

Specific Gravity and Mechanisms for Its Control in Tadpoles of Three Anuran Species from Different Water Strata

Ming-Chung Tu*, Chia-Wei Chu and Kuang-Yang Lue

Department of Biology, National Taiwan Normal University, Taipei, Taiwan 116, R.O.C.

Tel: 886-2-29336875 ext. 321. Fax: 886-2-29312904. E-mail: Biofv026@scc.ntnu.edu.tw

(Accepted October 13, 1998)

Ming-Chung Tu, Chia-Wei Chu and Kuang-Yang Lue (1999) Specific gravity and mechanisms for its control in tadpoles of three anuran species from different water strata. *Zoological Studies* 38(1): 76-81. Buoyancy regulation is crucial for the survival of aquatic animals because it can save appreciable energy. Although anuran larvae are generally benthic, a few species live either at the surface or in mid-water. We have predicted that the specific gravity (sp. gr.) of tadpoles in surface layers should be 1.0 or slightly less, and that of mid-level tadpoles should be 1.0, whereas the sp. gr. of benthic tadpoles should be greater than 1.0. We tested this prediction in tests using larvae of *Microhyla heymonsii*, *M. ornata*, and *Rana kuhlii* as representatives of surface, mid-waters, and benthic tadpoles, respectively. Mean larval sp. grs. were 0.993 for *M. heymonsii*, 0.998 for *M. ornata*, and 1.041 for *R. kuhlii*. Although the mean sp. gr. ($\bar{x} = 0.998$) of mid-level tadpoles partially contradicts our prediction (1.0), larval sp. grs. of the other 2 species did fit our prediction. The general trend for benthic tadpoles to have a greater sp. gr. than those of mid-layer or surface tadpoles was supported by our experiments.

Lungs seem to play an important role in buoyancy regulation for anuran larvae. To check this prediction, we measured and compared sp. grs. of the same individuals with and without lungs. Also, observations were made on larvae of surface and mid-water species after being forcibly kept from contacting surface air. Results of these experiments confirmed the importance of lungs in buoyancy regulation in both *M. heymonsii* and *M. ornata* tadpoles. The larvae of *M. heymonsii* recovered from the deprivation of surface air treatment much better than did those of *M. ornata*. This phenomenon might be correlated with their different habitats.

Key words: Buoyancy, Anuran larvae, Amphibian.

Buoyancy control is critical for reduction of energy expenditure in aquatic animals. If an animal can maintain neutral buoyancy, it can save the energy expended on avoiding sinking or rising. Therefore, many aquatic organisms have developed various ways to stay at a desired position in the water column (Knut 1997). Although tadpoles live in various strata of still water or in lotic environments (Duellman and Trueb 1986, Pough et al. 1989) that should impose various selective pressures upon their buoyancy, there are few studies directly addressing this problem (Gee and Waldick 1995).

The specific gravity of fresh water is 1.0, and so, mid-water tadpoles should have a sp. gr. close to 1.0. Positive buoyancy might be adequate for a

surface dweller, but it may hinder escape from predators (Gee and Waldick 1995). Therefore, the sp. gr. of a surface tadpole should still be 1.0 or only slightly less. On the contrary, negative buoyancy by greater sp. gr. should be selected for benthic larval anurans.

Because cytoplasm is usually slightly denser than freshwater, the sp. gr. of aquatic animals is normally greater than 1.0 (Withers 1992). Therefore, no mechanism is required for organisms to sink. However, to stay afloat without expending energy, certain strategies are necessary for animals to reduce their overall density. Among the various possible mechanisms, lipid and, especially, gas storages are well known and commonly used to reduce body density by vertebrates. For

*To whom correspondence and reprint requests should be addressed.

example, lipids stored in the liver are known to reduce the sp. gr. of many sharks, and many teleost fish use their air bladder to reach neutral buoyancy (Knut 1997).

Pulmonary volume adjustment is the major method of buoyancy regulation in reptiles (Seymour 1982). Lung gas has long been suspected to play a hydrostatic role in the larvae of urodeles and anurans. Based on the observations of lungs of some urodeles that lack vascularization and alveoli, Goodrich (1930) and Noble (1931) concluded that amphibian lungs had a hydrostatic function. Lannoo and Bachman (1984) also demonstrated that lung ventilation is related not only to respiration but also to buoyancy regulation in larval *Ambystoma tigrinum*. *Rana pipiens* larvae have small lungs and are not buoyant, whereas *Pseudacris triseriata*, with large lungs, are buoyant and are often found at the surface (Wassersug and Seibert 1975). Burggren (1989), however, has argued that, although lungs had been considered as playing a role in buoyancy regulation in amphibian larvae, no experimental evidence was available. In fact, only a limited number of studies have clearly shown the buoyancy function of lungs in larval anurans (Feder and Wassersug 1984, Campeny and Casinos 1989, Pronych and Wassersug 1994, Gee and Waldick 1995, Snetkova et al. 1995). Thus, we conducted a series of experiments to investigate the role of the lungs in the maintenance of buoyancy in anuran tadpoles.

MATERIALS AND METHODS

According to our field experiences and a report of Chou and Lin (1997), tadpoles of *Microhyla heymonsi* have upturned funnel-like oral discs and float just below the water surface most of the time, whereas the larvae of *M. ornata*, with transparent bodies, usually stay in mid-water. We thus chose larvae of these 2 species as representatives of surface and mid-water dwellers, respectively. We used larvae of *R. kuhlii* to represent benthic tadpoles.

A few days before the experiments, we collected tadpoles from the field. Each individual was placed in a 15-cm water column, which was divided into bottom (0-1 cm from the bottom), middle (1-14 cm) and surface (14-15 cm) layers. The position in the column was continuously observed for 1 h each in the morning, around noon, and at night. The time that each tadpole spent in each layer of water within each 1 h observation

period was recorded with a stopwatch.

Larvae used for measuring specific gravity were within Gosner's (1960) stages 25-36. Initially, we killed tadpoles by putting them in a freezer for about 40 min, but we found that this method significantly affected the lung volume in *M. heymonsi*. We thus changed to another method, treatment with an anesthetic (2-phenoxyethanol), for this purpose subsequently. Dead tadpoles were then placed in a cup of tap water at 20.5 °C to 25.2 °C. The method we adopted to measure sp. grs. of tadpoles was first developed by Coombs (1981), who placed samples in a series of sea water of different concentrations. Because the original version of this method could only measure sp. gr. larger than 1.0, we modified it by using a mixture of alcohol and sodium chloride solution to determine the densities of tadpoles that might be lower than 1.0. When a given tadpole neither floated nor sank in the solution (i.e., an indication that its density was equivalent to that of the mixture), the density of the mixture was then measured by a hydrometer. We used 2 hydrometers, one for 0.940 to 1.000 and the other for 1.000 to 1.060, and their accuracies were within 0.002.

Dead tadpoles were dissected to check the lung condition. The sp. gr. of dissected tadpoles without lungs was determined again. In another treatment, 5 individuals of *M. heymonsi* larvae and 5 *M. ornata* larvae were kept in separate containers with screens to prevent their access to air. Both containers were then sunk in a larger container with water aerated by an air pump. The behaviors of the tadpoles were then recorded.

One-way ANOVA, combined with the Student-Newman-Keuls test, was first used to evaluate the statistical significances in observed differences between values of separate and those of more than 2 groups. If normality or equal variance tests failed, Dunn's test was chosen instead of Student-Newman-Keuls test. Student's *t*-test indicated if the larval sp. gr. of each species was different from 1.0, including differences in sp. gr. between larvae killed by cold and by anesthetic.

RESULTS

No significant differences ($p > 0.05$) were found in the time that tadpoles spent in each stratum of water among different hours of a day (morning, mid-day, and night). Thus, results of 3 h of observations were pooled. The larvae of *M.*

heymonsi spent 71.1% of the time at the surface, whereas those of *M. ornata* spent 54.8% of the time in mid-layer water. Larvae of *R. kuhlii* spent 97.1% of the time in the bottom stratum (Fig. 1).

Although the mean larval specific gravity of *M. ornata* ($\bar{x} = 0.998$) was very close to 1.0, it was still significantly smaller than 1.0 ($p < 0.05$). When killed with anesthetic, the mean larval sp. grs. of *M. heymonsi* and *R. kuhlii* were 0.993 and 1.041, respectively, which are also significantly different from 1.0 ($p < 0.001$; $p < 0.001$). When tadpoles were killed by freezing, the mean larval sp. gr. of *M. heymonsi* significantly increased to 1.015 ($p < 0.0001$). However, larval sp. grs. of *M. ornata* and *R. kuhlii* did not change significantly ($p = 0.6285$; $p = 0.0881$, respectively) when they were killed by different methods (Table 1). Lungs filled with air were found in both thawed dead, and euthanized

tadpoles of *M. ornata* and euthanized *M. heymonsi*. Thawed dead tadpoles of *M. heymonsi* had lungs only partially filled with gas. By contrast, such a condition was not observed in tadpoles of *R. kuhlii* killed in either way.

After lungs were removed, mean larval sp. grs. of *M. heymonsi*, *M. ornata*, and *R. kuhlii* increased to 1.025, 1.018, and 1.046, respectively (Table 1). However, in Dunn's all pairwise multiple comparison, only *M. heymonsi* showed a significantly higher sp. gr. ($p < 0.05$) after this manipulation. The mean larval sp. gr. of *R. kuhlii* was, irrespective of the presence or absence of lungs, significantly higher ($p < 0.05$) than those of the other species except for that of *M. heymonsi* without lungs (Fig. 2).

The tadpoles of both *M. heymonsi* and *M. ornata* sank to the bottom of the container if they could not get access to surface air. They lay on their sides and made slow buccal movements. Occasionally they struggled toward the surface with vigorous movements of the tail but sank to the bottom again as soon as tail movements ceased. However, they survived for 1 wk in this condition. One week later when tadpoles were allowed access to the surface air again, *M. heymonsi* larvae resumed staying in the surface layer as soon as they contacted surface air. By contrast, larvae of *M. ornata* could not stay near the surface as they ordinarily do. Although they swam up to the surface and gulped air occasionally, air bubbles could only be held in their oral cavities (i.e., not in their lungs: as observed through their transparent bodies). When an air bubble was held in the mouth, they floated on the surface in an un-

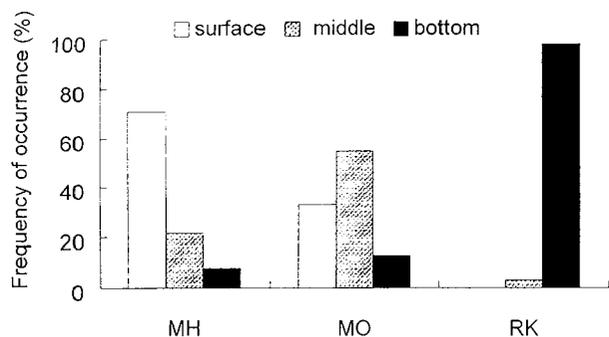


Fig. 1. Mean percentages of time that tadpoles of *Microhylya heymonsi* (MH), *M. ornata* (MO), and *Rana kuhlii* (RH) spent in a 15-cm water column, which was divided into surface (15-14 cm from the bottom), middle (14-1 cm) and bottom (1-0 cm) layers.

Table 1. Specific gravity, body length, and developmental stages of tadpoles of three anuran species

Species	<i>Microhylya heymonsi</i>			<i>Microhylya ornata</i>			<i>Rana kuhlii</i>		
	Intact#	intact	without lungs	Intact#	intact	without lungs	Intact#	intact	without lungs
Sample size	12	10	10	20	15	15	20	15	15
Temperature range (°C)	20.5-25.2	22.3-24.9	22.3-24.9	23.2-24.8	23.2-24.7	23.2-24.7	22.8-24.6	22.3-23.3	22.3-23.3
Development stages	29-36	29-36	29-36	25-35	25-28	25-28	29-36	25-36	25-36
Range of total length (mm)	11.65-20.90	15.90-19.95	15.90-19.95	11.30-16.50	10.65-14.90	10.65-14.90	19.90-28.45	14.50-26.00	14.50-26.00
Range of snout-vent length (mm)	5.35-7.45	5.20-6.30	5.20-6.30	4.60-5.95	4.70-5.90	4.70-5.90	7.50-10.40	5.00-8.40	5.00-8.40
Range of sp. gr.	1.004-1.023	0.980-0.999	1.020-1.032	0.994-1.002	0.995-0.999	1.013-1.024	1.025-1.060	1.025-1.058	1.040-1.058
Mean of sp. gr.	1.015	0.993	1.025	0.998	0.998	1.018	1.043	1.041	1.046
Standard deviation of sp. gr.	0.006	0.007	0.003	0.002	0.001	0.004	0.009	0.009	0.006
t-test	$p < 0.0001$			$p = 0.6285$			$p = 0.0881$		

#: Tadpoles killed by freezing.

balanced position. Usually they released the air during subsequent swimming and then sank down to the bottom again. Tadpoles of *M. ornata* mostly continued to lie on their sides at the bottom for more than 1 wk thereafter.

DISCUSSION

Different methods used to prepare materials for specific gravity measurements may have substantially influenced the resultant values. *M. heymonsi* tadpoles killed by freezing had significantly higher sp. grs. than those killed by anesthetic. We suspect that the tadpoles of the former group had expelled some gas from their lungs during the freezing process. However, tadpoles of *M. ornata* and *R. kuhlii* did not seem to release gas during the same treatment.

We frequently observed tadpoles of *M. ornata* staying in mid-water with only the flagellum of their tails swinging quickly. If these tail flagellum movements provide a downward propulsion, this will counteract the tadpole's slight higher buoyancy. Nevertheless, if these movements provide an upward propulsion, the sp. gr. of live *M. ornata* tadpoles should slightly exceed 1.0. In this case, the 0.998 sp. gr. value of dead *M. ornata* tadpoles obtained in this study actually is an underestimation of the real sp. gr. in life. Muscle tone

may contribute to their slight positive buoyancy. Even when a muscle is at rest, a certain amount of sustained contraction occurs in its fibers (Hole 1990). The resistance of a muscle to stretch is called muscle tone. If the motor nerve to a muscle is cut, the muscle tone decreases and the muscle becomes flaccid (Ganong 1981). Consequently, if the tension of muscles that surround the lungs decrease a little after the tadpole's death, the volume of the lungs may increase slightly, resulting in an increase of positive buoyancy. However, the real reason why their sp. grs. after death are slightly less than 1.0 remains obscure. Further investigation is needed.

In the field, tadpoles of *M. ornata* are sympatric with those of *M. heymonsi*. So, occupancy of different water strata enables tadpoles to partition activity area, as well as food, which may be important for reducing competition between these 2 species. Many benthic tadpoles rasp their food from the substrate, whereas suspension feeders like *M. ornata* larvae feed on phytoplankton suspended in the water column. The tadpole of *M. heymonsi* is a typical neustonic feeder (Chou and Lin 1997) that feeds from the surface. These different feeding modes will certainly enhance the time period that they stay in a specific water stratum (Fig. 1), thus, increasing the selection pressure for buoyancy regulation. Slight positive buoyancy of *M. ornata* larvae seemingly contradicted our hypothesis. By contrast, the sp. grs. of the other 2 species did fit our prediction. The general trend for benthic tadpoles to have a greater specific gravity than those of mid-layer or surface tadpoles was supported by our experiments. More studies of other amphibian larvae with distantly related phylogeny but inhabiting the same water stratum or on closely related species but using different water strata may strengthen our hypothesis. Living habitat should play a more important role than phylogenetic position in shaping larval sp. grs. Gee and Waldick (1995) found that the ontogenetic buoyancy change in anuran larvae was correlated with their preferred habitats. In their observation, larvae were negatively buoyant when they were at stages of being active or sedentary on the bottom, whereas they showed nearly neutral buoyancy at stages of being active in mid-water. In urodeles, lung reduction in larvae will reduce buoyancy, and might be regarded as an adaptation for rheotaxis in lotic environments (Bruce et al. 1994).

It is known that lungs play an important role in the buoyancy regulation of certain anuran larvae (Pronych and Wasserug 1994, Gee and Waldick

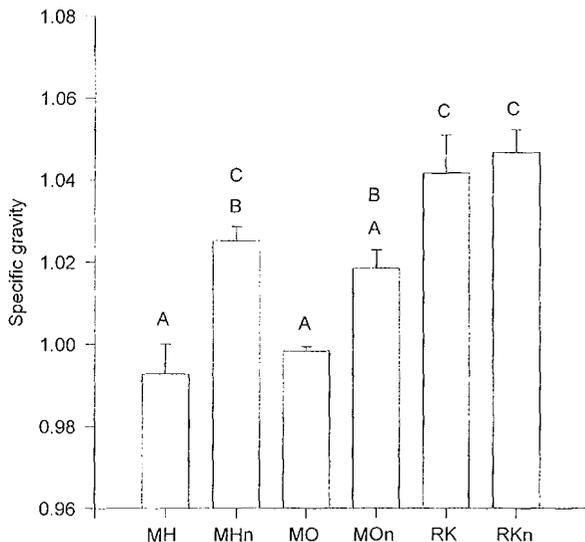


Fig. 2. Specific gravities (mean+SD) in larvae of *Microhyla heymonsi* intact (MH), *M. heymonsi* without lungs (MHn), *M. ornata* intact (MO), *M. ornata* without lungs (MOn), *Rana kuhlii* intact (RK), and *R. kuhlii* without lungs (RKn). Different letters on top of the bars indicate statistical differences by Dunn's all pairwise multiple comparison.

1995, Snetkova et al. 1995). To fill their lungs, tadpoles of either *M. heymonsi* or *M. ornata* need to swim to the surface to gulp air periodically. Our results indicate that if the tadpoles are isolated from the air, they will soon lose their neutral buoyancy and sink to the bottom. Similar results were found in the larvae of clawed frogs (Feder and Wassersug 1984, Pronych and Wassersug 1994). When lungs were removed from larvae of the above 2 species, their sp. grs. increased but only that of *M. heymonsi* reached a statistically significant level. The larval sp. gr. of *M. ornata* without lungs was still significantly lower than that of *R. kuhlii*. This implies that either other mechanisms have contributed to the increase of the lift for mid-layer tadpoles, or heavy structures such as calcified denticles have increased the body weight in tadpoles, increasing their sp. grs.

After being allowed to reaccess the air, tadpoles of *M. heymonsi* settled in the surface layer quickly while *M. ornata* did not in the middle layer. Also, during the freezing treatment, *M. heymonsi* expelled air while *M. ornata* did not. Larval *M. heymonsi* treated with anesthetic showed a greater variation in their sp. grs. However, when the lungs were removed, the variation decreased and became closer to that of *M. ornata* larvae without lungs (Fig. 2). All of the above phenomena suggest that larval *M. heymonsi* move air into and out of their lungs more frequently than does *M. ornata*. This may be related to the surface dwelling habits of the former (Fig. 1), which should require tadpoles to possess a greater ability of escape from predators by releasing lung air more quickly.

Acknowledgments: We thank Dr. W. H. Chou for helping us with identifications of tadpoles. Appreciation also goes to Dr. V. H. Hutchison and Dr. P. S. Hill for their critical opinion and improvement of English of our early draft.

REFERENCES

- Bruce RC, CK Beachy, PG Lenzo, SP Pronych, RJ Wassersug. 1994. Effects of lung reduction on rheotactic performance in amphibian larvae. *J. Exp. Zool.* **268**: 377-380.
- Burggren WW. 1989. Lung structure and function. In SC Wood, ed. Comparative pulmonary physiology. Vol. 39, Lung biology in health and disease. New York: Marcel Dekker, pp. 153-191.
- Campeny R, A Casinos. 1989. Densities and buoyancy in tadpoles of midwife toad, *Alytes obstetricans*. *Zool. Anz.* **223** (1/2): 6-12.
- Chou WH, JY Lin. 1997. Tadpoles of Taiwan. National Museum of Natural Science Special Publ. No.7. Taichung: National Museum of Natural Science.
- Coombs SH. 1981. A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs of the mackerel *Scomber scombrus*. *Mar. Biol.* **63**: 101-106.
- Duellman WE, L Trueb. 1986. Biology of amphibians. New York: McGraw-Hill.
- Feder ME, RJ Wassersug. 1984. Aerial versus aquatic oxygen consumption in larvae of the clawed frog, *Xenopus laevis*. *J. Exp. Biol.* **108**: 231-245.
- Ganong WF. 1981. Review of medical physiology. Los Altos: Lange Medical Publ.
- Gee JH, RC Waldick. 1995. Ontogenetic buoyancy changes and hydrostatic control in larval anurans. *Copeia* **1995**: 861-870.
- Goodrich ES. 1930. Studies on the structure and development of vertebrates. London: MacMillan Press.
- Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183-190.
- Hole JW Jr. 1990. Human anatomy and physiology. Dubuque: WCB Publishers.
- Knut SN. 1997. Animal physiology: adaptation and environment. Cambridge: Cambridge Univ. Press.
- Lannoo MJ, MD Bachman. 1984. Aspects of cannibalistic morphs in a population of *Ambystoma t. tigrinum* larvae. *Amer. Midl. Nat.* **112**: 103-110.
- Noble GK. 1931. The biology of the amphibia. New York: McGraw-Hill.
- Pough FH, JB Heiser, WN McFarland. 1989. Vertebrate life. New York: Macmillan Publ.
- Pronych S, RJ Wassersug. 1994. Lung use and development in *Xenopus laevis* tadpoles. *Can. J. Zool.* **72**: 738-743.
- Seymour RS. 1982. Physiological adaptations to aquatic life. In C Gans, FH Pough, eds. Biology of the reptilia. Vol.13, Physiology. London: Academic Press, pp. 1-51.
- Snetkova E, N Chelnaya, L Serova, S Saveliev, E Cherdanzova, S Pronych, R Wassersug. 1995. The effects of space flight on *Xenopus laevis* larval development. *J. Exp. Zool.* **273**: 21-32.
- Wassersug RJ, EA Seibert. 1975. Behavioral responses of amphibian larvae to variation in dissolved oxygen. *Copeia* **1975**: 86-103.
- Withers PC. 1992. Comparative animal physiology. Fort Worth: Saunders College Publ.

蝌蚪比重與生活水層關係之研究

杜銘章¹ 朱家蔚¹ 呂光洋¹

對於水裡生活的動物而言，浮力調整得當，可以節省許多能量，因此身體比重的調節，直接關係著其生存適應的問題，雖然多數的蝌蚪屬於底棲型，多半時間生活在水塘的底部，但有些種類的蝌蚪，如小雨蛙 (*Microhyla ornata*)，卻一直保持在水層中間，而黑蒙西氏小雨蛙 (*M. heymonsi*) 的蝌蚪，則多半懸浮在水表面下。我們推測上層型的蝌蚪，其比重應為 1.0 或比 1.0 稍小，而中層型的蝌蚪，其比重應為 1.0，底棲型的蝌蚪，比重則應大於 1.0。我們以黑蒙西氏小雨蛙、小雨蛙和古氏赤蛙 (*Rana kuhlii*) 的蝌蚪，分別代表上、中和底層的蝌蚪，結果發現其比重分別是 0.993、0.998 和 1.041，此和我們的預期相當吻合，而且在摘肺後測量其比重，以及在防止上層和中層蝌蚪取得水面上的空氣試驗下，結果都顯示肺對於黑蒙西氏小雨蛙和小雨蛙蝌蚪的浮力調整相當重要，不過此兩種蝌蚪在無法吸取水面上的空氣達一週後，讓牠們再度接觸空氣，發現黑蒙西氏小雨蛙復原情形較小雨蛙為佳，此現象可能和牠們棲地環境的不同有關。

關鍵詞：浮力，蝌蚪，兩生類。

¹國立臺灣師範大學生物系