Feedback between size balance and consumption strongly affects the consequences of hatching phenology in size-dependent predator–prey interactions

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Abstract

In many size-dependent predator–prey systems, hatching phenology strongly affects predator–prey interaction outcomes. Early-hatched predators can easily consume prey when they first interact because they encounter smaller prey. However, this process by itself may be insufficient to explain all predator–prey interaction outcomes over the whole interaction period because the predator–prey size balance changes dynamically throughout their ontogeny. We hypothesized that hatching phenology influences predator–prey interactions via a feedback mechanism between the predator–prey size balance and prey consumption by predators. We experimentally tested this hypothesis in an amphibian predator–prey model system. Frog tadpoles (*Rana pirica*) were exposed to a predatory salamander larva (*Hynobius retardatus*) that had hatched 5, 12, 19, or 26 days after the frog tadpoles hatched. We investigated how the salamander hatch timing affected the dynamics of prey mortality, size changes of both predator and prey, and their subsequent life history (larval period and size at metamorphosis). The predator–prey size balance favoured earlier hatched salamanders, which just after hatching could successfully consume more frog tadpoles than later hatched salamanders. The early-hatched salamanders grew rapidly and their accelerated growth enabled them to maintain the predator-superior size balance; thus, they continued to exert strong predation pressure on the frog tadpoles in the subsequent period. Furthermore, frog tadpoles exposed to the early-hatched salamanders were larger at metamorphosis and had a longer larval period than other frog tadpoles. These results suggest that feedback between the predator-superior size balance and prey consumption is a critical mechanism that strongly affects the impacts of early hatching of predators in the short-term population dynamics and life history of the prey. Because consumption of large nutrient-rich prey items supports the growth of predators, a similar feedback mechanism may be common and have strong impacts on phenological shifts in size-dependent trophic relationships.

Introduction

In mid- and high-latitudinal areas, the timing of key life-history events, such as spawning, hatching, and metamorphosis in species with complex life cycle, is intimately associated with the season. The association of life-history events with seasonal changes is called phenology. Phenology is an important field of study in both ecological and environmental sciences because the influence of environmental conditions on the timing of life-history events is species specific (Visser and Both 2005, Both et al. 2009). Hence, by studying phenological shifts of individual species, we gain knowledge about the demography of that species, and by understanding the consequences of species-specific phenological responses to environmental conditions in a community context, we can gain insight into the ecological impacts of climate change (Yang and Rudolf 2010, Nakazawa and Doi 2012).

In many animal species, one important life-history event, from the perspective of species fitness, that is sensitive to environmental change is hatch timing (Van Dongen et al. 1997, Philippart et al. 2003). The environmental sensitivity of hatch timing varies among species, and the species-specific responses of hatch timing can significantly affect interspecific interactions and the resultant community dynamics (Alford and Wilbur 1985, Nakazawa and Doi 2012). When a community includes hatchlings (larvae) of predator and prey species, the relative hatch timing of these species may determine the period of their trophic interaction and the ecological characteristics of the two species (Van Dongen et al. 1997, Philippart et al. 2003). Theoretical studies have proposed two mechanisms by which the hatch-timing might affect the strength of larval predator–prey interactions. First, it might modify the interaction period, thereby changing the strength or duration of the trophic interaction (Cushing 1990, Durant et al. 2005, Visser and Both 2005, Miller-Rushing et al. 2010). We refer to this as the 'classic' hypothesis. Predator–prey interactions are strongest when the timing of the predator's peak energy requirement coincides with the peak availability of prey (Cushing 1990). Such a 'match' occurs proportionally to the duration of their period of interaction, which is determined by the relative hatching phenology of the interacting species. More recently,

(Yang and Rudolf 2010) have hypothesized that the per capita interaction strength is modified by changes in the ontogenetic stages of the predator and prey species during the interaction period. Ontogenetic stage can affect the strength of predator–prey interactions through its effect on the size of individual organisms, which is a main factor determining the strength of predator–prey interactions (De Roos et al. 2003). For example, predation pressure likely increases over the ontogeny of predators because their physical performance increases with their size (Rudolf 2012). In contrast, predation pressure likely decreases over the ontogeny of prey because as a prey's body size increases, so does its ability to protect itself against predator attacks. Hence, changes in relative hatch timing between a predator and a prey species, by modifying their ontogenetic interaction, can have significant consequences (Post 2003, Rasmussen et al. 2014). Compared with the first mechanism, the second mechanism is much less well understood. Moreover, its importance remains largely unrecognized, because the interactive nature of size changes of both predator and prey make the consequences of those changes in predator–prey systems more complicated and difficult to unravel.

Several empirical studies have suggested that the hatching phenology of predator and prey species affects their ontogenetic interactions (Keast 1985, Sredl and Collins 1991, Borchertding et al. 2010, Rasmussen et al. 2014). For example, Rasmussen et al. (2014) showed that large differences in hatch timing between two dragonfly species led to a larger predation impact by nymphs of the earlier hatched species on those of the later hatched species. These studies showed that greater size asymmetry between the predator and prey species during the period of their first interaction, caused by earlier hatching of the predator species relative to that of the prey species, leads to stronger predator–prey interactions. Although there seems to be little doubt that the relative size balance between predator and prey during this first interaction period is critical for initiating the predator–prey relationship, the size balance alone may be insufficient to explain the outcome of predator–prey interactions over a longer period, because the relative sizes of predator and prey change dynamically (Urban 2007).

The average size of individuals of both the predator and prey populations increases over the

course of a season if migration and reproduction are negligible. In particular, the size of individuals in the hatching cohort of both predator and prey in a size-dependent predator–prey relationship can dramatically increase. The predator can increase its size not only through ordinary isometric growth (Borcherding et al. 2000, Urbatzka et al. 2008) but also through allometric growth due to a plastic response known as an inducible offence (Kopp and Tollrian 2003, Kishida et al. 2010). The prey size can be enhanced by ordinary growth, by a plastic response known as an inducible defence such as body size enlargement (Brönmark and Miner 1992, Kishida and Nishimura 2004), and by size-selective mortality (Takatsu and Kishida 2013). Because in a size-dependent predator–prey relationship, size increments of predator and prey operate antagonistically, changes in the relative size relationship between predator and prey can change their interaction dynamics and therefore has fitness consequences. Hence, to better understand the consequences of phenological changes in predator and prey, it is important to examine the dynamic aspects of ontogenetic interactions throughout their interaction period (Yang and Rudolf 2010). Nevertheless, the dynamic nature of ontogenetic interactions has received markedly little attention, despite the growing recognition of the importance of ontogeny in ecological interactions (Rasmussen et al. 2014).

In this study, we experimentally investigated how the hatching phenology of an amphibian predator relative to that of its amphibian prey affected their ontogenetic interactions during the larval period and its consequences for their later life history. As our model system, we used tadpoles of the Japanese brown frog (*Rana pirica* [Matsui]) and the predatory larvae of a salamander (*Hynobius retardatus* [Dunn]). These species constitute an excellent predator–prey system for studying the ecological consequences of hatching phenology and its underlying mechanisms, because they are typical seasonal breeders that can be observed even in small ponds (less than 1 m²), making it practical to conduct small-spatial-scale experiments with adequate numbers of replicates. In addition, the size-dependent nature of the trophic interaction of these two species has already been well studied (reviewed in Kishida et al. 2010). Although *R. pirica* generally hatches earlier than *H. retardatus*, the size of the hatch timing gap differs among ponds (see Materials and methods). We

experimentally exposed frog tadpoles to predatory salamander larvae with different hatch timings to evaluate how hatch timing gaps of different sizes affected larval interactions and to investigate its mechanism. We showed that the predation success of *H. retardatus* depended initially on the predator–prey size balance during their first interaction period, and that the initial predation success determined their subsequent size balance and thus the later predation success of the salamander. Hatching phenology thus strongly influenced predator–prey interactions via feedback between the predator–prey size balance and successful predation (i.e. consumption of frog tadpoles) by the larval salamander predators. Moreover, the phenological impact of this predator–prey interaction also had an impact on other life-history traits of the frogs, namely, the timing of their metamorphosis and their size at metamorphosis. Thus, the size of the hatch timing gap may critically affect the survival and fitness of the frogs during both their aquatic and terrestrial life-history stages.

Materials and methods

Study system and hypotheses

Hynobius retardatus salamanders and *Rana pirica* frogs are distributed throughout Hokkaido Island, Japan. Because these two amphibian species both spawn in mountain ponds in early spring, the larvae of both species frequently coexist in the same pond at high densities and can thus interact during their larval period. In general, *R. pirica* tadpoles hatch earlier than *H. retardatus* larvae, because the frogs lay their eggs along the pond edges, where it is relatively warm, whereas the salamanders deposit their egg clusters in the relatively deep central part of the ponds, where the water temperature is relatively low. In 2013, we carried out field observations of 17 ponds in the Teshio Experimental Forest of Hokkaido University, which is in the northern-most part of Hokkaido Island. During this survey, we found that the initial hatching date of the frog tadpoles ranged from 5 to 18 days before that of the salamander larvae (12 ± 5.3 days, mean $\pm$ SD, $n = 17$ ponds). In general, the larvae of this salamander feed on aquatic invertebrates such as chironomid larvae and tubifex, *R. pirica* tadpoles, and small conspecific larvae (Kishida et al. 2009b). However, because their

invertebrate prey is scarce and inactive in spring, the primary prey items of the salamander larvae from late spring to early summer are often other amphibian larvae, including frog tadpoles. Frog tadpoles are relatively large prey items for the salamander larvae, which are gape-limited predators, so predator–prey interactions in this system are determined by the size balance between them. One factor which determine the size-dependent predator–prey interaction is the antagonistic phenotypic plasticities exhibited by these two species (Kishida et al. 2009a). In the presence of relatively small frog tadpoles, the salamander larvae develop a broadened gape during their early ontogeny that enhances their ability to swallow the frog tadpoles (Michimae and Wakahara 2002, Takatsu and Kishida 2013). In contrast, frog tadpoles that are exposed to predation risk from salamander larvae develop an enlarged body and tail by thickening their epithelium tissue, and this bulgy phenotype makes it harder for the salamander larvae to swallow them (Kishida and Nishimura 2004).

On the basis of these findings, we predicted that when the gap in hatch timing between the frogs and salamanders was smaller (i.e. when the salamander hatched soon after the frogs), the predator–prey interaction between them would be stronger. Furthermore, we hypothesized that the following sequential mechanisms to explain this result: first, when the gap in hatch timing is small, the salamander hatchlings should be more able to consume frog tadpoles during their first interaction period (i.e. just after the salamanders hatched), because they encounter tadpoles that are less grown and thus smaller. Next, owing to the advantages of utilizing this nutrient-rich prey, the salamander larvae should grow rapidly, increasing not only their overall size but also, through phenotypic plasticity, their gape width (Michimae and Wakahara 2002, Kishida et al. 2014). As a result, even though the surviving frog tadpoles also become larger, we hypothesized that the increase in the salamander gape should enable the salamander larvae to maintain their size advantage, and the early-hatched salamander larvae should thus be able to continue to impose strong predation pressure on the tadpoles during the subsequent interaction period.

Experimental design, settings, and measured traits

Experimental design

Although many field studies have suggested that the size of the gap in hatch timing between predator and prey strongly influences their interaction strength, these studies did not exclude alternative, seasonal factors, such as temperature and resource availability, which can affect the demography of predator and prey (Sredl and Collins 1991, Boone et al. 2002). So that we could rigorously test our hypotheses, we designed an indoor experiment that allowed us to control for the possible effects of environmental factors other than hatch timing on predator–prey interactions as well as to closely trace dynamic changes in both the size balance of the focal species and predation pressure.

The experiment consisted of five treatments: four 'Salamander' treatments included a predatory salamander larva, hatched 5, 12, 19, or 26 days after the frog tadpoles (i.e. 5d, 12d, 19d and 26d Salamander treatments), and a fourth 'No-salamander' treatment, which did not include a salamander larva (Supplementary material Appendix A1 Table A1).

Animal collection and experimental setting

During 27–29 May 2013, we collected 10 *R. pirica* egg masses and six *H. retardatus* egg clusters from several ponds in the Teshio Experimental Forest of Hokkaido University. The frog egg masses were placed in stock tanks filled with aged tap water and incubated in our experimental room, which was maintained at 15 °C with a natural light–dark regime. For each experimental treatment we used a 22-L tank (43.6 cm L × 28.4 cm W × 14.1 cm D) filled with 4 L of aged tap water. Five days after the frog tadpoles hatched, we randomly assigned 50 similar-sized hatchlings (body length, average ± SD, 5.36 ± 0.19 mm, n = 45) to each tank. Then we assigned one salamander hatchling to each Salamander treatment tank according to the hatching schedule (Supplementary material Appendix A1 Table A1). The densities of hatchlings were within the range of those in their natural habitat (Kishida and Nishimura 2006). We performed 10 replicates of each Salamander treatment, and 5 replicates of the No-salamander treatment.

To avoid confounding genetic effects with hatching dates, we used the salamander

hatchlings derived from the same clusters in all Salamander treatments by controlling their hatch timing. Each of the six collected salamander embryo cluster (i.e. full or half sibs) was divided into fourths, and each quarter was designated for one of the four Salamander treatments. To control their hatch timing, we incubated the embryos in a refrigerator maintained at about 5 °C for appropriate periods. Because the salamander hatchlings are highly vulnerable and less active for several days after they hatch, we added the salamander hatchlings to the treatment tanks two days after they hatched. Salamander hatchlings from different egg clusters were randomly assigned to the tanks in each treatment. It is possible that keeping the embryos at low temperature could have a confounding effect on their growth. To evaluate this effect, we reared salamander larvae with different hatch timings without frog tadpoles and compared their growth. Seven salamander larvae that hatched at 5, 12, 19, or 26 days after the frog tadpoles hatched (i.e. a total of 28 larvae) were each reared alone in small cases (7.9 cm L × 7.9 cm W × 5.0 cm D) filled with 300 ml of aged tap water (i.e. as in the No-tadpole treatment). The hatch timing itself had no impact on larval growth, as assessed by the snout-vent length (SVL), of salamander larvae reared alone during days 0–14 (one-way ANOVA: $F_{3,23} = 0.77$, $p = 0.52$) or during days 15–28 ($F_{3,23} = 1.74$, $p = 0.19$) after they hatched. This result indicates that the low-temperature procedure used to control the hatch timing of the salamanders probably did not have any confounding effect on our experimental results.

Throughout the experiment, we exchanged the rearing water every two days. The frog tadpoles were fed rabbit chow ad libitum and the salamander larvae were fed five tubifex, as an alternative food source, every two days. The experiment was conducted in a laboratory.

Unfortunately, two salamander larvae in the 12d Salamander treatment and one in the 26d No-tadpole treatment accidentally died shortly after they were assigned to the treatment tank. Therefore, these tanks were removed and their data were unavailable for the statistical analyses.

Predator–prey interactions, morphology, and growth of predators and prey

After the first assignment of the salamander hatchlings, we took digital photographs of all surviving frog tadpoles and salamander larvae ventrally every seven days by using a scanner

(CanoScan 9000F, Canon Co., Ltd., Japan). We counted the number of surviving frog tadpoles in the photographs. We defined the strength of the predator–prey interaction as number of frog tadpoles consumed by the salamander larvae, which we calculated by subtracting the “average number of dead frog tadpoles in the No-salamander treatment” from “the number of dead frog tadpoles in each tank of the four Salamander treatments”. No consumption of frog tadpoles by the salamander larvae occurred in any Salamander treatment after around 82 days after the frog tadpoles hatched; therefore, we did not scan any animals after that day.

We used ImageJ 1.45s software to measure gape width (i.e. the head width at eye level, GW), the largest head width (HW), and the SVL of all salamander larvae, and to measure the largest body width (BW) and SVL of 10 frog tadpoles randomly selected from each tank. Because predation success greatly depends on the size balance between the gape width of the salamander larvae and the body size of the frog tadpoles (Kishida and Nishimura 2004), we focused particularly on the absolute size and changes (growth) of gape width in the salamander larvae and body width in the frog tadpoles. We defined changes of gape width in the salamander larvae and of body width in the frog tadpoles in a given period as the increment of those traits in that period (e.g. two weeks). We also quantified the antagonistic inducible offences and defences of the predators and prey. (Michimae and Wakahara 2002) showed that the ratio of gape width (GW) to largest head width (HW) in larvae of this salamander can be used as an index of the inducible offensive morph. A larger value of this index means stronger expression of the offensive morph. Because the inducible bulgy tadpole morph has transparent, thickened epithelium tissues, we measured the length of the thickened epithelium tissue (TL) along the line of the greatest body width. Then, we calculated the ratio $BW / (BW - TL)$ as the index of the inducible bulgy morph. Here, an index value of one means no induction of the defensive morph and higher positive values mean greater expression of the bulgy morph. In our analyses, we used individual trait data for the salamander larvae, because each treatment tank contained only one salamander, and for the frog tadpole trait data, we used tank means. To calculate the mean body width and mean changes of body width in the frog tadpoles of a tank, we used only the data from the

40% of the frog tadpoles with the smallest body width (i.e. four individuals) among the 10 measured frog tadpoles per tank. We considered this approach to be appropriate because salamander larvae selectively consume smaller frog tadpoles (Kishida et al. 2009a, Takatsu and Kishida 2013). To characterize the size balance between the salamander larva and the frog tadpoles in each treatment tank, we calculated the ratio of the gape width of the salamander larva to the mean body width of the frog tadpoles (i.e. GW/BW).

Life history of the salamanders and frogs

Timing of metamorphosis (or duration of the larval period) and size at metamorphosis are important life-history traits in amphibians because they have fitness consequences, and both of these traits are affected by the strength of the predator–prey interaction (Benard 2004, Relyea 2007). We expected that the hatch timing of the salamanders would affect the life-history traits of the amphibians by changing the strength of their predator–prey interaction. We defined the timing at metamorphosis in the frogs as the first day on which their forelimbs were completely emerged. In the salamanders, we defined the timing at metamorphosis as the day when the external gills were found to be completely absorbed. We observed the tanks daily to check for the occurrence of metamorphosis in both amphibian species and removed the metamorphs when we found them. We also measured the wet body mass of each collected metamorph.

We defined the total coexistence time, during which both salamander larvae and frog tadpoles coexisted in a tank, as the number of days that the larval periods of the frogs and salamanders in the tank overlapped. Here, we used the median day to metamorphosis of the frogs in each tank as the larval period of the frogs.

Statistical analyses

Because the variance of most of the measured traits differed greatly among the treatments (O'Brien test: $p < 0.01$), we used the nonparametric Kruskal-Wallis test for comparison of traits with unequal variance among treatments. If the Kruskal-Wallis test showed significant differences among

the treatments, we used the Wilcoxon test to perform pairwise comparisons and adjusted the statistical significance of the result of each pairwise comparison by the Bonferroni method, using a significance level of $\alpha = 0.05$. For variables that satisfied the assumptions of a parametric test, we used one-way ANOVA to evaluate treatment effects. If we found significant treatment effects, we used the Tukey-Kramer HSD test to perform pairwise comparisons. In addition to the among-treatments comparisons, we used linear regression to analyse the data of individual tanks within certain treatments as necessary to test our specific hypotheses.

Results

One-way ANOVA for SVL at the hatching time of the frog tadpoles in the Salamander and No-salamander treatments showed that the initial body size of the frog tadpoles did not differ among the treatments ($F_{4,40} = 0.73$, $p = 0.58$). Similarly, Kruskal-Wallis test for SVL at the hatching time of the salamanders indicated that the size of the assigned salamander hatchlings did not differ among the four Salamander treatments (Kruskal-Wallis test: $\chi^2_3 = 6.91$, $p = 0.07$).

Predator–prey interaction strength

The hatch timing of the salamanders affected the total number of frog tadpoles consumed by the salamander larvae (Kruskal-Wallis test of the four Salamander treatments: $\chi^2_3 = 20.53$, $p = 0.0001$). When the salamander hatched earlier, the strength of the predator–prey interaction was stronger (Fig. 1A).

Hypothesized mechanism 1: Importance of the interaction period

We hypothesized that the hatch timing of the salamanders would affect the predation strength through changes in the interaction period. If this hypothesis is correct, then the number of frog tadpoles consumed should be proportional to the time they coexisted with the salamander larva in a tank. Kruskal-Wallis test results indicated that among the four Salamander treatments, the

coexistence time was longer in the early-hatch treatments (i.e. the 5d and 12d Salamander treatments) than in the late-hatch treatments (i.e. the 19d and 26d Salamander treatments) ($\chi^2_3 = 28.88$, $p < 0.0001$; coexistence time, mean $\pm$ SD, in the 5d, 12d, 19d, and 26d Salamander treatments was 62.2 ± 7.7 , 60.7 ± 2.6 , 51.6 ± 3.3 , and 43.1 ± 3.7 days, respectively). Thus, there is a relationship between coexistence time and predation mortality, consistent with the hypothesis. However, further analysis revealed that this relationship is spurious. In all treatments, most of the predation occurred in the first 42 days after the salamander hatched (Fig. 1B), and the frog tadpoles experienced very little mortality between day 42 and the end of the experiment (i.e. 103 days after the frog tadpoles hatched, when all surviving amphibians in all tanks had completed metamorphosis; Supplementary material Appendix A1 Table A1). The number of frog tadpoles consumed after day 42 did not differ among the Salamander treatments (Kruskal-Wallis test: $\chi^2_3 = 1.08$, $p = 0.78$). Hence, the substantial variation in the mortality of the frog tadpoles among the four Salamander treatments occurred during the same time period (i.e. the first 42 days after the salamanders hatched: Kruskal-Wallis test, $\chi^2_3 = 17.69$, $p = 0.0005$). These results clearly indicate that although the salamander hatch timing affected the coexistence time, the mechanism of the effects of hatch timing on the predation strength was not the duration of the interaction period.

Hypothesized mechanism 2: Ontogenetic interaction

Alternatively, we hypothesized that feedback occurs between the predator-prey size balance and prey consumption. This feedback consists of two sequential processes associated with size-dependent predation. As the initial process, we hypothesized that early hatching gives the salamander larvae more opportunities during the period of their first interaction to consume frog tadpoles than late hatching, because during the first interaction period the size balance is likely to favour the salamander larva. As the second process, we hypothesized that salamander larvae that successfully consume frog tadpoles grow rapidly, and that their enlarged size allows the salamander larvae to continue to consume frog tadpoles more easily.

In the following analyses, we compare size relationships in relation to the salamander's age (i.e. days after the salamander hatched, not the calendar date or days after the frogs hatched). In this way, we were able to compare ontogenetic interactions among the treatments with salamander larva and frog tadpoles that had been coexisting in each tank for the same amount of time. We found that the ratio of salamander GW to frog tadpole BW at the hatching time of the salamanders differed significantly among the four Salamander treatments (Kruskal-Wallis test: $\chi^2_3 = 24.31$, $p < 0.0001$, Fig. 2A). The initial size balances in the early-hatch Salamander treatments, which favoured the salamander larvae more than those of the late-hatch Salamander treatments, resulted in greater predation mortality of the frog tadpoles during the first two weeks that the two species coexisted in the tank (Kruskal-Wallis test: $\chi^2_3 = 26.15$, $p < 0.0001$, Figs 1B and 2A). The importance of the initial size balance for the interaction strength in the initial period was additionally supported by the linear regression analysis results, based on the individual tank data of the 5d and 12d Salamander treatments (Fig. 2A). The GW/BW ratio at the hatch time of the salamanders significantly explained the number of frog tadpoles consumed during the subsequent two weeks in the 5d Salamander treatment ($F_{1,8} = 5.59$, $p = 0.02$, $R^2 = 0.41$), and it marginally did so in the 12d Salamander treatment ($F_{1,6} = 3.34$, $p = 0.12$, $R^2 = 0.38$).

Our second hypothesized process was strongly supported by the following analysis results. We predicted that the early-hatched salamanders, which consumed more frog tadpoles in the early experimental period, would exhibit more rapid growth and greater consumption of frog tadpoles in subsequent periods, than the later-hatched salamanders would. The one-way ANOVA ($F_{3,36} = 12.97$, $p < 0.0001$) and Tukey-Kramer HSD test results for the four Salamander treatments revealed that the salamander growth during the first two weeks after they hatched was significantly larger in the 5d and 12d Salamander treatments than in the 19d and 26d Salamander treatments (GW growth, mean $\pm$ SD, in the 5d, 12d, 19d, and 26d treatments, 4.0 ± 1.0 , 3.6 ± 0.9 , 2.5 ± 0.7 , and 2.0 ± 0.6 mm, respectively; see Supplementary material Appendix A1 Fig. A1). Hence, the differential growth among the treatments caused the variation in the predator-prey size balance among the treatments to

be larger two weeks after the salamanders hatched (Kruskal-Wallis test of the GW/BW ratio: $\chi^2_3 = 17.98$, $p = 0.004$, Fig. 2B). The GW/BW ratio of the 5d Salamander treatment was largest among the Salamander treatments, followed by the 12d, 19d, and 26d Salamander treatments. The importance of frog tadpole consumption in maintaining the salamander-superior size balance in the early-hatch treatments is additionally evidenced by a significant result of the linear regression in which the number of consumed frog tadpoles during the first two weeks was the explanatory variable and the predator-prey size balance two weeks after the salamander hatched in the 5d Salamander treatment was the response variable ($F_{1,8} = 44.83$, $p = 0.0002$, $R^2 = 0.85$, data not shown). The Kruskal-Wallis test results for the four Salamander treatments revealed that the salamander hatch timing influenced the number of frog tadpoles consumed during the second two-week period (i.e. 15–28 days after the salamander hatched) ($\chi^2_3 = 11.12$, $p = 0.01$, Fig. 2B). These test results indicate that the predation impact during this second period reflects the size balance at the end of the first period (at 14 days after the salamander hatched) (Fig. 2B). Thus, the size balance resulting from the past predator-prey interaction determined subsequent predator-prey interactions. Furthermore, linear regression analyses of individual tank data of the 5d and 12d Salamander treatments showed that the GW/BW size ratio on day 14 after the salamander hatched significantly explained the number of frog tadpoles consumed in the subsequent two weeks (5d Salamander treatment, $F_{1,8} = 15.56$, $p = 0.004$, $R^2 = 0.66$; 12d Salamander treatment, $F_{1,6} = 12.46$, $p = 0.012$, $R^2 = 0.67$, Fig. 2B).

Impacts of hatching phenology on the morphology and life history of predator and prey

Antagonistic inducible offence and defence

The degree of expression of the offensive morph of the salamander larvae differed among the four Salamander treatments at two weeks after the salamander hatched (one-way ANOVA: $F_{3,34} = 8.56$, $p = 0.0002$, Fig. 3A). Early-hatched salamander larvae had a more offensive morph than later-hatched individuals (Fig. 3A). We also found significant differences in the degree of expression of the defensive morph of the frog tadpoles among the four salamander treatments at this time

(Kruskal-Wallis test: $\chi^2_3 = 17.51$, $p = 0.0006$). The frog tadpoles exposed to the earliest hatched salamanders developed the defensive morph to a greater degree (Fig. 3B).

Life history of the salamanders and frogs

Frog tadpoles exposed to the early-hatched salamanders were larger at metamorphosis (one-way ANOVA: $F_{4,38} = 21.97$, $p < 0.0001$, Fig. 3C) and their larval period lasted longer (one-way ANOVA: $F_{4,38} = 14.11$, $p < 0.0001$, Fig. 3D) than frog tadpoles exposed to the later-hatched salamanders. There was no significant treatment effect on size at metamorphosis of the salamander larvae (one-way ANOVA: $F_{3,32} = 1.90$, $p = 0.15$). Also, there was no significant difference in the duration of the larval period of the salamanders (Kruskal-Wallis test: $\chi^2_3 = 5.67$, $p = 0.13$). The early-hatched salamander larvae metamorphosed at an earlier calendar date than the later-hatched salamander larvae (Kruskal-Wallis test: $\chi^2_3 = 16.91$, $p = 0.0007$), indicating that the salamander hatch timing had no effect on the duration of their larval period.

Discussion

Previously, alteration of the duration of the interaction period was focused as the primary mechanism offered to explain the effects of phenological shifts of the predator and prey species on their interactions (Cushing 1990, Durant et al. 2005, Visser and Both 2005, Miller-Rushing et al. 2010). Some studies, however, suggested that the hatching phenology of predator and prey also strongly affected their interactions by changing their size relationship (Keast 1985, Sredl and Collins 1991, Borchertding et al. 2010, Rasmussen et al. 2014). These latter studies focused on the size balance between predator and prey during their first interaction period. The initial size balance may be a critical determinant of the interaction strength at the initial time, but it may not be enough to explain the outcome of predator–prey interactions for the entire duration of their interaction. The initial size balance cannot be maintained over the long term because the sizes of both the predator and prey species change with time through the combined effects of ordinary growth, phenotypic plasticity, and size-selective mortality (Olson 1996, Kishida et al. 2009a, Kishida et al. 2011, Takatsu

and Kishida 2013). Even if the initial size balance favours the predator, the impacts of the predator on the prey will be transient if the growth of prey relative to that of the predator is high (i.e. if the prey can readily enter a size refuge) (Urban 2007, Rasmussen et al. 2014). Despite this possible ephemerality of the predator–prey size balance, the fact that hatching phenology has a considerable impact on predator–prey interactions (Sredl and Collins 1991, Eitam et al. 2005, Borchertding et al. 2010, Rasmussen et al. 2014) suggests that some mechanism would exist to maintain or strengthen the predator-superior size balance.

Here, we experimentally demonstrated that predator hatch timing dramatically affects trophic interactions between larval amphibians. In contrast to the classic view, we found that the duration of the period of coexistence is not an important mechanism of this effect; rather, the initial size balance between the salamander larva and the frog tadpoles, determined by the predator hatch timing, was the key driver of the predator–prey interactions. Our focus on mechanisms showed that early hatching of the salamanders drives the interplay between salamander-superior size balance and prey consumption. Compared to late hatching, early hatching of the salamanders led to a predator-superior size balance that allowed more salamander larvae to successfully consume frog tadpoles just after the salamanders hatched. This consumption of frog tadpoles made it possible for the early hatched salamanders to grow rapidly, and thus maintain the initial predator-superior size balance in many tanks of the early-hatch treatments. As a result, the early-hatched salamander larvae imposed strong predation pressure on frog tadpoles during the subsequent period, whereas the frog tadpoles suffered very little mortality from the later-hatched salamanders at any time during the experiment. Another possible mechanism influencing the maintenance of the predator-superior size balance might be frog tadpole size changes, because the size-balance dynamics reflect both predator and prey size changes. In this case, this mechanism did not operate because the differences in tadpole body width among the four Salamander treatments gradually decreased with time (Fig. 4). The body width of the frog tadpoles differed very little among the treatments at two weeks after the salamander hatched, because the frog tadpole body width in the early-hatch Salamander treatments increased at a

much faster rate than it did in the later-hatch Salamander treatments (one-way ANOVA, growth in BW of frog tadpoles during the first two weeks after salamander hatched among the four Salamander treatments: $F_{3,34} = 121.25$, $p < 0.0001$, Supplementary material Appendix A1 Fig. A1). This fast rate of increased was due to the expression of the inducible bulgy morph and, possibly, size-selective mortality (see also Takatsu and Kishida 2013). Therefore, feedback between the size balance and prey consumption mediated by the rapid growth of predators was the key mechanism explaining why the early hatching of the salamanders resulted in the continuation of a strong predator-prey relationship between the salamander larvae and frog tadpoles over a relatively long time period.

Enlargement of the capturing organ of the salamander larvae (i.e. the gape) can reflect both ordinary isometric growth and allometric growth due to the plastic response of an inducible offence (Michimae and Wakahara 2002). At two weeks after the salamanders hatched, the early-hatched salamander larvae had developed the inducible offensive morph to a greater degree than the later-hatched ones had (Fig. 3A). Since salamander larvae with the offensive morph consume more frog tadpoles than those with the non-offensive morph even if their canonical size (body size) is similar (Takatsu and Kishida 2013, Kishida et al. 2014), we can infer that the allometric growth due to the inducible offence itself contributed to the predation success of the early-hatched salamander larvae in this experiment, independently of ordinary growth. Inducible offences are widespread among predator taxa (reviewed by Kishida et al. 2010) and theory suggests that the evolution of inducible offences is part of an adaptive phenotypic plasticity strategy of predators to overcome the inducible defences of their prey (Mougi et al. 2011). Despite the prevalence of inducible morphs, our understanding of the outcomes due to inducible offences in ecological interactions remains very limited (Kopp and Tollrian 2003, Banerji and Morin 2013). Thus, other systems that include predator species that adopt an inducible offensive strategy should be studied to deepen our knowledge about how phenotypic plasticity in predators affects the ecological consequences of phenological shifts of predators and prey. Ordinary isometric growth is also likely to be important in mediating the effects of hatching phenology on predator-prey interactions. In this study, at 14 days after the salamander

hatched, the SVL (the index of canonical size) of the salamander larvae in the 5d Salamander treatment was 16.4%, 14.2%, and 14.1% larger than that of the salamander larvae in the 12d, 19d, and 26d Salamander treatments, respectively (one-way ANOVA: $F_{3,34} = 7.60$, $p = 0.0005$, data not shown). This larger growth increment of the early-hatched salamander larvae might enable them to consume more frog tadpoles not only by enhancing the predator-superior size balance but also by increasing the energy requirement of individuals (Fonds et al. 1992). Such effects of ordinary growth may be more common than those of inducible offences because most organisms substantially change their size in the course of their development and prey-capturing ability and food-intake amount both scale with predator size (Werner and Gilliam 1984).

Some studies have suggested that the hatching phenology of predators and prey strongly influences the outcomes of their interactions (Keast 1985, Sredl and Collins 1991, Post 2003, Eitam et al. 2005, Borchertding et al. 2010). For example, Borchertding et al. (2010) reported that when perch (*Perca fluviatilis* [Linne]) (a gape-limited predator) hatch earlier than their prey (bream, *Abramis brama* [Linne]) does, the size of most individual perch is large enough for them to prey on bream at their first encounter. This size relationship is associated with a decrease in the duration of the period during which bream are found in the stomachs of perch, suggesting that when perch hatch early they rapidly overexploit the bream. In addition, there may be feedback between the predator-superior size balance and predation success under the inferred strong top-down effect of perch on bream because the consumption of prey fish sometimes leads to intensive acceleration of predator growth (DeAngelis et al. 1980). Post (2003) reported that the earliest hatched largemouth bass (*Micropterus salmoides* [Lacepede]) can prey on later-hatched conspecifics because of the large size difference between them when they first meet. In addition, they found that during the predator's growth season, individual predators continued to grow at a higher rate and the predator-superior size balance and cannibalism were maintained, regardless of increases in the size of prey individuals through the season. These findings are good evidence that feedback between the size balance and prey consumption is a key process determining the effects of hatching phenology on predator-prey

interactions.

Why does hatching phenology strongly influence predator–prey interactions? If feedback between size balance and prey consumption is necessary for phenological shifts to have a strong impact on predator–prey interactions, then the nutrient richness of prey individuals is likely key to explaining variation of the outcome. If a predator species utilizes a prey species that is sufficiently nutrient-rich to promote predator growth that surpasses increases in the prey size that would allow it to escape into a size refuge, the predator-superior size balance resulting from relatively early hatching of predators is likely to be maintained or strengthened. Larger prey items have a greater nutrient content, and predator species interact with relatively large prey items in size-dependent predator–prey interactions. These facts suggest that feedback between the predator–prey size balance and prey consumption likely occurs in size-dependent predator–prey interactions. In turn, shifts in hatching phenology may commonly have a strong impact on size-dependent predator–prey interactions.

We investigated the impacts of hatching phenology on the life-history traits of predator and prey in an amphibian system. Variation in the size at metamorphosis and the timing of metamorphosis is common in organisms with complex life histories (Benard 2004) and can be an important driver of population and community dynamics (Kishida et al. 2010). In this study, we found that the salamander hatch timing affected the size of frogs at metamorphosis and the timing of their metamorphosis. For example, the earliest hatched salamanders (i.e. 5d) caused the frog tadpoles to be 26.5% larger at metamorphosis and they metamorphosed 8.9% later compared with the latest hatched salamanders (i.e. 26d). Differential effects on life-history traits among the treatments could reflect differences in predation pressure among them. The results of a recent field study (Kishida et al. 2014), in which the presence or absence of offensive and non-offensive morphs of salamander larvae were experimentally manipulated, support this possibility. Kishida et al. (2014) showed that the frog tadpoles were larger at metamorphosis and they metamorphosed later when they were exposed to stronger predation pressures from offensive-morph salamander larvae. Although most models predict

a smaller size at metamorphosis and earlier timing of metamorphosis in response to predation risk (Benard 2004), several mechanisms can explain our results, including the role of basal resources, plasticity, and size refugia (Relyea 2002, Vonesh 2005, Takatsu and Kishida 2013).

Regardless of the specific mechanism accounting for changes in the frog's size at metamorphosis and the date of their metamorphosis, these changes may affect frog fitness. In amphibians, a larger size at metamorphosis is associated with increased adult growth and survivorship, increased body size at maturity, and increased reproductive success (Smith 1987, Altwegg and Reyer 2003). Conversely, later-metamorphosed adults may have a lower survival rate and growth (Altwegg and Reyer 2003). Moreover, later metamorphosis may greatly affect survivorship at metamorphosis because amphibian species that breed in lentic habitats often face total destruction (death of all larvae) if the pond dries up before the larvae complete metamorphosis (Semlitsch et al. 1988). In our sampling field in 2013, 57.6% of a total of 99 ponds in which our focal species spawn in the early spring had dried up by two months after the eggs hatched. Thus, delayed metamorphosis (i.e. a longer larval period) of amphibians breeding in temporary ponds may lead to a considerable reduction in the number of metamorphosed adults. The hatching phenology of *H. retardatus* and *R. pirica* is likely to have complex effects on the survivorship and fitness of the frogs at both their aquatic and terrestrial stage. Early hatching of the frogs relative to the hatching of the salamanders may reduce their predation mortality during their larval period, but the resultant small size at metamorphosis of the frogs might negatively affect their fitness as adults. In contrast, although relatively late hatching frogs are exposed to strong predation pressure from the salamander larvae, the surviving frogs may perform better after metamorphosis because of their larger size. In general, our results suggest that in size-dependent predator–prey amphibian systems, the effect of hatching phenology on the prey population is mediated not only by changes in survival but also by changes in life-history traits. We expect that similar processes may operate, with similar results, in other trophic systems with a gape-limited predator and a prey with a complex life history, such as in other larval amphibian predator–prey systems.

This study demonstrated that the growth of predator individuals plays a key role that links hatching phenology to predator–prey interactions. The growth of individuals is affected by various biotic and abiotic environmental factors such as alternative prey (resource) availability, predation risk, and temperature, and the impact of hatching phenology on ecological interactions is likely to be modified by these factors. For example, the availability of alternative prey for the predator species seems to strongly affect the consequences of phenological shifts. Post (2003) found that in early-hatched largemouth bass both hatch timing and the availability of alternative prey for the earliest hatched largemouth bass are critically important for initiating cannibalism dynamics in the population. In our system as well, alternative prey may be important, because salamander hatchlings are unlikely to predate on frog tadpoles without also consuming alternative prey items such as small invertebrates and conspecifics (Kishida, unpublished data). The impact of hatching phenology can also be influenced by the presence of top predatory insects. The larvae of *H. retardatus* strongly reduce their foraging activity and decrease their growth in the presence of larval dragonfly risk cues (Kishida et al. 2009a, Kishida et al. 2011). Even if the salamanders hatch early relative to the frogs, they may not become substantial predators in the subsequent period if larval dragonflies are present. To improve our ability to predict the impact of phenological shifts, future studies should further consider how changes in the community composition can change the outcome of shifts in hatching phenology.

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- Supplementary material (available online as Appendix oik-... at <www.oikosoffice.lu.se/appendix>).
- Appendix 1.

Figure legends

Figure 1. Effects of salamander hatch timing on (A) total predation mortality during the experiment and (B) predation mortality of the frog tadpoles per each 14-day period of coexistence (i.e. days after the salamander larva hatched) (mean $\pm$ SE). Error bars in panel (A) represent 95% confidence intervals. Means not sharing the same letter are significantly different. Asterisks in panel (B) denote significant differences in predation mortality among the treatments ($p < 0.001$; Kruskal-Wallis test).

Figure 2. Relationship between prey consumption and the predator-prey size balance. GW/BW is the ratio of the gape width (GW) of the salamander larva to the average body width (BW) of the frog tadpoles. Each circle represents data for an individual tank and squares denote the mean size balance and predation mortality of each treatment. Error bars denotes 95% confidence intervals. (A) Relationship between the initial size balance and predation mortality of the frog tadpoles during the first 2 weeks. The solid ($y = -25.44 + 20.63x$, $R^2 = 0.41$) and dashed ($y = -5.25 + 9.77x$, $R^2 = 0.36$) lines show the linear regression results for the 5d and 12d Salamander treatments, respectively. (B) Relationship between the size balance at two weeks (14 days) after the salamander hatched and the predation mortality of the frog tadpoles during the subsequent 2 weeks (i.e. days 15–28). The solid ($y = -22.29 + 24.49x$, $R^2 = 0.66$) and dashed ($y = -8.99 + 10.68x$, $R^2 = 0.67$) lines show the linear regression results for the 5d and 12d Salamander treatments, respectively.

Figure 3. Impact of hatching phenology on traits of the salamanders and frogs. (A) Degree of offensive morph development in the salamander larvae and (B) degree of defensive morph development in the frog tadpoles at 14 days after the salamanders hatched. (C) Size at and (D) time (days) to metamorphosis of frog tadpoles. Abbreviations: 5d, 12d, 19d, 26d, and No sal indicate the 5d, 12d, 19d, and 26d Salamander treatments and No-salamander treatment, respectively. Error bars denote 95% confidence intervals. Means not sharing the same letter are significantly different.

Figure 4. Changes in the size relationship between salamander gape width and frog tadpole body width from days 0 to 14 after the salamanders hatched. The four lower circles (at the left end of each connecting lines) and the four upper circles (at the right end) represent size relationships on the hatch date of the salamanders (i.e. day 0) and on day 14 after the salamanders hatched, respectively. The size of the lower (upper) circles indicates the number of frog tadpoles consumed during days 0–14 (days 15–28) after the salamanders hatched. Shading shows the area of a salamander-superior size balance (i.e. salamander gape width is bigger than frog tadpole body width). Error bars denote 95% confidence intervals.

Figure 1

Figure 2

Figure 3

Figure 4

Appendix 1

Table A1. Frog–salamander coexistence periods during the experiment. Days after the frog hatch date and the total duration of the experiment (day) are shown in boldface (based on the calendar date).

Days after the salamander hatch date, shown in normal type, indicate the coexistence period of the salamander larvae and frog tadpoles in each treatment over the course of the experiment.

Abbreviations: 5d, 12d, 19d, 26d, and No sal indicate the 5d, 12d, 19d, and 26d Salamander treatments and the No-salamander treatment, respectively.

Days after the frog hatch date		0	5	12	19	26	33	40	...	103
Days after the salamander hatch date	5d		0	7	14	21	28	35	...	
	12d			0	7	14	21	28	...	All survived amphibian larvae completed metamorphosis
	19d				0	7	14	21	...	
	26d					0	7	14	...	
	No sal								...	

Figure A1

Figure A1. Growth of (A) gape width (GW) of the salamander larvae and (B) body width (BW) of the frog tadpoles during the first two weeks after the salamanders hatched in the four Salamander treatments. Means not sharing the same letter are significantly different. Error bars denote 95% confidence intervals.