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Hematology as Related to Diving Characteristics of *Elaphe obsoleta*, *Nerodia erythrogaster*, *Nerodia fasciata* and *Nerodia rhombifera*

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ABSTRACT

The diving capabilities of *Nerodia erythrogaster flavigaster* and *Nerodia fasciata confluens* were investigated and the results were compared with similar studies on *Nerodia rhombifera rhombifera* and *Elaphe obsoleta obsoleta* (Baeyens et al., 1978). In addition, morphological and hematological parameters contributing to underwater survival were examined.

The duration of underwater survival for *N. erythrogaster* and *N. fasciata* was approximately one hour with no difference between the species. The lung volumes of the two species were also similar but were significantly less than lung volumes reported for *E. obsoleta*. There were no significant differences in hemoglobin concentration, red blood cell count or hematocrit between *N. rhombifera*, *N. erythrogaster*, *N. fasciata*, and *E. obsoleta*. Based on similarities in underwater tolerance, lung morphology and hematology, *Nerodia* more closely resembles the terrestrial *E. obsoleta* than those reptiles specifically adapted to an underwater existance.

INTRODUCTION

Certain representatives from all the vertebrate classes display pronounced respiratory and cardiovascular changes in response to submergence. These animals, collectively referred to as the diving animals, are physiologically and morphologically suited for underwater survival. Most of the experimentation to date has focused on the diving mammals and birds, and relatively little is known of the physiology of diving reptiles.

This study represents a continuation of work reported earlier (Baeyens et al., 1978) and has two specific purposes. First, to compare the diving abilities of two species of water snakes (*Nerodia erythrogaster flavigaster*, and *Nerodia fasciata confluens*) with the diving ability of the terrestrial black rat snake. Second, to explore some of the hemotological and morphological attributes which might contribute to underwater survival in *N. erythrogaster*, *N. faciata*, *N. rhombifera* and *E. obsoleta*.

MATERIALS AND METHODS

Specimens of *N. erythrogaster*, *N. fasciata* and *N. rhombifera* were collected at night from various minnow farms in Lonoke County, Arkansas. *E. obsoleta* were collected during the day from wooded areas in Pulaski County, Arkansas. Weights of all the experimental animals ranged between 450 and 800 g. Most snakes were utilized within one week of capture. Snakes kept in captivity over two weeks were fed leopard frogs and minnows (*Nerodia*) or small mice (*Elaphe*). Most experiments were carried out between March and September, a period during which snakes are most active in Arkansas. For statistical analysis a Student-Newman-Keuls test was used to make multiple comparisons among means, and values considered significantly different have a p value of 0.05 or less (Sokal and Rohlf, 1969).

The duration of underwater survival for *N. erythrogaster* and *N. fasciata* was determined by subjecting the snakes to involuntary dives as previously described (Baeyens et al., 1978). The dive began the instant the animal voluntarily submerged its external nares at which time the cage containing the animal was totally submerged. The snakes were kept under close observation throughout the dive. Termination of the dive occurred at the first sign of stress. The outward indications of stress varied from snake to snake but were usually associated with a release of air from the lung and a sudden increase in activity in an attempt to reach the surface. Snakes remained under close observation after surfacing to insure that they were not seriously impaired as a result of the dive.

Lung volumes were determined for *N. erythrogaster* and *N. fasciata* by injecting air into the lung until the point of maximal lung expansion was reached (Baeyens et al., 1978). The lung capacities are expressed in terms of ml per kg body weight.

In all four species blood samples were collected from the hepatic portal vein with a 23 gauge needle fitted to a 5 cc syringe containing EDTA as an anticoagulant. Approximately 4 ml of blood were drawn from each animal. Hemoglobin concentration was measured as cyanmethemoglobin using Drabkin's diluent (Davidsohn and Wells, 1963). For hematocrit determinations, nonheparinized capillary tubes were filled with blood and one end was sealed with clay. The tubes were immediately centrifuged at approximately 10,000 RPM for ten minutes. Red blood cells were counted with a standard Neubauer counting chamber with Hayem's solution as the diluent (Davidsohn and Wells, 1963). Five red blood cell counts were made for each snake and the average was recorded.

RESULTS

Dive Times - Snakes were closely watched throughout the dive and at the first sign of stress the dive was terminated. When a snake was severely stressed as a result of a dive, it would be comatose and would take several hours to recover. Dive times were recorded only for those snakes which showed no signs of hypoxic stress upon surfacing.

Members of each species tolerated dive periods exceeding one hour (Table 1). There were no significant differences in the submergence times of the two species.

Lung Morphology - In both *N. erythrogaster* and *N. fasciata* the left lung has been lost, and the right lung is a simple tubular-shaped structure. The general lung structure of both species was similar to that described for *N. rhombifera* and *E. obsoleta* (Baeyens et al., 1978). The vascular portion of the lung comprised 24% of the total lung length in *N. erythrogaster* and *N. fasciata*, and there were no significant differences in total lung volume between the species. The lung volumes of *N. erythrogaster* and *N. fasciata* were similar to values reported for *N. rhombifera* but were significantly less than values reported for *E. obsoleta* (Table 1).

Hemotology - The results of the hematology studies are given in Table 2. Statistical analysis revealed no significant differences within or between species in any of the hematological values measured.

Table 1. Dive times and lung volumes for *Nerodia* and *Elaphe*.

Species	Dive Time (min) Mean ± S E (N)	Lung Volume (ml/kg body wt) Mean ± S E (N)
N. erythrogaster	78.4 ± 17.6 (10)	64.0 ± 12.2 (10)
N. fasciata	59.3 ± 20.2 (5)	61.4 ± 9.2 (5)
N. rhombifera[1]	68.3 ± 9.2 (10)	51.7 ± 11.1 (12)
E. obsoleta[1]	70.5 ± 8.3 (9)	80.2 ± 12.7 (6)*

*Significant difference from *Nerodia* at the 5% level.

[1]From Baeyens et al. 1978.

Table 2. Hematocrits (PCV), hemoglobin concentrations (Hb) and red blood cell counts (RBC) for *Nerodia* and *Elaphe*.

Species	Number of Snakes	PCV%	Hb g%	RBC/mm^3(X10^6)
N. erythrogaster	10	28.8 ± 4.5	10.7 ± 1.2	0.71 ± 0.07
N. fasciata	5	29.0 ± 6.9	9.7 ± 1.6	0.57 ± 0.03
N. rhombifera	10	32.3 ± 3.5	10.9 ± 2.1	0.75 ± 0.09
E. obsoleta	7	31.6 ± 3.5	11.5 ± 2.2	0.69 ± 0.04

DISCUSSION

Two areas which may be utilized for oxygen storage during a dive are the lungs and the blood. The lung and air sac volumes of diving mammals and birds are not significantly larger than those of their terrestrial counterparts (Scholander and Irving, 1941). Andersen (1961) found that the lung volume of the alligator, an excellent reptilian diver, ranges from 76 to 102 ml/kg. This is somewhat higher than the values reported for diving mammals, but lower than those reported for diving birds (Eliassen, 1960). The lung of the completely aquatic marine snake *Pelamis platuris* fills the entire coelomic cavity extending from the neck to the vent (Heatwole and Seymour, 1975). The immensity of the lung in this species is also reflected by its large volume (580 ml/kg body weight). In contrast, the lung volumes were much smaller in the two species we studied (Table 1). Since the lung volume of *Nerodia* is somewhat smaller than the terrestrial *Elaphe*, it does not appear that the lung of *Nerodia* is particularly adapted as an important oxygen storage organ for underwater activity.

The role of elevated hemoglobin concentration for oxygen storage has been shown to be of importance in diving mammals and birds (Andersen, 1966). The importance of hemoglobin concentration in diving reptiles has been difficult to ascertain because of the wide variations in reported values (Seymour and Webster, 1975; Dessauer, 1970). The present study indicates a similarity in hemoglobin concentration between *Nerodia* and *Elaphe*. These values fall within the range of hemoglobin concentrations found in sea snakes (Seymour and Webster, 1975). Red blood cell counts and hematocrits were also similar in the four species studied. These similarities agree with Hutton's (1958) findings of no consistent differences in either red blood cell counts or hematocrits between aquatic and terrestrial snakes.

Based on the parameters examined, *Nerodia* appears to be no better adapted for an aquatic existence than is the terrestrial *Elaphe* (Baeyens et al., 1978). The survival time of *Nerodia* when forcibly submerged is no longer than that of *Elaphe*, and is much shorter than for some turtles and sea snakes which can remain underwater for several hours (Pickwell, 1972; Graham, 1974). The similarities in hemoglobin concentration in *Nerodia* and *Elaphe* suggest that the blood does not have a unique oxygen storage capacity to facilitate underwater survival in *Nerodia*. In the same regard, the lung volume of *Nerodia* is smaller than that of *Elaphe*, and a great deal smaller than the lung volume of sea snakes. Based on the similarities in lung structure, hematology and underwater tolerance between *Nerodia* and *Elaphe*, it would seem that some of the physiological potential for an aquatic existence is already present in the black rat snake and presumably in other terrestrial species as well.

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