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Some Physiological and Morphological Adaptations for Underwater Survival in *Natrix rhombifera* and *Elaphe obsoleta*

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ABSTRACT

The submergence times of the diamondback water snake *(Natrix rhombifera)* and black rat snake *(Elaphe obsoleta)* were compared. Both species could easily survive underwater for periods greater than one hour. Furthermore, there was no difference in time of underwater survival in the two species.

Some physiological and morphological parameters which may contribute to the ability of *N. rhombifera* and *E. obsoleta* to remain submerged were also examined. *E. obsoleta* was found to have a greater lung volume and larger and more numerous alveoli than *N. rhombifera*. Both species demonstrated a bradycardia upon submergence but it was less pronounced than the bradycardia of the true diving animals. It is concluded that *N. rhombifera* has few physiological adaptations for diving and that some of the physiological attributes for an aquatic existence have already developed in *E. obsoleta*.

INTRODUCTION

Representatives of all the major vertebrate groups display pronounced respiratory and cardiovascular changes when entering an aquatic environment. Many air breathers have the capacity to remain submerged for long periods of time and are physiologically and anatomically well suited to the diving situation. Most of the experimentation involving diving animals has been performed on birds and mammals, and virtually nothing is known of the diving physiology of water reptiles.

The ability of diving mammals and birds to remain submerged for prolonged periods of time is based on several interacting mechanisms. Pulmonary and myoglobin oxygen storage is greater in divers than nondivers (Scholander and Irving, 1941). There is a relative insensitivity of the respiratory center to carbon dioxide and lactic acid in divers (Irving, Scholander and Grinnell, 1941). An important circulatory adaptation in many diving birds and mammals is a very pronounced bradycardia (heart slowing) which occurs immediately after submergence. For example, in the seal the heart slows from a resting rate of about 80/min. to 10/min. during a dive (Scholander and Irving, 1941). Various circulatory shunts which maintain blood supply to the head and heart while occluding the supply to the rest of the body during submergence are found in some mammalian divers. The relative importance of all of these adaptations varies among diving mammals and birds.

The purpose of our study was two-fold. First, to discern if the diamond-back water snake, *Natrix rhombifera*, is better able to exist underwater than the terrestrial black rat snake *(Elaphe obsoleta)*. Second, to examine some physiological and morphological parameters which may contribute to the ability of the two species to remain submerged.

METHODS AND MATERIALS

Natrix rhombifera were collected at night from various minnow farms located in Lonoke County, Arkansas. *Elaphe obsoleta* were collected during the daytime from wooded areas in Pulaski County, Arkansas. The weights of all experimental animals ranged between 500 and 800 g. Most of the snakes were utilized within one week of capture. Long term captives were fed leopard and bull frogs (Natrix) or small mice *(Elaphe)*. All experiments were carried out between March and September, 1977, a period during which most snakes are normally active in Arkansas. The results of all experiments were analyzed by employing a Student's t-test. Values considered significant have a p value of 0.05 or less.

Dive Time Determinations

A 50 gallon tank was filled with tap water at 20°C. The snake was placed in a wire-mesh minnow cage which was then wired securely closed. The cage was lowered into the water leaving enough air at the top to allow the animal to breathe. Snakes were allowed normal activity which shortly would include diving. Time zero was recorded at the instant the snake voluntarily submerged its head at which point the cage was completely immersed. Animals were carefully observed throughout the experimental period. At the first sign of stress, which was always associated with frenzied attempts to reach the surface, the dive was terminated. After diving, the snakes were kept under close observation for approximately 30 min. to insure that they were not impaired as a result of the dive.

Lung Volume Determinations

Snakes were killed by exposure to chloroform. A mid-ventral incision was extended from the neck region, just posterior to the head, to the beginning of the small intestine. The heart, major blood vessels and a portion of the esophagus overlying the ventral aspect of the lung were then excised by blunt dissection. In most of the snakes dissected there was an intimate connection between the pleural membrane and the visceral membrane surrounding the liver. This often resulted in a perforation of the lung while trying to free it from the hepatic tissue. Consequently, the liver was left in place and didn't seem to interfere with the volume determinations.

Sealing the lungs to measure lung volume was accomplished by ligating the trachea just anterior to the lung with a piece of thread. A 50-ml syringe fitted with a 23 gauge needle was carefully inserted into the vascular portion of the sealed lung and any residual air remaining in the lung was slowly evacuated. After the lung was completely collapsed, the syringe was filled with exactly 50 ml of air and a second syringe (also filled with 50 ml of air) was inserted into the vascular portion of the lung at a distance of approximately 2 cm from the first syringe. Air from one of the syringes was slowly injected into the lung and if maximal lung expansion was not attained, the second syringe was slowly emptied to the point of maximal lung expansion. The point of maximal expansion was determined when there was a noticeable increase in resistance to the further addition of air. Overinflation was discernable by the formation of bulges or outpocketings particularly in the saccular portion of the lung. Lung volumes were expressed in terms of ml per g body weight.

Histology

The lungs were removed and rinsed in saline and then cut into small sections of approximately 1 mm thickness. The sections were placed in either Zenker's or AFA fixative for 72 hours. After fixation, the tissues were dehydrated with ethanol, cleared in xylene, and embedded in paraplast. Sections 8μ in thickness were cut with a rotary microtome and placed on slides. At this time the tissues that were fixed in Zenker's were stained with Mallory's triple connective tissue stain and the tissues that were fixed in AFA were stained with Harris' hematoxylin.

Heart Rate Determinations

The electrical activity of the heart was measured by means of EEG disc electrodes (Narco Bio-Systems, Inc., Houston, Texas). After experimenting with several different types of electrodes it was found that the EEG silver discs gave the most distortion free recording, were the easiest to attach, and caused the least discomfort to the animal. The two recording electrodes were placed approximately 1 cm lateral to the mid-dorsal line. Placement was such that one electrode was located directly above the thirty-second scute from the animal's anterior. The other electrode was lined up with the thirty-sixth scute from the animal's anterior. Previous dissections had revealed that the heart was located in the region between 32 and 40. A third electrode was placed on the animal's back more posteriorly to ground the system. The electrodes were connected to a cardiac preamplifier. The output from the preamplifier was directed into a Physiograph (Narco Bio-Systems, Inc., Houston, Texas) for recording.

The snakes were restrained with masking tape loosely wrapped around the body at intervals and then attached firmly to a flat surface. The head of the snake was inserted through a pliable rubber dam into a 2.5 cm diameter hollow glass tube. The tube was covered with a piece of dark cloth to reduce the possibility of disturbing the animal during the experiment.

RESULTS

Dive Times

There was no significant difference in the submergence time of the two species (Table I). Rat snakes maintained a high level of activity, swimming about the cage looking for a means of escape, throughout the dive period. The water snakes usually remained relatively quiescent until hypoxic stress became evident at which time there was increased activity in an attempt to reach the surface. In each species high levels of activity resulted in a decreased time of submergence.

The possibility of gas exchange across the skin was also investigated. In order to prevent possible cutaneous respiration, some snakes of both species were heavily coated with Vasoline Petroleum Jelly. In no instance did the Vasoline treatment reduce the submergence time, indicating the unlikelihood of cutaneous respiration.

Table 1. Dive times and lung volumes of *Elaphe obsoleta* and *Natrix rhombifera*

Species	Dive Time (min) mean ± SE (N)	Lung Vol. (ml/g body wt.) Mean ± SE (N)
E. obsoleta	70.5 ± 8.3 (9)	0.0803 ± 0.0125 (3)
N. rhombifera	68.3 ± 9.2 (10)	0.0510 ± 0.0111 (12)*

*Significant difference in lung volume at the 5% level.

Lung Morphology

In some primitive snakes the lungs are paired structures, whereas in other snakes there is a radical departure from this plan in that the left lung has been lost or is rudimentary with the right one remaining as the only respiratory unit. In *N. rhombifera* and *E. obsoleta* the right lung is a single tubular-shaped structure, and there are no remaining vestiges of the left lung.

A cartilagenous trachea begins just posterior to the head and extends into the pulmonary tissue. In the cardiac region there is an expansion of the respiratory tube which is the beginning of the lung. The first part of the lung is characterized by having a highly vascularized wall lacking any cartilagenous supports. Due to the rich blood supply, this first part of the lung is bright red in color and is called the vascular portion. The vascular portion of the lung comprised 27% of the total lung length in *N. rhombifera* and 36% of the total lung length in *E. obsoleta*.

Posteriorly, the vascular portion of the lung leads directly into the saccular portion. The saccular portion is also a cylindrical tube, slightly smaller in diameter than the vascular portion. It is poorly vascularized and appears white in color. Posteriorly, the saccular portion of the lung extends to different lengths in the two species. In *E. obsoleta* it was greatly extended, reaching to the gallbladder, whereas in *N. rhombifera* it reached its maximum extension several cm anterior to the gallbladder. *E. obsoleta* was found to have a 36.5% greater total lung volume than *N. rhombifera* (Table 1).

All of the tissue within the vascular portion of the lung surrounded one large chamber without internal septa. Scanning electron microscopy revealed that the inner surface of the vascular portion had a grid-like appearance. Light microscopy demonstrated that the grids were bundles of smooth muscle and the spaces between the grids were diverticula that extended into the lung tissue. The diverticula branched, became smaller, and eventually terminated in alveoli.

The alveoli in both species were polygonal in shape and consisted of a single layer of squamous epithelium. Surrounding the alveoli was an extensive capillary network. A very thin layer of connective tissue was located between the epithelium of the alveoli and the endothelium of the capillaries. The alveoli of *E. obsoleta* were both larger and more numerous than those of *N. rhombifera* (Table 2).

Table 2. Summary of alveoli characteristics in *Elaphe obsoleta* and *Natrix rhombifera*

Species	Number (mm^2) Mean ± SE (N)	Diameter (μ) Mean ± SE (N)
E. obsoleta	28 ± 4.5 (10)*	182 ± 22.6 (10)*
N. rhombifera	18 ± 6.6 (10)	84 ± 12.7 (10)

*Significant difference at the 5% level.

The saccular portion contained no alveoli and was composed primarily of connective tissue and a layer of muscle surrounding a single undivided lumen. The muscle layer was composed of smooth muscle cells interspersed with connective tissue.

Diving Bradycardia

Insertion of the snake's head through the rubber dam invariably resulted in a temporary tachycardia. This was particularly true the first time a particular snake was measured. After the first measurement the snakes demonstrated a remarkable acclimation to the experimental procedure, and the increase in heart rate was barely perceptable by the third or fourth measurement. The heart rate was always allowed to stabilize at its lowest value before any data was recorded.

After the heart rate stabilized, approximately 100 ml of tap water (20°C) was poured into the open end of the glass tube. This volume of water was sufficient to completely immerse the snake's head. Recording began immediately upon submergence and continued for 15 min. or until the animal became active. A pronounced bradyardia varied between snakes and even varied somewhat in the same snake

on different dives. In all cases, however, bradycardia developed within one minute of submergence.

During the first three minutes of the dive there was considerable variation in the interval between heart beats, but after four minutes this variation disappeared. Within the first minute of submergence all the snakes exhaled. The bradycardia became much more noticeable after the initial exhalation and this could explain the variation in the onset time of bradycardia. In addition, any activity by the snake during the dive resulted in an increased heart rate which remained high for several minutes. Whenever a snake demonstrated any physical activity during a dive the experiment was terminated, and no data were recorded. Table 3 shows the percent reduction in heart rate of the two species 1 and 5 min. after submergence.

Table 3. One and five minute post-submergence bradycardia in *Elaphe obsoleta* and *Natrix rhombifera*. Heart rates (beats per min) are expressed as percent reduction from pre-dive rates.

Species	1 min.* Mean ± SE (N)	5 min.* Mean ± SE (N)
E. obsoleta	32.9 ± 9.6 (5)	44.7 ± 10.3 (5)
N. rhombifera	39.9 ± 8.6 (10)	57.2 ± 6.7 (10)

*Significant reduction from pre-dive heart rate at the 5% level.

DISCUSSION

Species Differences

It was commonly observed that inactive snakes could remain submerged for greater periods of time than could active ones under otherwise similar conditions. Submergence time seems to show an inverse relation to metabolic rate. For example, the submergence time was shortened by increased activity because of the higher metabolic rate under these conditions. During voluntary dives *N. rhombifera* was commonly observed quietly resting on the bottom for as long as 45 minutes. Even during the forced dives *Natrix* remained fairly inactive whereas the rat snake, in spite of a 36.5% larger lung volume, could not exceed the ability of the water snake to remain submerged.

Pickwell (1972) found that the sea snake *Pelamis platuris* tolerated 5 hours of being restrained underwater. Graham (1974) observed that some species of sea snakes had survival times ranging from 1.9 to 5 hours when they were held underwater in a wire cage. Interestingly, Graham found that the underwater survival time was directly porportional to the aquatic oxygen tension, which indicates that *P. platurus* can respire aquatically. It could take up to 30% of its total oxygen through the skin and could release carbon dioxide into the water at slightly higher rates. Our studies in which the snakes were coated with vasoline indicated that cutaneous respiration was not an important factor in prolonging underwater survival in either *N. rhombifera* or *E. obsoleta*.

The lung of *P. platuris* fills the coelomic cavity from the neck to the vent (Heatwole and Seymour, 1975). The lung volume in this species was found to be 0.58 ml/g body weight with the vascular portion comprising about 88% of the total lung length. In contrast, the lung volumes were much smaller in the two species we studied, and the vascular portion comprised only 27% and 36% of the total lung length in *N. rhombifera* and *E. obsoleta* respectively.

The involuntary submergence times in both *N. rhombifera* and *E. obsoleta* were much shorter than the reported values for sea snakes. The increased diving times of sea snakes are probably the result of specialized morphological and physiological adaptations not common to the species we studied. For example, the tremendous lung volume of sea snakes allows more oxygen storage for use underwater than is possible in either *N. rhombifera* or *E. obsoleta*. In addition, sea snakes can engage in a much higher rate of cutaneous respiration than can other snakes, again contributing to prolonged underwater survivability.

In both *N. rhombifera* and *E. obsoleta* the lung was clearly divided into two parts; a vascular portion and a saccular portion. All of the alveoli were located in the vascular portion which means that all gas exchange takes place in this portion. In reptiles it has been observed that lung volume increases in proportion to metabolic rate (Tenney and Tenney, 1970). The larger lung volume coupled with the larger and more numerous alveoli in the rat snake probably reflects a greater need for gas exchange in this active animal than in the relatively sedentary water snake.

The saccular portion of the lung contained no alveoli and, therefore, is not immediately involved in gas exchange. Brattstrom (1959) suggested the following functions for the saccular portion of the lung in snakes generally: (1) respiration, including both air storage and a flow through system, similar to the avian lung, permitting repassing of air over vascular areas during expiration; (2) cooling of the gonads by bringing cool air internally; and (3) body support, including a direct mechanical support and as a buoy in water. The air storage and buoyancy functions would be particularly important to water snakes.

McDonald (1959) suggested another possible significance of the saccular portion of the lung. He pointed out that if a cylindrical sac is elongated but the surface area is kept constant, its volume decreases. Thus, an elongation of the vascular portion of the lung imposed by the snake's body shape would limit the tidal volume and, hence, the amount of oxygen inspired, even though the total vascular surface might be adequate for gas exchange. McDonald suggested that the saccular portion provides a mechanism for increasing tidal volume and, thereby, compensating for the relative loss of lung volume in the elongated vascular portion. Furthermore, these considerations would apply equally well to water and terrestrial snakes and would explain the presence of a saccular portion in the lung of *E. obsoleta*.

The generalized lung plan of the snake might also be adaptive in other ways. For example, a large food bolus in the stomach might occlude the saccular portion of the lung, and an anterior shift of the vascular tissue would be an advantage. In addition, both *N. rhombifera* and *E. obsoleta* ingest large prey, and a saccular portion used for oxygen storage would be an advantage to both species during the swallowing process when respiration is interrupted. This is particularly true in view of the fact that the saccular portion contains smooth muscle which would allow a redistribution of air to the vascular portion during swallowing. The smooth muscle in the saccular portion may also be important in redistributing air from the saccular portion where it is stored to the vascular portion where gas exchange occurs during a prolonged dive.

Diving vertebrates including mammals, birds, reptiles, and amphibians display marked reductions in heart rate during submergence. This same response even occurs in non-divers such as man. Reptiles which have been shown to exhibit diving bradycardia include the crocodilians, fresh-water and marine turtles, terrestrial, semi-aquatic and marine lizards and several species of aquatic snakes (Wilber, 1960; Belkin, 1964; Bartholomew and Lasiewski, 1965; Jacob and McDonald, 1976; Irvine and Prange, 1976; Heatwole, 1977).

In *N. rhombifera* and *E. obsoleta* there was a reduction in heart rate within one minute after submergence. The fact that the bradycardia was somewhat more pronounced in the water snake than in the rat snake is probably because submergence is a common occurrence in water snakes whereas it represents a rather unnatural event to the terrestrial rat snake.

The diving bradycardia exhibited by the "true" diving mammals such as seals and whales appears to be initiated by a nerve reflex since it develops within one heartbeat of submersion. The vagus nerve appears to be the efferent limb for this reflex because the response is prevented by the administration of parasympatholytic drugs like atropine (Prosser, 1973).

The changes in heart rate reported here probably do not represent a true diving bradycardia. True diving bradycardia is known to be insensitive to activity. In our experiments the diving heart rate was elevated in both species by moderate increases in activity. In addition, in true diving animals the bradycardia is extreme. For example, the heart rate of the South American caiman drops from a resting level of 25 to less than 5 beats per minute during a dive (Gaunt and Gans, 1969). In our study the reduction in heart rate was only 45 to 57% of normal, values comparable to non-diving animals including man. Finally, in true diving animals the bradycardia begins within

one heartbeat of submersion, whereas in our study bradycardia sometimes did not become noticeable until one or two minutes after the dive began, and was never evident immediately after the first heart beat in either species. Thus, the diving heart rate in *N. rhombifera* and *E. obsoleta* should not be considered comparable to the bradycardia exhibited by the true diving animals.

In summary, the surprising thing about the water snake, *N. rhombifera*, is that is appears to have few physiological adaptations for diving. The survival time of *Natrix* when forceably submerged is no longer than that of the terrestrial rat snake, and is much shorter than some fresh-water and marine turtles and the sea snakes which can remain under water for several hours. Furthermore, the lung volume in *Natrix* is somewhat smaller than that of terrestrial snakes and is a great deal smaller than that of the sea snakes. In addition, *Natrix* does not seem to have any capacity for cutaneous respiration as do several species of sea snakes. Finally, the bradycardia recorded in *Natrix* during a dive was similar to that of the terrestrial rat snake and was much less than the reductions in the heart beat demonstrated by the true diving reptiles.

Based on lung morphology, underwater tolerance, and diving bradycardia it would seem that much of the physiological potential for an aquatic existence has already developed in the rat snake and presumably in other terrestrial species as well. In this regard, it is surprising that the evolutionary transition from a terrestrial to an aquatic existence has occurred so infrequently among the snakes.

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