Temporal patterns in bullfrog (*Rana catesbeiana*) tadpole activity: a mesocosm experiment on the effects of density and bluegill sunfish (*Lepomis macrochirus*) presence

Geoffrey R. Smith, Amber A. Burgett, Kathryn A. Sparks, Kathleen G. Temple & Kristen E. Winter

Department of Biology, Denison University, Granville, Ohio, USA

The presence of predators or predator cues has an effect on the behaviour of tadpoles of several species of anurans. We used a mesocosm experiment to examine whether bullfrog (*Rana catesbeiana*) tadpoles might 1) alter their activity levels in response to variations in density and the presence of bluegill sunfish (*Lepomis macrochirus*), and 2) alter their daily activity cycle in response to variations in density and the presence of bluegill. The bullfrog tadpoles in our experiments showed a clear temporal pattern of activity, with activity always peaking during the evening or nighttime hours. Tadpole density had no effect on activity levels. We observed depressed activity levels in the presence of bluegill in the first observation period (30 June–1 July). Bluegill tended to depress activity levels in the second observation period (7–8 July), but this was not statistically significant. We observed elevated activity levels in tadpoles in the presence of bluegill during the third observation period (14–15 July). Neither bluegill nor tadpole density had an effect on the temporal pattern of activity in the bullfrog tadpoles. In addition to showing that fish can mediate activity in bullfrog tadpoles, our results suggest that the responses of tadpoles to the presence of predators can vary over time, and thus using a single observation could influence the conclusions that may be drawn.

Key words: diel activity, predation, predator–prey relationships

INTRODUCTION

The presence of predators or predator cues has an effect on the behaviour of tadpoles of several species of anurans. In many cases, activity levels decrease in the presence of predators or predator cues (e.g. Peacor & Werner, 1997; Laurila, 2000; Richardson, 2001; Relyea, 2002b; Parris et al., 2006). In some species habitat use changes (e.g. Lawler, 1989; Teplitsky et al., 2003). Fewer studies have examined whether tadpoles may adjust their behaviour in response to predators or predator cues by changing the temporal pattern of activity (e.g. Peacor & Werner, 2001; Peacor, 2002; McIntyre et al., 2004). For example, one could expect prey might shift away from diurnal activity to nocturnal activity in the presence of a visually-oriented predator, and thus a predator could alter the temporal activity pattern observed in their prey.

In some species, the number of conspecifics in a group can influence the activity or behaviour of tadpoles (e.g. Griffiths & Foster, 1998; Golden et al., 2001; Relyea, 2002a; Awan & Smith, 2007a), suggesting that tadpoles may use group size as a cue to assess predation risk (see also Peacor, 2003), or may increase activity in larger groups in response to increased numbers of potential competitors (see also Relyea, 2002a). For example, Relyea (2004) found that activity of wood frog tadpoles in the presence of odonate predators increased with increasing density of conspecific tadpoles.

We used a mesocosm experiment to examine whether bullfrog (*Rana catesbeiana*) tadpoles might 1) alter their activity levels in response to variation in tadpole density and the presence of bluegill sunfish (*Lepomis macrochirus*), and 2) alter their temporal activity patterns in response to variation in tadpole density and the presence of bluegill. In general, bullfrog tadpoles are unpalatable to fish (Kruse & Francis, 1977; Kats et al., 1988); however, they do sometimes exhibit behavioural responses to the presence of fish or fish cues (e.g. Relyea & Werner, 1999; Eklöv, 2000; Boone & Semlitsch, 2003), so we expected bullfrog tadpoles might decrease activity in the presence of fish. We expected that tadpoles at higher densities would be more active, either because the presence of more conspecifics would dilute or reduce predation risk (e.g. Spieler, 2005) thus allowing tadpoles to engage in riskier behaviour, or increased numbers of potential competitors might itself influence activity (e.g. Griffiths and Foster, 1998). Laboratory experiments on the behavioural response of bullfrog (*Rana catesbeiana*) tadpoles to the presence of bluegill suggest that the density of the tadpoles affects their response to predator cues (G.R. Smith & A.R. Awan, unpubl. data). We expected bullfrogs to decrease their activity during the day to avoid the visually-oriented fish predator.

MATERIALS AND METHODS

Egg masses of *R. catesbeiana* were collected from a small pond that lacks bluegill on the Denison University Biological Reserve located in Granville, Licking Co., Ohio, USA, and transported back to the laboratory where they were incubated in aged tapwater at 17–19 °C. Upon hatching, tadpoles from each egg mass were maintained in their

Correspondence: Geoffrey R. Smith, Department of Biology, Denison University, Granville, Ohio 43023, USA.
E-mail: smithg@denison.edu

own large plastic container at relatively high densities and fed ground Purina Rabbit Chow daily until they were introduced into the experiment.

On 21 June 2004 tadpoles were introduced into the experiments. Tadpoles were free-swimming and feeding (stage 25; Gosner, 1960). At their introduction into the experiment, the mean body mass (±1 SE) of 10 haphazardly selected tadpoles was 0.009±0.0004 g.

We used 1135 l cattletanks (n=24) filled with 800 l (depth = 44 cm) of well water. All mesocosms were inoculated with zooplankton and phytoplankton concentrates from local ponds. Deciduous leaf litter (20 l; mostly maple leaves, *Acer* spp.) was added to provide nutrients and structure to the mesocosm. We also added 30 g of Purina Rabbit Chow pellets to provide nutrients for the zooplankton and phytoplankton, as well as initial food for the tadpoles. Colonization by macroinvertebrates and other amphibians was prevented by fibreglass window screening placed over the top of the cattletanks.

The experimental design consisted of a 2 × 3 factorial experiment with two predator treatments (no or one bluegill present) and three density treatments (25, 50 or 100 tadpoles). Each treatment combination was replicated four times. Tadpole densities were at the low end of naturally occurring densities of tadpoles of this and other species observed in local ponds (e.g. Smith et al., 2003, 2005). The bluegill were on average 61.3±1.9 mm total length and 7.6±0.7 g.

Observations were made every two hours on 30 June and 1 July 2004 from 0800 to 2000. On 7–8 July and 14–15 July 2004, we observed bullfrog tadpole activity every two hours from 0600 to 2400. Sunset occurred around 2100, thus observations at 2000 could be considered dusk and those at 2200 and later considered night. Activity level was determined by counting the number of tadpoles that were active in the top 28 cm of the water column of the tanks. We chose to use only tadpoles at the top of the water column because we were able to observe tadpoles to this depth in all tanks at all times of the day. Observations after dark used flashlights.

We analysed activity levels (as proportion of tadpoles active) for each pair of days separately (30 June–1 July, 7–8 July and 14–15 July) using a repeated measures ANOVA with fish presence and tadpole density as factors, and time of day as the repeated measure. We used the mean proportion of tadpoles active in each mesocosm for the pair of days as the dependent variable. We arcsine-square-root transformed proportion data prior to analyses. Separate analyses were used because 1) the hours of observation were not the same for all six days, thus precluding an overall analysis that would allow all data to be used, and 2) by separating the analyses, we can examine for potential changes in responses between the three different observation periods that were each separated by a week, perhaps due to changes in tadpole size or age.

RESULTS

30 June–1 July. Bullfrog tadpoles were less active in the presence of bluegill than in their absence (Fig. 1A; $F_{1,18}$=24.8, P<0.0001). Activity was not affected by tadpole density ($F_{2,18}$=1.65, P=0.22) or by the fish by tadpole density interaction ($F_{2,18}$=1.12, P=0.35).

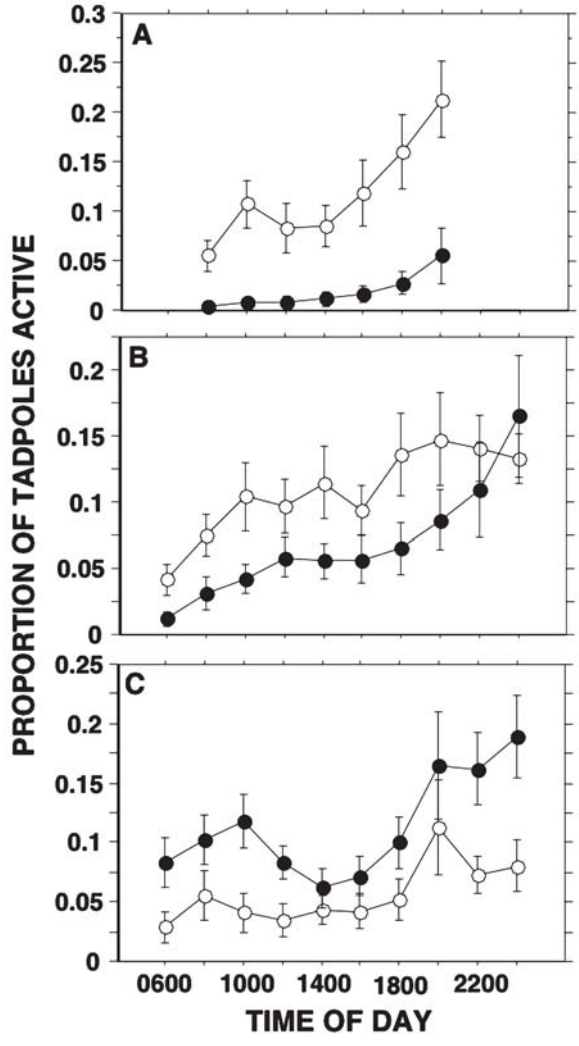

Fig. 1. Diel variation in the proportion of bullfrog (*Rana catesbeiana*) tadpoles in the presence (open circles) and absence (closed circles) of bluegill (*Lepomis macrochirus*) for three observation periods: A) 30 June–1 July, B) 7–8 July and C) 14–15 July. Means are pooled for all density treatments. Means are given ±1 S.E.

Activity levels tended to increase throughout the day (Fig. 1A; $F_{6,108}$=14.1, P<0.0001). No interactions with time were significant (all P>0.09).

7–8 July. Bullfrog tadpoles were less active in the presence of bluegill than in their absence, but the difference was not statistically significant (Fig. 1B; $F_{1,18}$=2.87, P=0.11). The proportion of tadpoles active was not affected by tadpole density ($F_{2,18}$=0.40, P=0.68). The interaction of fish and tadpole density also had no effect on tadpole activity ($F_{2,18}$=1.51, P=0.25).

Tadpole activity increased from the morning to the nighttime observations (Fig. 1B; $F_{9,162}$=19.0, P<0.0001).

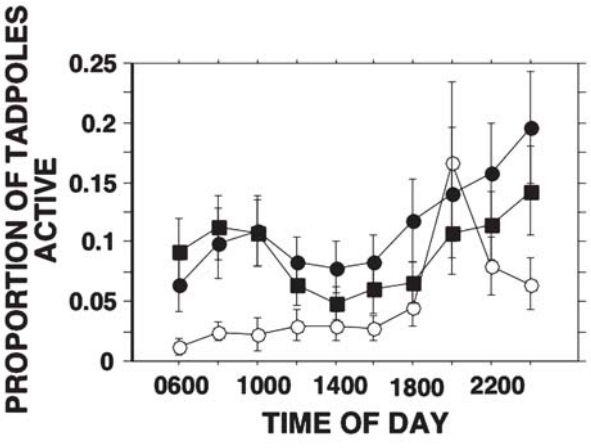

Fig. 2. Diel variation in the proportion of bullfrog (*Rana catesbeiana*) tadpoles as a function of tadpole density (open circles = 25 tadpoles, closed circles = 50 tadpoles, closed rectangles = 100 tadpoles) during the 14–15 July observation period. Means are given ±1 S.E.

There were no significant interactions with time (P>0.15 in all cases).

14–15 July. For this time period, activity of bullfrog tadpoles was actually higher in mesocosms with fish than in those without fish (Fig. 1C; $F_{1,18}$=5.77, P=0.027). Tadpole density had no effect on activity ($F_{2,18}$=2.55, P=0.11). The interaction between fish and tadpole density was also not significant ($F_{2,18}$=0.76, P=0.48).

Activity level varied with the time of the day the observation was made, with activity showing a slight bimodal pattern but still peaking in the evening and nighttime hours (Fig. 1C; $F_{9,162}$=9.30, P<0.0001). The interaction between time and fish was not significant ($F_{9,162}$=1.10, P=0.37). There was a statistically significant time by tadpole density interaction; tadpoles in the highest density treatments were more active during the day than at the other densities, but this difference disappeared in the nighttime hours (2200 and 2400) (Fig. 2; $F_{18,162}$=1.70, P=0.045). The three way interaction between time, fish and tadpole density was not significant ($F_{18,162}$=0.73, P=0.77).

DISCUSSION

The bullfrog tadpoles in our experiments showed a clear temporal pattern of activity, with activity always peaking during the evening or nighttime hours. Later in the experiment there was a second, minor peak in the mid-morning hours. There is no evidence that the presence of fish altered the temporal activity pattern of the bullfrog tadpoles (i.e. no significant time by fish interactions). In other species of amphibian larvae, the presence of predators has been shown to alter the diel activity cycle. Larval *Ambystoma gracile* are active only at night in lakes with fish, but are active throughout the day in lakes without fish (Taylor, 1983). Peacor & Werner (2001) found that the presence of *Anax* predators depressed the activity of small bullfrog tadpoles during the day, but had no effect at night (see also Peacor, 2002). Activity levels of tadpoles of *Rana palmipes* were higher in the night than the day, but activity of tadpoles in the presence of *Belastoma* water bugs at night was not as high as controls, although activity in the presence of the predator was higher at night (McIntyre et al., 2004).

Despite the fact that bullfrog tadpoles are unpalatable to fish (Kruse & Francis, 1977; Kats et al., 1988; G.R. Smith and A.R. Awan, pers. obs.), activity levels were depressed in the presence of bluegill in the first observation period (30 June–1 July), and tended to be depressed in the second observation period (7–8 July). However, we observed elevated activity levels in the presence of bluegill during the third observation period (14–15 July). Previous studies on the effects of the presence of sunfish (*Lepomis* spp.) on bullfrog tadpole activity have found a range of responses. Boone and Semlitsch (2003) found that the presence of bluegill depressed the activity of bullfrog tadpoles, but only late (42 days) in their experiment. Bluegill reduced the activity of small bullfrog tadpoles but not large bullfrog tadpoles (Eklöv, 2000). Relyea & Werner (1999) found no effect of bluegill on the activity of bullfrog tadpoles in laboratory experiments. Bullfrog tadpoles were slightly more active in the presence of *Lepomis gibbosus* than in the absence of the fish (Richardson, 2001).

The changes in activity we observed could have broader implications. First, low activity by tadpoles in the presence of fish can decrease the chances a tadpole will be consumed by the fish (e.g. Lefcort, 1996; Anholt et al., 2005). However, low activity levels have a cost in that reductions in activity frequently result in decreased foraging and growth (e.g. Skelly & Werner, 1990; Relyea & Werner, 1999). Second, reduced activity by bullfrog tadpoles in the presence of bluegill may also have consequences for the effects of bullfrog tadpoles on other members of the anuran larval community by reducing their ability to forage, thereby reducing their competitive impact, as was observed for small bullfrog tadpoles that reduced their activity in the presence of dragonfly larvae (Werner & Anholt, 1996). Bullfrog tadpoles have been shown to have negative competitive effects on other tadpole species (e.g. Werner, 1994; Werner & Anholt, 1996; Smith et al., 2006), and thus changes in activity in the presence of predators could influence anuran larval community dynamics.

The changes in activity and the response to the presence of the bluegill over the course of our experiment suggest that bullfrog tadpoles may alter their behaviour as a function of age or size (i.e. depressed activity in the presence of bluegill on 30 June–1 July, non-significant tendency for depressed activity on 7–8 July, and elevated activity on 14–15 July). Other species of tadpoles appear to alter their behaviour over ontogeny as well. Small American toad (*Bufo americanus*) tadpoles reduce activity in the presence of predators more than large tadpoles (Anholt et al., 1996). Laurila et al. (2004) found that the effects of predator cues (*Aeschna* dragonfly larva) changed with tadpole age in *Rana temporaria*, but found

that the response increased with tadpole size. Activity of small bullfrog tadpoles was depressed by the presence of *Anax* predators, but had no effect on large bullfrog tadpole activity (Peacor & Werner, 2001; Peacor, 2002). Changes in activity with ontogeny may be related to changes in perceived predation risk due to changes in 1) the ability of tadpoles to swim and/or escape predators (e.g. Wassersug & Sperry, 1977; Huey, 1980; Brown & Taylor, 1995), 2) palatability (Lawler & Hero, 1997; but palatability can sometimes increase with development: Brodie & Formanowicz, 1987), or 3) the size of the tadpoles providing a size refuge from predators (Brodie & Formanowicz, 1983; Crump, 1984; Travis et al., 1985; Formanowicz, 1986; Semlitsch & Gibbons, 1988; Richards & Bull, 1990). Further experimentation is necessary to tease apart the possible explanations for the change in behaviour that we observed in the bullfrog tadpoles in our experiment.

Tadpole density had very little effect on the activity of the bullfrog tadpoles in our experiment (density also did not affect mortality; G.R. Smith et al., unpubl. data). The only significant effect was the time by tadpole density interaction during the 14–15 July observation period, and even that was a relatively small effect. Other studies on the effects of density or group size on tadpole behaviour have found significant influences on activity, with activity increasing with density (Griffiths & Foster, 1998; Golden et al., 2001; Relyea, 2002a, 2004; Awan & Smith, 2007a). However, just as we found in the bullfrog tadpoles, not all species show an increase in activity with density (Golden et al., 2000; Awan & Smith, 2007b), but since our experimental densities were at the low end of natural densities (see Materials and Methods), it may be that higher densities would have resulted in changes in tadpole behaviour.

In addition to showing that fish can mediate activity patterns in bullfrog tadpoles, our results suggest that the responses of tadpoles to the presence of predators can be quite variable, and thus conclusions based on a single observation at any one moment in an experiment, or even one time during the day, could influence the conclusions that are drawn. For example, if we had only observed tadpole behaviour on 14 or 15 July, then we might have concluded that fish increased the activity of bullfrog tadpoles, but if we had only observed activity during the other observation periods, we would come to a different conclusion. One could also find similar examples depending on the time of day observations were made. We therefore suggest that multiple observations of tadpole behaviour be made throughout an experiment and throughout the day to provide a more complete understanding of tadpole activity patterns and responses to predators.

ACKNOWLEDGEMENTS

We thank J.E. Rettig for helping to collect the bluegill, and M. Fraker and an anonymous reviewer for comments on the manuscript. This research was supported by the Howard Hughes Medical Institute and the Denison University Research Foundation. This experiment was conducted under permits from the Ohio Department of Natural Resources and under Denison University IACUC protocol 2004-01.

REFERENCES

Anholt, B.R., Negovetic, S., Rauter, C. & Som, C. (2005). Predator complement determines the relative success of tadpoles of the *Rana esculenta* complex. *Evolutionary Ecology Research* 7, 733–741.

Anholt, B.R., Skelly, D.K. & Werner, E.E. (1996). Factors modifying antipredator behavior in larval toads. *Herpetologica* 52, 301–313.

Awan, A.R. & Smith, G.R. (2007a). The effect of group size on the activity of leopard frog (*Rana pipiens*) tadoles. *Journal of Freshwater Ecology* 22, 355–357.

Awan, A.R. & Smith, G.R. (2007b). The effect of group size on the responses of wood frog tadpoles to fish. *American Midland Naturalist* 158, 79–84.

Boone, M.D. & Semlitsch, R.D. (2003). Interactions of bullfrog tadpole predators and an insecticide: predator release and facilitation. *Oecologia* 137, 610–616.

Brodie, E.D. Jr. & Formanowicz, D.R. Jr. (1983). Prey size preference of predators: differential vulnerability of larval anurans. *Herpetologica* 39, 67–75.

Brodie, E.D. Jr. & Formanowicz, D.R. Jr. (1987). Antipredator mechanisms of larval anurans: protection of palatable individuals. *Herpetologica* 43, 369–373.

Brown, R.M. & Taylor, D.H. (1995). Compensatory escape mode trade-offs between swimming performance and maneuvering behavior through larval ontogeny of the wood frog, *Rana sylvatica*. *Copeia* 1995, 1–7.

Crump, M.L. (1984). Ontogenetic changes in vulnerability to predation in tadpoles of *Hyla pseudopuma*. *Herpetologica* 40, 265–271.

Eklöv, P. (2000). Chemical cues from multiple predator–prey interactions induce changes in behavior and growth of anuran larvae. *Oecologia* 123, 192–199.

Formanowicz, D.R. Jr. (1986). Anuran tadpole/aquatic insect predator–prey interactions: tadpole size and predator capture success. *Herpetologica* 42, 367–373.

Golden, D.R., Smith, G.R. & Rettig, J.E. (2000). Effects of age and group size on habitat selection and activity level in *Xenopus laevis* tadpoles. *Transactions of the Nebraska Academy of Science* 26, 23–27.

Golden, D.R., Smith, G.R. & Rettig, J.E. (2001). Effects of age and group size on habitat selection and activity level in *Rana pipiens* tadpoles. *Herpetological Journal* 11, 69–73.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.

Griffiths, R.A. & Foster, J.P. (1998). The effect of social interactions on tadpole activity and growth in the British anuran amphibians (*Bufo bufo*, *B. calamita*, and *Rana temporaria*). *Journal of Zoology* 245, 431–437.

Huey, R.B. (1980). Sprint velocity of tadpoles (*Bufo boreas*) through metamorphosis. *Copeia* 1980, 537–540.

Kats, L.B., Petranka, J.W. & Sih, A. (1988). Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* 69, 1865–1870.

Kruse, K.C. & Francis, M.G. (1977). A predation deterrent in larvae of the bullfrog, *Rana catesbeiana*. *Transactions of the American Fisheries Society* 106, 248–252.

Laurila, A. (2000). Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos* 88, 159–168.

Laurila, A., Järvi-Laturi, M., Pakkasmaa, S. & Merilä, J. (2004). Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. *Oikos* 107, 90–99.

Lawler, K.L. & Hero, J.-M. 1997. Palatability of *Bufo marinus* tadpoles to a predatory fish decreases with development. *Wildlife Research* 24, 327–334.

Lawler, S.P. (1989). Behavioral responses to predators and predation risk in four species of larval anurans. *Animal Behaviour* 38, 1039–1047.

Lefcort, H. (1996). Adaptive, chemically mediated fright response in tadpoles of the southern leopard frog, *Rana utricularia*. *Copeia* 1996, 455–459.

McIntyre, P.B., Baldwin, S. & Flecker, A.S. (2004). Effects of behavioral and morphological plasticity on risk of predation in a neotropical tadpole. *Oecologia* 141, 130–138.

Parris, M.J., Reese, E. & Storfer, A. (2006). Antipredator behavior of chytridiomycosis-infected northern leopard frog (*Rana pipiens*) tadpoles. *Canadian Journal of Zoology* 84, 58–65.

Peacor, S.D. (2002). Positive effect of predators on prey growth rate through induced modifications of prey behaviour. *Ecology Letters* 5, 77–85.

Peacor, S.D. (2003). Phenotypic modifications to conspecific density arising from predation risk assessment. *Oikos* 100, 409–415.

Peacor, S.D. & Werner, E.E. (1997). Trait-mediated indirect interactions in a simple aquatic food web. *Ecology* 78, 1146–1156.

Peacor, S.D. & Werner, E.E. (2001). The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences* 98, 3904–3908.

Relyea, R.A. (2002a). Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* 72, 523–540.

Relyea, R.A. (2002b). Costs of phenotypic plasticity. *American Naturalist* 159, 272–282.

Relyea, R.A. (2004). Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85, 172–179.

Relyea, R.A. & Werner, E.E. (1999). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* 80, 2117–2124.

Richards, S.J. & Bull, C.M. (1990). Size-limited predation on tadpoles of three Australian frogs. *Copeia* 1990, 1041–1046.

Richardson, J.M.L. (2001). A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behavioral Ecology* 72, 51–58.

Semiltsch, R.D. & Gibbons, J.W. (1988). Fish predation in size-structured populations of treefrog tadpoles. *Oecologia* 75, 321–326.

Skelly, D.K. & Werner, E.E. (1990). Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* 71, 2313–2322.

Smith, G.R., Dingfelder, H.A. & Vaala, D.A. (2003). Distribution and abundance of amphibian larvae within two temporary ponds in central Ohio, USA. *Journal of Freshwater Ecology* 18, 491–496.

Smith, G.R., Temple, K.G., Dingfelder, H.A. & Vaala, D.A. (2006). Effects of nitrate on the interactions of the tadpoles of two ranids (*Rana clamitans* and *R. catesbeiana*). *Aquatic Ecology* 40, 125–130.

Smith, G.R., Vaala, D.A. & Dingfelder, H.A. (2005). Abundance of vertebrates and macroinvertebrates one and two years after a winterkill in a small Ohio pond. *Journal of Freshwater Ecology* 20, 201–203.

Spieler, M. (2005). Can aggregation behaviour of *Phrynomantis microps* tadpoles reduce predation risk? *Herpetological Journal* 15, 153–157.

Taylor, J. (1983). Orientation and flight behavior of a neotenic salamander (*Ambystoma gracile*) in Oregon. *American Midland Naturalist* 109, 40–49.

Teplitsky, C., Plénet, S. & Joly, P. (2003). Tadpoles' responses to risk of fish introduction. *Oecologia* 134, 270–277.

Travis, J., Keen, W.H. & Juilianna, J. (1985). The role of relative body size in a predator–prey relationship between dragonfly naiads and larval anurans. *Oikos* 45, 59–65.

Wassersug, R.J. & Sperry, D.G. (1977). The relationship of locomotion to differential predation on *Pseudacris triseriata*. *Ecology* 58, 830–839.

Werner, E.E. (1994). Ontogenetic scaling and competitive relations: size-dependent effects and responses in two anuran larvae. *Ecology* 75, 197–213.

Werner, E.E. & Anholt, B.R. (1996). Predator-induced behavioral indirect effects, consequences to competitive interactions in anuran larvae. *Ecology* 77, 157–169.

Accepted: 18 July 2007