Oecologia (2000) 125:301–308
DOI 10.1007/s004420000451

Peter B. McIntyre · S. Andy McCollum

Responses of bullfrog tadpoles to hypoxia and predators

Received: 12 June 1999 / Accepted: 24 April 2000 / Published online: 8 July 2000
© Springer-Verlag 2000

Abstract Low dissolved oxygen concentrations present numerous challenges for non-air-breathing aquatic organisms. Amphibian larvae and their predators can respond to oxygen levels by altering their behavior and physiology, but the ecological consequences of these responses are generally unknown. We conducted two laboratory experiments to study the effects of dissolved oxygen on respiratory behavior and susceptibility to predation of larval bullfrogs (*Rana catesbeiana*). In the first, we exposed small, lungless tadpoles to a predatory salamander larva (*Ambystoma tigrinum*) under high and low oxygen conditions. More tadpoles were consumed in high oxygen tanks than in low ones, presumably because salamanders remained near the surface in the low oxygen tanks while most tadpoles rested on the bottom. Tadpole activity depended on both oxygen and predator presence: swimming decreased after addition of salamanders under high oxygen, but increased under low oxygen. In the second experiment, we examined the effect of predator chemical cues on the air-breathing rate of large tadpoles with well-developed lungs under low oxygen conditions. In the presence of chemical cues produced by dragonfly larvae consuming bullfrog tadpoles, air-breathing and swimming were significantly reduced relative to controls. These experiments demonstrate the potential impact of dissolved oxygen on predator-prey interactions, and suggest that outcomes depend on the respiratory ecology of both predator and prey.

P.B. McIntyre
Harvard University, Cambridge, MA 02138, USA

S.A. McCollum
University of Michigan, Department of Biology,
Ann Arbor, MI 48109-1048, USA

Present address: P.B. McIntyre
Department of Ecology and Evolutionary Biology,
Corson Hall, Cornell, University, Ithaca, NY 14853-2701, USA

Present address: S.A. McCollum
Department of Biology, Cornell College,
Mount Vernon, IA 52314, USA

Key words Air-breathing · *Rana catesbeiana* · Dissolved oxygen · *Ambystoma tigrinum* · Hypoxia

Introduction

Aquatic habitats vary widely in many chemical characteristics, but for non-air-breathing animals dissolved oxygen (DO) concentration is among the most important. Ponds are subject to large DO fluctuations on a daily and seasonal basis (Noland and Ultsch 1981; Nie et al. 1999; Werner and Glennemeier 1999). DO is generated by atmospheric diffusion, surface mixing, and photosynthesis, and consumed by aerobic respiration. As a result, the concentration of DO is typically highest during the afternoon and early evening, when photosynthesis can generate enough oxygen to saturate much of the water column, and lowest during the early morning. DO also varies seasonally with changes in ambient light, temperature, and abundance of decaying organic material (Junk et al. 1983; Chapman et al. 1998; Werner and Glennemeier 1999). Within the water column, DO often declines sharply near the substrate (Ultsch 1971; Nie et al. 1999) or below a thermocline (Wetzel 1983; Rahel and Nutzman 1994). Taken together, these factors create complex spatial and temporal patterns of DO concentration.

Variation in abiotic factors, such as DO, can have important effects on community organization and interspecific interactions (Dunson and Travis 1991; Wellborn et al. 1996). The nature of these effects will depend on the particular responses of each species to DO concentration (Kolar and Rahel 1993). While low DO imposes severe demands on respiratory systems and generally constrains activity, it may create a refuge for hypoxia-tolerant prey species if their predators are unable to tolerate hypoxia (Ultsch 1971; Poulin et al. 1987; Chapman et al. 1996). Hypoxia-tolerant predators may forage efficiently despite variation in DO (Rahel and Nutzman 1994), and avoiding low DO may increase the exposure of some prey species to predators (Rahel and Kolar 1990; Kolar and Rahel 1993).

Amphibian larvae present an ideal opportunity to study the influence of physiological requirements on species interactions (Feder 1984; Burggren and Infantino 1994). Larval anurans show a variety of physiological responses to low DO, including altering blood flow, modifying gill ventilation rates, and changing lung, skin, and gill morphology (reviewed in Burggren 1984; Feder 1984; Burggren and Infantino 1994). However, air-breathing is their most rapid and vital response; though non-air-breathing tadpoles can survive low DO conditions for short periods (Wassersug and Seibert 1975; Noland and Ultsch 1981), atmospheric oxygen is probably necessary for long-term survival under sustained hypoxia. Air-breathing requires lungs, and there is considerable phylogenetic variation in the timing of lung development (Wassersug and Seibert 1975). For instance, larval bufonids and *Ascaphus* do not inflate their lungs until just prior to completing metamorphosis, whereas many ranids develop and use their lungs for pulmonary respiration fairly early in development (Feder 1984). The ontogenetic development of air-breathing also varies within species (Feder 1981).

The interplay between air-breathing and susceptibility of tadpoles to predation has been a topic of considerable speculation but relatively little empirical research (Burggren 1984; Feder 1984; Burggren and Infantino 1994). Surfacing to breathe may attract the attention of visual predators. Turtles are more likely to attack and consume actively swimming *Rana berlandeiri* tadpoles than stationary ones, though air-breathing tadpoles are not at greater risk than other active individuals (Feder 1983b). Air-breathing enhances the swimming stamina of *R. berlandeiri* tadpoles (Wassersug and Feder 1983), but many predators of tadpoles do not chase their prey for long distances, so swimming stamina may have little influence on risk of predation (Feder 1984). Thus, the effects of low DO on predator-prey interactions remain unclear.

Here, we investigate the relationship between hypoxia and risk of predation for tadpoles both before and after they develop lungs. The early developmental stages (stages 25–32, Gosner 1960) of many species have poorly developed lungs (reviewed in Feder 1984), and small tadpoles are more vulnerable to predators than large ones (Caldwell et al. 1980; Brodie and Formanowicz 1983; Formanowicz 1986; Semlitsch 1990; Tejedo 1993). Thus, physiological and behavioral responses to low DO may influence risk of predation most strongly when tadpoles are small. Our first experiment explores the susceptibility of small, non-air-breathing *Rana catesbeiana* tadpoles to predation by larval salamanders (*Ambystoma tigrinum*) under high and low DO. Tadpoles rely heavily upon chemical cues to inform them about their environment, and they can rapidly alter their behavior (Stauffer and Semlitsch 1993; Petranka et al. 1987) and morphology (McCollum and Van Buskirk 1996) in response to chemical cues from predators. In our second experiment, we use chemical cues from dragonfly larvae consuming conspecific tadpoles to elucidate the interplay between perceived risk of predation and air-breathing frequency in large *R. catesbeiana*. In light of our results, we reevaluate the adaptive value of aerial respiration in tadpoles.

Materials and methods

The species

Rana catesbeiana tadpoles inhabit permanent ponds, rivers, and swamps, where larvae overwinter for 0–3 years (Collins 1979; Crowder et al. 1998). Bullfrog tadpoles are distasteful to fish, but are consumed by many common predators such as salamander and dragonfly larvae (Werner and McPeek 1994). Their lungs develop between Gosner (1960) stages 25–39 (Feder 1984; Crowder et al. 1998), but this process is influenced by many factors (Feder 1981; Burggren 1984). Generally, aerial respiration is not an option for the first several months or longer after hatching. Among the tadpoles used here, air-breathing was rare among individuals under 1 g wet mass (corresponding to Gosner (1960) stages in the low 30 s), but all tadpoles weighing more than 1 g breathed air readily. Under hypoxia, air-breathing becomes the sole source of oxygen (Burggren and West 1982; Burggren et al. 1983), overall oxygen consumption is reduced, and lactate accumulates (Feder 1983a; Hastings and Burggren 1995; Crowder et al. 1998).

Tiger salamander (*Ambystoma tigrinum)* larvae are voracious predators on invertebrates and larval amphibians, including bullfrog tadpoles (Werner and McPeek 1994; Petranka 1998). Like tadpoles, *Ambystoma* larvae rely on air-breathing when DO is low (Heath 1976; Branch and Taylor 1977). Air-breathing frequency increases with size (Wassersug and Seibert 1975), and may be related to food availability and ambient light (Lannoo and Bachman 1984). Denying surface access usually kills larval *Ambystoma* under hypoxia (Weigmann and Altig 1975; but see Heath 1976).

Anax longipes is a large aeshnid dragonfly found in permanent and semi-permanent ponds. Larval *Anax* are important predators on tadpoles (Caldwell et al. 1980; Werner and McPeek 1994). Ranid tadpoles reduce their activity and grow more slowly when exposed to chemical cues produced as dragonflies consume conspecific tadpoles (Peacor and Werner 1997). Hypoxia forces *Anax* to remain near the surface, where they position their rectal gills at or near the water surface (Corbet 1999).

This research was conducted at the University of Michigan's E.S. George Reserve (ESGR). Tadpoles were collected at the Michigan Department of Natural Resources' Saline Pond Facility. Small tadpoles were raised from eggs and kept in predator-free plastic wading pools until their use here. Large tadpoles were collected from a pond in which they had overwintered, and were maintained in a 1000 l plastic cattle tank. *Ambystoma* eggs and *Anax* larvae were collected from fishless, artificial ponds on the ESGR and maintained in the laboratory.

Experiment 1

Experiment 1 was designed to elucidate the effect of DO on the susceptibility of small, functionally-lungless *R. catesbeiana* tadpoles to predation by larval *A. tigrinum*. The experiment was conducted in the laboratory, using 24 10-gallon (38 l) glass aquaria in two rows of six on each of two shelves. Tanks were illuminated on a 14L:10D cycle by a bank of fluorescent lights above each shelf. Two treatments, low and high DO, were replicated 12 times each. One tank on each shelf was randomly assigned to be either a high or low oxygen treatment, and DO treatments were alternated for the remainder of the aquaria on that shelf.

Tanks were filled 24.5 cm deep with aged well water. Prior to filling, one end of each tank was fitted with a 13×25.5 cm hood of plastic sheeting descending from approximately 27 cm high at the end of the aquarium to 23.5 cm towards its center. When the aquaria were filled, a region of air trapped under the hood covered 25% of the water's surface. High or low oxygen levels were

achieved by continuously bubbling either atmospheric air from a compressor or N_2 gas into the covered region of each tank. This method rapidly stabilized DO at 8.5–8.9 mg/l in high oxygen tanks and 1.0–1.4 mg/l in low oxygen tanks, representing the extremes of DO experienced by the experimental species near the ESGR. DO and temperature were measured in each tank at the beginning and conclusion of the experiment with a YSI Model 57 meter; temperature ranged from 22.8–23.7°C in both treatments. Each tank received one bundle of nylon ropes (four 91 cm pieces tied together in the center) to provide perches and hiding places, and 100 mg of ground food (3:1 rabbit food pellets to fish food flakes) for the tadpoles.

To obtain uniform-sized experimental animals, tadpoles were gently passed through a series of sieves. Tadpoles of the same size were haphazardly divided into 34 groups of 10 individuals, and 10 groups were used to estimate the developmental stage and mass of experimental tadpoles (Gosner (1960) stage 25–26; wet mass =846±74 (SD) mg/10 tadpoles). The remaining 24 groups were randomly assigned to experimental aquaria and added to their respective tanks the night before the experiment to acclimate. All tadpoles were alive and vigorous at the beginning of the experiment. Salamanders were also acclimated to high or low DO overnight in non-experimental tanks. Mean salamander mass was 1.561±0.268 g, and individuals were haphazardly assigned to aquaria.

The experiment began when one pre-weighed *A. tigrinum* larva was added to each tank at 8:00 a.m. We recorded tadpole swimming activity and position twice before and five times after adding the salamanders. These observations were made one half-hour apart before adding the predators, and every two hours thereafter (beginning in the second hour). To assess activity, we rapidly surveyed each tank and estimated the proportion of tadpoles that were swimming. Position within tanks was measured as the proportion of tadpoles near the surface (within 3 mm), touching the bottom, and in the water column. The location of the salamander in each tank (top vs bottom half) was also recorded hourly. The experiment lasted for 10 h, after which we recorded the proportion of tadpoles that were still alive.

All proportions were arcsin-square root transformed for analysis, and each of the four rows of tanks was treated as a block. We used ANCOVA to estimate the effects of DO level, block, DO × block interaction, and salamander mass (the covariate) on tadpole survival to the conclusion of the experiment. Preliminary analysis indicated that there was no interaction between DO and salamander mass ($p>0.15$), thus the data met the homogeneity of slopes assumption of ANCOVA.

Repeated measures ANOVA was used to analyze the main effects and interaction of DO and salamander introduction on the proportion of tadpoles swimming, resting on the bottom, and touching the surface. These analyses compared the means of observations made before (n=2) and after (n=5) introducing the salamander. The repeated measure thus represents a change between the two time periods (before vs after). ANCOVA was used to assess the influence of DO, block, DO × block interaction, and salamander size on the proportion of observations in which a salamander was in the upper half of the tank.

Experiment 2

Experiment 2 examined the effects of predator chemical cues on the swimming activity and air-breathing frequency of large *R. catesbeiana* under low DO. Sixteen aquaria were placed in two rows of four on each of two shelves. All were aerated with N_2 gas using the method described above, maintaining DO levels between 0.7 and 1.4 mg/l and temperatures of 21.0–21.9°C. For 5 days prior to the experiment, a screened cage containing 0 or 1 larval *Anax longipes* was floated in each tank. Treatments were alternated in adjacent tanks. Small bullfrog tadpoles were fed to the caged *Anax* on the first, third, and fourth days to produce chemical cues of predation. All tadpoles were consumed and the cages were removed on the fifth day.

Forty-eight large, air-breathing bullfrog tadpoles were selected by matching like-sized individuals based on wet mass. Their masses ranged from 1179 to 3060 mg (stages 31–36; Gosner 1960), and the mean difference between paired tadpoles was 21±17 mg (54 mg maximum). Each pair of tadpoles was haphazardly assigned to two adjacent tanks, such that one member of each pair was in a tank containing *Anax* chemical cues and the other was not. Pairs were used in haphazard order, and tadpoles were allowed to acclimate in their tanks for 45 min prior to trials.

Trials consisted of watching four tanks simultaneously and recording the number of air-breaths taken by each tadpole over a 15-min period. We also recorded the proportion of time spent swimming by the last 16 tadpoles during two 2-min periods. After each trial, tadpoles were removed and DO and temperature were measured for each tank. Each tank was used in three trials, for a total of 48 trials (24 pairs of tadpoles). All trials took place on the same day.

The frequency of air-breaths per minute was analyzed for effects of predator cues using ANCOVA, with predator cues and tanks as categorical variables and tadpole mass as a covariate in the model. The arcsine-square root transformed proportion of time spent swimming by each of the last 16 tadpoles was analyzed (ANCOVA) for effects of predator cues and tadpole mass. To test whether individual variation in swimming activity was responsible for differences in air-breathing frequency, swimming was added as a covariate in the ANCOVA model of air-breathing for the last 16 tadpoles. In each of these analyses, the interaction between tadpole mass and predator cues was non-significant ($P>0.15$) and was removed from the final model.

All analyses were conducted using SYSTAT (SPSS 1994).

Results

Experiment 1

Both DO and salamander size influenced the survival of tadpoles (Table 1, Fig. 1). Tadpole survival to the end of the experiment was greater under low DO than high DO (mean ± SE: high DO =0.267±0.014, low DO =0.383± 0.013). Larger salamanders were more effective predators under both DO regimes (Fig. 1), and there was no interaction between salamander mass and DO ($p>0.15$).

Repeated measures ANOVA of tadpole swimming before and after addition of the salamander revealed no main effect of either DO or time, but there was a significant interaction between the two (Table 2). This was because mean tadpole swimming activity decreased slightly after introduction of the predator in the high DO tanks, while it increased considerably in the low DO tanks (Fig. 2). Tadpoles were most often observed on the bottom of tanks, and very few (<5%) were observed at

Table 1. ANCOVA of small tadpole survival in experiment 1 (see Fig. 1). Salamander wet mass was the covariate in the analysis, and there was no interaction between DO and salamander mass ($P>0.15$)

Source	*df*	MS	*F*	*P*
DO	1	0.212	9.18	0.008
Block	3	0.016	0.67	0.582
DO × Block	3	0.021	0.91	0.458
Salamander mass	1	0.194	8.39	0.011
Error	15	0.023		

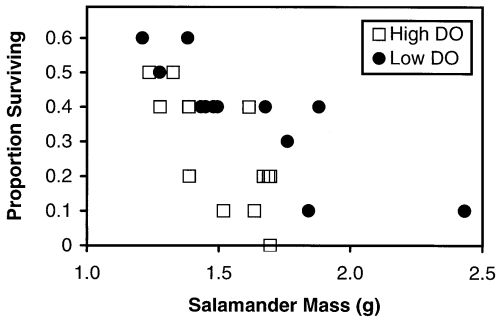

Fig. 1 Survival of small (<0.15 g) *R. catesbeiana* tadpoles in low (1.0–1.4 mg/l DO) and high (8.5–8.9 mg/l) dissolved oxygen as a function of predator (larval *A. tigrinum*) mass (see Table 1). Each point represents the proportion of 10 tadpoles that survived over 10 h in a 10 gallon aquarium with a single predator

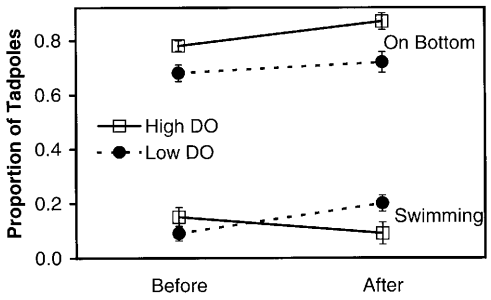

Fig. 2 Effect of dissolved oxygen on the mean proportion of small (<0.15 g) *R. catesbeiana* tadpoles swimming and resting on the bottom in experiment 1 (see Table 2). "*Before*" signifies the mean of observations made before adding a predator (larval *A. tigrinum*) (*n*=2 observations per tank); "*After*" represents the mean of those made thereafter (*n*=5 observations per tank). Means (+ SE) for 12 tanks per treatment are shown

the surface. A greater proportion of tadpoles rested on the bottom in the high DO than the low DO treatment (Table 2). There were also significantly more tadpoles resting on the bottom after predators were added to the tanks than before (Table 2, Fig. 2). There was no effect of DO or time on the proportion of tadpoles at the surface.

Ambystoma were usually observed in the top half of tanks under low DO (mean proportion in top half ± SE: 0.71±0.11), but were never recorded there when DO was high. Thus, the location of salamanders differed significantly between DO levels ($F_{1,15}$=30.94, P<0.001), but was unrelated to salamander size ($F_{1,15}$=0.471, P=0.503). Salamanders took breaths of air frequently in hypoxic tanks and rarely in normoxic ones, but we did not quantify this difference. We also observed differences in the gill coloration of salamanders in the two treatments, which we presume were related to blood flow (Bond 1960; Burggren 1984). Salamanders in the high DO tanks had bright red gill filaments, while those in low DO tanks had dull, brown filaments.

Experiment 2

Tadpoles responded strongly to predator cues in the second experiment (Table 3). The proportion of time spent swimming was reduced from 0.42±0.10 (SE) to 0.14±0.05 in the presence of chemical cues from *Anax*, and the frequency of air-breaths was halved (Fig. 3). Neither response depended on tadpole mass. Incorporating time spent swimming as a covariate in the analysis of air-breathing frequency did not improve the model (Table 3), suggesting that time spent swimming and air-breathing frequency were independent.

Table 2 Repeated-measures ANOVA of the effects of DO and time on tadpole behavior in experiment 1 (see Fig. 2). Data are tank means before (*n*=2) and after (*n*=5) introducing the salamander. The "Between-tank" comparison corresponds to the effect of DO level; the "Within-tank" comparisons assess differences in behavior before versus after adding the salamander (i.e. "Time" effect), and the interaction between time and DO level

Variable	Source	*df*	MS	*F*	*P*
Swimming	Between-tank				
	DO	1	0.078	1.49	0.235
	Error (tanks within DO)	22	0.053		
	Within tank				
	Time	1	0.012	0.35	0.558
	Time × DO	1	0.391	11.57	0.003
	Error (within tank)	22	0.034		
Bottom use	Between-tank				
	DO	1	0.347	11.95	0.002
	Error (tanks within DO)	22	0.29		
	Within tank				
	Time	1	0.169	7.21	0.014
	Time × DO	1	0.041	1.76	0.198
	Error (within tank)	22	0.023		
Surface use	Between-tank				
	DO	1	0.036	1.92	0.180
	Error (tanks within DO)	22	0.019		
	Within tank				
	Time	1	0.006	1.09	0.309
	Time × DO	1	0.001	0.20	0.660
	Error (within tank)	22	0.005		

Table 3 ANCOVA of the effect of Anax chemicals on the proportion of time spent swimming and the frequency of air-breathing by large tadpoles in experiment 2 (see Fig. 3). Swimming was measured for only 16 of the 48 tadpoles. Swimming and air-breathing were analyzed separately, then swimming was used as a covariate in an ANCOVA of air-breathing for the 16 tadpoles for which both data were recorded

Variable	Source	*df*	MS	*F*	*P*
Swimming (16 tadpoles)	Predator cues	1	0.578	7.41	0.017
	Tadpole mass	1	0.157	2.01	0.180
	Error	13	0.078		
Air breathing (all 48 tadpoles)	Predator cues	1	1.581	62.55	<0.001
	Tank	14	0.057	2.27	0.028
	Tadpole mass	1	0.010	0.38	0.543
	Error	31	0.025		
Air breathing (16 tadpoles with data on swimming)	Predator cues	1	0.300	8.13	0.015
	Tadpole mass	1	0.015	0.42	0.530
	Swimming	1	0.023	0.62	0.448
	Error	12	0.037		

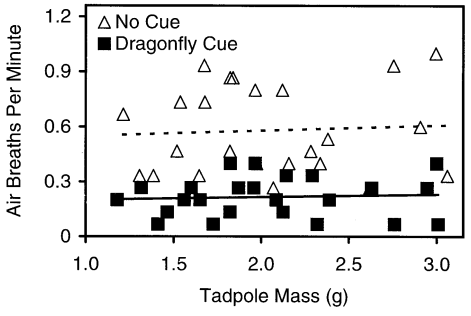

Fig. 3 Frequency of air breathing by large (1–3 g) *R. catesbeiana* tadpoles under hypoxic conditions (0.7–1.3 mg/l DO) as a function of tadpole mass in the presence (*solid line*) and absence (*dashed line*) of dragonfly chemical cues (see Table 3). Each point represents a single tadpole, and the lines are based on linear regressions

Air breathing rates also differed significantly between tanks (Table 3). Tanks varied in temperature and DO ($F_{15,32}$=55.54, P<0.001; $F_{15,32}$=3.51, P=0.001, respectively), although these differences were small. Preliminary analyses using temperature and DO as covariates indicated that variation in these factors had no influence on time spent swimming or air-breathing frequency (P>0.1 in all cases), so we dropped these terms from the final models.

Discussion

These two experiments underscore the potential strength of interactions between biotic and abiotic factors in aquatic ecology (Dunson and Travis 1991). Manipulation of one abiotic factor– DO– within the context of a simple community– one predator and one prey species– significantly altered each species' behavior and the outcome of predator-prey interactions.

Experiment 1 addressed the effect of DO on predator-prey interactions between small tadpoles and predatory salamanders. Risk of predation was lower under hypoxia than normoxia, and was apparently mediated by differences in the behavioral responses of *A. tigrinum* and *R. catesbeiana* to DO concentration. Whereas most tadpoles remained near the bottom of the tank regardless of DO (Fig. 2), salamanders spent most of their time near the surface under hypoxic conditions but were always near the bottom when DO was high. Thus, salamanders probably encountered more tadpoles under high DO than low, resulting in an increased predation rate. From the standpoint of the salamander, respiratory requirements apparently constrain hunting capability. For the tadpoles, low DO reduced risk of predation through its effects on predator behavior. In addition, the increase in the proportion of tadpoles resting on the bottom after introduction of the salamander may reflect an adaptive predator-avoidance response.

The increased swimming by tadpoles in low DO tanks following the introduction of the salamander is difficult to explain. Tadpoles often become less active in the presence of predators (e.g., Werner and McPeek 1994; McCollum and Van Buskirk 1996; Peacor and Werner 1997), as we observed in high DO tanks. Presumably, this response reduces the risk of detection by visual predators. Thus, it was striking that the tadpoles doubled their swimming after salamanders were added to low DO tanks. Since activity increases oxygen consumption (Wassersug and Feder 1983), we expected tadpoles without functional lungs to reduce their swimming even further under low DO in order to conserve oxygen.

Increased swimming in the presence of predators in low DO may be an effort to maximize their potential to escape predators. Tadpoles may move in search of higher DO concentrations (Costa 1967), in which aerobic flight from predators might be more easily sustained (Wassersug and Feder 1983). If so, we would expect predators to induce tadpoles to spend less time on the bottom, where DO concentrations are typically lowest in natural ponds, and more time near the surface, where DO is usually highest. Our data do not support this hypothesis. Constant bubbling kept our tanks well mixed, and only a thin surface layer of water could have been well-oxygenated in our low DO tanks. Under these conditions, small *Hyla versicolor* tadpoles increase their contact with the surface (McIntyre and McCollum, unpublished data), but the small *R. catesbeiana* tadpoles in experiment 1 made little use of the surface layer. An alternative function of swimming may be to prevent the formation of an anoxic boundary layer along the skin

(Pinder and Feder 1990; Feder and Booth 1992). Boundary layers compromise cutaneous respiration, and may lower the capacity for aerobic swimming (Feder 1983b, 1984). Swimming may also enhance branchial respiration by increasing flow over the gills (West and Burggren 1982; Feder 1983a).

Unfortunately, experiment 1 did not include an independent, predator-free treatment. All predator-free observations were made at the beginning of trials, while responses to the predator were recorded later, confounding the effects of time and predators. However, the only clear shift in tadpole behavior occurred soon after introduction of the salamander, so it is unlikely that prolonged hypoxia or other factors were responsible.

Experiment 2 addressed the effect of perceived risk of predation on air-breathing and swimming by large tadpoles under hypoxic conditions. Although large *R. catesbeiana* tadpoles are not invulnerable to predators, they are less susceptible than smaller conspecifics (Formanowicz 1986; Tejedo 1993), and large tadpoles tend to respond less strongly to predator cues than small individuals (e.g., Werner and Anholt 1996; Puttlitz et al. 1999). However, under hypoxia, the large tadpoles in experiment 2 were less active in the presence of chemical cues from *Anax* than in control trials, while the small tadpoles in experiment 1 became more active in the presence of *Ambystoma*.

This disparity between the responses of small and large tadpoles to predators in our experiments may be attributable to differences in previous experience with predators or simply different responses to cues from a caged dragonfly and a free-roaming salamander. However, differences in the respiratory pathways available to these two size classes of tadpoles may also have been important. Large, air-breathing tadpoles can rely exclusively upon atmospheric air as a source of oxygen (Feder 1983a), so they only need to swim enough to reach the surface to breathe. Smaller individuals have a proportionately smaller total oxygen budget (Feder 1982; Crowder et al. 1998), but they must rely upon branchial and cutaneous oxygen uptake until they develop lungs. Thus, it may be necessary for lungless tadpoles to swim frequently in order to eliminate boundary layers and maximize flow over the gills, while air-breathers may be more flexible because of the decoupling of respiration and swimming.

More profound, we believe, than the reduction in swimming by large tadpoles was the reduction in air-breathing rate in the presence of *Anax* cues. Reducing air-breathing frequency entails a major shift in both the source and amount of oxygen obtained. Aquatic respiration alone is insufficient to meet a large tadpole's respiratory requirements in severely hypoxic water, and lactate accumulation is greater under hypoxia even when tadpoles can reach the surface (Crowder et al. 1998). Denying tadpoles access to the surface drastically reduces their oxygen budgets and causes lactate to build up from anaerobic metabolism (Feder 1983a). Thus, reducing the frequency of air-breaths in response to predator cues has implications for both risk of predation (Feder 1983b; Wolf and Kramer 1987) and capacity for aerobic activity. Air-breathing enhances tadpoles' swimming endurance under hypoxic conditions (Wassersug and Feder 1983), so tadpoles may be less able to escape predators or sustain normal aerobic metabolism when they reduce air-breathing in the presence of predator cues. In the long term, this could affect growth and developmental rates (e.g., Weber and Kramer 1983), as well as survival.

The frequency of air-breathing did not covary with time spent swimming (Table 3), suggesting that lower breathing frequency is a direct response to perceived risk of predation rather than a by-product of lower oxygen demand due to reduced activity. If this is the case, the reduction in air-breathing in response to predator chemical cues implies an association between air-breathing and risk of predation. Two of the most common predators on bullfrog tadpoles, aeshnid dragonfly larvae and *Ambystoma*, both reside higher in the water column under low than high DO, so minimizing trips to the surface appears adaptive for air-breathing tadpoles (Feder 1983b). Although we did not quantify the proportion of time spent near the surface by tadpoles in experiment 2, it was noticeably lower in the predator treatment. The results of experiment 1 suggest that this spatial separation of predator and prey may strongly influence predation rates. Reducing the time spent near the surface may be necessary to realize the benefits of curtailed air-breathing; one strategy may not be effective without the other (Wolf and Kramer 1987). This is particularly relevant for tadpoles that linger at or near the surface under hypoxia without actually air-breathing (Wassersug and Seibert 1975; Crowder et al. 1998).

The lack of a relationship between air-breathing frequency and tadpole mass in experiment 2 was surprising. Under normoxia, oxygen consumption typically increases with mass for a variety of taxa (Feder 1981, 1982; Hastings and Burggren 1995; Crowder et al. 1998), as does air-breathing frequency (Wassersug and Seibert 1975; Wassersug and Feder 1983; Feder and Wassersug 1984; but see Crowder et al. 1998). However, mass had no effect on air-breathing frequency in our experiment (Table 3), despite the wide range of sizes used (1–3 g). The air-breathing rates we observed were within the range published elsewhere for *R. catesbeiana* (Crowder et al. 1998) and *R. pipiens* (Wassersug and Seibert 1975).

For hypoxia-tolerant species, low DO may create refuges from predators (Ultsch 1971; Poulin et al. 1987; Chapman et al. 1996). Nie et al. (1999) found that bullfrog tadpoles often use low DO microhabitats when oxygen concentrations are higher nearby, and our results suggest that such areas may serve as refuges from hypoxia-intolerant predators. In other cases, low DO can make prey species more susceptible to predators by forcing them to increase activity or leave protective cover (Wolf and Kramer 1987; Rahel and Kolar 1990; Kolar and Rahel 1993). Feder (1983b) suggested that air-breathing tadpoles may be subject to this trade-off, since visual predators may notice air-breathers more frequent-

ly than non-air-breathers. The results of experiment 2 support an association between risk of predation and air-breathing, but it is important to note that responses by prey to hypoxia and predators may depend strongly on both species and developmental stage (Wassersug and Seibert 1975; Huuskonen and Karjalainen 1997). Similarly, differences in the physiological requirements and behaviors of predator species may influence the effects of DO on their foraging efficiency. Responses of predators to DO may also be mediated by many other factors, such as ambient light, prey type, and prey density (Hassinger and Anderson 1970; Lannoo and Bachmann 1984).

The question remains as to why tadpoles should develop lungs and air-breathe far in advance of metamorphosis (Feder 1983b, 1984). Our results from experiment 1 and similar experiments with other tadpole and predator species suggest that low DO often reduces risk of predation for non-air-breathing tadpoles (McIntyre and McCollum unpublished data), whereas air-breathing probably increases risk of predation in most cases (Feder 1983b). Since non-air-breathing tadpoles can survive low DO conditions for at least short periods of time (Wassersug and Seibert 1975; Noland and Ultsch 1981), functional lungs may be a necessity only in chronically hypoxic habitats. One advantage of precocious air-breathing may be to facilitate metamorphic plasticity. Metamorphosis requires the possession of well developed lungs, and the process of air-breathing enhances pulmonary development (Bruce et al. 1994; Pronych and Wassersug 1994) and maintenance (Crowder et al. 1998). Thus, the benefits of metamorphic plasticity (Wilbur and Collins 1973; Werner 1986; Newman 1992) in response to deteriorating environmental conditions may outweigh the costs, such as increased risk of predation, of premetamorphic air-breathing.

Acknowledgements We are grateful to Scott Peacor for supplying the bullfrog tadpoles, to Kerry Yurewitz and Neil Kubica for assistance in capturing and maintaining the salamanders, and to Karen Glennemeier for the loan of her DO meter. We also thank The University of Michigan Museum of Zoology and Ron Nussbaum for permission to work at ESGR. The manuscript was improved by comments from Richard Wassersug, Earl Werner, John Cadle, Dana Hawley, Craig Osenberg, and an anonymous reviewer. PBM was supported by an REU supplement to NSF DEB 9408397 to S.A. McCollum, J. Van Buskirk, and E.E. Werner.

References

Bond AN (1960) An analysis of the response of salamander gills to changes in the oxygen concentration of the medium. Dev Biol 2:1–20

Branch LC, Taylor DH (1977) Physiological and behavioral responses of larval spotted salamanders (*Ambystoma maculatum*) to various concentrations of oxygen. Comp Biochem Physiol 58A:269–274

Brodie ED Jr, Formanowicz DR Jr (1983) Prey size preference of predators: differential vulnerability of larval anurans. Herpetologica 39:67–75

Bruce RC, Beachy CK, Lenzo PG, Pronych SP, Wassersug RJ (1994) Effects of lung reduction on rheotactic performance in amphibian larvae. J Exp Zool 268:377–380

Burggren WW (1984) Transition of respiratory processes during amphibian metamorphosis: from egg to adult. In: Seymour RS (ed) Respiration and metabolism of embryonic vertebrates. Junk, Dordrecht, pp 31–53

Burggren WW, Infantino RL Jr (1994) The respiratory transition from water to air breathing during amphibian metamorphosis. Am Zool 34:238–246

Burggren WW, West NH (1982) Changing respiratory importance of gills, lungs, and skin during metamorphosis in the bullfrog *Rana catesbeiana*. Respir Physiol 47:151–164

Burggren WW, Feder ME, Pinder AW (1983) Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandeiri* and *Rana catesbeiana*. Physiol Zool 56: 263–273

Caldwell JP, Thorp JH, Jervey TO (1980) Predator-prey relationships among larval dragonflies, salamanders, and frogs. Oecologia 46:285–289

Chapman LJ, Chapman CA, Ogutu-Ohwayo R, Chandler M, Kaufman L, Keiter AE (1996) Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. Conserv Biol 10:554–561

Chapman LJ, Chapman CA, Crisman TL (1998) Limnological observations of a papyrus swamp in Uganda: implications for fish faunal structure and diversity. Verh Int Verein Limnol 26:1821–31

Collins JP (1979) Intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeiana*. Ecology 60:738–749

Corbet PH (1999) Dragonflies: behavior and ecology of Odonata. Comstock, Ithaca, NY

Costa HH (1967) Avoidance of anoxic water by tadpoles of *Rana temporaria*. Hydrobiologia 30:374–384

Crowder WC, Nie M, Ultsch GR (1998) Oxygen uptake in bullfrog tadpoles (*Rana catesbeiana*). J Exp Zool 280:121–134

Dunson WA, Travis J (1991) The role of abiotic factors in community organization. Am Nat 138:1067–1091

Feder ME (1981) Effect of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. Comp Biochem Physiol 70A:497–508

Feder ME (1982) Effect of developmental stage and body size on oxygen consumption of anuran larvae: a reappraisal. J Exp Zool 220:33–42

Feder ME (1983a) Responses to acute hypoxia in larvae of the frog *Rana berlandieri*. J Exp Biol 104:79–95

Feder ME (1983b) The relation of air breathing and locomotion to predation on tadpoles, *Rana belandieri*, by turtles. Physiol Zool 56:522–531

Feder ME (1984) Consequences of aerial respiration for amphibian larvae. In: Seymour RS (ed) Respiration and metabolism of embryonic vertebrates. Junk, Dordrecht, pp 71–86

Feder ME, Booth DT (1992) Hypoxic boundary layers surrounding skin-breathing aquatic amphibians: occurrence, consequences, and organismal responses. J Exp Biol 166:237–251

Feder ME, Wassersug RJ (1984) Aerial versus aquatic oxygen consumption in larvae of the clawed frog, *Xenopus laevis*. J Exp Biol 108:231–245

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

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

Hassinger DD, Anderson JD (1970) The effect of lunar eclipse on nocturnal stratification of larval *Ambystoma opacum*. Copeia 1970:178–179

Hastings D, Burggren WW (1995) Developmental changes in oxygen consumption regulation in larvae of the South African clawed frog *Xenopus laevis*. J Exp Biol 198:2465–2475

Heath AG (1976) Respiratory responses to hypoxia by *Ambystoma tigrinum* larvae, paedomorphs, and metamorphosed adults. Comp Biochem Physiol 55A:45–49

Huuskonen H, Karjalainen J (1997) Predator-induced respiratory responses in juveniles of vendace *Coregonus albula*, whitefish *C. lavaretus*, perch *Perca fluviatilis* and roach *Rutilus rutilus*. Env Biol Fish 49:265–269

Junk WJ, Soares GM, Carvalho FM (1983) Distribution of fish species in a lake of the Amazon River floodplain near Manaus (Lago Camaleao), with species reference to extreme oxygen conditions. Amazoniana 4:397–431

Kolar CS, Rahel FJ (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210–219

Lannoo MJ, Bachmann MD (1984) On floatation and air breathing in *Ambystoma tigrinum* larvae: stimuli for and relationship between these behaviors. Can J Zool 62:15–18

McCollum SA, Van Buskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution 50:583–593

Newman RA (1992) Adaptive plasticity in amphibian metamorphosis. Bioscience 42:671–678

Nie M, Crim JD, Ultsch GR (1999) Dissolved oxygen, temperature, and habitat selection by bullfrog (*Rana catesbeiana*) tadpoles. Copeia 1999:153–162

Noland R, Ultsch GR (1981) The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). Copeia 1981:645–652

Peacor SD, Werner EE (1997) Trait-mediated indirect interactions in a simple aquatic food web. Ecology 78:1146–1156

Petranka JW (1998) Salamanders of the Unites States and Canada. Smithsonian, Washington, DC

Petranka JW, Kats LB, Sih A (1987) Predator prey-interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Anim Behav 35:420–425

Pinder AW, Feder ME (1990) Effect of boundary layers on cutaneous gas exchange. J Exp Biol 143:67–80

Poulin R, Wolf NG, Kramer DL (1987) The effect of hypoxia on the vulnerability of guppies (*Poecilia reticulata*, Poeciliidae) to an aquatic predator (*Astronotus ocellatus*, Cichlidae). Environ Biol Fish 20:285–292

Pronych S, Wassersug R (1994) Lung use and development in *Xenopus laevis* tadpoles. Can J Zool 72:738–743

Puttlitz MH, Chivers DP, Kiesecker JM, Blaustein AR (1999) Threat-sensitive predator avoidance by larval Pacific treefrogs (Amphibia, Hylidae). Ethology 105:449–456

Rahel FJ, Kolar CS (1990) Trade-offs in the response of mayflies to low oxygen and fish predation. Oecologia 84:39–44

Rahel FJ, Nutzman JW (1994) Foraging in a lethal environment: fish predation in hypoxic waters of a stratified lake. Ecology 75:1246–1253

Semlitsch RD (1990) Effects of body size, sibship, and tail injury on susceptibility of tadpoles to dragonfly predation. Can J Zool 68:1027–1030

Stauffer H, Semlitsch RD (1993) Effects of visual, chemical, and tactile cues of fish on the behavioral responses of tadpoles. Anim Behav 46:355–364

SPSS (1994) SYSTAT version 5.05 for Windows. SPSS, Chicago

Tejedo M (1993) Size-dependent vulnerability and behavioral responses of tadpoles of two anuran species to beetle larvae predators. Herpetologica 49:287–294

Ultsch GR (1971) The relationship between dissolved carbon dioxide and oxygen to microhabitat selection in *Pseudobranchus striatus*. Copeia 1971:247–252

Wassersug RJ, Feder ME (1983) The effects of aquatic oxygen concentration, body size, and respiratory behavior on stamina of the obligate aquatic (*Bufo americanus*) and facultative air-breathing (*Xenopus laevis* and *Rana berlandeiri*) anuran larvae. J Exp Biol 105:173–190

Wassersug RJ, Seibert EA (1975) Behavioral responses of amphibian larvae to variation in dissolved oxygen. Copeia 1975: 86–103

Weber JM, Kramer DL (1983) Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can J Fish Aquat Sci 40:1583–1588

Weigmann DL, Altig R (1975) Anoxic tolerances of three species of salamander larvae. Comp Biochem Physiol 50A:681–684

Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. Annu Rev Ecol Syst 27:337–363

Werner EE (1986) Amphibian metamorphosis: growth rate, predation risk and the optimal size at transformation. Am Nat 128: 319–341

Werner EE, Anholt BR (1996) Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. Ecology 77:157–169

Werner EE, Glennemeier KS (1999) Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia 1999:22–33

Werner EE, McPeek MA (1994) Direct and indirect effects of predators on two anuran species along an environmental gradient. Ecology 75:1368–1382

West NH, Burggren WW (1982) Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole. Respir Physiol 47:165–176

Wetzel RG (1983) Limnology. Saunders, Philadelphia, Pennsylvania

Wilbur HM, Collins JP (1973) Ecological aspects of amphibian metamorphosis. Science 182:1305–1314

Wolf NG, Kramer DL (1987) Use of cover and the need to breathe: the effects of hypoxia on vulnerability of dwarf gouramis to predatory snakeheads. Oecologia 73:127–132