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Giant cannibals drive selection for inducible defence in heterospecific prey

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Predation can strongly influence the evolution of prey species by selecting for defensive phenotypes that reduce the risk of predation. Although cannibalism is frequent in predators and is known to strongly influence predator–prey dynamics, it is largely neglected when studying the evolution of phenotypic defences in prey. Changes in cannibalism can alter the abundance, size-structure, and even phenotypes within predator populations, and thereby change the numerical and per-capita effects driving the selection pressure on defensive phenotypes in prey. In the present study, we experimentally manipulated the presence and absence of cannibalism in predatory salamander larvae *Hynobius retardatus* (Dunn) and examined how this affects the expression of and selection pressure on inducible defensive phenotype (bulgy phenotype) in its prey, *Rana pirica* (Matsui) tadpoles. In the absence of cannibalism, salamanders were generally not sufficiently large to consume tadpoles, tadpoles did not express the defensive phenotype, and all phenotypes had equally high survival. By contrast, cannibalism of salamanders accelerated growth rates, leading to the development of 'giant' cannibalistic morphs that were able to consume tadpoles. Importantly, only in the presence of these giant cannibals did tadpoles express a defensive 'bulgy' phenotype that had significantly higher survival rates than phenotypes without this defence, indicating that cannibalism altered the selective regime for defensive traits in prey. The results of the present study demonstrate that cannibalism can drive trait evolution in predator–prey systems and suggest that we cannot predict how changes in numerical effects in predator populations affect selection regimes on prey traits without accounting for concurrent changes in predator per-capita effects. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2017, **120**, 675–684.

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INTRODUCTION

The evolution of anti-predator traits is one of the best examples demonstrating the importance of species interactions for the evolution of life-history traits (Hoso *et al.*, 2010; Kosloski & Allmon, 2015). The strength of the selection pressure on prey defences is determined by the mortality imposed by the predator, which depends on both the predators' population size (i.e. density) and per-capita consumption rates (Abrams, 2000; Takatsu & Kishida, 2013). In general, decreasing the predator density or per-capita consumption rate reduces the total number of predation events, although how such changes in predator population influence prey evolution depends on how changes in the density and per-capita consumption rates of predators are correlated. Hence, exploring factors that modify predator demography and individual phenotype is imperative for obtaining a better understanding of the evolutionary processes of prey defences (Turcotte, Corrin & Johnston, 2012; Walsh, 2013; terHorst *et al.*, 2015). Despite considerable progress, little is known on how intraspecific interactions in predator populations influence selection pressure on prey defences. Yet, intraspecific interactions among predators are likely to simultaneously affect both the demography and individual

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phenotypes of predators, and they can vary considerably across time and space and thus lead to concurrent variation in selection pressure on prey defences.

Cannibalism is a key interaction driving the demographic structure and even the morphology of predator populations (Fox, 1975; Polis, 1981; Claessen, De Roos & Persson, 2004), although its effect on the evolution of heterospecific prey is largely unknown (Rudolf, Sorrell & Pedersen, 2012). Predicting how cannibalism in predator populations affects prey evolution is challenging because it can have multiple and contrasting effects on the numerical and per-capita effects of the predator on its prey. Cannibalism in a predator can reduce predation rates on heterospecific prey via the consumption of conspecific predators (i.e. numerical effects) or changes in the foraging behaviour of conspecific victims or cannibals (i.e. per-capita effects) (Rudolf, 2006, 2007, 2012). These scenarios suggest that cannibalistic interactions in a predator should dampen the selection pressure on defensive phenotypes of heterospecific prey. By contrast, when interactions occur among growing predators and prey, cannibalism of predators can intensify predation rates on heterospecific prey if cannibalism allows predators to rapidly increase in growth and thereby improve the per-capita predation ability of cannibalistic predators (Takatsu & Kishida, 2015). In this scenario, cannibalism could increase selection pressure favouring the more defensive phenotypes of its heterospecific prey.

In the present study, we used two complementary experiments to test when and how cannibalism in a growing predator species alters selection for defensive phenotype of its heterospecific prey using a gape-limited predator–prey interaction between cannibalistic salamander larvae [*Hynobius retardatus* (Dunn)] and frog tadpoles [*Rana pirica* (Matsui)] as a model system. Specifically, we first examined whether cannibalism in salamander hatchlings produces selection pressures favouring the defensive ('bulgy') phenotype of tadpoles. In addition, we investigated whether expression of the defensive phenotype is associated with the occurrence of salamander cannibalism. If salamander cannibalism has played a key role in evolution of the inducible defence, tadpoles should express more defensive (bulgy) phenotypes in the presence versus absence of cannibalism in the salamanders.

MATERIAL AND METHODS

Study system

Hynobius retardatus (Dunn) salamanders and *R. pirica* (Matsui) frogs usually spawn in small temporary ponds in early spring in Hokkaido, Japan. Although salamander larvae are carnivores, the trophic relationship with frog tadpoles is not always established, even if adult salamanders and frogs lay their eggs in the same ponds. Strong predator–prey interactions can occur in two alternative scenarios. The first scenario is that salamander hatchlings exhibit an offensive phenotype, which is characterized by a wider gape for the effective consumption on frog tadpoles in the presence of 'small' frog tadpoles (Michimae & Wakahara, 2002; Takatsu & Kishida, 2013; Kishida *et al.*, 2015). This scenario can occur when the hatch timings of salamanders and frogs are very close (within 2 weeks) (Kishida, Trussell & Nishimura, 2009; Takatsu & Kishida, 2013; Nosaka, Katayama & Kishida, 2015). However, such hatching phenology is not common because frog tadpoles typically hatch 3–4 weeks earlier than salamanders. In the typical hatching phenology, frog tadpoles are too large to be consumed by salamander hatchlings (Nosaka *et al.*, 2015). A strong predator–prey interaction can be established only when salamander larvae grow rapidly. Importantly, cannibalism among salamander hatchlings plays a critical role with respect to salamanders becoming substantial predators for tadpoles because salamander hatchlings that successfully cannibalize conspecifics grow at a much faster rate and develop into 'giants' with a much larger body size and offensive phenotype (i.e. wider gape width) (Wakahara, 1995; Kishida *et al.*, 2011). The increase in size and change in morphology allows cannibalistic 'giants' to consume the earlier-hatched tadpoles that are too large for their non-cannibalistic conspecifics (Takatsu & Kishida, 2015). This is the second scenario and, in the present study, we focus on this common case.

Importantly, in this system, tadpoles exhibit inducible morphological defences. When exposed to a strong predation risk from salamander larvae, tadpoles develop an enlarged body and tail by thickening their epithelium tissue. This defensive 'bulgy' phenotype makes it harder for the salamander larvae to swallow them (Kishida & Nishimura, 2004). Based on this relationship, we hypothesized that cannibalism in the salamander hatchlings could maintain (or even drive evolution of) the inducible defensive (bulgy) phenotype of tadpoles.

Experiment summary

The present study was composed of two experiments. A schematic diagram of the experiments is provided in the Supporting information (Fig. S1). In the first experiment (Experiment 1: Establishment of cannibalistic and non-cannibalistic situations), we controlled for the occurrence of cannibalism of salamander hatchlings. This allowed us (1) to

establish different ecological situations with and without cannibalism in the predator and (2) to examine how these different cannibalism scenarios in the predator influence the expression of the defensive 'bulgy' phenotype of tadpoles. Finally, we conducted the second experiment (Experiment 2: Selection trials) using the cannibalistic and non-cannibalistic situations established in the first experiment to (3) quantify how cannibalism in the predator alters the strength of selection pressure on the defensive bulgy phenotype by comparing relative survival of the tadpoles with more- and less-bulgy phenotypes among these situations. In the experiments, we used *R. pirica* tadpoles and *H. retardatus* larvae derived from eggs collected from natural ponds. The methods of collection and the husbandry of the amphibian eggs are described in the Supporting information (Appendix S1).

Experiment 1: Establishment of cannibalistic and non-cannibalistic situations

We conducted the experiment in the laboratory using a semi-transparent polypropylene tank (43.6 cm × 28.4 cm × 14.1 cm high) filled with 5 L of aged tap water as an experimental unit. Just before assigning tadpoles to the experimental units, we mixed all of the tadpoles derived from 15 egg masses (i.e. 2-week-old at stage 25–30; Gosner, 1960) (see Supporting information, Appendix S1). On 15 May 2013, we haphazardly assigned 45 tadpoles from this mix to each of 46 tanks. The mean ± SD ($N = 20$; a subsample of the tadpoles used) snout–vent length (hereafter referred to as body length) of the assigned tadpoles was 11.75 ± 0.72 mm.

The occurrence of cannibalism in salamander hatchlings depends greatly on size asymmetry between interacting individuals (Kishida *et al.*, 2015). Thus, to control for the occurrence of cannibalism, we manipulated population size structure (i.e. the presence and absence of early or late hatchlings) in salamander hatchlings at the same time as keeping the total initial density of salamanders constant across the treatments. We obtained the early and late salamander hatchlings by manually controlling the water temperature experienced by the embryos from a single egg cluster. The difference in hatch timing between early- (14 May) and late- (21 May) hatchlings was 7 days. The salamander hatchlings were assigned to the relevant treatments 1 day after they hatched. The method for obtaining the early- and late- hatchlings is same as that described in Takatsu & Kishida (2015).

The experiment consisted of four treatments: (1) the 'Cannibalism' treatment received five early and 15 late hatchlings; (2) the 'No-cannibalism-early' treatment received 20 early hatchlings; (3) the 'No-cannibalism-late' treatment received 20 late hatchlings; and (4) the 'No-salamander treatment' received no salamander hatchlings, thus serving as a control treatment. We replicated treatment (1) 20 times, treatments without cannibals (2 and 3) 10 times and control treatments (4) six times. We adopted the unbalanced replication design to avoid excessive use of the animals because our previous studies showed that mortality of tadpoles in the absence of predators was very low (Takatsu & Kishida, 2013; Nosaka *et al.*, 2015) and variance of the demographic and trait level consequences was larger in the Cannibalism treatment compared to the No-cannibalism treatments (Takatsu & Kishida, 2015). Each replicate was randomly assigned to one of the 46 tanks. Densities of frog tadpoles (363 individuals m^{-2}) and salamander hatchlings (162 individuals m^{-2}) are within their natural ranges (Michimae, 2006).

We defined the day on which the early salamander hatchlings and frog tadpoles were assigned into the tanks as day 1 of the experiment, and assigned the late hatchlings into the relevant tanks on day 8. At day eight, mean ± SD ($N = 20$) body length of the early salamander hatchlings (stage 49–50; Iwasawa & Yamashita, 1991) in the Cannibalism and No-cannibalism-early treatment was 15.44 ± 0.76 and 15.24 ± 0.54, respectively, and the body length of the late salamander hatchlings (stage 43–44; Iwasawa & Yamashita, 1991) in the Cannibalism and No-cannibalism-late treatments was 12.61 ± 0.58 and 12.25 ± 0.63, respectively. The timing of hatching of both frogs and salamanders is typical in natural habitats (Nosaka *et al.*, 2015). Throughout the experiment, we added one piece of rabbit chow (dry weight: 0.2 g) and 20 frozen *Chironomid* larvae to all tanks every 2 days as an alternative food for the tadpoles and the salamanders, respectively. Note that *Chironomid* larvae do not affect gigantism and the expression of offensive phenotypes of salamanders (Kishida *et al.*, 2009). Throughout the experiments, we exchanged the water every 2 days.

At day 31, we counted all surviving tadpoles and salamanders. We then scanned the ventral aspect of all surviving salamanders using a computer scanner (CanoScan 8800F; Canon, Japan). At this point, most tanks only in the Cannibalism treatment had 'giant' cannibals and salamanders had started to engage in cannibalism and the consumption of tadpoles. We examined the effects of cannibalism treatments on mortality rate of tadpoles and salamanders (i.e. number of dead individuals during 30 days divided by the number of individual at the start of experiment) using generalized linear models with binomial error distribution, followed by pairwise comparisons. We adjusted significance levels ($\alpha = 0.05$) of post-hoc

tests using the sequential Bonferroni method (Holm, 1979). Using the scanned images, we measured gape width and body length of the all salamanders. To examine whether cannibalism of salamander hatchlings caused gigantism of the salamanders in the Cannibalism treatment, we compared body length and gape width of the salamander with the largest body length within a tank among the treatments using a Kruskal–Wallis test followed by Wilcoxon tests for pairwise comparisons. We focused on the largest individual within a tank because only a few salamanders become giants in cannibalistic population (Kishida *et al.*, 2011; Takatsu & Kishida, 2015).

To examine whether the expression of the bulgy phenotype was associated with salamander cannibalism, we compared the proportion of tadpoles expressing the bulgy phenotype among the four treatments. Because the bulgy phenotype is characterized by the transparent, thickened epithelium tissues (Kishida & Nishimura, 2004), the ratio of width of transparent tissue to body width is a good index of the phenotype. We defined the tadpoles whose width of transparent tissue exceeds 10% of body width along the line of the greatest body width as tadpoles having a bulgy phenotype. By using the scanned images of ventral side of tadpoles, we measured maximum body width and transparent tissue width of 10 tadpoles randomly selected from each tank and calculated the ratio of each tadpole. Then, we calculated proportion of tadpoles having bulgy phenotype in each tank. A Kruskal–Wallis test was used to examine whether the proportion of tadpoles having bulgy phenotype was different among the four treatments. When we found significant difference among the treatments, we performed pairwise comparisons using the Wilcoxon test with sequential Bonferroni method.

Experiment 2: Selection trials

Thirty-one days after start of Experiment 1, we conducted selection trials to examine whether salamander cannibalism leads to selective regimes favouring defensive bulgy phenotype of tadpoles. We used the same four experimental treatments (i.e. Cannibalism, No-cannibalism-early, No-cannibalism-late, and No-salamander treatments) from Experiment 1 as possible selective environments. Because we were limited by the number of more- and less-bulgy tadpoles, we randomly selected nine replicate tanks from each of the three salamander treatments (1–3) and we used all six replicate tanks from the No-salamander control treatment for conducting the Selection trials. To measure selection strength in each treatment, we used two types of tadpoles with different degrees of defensive bulgy phenotype (more- and less-bulgy tadpoles) instead of the surviving tadpoles in Experiment 1 (see Supporting information, Fig. S1). Details of the method for the preparation of the more- and less-bulgy tadpoles are provided in the Supporting information (Appendix S2).

We first removed all surviving tadpoles from each of the selected 33 tanks. Next, we assigned 10 more-bulgy and 10 less-bulgy tadpoles into each of the selected tanks. Before assigning tadpoles to tanks, we marked the tails of more- and less-bulgy tadpoles differently to track the survival of each phenotype separately. In half of the replications of each treatment, we cut a small piece of the upper side of the tails of the more-bulgy tadpoles and the lower side of the tails of less-bulgy tadpoles, whereas we carried out the opposite procedure in the other half of the replicates. This tail-cutting method does not influence the mortality of tadpoles (i.e. all tadpoles survived in the absence of salamanders). The marking method was important to discriminate between phenotypes during the experiment because morphological differences between the more- and less-bulgy phenotypes disappeared as a result of the stronger expression of the defensive phenotype in the less-bulgy tadpoles compared to the more-bulgy tadpoles during the trial period. We added one piece of rabbit chow (dry weight: 0.2 g) and 20 *Chironomid* larvae into all tanks every 2 days as alternative food for the tadpoles and salamanders, respectively.

We counted the numbers of surviving tadpoles in the more- and less-bulgy tadpole category every day. The experiment was terminated 6 days after starting the trials because the morphological (and thus functional) differences between more- and less-bulgy tadpoles had almost disappeared at that time. We calculated the relative survival rate of the two groups by dividing the number of surviving more-bulgy tadpoles by the number of surviving less-bulgy tadpoles in each tank on each day. We used the Wilcoxon signed-rank test to determine whether the ratios were larger or smaller than one on each day because the data did not satisfy parametric test assumptions. When the ratio was significantly > 1, we inferred that the salamander selected for more-bulgy tadpoles, whereas, when the ratio was significantly < 1, we inferred that they selected for less-bulgy tadpoles.

RESULTS

Experiment 1: Establishment of cannibalistic and non-cannibalistic situations

Survival and morphology of salamanders

We found significant differences in the mortality of the salamanders during 30 days among three

salamander treatments (i.e. Cannibalism and two No-cannibalism treatments) ($\chi^2_2 = 21.98$, $P < 0.0001$). Salamander mortality in the Cannibalism treatment was 17.5- and 23.3-fold higher than in No-cannibalism-early or No-cannibalism-late treatments, respectively (Fig. 1A). Only approximately 4% and 3% of salamanders were dead in No-cannibalism-early and No-cannibalism-late treatments, respectively, and thus there were no statistical difference between the two No-cannibalism treatments ($\chi^2_1 = 0.015$, $P > 0.90$). We found significant differences in body length (i.e. snout–vent length; ($\chi^2_2 = 27.89$, $P < 0.0001$) and gape width ($\chi^2_2 = 25.92$, $P < 0.0001$) of the largest salamander at day 30 among the three salamander treatments (Fig. 2). Body length of the largest salamander in the Cannibalism treatment was 41% and 48% larger compared to those in the No-cannibalism-early treatment and No-cannibalism-late treatment, respectively. Furthermore, gape width of the largest salamander was 54% and 68% larger compared to those in the No-cannibalism-early treatment and No-cannibalism-late treatment, respectively. There were no differences in these traits between the two No-cannibalism treatments (body length, $Z = -1.78$, $P = 0.076$; gape width, $Z = -1.70$, $P = 0.089$).

Survival and expression of bulgy phenotype of tadpoles

Salamander treatments significantly affected the mortality of tadpoles at day 30 ($\chi^2_3 = 12.67$, $P = 0.0054$). Tadpole mortality in the Cannibalism treatment was 13.3- and 20-fold higher than in the No-cannibalism-early and No-cannibalism-late treatments, respectively (Fig. 1B). Because only very few tadpoles died in the two No-cannibalism treatments

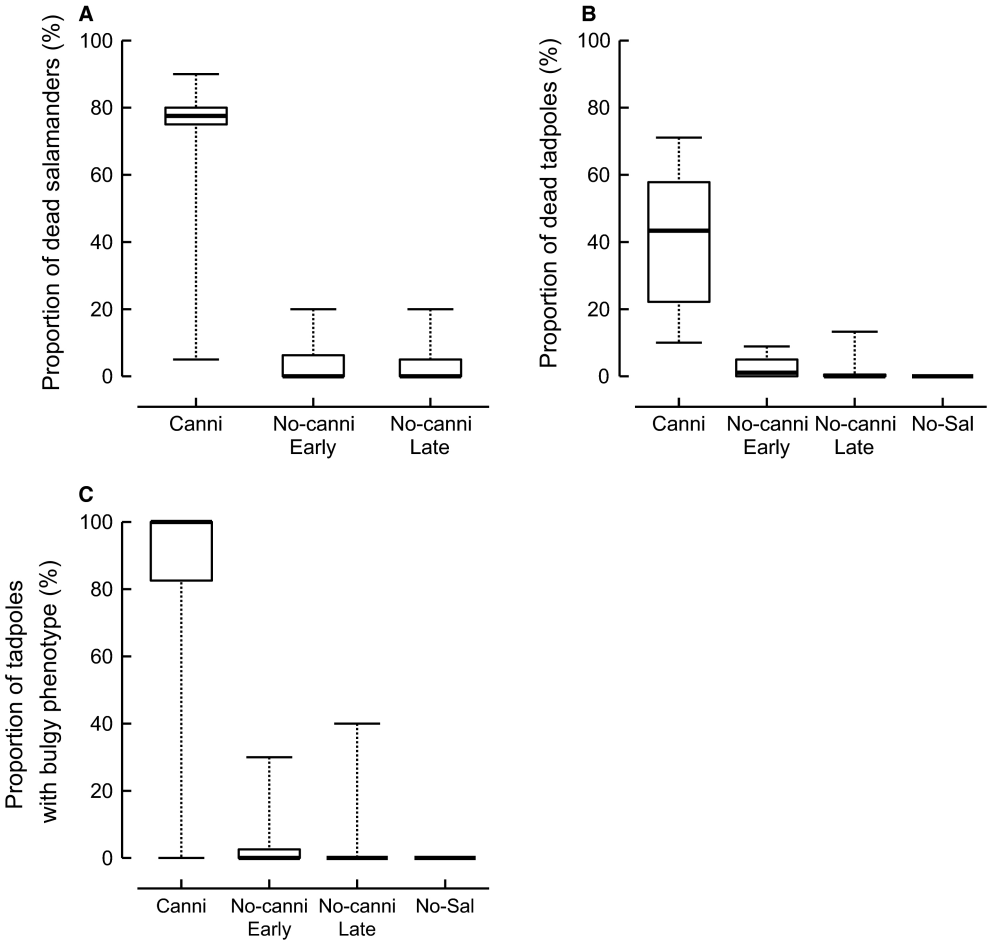

Figure 1. Proportions of dead salamander larvae (A), dead frog tadpoles (B), and bulgy (defensive) frog tadpoles (C) at the end of Experiment 1. Canni, Cannibalism; No-canni, No-cannibalism; No-sal, No-salamander (i.e. control). 'Early' and 'Late' in the No-cannibalism treatment represent the hatch timing of the salamanders used in the treatments. Thick horizontal bar indicates the median, box contains 50% of the data, and whisker indicates the range.

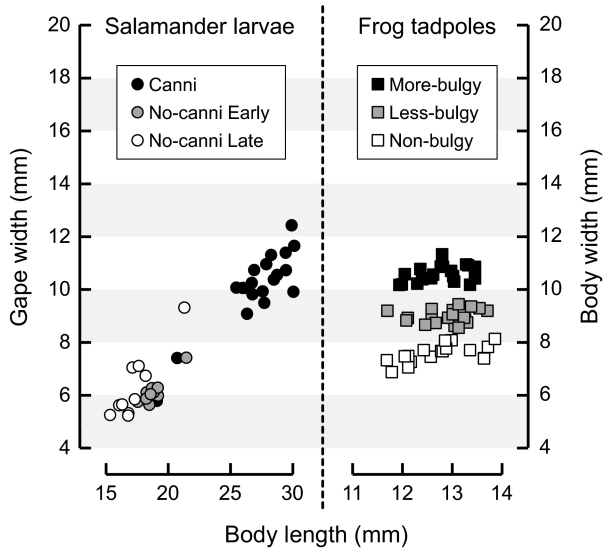

Figure 2. Gape width of the largest salamanders within each tank (left panel) and body width of tadpoles (right panel) used in the Selection trials versus their respective body length. Canni, Cannibalism; No-canni Early, No-cannibalism-early; No-canni Late, No-cannibalism-late. 'More-bulgy', 'Less-bulgy', and 'Non-bulgy' represents the degree to which the defensive 'bulgy' phenotype of tadpoles was expressed.

and No-salamander treatment, we found no significant differences in any pairwise comparisons of these treatments ($P > 0.61$). The proportion of tadpoles having the bulgy phenotype was different among salamander treatments ($\chi^2_3 = 32.25$, $P < 0.0001$). The proportion of bulgy tadpoles in the Cannibalism treatment (mean ± SD: 83.5 ± 31.8%) was significantly higher than those in the other three treatments (pairwise comparisons, $P < 0.002$) and we found no significant difference among the remaining three treatments (Fig. 1C). Indeed, no tadpoles expressed bulgy phenotype in the No-salamander treatment and only approximately 4% of tadpoles expressed the bulgy phenotypes in the No-cannibalism-early and No-cannibalism-late treatments (Fig 1C).

Experiment 2: Selection trials

How did salamander cannibalism shape selection regimes?

In the two No-cannibalism and No-salamander treatments, all but one tadpole survived the duration of the selection experiment (Fig. 3A). Hence, there were no differences in relative survival rates between more-bulgy tadpoles and less-bulgy tadpoles in these treatments. By contrast, 81.1 ± 11.6% of the more-bulgy tadpoles and 50.0 ± 18.7% of the less-bulgy tadpoles survived to the end of the experiment in the Cannibalism treatment (Fig. 3A). Thus, the proportion of the surviving more-bulgy tadpoles was significantly ($P < 0.012$) higher (approximately 62%) than that of surviving less-bulgy tadpoles (Fig. 3B) in the Cannibalism treatment. These results suggest that the defensive (bulgy) phenotype of frog tadpoles only has a higher fitness in the presence of 'giant' cannibalistic salamander larvae.

DISCUSSION

Cannibalism in predators is increasingly recognized as a critical factor determining the demography of their prey, although little is known about how cannibalism in a predator shapes the evolution of the defensive traits of its prey (Rudolf *et al.*, 2012). Using a combination of experiments, we demonstrate that cannibalism in a predator can determine the selection regime for inducible defence in its prey. Specifically, we found that only in the presence of cannibalism in the predatory salamander (1) tadpoles were exposed to strong predation by 'giant' cannibals; (2) tadpoles expressed the defensive (bulgy) phenotype in the presence of cannibalistic salamanders; and (3) the proportion of surviving tadpoles with a defensive (bulgy) phenotype was significantly higher in the presence of cannibalistic salamanders. To the best of our knowledge, these results provide the first evidence that the presence or absence of cannibalism in a predator can determine the selection pressure favouring defensive versus normal phenotypes in their prey.

Although the selection trial (Experiment 2) was only 6 days in duration, the selection pressure is expected to operate over most of the larval period of frog tadpoles in natural habitats. Once the salamanders consume tadpoles, the salamanders continue to impose strong predation pressure on tadpoles until they metamorphose and leave the aquatic habitat (Nosaka *et al.*, 2015; Takatsu & Kishida, 2015). Even without cannibalism, salamanders can become predatory giants when the salamanders interact with small tadpoles at their hatchling stage (Michimae & Wakahara, 2002; Kishida *et al.*, 2009). However, such a scenario is only realized when salamanders hatch substantially before tadpoles or the two species hatch at the same time, both of which rarely occur under natural conditions (Nosaka *et al.*, 2015). Therefore, we can conclude that cannibalism among the salamander hatchlings is a substantial factor causing the selection pressure favouring the defensive phenotype of tadpoles.

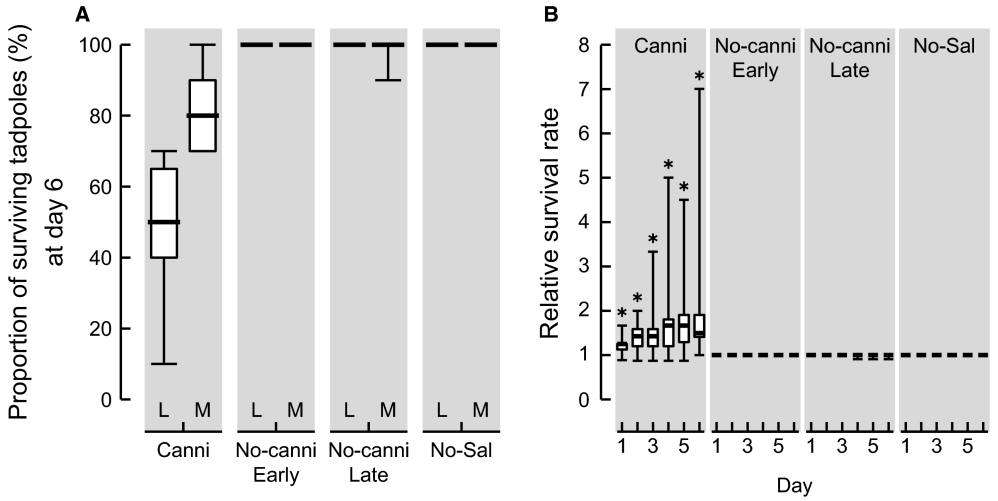

Figure 3. A, proportion of surviving less-bulgy (L) and more-bulgy (M) tadpoles at the end of Selection trials after 6 days. B, relative survival rates of more-bulgy tadpoles to less-bulgy tadpoles during the experimental period. Thick horizontal bar indicates the median, box contains 50% of the data, and whisker indicates the range. Canni, Cannibalism; No-canni, No-cannibalism; No-sal, No-salamander. 'Early' and 'Late' in the No-cannibalism treatment represent hatch timing of the salamanders used in the treatments. Asterisks indicate that the relative survival rates were significantly different from 1 ($P < 0.012$).

The present study generally suggests that paying attention to the relative importance of numerical and per-capita effect of ecological interactions is useful for better understanding how ecological interactions influence trait evolution. Although cannibalism in a predator can dampen predation pressure on its heterospecific prey via a reduction of predator density (i.e. numerical effect) or the foraging activity of cannibalistic victims on their heterospecific prey (i.e. per-capita effect of cannibalistic victim) (Rudolf, 2006, 2007, 2012), it can intensify the selective predation pressure via an improvement of the predation ability of cannibalistic predators by promoting their rapid growth (i.e. per-capita effect of cannibalistic predator). Hence, the net effect of predator cannibalism depends on the relative importance of the numerical and per-capita effects. Although the number of surviving salamanders in the Cannibalism treatment was equivalent to 31% of the number of salamanders in the No-cannibalism treatments at the beginning of the Selection trials, selective predation pressure on prey tadpoles emerged only in the Cannibalism treatment. This clearly indicates that the change in per-capita effect of cannibalistic predator was the more important factor shaping selection pressure on prey defence than the other two effects. The change in the per-capita effect was driven by the accelerated growth of cannibalistic predators; at the end of the induction experiment (i.e. at the beginning of the Selection trials), the body length and gape width of the largest salamanders were, respectively, 1.5-fold and 1.6-fold larger in the Cannibalism treatment compared to the No-cannibalism treatments. In the Cannibalism treatment, the mean gape width of the largest salamanders was 11% greater than the mean body width of less-bulgy tadpoles but 5% smaller than the mean body width of more-bulgy tadpoles (Fig. 2). A salamander can consume a tadpole when its gape width is more than 10% larger than the tadpole's body width (Nosaka *et al.*, 2015). Thus, giant cannibalistic salamanders could consume less-bulgy tadpoles, although they were mostly unable to consume more-bulgy tadpoles. On the other hand, gape width of the largest salamanders in the No-cannibalism treatments was at least 27% smaller than the mean body width of tadpoles regardless of their phenotype (Fig. 2). Thus, without the growth boost from cannibalism, salamander larvae were unable to consume either phenotype of tadpoles and consequently did not select for defensive phenotypes even though salamander density was three times higher without cannibalism. Indeed, the reduction in predator density was necessary to increase the per-capita effect of predators on the prey. Because changes in predator density had little to no effect, the increase in per-capita consumption rates of giant cannibalistic predators resulted in a net increase in prey mortality and in a selection regime that strongly favoured expression of the defensive phenotype of its prey. These results emphasize that we cannot predict how changes in numerical effects in predator populations

affect selection regimes on prey traits without accounting for concurrent changes in predator per-capita effects.

We saw clear differences in the expression of defensive phenotypes in the prey between treatments with and without salamander cannibalism (Experiment 1). Although very few tadpoles expressed a bulgy (defensive) phenotype in the two No-cannibalism treatments, the vast majority (84%) of tadpoles developed bulgy phenotype in the Cannibalism treatment. This suggests that the tadpoles did not simply express a defensive phenotype when salamanders were present but, instead, only when predators engaged in significant levels of cannibalism. Because tadpoles develop bulgy phenotype in response to the close proximity of the salamander larvae (Kishida & Nishimura, 2004), the tadpoles might recognize the phenotypes of the salamanders by means of phenotype-specific aggressiveness (i.e. predation attempt by the salamanders) or nondiffusible chemicals of salamanders, perhaps even by direct contact (Kishida, Mizuta & Nishimura, 2006). Regardless of the precise mechanism, the risk-dependent expression of the bulgy phenotype of tadpoles suggests adaptive phenotypic plasticity as a cost saving strategy because the defensive phenotype is likely costly to produce or maintain (Mori *et al.*, 2009; Kishida *et al.*, 2010) and the emergence of predatory giant salamanders in salamander populations varies with abiotic and biotic conditions (Kishida *et al.*, 2009, 2011; Nosaka *et al.*, 2015). For example, the presence of the top predator, *Aeshna nigroflava* (Martin) dragonfly larvae, strongly suppresses cannibalism in *Hynobius retardatus* (Dunn) salamander hatchlings and thereby greatly reduces the likelihood of the emergence of giant cannibals (Kishida *et al.*, 2011). This implies that variation in community structure can play a key role in evolution of the inducible bulgy phenotype by altering cannibalism within predator populations.

Cannibalism is widespread in a variety of predator species and is known to play a prominent role in the dynamics of prey communities (Polis, 1981; Persson *et al.*, 2003; Claessen *et al.*, 2004; Persson, De Roos & Bertolo, 2004; Woodward, Speirs & Hildrew, 2005; Rudolf, 2007). By contrast to our system, in many of the trophic systems between cannibalistic predator species and their heterospecific prey documented so far, cannibalism in predator populations weakens the consumption rates of heterospecific prey by reducing density and foraging the activity of predators (Persson *et al.*, 2003; Crumrine, 2005; Rudolf, 2006, 2008; Law & Rosenheim, 2011). In these systems, cannibalism of a predator species should reduce the selection pressure on defensive phenotypes in heterospecific prey. However, the present study indicates that this effect may be reduced or even reversed in systems where cannibalism can alter the phenotype of predators by allowing cannibalistic individuals to grow rapidly and greatly improve their predatory ability. Given that cannibalism can strongly increase growth rates in many carnivorous insects, fishes, and amphibian species (DeAngelis, Cox & Coutant, 1980; Sogard & Olla, 1994; Fangan & Odell, 1996; Ziemba & Collins, 1999; Hardie & Hutchings, 2014), our results suggest that cannibalism can be an important factor driving the evolution of defensive traits in heterospecific prey species in a wide range of terrestrial and aquatic predatory prey systems. Therefore, future studies that examine how cannibalism affects phenotypic characteristics of predators and their per-capita effect on the prey promise to be a fruitful venue for gaining a better understanding of the evolution of prey defences.

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REFERENCES

Abrams PA. 2000. The evolution of predator–prey interactions: theory and evidence. *Annual Review of Ecology Systematics* **31:** 79–105.

Claessen D, De Roos AM, Persson L. 2004. Population dynamic theory of size-dependent cannibalism. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271:** 333–340.

Crumrine PW. 2005. Size structure and substitutability in an odonate intraguild predation system. *Oecologia* **145:** 132–139.

DeAngelis DL, Cox DK, Coutant CC. 1980. Cannibalism and size dispersal in young-of-the-year largemouth bass: experiment and model. *Ecological Modeling* **8:** 133–148.

Fangan WF, Odell GM. 1996. Size-dependent cannibalism in praying mantids: using biomass flux to model size-structured populations. *American Naturalist* **147:** 230–268.

Fox LR. 1975. Cannibalism in natural populations. *Annual Review of Ecology Systematics* **6:** 87–106.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16:** 183–190.

Hardie DC, Hutchings JA. 2014. Cannibalistic growth polyphenism in Atlantic cod. *Evolutionary Ecology Research* **16:** 569–580.

Holm S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6:** 65–70.

terHorst CP, Lau JA, Cooper IA, Keller KR, Rosa RJL, Royer AM, Schultheis EH, Suwa T, Conner JK. 2015. Quantifying nonadditive selection caused by indirect ecological effects. *Ecology* **96:** 2360–2369.

Hoso M, Kameda Y, Wu SP, Asami T, Kato M, Hori M. 2010. A speciation gene for left-right reversal in snails results in anti-predator adaptation. *Nature Communications* **1:** 133.

Iwasawa H, Yamashita K. 1991. Normal stages of development of a hynobiid salamander, *Hynobius nigrescens* Stejneger. *Japanese Journal of Herpetology* **14:** 39–62.

Kishida O, Nishimura K. 2004. Bulgy tadpoles: inducible defense morph. *Oecologia* **140:** 414–421.

Kishida O, Mizuta Y, Nishimura K. 2006. Reciprocal phenotypic plasticity in a predator–prey interaction between larval amphibians. *Ecology* **87:** 1599–1604.

Kishida O, Trussell GC, Nishimura K. 2009. Top-down effects on antagonistic inducible defense and offense. *Ecology* **90:** 1217–1226.

Kishida O, Trussell GC, Mougi A, Nishimura K. 2010. Evolutionary ecology of inducible morphological plasticity in predator–prey interaction: toward the practical links with population ecology. *Population Ecology* **52:** 37–46.

Kishida O, Trussell GC, Ohno A, Kuwano S, Ikawa T, Nishimura K. 2011. Predation risk suppresses the positive feedback between size structure and cannibalism. *Journal of Animal Ecology* **80:** 1278–1287.

Kishida O, Tezuka A, Ikeda A, Takatsu K, Michimae H. 2015. Adaptive acceleration in growth and development of salamander hatchlings in cannibalistic situations. *Functional Ecology* **29:** 469–478.

Kosloski ME, Allmon WD. 2015. Macroecology and evolution of a crab 'super predator', *Menippe mercenaria* (Menippidae), and its gastropod prey. *Biological Journal of the Linnean Society* **116:** 571–581.

Law YH, Rosenheim JA. 2011. Effects of combining an intraguild predator with a cannibalistic intermediate predator on a species-level trophic cascade. *Ecology* **92:** 333–341.

Michimae H. 2006. Differentiated phenotypic plasticity in larvae of the cannibalistic salamander *Hynobius retardatus*. *Behavioral Ecology and Sociobiology* **60:** 205–211.

Michimae H, Wakahara M. 2002. A tadpole-induced polyphenism in the salamander *Hynobius retardatus*. *Evolution* **56:** 2029–2038.

Mori T, Kawachi H, Imai C, Sugiyama M, Kurata Y, Kishida O, Nishimura K. 2009. Identification of a novel uromodulin-like gene related to predator-induced bulgy morph in anuran tadpoles by functional microarray analysis. *PLoS ONE* **4:** e5936.

Nosaka M, Katayama N, Kishida O. 2015. Feedback between size balance and consumption strongly affects the consequences of hatching phenology in size-dependent predator–prey interactions. *Oikos* **124:** 225–234.

Persson L, De Roos AM, Claessen D, Byström P, Lövgren J, Sjögren S, Svanback R, Wahlstrom E, Westman E. 2003. Gigantic cannibals driving a whole-lake trophic cascade. *Proceedings of the National Academy of Sciences of the United States of America* **100:** 4035–4039.

Persson L, De Roos AM, Bertolo A. 2004. Predicting shifts in dynamics of cannibalistic field populations using individual-based models. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271:** 2489–2493.

Polis GA. 1981. The evolution and dynamics of intraspecific predation. *Annual Review of Ecology Systematics* **12:** 225–251.

Rudolf VHW. 2006. The influence of size-specific indirect interactions in predator–prey systems. *Ecology* **87:** 362–371.

Rudolf VHW. 2007. Consequences of stage-structured predators: cannibalism, behavioral effects, and trophic cascades. *Ecology* **88:** 2991–3003.

Rudolf VHW. 2008. The impact of cannibalism in the prey on predator–prey systems. *Ecology* **89:** 3116–3127.

Rudolf VHW. 2012. Seasonal shifts in predator body size diversity and trophic interactions in size-structured predator–prey systems. *Journal of Animal Ecology* **81:** 524–532.

Rudolf VHW, Sorrell I, Pedersen AB. 2012. Revenge of the host: cannibalism, ontogenetic niche shifts, and the evolution of life-history strategies in host-parasitoid systems. *Evolutionary Ecology Research* **14:** 31–49.

Sogard SM, Olla BL. 1994. The potential for intracohort cannibalism in age-0 walleye pollock, *Theragra chalcogramma*, as determined under laboratory conditions. *Environmental Biology of Fishes* **39:** 183–190.

Takatsu K, Kishida O. 2013. An offensive predator phenotype selects for an amplified defensive phenotype in its prey. *Evolutionary Ecology* **27:** 1–11.

Takatsu K, Kishida O. 2015. Predator cannibalism can intensify negative impacts on heterospecific prey. *Ecology* **96:** 1887–1898.

Turcotte MM, Corrin MSC, Johnston MTJ. 2012. Adaptive evolution in ecological communities. *PLoS Biology* **10:** e1001332.

Wakahara M. 1995. Cannibalism and the resulting dimorphism in larvae of a salamander *Hynobius retardatus*, inhabited in Hokkaido, Japan. *Zoological Science* **12:** 467–473.

Walsh MR. 2013. The evolutionary consequences of indirect effects. *Trends in Ecology and Evolution* **28:** 23–29.

Woodward G, Speirs DC, Hildrew AG. 2005. Quantification and resolution of a complex, size-structured food web. *Advances in Ecological Research* **36:** 85–135.

Ziemba RE, Collins JP. 1999. Development of size structure in tiger salamanders: the role of intraspecific interference. *Oecologia* **120:** 524–529.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Schematic diagram of the experiments.
Appendix S1. Collection and husbandry methods of *Rana pirica* and *Hynobius retardatus* eggs.
Appendix S2. Preparation of phenotypic variants of tadpoles as selection targets.