

On flotation and air breathing in *Ambystoma tigrinum* larvae: stimuli for and the relationship between these behaviors¹

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Laboratory and field tests were conducted to show the roles of pelagic prey availability and nocturnal darkness in inducing floating and air-breathing behaviors in *Ambystoma tigrinum* larvae. Both pelagic prey availability and nocturnal darkness independently increase the frequencies of floating and air breathing. Air breathing and floating are correlated. Lungs are necessary for larvae to remain suspended and feed in the water column. Results suggest larvae float in response to darkness to feed on pelagic prey.

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Des tests en laboratoire et en nature ont été mis au point afin de déterminer si la disponibilité des proies pélagiques et l'obscurité nocturne ont un rôle à jouer dans le déclenchement des comportements de flottaison et de respiration aérienne chez les larves d'*Ambystoma tigrinum*. Les deux facteurs contribuent de façon indépendante à augmenter la fréquence de ces comportements. Il y a corrélation entre la respiration aérienne et la flottaison. Les poumons permettent aux larves de rester suspendues dans la colonne d'eau et de s'y nourrir. Les résultats indiquent que les larves restent entre deux eaux en réaction à l'obscurité, pour se nourrir de proies pélagiques.

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Introduction

Nocturnally active *Ambystoma* larvae float in the water column as they feed on zooplankton and other pelagic prey (Anderson and Graham 1967; Branch and Altig 1981). It has been suggested that this floating behavior is a response to at least two environmental stimuli: darkness (Anderson and Graham 1967; Hassinger and Anderson 1970; Anderson and Williamson 1974) and presence of pelagic prey (Henderson 1973; Petranka and Petranka 1980); but neither the causes nor mechanisms of floating have been experimentally established.

Floating *Ambystoma* larvae breathe air frequently (personal observation). In poorly oxygenated water, air breathing (*sensu* Spurway and Haldane 1953) is a well-known mechanism for increasing oxygen uptake in many amphibian larvae (e.g., *Pseudobranchius striatus* and *Notophthalmus viridescens*, Ultsch 1976; *Rana pipiens*, *Scaphiopus bombifrons*, and *A. tigrinum*, Wassersug and Seibert 1975; *A. tigrinum*, Heath 1976; *Rana clamitans*, West and Burggren 1982), but we have observed that floating *Ambystoma* larvae also breathe air when the water is hyperoxic. Air breathing may then be a method of regulating floating as well as a mode of respiration.

The objective of the present study is to experimentally determine why and how *Ambystoma* larvae float. Specifically, we test whether floating behavior can be induced by the stimuli of nocturnal darkness and pelagic prey availability and examine whether air breathing has a function in floating.

Materials and methods

General methods

Ambystoma tigrinum eggs and larvae used in these tests were collected from a marsh 1.5 km SE of Iowa Lakeside Laboratory (Dick-

inson County, IA, 43°23' N, 95°11' W). All larvae tested were between 17 and 59 mm standard snout-vent length and, except where noted otherwise, were observed in groups of three or four per 5.7-L aquarium for ½ h. All laboratory observations were made with animals in hyperoxic water (10–11 mg/L dissolved oxygen). Night observations were made with the aid of a flashlight with red filter which did not appear to disturb the larvae. Temperatures were recorded for all tests. Data were analyzed using PROC ANOVA or PROC GLM (Statistical Analysis System, Helwig and Council 1979). In all cases when larvae were observed in groups, group totals were divided by the number of larvae per group and this number was used in our statistical analysis.

Individual tests

A preliminary test was designed to show whether air breathing in floating larvae functions in buoyancy. *Ambystoma tigrinum* eggs were hatched in the laboratory. Newly hatched larvae were fed only zooplankton for 2 weeks and then introduced, under lighted conditions, to pelagic (*Chaoborus* sp.), benthic (*Chironomus* sp.), or pelagic and benthic (*Culiseta* sp.) dipteran prey. (For additional details of prey behavior see Lannoo (1982, p. 51).) All prey used are routinely eaten by *A. tigrinum* larvae in nature (e.g., Dincen 1955; Dodson and Dodson 1971). Numbers of prey ingested and air breaths were recorded on each of 4 consecutive days as larvae became experienced with the dipterans. To compare air breaths between larvae feeding on the different dipterans and avoid the statistical problem of nonindependent samples, one grand mean across all days for each group was used in our statistical analysis. Our test design assumes energetic costs of pelagic and benthic feeding are similar and makes use of observations that *Ambystoma* larvae learn (i.e., functionally respond to novel stimuli) (Kuntz 1923; Schneider 1968; Ray 1970; Henderson 1973). As larvae learn and take more prey, two air-breathing responses, representing the extremes, are possible. Either air-breathing rates increase with increasing numbers of prey taken (a response to increased activity associated with feeding behavior) and are independent of prey position, or air breathing is dependent on prey position and independent of numbers of prey taken. If air breathing in feeding larvae is dependent on feeding activity, it should imply that the primary function of air breathing is pulmonary respiration to meet the demands of increased activity. But if air breathing is dependent on prey position, then buoyancy would seem the more likely function. Because a buoy-

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ancy function was indicated (see Results), air breaths and floating were recorded concurrently in subsequent tests.

To determine whether pelagic prey availability influences floating and air breathing, salamanders were fed either pelagic *Chaoborus* or benthic *Chironomus* larvae. (Salamanders had had previous experience with prey of these types.) A third set of salamanders was observed without prey. A salamander was recorded as floating if it was off the substrate either suspended motionless or swimming. (See Appendix for a short discussion of the term floating.) Larvae were recorded as floating or not floating at 1-min intervals and, after statistical analysis, data were converted to percentages.

To assess whether nocturnal darkness also influences floating and air breathing, larvae were observed both day and night in a 57-L aquarium in their marsh and in the laboratory. Marsh observations were made with animals in an enclosure where dissolved oxygen concentration and prey availability could not be controlled. To eliminate these variables we withheld prey and used hyperoxic water in the laboratory tests.

The relationship between floating and air breathing was described using a least-squares regression on data collected during the pelagic prey and darkness tests. To examine causality in the air-breathing–floating relationship we surgically removed both lungs of experimental larvae. Animals were first anesthetized with buffered 0.03% MS-222 (tricaine methanesulfonate). Bilateral incisions were made dorsocaudally to the articulations of front limbs and lungs were exposed. Lungs were tied rostrally and removed immediately caudal to the ties. Incisions were stitched to speed healing. Sham-control animals were incised identically to the experimentals. A small piece of thread was placed lateral to each lung, simulating the thread used to tie off lungs of experimental larvae, and incisions were stitched. Experimental and control larvae were placed in individual aquaria containing *Daphnia* sp. (at a concentration of between 1000 and 4000 individuals/L) to induce floating, and were observed separately for 1 h. After the first trial, floating larvae were recorded as either being suspended (not moving) or swimming.

Results and conclusions

Air-breathing rates are high when larvae feed on pelagic prey (*Chaoborus* sp.), intermediate when they feed on pelagic and benthic prey (*Culiseta* sp.), and low when they feed on benthic prey (*Chironomus* sp.) (Fig. 1). From these data we concluded that it is possible for air breathing to have a buoyancy function and decided to record air breaths and floating concurrently to elucidate this relationship.

Pelagic prey availability significantly increases the frequency of both air breathing and floating when compared with conditions of prey absence and benthic prey presence (Table 1), although we found floating was high in test aquaria in the absence of prey. Nocturnal darkness also increases the frequency of air breathing and floating both in the field and in the laboratory, although the effect was more prominent in the field (Table 2).

Floating and air breathing are positively correlated (Fig. 2). While variation in this relationship is high ($r^2 = 0.45$), the regression line fit to these data is significant ($p < 0.001$).

Results of ablation experiments show that lung-excised animals cannot suspend themselves in the water column (Table 3). Behavioral observations indicate that lung-excised larvae can be off the substrate as long as they swim, but sink to the bottom when they stop swimming and splay their limbs in an effort to float. One lung-excised larva was able to suspend itself but its behavior was unusual; after air breathing its head was buoyant, the result of having air in its mouth, but its abdomen and tail were not buoyed and hung vertically. Differences in air-breathing rates were not statistically significant owing to variation and the small numbers of animals observed. Never-

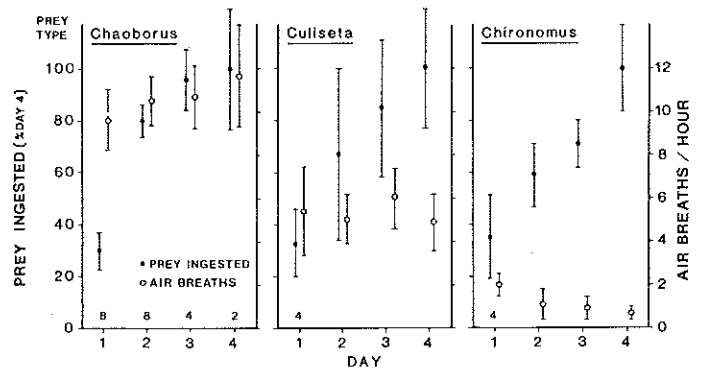


FIG. 1. Feeding and air-breathing rates for *A. tigrinum* larvae initially experiencing novel dipteran prey. *Chaoborus* is pelagic, *Culiseta* is both pelagic and benthic, and *Chironomus* is benthic. Numbers above days are groups observed per day, which was constant for *Culiseta* and *Chironomus*. Because *Chaoborus* was unavailable, not all groups were observed on days 3 and 4. Three larvae were observed per group when feeding on *Chaoborus* and *Chironomus*, four per group when fed *Culiseta*. Mean numbers of air breaths per larva per hour were 11.2, 5.2, and 1.2 for *Chaoborus*, *Culiseta*, and *Chironomus*, respectively. Means were calculated from within group means and significantly different ($F = 58.05$, $P > F < 0.001$). Error bars are 1 SE.

theless, lung-excised larvae surfaced to air breathe more often than controls (Table 3). Temperatures ranged from 19 to 26°C and were not correlated with any trends in results.

Discussion

Both increased pelagic prey availability (Table 1) and darkness (Table 2) independently increase the frequencies of floating and air-breathing behaviors in *A. tigrinum* larvae. Floating in response to pelagic prey availability supports the observations of Attar and Maly (1980) for salamandrids that adult (aquatic) *N. viridescens* will switch position from the vicinity of less numerous to more numerous prey. Our data also support Henderson's (1973) study on *Ambystoma gracile* showing that by increasing the encounter rate with a prey type in association with a particular habitat larvae learn to select that habitat.

Based on our observations that larvae floated in response to nocturnal darkness in the laboratory in the absence of either pelagic or benthic prey, we agree with Anderson and Graham (1967) that under natural conditions, nocturnal darkness, and not pelagic prey, is the proximal cue triggering floating in these animals. Because most animals used in our floating tests were floating in the marsh prior to being captured, we feel larvae retained their tendency to respond to nocturnal darkness in "expectation" of feeding on pelagic prey. In other words, larvae had learned from experience that nocturnal floating leads to food. Larvae continued to respond to nocturnal darkness independent of pelagic prey in our laboratory tests because they had not yet learned this behavior was unproductive.

Salamanders also floated under lighted conditions in response to pelagic prey in the laboratory (Table 1). These results contradict field observations that indicate both pelagic prey and darkness must be present before floating occurs (Anderson and Graham 1967; Hassinger and Anderson 1970; Hassinger et al. 1970; Henderson 1973). In many wetland communities this distinction is of no functional consequence because predominant freshwater invertebrates commonly exhibit diurnal vertical migrations (e.g., *Daphnia* sp. and *Chaoborus* sp., Pennak 1978) or are nocturnal (e.g., *Hyallolella azteca*, Pennak 1978),

TABLE 1. Mean rates of larval air breathing (breaths per larva per hour) and percentage of time a larva was counted as floating per hour while feeding on *Chaoborus*, not feeding, or feeding on *Chironomus*

	<i>Chaoborus</i> (4) ^a		Prey absent (6) ^a		<i>Chironomus</i> (4) ^a		F	P>F
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE		
Air breathing	10.6	1.4	3.6	0.8	0.2	0.2	26.18	0.001
Floating (%)	39.3	9.0	33.0	3.3	7.0	2.7	9.65	0.004

NOTE: Means were calculated from within group means. All groups contained three larvae.
^aValues in parentheses are the number of groups.

TABLE 2. Mean rates of larval air breathing (breaths per larva per hour) and percentage of time a larva was counted as floating per hour under day and night conditions in the field and in the laboratory

Group	Day		Night		F	P>F
	\bar{x}	SE	\bar{x}	SE		
Field (5)						
Air breathing	1.4	0.6	7.8	1.4	16.67	0.004
Floating (%)	19.7	7.0	58.0	4.6	12.84	0.007
Lab (4)						
Air breathing	3.6	0.8	7.4	1.6	4.36	0.082
Floating (%)	21.7	9.3	55.3	11.3	7.32	0.035

NOTE: Means were calculated from within group means. All groups contained three larvae.

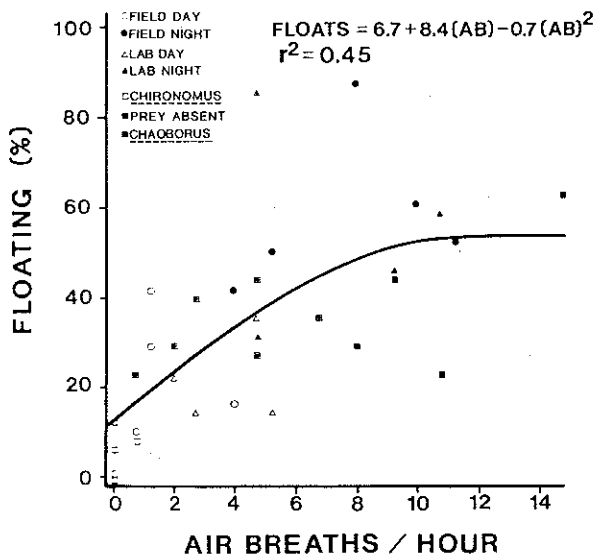


FIG. 2. Floating as a function of air-breathing rate for *A. tigrinum* larvae. Data represented by squares are given in Table 1, circles and triangles represent data in Table 2. For the equation $F = 12.1$, $P > F < 0.001$.

but it is important if one is interested in how salamanders perceive and respond to their environment. Diel activity, including floating, in nature is compromised presumably by the need of salamanders to avoid predators (e.g., Hassinger et al. 1970). In the shallow water of a drying marsh the senior author (M.J.L.) has observed diel predation on *A. tigrinum* larvae and *R. pipiens* tadpoles by common grackles (*Quiscalus quiscula*) and a belted kingfisher (*Megaceryle alcyon*). Shallow water is functionally similar to diurnal floating in locating animals near the water surface where they are most conspicuous to aerial

predators. We suggest that floating under lighted conditions in the laboratory was the result of reduced light intensity as a result of being indoors (i.e., under these conditions the animals would not be conspicuous). Indeed, Hassinger and Anderson (1970) report floating in nature in response to overcast skies during the day.

Hassinger and Anderson (1970) also reported *Ambystoma* larvae floated at night during the period of a lunar eclipse but did not float before or after the eclipse in response to the full moon. These observations suggest that circadian rhythm effects are not as important as proximal cues in initiating floating.

We show that air breathing is correlated with prey position in the water column (Fig. 1). Based on our preliminary test, the precise reasons for this relationship are not clear. For example in addition to the relationship proposed between air breathing and buoyancy, energy demands and subsequent changes in oxygen requirement when feeding on different prey types or at different levels in the water column may have accounted for the observed pattern. However, in subsequent tests we do show that air breathing is correlated with floating (Fig. 2). More importantly we also reveal the cause and effect nature of this relationship by showing that lung ablation, which negates the effects of air breathing, negatively effects floating (Table 3). It is curious that lung ablation has little effect on salamander swimming performance but greatly inhibits their ability to suspend themselves, and thus float, in the water column.

Energy and time are involved in surfacing to breathe air. These costs have been discussed in reference to ambystomatid respiration (Rose et al. 1971), salamandrid mating (Halliday 1977), and larval anuran stamina in currents (Wassersug and Feder 1983). Potential costs involved in air breathing in ambystomatids are reduced owing to the floating animal's position already near the water surface. The benefits of air breathing have already been mentioned: by enabling an animal to float and maintain a position in the water column *Ambystoma* larvae are able to feed on large quantities of small pelagic prey which are inaccessible to benthic animals. We agree with Branch and Altig (1981) that, given the constraints of nocturnality imposed by predators, if large numbers of zooplankton are available floating to feed is probably the most efficient foraging method that can be employed by larval ambystomatids.

In summary, our data show that both pelagic prey availability and darkness independently stimulate both floating and air-breathing behaviors in *A. tigrinum* larvae. Because larvae respond to darkness in the absence of pelagic prey we propose that in nature darkness is the proximal cue initiating floating in these animals. Floating and air breathing are correlated. Because lung-excised animals cannot suspend themselves in the water column, lungs are serving a buoyancy function and air breathing is essential for floating.

TABLE 3. Mean numbers of air breaths and percentage of floats per hour for lung-excised and sham-control larvae

Larvae	Lung excised			Sham control			F	P>F
	\bar{x}	SE	n	\bar{x}	SE	n		
Floating (%)	18.2	4.7	7	42.7	6.7	7	9.07	0.011
Suspended (%)	3.3*	3.3	6	20.8	7.7	6	4.30	0.065
Swimming (%)	13.7	4.5	6	19.7	2.0	6	1.52	0.246
Air breathing	3.9	0.9	7	1.9	1.2	7	1.72	0.214

NOTE: Means were calculated from individuals. In this test, six out of the seven floating larvae were subcategorized into swimming and suspended animals.

*Due to one animal.

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Appendix

Nocturnal floating in *Ambystoma* larvae is commonly called stratification because initial reports (Anderson and Graham 1967; Hassinger and Anderson 1970) indicated larvae float in layers or strata. Branch and Altig (1981) found no statistical support for larval stratification nor have we observed distinct layering. We prefer to use the term floating for the general phenomenon of aquatic salamanders off a substrate and suggest using the term stratification only when layering can be quantitatively shown.

- ANDERSON, J. D., and R. E. GRAHAM. 1967. Vertical migration and stratification of larval *Ambystoma*. *Copeia*, 1967: 371-374.
- ANDERSON, J. D., and G. K. WILLIAMSON. 1974. Nocturnal stratification in larvae of the mole salamander, *Ambystoma talpoideum*. *Herpetologica*, 30: 28-29.
- ATTAR, E. N., and E. J. MALY. 1980. A laboratory study of preferential predation by the newt *Notophthalmus v. viridescens*. *Can. J. Zool.* 58: 1712-1717.
- BRANCH, L., and R. A. ALTIG. 1981. Nocturnal stratification of three species of *Ambystoma* larvae. *Copeia*, 1981: 870-873.
- DINEEN, C. F. 1955. Food habits of the larval tiger salamander (*Ambystoma tigrinum*). *Proc. Indiana Acad. Sci.* 65: 231-233.
- DODSON, S. I., and V. E. DODSON. 1971. The diet of *Ambystoma tigrinum* larvae from western Colorado. *Copeia*, 1971: 614-624.
- HALLIDAY, T. R. 1977. The effect of experimental manipulation of breathing behaviour on the sexual behaviour of the smooth newt, *Triturus vulgaris*. *Anim. Behav.* 25: 39-45.
- HASSINGER, D. D., and J. D. ANDERSON. 1970. The effect of lunar eclipse on nocturnal stratification of larval *Ambystoma opacum*. *Copeia*, 1970: 178-179.
- HASSINGER, D. D., J. D. ANDERSON, and G. H. DALRYMPLE. 1970. The early life history and ecology of *Ambystoma tigrinum* and *Ambystoma opacum* in New Jersey. *Am. Midl. Nat.* 84: 474-495.
- HEATH, A. G. 1976. Respiratory responses to hypoxia by *Ambystoma*

- tigrinum* larvae, paedomorphs, and metamorphosed adults. *Comp. Biochem. Physiol. A*, 55: 45-49.
- HELWIG, J. T., and K. A. COUNCIL (Editors). 1979. SAS user's guide. SAS Institute, Raleigh, NC.
- HENDERSON, B. A. 1973. The specialized feeding behavior of *Ambystoma gracile* in Marion Lake, British Columbia. *Can. Field-Nat.* 87: 151-154.
- KUNTZ, A. 1923. The learning of a simple maze by the larvae of *Ambystoma tigrinum* (Green). *Univ. Iowa Stud. Nat. Hist.* 10(2): 27-35.
- LANNOO, M. J. 1982. Food habits and feeding behavior of tiger salamander larvae (*Ambystoma tigrinum*) in northwestern Iowa. M.S. thesis. Iowa State University, Ames, IA.
- PENNAK, R. W. 1978. Freshwater invertebrates of the United States. 2nd ed. Interscience Publishers, John Wiley & Sons, New York.
- PETRANKA, J. W., and J. G. PETRANKA. 1980. Selected aspects of the larval ecology of the marbled salamander *Ambystoma opacum* in the southern portion of its range. *Am. Midl. Nat.* 104: 352-363.
- RAY, A. J., JR. 1970. Instrumental avoidance learning by the tiger salamander *Ambystoma tigrinum*. *Anim. Behav.* 18: 73-77.
- ROSE, F. L., D. ARMÉNTROUT, and P. ROPER. 1971. Physiological responses of paedogenic *Ambystoma tigrinum* to acute anoxia. *Herpetologica*, 27: 101-107.
- SCHNEIDER, C. W. 1968. Avoidance learning and the response tendencies of the larval salamander *Ambystoma punctatum* to photic stimulation. *Anim. Behav.* 16: 492-495.
- SPURWAY, H., and J. B. S. HALDANE. 1953. The comparative ethology of vertebrate breathing. I. Breathing in newts, with a general survey. *Behaviour*, 6: 8-34.
- ULTSCH, G. R. 1976. Respiratory surface area as a factor controlling the standard rate of O₂ consumption of aquatic salamanders. *Respir. Physiol.* 26: 357-369.
- WASSERSUG, R. J., and M. E. FEDER. 1983. The effects of aquatic oxygen concentration, body size and respiratory behaviors on the stamina of obligate aquatic (*Bufo americanus*) and facultative air breathing (*Xenopus laevis* and *Rana berlandieri*) anuran larvae. *J. Exp. Biol.* 105: 173-190.
- WASSERSUG, R. J., and E. A. SEIBERT. 1975. Behavioral responses of amphibian larvae to variation in dissolved oxygen. *Copeia*, 1975: 86-103.
- WEST, N. H., and W. W. BURGREN. 1982. Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole. *Respir. Physiol.* 47: 165-176.