander size distribution (size homogenous versus heterogeneous), since we expect *a priori* that the offensive phenotype should occur only when salamander hatchlings are able to cannibalize smaller individuals through larger size heterogeneity (Kishida et al. 2015). Each treatment was replicated ten times.

We put 30 tadpoles and 15 salamanders in each tank. We haphazardly placed three-week-old tadpoles into each of our 80 tanks on 18 May 2018 (day 1, see Fig. 1). Tadpoles originated either all from Erimo (mean±SD snout-vent length, 7.32±0.60 mm, $N = 20$) or all from Chitose (7.10±0.53 mm, $N = 20$). Following well-established methods (Takatsu and Kishida 2015, Takatsu et al. 2017), we manipulated size structure of salamander hatchlings by using individuals that hatched at different times (early- and late-hatchlings, placed into tanks at day 1 and 8, respectively) while keeping the total initial salamander density constant across treatments ($N = 15$). Briefly, we obtained early- and late-hatchlings by controlling the water temperature experienced by embryos; late-hatchlings resulted from longer exposure to low water temperature during the embryonic stages compared to early-hatchlings (see Takatsu and Kishida 2015). We produced the following size distribution treatment levels: a size heterogeneity treatment which included 5 early- and 10 late- salamander hatchlings and a homogeneity treatment with either 15 early- or 15 late- salamander hatchlings (Fig. 1). Our conditions for hatch timing and density of the two amphibian species were within the range found in natural habitats (Michimae 2006; Nosaka et al. 2015). Although the size homogeneous treatment level included two conditions (early or late hatchlings)—each condition was replicated 5 times for each combination of the two geographic populations of salamanders and tadpoles—we pooled all data within this treatment level because preliminary analyses revealed no difference between the two conditions in

morphology or mortality of either tadpoles or salamanders (Appendix S1). In all cases, salamanders within each tank were siblings to standardize genetic variance among treatments.

Previous studies repeatedly report negligible tadpole mortality in the absence of predatory salamanders in similar experimental settings (e.g., Takatsu and Kishida 2015, 2020). Thus, to avoid excessive use of animals, we did not include a tadpole-only treatment to estimate background mortality. Throughout the experiment, we added a piece of rabbit chow (dry weight: 0.2 g) and 100 frozen Chironomid larvae to each tank three times per week as alternative food for tadpoles and salamanders, respectively. We omitted 1 replicate for Erimo salamander–Chitose tadpole–size heterogenous and 2 for Chitose salamander–Chitose tadpole–size homogenous treatments in the all analyses because of a counting error.

At day 11, 18, 25 and 32, we counted all surviving tadpoles and salamanders. From the count data, we calculated cumulative salamander mortality by day 18 (just before predation by salamanders on tadpoles began) and cumulative tadpole mortality at the end of experiment (day 32)**.** On day 18, we also photographed the dorsal side of surviving animals using a digital camera (Panasonic Lumix DC-TZ90). The experiment ceased on day 32 as metamorphosis of tadpoles began in several tanks.

Phenotyping

To dissect mechanistic details of the trophic interactions, we assessed morphological traits of salamanders and tadpoles. From dorsal side photographs of the surviving amphibians at day 18, we measured salamander trunk (heart-vent) length—which controls for the disproportionate scaling of offensive salamander heads—and gape width, as well as maximum body width and snout-vent length of tadpoles (Kishida and Nishimura 2004, Kishida et al. 2014), using Image J

249 software (Schneider et al. 2012). For tadpoles, we measured as many individuals as possible, but
250 we could not assess morphology in 11 tanks because tadpoles were moving or inclining (Table
251 S1). We also focused on the morphology of the salamander with the largest body length in each
252 tank, by visually selecting and measuring the four largest salamanders per tank and then
253 excluding data on all but the largest individual, because typically only one salamander per tank
254 expresses the offensive giant phenotype if cannibalism occurs (Kishida et al. 2011, Takatsu and
255 Kishida 2015). We measured trunk length of salamanders and snout-vent length of tadpoles as
256 proxies for body size (Kishida et al. 2015). Finally, we calculated relative gape width of
257 salamanders (gape width / trunk length) and relative body width of tadpoles (maximum body
258 width / snout-vent length per tadpole) as measures of the salamander offensive phenotype and
259 the tadpole defensive phenotype, respectively (Takatsu and Kishida 2013).
260 We measured these morphological variables at day 18—prior to the start of tadpole
261 predation by salamanders with the offensive phenotype—as this allows us to infer the phenotypic
262 mechanisms underlying any observed salamander-tadpole trophic interactions. We calculated
263 mean values of the tadpole variables in each tank for use in statistical analyses (6-10
264 tanks/treatment, see Table S1), whereas to analyze treatment effects on salamander phenotype,
265 we used only the data from the largest salamander per tank. Moreover, we used the above
266 morphological data to calculate the number of *potential predators* per tank, defined as the
267 number of salamanders whose gape width exceeded mean tadpole body width. This count data
268 allows us to test whether the size balance between salamander gape and tadpole body affects the
269 propensity for salamanders to consume tadpoles (Takatsu and Kishida 2015).
270
271 *Statistical analysis*

To check for treatment effects on tadpole and salamander morphological traits, we first conducted three-way ANOVA after confirming that morphology data met the assumptions of normality and homogeneity of variances. In each case, we used tadpole population (Tad_{pop}), salamander population (Sal_{pop}), salamander size distribution ($Size_{sal}$), and their three-way interaction as predictor variables. The following response variables were modelled separately: tadpole mean body width, mean body size (snout-vent length), and mean relative body width, and salamander gape width, body size (trunk length), and relative gape width of the largest (by trunk length) salamander per tank. Where ANOVA results revealed significant treatment effects, we conducted a Tukey HSD post hoc test to examine how variables differ among treatment levels.

We next asked whether tadpole population, salamander population, and salamander size distribution affected the strength of the predator-prey relationship within and between species. Specifically, we tested for the effect of a three-way interaction between size distribution and both salamander and tadpole populations. We first tested whether salamander mortality (by cannibalism) differed among treatments. A score test for zero inflation (van den Broek 1995) revealed that our salamander mortality data was significantly zero-inflated (Score = 202.5, $p < 0.001$). To test for treatment effects on salamander mortality prior to tadpole predation, we then used a Scheirer–Ray–Hare (SRH) test on salamander mortality at day 18. The SRH test is a nonparametric equivalent of multifactorial analysis of variance (ANOVA) that extends the rank-based Kruskal-Wallis test to allow consideration of more than one factor (and potential interactions) as predictor variables (Scheirer et al. 1976). The SRH test is a conservative estimate of among group differences—the test strength is considerably lower than the equivalent parametric ANOVA (Dytham 2003)—so observed differences are likely to be true effects (i.e.

low Type I error rate at the expense of increased Type II error). Where we identified significant terms, we made pairwise post hoc comparisons of treatment levels using a Tukey HSD test on the ranked data from our SRH test (Tukey 1949).

We also conducted the above analyses (multifactorial comparisons and post hoc Tukey HSD on significant terms) to determine whether treatment groups differed in their tadpole mortality values. Tadpole mortality was zero-inflated (Score = 64.8, $P < 0.001$), so we again used the SRH test which is robust to the underlying data distribution. We modelled cumulative tadpole mortality at the end of experiment against tadpole population, salamander population, salamander size distribution, and their pairwise and three-way interactions. All analyses were conducted in R (ver 4.0.2) using packages *MASS* (ver 7.3-51.6; Venables and Ripley 2002) and *Stats* (R Core Team 2020).

Results

Salamander and tadpole morphology

Our ANOVA test of treatment effects on the morphology of the largest salamander per tank revealed that the offensive giant salamander phenotype was most strongly expressed in the Erimo salamander population when hatchlings grew under a heterogeneous size distribution (Fig. 2A–C). While trunk length was larger in Erimo salamanders irrespective of initial size distribution (ANOVA: Sal_{pop} $F = 53.77$, $P < 0.001$; Fig. 2B), the offensive phenotype expression—large gape relative to trunk length—and gape width was determined by salamander population, initial size distribution and their interaction (Gape width: Sal_{pop} $F = 135.68$, $P < 0.001$; $Size_{sal}$ $F = 89.6$, $P < 0.001$, $Sal_{pop} \times Size_{sal}$ $F = 24.43$, $P < 0.001$; Fig. 2A. Relative gape

width: Sal_{pop} $F = 47.19$, $P < 0.001$; $Size_{sal}$ $F = 71.17$, $P < 0.001$; $Sal_{pop} \times Size_{sal}$ $F = 10.68$, $P < 0.001$; Fig. 2C). A post hoc Tukey HSD test on the significant $Sal_{pop} \times Size_{sal}$ interaction revealed that Erimo salamanders under the size heterogeneous treatment had larger relative gape widths than Erimo salamanders reared under a homogenous size distribution (Tukey HSD: $P_{adj} < 0.001$) and than Chitose salamanders under the size heterogeneous treatment ($P_{adj} < 0.001$; Fig. 2C). This was also true for salamander gape width (Tukey HSD $P_{adj} < 0.001$ in both cases). Tadpole population did not affect salamander morphology (ANOVA: $P > 0.05$).

Three-way ANOVA on tank-averaged tadpole phenotypes revealed that Erimo tadpoles had a larger body than Chitose tadpoles (Tad_{pop}: body width $F = 28.24$, $P < 0.001$; body length, $F = 20.76$, $P < 0.001$; Fig. 2D,E). These interpopulation difference in body length and width were greater in the presence of Erimo salamanders than Chitose salamanders ($Sal_{pop} \times Tad_{pop}$: body width $F = 10.08$, $P = 0.002$; body length $F = 5.76$, $P = 0.021$) and tended to be greater under heterogeneous salamander size distributions ($Tad_{pop.} \times Size_{sal}$: body width $F = 8.43$, $P = 0.005$; body length $F = 3.26$, $P = 0.076$). Indeed, Erimo tadpoles exposed to Erimo salamanders grown under cannibalistic conditions had the widest bodies among all treatments (pairwise Tukey HSD comparisons: $P_{adj} < 0.036$), while the remaining treatments did not differ in body width (pairwise Tukey HSD comparisons: $P_{adj} > 0.05$) (Fig. 2D). However, a post hoc Tukey HSD test revealed body length was similar across treatments (Fig. 3E). The discordance between body width and size arose because tadpoles expressed the more defensive ‘bulgy’ phenotype (greater body width:length ratio) when faced with Erimo salamanders than with Chitose salamanders (ANOVA Sal_{pop}: $F = 11.78$, $P = 0.001$; Fig. 3F). There was also a tendency for Erimo tadpoles to express the defensive phenotype more strongly in the presence of Erimo salamanders reared under a heterogeneous size distribution than a homogeneous one (i.e. a three-way $Tad_{pop.} \times Sal_{pop} \times$

341 $Size_{sal}$ interaction; Fig. 3F), though this interaction only approached statistical significance (F =
342 3.04, P = 0.086).

343 At day 18, just before tadpole predation began, *potential predators* (salamanders with gape
344 width larger than mean tadpole body width; Nosaka et al. 2015) appeared only in the Erimo
345 salamander treatment under heterogeneous size distribution (for Chitose and Erimo tadpoles,
346 present in 6/8 and 4/10 tanks [1.38 and 0.5 potential predator/tank on average], respectively).
347
348 *Salamander mortality*
349 A Sheirer-Ray-Hare test of among-group differences in salamander mortality at day 18
350 found a significant effect of initial salamander size distribution and its interaction with
351 salamander population (Fig. 3A), though salamander population alone was marginally
352 nonsignificant (SRH = 3.28, P = 0.07). Salamander mortality was significantly higher in the
353 heterogeneous salamander size distribution treatment than the homogeneous treatment ($Size_{sal}$:
354 SRH = 35.1, $P < 0.001$). A post hoc Tukey HSD test on the significant $Sal_{pop} \times Size_{sal}$ interaction
355 (SRH = 8.3, P = 0.004) revealed that salamander mortality was higher under the heterogenous
356 than the homogeneous size distribution conditions for both the Erimo salamander treatment
357 (Tukey HSD: $P_{adj} < 0.001$) and the Chitose treatment (P_{adj} = 0.007; Fig. 3A). Moreover, Erimo
358 salamanders experienced higher mortality than Chitose salamanders under heterogenous size
359 distribution treatments (Tukey HSD: $P_{adj} < 0.001$), while there was no difference in mortality
360 between salamander populations under the homogeneous size distribution (P_{adj} = 0.62; Fig. 3A).
361
362 *Tadpole mortality*

Our Sheirer-Ray-Hare test of among-group differences revealed significant effects of salamander population, salamander size distribution, and their interaction, on tadpole mortality (Fig. 3B). Tadpole mortality was significantly higher in the heterogeneous salamander size distribution treatment than the homogeneous treatment ($Size_{sal}$: SRH = 14.1, $P < 0.001$), and was higher in the Erimo salamander treatment than the Chitose treatment (Sal_{pop}: SRH = 24.8, $P < 0.001$). A post hoc Tukey HSD test on the significant $Sal_{pop} \times Size_{sal}$ interaction (SRH = 8.42, $P = 0.004$) identified significantly higher tadpole mortality under the heterogeneous than the homogeneous salamander size distribution treatment for the Erimo salamander population treatment (Tukey HSD: $P_{adj} < 0.001$), but there was no difference between size distribution treatment levels for the Chitose salamander population ($P_{adj} = 0.69$; Fig. 3B). We also found that for the heterogeneous salamander size distribution treatment, tadpole mortality was significantly higher under Erimo than Chitose salamanders (Tukey HSD: $P_{adj} < 0.001$), but there was no difference between Erimo and Chitose salamander treatments under homogeneous salamander size distribution ($P_{adj} = 0.12$; Fig. 3B). We did not find statistical significance for the main and interactive effects of tadpole populations on the tadpole mortality.

Discussion

Although there is increasing recognition of genetic variation in the conditional development of functional traits (Pigliucci 2005, Winterhalter and Mousseau 2007), little is known about how genetic differences in development of predator and prey functional traits influence their trophic interaction strengths. Through an experimental study using predatory *Hynobius retardatus* salamander and prey *Rana pirica* frog tadpoles from two geographic

386 regions (Eriomo and Chitose), we found that population genetic differences in the inducible
387 offense of predatory salamanders shapes predator-prey interaction strengths. Our experiment
388 revealed that tadpole survival was significantly reduced during their larval stage only when they
389 were exposed to one population (Erimo), and hence genotype, of predatory salamanders under a
390 particular environmental context (heterogeneity in initial predator size distribution). That is,
391 predator-prey interaction strengths between the two amphibian species were jointly determined
392 by salamander genotype and environmental conditions (Fig. 3B). Further, we showed that this
393 interactive effect on salamander predation was explained by the phenotypic differentiation of the
394 salamanders among treatments. Due to cannibalism during the early life stages of salamanders
395 (Fig. 3A), the largest Erimo salamanders in the size heterogeneous treatment attained a far wider
396 gape and larger body than those in the other treatments before the onset of the salamander-
397 tadpole trophic interaction (Fig. 1A). Consequently, the induced offensive phenotype of Erimo
398 salamanders in the size heterogeneous treatment successfully consumed frog tadpoles that would
399 otherwise be, due to size constraints, unviable prey items. The causal relationship between
400 salamander phenotype and interaction strength (predation) is also evidenced by the exclusive
401 appearance of potential predators in the Erimo salamander population only under initial size
402 heterogeneity. We thus revealed the potential of genetic variation in predator inducible offense to
403 mechanistically shape predator-prey interaction strengths.

404 The antagonistic nature of predator-prey interactions produces the intuitive expectation that
405 genotypes of both predators and prey influence interaction strengths (Hiltunen and Becks 2014,
406 Bassar et al. 2017). However, we detected significant population effects on the interaction
407 strength only in for predatory salamanders (Fig. 3A). Though differential expression of defensive
408 bulgy phenotypes of Erimo and Chitose tadpoles resulted in a significant difference between

409 tadpole populations in their body width under high predation risk (i.e. Erimo salamanders and
410 initial salamander size heterogeneity; Fig. 2D), this did not translate into variation in tadpole
411 mortality. Asymmetry between predator and prey population effects on interaction strengths may
412 result from differences in the timing of inducible offense and defence. While salamanders
413 express offensive phenotype before predating tadpoles, tadpoles exhibit inducible defence in
414 response to the emergence of giant salamanders (Takatsu and Kishida 2015, Takatsu et al. 2017).
415 Due to this time lag between the trait expression of potential prey and their would-be predators,
416 population-level differences in offensive trait expression by predatory salamanders should more
417 strongly influence interaction strengths than population differences in tadpoles inducible
418 defence.

419 We argue that population-specific morphological plasticity of predators underlies the
420 observed salamander population-by-size distribution (G×E) interaction in our model trophic
421 interaction. Although population-specific behavioural plasticity (e.g. development of
422 aggressiveness) can offer an alternative explanation (Bell and Stamps 2004), its importance is
423 negligible here since densities of experimental animals are relatively high. In such a context, our
424 salamanders and tadpoles should frequently encounter each other, providing ample opportunity
425 for salamander predation. As such, predation success should exclusively depend on the balance
426 between salamander gape and tadpole body size, since *H. retardatus* is a representative
427 swallowing-type predator and *R. pirica* tadpoles are their largest prey items (Takatsu and Kishida
428 2013).

429 Our findings suggest that interaction strengths vary across species' geographic ranges.
430 When comparing predation between naturally co-occurring salamanders and tadpoles, the Erimo
431 pair (Erimo salamanders and Erimo tadpoles) differed in predator-prey interaction strengths

between the size heterogeneous and homogeneous salamander treatments, while the Chitose pair did not (Fig. 3B). That is, while the Erimo and Chitose pairs showed similarly weak interactions in the absence of salamander cannibalism (i.e. under homogeneous size distribution), the Erimo pair exhibited stronger predator-prey interactions than the Chitose pair where early-life cannibalism resulted in salamander gigantism (Fig. 3B). As one of the factors involving salamander cannibalism, our study featured salamander size distributions resulting from hatch timing variation of salamander hatchlings, which should vary among ponds within geographic regions (Nosaka et al. 2015). Likewise, other prospective factors affecting cannibalism (e.g. conspecific density, alternative prey abundance, habitat complexity, and predation pressure: Fox 1975, Polis 2003, Kishida et al. 2011) are generally spatially heterogeneous within geographic regions. If within-region spatial heterogeneity in such factors is equivalent across regions, interaction strengths should be more variable in Erimo (where there is potential for strong predator-prey interactions) than in the Chitose region (potential only for weaker interactions) for these species. Interaction strength variability across ponds may further impact pond communities due to the large biomass of the two amphibian species (Kishida and Nishimura 2006, Michimae 2011); predation by salamanders on tadpoles can shift the densities and trait distributions of both species, with knock-on consequences for pond communities (Petranka and Kennedy 1999). Hence, population-specific condition-dependent expressions in functional traits (i.e. reaction norms) of salamanders can create regional variation in the heterogeneity of their trophic interaction, with the potential to further shape regional variation in β-diversity of natural pond communities.

Condition-dependent development of morphological traits as a reaction norm can be the target of natural selection (Urban 2008, 2010). Our finding of geographic variation in the

455 inducible offense of predators and inducible defence of prey may be due to differences in the
456 coevolutionary history of our model species. While larger body size is necessary for tadpoles to
457 avoid predation by giant salamanders, much larger gape is required for salamanders to consume
458 tadpoles expressing the ‘bulgy’ defensive phenotype (Takatsu and Kishida 2015, Takatsu et al.
459 2017). Although we only examined two geographic populations, the inter-population variation in
460 predator inducible offense and prey inducible defence suggests an arms race scenario; Erimo
461 salamanders more frequently expressed the offensive phenotype than Chitose salamanders, and
462 Erimo tadpoles grew more rapidly and more commonly expressed the defensive phenotype than
463 Chitose tadpoles. This phenotypic pattern implies a geographic mosaic of coevolution
464 (Thompson 1999, Thompson and Cunningham 2002), with Erimo as a coevolutionary hotspot (a
465 region where coevolution is escalated). In this scenario where evolutionary enhancement of
466 antagonistic phenotype expression imposes stronger selective pressure on the opponent,
467 ecological interactions should be strongest in coevolutionary hotspots. Describing geographic
468 patterns of antagonistic phenotypic plasticity as we have here and testing coevolutionary
469 hypotheses should therefore advance our understanding of the mechanisms underpinning
470 interspecific interaction strengths.

471 Our study suggests that genetic variation in expression of offensive phenotypes of
472 predators shapes variation in predation pressure within and across regions. Under different
473 selection regimes, populations diverge their reaction norms for their functional traits (e.g. plastic
474 versus fixed phenotypes) along environmental gradients (Kishida et al. 2007, Winterhalter and
475 Mousseau 2007). Investigating geographic variation in reaction norms of functional traits for
476 interacting species and the consequences of pairwise combinations of such developmental

reaction norms shows promise in disentangling complex geographic mosaics of ecological interactions around the globe.

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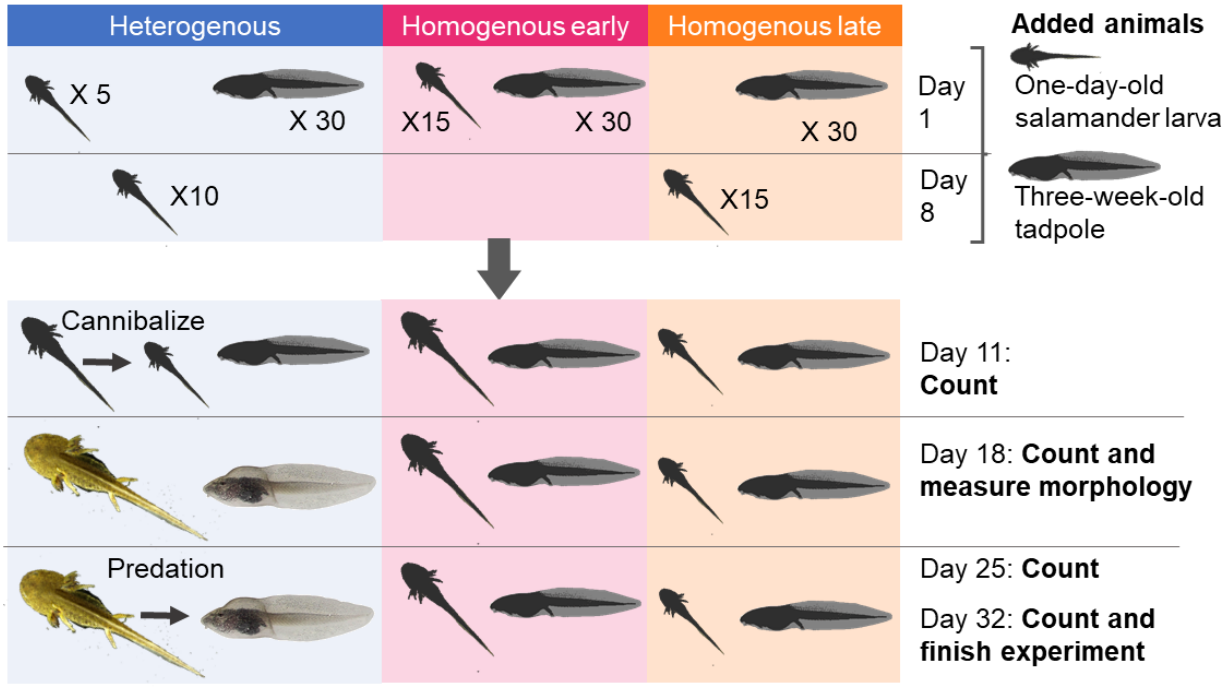
617

618 **FIGURE LEGENDS**

619



620

621 Fig. 1. Timeline of our experiment. We illustrate a typical scenario for salamander cannibalism,
622 followed by salamander gigantism with offensive phenotype, tadpole predation and tadpole
623 induced defense.

624

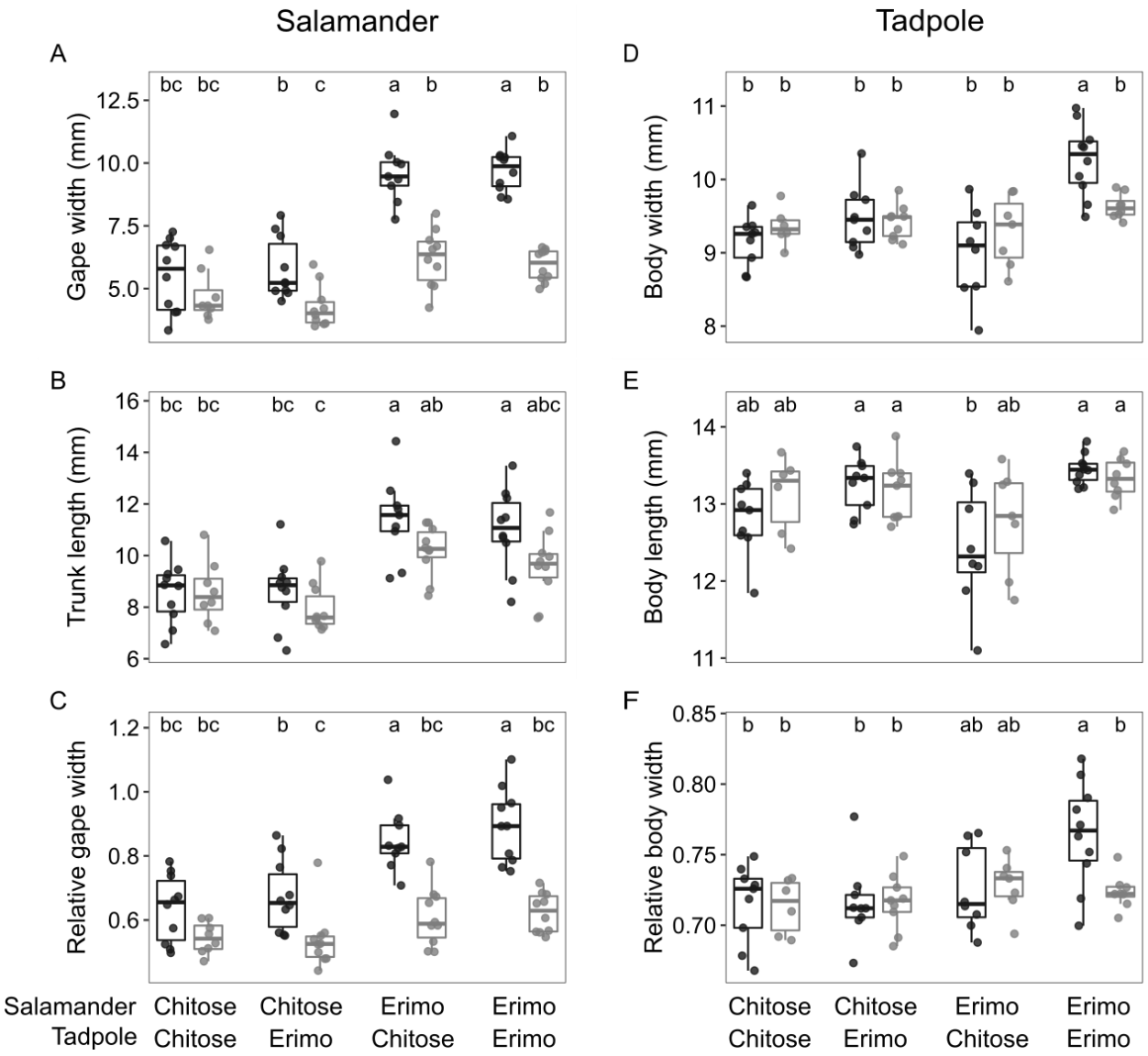


Fig. 2. Treatment effects on salamander and tadpole morphology (functional traits). Salamander gape width (A), trunk length (B), and relative gape width (C), and tadpole body width (D), body length (E), and relative body width (F) for each combination of geographic populations (Chitose and Erimo) and initial salamander size distribution treatments (heterogeneous size distribution [to facilitate salamander cannibalism] = black, homogeneous [to suppress cannibalism] = grey). Boxplots represent the median and interquartile range of treatment groups, with points showing

individual tanks. The largest salamander in each tank and all measurable tadpoles were measured at day18, prior to the start of predation (see Materials and Methods).

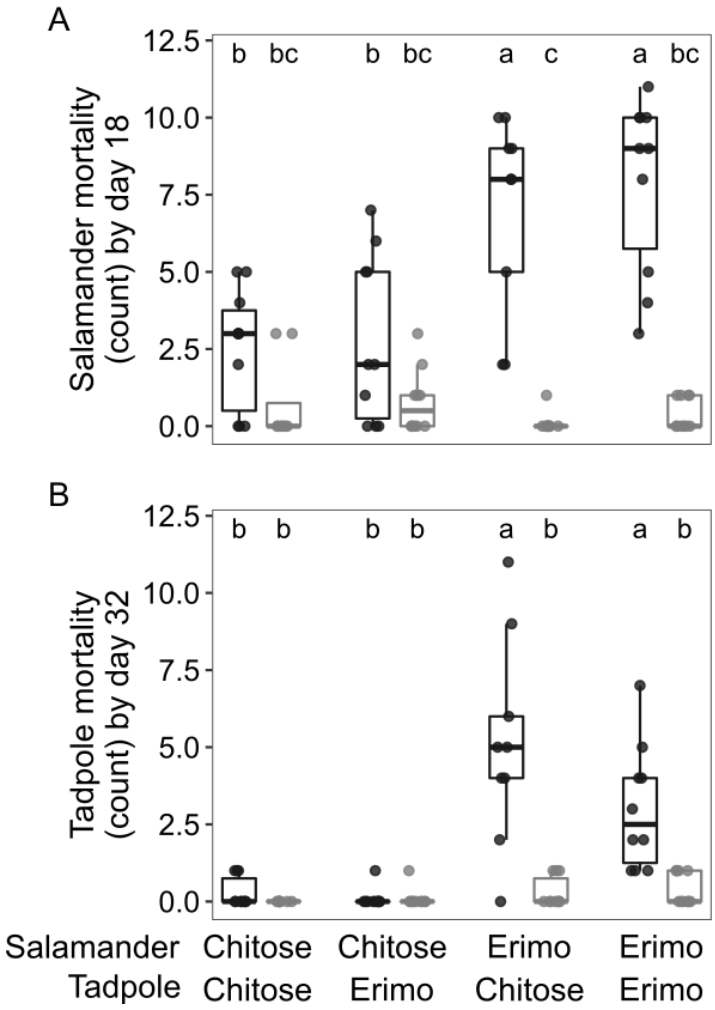


Fig. 3. Mortality (count) of predatory salamanders by day 18 (A), prior to the onset of predation, and prey tadpoles by the end of experiment (B), for each combination of geographic populations (Chitose and Erimo) and initial salamander size distribution treatments (heterogeneous size distribution [to facilitate salamander cannibalism] = black, homogeneous [to suppress cannibalism] = grey). Boxplots represent the median and interquartile range of treatment groups,

641 with points showing individual tanks. Salamander and tadpole mortality largely reflect
642 cannibalism and tadpole consumption by salamanders, respectively.
643

644 **Appendix S1.**

645

646 By manipulating salamander hatch timing, we established an initial size heterogeneity treatment
647 and two initial size homogenous sub-treatments differing in the hatch timing (early and late) at
648 the beginning of the experiment. We expected that salamander and tadpole mortality did not
649 differ between the size homogenous sub-treatments within salamander–tadpole population pairs
650 because salamander cannibalism should be rare and thus the offensive giant salamanders should
651 also rarely emerge in those treatments. As salamanders in size homogenous treatments are
652 expected to be harmless for tadpoles, we also anticipated that tadpole morphology did not differ
653 between the size homogenous sub-treatments in each salamander–tadpole population pair (i.e.
654 following four pairs are our interest: Chitose salamander– Chitose tadpole–early hatch vs. –late
655 hatch; Chitose salamander– Erimo tadpole–early hatch vs. –late hatch; Erimo salamander–
656 Chitose tadpole–early hatch vs. –late hatch; Erimo salamander–Erimo tadpole–early hatch vs. –
657 late hatch). If these expectations—mortality and morphology of both species did not differ
658 between sub-treatments within salamander–tadpole populations pair—are satisfied, we can pool
659 data of the sub-treatments within each salamander-tadpole populations pair.
660 We employed a one-way ANOVA test or Scheirer–Ray–Hare (SRH) test followed by
661 Tukey HSD test to compare the sub-treatments across all population pairs. We used the same
662 data as for the main analyses: salamander mortality by day 18; tadpole mortality by day 32; and
663 morphology of both species at day 18. While we used an ANOVA for morphological data, we
664 used a SRH test for the mortality data because the mortality of both species were highly zero-
665 inflated (see Materials and Methods, *Statistical analyses*).
666 In the mortality of both species, SRH detected significant heterogeneity across treatments
667 (salamander, $SRH = 49.40$, $P < 0.001$; tadpole, $SRH = 49.61$, $P < 0.001$). However, the post-hoc
668 Tukey HSD test did not detect any differences between the sub-treatments (Fig. S1A and S1B for
669 salamander and tadpole, respectively). ANOVA detected significant heterogeneity across
670 treatments in salamander morphology (gape width, $F = 28.19$, $P < 0.001$; trunk length, $F = 8.03$,
671 $P < 0.001$; relative gape width, $F = 13.01$, $P < 0.001$) and tadpole morphology (body width, $F =$
672 5.38, $P < 0.001$; body length, $F = 4.34$, $P < 0.001$; relative body width, $F = 2.82$, $P = 0.006$). As
673 with mortality, however, the post-hoc Tukey HSD test did not detect any morphological
674 differences between the sub-treatments in each population pair (salamander gape width, Fig.

675 S2A; salamander trunk length, Fig. S2B; salamander relative gape width, Fig. S2C; tadpole body
676 width, Fig. S3A; tadpole body length, Fig. S3B; tadpole relative body width, Fig. S3C).
677 Overall, the mortality and morphology of both species did not differ between the two
678 alternative hatch timing conditions within each population pair. We therefore pooled the data of
679 the two initial size homogenous sub-treatments.
680

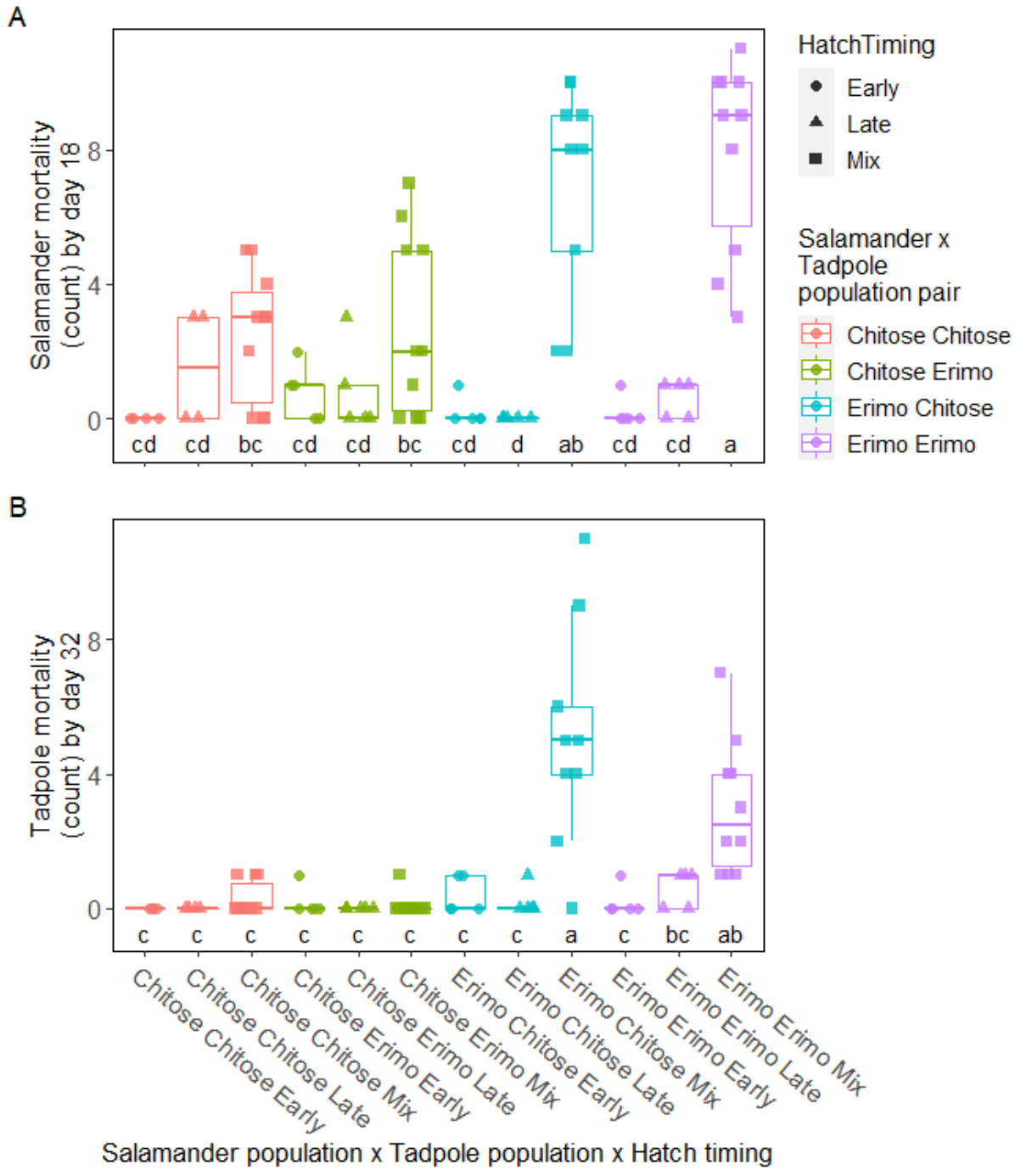
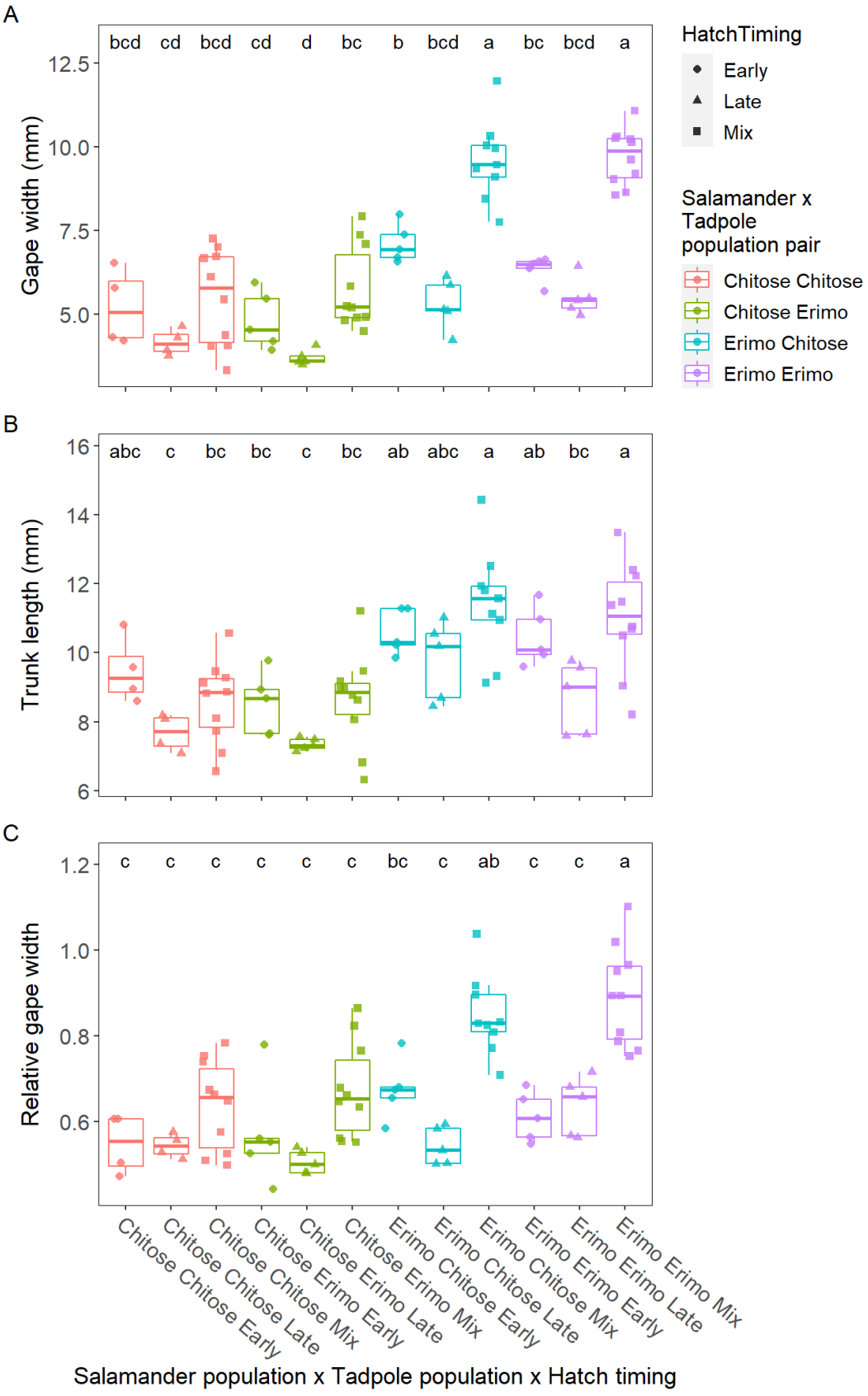
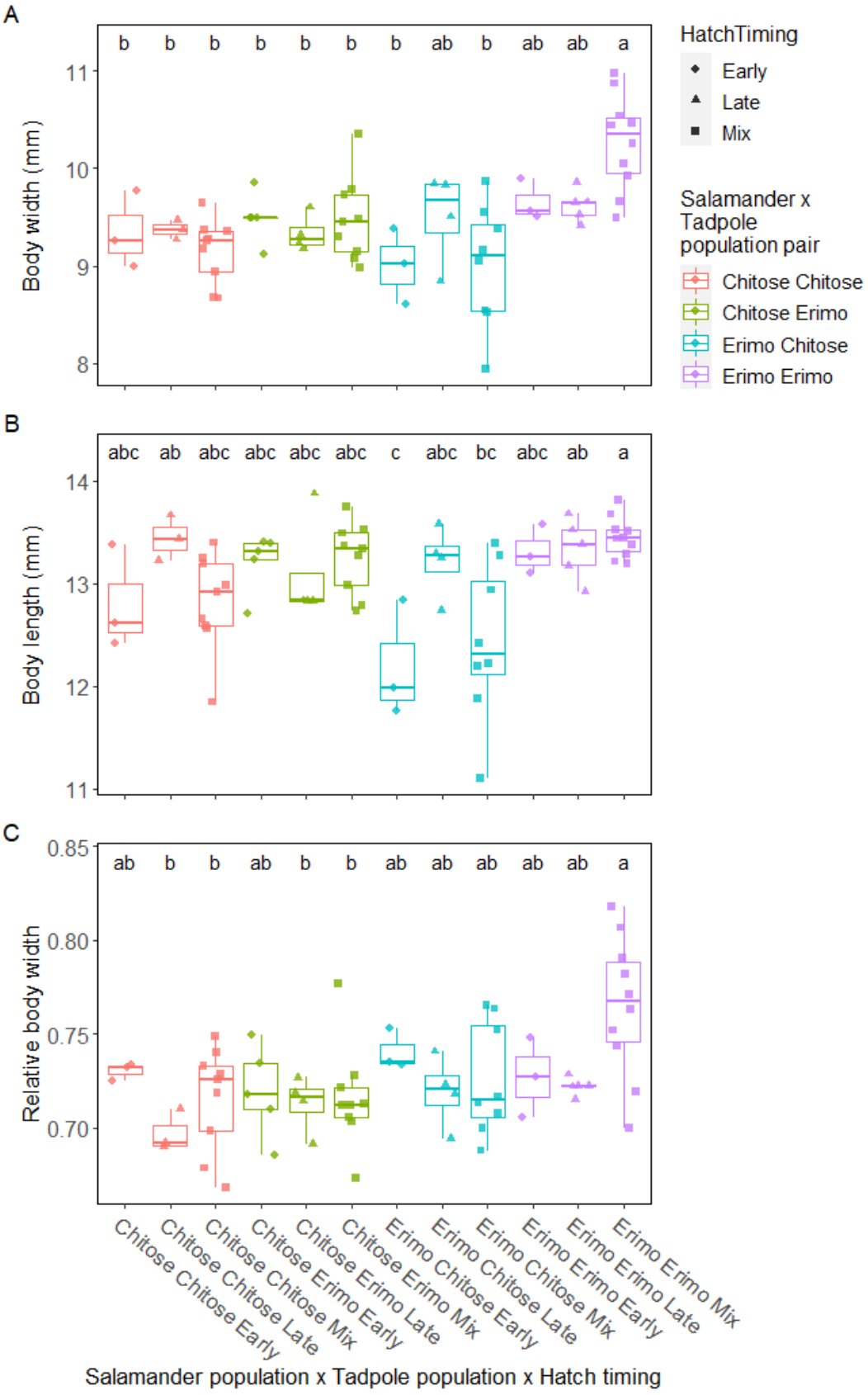


Fig. S1 Salamander mortality by day 18 (**A**) and tadpole mortality by day 32 (**B**), shown by boxplot with raw data points. "Early", "Late", and "Mix" hatch timing indicates initial size homogenous treatments with early hatch timing and late hatch timing, and initial size heterogenous treatment, respectively. Treatments sharing the same alphabet letter are not significantly different (Tukey's HSD for ranked variable).


A
bcd cd bcd cd d bc b bcd a bc bcd a
Gape width (mm)
12.5
10.0
7.5
5.0
B
abc c bc bc c bc ab abc a ab bc a
Trunk length (mm)
16
14
12
10
8
6
C
c c c c c c bc c ab c c a
Relative gape width
1.2
1.0
0.8
0.6
Chitose Chitose Early
Chitose Chitose Late
Chitose Chitose Mix
Chitose Erimo Early
Chitose Erimo Late
Chitose Erimo Mix
Erimo Chitose Early
Erimo Chitose Late
Erimo Chitose Mix
Erimo Erimo Early
Erimo Erimo Late
Erimo Erimo Mix
Salamander population x Tadpole population x Hatch timing
HatchTiming
Early
Late
Mix
Salamander x Tadpole population pair
Chitose Chitose
Chitose Erimo
Erimo Chitose
Erimo Erimo

687

688 **Fig. S1** Salamander morphology (**A**, gape width; **B**, trunk length; **C**, relative gape width) at day
689 18, shown by boxplot with raw data points. See Fig. S1 for hatch timing. Treatments sharing the
690 same alphabet letter are not significantly different (Tukey's HSD).
691


A
B
C
Body width (mm)
Body length (mm)
Relative body width
b b b b b b b ab b ab ab a
abc ab abc abc abc abc c abc bc abc ab a
ab b b ab b b ab ab ab ab ab a
HatchTiming
Early
Late
Mix
Salamander x Tadpole population pair
Chitose Chitose
Chitose Erimo
Erimo Chitose
Erimo Erimo
Chitose Chitose Early
Chitose Chitose Late
Chitose Chitose Mix
Chitose Erimo Early
Chitose Erimo Late
Chitose Erimo Mix
Erimo Chitose Early
Erimo Chitose Late
Erimo Chitose Mix
Erimo Erimo Early
Erimo Erimo Late
Erimo Erimo Mix
Salamander population x Tadpole population x Hatch timing

692

693 **Fig. S2** Tadpole morphology (**A**, body width; **B**, body length; **C**, relative body width) at day 18,
694 shown by boxplot with raw data points. For hatch timing, see Fig. S1. Treatments sharing the
695 same alphabet letter are not significantly different (Tukey's HSD).