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Effects of light and group size on the activity of wood frog tadpoles (*Rana sylvatica*) and their response to a shadow stimulus

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Abstract. Tadpoles are known to behaviorally respond to cues from aquatic predators. However, there are several additional factors that might affect tadpole behavior. We examined the influence of light conditions and group size on the activity of wood frog (*Rana sylvatica*) tadpoles and their response to a simulated non-aquatic predator (i.e., a shadow stimulus). Activity levels of undisturbed wood frog tadpoles were higher in larger groups (15 tadpoles) than in the smaller groups (5 tadpoles). Activity following exposure to a simulated aerial predator (i.e., a shadow stimulus) was also higher in the larger groups of tadpoles than in the smaller groups. Light conditions did not influence activity level in undisturbed tadpoles, but did affect the response to the shadow stimulus, with the greatest responses being observed under bright light conditions. Our results suggest that the factors influencing tadpole activity can include a diverse range of factors and cues, including lighting conditions and group size.

Keywords. *Rana sylvatica*, antipredator response, environmental effects.

Several studies have examined the activity levels of tadpoles in response to predators (Anholt et al., 2000; Eidietis, 2005; Relyea, 2005; Smith et al., 2008; Smith and Awan, 2009), with many finding that tadpoles are generally less active when predation risk is high (Relyea, 2004; Eidietis, 2005). However, there are several potential factors that might affect tadpole behavior in addition to cues from aquatic predators. For example, visual cues from non-aquatic predators (e.g., shadows) may represent an immediate threat to tadpoles and lighting conditions and group size may mediate perceived risk (e.g., group size dilutes predation risk, see Spieler, 2005 for example in tadpoles) or the ability to actually perceive predation risk. One might expect non-aquatic or terrestrial predators to induce a startle or flight response as opposed to freezing or reduction in activity levels. Similarly, shadows or visual stimuli indicate a predator is in the immediate vicinity whereas chemical cues indicate the presence of predators, with relatively little precision either spatially or temporally. Thus, a flight response might be expected to a shadow stimulus rather than a decrease in activity. We examined the influence of light conditions and group size on the activity of wood frog (*Rana sylvatica*) tadpoles, and their response to a simulated non-aquatic predator (i.e., a shadow stimulus).

Multiple wood frog egg masses were collected from a local pond and hatched in the laboratory. Tadpoles were Gosner Stage 26 (Gosner, 1960) when used in our experimental trials. For each trial, 5 (low group size) or 15 (high group size) tadpoles were placed in plastic containers (33 × 17 × 11 cm) containing 4 L of aged tapwater. No individual tadpole was used in more than 1 trial. Each treatment combination was replicated 12 times. Trials were conducted from 1300 h to 1700 h.

Trials were conducted under low light (0.35 μmol photons s^{-1} m^{-2}), medium light (2.90 μmol photons s^{-1} m^{-2}), or high light (213.0 μmol photons s^{-1} m^{-2}) conditions. These light conditions were chosen to provide a range of illumination likely to occur throughout the day, including dawn and dusk, when visually oriented terrestrial predators (e.g., birds) are active. Lighting conditions were created by manipulating overhead lighting, and using a 100 W standard light bulb suspended at a constant height over the experimental container. After a 15 minute acclimation period, undisturbed tadpole activity was determined as the proportion of tadpoles moving (i.e., a scan sample of activity; Lehner, 1996). A cut-out of an aerial predator was then passed over the container at a constant speed and tadpole activity immediately determined by a scan-sample of the proportion of tadpoles active.

We used two-way ANOVAs on the arcsine-square root transformed proportional data for undisturbed activity and the shadow-stimulated activity with group size and lighting condition as independent variables. We used Fisher's Protected Least Squares Difference post-hoc tests to further explore significant effects when appropriate.

Undisturbed activity levels were higher in the high density tadpole groups than in the low density groups (Fig. 1A; $F_{1,66} = 6.74$, $P = 0.012$). Light conditions had no effect on undisturbed activity levels (Fig. 1A; $F_{2,66} = 1.16$, $P = 0.32$). The interaction between light conditions and group size was not significant ($F_{2,66} = 0.23$, $P = 0.80$).

Activity in response to the shadow stimulus was higher in the high density groups of tadpoles compared to the low density groups (Fig. 1B; $F_{1,66} = 6.46$, $P = 0.013$). Activity induced by the shadow stimulus was highest under bright light conditions (Fig. 1B; $F_{2,66} = 4.27$, $P = 0.018$; Fisher's PLSD: high vs. medium: $P = 0.019$; high vs. low: $P = 0.010$; low vs. medium: $P = 0.80$). The interaction between light conditions and group size was not significant ($F_{2,66} = 1.88$, $P = 0.16$).

In the absence of any predator cues, activity levels of the wood frog tadpoles were higher in the larger groups than in the smaller groups. In addition, activity in response to the shadow stimulus was higher in the larger groups of tadpoles than in the smaller groups of tadpoles. Rot-Nikcevic et al. (2006) found that activity of *R. sylvatica* tadpoles increases in the presence of higher numbers of conspecifics, either real or simulated using mirrors (see also Relyea, 2002). However, Awan and Smith (2007) found no effect of group size on wood frog tadpole activity level, although they tested groups of up to 8 tadpoles. It thus appears that wood frogs in general increase activity with tadpole density, with one or two exceptions. Such a response may reflect lower perceived predation risk in larger groups (e.g., Peacor, 2003) or a response to increased numbers of conspecifics (i.e., potential competitors (e.g., Relyea, 2002). The higher activity in response to the shadow stimulus in larger groups may reflect the startle response of one or a few individual tadpoles being transmitted to others in the group, suggesting a potential benefit to larger tadpole group sizes (e.g., the confusion effect, Miller, 1992; Krakauer, 1995; Krause and Ruxton, 2002).

Light conditions did not influence activity level in the undisturbed tadpoles, but did affect the response of tadpoles to the shadow stimulus, with the greatest responses being

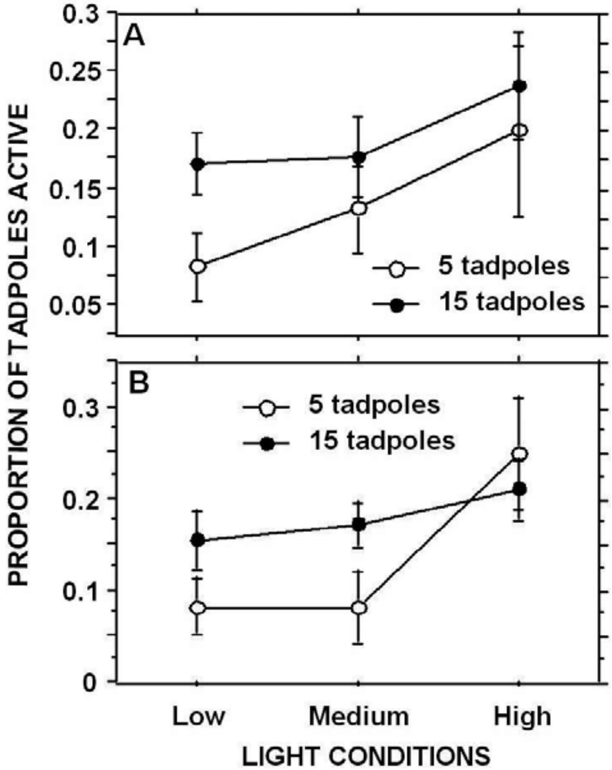

Fig. 1. The effect of lighting conditions and group size on A) the activity level under undisturbed conditions, and B) the responses of wood frog (*R. sylvatica*) tadpoles to a shadow stimulus. Means are given ± 1 SE.

observed under bright light conditions. Increased responses to predators in brighter conditions likely result from an increase in the ability of the tadpoles to visually perceive the predator. Previous studies on tadpole activity suggest light conditions can influence activity patterns in tadpoles. *Bufo rufus* tadpoles are inactive at night, but when a light is shined on them they become active (Eterovick and Sazima, 1999). *Xenopus laevis* tadpoles alter their behavior as lighting conditions change (Jamieson and Roberts, 2000). Activity level of *Bufo americanus* tadpoles increases as light increases, and decreases on overcast days (Beiswenger, 1977). Similarly, activity of *Bufo bufo* tadpoles is concentrated during the day (Griffiths et al., 1988). In contrast, activity of Bullfrog (*Rana catesbeiana*) tadpoles is highest at dusk or at night (Smith et al., 2007). In addition, lighting conditions may affect how tadpoles respond to predators. For example, Fraker (2008) found that Green Frog (*Rana clamitans*) tadpoles reduced their activity more in the presence of a predator during daylight hours than at other times of the day.

In conclusion, our results suggest that the factors influencing tadpole activity can include a diverse range of factors and cues. In particular, group size can influence undisturbed activity and activity levels following a visual stimulus. Lighting conditions can influence activity in response to a shadow stimulus.

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