JOURNAL OF HERPETOLOGY

Volume 15 *31 October 1981* *Number 4*

The Effects of Progressive Hypoxia and Rocking Activity on Blood Oxygen Tension for Hellbenders, *Cryptobranchus alleganiensis*

Richard A. Harlan and Robert F. Wilkinson

Life Sciences Department, Southwest Missouri State University,
Springfield, Missouri 65802, USA

ABSTRACT—The effects of progressive hypoxia and rocking activity on blood gases and pH were measured for *Cryptobranchus alleganiensis*. Measurements were taken at 100%, 75%, 50%, 25% and 0% water O_2 content while temperature was held constant at 12°C. The data show animals that are rocking maintain constant blood O_2 tensions until water O_2 content drops below the 25% saturation level. Abolishing rocking activity results in a loss of ability to maintain constant blood O_2 tensions as water O_2 content drops below the 75% saturation level. The frequency of rocking activity increases as the water O_2 content drops below the 75% saturation level.

* * *

INTRODUCTION

Emergence of primitive vertebrates from aquatic to terrestrial ecosystems was associated with important changes in their respiratory systems. The processes of evolution associated with respiratory change and terrestrial emergence are thought to be closely related to ambient oxygen concentration, carbon dioxide elimination and temperature.

Fossil evidence indicates a gradual transition from strictly aquatic gill-breathers to purely terrestrial lung-breathers. Many of the transitional species have utilized bimodal gas exchange for respiration. The successful transition to special habits has generally resulted in the selection of one mode or a combination of two modes as the principle mechanism for respiratory gas exchange.

Studies of gas exchange for totally aquatic amphibians with predominantly cutaneous respiration were lacking till the investigations of Guimond (1970). Guimond presented an extensive study of gas exchange characteristics for four species of aquatic salamander, including *Cryptobranchus*. His data show external respiration for *Cryptobranchus* is predominantly cutaneous at all temperatures tested.

A characteristic rocking and swaying behavior of *Cryptobranchus* has been observed under various conditions of captivity (Willey, 1920; Noble, 1925; Bishop, 1941). There is no record of this behavior pattern occurring in its natural habitat. It has been suggested that *Cryptobranchus* rocking activity could improve respiratory efficiency (Guimond and Hutchinson, 1973; Nickerson and Mays, 1973). A proposed relationship between rocking activity and respiratory gas exchange has both morphological and physiological support.

The skin of *Cryptobranchus* is modified by numerous longitudinal folds on the dorsal and lateral aspects of the body surface. Noble (1925) and Bernstein (1953) have shown these lateral folds, as well as the entire cutaneous surface, are highly vascularized structures. Thus, with these modifications, the skin could function as a gas exchange unit for external aquatic respiration.

Beffa (1976) was the first to present data relating rocking activity to dissolved oxygen content of water. His data showed periods of rocking and frequency of rocking increase as dissolved oxygen content of water decreased. His data also showed rocking activity increased significantly as water temperature increased. There is an increase in rocking activity at all water O_2 content levels tested as water temperature is raised from 5°C to 12°C. When water temperature is raised to 20°C, rocking activity inceases for salamanders in water O_2 contents of 25% and 50% saturation but decreases for salamanders in water O_2 contents of 75% and 100% saturation.

The purpose of this study was to measure the effects of progressive hypoxia and rocking activity in blood oxygen tension. The experimental water temperature of 12°C was chosen because of the paradoxical response for rocking activity to water temperatures greater than 12°C when water O_2 content is greater than 50% saturation.

MATERIALS AND METHODS

The hellbenders were collected from the Niangua River, Dallas Co., Missouri between June and October. They were acclimated to 12°C in aerated aquaria for a minimum of eight days, which should have been an adequate acclimation time according to Hutchison et al. (1973). No food was offered during acclimation or testing. All tests were run at 12°C ± 0.5°C.

Each salamander was anesthetized in a solution containing 0.5 g Tricaine (Ethyl-M-Amino Benzoate, Sigma Chemical) dissolved in 4.0 l of tap water. A small section of polyethylene tubing containing a section of nichrome wire was inserted into a lateral fold of the animal and secured. This facilitated connecting the salamander to a transducer so the rocking activity of the animal could be electronically monitored, as described by Beffa (1976).

The test animals were placed in individual aquaria. Water O_2 content was maintained at the desired saturation levels by bubbling air and nitrogen into the aquaria water. Water O_2 content of the aquaria was monitored continuously during the experimental period with a portable O_2 meter and probe (Yellow Springs Instrument Company). Each saturation level was maintained at ± 5% of the equilibrated value. The rocking activity of the salamander was recorded on a three channel E and M Physiograph using "B" type transducers at a paper speed of 0.1 cm/second.

Nine animals were tested at 100%, 75%, 50%, 25% and 0% water O_2 content levels. The testing period was begun by recording rocking activity for each animal for 30 minutes at 100% O_2 saturation. The animal was then removed from the aquarium and a blood sample taken by cardiac puncture. The animal was then placed into an aquarium previously equilibrated to 75% oxygen saturation. Rocking activity was again recorded for 30 minutes at the end of which another blood sample was taken. This procedure was repeated at each of the 50%, 25% and 0% oxygen saturation levels.

Upon completion of this initial testing, each animal was placed in a 12°C solution of 0.025% Tricaine equilibrated to 100% oxygen saturation. The animal was considered to be sufficiently anesthetized when tactile stimulation failed to produce a response. The salamander was then placed into an aquarium previously equilibrated to 100% oxygen saturation. After 30 minutes in aquaria water 100% saturated with oxygen, a blood sample was taken. This procedure was carried out for each salamander at 75%, 50%, 25% and 0% oxygen satuation levels. The Tricaine-treated salamanders were unable to rock or sway during the experimental period.

Another group of six hellbenders was tested for the effect of simulated rocking activity on blood oxygen tension. Each of the six salamanders was placed in an aquarium previously equilibrated to 50% oxygen saturation. The water temperature was maintained at 12°C ± 0.5°C. After 30 minutes natural rocking activity, a blood sample was taken. Following this initial testing procedure, each of the six salamanders was anesthetized with a solution of 0.025% Tricaine. The Tricaine-treated salamander was placed on a mechanical stage which could be made to rock in a manner similar to natural activity. The mechanical stage with attached salamander was placed into an aquarium containing water previously equilibrated to 50% oxygen saturation. The salamander was mechanically rocked for 30 minutes at a frequency of six per minute. At the completion of simulated rocking activity, a blood sample was taken for blood gas and pH analysis.

All blood gas and pH analyses were run on an Instrumentation Laboratory 513 pH/blood gas analyzer. The IL-513 electrodes were thermostatted to 37°C. Thus, the data shown has not been temperature corrected for the 12°C experimental water. A commercial quality control system was used to check accuracy and precision of the instrument before and after each analysis.

The data for the effects of various water O_2 content levels on blood gas tensions and pH for anesthetized versus unanesthetized salamanders were analyzed with two-way analysis of variance. These data were further analyzed with Duncan's New Multiple Range Test to determine which of the means calculated for blood gas tension and pH were different from each other if the ANOVA indicated a significant difference was present.

RESULTS AND DISCUSSION

The effects of dissolved oxygen content of water on blood oxygen tension and rocking frequency are shown in Fig. 1. The data indicate that rocking frequency and blood oxygen tension are affected by dissolved oxygen content of water; furthermore non-rocking hellbenders are not able to maintain normal blood oxygen tensions in water with lowered oxygen content. Analysis of variance for the data from rocking and non-rocking salamanders shows the effect of decreased dissolved oxygen content on hellbenders was significant ($P < .05$).

Two graphs with results.

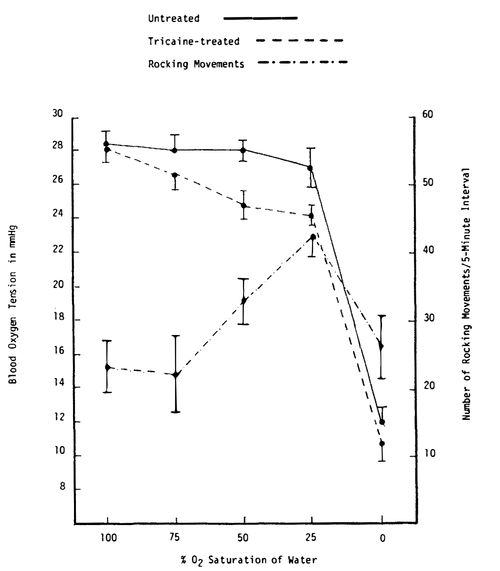

FIGURE 1. Effects of water O_2 content on blood oxygen tension and rocking activity for untreated and Tricaine treated salamanders. Points are Means ± SE.

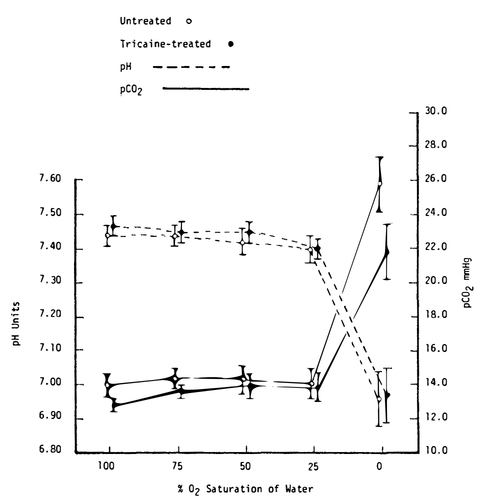

FIGURE 2. Effects of water O_2 content on blood carbon dioxide tension and pH for untreated and Tricaine treated salamanders. Points are Means ± SE. Data points for levels of % saturation have been off-set for clarity.

There was a two-fold increase in rocking frequency as the oxygen content of water was lowered from 75% to 25% saturation. Beffa (1976) suggested the increase in rocking frequency was a response to respiratory stress. His conclusions were based on data which showed both periods and frequency of rocking increase as dissolved oxygen content of water is decreased. Our results confirm Beffa's findings that rocking increases as water oxygen content decreases. Our data further show there is a significant difference in the blood oxygen tensions of rocking and non-rocking hellbenders as the water oxygen content is lowered from 75% to 25% saturation ($P < .05$). Thus our study supports Beffa's conclusions in as much as non-rocking hellbenders do not appear to be extracting aquatic oxygen as efficiently as they could if they were rocking.

Similar effects on blood pCO_2 and pH for non-rocking hellbenders were not significant (Fig. 2). The response of *Cryptobranchus* to near total anoxia in water is not unlike that seen for other ectotherms under similar conditions (Lenfant and Johansen, 1967). The blood pH shows marked acidemia which has been shown to be of both respiratory and metabolic origin (Boutilier et al., 1980). At least 50% of anaerobic CO_2 accumulation in totally aquatic vertebrates is known to result from acidification of body bicarbonate pools (Burggren and Cameron, 1980). In addition to bicarbonate acidification, Thillart and Kesbeke (1978) suggest as much as 50% of anaerobic CO_2 accumulation may arise from metabolic origins. A likely site of CO_2 generation via metabolism is dehydrogenation of pyruvate, alphaketoglutarate, or other selected substrates (Hochachka and Somero, 1973).

Recent studies by Boutilier et al. (1980) and Moalli et al. (1981) show blood gas tension and pH values for *Cryptobranchus* which are at variance with our study. The discrepancy between their studies for *Cryptobranchus* in water 100% saturated with oxygen and our study are probably due to differences in experimental protocol. Method of taking blood from salamander, temperature correction of blood gas and pH data, and use of flow-through aquaria could all account for the differences observed between our study and theirs.

The effects of simulated rocking activity on blood gas tensions and pH at 50% O_2 saturation of water are summarized in Table 1. Analysis of the data with paired t-test shows there is no significant difference between natural and simulated rocking activity with respect to blood gas tensions and pH ($P > .05$). Since the blood oxygen tension of Tricaine-treated hellbenders could be normalized with a simulated rocking procedure, we assume any pharmacological affect of Tricaine on blood oxygen tension is negligible.

From the data obtained in this study, we conclude that abolishing the rocking response of *Cryptobranchus* impairs its ability to maintain normal blood oxygen tensions in water containing oxygen in reduced amounts.

The adaptive significance of rocking activity lies in its potential for increasing cutaneous oxygen uptake in hypoxic environments. Because of is rich capillary network, the cutaneous surface could function as a more efficient gas exchanger when the boundary layer of the water-skin interface is disrupted. Thus the rocking activity serves to avail the cutaneous surfaces to a constantly renewable source of oxygenated water. Since rocking activity changes as temperature and water oxygen content change, the potential to adapt to any one or both of these variables is enhanced by the rocking activity of the hellbenders.

TABLE 1. The effects of mechanically simulated rocking activity on blood gas tensions and pH of hellbenders. Figures are Means ± 95% CI.

Rocking Condition	N	pH	pCO_2	pO_2
Natural activity (50% water O_2 content)	6	7.47 ± .05	11.1 ± 2.6	31.4 ± 3.1
Simulated activity (50% water O_2 content)	6	7.44 ± .05	10.2 ± 1.8	27.8 ± 2.3

ACKNOWLEDGMENTS

We wish to acknowledge Milton S. Topping for his statistical advice and members of the Bobby Witcher Society for help in collecting hellbenders.

LITERATURE CITED

Beffa, D. A. 1976. Responses of *Cryptobranchus alleganiensis alleganiensis* to different oxygen concentrations, temperature, and photoperiod. MA Thesis. Southwest Missouri State University. 37 pages.

Bernstein, H. 1953. Structural modifications of the amphibian skin. Proc. Acad. Natur. Sci. Philadelphia. 27:207–211.

Bishop, S. C. 1941. The salamanders of New York. N.Y. State Mus. Bull. No. 324, 365 pp.

Boutilier, R. G., D. G. McDonald and D. P. Toews. 1980. The effects of enforced activity on ventilation, circulation and blood acid-base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis;* a comparison with the semi-terrestrial anuran, *Bufo marinus*. J. Exp. Biol. 84:289–302.

Burggren, W. W. and James N. Cameron. 1980. Anaerobic metabolism, gas exchange, and acid-base balance during hypoxic exposure in the channel catfish, *Ictalurus punctatus*. J. Exp. Zool. 213:405–416.

Guimond, R. W. 1970. Aerial and aquatic respiration in four species of paedomorphic salamanders: *Amphiuma means means, Cryptobranchus alleganiensis alleganiensis, Necturus maculosus maculosus,* and *Siren lacertina.* Ph.D. Dissertation. University of Rhode Island.

———— and V. H. Hutchison. 1973. Aquatic respiration: An unusual strategy in the hellbender, *Cryptobranchus alleganiensis alleganiensis* (Daudin). Science. 182:1263–1265.

Hockachka, Peter W. and George N. Somero. Strategies of Biochemical Adaptation. Philadelphia: W. B. Saunders Co.; 1973.

Lenfant, C. and K. Johansen. 1967. Respiratory adaptations in selected amphibians. Respir. Physiol. 2:247–260.

Moalli, Richard, Roy S. Meyers, Gordon R. Ultsch and Donald C. Jckson. 1981. Acid-base balance and temperature in a predominantly skin-breathing salamander, *Cryptobranchus alleganiensis.* Respir. Physiol. 43:1–11.

Nickerson, M. A. and C. E. Mays. 1973. The hellbenders: North American "Giant Salamanders." Publications in Biology and Geology. No. 1. Milwaukee Public Museum. 106 pages.

Noble, G. K. 1925. Integumentary, pulmonary, and cardiac modifications correlated with increased cutaneous respiration in the amphibia: a solution to the "hairy" frog problem. J. Morphol. Phsyiol. 40:341–416.

Van den Thillart, G. and Fanja Kesbeke. 1978. Anaerobic production of carbon dioxide by goldfish *Carassius auratus*. Comp. Biochem. Physiol. 59A:393–400.

Willey, A. 1920. Remarks on the respiratory movements of *Necturus* and *Cryptobranchus.* Proc. Zool. Soc. London. 43:649–651.

Accepted 8 June 1981