Predator Effects on Metamorphosis: The Effects of Scaring Versus Thinning at High Prey Densities

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Organisms with complex life cycles face the challenge of when to switch between habitats and foraging strategies over ontogeny in ways that improve their fitness. Metamorphosis is a well-studied life history event in animals, and ecologists have spent decades trying to understand how the size at and time to metamorphosis are altered by natural stressors such as competition and predation. The challenges in interpreting the effects of predators on metamorphic decisions include the need to compare predator species that pose different levels of risk, compare the roles of predators inducing fear versus thinning of the density of prey, and examine prey life history traits and behavior over ontogeny. We addressed these challenges in a mesocosm experiment in which we introduced a high initial density of hatchling Northern Leopard Frogs (*Rana pipiens*) and exposed them to three different species of caged predators (to induce three different levels of fear), three rates of hand-thinning (to mimic the thinning effect of each predator), or three species of lethal predators (to cause induction and thinning). Under these initial high densities, we found that caged predators had no effects on tadpole activity, growth, and development. This outcome was likely due to the high density of tadpoles causing high competition, which can inhibit anti-predator responses. High rates of hand thinning caused decreased tadpole activity, greater mass, and faster development. Interestingly, lethal predators caused phenotypic changes that were largely in line with the hand-thinning effects alone. These results suggest that at high initial prey densities, the thinning process of predation appears to play a much more important role in prey metamorphosis than induction from predatory chemical cues.

THERE is a long history of biologists trying to understand the conditions and processes responsible for causing remarkable changes between life stages in metamorphic animals (Gilbert and Frieden, 1981; Kaltenbach, 1996; Hall and Wake, 1999; Truman and Riddiford, 2002). Ecologists have given a great deal of attention to how animals make decisions about when and at what size to metamorphose under different ecological conditions including ephemeral habitats, competition, and predation (Semlitsch et al., 1988; Newman, 1992; Altwegg, 2002). Predation has received a particularly large amount of attention from both theoretical and empirical perspectives. Theoretical work on predator impacts on metamorphosis has spanned several decades and produced a wide variety of predictions about when, and at what size, an individual should metamorphose depending upon the assumptions employed (Wilbur and Collins, 1973; Werner, 1986; Ludwig and Rowe, 1990; Rowe and Ludwig, 1991). Two reviews of experimental work (Benard, 2004; Relyea, 2007) have concluded that predators can impact the time to, and size at, metamorphosis by inducing prey to want to leave the larval environment, by inducing prey to change their behavior and morphology (which come with associated growth and development costs), and by thinning the prey population (thereby reducing prey competition). Because multiple processes associated with predation can simultaneously affect metamorphic decisions, it is difficult to understand the underlying mechanisms based on "input-output" experiments that observe growth and developmental rate at the end of an experiment.

A large number of studies have investigated either the effect of restrained (i.e., caged) predators, which can induce anti-predator defenses, or the effect of lethal predators, which simultaneously induce anti-predator defenses and thin the prey population. However, we lack experiments that directly compare the two scenarios for metamorphs (for analogous larval experiments, see Van Buskirk and Yurewicz, 1998; Relyea, 2002a). Because lethal predators cause thinning, we need to isolate the process of thinning the prey population, in the absence of predator induction, to know the relative role that thinning plays in metamorphic decisions. To better interpret how organisms arrive at a particular time to and size at metamorphosis, it is critical to quantify associated changes in behavior, growth, and development of individuals at multiple points in ontogeny prior to metamorphosis. Doing so allows insight into the mechanisms underlying a particular metamorphic outcome since the final metamorphic outcome can be achieved via numerous different larval trajectories. Finally, using multiple predator species would determine how induction and thinning impact metamorphosis across a wide range of predation risk and predator thinning rates.

We addressed these challenges using a mesocosm experiment that sought to examine the roles of induction by different predators, thinning rates of different predators, and the lethal presence of different predators on metamorphosis in Northern Leopard Frogs (*Rana pipiens*). By examining the behavior, growth, and development of the animals at several points in ontogeny, we sought to test the following hypotheses: 1) the induction effects of predators that pose increasing levels of risk to prey will cause increasingly lower activity, slower growth, and longer times to metamorphosis in the prey; 2) the thinning effects of predators that pose increasing levels of risk to prey will cause increasingly lower activity, faster growth, and shorter times to metamorphosis of the prey; and 3) the impact of lethal predators will reflect the combination of induction and thinning effects caused by each species of predator.

MATERIALS AND METHODS

The experiment was conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology located in

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northwestern Pennsylvania. We used a completely randomized design containing ten treatments replicated four times for a total of 40 experimental units. The experimental units were 1300 L cattle watering tank mesocosms that were filled with 1000 L of well water on 5–9 May 2005. We then added 300 g of leaves (primarily *Quercus* spp.) and 25 g of rabbit chow to provide substrate and nutrients for periphyton growth (the tadpole's food source). To each tank, we also added a 0.12 L aliquot of water, collected from four local ponds and screened for invertebrate predators, to serve as a source of zooplankton and algae. The aliquots were haphazardly assigned from a well-mixed bucket of pond water. To ensure sufficient zooplankton, four days later (13 May) we dosed each tank with an additional 0.12 L aliquot of zooplankton that was collected from three of the same four local ponds. We then waited 10 d for the mesocosm communities to develop. Each mesocosm was covered with 60% shade cloth to prevent colonization by other organisms.

Our ten treatments included a control, three caged-predator treatments, three hand-thinning treatments, and three lethal-predator treatments. The three caged-predator treatments consisted of three species that represent low, medium, and high risk of mortality to tadpoles, and, as a result, they induce small, medium, and large phenotypic changes in tadpoles (Relyea, 2003): adult water bugs (*Belostoma flumineum*); adult Red-Spotted Newts (*Notophthalmus viridescensi*); or larval dragonflies (*Anax junius*). The three lethal-predator treatments, which used the same three predator species, consisted of a single predator that was free to consume the tadpoles. The three hand-thinning treatments were designed to span a range of expected predator thinning rates (low thinning to represent water bugs, medium thinning to represent newts, and high thinning to represent dragonflies).

Our prey were leopard frog tadpoles, which are very responsive to a variety of predators (Relyea, 2001a; Schoeppner and Relyea, 2009a). We collected the tadpoles as five masses of newly oviposited eggs from a single population to ensure that the tadpoles were predator-naïve at the start of the experiment. We placed the eggs into outdoor wading pools containing aged well water and covered with 60% shade cloth to prevent the colonization of other organisms. Once the eggs hatched, we fed the tadpoles rabbit chow *ad libitum* until they were ready for the experiment. On 19 May 2005, we added 100 leopard frog tadpoles to each tank from a mixture of the five clutches of tadpoles (initial mean mass$\pm$1 SE = 37$\pm$2 mg). This corresponded to a density of 40 tadpoles/m^2, which is on the high end of typical tadpole densities (although densities of hatchlings can be much higher; Relyea, pers. obs.). However, we wanted to begin with high densities to ensure that at least a few tadpoles survived to metamorphose in the presence of the most lethal predator. A sample of 20 tadpoles was set aside to estimate mortality due to handling. The 24 hr survival of this sample was 100%.

Based on previous evidence that predation on tadpoles follows a negative exponential relationship over time (Van Buskirk and Yurewicz, 1998; Relyea, 2002a), we set a thinning schedule to remove either 15, 40, or 80% of the initial number of stocked prey using an aquarium net (Fig. 1). Our expectation was that these three thinning rates would be similar to the consumption rates of the lethal water bugs, newts, and dragonflies, respectively. Thinning was approximately random in that we attempted to remove tadpoles without bias relative to size or behavior and tadpoles rarely escaped our nets. While predators cause selection on tadpole

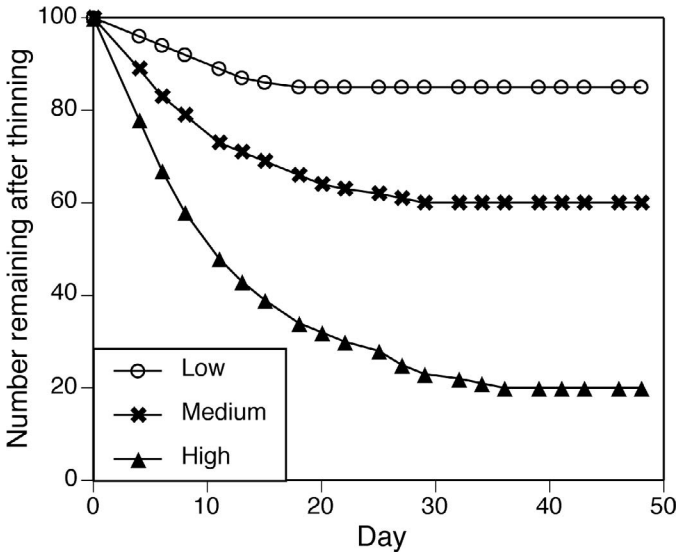

Fig. 1. The thinning schedule of tadpoles over time. Tadpoles were removed at three different rates to simulate the thinning effects of low-risk predators (e.g., adult water bugs), medium-risk predators (adult newts), and high-risk predators (larval dragonflies).

phenotypes in short-term experiments (i.e., 24 hrs; Van Buskirk and Relyea, 1998), longer-term experiments (i.e., 13 d) have shown that selection is unimportant in affecting the final phenotype relative to the effects of induction and thinning (Relyea, 2002a).

Each tank was equipped with a single predator cage constructed of 10 $\times$ 10 cm well pipe with window screen on both ends affixed by rubber bands. Because tadpole predators are largely detected by the chemical cues emitted by predators during their consumption of prey (Laurila et al., 1998; Relyea, 2002a; Schoeppner and Relyea, 2009b), these cages allowed chemical cues from predators to diffuse throughout the mesocosm without killing any of the target animals. For the three caged-predator treatments, the cage held either a single water bug, newt, or dragonfly. Each predator was fed 300 mg of tadpoles three times per week. Past work has demonstrated that tadpoles can detect and respond to a single fed predator in 1,000 L of water (Schoeppner and Relyea, 2009a, 2009b). Because our leopard frog tadpoles were in relative short supply, we fed the predators Wood Frog tadpoles (*R. sylvatica* LeConte, 1825); past work has shown that tadpoles do not discriminate between predators consuming conspecific tadpoles and predators consuming congeneric tadpoles (Laurila et al., 1997; Schoeppner and Relyea, 2005, 2009b). If a predator did not eat for two consecutive feedings, it was replaced, the uneaten prey were left in the cage, and we continued on the same feeding schedule. In treatments that lacked caged predators, predator cages remained empty; at each feeding, the cages were lifted to equalize disturbance across all mesocosms.

One issue we faced in this experiment was that the leopard frog tadpoles could grow to sizes that eventually allowed them to attain a size refuge from their predators. Based on observations on the consumption of tadpoles in the cages, laboratory experiments that determined the largest leopard frog tadpole that could be consumed by each predator, and the size of leopard frog tadpoles in the lethal-predator treatments of the mesocosms, it was clear that our leopard frogs grew to a size refuge from water bug predators by day

20, from newts by day 32, and from dragonflies by day 36. Because the lethal predators should face the same size refuge constraints as the caged predators, we stopped feeding the caged predator species on each of the above dates so that caged-predator cues were emitted for the same number of days as lethal predator cues. We assumed that the lethal (i.e., uncaged) predators in the tanks also would no longer be consuming tadpoles and emitting tadpole-specific predator cues. Our hand-thinning treatments coincided to end on approximately the same dates so that thinning by hand and thinning by lethal predators would occur over the same span of time. To prevent the death of unfed predators in the cages, we rotated recently starved predators into the cages until day 48 when we removed all predators from the cages.

A second issue we considered was whether to account for the increase in nutrients that consumed prey would add to the system in the caged-predator and lethal-predator treatments, but not in the hand-thinning treatments (since the tadpoles were removed from the tanks). The impact of these nutrients would be to potentially increase the periphytic algae for tadpoles, which would cause a decrease in foraging activity and an increase in growth and development. We saw no such impacts in our caged-predator treatments compared to the control, which suggests that the nutrient addition did not play an important role in determining prey responses.

Amphibian response variables.—We observed tadpole behavior three times throughout the course of the experiment (on days 12, 26, and 34). For each tank, we counted the number of visible tadpoles in each tank and, of those visible, the number that were moving (i.e., swimming or feeding). By dividing the latter by the former, one can derive the percentage of visible tadpoles that are active, a protocol that has proven to be very effective in past studies (Peacor and Werner, 1997; Relyea, 2002b). We observed each mesocosm eight times on each of the three observation days. Our behavioral response variable was the mean percent active for each mesocosm on each sample date.

In addition to behavioral data, we examined tadpole growth and development. Within 1 d of collecting the behavioral data (days 13, 27, and 33), we randomly collected ten tadpoles from each mesocosm, weighed all ten, and then recorded the Gosner development stage on two individuals (Gosner, 1960). Only two tadpoles were staged due to time constraints. After collecting these data, all tadpoles were returned to their respective mesocosms.

The leopard frogs began to metamorphose on day 41 and the tanks were subsequently checked daily for metamorphs. Metamorphs were collected when their tail was less than one-fourth as long as their body. They were held in the laboratory in 1 L plastic tubs containing damp sphagnum moss until the tails were fully absorbed (Gosner stage 46). On this day, we noted the mass at metamorphosis and the time to metamorphosis (defined as the number of days since being added to the mesocosms). All metamorphosed animals were returned to their native pond.

Seasonal time constraints forced us to terminate the experiment on days 91–92 (i.e., 18–19 August). Rather than simply defining a given day as a dry pond, we gradually drew down the water level in each tank to mimic the slow drying of a pond because tadpoles can detect a drying pond (based on the swimmable volume of water; Denver et al., 1998). Beginning on day 79, we removed ~60 L of water from each tank per day, leading to a drawdown that lasted for 12 d. On days 91–92, all remaining tadpoles were removed from each tank, counted, and returned to the pond from which they were collected. Any frogs possessing at least one emerged forelimb were considered successful metamorphs and allowed to complete their metamorphosis in the laboratory tubs. The remaining animals were considered to have died due to pond drying.

Statistical analysis.—We analyzed the data using analysis of variance using tank means as our response variables (alpha = 0.05 for all tests). Because amphibian activity, mass, and development were measured at multiple time points during the experiment, these data were analyzed using separate repeated-measured analyses of variance (rm-ANOVAs) to determine the effects of treatment, time, and the treatment-by-time interaction. Because amphibian survival was measured only at the end of the experiment, this response variable was analyzed using a separate ANOVA. Significant univariate tests were followed by Tukey mean comparison tests. Of particular interest were mean comparisons between the control and the other nine treatments and mean comparisons between each hand-thinning treatment and the corresponding species of lethal predator. Late in the experiment (day 56), one tank developed a film on the water's surface that was associated with several dead animals observed in the mesocosm, and this replicate was removed from the analyses. Two other tanks were outliers that caused heteroscedastic errors and were removed as well (one caged-dragonfly treatment and one medium-thinning treatment). Tests of assumptions indicated that all data were normally distributed (mass at metamorphosis was log-transformed) and that the variances were either homogeneous or within the range of variance for which ANOVA tests are robust (Quinn and Keough, 2002). In only two cases (activity on day 33 and the survival of metamorphs plus remaining tadpoles) was this not the case. These two response variables could not be successfully transformed nor did they contain any significant outliers. In these cases, non-parametric tests were consistent with the parametric tests; thus, we report the latter test.

RESULTS

Tadpole activity.—The rm-ANOVA on tadpole activity found effects of treatment ($F_{9,27} = 7.1$, $P < 0.001$), time ($F_{2,54} = 51.6$, $P < 0.001$), and a treatment-by-time interaction ($F_{18,54} = 201$, $P = 0.025$; Fig. 2). On day 12, the treatments affected tadpole activity ($F_{9,27} = 4.7$, $P = 0.002$). Compared to the control, only the lethal dragonfly caused lower activity ($P = 0.005$). Comparing hand thinning to the lethal predators, we found no differences between low thinning and lethal water bugs ($P = 0.962$) or medium thinning and lethal newts ($P = 0.753$). There was a trend for higher activity between high thinning and lethal dragonflies, but it was marginally non-significant ($P = 0.086$).

On day 26, the treatments affected tadpole activity ($F_{9,27} = 3.6$, $P = 0.005$). Compared to the control, only high thinning caused lower activity ($P = 0.024$). Comparing thinning versus lethal predators, we found no differences in any of the three comparisons (all $P > 0.9$).

On day 34, the treatments still affected tadpole activity ($F_{9,27} = 5.4$, $P < 0.001$). Compared to the control, only high thinning caused lower activity, but the effect was marginal ($P = 0.070$). There also were no differences between each thinning treatment and the corresponding lethal-predator treatment (all $P > 0.5$).

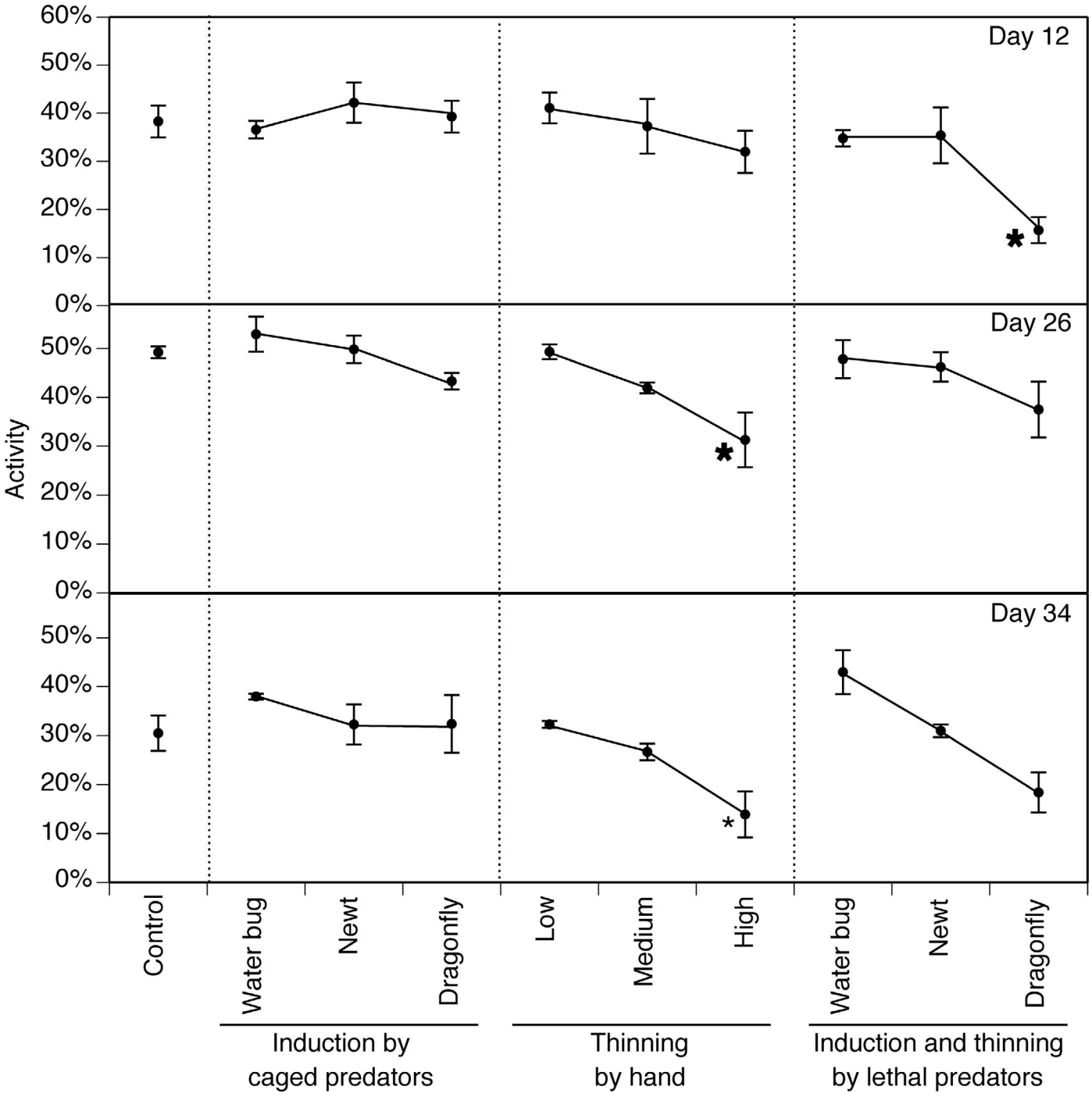

Fig. 2. The activity of leopard frog tadpoles exposed to either no predatory effects (i.e., control), chemical cues from three caged predators, hand-thinning rates to simulate the thinning rates of the three predators, and three lethal predators which simultaneously produce chemical cues and cause thinning of the prey populations. Large asterisks (*) indicate significant differences ($P \leq 0.05$) between the control and any of the other nine treatments, whereas small asterisks indicate marginally significant differences ($P \leq 0.07$).

Tadpole mass.—The rm-ANOVA on tadpole mass found effects of treatment ($F_{9,27} = 23.7$, $P < 0.001$), time ($F_{3,81} = 802$, $P < 0.001$), and a treatment-by-time interaction ($F_{27,81} = 18.6$, $P < 0.001$; Fig. 3). As a result, we examined mass on each of the sample dates. On day 13, there was a marginal treatment effect ($F_{9,27} = 2.0$, $P = 0.084$).

On day 27, there was a treatment effect on tadpole mass ($F_{9,27} = 17.3$, $P < 0.001$). Compared to the control, only the high-thinning and lethal-dragonfly treatments produced larger tadpoles (all $P < 0.001$). Comparing hand-thinning versus lethal-predator treatments, we found no differences for any of the three comparisons ($P > 0.2$).

On day 33, mass continued to exhibit a treatment effect ($F_{9,27} = 24.2$, $P = 0.001$). Once again, compared to the control, only high thinning and lethal dragonflies caused greater tadpole mass (all $P < 0.001$). Comparing hand thinning versus lethal predators, we found no differences between low thinning and lethal water bugs ($P = 0.982$) or between medium thinning and lethal newts ($P = 0.135$), but high thinning produced larger tadpoles than lethal dragonflies ($P = 0.001$).

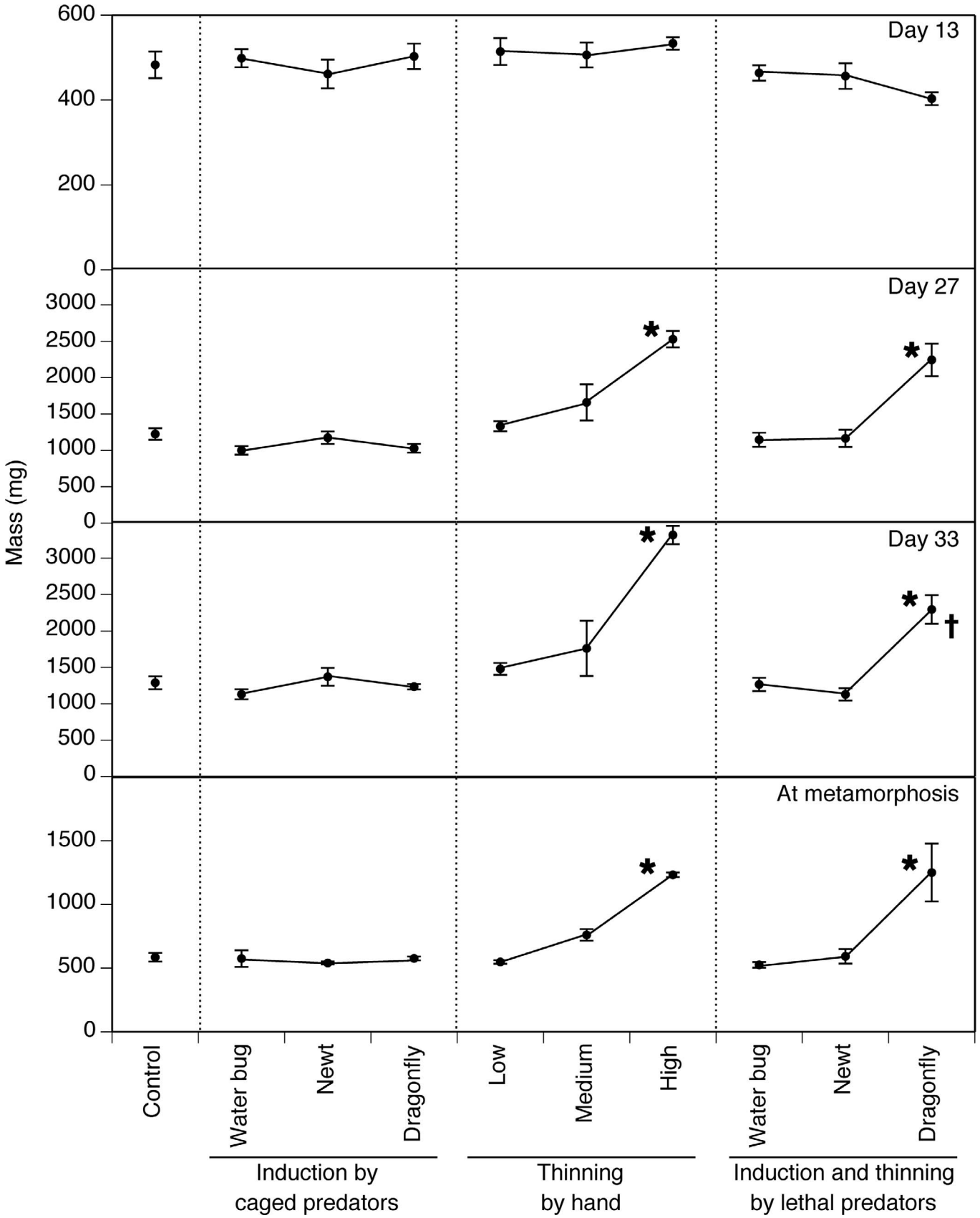

Fig. 3. The mass of leopard frog tadpoles exposed to either no predatory effects (i.e., control), chemical cues from three caged predators, hand-thinning rates to simulate the thinning rates of the three predators, and three lethal predators which simultaneously produce chemical cues and cause thinning of the prey populations. Large asterisks (*) indicate significant differences ($P \leq 0.05$) between the control and any of the other nine treatments. Cross symbols (†) indicate significant differences ($P \leq 0.05$) between either low thinning and lethal water bugs, medium thinning and lethal newts, or high thinning and lethal dragonflies.

At metamorphosis, leopard frogs also exhibited treatment differences ($F_{9,27} = 11.7$, $P = 0.001$). Compared to the control, only the high-thinning and lethal dragonflies produced larger metamorphs (all $P < 0.001$). Comparing hand thinning versus the corresponding lethal predators, we found no differences in mass ($P > 0.9$).

Tadpole development.—The analysis of tadpole development found effects of treatment ($F_{9,27} = 9.3$, $P < 0.001$), time ($F_{3,81} = 12356$, $P < 0.001$), and a treatment-by-time interaction ($F_{27,81} = 5.4$, $P < 0.001$; Fig. 4). On day 13, there was a marginally significant treatment effect ($F_{9,27} = 1.8$, $P = 0.058$). Compared to the control, however, none of the other nine treatments differed (all $P > 0.3$).

On day 27 there was a treatment effect ($F_{9,27} = 8.5$, $P < 0.001$). Compared to the control, only high thinning caused more rapid development ($P = 0.006$). Comparing hand thinning and lethal predators, we found no differences between each thinning treatment and its corresponding lethal-predator treatment (all $P > 0.12$).

On day 33, there continued to be a treatment effect ($F_{9,27} = 6.1$, $P < 0.001$). Compared to the control, only high thinning caused marginally more rapid development ($P = 0.062$). Comparing hand-thinning and the corresponding lethal predators, we found no differences in tadpole development ($P > 0.4$).

At metamorphosis, development rate (i.e., 1/larval period) also showed a treatment effect ($F_{9,27} = 6.1$, $P < 0.001$). Compared to the control, development rate was only affected by high thinning ($P < 0.001$). Comparing hand-thinning and lethal predators, we found no differences between each thinning treatment and the corresponding lethal-predator treatment (all $P > 0.12$).

Tadpole survival.—The analysis of survival to metamorphosis found no treatment effect ($F_{9,27} = 1.6$, $P < 0.167$; Fig. 5). When we combined the number of successful metamorphs plus the number of tadpoles remaining in the mesocosms on the final day of drying, however, we found treatment differences ($F_{9,27} = 24.8$, $P < 0.001$). Compared to the control, the proportion of metamorphs plus tadpoles was not affected by the caged predators (all $P > 0.9$). Of the three hand-thinning treatments, survival was not reduced with low thinning ($P > 0.9$), but was reduced with medium and high thinning (both $P < 0.005$). Of the lethal predators, survival was not reduced with lethal water bugs ($P = 0.782$) or lethal newts ($P = 0.249$), but was reduced with lethal dragonflies ($P < 0.001$). Comparisons between the three hand-thinning treatments and the lethal predators found no differences in any of the three comparisons (all $P > 0.8$), suggesting that the three hand-thinning rates reduced survival to levels that were similar to each of the lethal predators.

DISCUSSION

Our experiment demonstrated that, in the absence of prey thinning, caged predators induced no impacts on the behavior and life history traits of leopard frogs when raised at relatively high densities. Applying hand thinning at rates that were representative of three different predators, we found decreases in prey activity and increases in mass and development. Finally, the effects of lethal predators (which emit cues and thin the tadpole population) occasionally caused changes in prey traits that differed from the effect of thinning alone. Collectively, these result suggest that—under the conditions of this experiment—the thinning process of predators played a dominant role in how predators affect the metamorphic traits.

There was a striking lack of responses to the three species of caged predators in behavior, mass, and development throughout ontogeny. This is interesting because a large number of studies have demonstrated that tadpoles typically respond to aquatic predator cues by reducing their foraging activity because less active tadpoles are at a lower risk of predation (Relyea, 2001a, 2001b; Van Buskirk, 2002). However, this comes at a cost of slower growth and development (Lardner, 1998; Vorndran et al., 2002). Even in multiple species of leopard frog tadpoles, researchers have observed reductions in activity with predator cues (Babbit, 2001; Relyea, 2001a). We have even observed predator-induced activity reduction for the same population of leopard frog tadpoles used in the current study (Schoeppner and Relyea, 2009a). Thus, it is clear that the population of leopard frogs used in this study possesses the ability to detect and respond to predator cues, yet they did not do so.

There are several potential explanations for why the leopard frog tadpoles did not respond to predator cues. First, it may be that the number of caged predators (or amount of prey fed to predators) was insufficient to induce anti-predator defenses. As a reminder, we used a single predator fed approximately 300 mg of tadpoles three times per week in 1,000 L of water. Past studies using have found that this amount of predation is sufficient to induce phenotypic changes (e.g., 300 mg of tadpoles fed to a single predator every 2 d in 1,000 L of water; Relyea, 2004; 200 mg of tadpoles fed to a single predator every 2 d in 700 L of water; Schoeppner and Relyea, 2008). While not conducted on leopard frogs, these studies suggest that a single caged predator in a mesocosm produces enough cue to induce phenotypic changes in tadpoles.

A second possibility for the lack of responses to the caged predators is that the leopard frogs do not respond to predators fed Wood Frogs. Past studies of tadpole responses to different predator diets have found that hylid tadpoles cannot discriminate among predators fed conspecific versus congeneric tadpoles (Schoeppner and Relyea, 2005, 2009b) and ranid tadpoles cannot discriminate between predators fed tadpoles from different families (Laurila et al., 1997). Thus, the existing evidence argues against the predators' diet being responsible for the lack of responses to the caged predators.

The more likely explanation is that the tadpoles were experiencing high competition. Predator induction of prey traits is commonly weak or non-existent when tadpoles face high competition compared to when they experience low competition (Laurila and Kujasalo, 1999; Nicieza, 2000; Babbit, 2001; Relyea, 2002a, 2004; Relyea and Hoverman, 2003). In Southern Leopard Frogs (*Rana sphenocephala*), for example, Babbit (2001) found that tadpole activity responses to cues from larval dragonflies were strong under high-food conditions, but weak under low-food conditions. This interactive effects of predator cues and per-capita food availability (i.e., competition) on prey responses is found in many other taxa as well, including zooplankton and gastropods (Weetman and Atkinson, 2002; Turner, 2004).

If high competition were the cause of leopard frogs not responding to caged predators, we would predict that predator cues (particularly cues from the most dangerous predators, the larval dragonflies) should induce behavioral

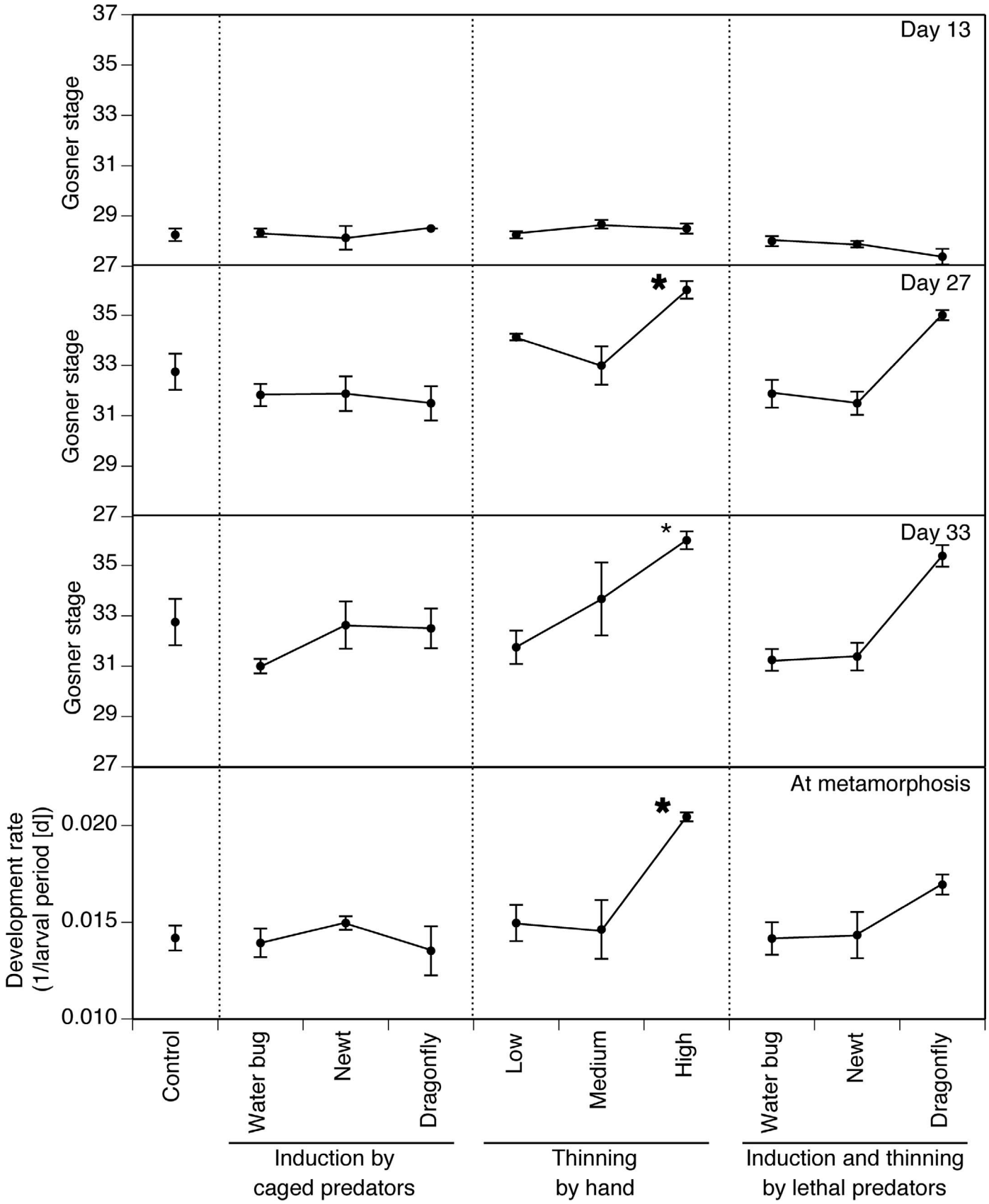

Fig. 4. The development of leopard frog tadpoles exposed to either no predatory effects (i.e., control), chemical cues from three caged predators, hand-thinning rates to simulate the thinning rates of the three predators, and three lethal predators which simultaneously produce chemical cues and cause thinning of the prey populations. The lowest panel is the developmental rate of metamorphs, defined as 1/larval period. Large asterisks (*) indicate significant differences ($P \leq 0.05$) between the control and any of the other nine treatments.

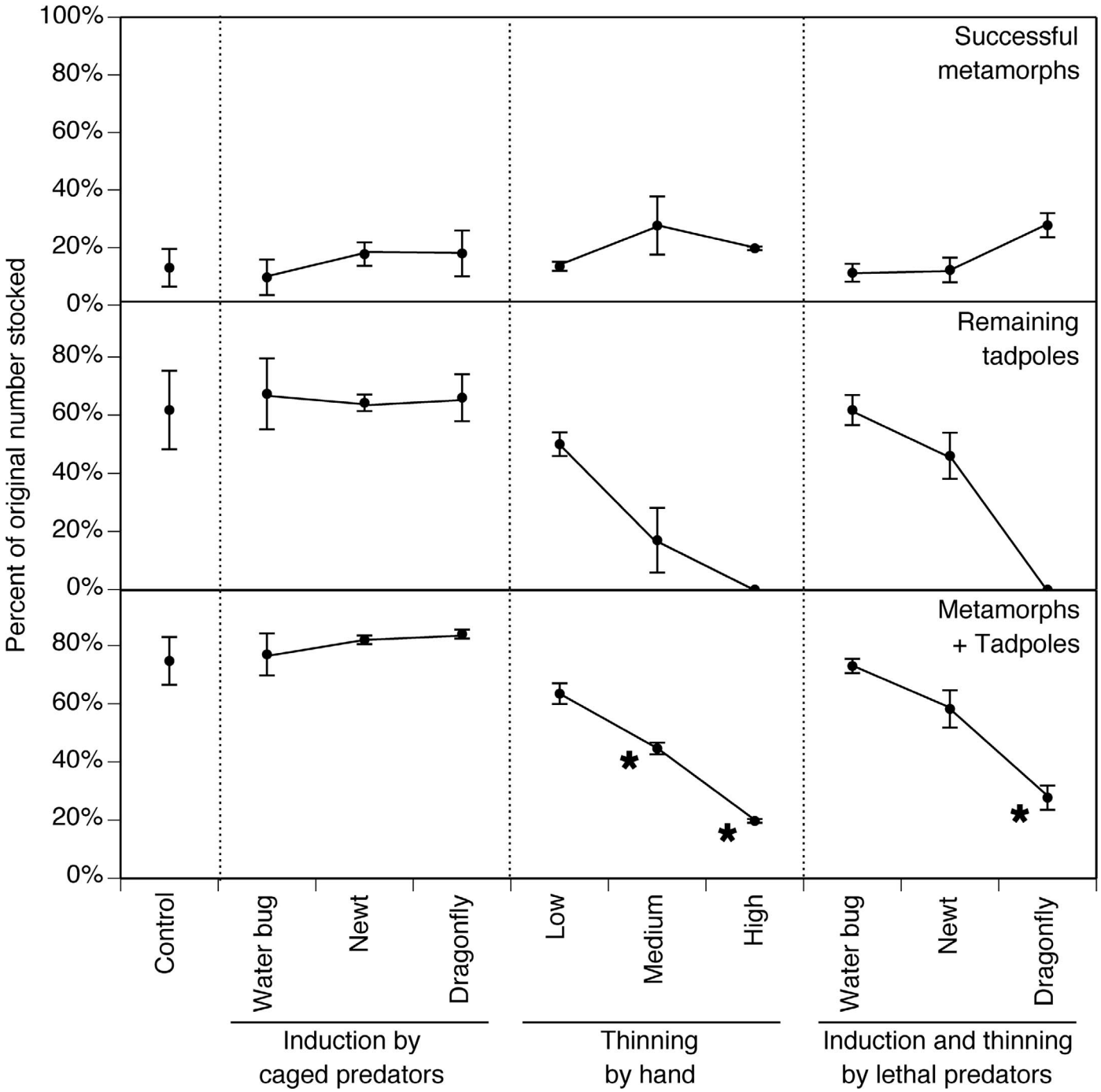

Fig. 5. The survival of leopard frogs exposed to either no predatory effects (i.e., control), chemical cues from three caged predators, hand-thinning rates to simulate the thinning rates of the three predators, and three lethal predators which simultaneously produce chemical cues and cause thinning of the prey populations. Survival reflects the number of metamorphs emerging, the number of tadpoles remaining in the mesocosms when the experiment was terminated, and the total survival of metamorphs + tadpoles remaining in the mesocosms when the experiment was terminated. Large asterisks (*) indicate significant differences ($P \leq 0.05$) between the control and any of the other nine treatments, whereas small asterisks indicate marginally significant differences ($P \leq 0.07$).

changes when combined with high thinning. We saw evidence of this in the greater activity reduction with lethal dragonflies versus high thinning on day 12. This suggests that the dragonflies were producing chemical cues that were detectable by the tadpoles, but the tadpoles only behaviorally responded when the amount of intraspecific competition declined with high rates of thinning.

One challenge in separating the effects of predator induction and predator thinning was to devise rates of thinning that could approximate the rates of predation by three predator species that differ widely in their predation rates (documented in Relyea, 2003). The shape of our hand-thinning schedule (a negative exponential relationship) was derived from past experiments (Van Buskirk and Yurewicz, 1998; Relyea, 2002a), and the final survival of the leopard frogs (tadpoles + metamorphs) was strikingly similar between the low thinning and lethal-water bug treatments, between the medium thinning and lethal-newt treatments, and between the high thinning and lethal-dragonfly treatments. Thus, the three hand-thinning treatments were successful at

mimicking the thinning caused by the three species of lethal predators.

The high hand-thinning treatment affected the behavior, mass, and development of the amphibians. High hand thinning caused a decline in tadpole activity that first became significant on day 26 and continued into day 34. This reduced activity is consistent with a reduction in competition, which has been repeatedly observed in larval amphibians (Anholt et al., 2000; Babbit, 2001; Relyea and Hoverman, 2003) as well as many other taxa (Lima and Dill, 1990; Kats and Dill, 1998). High hand thinning caused a concomitant increase in mass. Greater mass was first observed in the tadpoles on day 27 and continued through metamorphosis, at which point high hand thinning produced metamorphs that were nearly twice as large and experienced a shorter larval period. Collectively, these data demonstrate that high hand thinning reduced competition, reduced tadpole activity, and increased the growth and development of the prey.

The effects of the three lethal-predator treatments largely mirrored the effects of thinning alone. The lethal water bugs, for example, caused no impact on amphibian activity, mass, or development. Previous studies have found that water bugs are poor predators of amphibians due to relative low capture efficiency, long handling times, and a limited range of tadpole sizes that can be captured; due to this low risk, lethal water bugs typically induce weak or non-existent changes in tadpole behavior and relative morphology (Relyea, 2001a, 2001b, 2004).

Lethal newts generally pose a moderate risk of predation to tadpoles and, as a result, we expected them to induce moderate phenotypic changes in the prey. Past experiments have generally found that newts induce weak to moderate levels of spatial avoidance, reduced activity, and morphological change compared to more risky predators such as larval dragonflies (Wilson and Lefcort, 1993; Relyea, 2001a; Van Buskirk, 2001; Laurila et al., 2006). In the current experiment, cues from caged newts induced no changes in activity, mass, or development. When hand thinning occurred at a rate that was representative of thinning by newts, there was still no change in activity or development, suggesting that this moderate rate of thinning of newts on a high density of prey was not sufficient to allow the tadpoles to respond.

Larval dragonflies have been documented to be one of the most dangerous predators to tadpoles and, as a result, induced the strongest phenotypic changes in tadpole activity and morphology (Relyea, 2001a, 2003, 2004; Relyea and Auld, 2004, 2005). In the current experiment, the lethal dragonflies also caused the greatest tadpole mortality of the three predators. If the leopard frogs were constrained from responding to the caged predators due to high competition, then comparisons between the high-thinning treatment and the lethal-dragonfly treatment (which had equivalent survival) would present the best opportunity to observe predator induction. Consistent with this expectation, we observed lower activity with lethal dragonflies than with high thinning on day 12. Midway in the experiment (day 33), we also observed that tadpoles were less massive with lethal dragonflies than with high thinning. This suggests that the leopard frog tadpoles were capable of altering their activity and mass in response to cues from lethal dragonflies, but only when the dragonfly cues were paired with much lower prey densities. More extreme expression of anti-predator responses between lethal versus non-lethal predators has also been observed in other systems (Brodin and Johansson, 2002).

We also found the rather non-intuitive result that when faced with the challenge of a drying pond, communities containing the most dangerous lethal predator produced just as many surviving metamorphs as the control environment (with a trend toward more metamorphs). In fact, the lethal-dragonfly treatment was the only treatment in which every live tadpole in the mesocosms emerged as a metamorph. Although the control treatment had more individuals at the end of the experiment, most were not sufficiently developed to metamorphose and would therefore die in a naturally drying pond. In addition, metamorphs emerging from lethal-dragonfly environments were nearly twice as large as those emerging from the controls. This occurred because individuals fortunate enough to survive dragonfly predation enjoyed substantially reduced competition. A larger mass at metamorphosis is important to future fitness because it is associated with earlier time to sexual maturity, superior survival after metamorphosis, and the production of more and larger eggs in females (Howard, 1980; Berven, 1981; Berven and Gill, 1983; Smith, 1987; Semlitsch et al., 1988; Altwegg and Reyer, 2003). Thus, animals that survived the most dangerous predator would be expected to have high future fitness. Similar results have been observed in other taxa, including damselfly larvae (Brodin and Johansson, 2002).

Overall, the study sought to address the challenge of assessing how the separate processes of predator induction and predator thinning impact prey phenotypes including the size at and time to metamorphosis. Our results suggest when a prey population is at a relatively high density, the major impact of predators on behavior, growth, and metamorphosis is through the process of thinning rather than through the process of induction. While our experiment examined larval amphibians, it may be that these insights apply widely to a variety of taxa that either metamorphose or possess other forms of complex life cycles (Werner, 1986).

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