STUDIES ON RESPIRATORY
MECHANISMS OF THE SALAMANDER
PSEUDOBRANCHUS STRIATUS

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INTRODUCTION

General Consideration of Amphibian Respiration

The Amphibia have been accorded an interest by biologists disproportionate to their rank as the smallest class of tetrapods. This is due to the fact that the Amphibia show the characteristics of ontological and phylogenetic transition from aquatic to terrestrial existence. This unique position among the vertebrates in being adapted to two very different environments, land and fresh-water, is emphasized by all aspects of amphibian biology. Adaptations in respiratory morphology, physiology, and behavior are important characteristics that make any amphibian suited to the environment in which it is found.

The comparative physiology and morphology of respiratory mechanisms of both invertebrates and vertebrates has been treated by Krogh (1941). General information on respiration in the Amphibia is found in Noble (1931) and Angel (1947). The anatomy and evolutionary significances of the respiratory organs of amphibians have been discussed by Smith (1960) and Goin and Goin (1962). Since the Amphibia exploit two media, air and water, for external respiration the organs of gas exchange are varied in

structure and usage. The integument and buccopharyngeal epithelium are used in both air and water, while the lungs are used in aerial respiration and the external gills are used in aquatic respiration.

Krogh (op. cit.) delimits gills as those appendages which have no other major function except aquatic respiration. This definition is valid for the many varied structures called gills in invertebrates, but in the amphibians only those paired structures that are found in the branchial region should be called true gills. This obviously would exclude integumentary growths such as the papillae of Astylosternus and the skin folds of Cryptobranchus and Telmatobius from this category, although these structures are vascularized and utilized for respiration.

Gills are present at some stage in the life history of all amphibians except for those few which do not have an aquatic larval stage. Most amphibians metamorphose but the gills persist in sexually mature forms which were formerly referred to as the Perennibranchiata. This was not a natural group but members of several taxa which can be divided into two groups, the neotenic salamanders and the paedogenic perennibranchs. The neotenic salamanders include many ambystomids and some plethodontids. They metamorphose in response to the activation of their thyroid glands by iodine treatment or temperature change. Paedogenic

perennibranchs including the sirenids, proteids, and some plethodontids are not so stimulated to metamorphose but are genetically controlled to retain their gills beyond the immature stage.

The size, structure, and utilization of gills in larval forms of perennibranchs have been considered to be in direct relation to the oxygen content of the water (Noble, 1931 and Angel, 1947). The larvae of those species of salamanders or frogs which develop in water of low oxygen tension show the largest and most ramified gills and those larval forms of species found in well aerated conditions generally show greatly reduced gills. Conant (1958) says of the perennibranch Necturus maculosus, "Size and condition of gills, although subject to individual variation, usually reflect environment. They are most likely to be large, bushy, and kept in motion if the water is foul or warm; usually they are small and contracted if the water is cool and contains considerable oxygen in solution."

The lungs of amphibians vary greatly in size and structure. They are absent in salamanders of the family Plethodontidae and the brook species of the genus Hynobius. In Rhyacotriton and Salamandrina which are both found in well aerated cool streams the lungs are greatly reduced in size. The lungs of the cold stream-dwelling anuran Ascaphus

are much smaller than those of other frogs and toads. Such reductions in lungs are correlated with increased cutaneous or buccopharyngeal respiration.

In the caecilians the left lung is usually rudimentary, supposedly correlated, as in snakes, with their elongated body form. However, in other elongated amphibians such as Amphiuma, Siren, and Pseudobranchius both lungs are equally well developed.

The lungs of anurans show increased complexity of internal structure in the predominantly terrestrial forms. The drier skin of terrestrial animals and subsequent reduction of cutaneous respiratory surface necessitates a corresponding increase of internal respiration.

Cutaneous respiration takes place to some degree in all amphibians. It is the main form of gaseous exchange in those animals which have no lungs, those with reduced lungs, or in anurans which are hibernating in the bottoms of ponds or lakes. Buccopharyngeal respiration is most important to the lungless active terrestrial salamanders in which additional surface area is needed to supplement the gas exchange through the integument. It is also utilized by other forms. I have had a Cryptobranchius alleganiensis under observation for over three years and it uses its buccopharyngeal epithelium for both aerial and aquatic respiration. Most of the time the animal remains

submerged and in order to supplement its cutaneous respiration it draws water through its nostrils into its mouth cavity bringing about a distension of its gular skin. When it does surface it draws air through its nostrils or mouth and either retains it in its buccopharyngeal cavity, again distending the gular region, or forces the air into the lungs which buoys the animal to float at the surface.

The lunged perennibranchs represent that group of amphibians which utilize all of the respiratory structures of amphibians in a single individual. Thus a study of the respiration of a lunged perennibranch would yield information on all phases of amphibian respiration. Because of its availability in the Gainesville area, its relatively easy maintenance in the laboratory, and its intermediate size as an amphibian, Pseudobranchius striatus axanthus was selected as the perennibranch for this study.

Pseudobranchius

The perennibranch amphibians of the family Sirenidae are generally classified under the suborder Meantes in the order Caudata (Urodela). Recently, however, Goin and Goin (op. cit.) have reinstated the order Trachystomata thus separating the two living genera Siren and Pseudobranchius and the several fossil genera of the Sirenidae from the rest of the tailed, legged amphibians.

There is only one living species of the dwarf siren, Pseudobranchius striatus. Five subspecies have been described, P. s. striatus Leconte, P. s. axanthus Netting and Goin, P. s. spheniscus Goin and Crenshaw, P. s. lustricolus Neill, and P. s. belli Schwartz. These are restricted to the extreme southeastern United States with P. s. axanthus occupying most of the peninsula of Florida and intergrading with all of the other subspecies. Goin and Auffenberg (1955) recognize two extinct species, Pseudobranchius robustus from the Pleistocene of Florida based on five vertebrae and Pseudobranchius vestustus from the Pliocene of Florida based on six vertebrae.

In the geographic range of Pseudobranchius where the water hyacinth Eichornia crassipes has been introduced, the hyacinth communities of prairie ponds, sloughs, and ditches are the most important habitat of this species (Goin, 1943 and Conant, 1958). The fibrous root system of the hyacinths supports dense populations of the invertebrates which serve as a food supply for Pseudobranchius and at the same time offer refuge for Pseudobranchius from its predators such as the warmouth bass Chaenobryttus coronarius or the mud snake Farancia abacura. Pseudobranchius eggs are laid singly or in clutches of up to five eggs on the upper portions of the hyacinth roots. Carr (1940) reports finding Pseudobranchius eggs throughout the spring months attached

to the filamentous leaves of Cabomba and Ceratophyllum as well as water hyacinth roots. Goin (1941) found Pseudobranchus eggs from April to November and during wet years I have collected Pseudobranchus eggs in every month with the heaviest egg-laying period being the latter half of September and early October in the Gainesville, Florida area.

In areas devoid of hyacinths where Pseudobranchus has been found such as the Gulf Hammock region of peninsular Florida, its habitat is the mucky bottom of bog areas into which they retreat at the least disturbance (Neill, 1951). Noble (op. cit.) describes Pseudobranchus as a burrowing salamander and field observation by Harper (1935) and Freeman (1958) attest to this habit of Pseudobranchus when under drying conditions.

The floating hyacinth community can be thought of as a relatively recent habitat of the burrowing mud-dwelling Pseudobranchus which itself has been around since the Pliocene. It offers food supply, shelter, and egg-laying areas which before the introduction of the hyacinth were probably provided by two habitats, the muddy bottoms of ponds and filamentous parts of aquatic vegetation.

The advantage of an eel-shape to mud-dwelling amphibians is obvious to any collector who has watched Amphiuma, Siren, or Pseudobranchus wriggle from his grasp and disappear into the muck. The vestigial legs of Amphiuma

seem of little use to the animal but the limbs of Siren, and Pseudobranchus, though only two in number, seem to serve the young animals for balance and support and serve the adults as feeble aids in burrowing, swimming, and crawling through the hyacinths. These anterior limbs of Pseudobranchus are proportionally smaller than those of Siren and have three toes each compared to the four toes found in Siren. The other distinctive external morphological character of the Sirenidae, the gills, will be discussed later. The Sirenidae differ from other caudate amphibia by lacking maxillary bones and cloacal glands. They possess a Jacobson's organ and the lungs are elongated, well vascularized and have small septa thus giving the animals an effective internal respiratory surface. Pseudobranchus differs from Siren in size and body proportions. It is a genus of slimmer animals and reaches a maximum length in largest subspecies, P. s. axanthus, of only 250 mm (Freeman, 1959), while the largest known Siren lacertina measured 950 mm in length (Goin, 1961).

Most of the investigations of the biology of the genus Pseudobranchus have been of a taxonomic nature. Its close association with the water hyacinth community has been noted by numerous herpetologists and ecologists. Various phases of its natural history have been cited by Noble (1931), Carr (1940), Goin (1941), Bishop (1947),

Conant (1958), Cochran (1961) and Goin and Goin (1962). However, few references to physiological research in this genus are available.

Noble and Richards (1932) implanted pituitary glands in ten female Pseudobranchus to induce egg-laying and Goin (1941) excised the gills of several animals and observed the respiratory behavior of these individuals. Little other experimental work has been done on members of the genus.

It was thought that a study of respiration in Pseudobranchus with both physiological and ecological considerations would be a contribution to the knowledge of the biology of amphibians. Such a study would include an investigation of the blood as well as the respiratory organs of Pseudobranchus.

Some characteristics of the blood of organisms that have been shown to be related to respiration are red blood cell sizes and numbers, hemoglobin values, and oxygen dissociation curves of the blood or of the hemoglobin. None of these had been investigated in Pseudobranchus.

As Pseudobranchus is active throughout the year and subjected to considerable changes in temperature, a study of the relationship between oxygen consumption and temperature was considered important. The relative utilization of the available respiratory surfaces of Pseudobranchus at different tensions of oxygen and carbon

dioxide under experimental conditions would indicate how the animals respond to changes in dissolved respiratory gases in their environment.

Information gained concerning pH, temperature, and dissolved oxygen and carbon dioxide under the hyacinth mats and in the open areas of ponds was needed to indicate some of the environmental conditions encountered by Pseudobranchus.

MATERIALS AND METHODS

Collecting Methods

Specimens of Pseudobranchius striatus axanthus were collected from the hyacinth ponds of Payne's Prairie six miles southwest of Gainesville, Alachua County, Florida. If the water levels of the ponds were sufficiently high for the hyacinth mats to float freely, a screen dredge scooped under the roots of the plants yielded a good catch of Pseudobranchius (Goin, 1942). When the water level was too low for effective dredging, Pseudobranchius were found in the mud underneath the stranded hyacinths on the periphery of the ponds. Here a potato rake was used to scrape back the hyacinths and mud to reveal the burrowing Pseudobranchius and the animals were seized by hand or captured in a dip net.

Maintenance of Animals in the Laboratory

The experimental animals were housed singly or in small groups in spring water or filtered pond water at room temperature in one-gallon jars or three-gallon glass aquaria. It was found that the animals did not fare as

well in aged tap water, perhaps because of the added fluorides and chloramine.

The Pseudobranchus were fed about twice a week on aquatic oligochaetes, chironomid larvae, or the white worm Enchytraeus. No other maintenance was needed except an occasional change of the water in the containers. One Pseudobranchus lived for four years under such conditions.

On several occasions when room temperature rose above 35°C individuals died apparently because this temperature exceeded the range of their temperature tolerance. During the winter months when aquaria were left on the uninsulated floor, water temperatures dropped below 10°C and the animals showed no ill effects.

Physical Environment of the Hyacinth Ponds

The physical conditions of the water of the hyacinth ponds considered to be important in the respiration of Pseudobranchus were temperature, dissolved oxygen and carbon dioxide contents, and pH. Measurements of these factors were made in the open water of the ponds and in water under the hyacinth mats in order to compare these two habitats.

Temperature measurements were taken in degrees centigrade at six inch depths with a Spencer thermometer. Water samples for oxygen and carbon dioxide determinations

were taken at six inch depths in ground glass stoppered bottles. The determination of oxygen was by the Winkler method and free carbon dioxide analysis was done by titration with N/44 sodium hydroxide with phenolphthalein as an indicator (Welch, 1948). The pH of the water was determined with a Beckman Model N portable pH meter and with pHDrion test paper.

Aquatic Respiration and Temperature

Two procedures of oxygen determinations that have been used for measuring aquatic respiratory rates in amphibians are the chemical Winkler method and the manometric Van Slyke method. Helff (1927) used the Winkler method in a comparative study of several Ambystoma larvae. The Winkler method was used by Etkin (1934) to determine the respiratory rates of bullfrog tadpoles. Evans (1939) used the Winkler method for the aquatic tests in comparing consumptions of several plethodontid salamanders. Vernberg (1952 and 1955a) employed the Van Slyke method of oxygen determination for his studies of the respiration of several plethodontids. A critical analysis of the Winkler method was made by Allee and Oesting (1934). Both Oesting (1934) and Wilder (1937) found no significant differences in the results obtained from the methods of Winkler and Van Slyke in oxygen determination.

For reasons of simplicity of equipment and reasonable simplicity of procedure the Winkler method was chosen for determining aquatic oxygen consumption of Pseudobranchius. Deviations from the Winkler technique as described by Welch (op. cit.) were that the volumes of the water samples were 25 cc; the volumes of the reagents manganous sulfate, potassium hydroxide-potassium iodide, and sulfuric acid were 0.1 cc each; and the aliquots for titration were 20 cc.

The aquatic respiratory rates of five adult female Pseudobranchius, varying in weight from 3.0 grams to 4.5 grams, were measured at 15°C, 20°C and 25°C $\pm$ 1°C. The animals were run in succession at each temperature for a total of 15 trials in the experiment.

Each Pseudobranchius was starved for 24 hours and placed in a round battery jar aquarium containing 2075 cc of spring water. The animals was left for one-half hour and then transferred to a second aquarium containing an equal amount of water. This procedure was used to inoculate the first or control aquarium with bacteria that might contribute to the oxygen utilization in the aquarium containing the Pseudobranchius.

The Pseudobranchius was retained below the surface of the water by a circular piece of plastic-coated window screening. A similar piece of screening was placed in the control aquarium. Glass siphons were installed in both

aquaria and the surfaces were covered with 2.5 cm of heavy mineral oil. Allee and Oesting (op. cit.) reported no oxygen contamination in water of low oxygen content over a 24 hour period when the surface was covered with two to five centimeters of mineral oil.

Water samples were siphoned from both aquaria into 25 cc bottles allowing them to overflow twice the contents of the bottles. Oxygen determinations were made in terms of parts per million. The aquaria were placed in a water bath and maintained at the desired test temperature. After five hours oxygen determinations were again made and the differences from the first determinations were recorded. The mineral oil was siphoned from the aquarium containing the Pseudobranchius and the animal was removed and weighed. The differences between the determinations of the aquaria was converted to cubic millimeters of oxygen per gram per hour at standard temperature and pressure using the method of conversion as described by Welch (op. cit.).

Effects of Carbon Dioxide and Oxygen on Respiratory Behavior

The following procedures were used to subject experimental animals to different tensions of carbon dioxide and oxygen in order to observe the effects of these gases on

respiratory behavior. Filtered pond water was boiled for 15 minutes in a 2000 ml Erlenmeyer flask to rid it of dissolved gases. The flask was sealed and allowed to cool to room temperature. After the water had cooled the flask was opened and a siphon was installed to remove water samples for carbon dioxide and oxygen determinations. The boiling of the water lowered the contents of both gases to one part per million (10^{-3} ml/liter) or less.

The Pseudobranchus to be observed was placed in the flask. The patterns of respiratory behavior of the animals were subjectively determined to be of three categories. The first was that in which supplemental aerial respiration, characterized by frequent surfacing by the Pseudobranchus to gulp air was used in addition to aquatic respiration. In the second type the animal rarely surfaced and aquatic respiration through the skin and expanded gills was sufficient. The third category was also aquatic but in this case the gills were constricted and therefore only integumentary gas exchange was employed.

Five Pseudobranchus were observed singly and in groups of two. When the patterns of respiratory behavior of the animals was stable enough for such behavior to be placed in one of the three mentioned categories carbon dioxide and oxygen determinations were made of the water in the flasks.

The tensions of the gases in the flask were changed by the addition of more water to the flask and by bubbling the gases directly into the flask. The carbon dioxide source was a hydrochloric acid-sodium carbonate generator. The evolved gas was bubbled through a wash bottle before it was added to the water in the flask. The oxygen source was air pumped into the water by means of a New Rochelle Cosmic Piston Pump.

Erythrocyte Count

The number of red blood cells per cubic millimeter of blood in Pseudobranchius was determined by standard techniques using a Spencer Bright Line hemacytometer. Five-hundredths of a milliliter of 1:1000 heparin was introduced into the body cavity of each Pseudobranchius one hour before it was etherized and the blood was drawn from the heart into the diluting pipette. Five counts were made on each of five adult animals and the average of each set was taken as the erythrocyte count of each individual.

Erythrocyte Size

Measurements of the sizes of the erythrocytes were made by means of an American Optical micrometer. Samples

of the blood from the above five Pseudobranchus were used. Lengths and widths of 20 cells of each animal were measured in micra.

Hemoglobin Value

Grams of hemoglobin per 100 cc of blood of Pseudo-branchus were determined by the acid hematin test (Cohen and Smith, 1919) using a Bausch and Lomb Spectronic 20 colorimeter.

Pseudobranchus blood clots rapidly so each animal was injected with 0.05 ml of 1:1000 heparin one hour before it was etherized and the sample of blood was withdrawn. Each animal was either used 48 hours after it was captured or had been kept well fed under laboratory conditions for one to four weeks. After the specimen had been etherized the body fluid was withdrawn from a small puncture in its belly and then 20 cu mm of blood was withdrawn directly from the heart into a Sahli blood pipette. The blood was hemolyzed in 5.0 ml of 2 per cent hydrochloric acid and the hemolyzed red blood cells were allowed to settle for 5 hours. Thus they would not interfere with the colorimeter readings of the acid hematin solution. Readings were converted to grams of hemoglobin per 100 cc of blood using the handbook of methods and calibrations of the Spectronic 20 colorimeter.

RESULTS

Physical Conditions of Prairie Ponds

The mats of floating water hyacinths affect the water temperature, dissolved oxygen, free carbon dioxide, and to a lesser extent the pH of the water of the prairie ponds.

The accelerated vegetative growth and flowering of Eichornia coincided with the rising temperature in April and May in the Gainesville area. Air temperatures and water temperatures at six-inch depths were taken at 11 A.M. during this period and are recorded in Table 1. Figure 1 represents these readings.

The differences between the temperatures of the open water and the sub-hyacinth water increased during the period of the investigation. The water level dropped until the hyacinth mats were stranded on the mud bottom. This drop in water level could have been due to a lowering of the water table because of insufficient rainfall or to dredging and draining operations in conjunction with the construction of Interstate 75 across Payne's Prairie.

TABLE 1
TEMPERATURE READINGS OF HYACINTH PONDS

Date	Air Temperature	Open Water Temperature	Sub-Hyacinth Temperature	Difference Open-Hyacinth
4-12-63	28° C	23° C	22.5° C	0.5° C
4-16-63	26° C	25° C	22° C	3.0° C
4-18-63	28° C	24° C	20° C	4.0° C
5- 1-63	26° C	30° C	23° C	7.0° C
5-18-63	33° C	35° C	26° C	9.0° C
5-22-63	29.5° C	35° C	24° C	11.0° C

Fig. 1.-Temperature readings of the air, open water, and sub-hyacinth water of a prairie pond during April and May of 1963.

The shading effect of the hyacinth mat and the subsequent drop in photosynthetic activity of phytoplankton and rooted vegetation under the mat produced the expected inverse oxygen-carbon dioxide relationship in water under the hyacinths as compared with the open water. The oxygen content of the water was lower under the hyacinth than in the open water and at the same time the carbon dioxide content is higher. The relationships are graphed in Figure 2.

Aquatic Respiration and Temperature

As shown in Tables 2, 3, and 4, the mean oxygen consumption of the five Pseudobranchius tested at 15°C was 19.88 mm³/gm/hr, at 20°C the mean was 29.92 mm³/gm/hr, and at 25°C the mean was 37.76 mm³/gm/hr. A Q_{10} value of 1.98 was determined in this range.

Effects of Carbon Dioxide and Oxygen Concentrations on Respiratory Behavior

The observed differences in respiratory behavior at various carbon dioxide and oxygen tensions are recorded in Figure 3. A polygon enclosing the symbols for aquatic respiration with expanded gills encompasses only one symbol for another type of respiratory behavior.

Fig. 2.-Oxygen and carbon dioxide concentrations in open water and sub-hyacinth water in April and May of 1963.

TABLE 2
OXYGEN CONSUMPTION RATE OF PSEUDOBANCHUS AT 15 DEGREES CENTIGRADE

<u>Pseudobranchus</u>	Animal A	Animal B	Animal C	Animal D	Animal E
First reading, O ₂ content, parts per million	7.4	6.8	7.9	7.8	7.6
Second reading, O ₂ content, parts per million	6.9	6.5	7.6	7.3	7.1
Difference in parts per million	0.5	0.3	0.3	0.5	0.5
Control: first reading, O ₂ content, parts per million	7.7	6.8	8.1	7.8	8.1
Control: second reading, O ₂ content, parts per million	7.4	6.8	8.1	7.8	7.8
Control: difference in parts per million	0.3	0.0	0.0	0.0	0.3
Difference of differences: O ₂ parts per million	0.2	0.3	0.3	0.5	0.3

Table 2 (Cont'd)

<u>Pseudobranchius</u>	Animal A	Animal B	Animal C	Animal D	Animal E
(X) 698 times difference of differences	139.6	209.4	209.4	349.0	139.6
(Y) Titration value of sodium thiosulfate	0.90	0.89	0.89	0.89	0.90
(Z) Liters of water	2	2	2	2	2
X·Y·Z, Amount of O ₂ consumed in cubic millimeters	251.28	372.73	372.73	621.22	251.28
<u>Weight of Pseudobranchius</u> <u>in grams</u>	3.2	3.8	3.5	4.5	3.3
Time of test run in hours	5	5	5	5	5
Oxygen consumption rate, mm ³ /gm/hr	15.7	19.6	21.3	27.6	15.2

Mean consumption rate - 19.88 mm³/gm/hr.

TABLE 3

OXYGEN CONSUMPTION RATE OF PSEUDOBURRACHUS AT 20 DEGREES CENTIGRADE

<u>Pseudoburachus</u>	Animal A	Animal B	Animal C	Animal D	Animal E
First reading, O ₂ content, parts per million	7.9	6.2	7.9	6.0	6.95
Second reading, O ₂ content, parts per million	6.9	5.7	7.4	5.6	6.3
Difference in parts per million	1.0	0.5	0.5	0.4	0.65
Control: first reading, O ₂ content, parts per million	7.1	5.6	6.9	5.5	7.2
Control: second reading, O ₂ content, parts per million	6.5	5.4	6.9	5.5	6.9
Control: difference in parts per million	0.6	0.2	0.0	0.0	0.3
Difference of differences: O ₂ parts per million	0.4	0.3	0.5	0.4	0.35

Table 3 (Cont'd)

<u>Pseudobranchus</u>	Animal A	Animal B	Animal C	Animal D	Animal E
(X) 698 times difference of differences	279.2	209.4	349.0	279.2	244.3
(Y) Titration value of sodium thiosulfate	0.93	0.93	0.92	0.95	0.96
(Z) Liters of water	1.9	2.0	2.0	2.0	2.0
X·Y·Z, Amount of O ₂ consumed in cubic millimeters	493.35	389.48	642.16	530.48	469.06
Weight of <u>Pseudobranchus</u> in grams	3.2	3.7	3.0	4.0	3.3
Time of test run in hours	5	5	5	5	5
Oxygen consumption rate, mm ³ /gm/hr	30.8	21.1	42.8	26.5	28.4

Mean Consumption Rate - 29.92 mm³/gm/hr.

TABLE 4

OXYGEN CONSUMPTION RATE OF PSEUDOBANCHUS AT 25 DEGREES CENTIGRADE

<u>Pseudobranchus</u>	Animal A	Animal B	Animal C	Animal D	Animal E
First reading, O ₂ content, parts per million	7.2	7.3	7.2	7.0	7.0
Second reading, O ₂ content, parts per million	6.4	6.5	6.3	6.2	6.4
Difference in parts per million	0.8	0.8	0.9	0.8	0.6
Control: first reading, O ₂ content, parts per million	7.6	7.4	7.2	7.0	7.1
Control: second reading, O ₂ content, parts per million	7.3	7.0	6.9	6.8	6.9
Control: difference in parts per million	0.3	0.4	0.3	0.2	0.2
Difference of differences: O ₂ parts per million	0.5	0.4	0.6	0.6	0.4

Table 4(Cont'd)

<u>Pseudobranchus</u>	Animal A	Animal B	Animal C	Animal D	Animal E
(X) 698 times difference of differences	349.0	279.2	418.8	418.8	279.2
(Y) Titration value of sodium thiosulfate	0.90	0.90	0.91	0.90	0.92
(Z) Liters of water	2	2	2	2	2
X·Y·Z, Amount of O ₂ consumed in cubic millimeters	628.20	502.56	762.22	753.84	513.73
Weight of <u>Pseudobranchus</u> in grams	3.0	3.6	3.0	4.3	3.1
Time of test run in hours	5	5	5	5	5
Oxygen consumption rate, mm ³ /gm/hr	41.9	27.9	50.8	35.1	33.1
Mean Consumption Rate - 37.76 mm ³ /gm/hr.

Fig. 3.-Respiratory behavior of *Pseudobranchius* at different concentrations of oxygen and carbon dioxide.

Erythrocyte Count

The erythrocyte counts of five adult Pseudobranchus were 74000, 86000, 88000, 90000, and 92000 cells per cubic millimeter of blood with a mean of 86000 cells/mm³ and with a standard deviation of 7050.

Erythrocyte Size

The mean length of 100 red blood cells was 48.1 micra with a standard deviation of 1.36 micra and a coefficient of variation of 2.8 per cent. The mean width was 23.4 micra with a standard deviation of 0.73 micra and a coefficient of variation of 3.1 per cent. The product of the length times the width in the oval red blood cells of Amphibians while not giving the surface area of such cells does give a relative amount of surface if this value is calculated for all investigated species. The product of the mean length times the mean width in Pseudobranchus was 1125.5. The length/width ratio was 2.05.

The largest individual red blood cell measured 55.9 micra by 29.3 micra with a length times width value of 1637.9. The smallest red blood cell measured 34.5 micra by 20.7 micra with a length times width value of 714.2. The extremes of length/width ratio were 1.5 and 3.0. The

longest red blood cell was 62.8 micra and the shortest was 34.5 micra. The broadest red blood cell was 29.3 micra and the narrowest was 17.9 micra. These values represent a great range both in size and shape of the erythrocytes in Pseudobranchius, but a graph (Fig. 4) of the ranges, means, two standard deviations, and four standard errors helps to show the rather low coefficient of variation.

Hemoglobin Value

The hemoglobin values for eight adult Pseudobranchius were 3.8, 5.4, 5.8, 6.9, 7.2, 7.4, 7.6, and 10.2 grams per 100 cc of blood. These give a mean of 6.8 grams of hemoglobin per 100 cc of blood with a standard deviation of 1.76 and a standard error of 0.622. A coefficient of variation of 25.9 per cent was calculated for these determinations.

Fig. 4.-Erythrocyte size in *Pseudobranchius*. Vertical and horizontal lines indicate ranges of length and width; crossing of lines indicates means; one black and one white rectangle combined on each side of the mean, one standard deviation; white rectangle on vertical and black rectangle on horizontal, two standard errors.

DISCUSSION

Physical Conditions of Hyacinth Ponds

Payne's Prairie has had a history of fluctuating water levels which have varied this environment from that of a lake covering the entire basin to the relatively dry prairie with scattered hyacinth ponds of the present. The ponds could thus be considered semi-isolated remnants of a shallow dystrophic lake. Their water levels fluctuate greatly between drought periods and the heavy rainy seasons, with many ponds going completely dry at times. At other times the ponds may overflow their sometimes ill-defined banks and coalesce through shallow stretches of flooded prairie with neighboring ponds often of considerable distances away.

Such fluctuations in water level place limitations on the types of organisms that could successfully survive in the hyacinth pond habitat on a long term basis. The pond species could be divided into four categories, those whose individuals had moved into the pond during flood stages from other ponds, those that could move overland from other ponds, those that had survived by being able to

estivate for short periods, or those that pass through an extended dormancy over dry periods. Pseudobranchus seems to most often survive in any one pond by estivating during dry periods.

The hyacinth mats contribute to the lowering of the volume of water in the ponds by the accumulation on the bottom of the ponds of detritus from the floating plants. The hyacinth mats ameliorate living conditions for some aquatic animals. The modifying effect of the floating mats of vegetation keeps the sub-hyacinth water cooler than the open water, especially at low water levels, and no doubt enables some ectotherms to survive when otherwise they could not without the protection of their heat reflecting canopy of hyacinths. Body temperatures of aquatic salamanders usually are identical with the water temperature in which they are found. Brattstrom (1963) reports that some aquatic salamanders appear to select specific temperature regions or levels within ponds. His September, 1954 record for collecting Pseudobranchus from Payne's Prairie gave the temperature of the water at 24°C. The 35°C temperature reached in the open pond water is in the range of the critical thermal maxima of many salamanders (Hutchison, 1961) and could be avoided by Pseudobranchus by selecting the cooler water under the hyacinths. If the water levels of the ponds drop enough for the hyacinth mats to be

stranded their matted fibrous roots retain moisture more efficiently than mere mucky sand and such animals as Siren, Pseudobranchius, Seminatrix, annelids, water beetles, etc. are afforded a refuge that may retard their rates of desiccation when the ponds go dry.

The rather peculiarly spaced dates for the gathering of the data at the ponds were selected because of the meteorological changes that took place in the area just previous to the time of the readings. Climatological data from the Gainesville area for the months of April and May, 1963 are shown in Tables 5 and 6. The Beef Research Unit (BRU) is nearer Payne's Prairie than the official U. S. Weather Bureau station. The data of the temperatures in the prairie pond reflect the changes in the temperature recorded officially at the Beef Research Unit.

A general description of the weather in the pond area in the month of April, 1963 would be "fair and warm," but a cold front moved through the area during the middle of the month and the minimum temperature was 25°F lower on April 16 than on April 12. The air temperature at the pond dropped at that time (Fig. 1) and though the water temperature had dropped further on April 18, the air temperature on the 18th was higher than on the 16th. On both May 1 and May 21 heavy rain fell at the pond and the Beef Research Unit. The climatological data from the U. S. Weather Bureau for those

TABLE 5

CLIMATOLOGICAL DATA FROM THE GAINESVILLE AREA, APRIL, 1963**

Day	Gainesville, Florida 3 WSW												BRU	
	(T) Temperature °F				Rain In.	Pan Evap. In.	Wind m/da	Min. RH %	Air T. 5'		Rain In.			
	Air 5'		Soil 4"											
	Max.	Min.	Max.	Min.					Max.	Min.				
1	80	58	82	71	.24		25	46	82	56				
2	82	58	85	70	.23		15	45	81	55				
3	84	54	84	70	.20		10	45	82	52				
4	87	54	84	71	.19		5	42	86	51				
5	87	55	86	72	.26		5	48	87	56				
6	85	57	86	73	.28		35	41	84	56				
7	79	57	80	70	.30	T	60	56	85	58				
8	78	46	84	66	.16	.59 T	20	45	75	46	.67			
9	80	50	84	68	.28		50	48	79	48				
10	82	61	83	72	.16		60	56	80	62				
11	87	61	88	74	.28		45	45	82	63				
12	89	62	88	75	.25		25	44	88	65				
13	82	61	88	75	.34		25	24	88	61	.06			
14	77	47	85	70	.37		50	28	77	47				
15	77	37	84	66	.26		20	20	71	38				
16	81	38	86	66	.23		0	24	76	36				
17	85	46	88	69	.27		5	37	82	42				
18	87	49	90	71	.27		5	33	84	47				
19	87	51	90	72	.30		20	37	85	49				
20	86	60	92	77	.24		25	41	87	57				
21	89	58	92	77	.28		10	41	86	56				
22	89	60	91	78	.31		30	39	88	61				

Table 5 (Cont'd)

Gainesville, Florida 3 WSW											BRU
Day	(T) Temperature of Air 5'		Soil 4" Depth*		Rain In.	Pan Evap. In.	Wind m/da	Min. RH %	Air T. 5'		Rain In.
	Max.	Min.	Max.	Min.					Max.	Min.	
23	89	65	90	78		.31	60	44	87	66	
24	87	66	92	78		.31	20	23	88	67	
25	86	60	87	77	.02	.22	5	38	85	56	
26	84	60	90	74	"	.14	0	44	87	57	
27	85	58	90	76		.35	5	37	84	54	
28	83	61	88	77		.30	5	46	85	60	
29	84	59	89	76		.33	5	39	83	56	
30	82	63	82	77	.69	.26	0	53	83	63	
April Average											
1963	84.0	55.7	86.9	72.8	1.30	7.92	21.5	83.2	54.7	0.73	
1962	81.7	52.6			1.79	7.44	34.5	81.6	50.4	2.00	
51 yr. avg.	81.1	57.6			2.96						

Accumulative Rainfall Summary (Inches)

	Gainesville	BRU
From January 1963	10.59	9.58
From January 1962	9.19	7.19

* Under Short Pensacola Bahiagrass Sod

** Agronomy Department and U.S. Weather Bureau Cooperating

Note: Observation time at Gainesville is in 4:30 p.m. and at BRU (Beef Research Unit) is 8:00 a.m.

TABLE 6
CLIMATOLOGICAL DATA FROM THE GAINESVILLE AREA, MAY, 1963**

Gainesville, Florida 3 WSW													BRU	
Day	(T) Temperature of					Rain In.	Pan Evap. In.	Wind m/day	Min. RH %	Air T. 5' Max. Min.	Rain In.			
	Air 5'	Soil 4"	Depth	Max.	Min.									
1	77	62	85	72		.74	.27	5	30	79	62	1.38		
2	76	51	78	68		T	.26	0	44	76	46			
3	76	60	75	69		T	.14	5	60	76	58			
4	80	55	82	66		T	.18	5	42	76	50			
5	84	48	86	68			.30	15	28	80	48			
6	90	56	88	71			.21	5	31	84	60			
7	90	58	89	73			.29	5	35	90	57			
8	90	59	89	75			.25	0	40	90	57			
9	91	60	91	76			.28	5	36	90	57			
10	92	61	89	76			.32	5	34	90	65			
11	92	61	90	76			.32	10	45	91	65			
12	92	67	92	79			.31	10	50	92	67			
13	89	72	92	81			.28	15	52	92	72			
14	90	62	92	78			.26	5	44	87	58			
15	89	62	90	80			.21	5	50	90	59			
16	92	67	94	81			.25	5	46	92	65			
17	92	67	95	82			.35	5	42	92	67			
18	92	65	96	81			.37	20	40	92	69			
19	94	64	96	82			.28	5	36	91	60			
20	93	67	96	82			.31	10	34	93	63			
21	92	66	94	82			.28	5	40	91	62			
22	92	64	93	78		0.49	.22	0	42	94	62	2.20		

Table 6 (Cont'd)

Gainesville, Florida 3 VSW													BRU
Day	(T) Temperature of				Soil 4"			Pan Evap. In.	Wind m/da	Min. RH %	Air T. 5'		Rain In. .94
	Max.	Min.	Max.	Min.	Max.	Min.	Rain In.				Max.	Min.	
23	90	65	91	78			0.02	.23	5	42	93	64	
24	81	62	86	76			T	.18	15	64	91	61	
25	87	64	91	76				.26	10	50	90	62	
26	88	63	90	79				.22	10	53	86	60	
27	92	63	95	81			2.18	.76	10	48	90	66	.16
28	90	65	89	76			T	.11	5	52	91	64	
29	89	66	90	76			.08	.18	0	52	90	64	
30	88	64	90	76			T	.15	0	45	90	62	
31	88	68	88	78				.27	5	50	87	64	
May Average													
1963	88.3	62.3	89.7	76.5			3.51	8.30	7.0	43.7	88.2	61.2	4.96
1962	92.5	63.0	86.6	76.6			2.19	9.26	28.2	36.8	93.7	60.5	2.29
51 yr. avg.	87.2	63.8					3.21						
Accumulative Rainfall Summary (Inches)													
Gainesville BRU													
From January 1963			14.10				14.27						
From January 1962			11.38				9.48						
51 Year average			15.53										

Note: Observation time at
Gainesville is 4:30 p.m. and at
BRU (Beef Research Unit) is 8:00 a.m.

* Under Short Pensacola Bahia Sod

** Agronomy Department and U.S. Weather Bureau Cooperating

two dates indicate the differences in the causes for the precipitation. The lower temperatures after May 1 show the effects of a cold front. The high temperature of the latter part of May show that precipitation on the 21st was from an isolated thunderstorm. On both May 1 and May 22 the temperature of the open water was well above that of the air temperature by the time of the readings. These data indicate that though shower activity may cool off the air at the ponds the higher solar energy warms up the shallow open water of the ponds very rapidly. By May 22 the deepest part of the pond was only six inches. At this date the difference in the temperature of the open water and the water under the hyacinths was 11°C .

→ The lower oxygen and higher carbon dioxide contents of the water under the hyacinths could place respiratory stress on animals restricted to aquatic respiration.

Pseudobranchus, with its ability to utilize lungs as well as skin and gills, is not so affected. Even though the oxygen content of the water underneath the hyacinths may drop to below two parts per million the Pseudobranchus is not in respiratory difficulty for it can rise to the surface and gulp air. The lack of oxygen in the sub-hyacinth water poses no problem and the cooler temperature is an asset to an ectothermic amphibian.

Though the carbon dioxide content of the water under the hyacinths is higher than that of the open water there does not seem to be a significantly lower pH of the water under the hyacinths. As the pond waters dropped several large limestone boulders were revealed which could possibly contribute a buffering effect to the water of the pond.

Oxygen Consumption and Temperature

The respiratory rate of Pseudobranchius is relatively low when compared with that of other amphibians of approximately the same body weight. Wilder (1937) found the respiratory rate of Desmognathus fuscus at 20°C to be from 32.6 to 49.2 mm³/gm/hr and that for Eurycea bislineata to be 55.7 to 89.9 mm³/gm/hr. Vernberg (1952) measured the rate of oxygen consumption at 10°C of Eurycea bislineata at 38 to 44 mm³/gm/hr and of Plethodon cinereus at 30-37 mm³/gm/hr. At 20°C the rate of oxygen consumption of P. cinereus was 50.63 mm³/gm/hr while at the same temperature the rate for P. glutinosus was only 33.35 mm³/gm/hr (Vernberg, 1955a). In all the above cases the investigators concluded that the differences in the respiratory rates at equal temperatures were due to the more active behavior of the salamanders with the higher rates.

Pseudobranchius by such standards would be considered an amphibian of low activity rate. Though it does have brief periods of violent activity in eluding capture, for the most part it appears sluggish and quiet in aquaria. The waters of the prairie ponds or ditches are practically devoid of any currents that would impose a stress on the swimming ability of Pseudobranchius. Therefore it would seem that the only stimuli for extreme activity in its normal environment would be in escape from its enemies or in brief struggles subduing and ingesting larger invertebrate prey.

A summary of the data on respiratory rates of Pseudobranchius in the 15°C-25°C temperature range is shown in Table 7. A two-way analysis of variance on this oxygen consumption data showed no significant differences among the five animals but significant differences ($P < .01$) in oxygen consumptions at the different test temperatures. Since the temperatures were equally spaced, linear and quadratic components of the temperature response could be separated by orthogonal sub-division of the sum of squares. The method and coefficients were taken from Cochran and Cox (1957). This analysis (Fig. 5) showed that the response to temperature was linear in the temperature range of this experiment (15°-25°C).

TABLE 7
TEMPERATURES AND OXYGEN CONSUMPTION RATES

	15° C	20° C	25° C	
	t_1	t_2	t_3	
A	15.7 y_{11}	30.8 y_{12}	41.9 y_{13}	88.4 H_1
B	19.6 y_{21}	21.1 y_{22}	27.9 y_{23}	68.6 H_2
C	21.3 y_{31}	42.8 y_{32}	50.8 y_{33}	114.9 H_3
D	27.6 y_{41}	26.5 y_{42}	35.1 y_{43}	89.2 H_4
E	15.2 y_{51}	28.4 y_{52}	33.1 y_{53}	76.7 H_5
	T_1	T_2	T_3	$\sum y_{ij}$
	99.4	149.6	188.8	437.8

ANALYSIS

Sources	d.f.	S.S.	M.S.	V.R. (F)
Animals	4	409.43	102.36	3.11
Temperatures	2	803.27	401.64	12.20**
Linear	1	779.24	779.24	24.28**
Quadratic	1	4.03	4.03	0.12
Error	8	263.30	32.91	
Total	14	1475.99		

** $P \leq 0.01$

Fig. 5.-Analysis of oxygen consumption and temperature.

Fuhrman and Fuhrman (1959) found a linear relationship between oxygen consumption and temperature in the salamander Desmognathus escholtzii at 15°-22.8°C.

A Q_{10} value of 1.98 was calculated for oxygen consumption of Pseudobranchius in the 15°-25°C range. Apparently the Q_{10} value of oxygen consumption of caudate amphibians have not been calculated but the Q_{10} value of oxygen consumption for Pseudobranchius does not seem significantly lower than expected for sluggish poikilotherms (Prosser, et al. 1950).

Though the temperatures of the air and open water in the ponds reach extremes out of the 15°-25°C range the temperature range of the water under the hyacinth approximates that of the test. The hyacinth mat acts as a thermoregulating agent by reflecting the radiant heat of the sun during the day and as an insulating agent against re-radiation loss of heat at night. Thus under the moderating influence of the hyacinth mat the temperature fluctuation of the environment are not so great as to overtax the respiratory capacity of the ectothermic Pseudobranchius.

Effect of Oxygen and Carbon Dioxide on Respiratory Behavior

The primary respiratory stimulus of the terrestrial vertebrates is generally considered to be an increase in the partial pressure of carbon dioxide in the environment and subsequently in the animal's blood stream. Any respiratory stress is characteristically met in such animals by an increased rate of ventilation. In those aquatic vertebrates which have aerial means of respiration, stimulus and response usually are the same as in terrestrial animals. However, in most lower aquatic form, respiratory stimulation is more likely to be a result of low oxygen concentration in the water rather than high carbon dioxide concentration and the response of most gilled vertebrates is either an expansion of gill surface area or an accelerated rate of movement of water over the gills (Prosser, et al. 1950).

Studies of the respiratory behavior of amphibians under high and low concentrations of oxygen and carbon dioxide have for the most part failed to quantify the concentration of the gases at the time the respiratory behavior was noted. Drastich (1925) observed a greater development of the gills of larval Salamandra under low oxygen concentration than under high oxygen concentration. Noble (1927) attributed the proportionally large gills of

the tadpoles of Hyla rosenbergi to the poor oxygen supply in their breeding basins. He also states (Noble, 1931) that the larvae of both Siren and Pseudobranchius have the ability to reduce their gills if respiratory conditions are not suitable for gill functions. According to Brattstrom and McFarland (1955) specimens of the newt Taricha torosa were so unresponsive to a rapid increase in carbon dioxide in their aquatic environment that they lost their motility and did not attempt to either rise to the surface to gulp air or crawl out on rocks available to them. Savage (1952) suggests that low oxygen tension does have some influence on the pulmonary system of anurans. He says the early development of lungs in the life history of the frog Rana temporaria is an important adaptation to its life in ponds of low oxygen content.

Work of a quantitative nature on the respiration of an ectothermic vertebrate was done by Willmer (1934). He investigated the influences of different tensions of oxygen and carbon dioxide on the yarrow Erythrinus erythrinus, a tropical fish of acid swamps, which respire by means of an air bladder as well as gills. During periods of aerial respiration the yarrow came to the surface at three to four minute intervals with its gill chambers remaining closed by its opercula. Its gill chambers were open and the yarrow remained below the surface during periods of aquatic

respiration. Willmer also observed a third type of respiratory behavior which combined both aerial and aquatic respiration which he considered intermediate to the other two.

Willmer determined experimentally that the yarrow was forced to rely on aerial respiration when the oxygen content of the water was below 0.5 cc per liter or when the carbon dioxide content of the water was either above 35 cc per liter or below 5 cc per liter at a pH in the range of its natural swamp environment. As long as the dissolved oxygen was above 1.5 cc per liter, except when the carbon dioxide content was above 25 or 30 cc per liter or below 10 cc per liter, the yarrow was able to respire entirely by aquatic means. At intermediate concentrations of oxygen and carbon dioxide both aerial and aquatic respiration were employed.

Willmer explained forced aerial respiratory behavior with its permanently closed gill openings as a method of preventing the possible loss of oxygen to the water through the gills. Similarly at high carbon dioxide concentrations the fish might be compelled to keep the gill chambers closed to avoid absorbing carbon dioxide from its environment. At least 5 cc per liter of carbon dioxide was considered necessary to stimulate the yarrow to move its opercula and gills so below that level the fish was forced

to come to the surface to get air. Thus Willmer considered the yarrow to be responsive to both oxygen and carbon dioxide in its respiratory behavior.

Dehadrai (1962) ran experiments on the fish Notopterus similar to the ones done by Willmer on Erythrinus and the respiratory behavior of the two fishes was similar. Respiratory behavior in Notopterus is purely aerial when free carbon dioxide in the water is below 5 parts per million or above 60 parts per million at oxygen concentrations from 1 to 8 parts per million. At carbon dioxide concentration of 10 to 40 parts per million and oxygen from 2 to 8 parts per million the respiratory behavior of Notopterus was mainly aquatic. Between the conditions when either aquatic or aerial respiratory behavior was dominant there was a range in which the fish resorted to "intermediate respiration."

The phases of respiratory behavior in Pseudobranchus were not as easily delimited as in Erythrinus or Notopterus. Pseudobranchus obviously does not have an operculum to be used as an indication of change from aquatic to aerial means of respiration. The constriction of the external gills is not so obvious as to give a hairline demarcation in any individual between supplemental gill usage and reliance on skin surface alone. However, the data graphed in Figure 3 show a definite enough influence of oxygen and

carbon dioxide on respiratory behavior to warrant hypotheses on this relationship.

Brattstrom and McFarland (op. cit.) attribute the failure of carbon dioxide to stimulate respiration in Taricha to the fact that the gas does not accumulate in very high concentrations in its natural environment. This is not the case, of course, for Pseudobranchus, as the concentration of carbon dioxide may get quite high under the hyacinth mats in the prairie ponds due to the respiration of the sub-hyacinth plants and animals and the reduced rate of photosynthesis due to lack of sunlight. The effect of high carbon dioxide concentration of the water in which Pseudobranchus is found is one of stimulating pulmonary ventilation. This effect is most evident when oxygen tension is low, again the condition under the hyacinths.

The utilization of the lungs in some perennibranchs has been questioned on the basis of anatomy or behavior. Luckhardt and Carlson (1920) conclude that Necturus does not use its lungs for respiration. Noble (1931) mentions the poorly vascularized lungs and lack of alveoli in some newts and in the perennibranchs Proteus and Necturus. Krogh (op. cit.) shows diagrams of the lungs of Siren having simple septa as opposed to the smooth surfaces of the lungs of Proteus. Hilton (1952) describes the lungs of Siren as "quite simple sacs" but he remarked that the close

network of capillaries in the lungs indicated their respiratory function. Czopek (1962) found that in Siren intermedia the lung capillaries represent 61 per cent of the total capillaries of the respiratory organs. His study also showed the skin capillaries to be 36 per cent, the gills to be 2 per cent, and the mouth cavity to be 1 per cent of the respiratory surface. Whitford and Hutchison (1963) say that the lungs and buccopharyngeal mucosa of Ambystoma maculatum become more important as respiratory structures at temperatures above 15°C. The lungs of Pseudobranchius are anatomically and histologically similar to those of Siren and from the behavior of the animals under the influence of carbon dioxide are obviously important respiratory organs.

Pseudobranchius can survive on pulmonary respiration alone. I placed an adult individual into an Erlenmeyer flask containing 1500 milliliters of boiled spring water and covered the surface with mineral oil. After depleting the oxygen concentration of the water to a point below 0.5 parts million and raising the carbon dioxide concentration to 5 parts per million, the Pseudobranchius was able to survive for 72 hours by surfacing for air about seven times per hour. Unfortunately the animal also engulfed a fair amount of mineral oil during this period and had to be removed from the flask. A juvenile (75 mm) Pseudobranchius

lived for six days in a 250 ml beaker containing mineral oil one centimeter deep and no water. It was unable to swim in the oil but could flex its body enough to get its mouth above the surface to breathe. After it was returned to an aquarium it swam normally and within 15 minutes fed on an earthworm.

The size of gills in Pseudobranchus seems to be influenced by the oxygen content of the water. Though not shown in Figure 3, at lower oxygen concentration the gills are larger. Figures 6 and 7 show the gills of an individual at oxygen concentration of 7.7 and 2.8 parts per million, respectively. The gills of a Pseudobranchus may expand to a relatively tremendous size if the animal is exposed to a very low concentration of oxygen for an extended period. Figures 8 and 9 show an animal that had been retained below the surface of oil-coated water in which its aquatic respiration had reduced the oxygen content from 6.8 parts per million to less than 1 part per million in a 24 hour period. The Pseudobranchus was motionless apparently because of hypoxia for it again became active after one hour in fresh spring water after frequent proddings with a dissecting needle. Dehadrai (op. cit.) reported similar restoration to normal activity of asphyxiated Notopterus in fresh water. He was even able to practice "artificial

Fig. 6.-Pseudobranchius in water with oxygen concentration of 7.7 parts per million.

Fig. 7.-Same animal in water with oxygen concentration of 2.8 parts per million.

Fig. 8.-Pseudobranchius after prolonged retention in water of oxygen concentration of less than one part per million.

Fig. 9.-Same animal, enlarged view.

respiration" on such fish by injecting fresh air into its empty swimbladder by means of a hypodermic syringe.

Other factors affecting gill size are food and activity. After feeding, Pseudobranchus usually goes through a period of little activity in which its gills often increase in size, probably due to the increased metabolic activity of the digestive processes. After periods of agitation characterized by rapid and prolonged swimming by the Pseudobranchus its gills also expand. In this case probably the reason is because of the oxygen debt built up during the activity of the animal.

However, gills are at best respiratory conveniences because the animals can live without them. Those animals taken from the burrow habitats have no gills and when they are placed in water they may take up to three weeks to grow new ones. Goin (1941) removed the gills from several Pseudobranchus and placed the animals in aquaria. He concluded from his observations that those animals which were allowed to come to the surface and gulp air for a period before being retained under the water were able to later survive on skin respiration alone. Those animals that were immediately retained underwater after their gills had been excised did not survive. McCrady (1954) removed the gill rami from the salamander Gyrinophilus pallescens and reported the animals died without their gills having regenerated.

I removed the gills from four Pseudobranchus and put them into separate aquaria in which they were screened from the surface. They went into periods of bleeding and "shock" as described by Goin as well as a sloughing of their epidermis due to handling. After recovering from the "shock" conditions the animals were found to survive in spite of the fact that they were unable to reach the surface and utilize aerial breathing. The initial oxygen concentration in the water was 6.8 parts per million which may have been higher than that used by Goin and enabled my animals to survive without aerial breathing. The amount of blood lost, which could have easily been a survival factor, was not measured in either case.

Erythrocyte Size and Count

Amphibians have the largest erythrocytes of all the vertebrates. The largest known red blood cells with a mean of 61.8×36.7 micra have been described by Smith (1925). Amphibians also have the lowest red blood cell counts of all the vertebrates.

Though the red blood cell sizes of many species of amphibians have been measured there have been relatively few species in which both cell size and cell number have been investigated. Table 8 gives the mean erythrocyte

TABLE 8
ERYTHROCYTE COUNT IN AMPHIBIANS

Species	Source	RBC 1000/mm ³
<u>Hyla arborea</u>	Prosser, <u>et al.</u> , 1950	670
<u>Rana esculenta</u>	Prosser, <u>et al.</u> , 1950	530
<u>Rana temporaria</u>	Prosser, <u>et al.</u> , 1950	408
<u>Bufo vulgaris</u>	Prosser, <u>et al.</u> , 1950	380
<u>Desmognathus monticola</u>	Vernberg, 1955b	197
<u>Eurycea lucifuga</u>	Vernberg, 1955b	178
<u>Desmognathus ochrophaeus</u>	Vernberg, 1955b	169
<u>Triturus vulgaris</u>	Prosser, <u>et al.</u> , 1950	135
<u>Desmognathus quadramaculatus</u>	Vernberg, 1955b	131
<u>Triturus alpestris</u>	Prosser, <u>et al.</u> , 1950	110
<u>Diemyctylus viridescens</u>	Prosser, <u>et al.</u> , 1950	103
<u>Plethodon cinereus</u>	Vernberg, 1955b	91
<u>Gyrinophilus danielsi</u>	Vernberg, 1955b	88
<u>Pseudobranchius striatus</u>	Freeman, 1963	86
<u>Ambystoma tigrinum</u>	Vernberg, 1955b	81
<u>Eurycea bislineata</u>	Vernberg, 1955b	80
<u>Siren lacertina</u>	Freeman, 1963	68
<u>Amphiuma means</u>	Freeman, 1963	67
<u>Salamandra maculata</u>	Prosser, <u>et al.</u> , 1950	57
<u>Necturus maculosus</u>	Claypole, 1896	56
<u>Plethodon glutinosus</u>	Vernberg, 1955b	56
<u>Salamandra atra</u>	Prosser, <u>et al.</u> , 1950	54
<u>Ambystoma maculatum</u>	Vernberg, 1955b	53
<u>Necturus maculosus</u>	Harris, 1953	51
<u>Proteus anguineus</u>	Prosser, <u>et al.</u> , 1950	36
<u>Necturus maculosus</u>	Vernberg, 1955b	28

counts of 24 species of amphibians. The measurements of the red blood cell sizes of these species are shown in Table 9 which includes the product of the mean length times the mean width. In Table 10 the values of the products of the red blood cell counts times the length times the width are arranged in descending order. These data are taken from Claypole (1896), Prosser, et al (1950), Harris (1953), Vernberg (1955b), and my measurements in Pseudobranchius, Siren, and Amphiuma.

Several generalizations can be made from the data in these tables. One is that the smallest and most numerous cells are found in the anurans. A second is that the large aquatic forms have the largest cells. A third and discouraging observation is that the data from different investigators are quite inconsistent. The three series of measurements of the erythrocytes of Necturus maculosus vary greatly. Vernberg's (1955b) Necturus counts and measurements are so far below those of Claypole (1896) and Harris (1953) as to make questionable his data on the other salamanders as well. However, I measured the red blood cells of Desmognathus fuscus from the Gainesville area and found the mean size to be 31.4×16 micra, well within the ranges of the three species of Desmognathus measured by Vernberg. Since Vernberg's measurement of the erythrocyte width in Necturus was the same as that recorded by Harris it would

TABLE 9
ERYTHROCYTE SIZE IN AMPHIBIANS

Species	Source	RBC Length Micra	RBC Width Micra	Length x Width
<u>Amphiuma means</u>	Freeman, 1963	62.4	36.2	2258.9
<u>Proteus anguineus</u>	Prosser, et al., 1950	60.0	35.0	2100.0
<u>Necturus maculosus</u>	Claypole, 1896	58.4	31.1	1816.2
<u>Necturus maculosus</u>	Harris, 1953	54.4	28.2	1534.1
<u>Siren lacertina</u>	Freeman, 1963	48.5	24.6	1193.1
<u>Necturus maculosus</u>	Vernberg, 1955b	40.5	28.2	1142.1
<u>Pseudobranchius striatus</u>	Freeman, 1963	48.1	23.4	1125.5
<u>Ambystoma maculatum</u>	Vernberg, 1955b	37.9	23.9	905.8
<u>Salamandra atra</u>	Prosser, et al., 1950	41.7	19.8	825.7
<u>Eurycea lucifuga</u>	Vernberg, 1955b	36.4	21.9	800.1
<u>Triturus vulgaris</u>	Prosser, et al., 1950	32.0	25.0	800.0
<u>Gyrinophilus danielsi</u>	Vernberg, 1955b	37.4	21.0	785.4
<u>Salamandra maculata</u>	Prosser, et al., 1950	35.5	20.5	727.8
<u>Plethodon glutinosus</u>	Vernberg, 1955b	33.4	20.2	674.7
<u>Ambystoma tigrinum</u>	Vernberg, 1955b	34.5	19.0	655.5

Table 2 (cont'd)

Species	Source	RBC Length Micra	RBC Width Micra	Length x Width
<u>Diemictylus</u> <u>viridescens</u>	Prosser, et al., 1950	30.0	19.6	588.0
<u>Desmognathus</u> <u>quadramaculatus</u>	Vernberg, 1955b	32.0	16.7	534.4
<u>Plethodon cinereus</u>	Vernberg, 1955b	30.7	16.3	500.4
<u>Triturus alpestris</u>	Prosser, et al., 1950	36.0	13.0	468.0
<u>Desmognathus</u> <u>monticola</u>	Vernberg, 1955b	29.9	14.9	445.5
<u>Eurycea bislineata</u>	Vernberg, 1955b	30.0	14.8	444.0
<u>Desmognathus</u> <u>ochrophaeus</u>	Vernberg, 1955b	30.7	12.7	389.9
<u>Rana esculenta</u>	Prosser, et al., 1950	22.8	15.7	358.0
<u>Bufo vulgaris</u>	Prosser, et al., 1950	20.5	13.3	272.7
<u>Rana temporaria</u>	Prosser, et al., 1950	19.7	13.3	262.0
<u>Hyla arborea</u>	Prosser, et al., 1950	18.5	11.7	216.5

TABLE 10

ERYTHROCYTE SIZE AND COUNT IN AMPHIBIANS

Species	Source	RBC 1000/ mm ³	RBC Length Micra	RBC Width Micra	Length x Width	LxW Count Millions
<u>Rana</u> <u>esculenta</u>	Prosser, <u>et al.</u> , 1950	530	22.8	15.7	358.0	190
<u>Amphiuma</u> <u>means</u>	Freeman, 1963	67	62.4	36.2	2258.9	151
<u>Hyla</u> <u>arborea</u>	Prosser, <u>et al.</u> , 1950	670	18.5	11.7	216.5	145
<u>Eurycea</u> <u>lucifuga</u>	Vernberg, 1955b	178	36.4	21.9	800.1	142
<u>Triturus</u> <u>vulgaris</u>	Prosser, <u>et al.</u> , 1950	135	32.0	25.1	800.0	108
<u>Rana</u> <u>temporaria</u>	Prosser, <u>et al.</u> , 1950	408	19.7	13.3	262.0	107
<u>Bufo</u> <u>vulgaris</u>	Prosser, <u>et al.</u> , 1950	380	20.5	13.3	272.7	104
<u>Necturus</u> <u>maculosus</u>	Claypole, 1896	56	58.4	31.1	1816.2	102
<u>Pseudobranchius</u> <u>striatus</u>	Freeman, 1963	86	48.1	23.4	1125.5	97
<u>Desmognathus</u> <u>monticola</u>	Vernberg, 1955b	197	29.9	14.9	445.5	88
<u>Siren</u> <u>lacertina</u>	Freeman, 1963	68	48.5	24.6	1193.1	81
<u>Necturus</u> <u>maculosus</u>	Harris, 1953	51	54.4	28.2	1534.1	78

Table 10 (Cont'd)

Species	Source	RBC 1000/ mm ³	RBC Length Micra	RBC Width Micra	Length x Width	LxW Count Millions
<u>Proteus anguineus</u>	Prosser, et al., 1950	36	60.0	35.0	2100.0	76
<u>Desmognathus quadramaculatus</u>	Vernberg, 1955b	131	32.0	16.7	534.4	70
<u>Gyrinophilus danieli</u>	Vernberg, 1955b	88	37.4	21.0	785.4	69
<u>Desmognathus ochrophæus</u>	Vernberg, 1955b	169	30.7	12.7	389.9	66
<u>Diemictylus viridescens</u>	Prosser, et al., 1950	103	30.0	19.6	588.0	61
<u>Ambystoma tigrinum</u>	Vernberg, 1955b	81	34.5	19.0	655.5	53
<u>Triturus alpestris</u>	Prosser, et al., 1950	110	36.0	13.0	468.0	51
<u>Ambystoma maculatum</u>	Vernberg, 1955b	53	37.9	23.9	905.8	48
<u>Plethodon cinereus</u>	Vernberg, 1955b	91	30.7	16.3	500.4	46
<u>Salamandra atra</u>	Prosser, et al., 1950	54	41.7	19.8	825.7	45
<u>Salamandra maculata</u>	Prosser, et al., 1950	57	35.5	20.5	727.8	41
<u>Plethodon glutinosus</u>	Vernberg, 1955b	56	33.4	20.2	674.7	37
<u>Eurycea bislineata</u>	Vernberg, 1955b	80	30.0	14.8	444.0	36
<u>Necturus maculosus</u>	Vernberg, 1955b	28	40.5	28.2	1142.1	32

seen that the much shorter cell length as recorded in his data could be attributed either to a typographical error or perhaps to an error in calculation in converting optical micrometer readings into micra.

Chang, Chen, and Chen (1943) and Harris (*op. cit.*) report a decrease in red cell count in Necturus as a result of starvation, thus the extremely low average of Vernberg's red cell counts in Necturus could be due to nutritional deficiency in his specimens.

Vernberg (1955b) in his study of 12 species of salamanders said that there is a general correlation between cell size and body weight in that larger species have larger cells. However, he noted an exception to this in that the larger Ambystoma tigrinum has smaller cells than the smaller Ambystoma maculatum. The usual inverse relationship of the species having the larger cells also having the smaller number of cells shows an exception in comparing Eurycea lucifuga and Eurycea bislineata.

The cell size and number in Pseudobranchius could be interpreted as an exception to these generalities also if this genus were considered with the salamanders investigated by Vernberg. Its body weight would be no greater than an average Gyrinophilus, Ambystoma, or Desmognathus, but its red blood cell size is greater than all of the amphibians except the large aquatic forms, Cryptobranchus, Proteus,

Necturus, Amphiuma, and Siren. At the same time its cell count is higher than two species each of the Ambystomidae, Salamandridae, and Plethodontidae.

Thus in its red blood cell dimensions, Pseudobranchius is grouped with its co-familial genus Siren and other giant aquatic salamanders in having the largest red blood cells among the vertebrates. It appears that the large red blood cell size of Pseudobranchius is a reflection of its descent from larger sirenid forms. The known fossil Pseudobranchius (Goin and Auffenberg, 1955) were larger than the extant P. axanthus.

From the point of view of an increased surface-volume ratio it seems that small and numerous erythrocytes are most effective as oxygen carriers. This condition is seen in the jumping, climbing, active anurans and in the endotherms. That Pseudobranchius retains its ancestral giant erythrocytes in its present smaller body is indicative that there was no selective pressure on the oxygen carrying capacity of the blood that would make smaller and more numerous cells a more efficient morphological adaptation.

The values of length times width counts of erythrocytes would give comparative figures for the available surface areas for transportation of oxygen provided that hemoglobin activities of the various species are the same. McCutcheon and Hall (1937) have said that the oxygen affinity

of hemoglobins of various amphibians varies, the difference being correlated with respiratory mechanisms, hemopoietic apparatuses, limitations of the environment, and metabolic activities of the animals. In Table 10, where the animals are arranged in descending order of the length times width times count of the erythrocytes, there seems to be a lack of phylogenetic or ecological grouping except that the anurans are in the top one-third of the table.

Hemoglobin Values

Hemoglobin values of some representative amphibians are presented in Table 11. As noted in the table the bloods of anurans are recorded as having higher hemoglobin contents than the caudate amphibians. Also notable is the discrepancy between the two values found for Necturus by different investigators. This is not particularly surprising considering the great range of values shown in the measurements of Pseudobranchius hemoglobin.

The coefficient of variation of 25.9 for the hemoglobin values of Pseudobranchius blood may not be as unrealistic as it seems at first. Unpublished data from Goin and Jackson on blood of Bufo terrestris show a coefficient of variation of 26.9 in hemoglobin count of the blood. Stuart (1951) in his comparison of hemoglobin values

TABLE 11
HEMOGLOBIN CONTENT OF AMPHIBIAN BLOOD

Animal	Source	Hb-gm/100 cc Blood
<u>Bufo bocourti</u>	Stuart, 1951	10.6
<u>Bufo vulgaris</u>	Prosser, <u>et al.</u> , 1950	10.2
<u>Hyla arborea</u>	Prosser, <u>et al.</u> , 1950	10.2
<u>Necturus</u> <u>maculosus</u>	Prosser, <u>et al.</u> , 1950	9.8
<u>Rana esculenta</u>	Prosser, <u>et al.</u> , 1950	9.5
<u>Bufo marinus</u>	Stuart, 1951	8.7
<u>Salamandra</u> <u>maculata</u>	Prosser, <u>et al.</u> , 1950	8.0
<u>Pseudobranchius</u> <u>striatus</u>	Freeman, 1963	6.8
<u>Salamandra atra</u>	Prosser, <u>et al.</u> , 1950	6.8
<u>Triton alpestris</u>	Prosser, <u>et al.</u> , 1950	6.0
<u>Necturus</u> <u>maculosus</u>	Harris, 1953	4.2

in Bufo marinus and Bufo bocourti had great ranges of 6.5-11.00 gm/100 cc and 7.5-13.75 gm/100 cc for the two species, respectively. The range would have been even greater for B. marinus had Stuart not considered seven of his 79 individuals to be "anemics" and dropped them by statistical maneuvers from his analysis.

Though the acid hematin test may not be the ideal method for definitive studies of amphibian blood it does give some comparative values when applied to enough different species or individuals of the same species. From my investigations it seems that Pseudobranchius blood has a hemoglobin value in the expected range of the caudate amphibians and great differences between individuals are evident.

The mean hemoglobin value of Pseudobranchius was 6.8 gm/100 cc of blood. Each gram of hemoglobin carries 1.34 ml of oxygen at saturation (Kitchen and Pritchard, 1963) and therefore the oxygen capacity of Pseudobranchius hemoglobin could be expressed as 9.1 volumes per or 9.1 ml per 100 cc of blood.

In his book The Ecology and Life History of the Common Frog Savage (1962) states, "The respiratory arrangements in tadpoles underline what I believe to be points of fundamental importance in Anuran ecology - the great degree of specialized adaptation to the environment, which enables

the different species to colonize habitats in great variety, from tree holes to puddles, from lakes to cow-ponds, from mountain streams to dry land. The common feature of most of these habitats is that they confine the tadpoles within usually quite small limits. If the conditions in their pond turn out to be dangerous they cannot go in search of better ones, as animals that live on dry land or in the sea can do, but must possess reserve mechanisms that can be brought into play to deal with the often violent fluctuations in the habitats."

Similar conclusions are here proposed for the "respiratory arrangements" of Pseudobranchus. Its mechanisms for respiration, gills, skin, and lungs are utilized in different degrees when environmental changes occur. If the oxygen content of the water is lowered the gills of Pseudobranchus expand. If the carbon dioxide of the water is high at the same time that the oxygen tension is low the animal may respond by rising to the surface to gulp air. If the pond goes dry Pseudobranchus can estivate in the mud and survive presumably on skin and pulmonary respiration alone. There seems to be no special adaptations in the blood of Pseudobranchus that set it apart from other amphibians.

The lower oxygen and higher carbon dioxide of the water underneath the hyacinths due to the respiration of

the organisms in the water are accentuated by the shading effect of the hyacinths which decreases the photosynthetic activity of hydrocotyl and other underwater plants. This low oxygen and high carbon dioxide concentration increases the need for aerial respiration in Pseudobranchus. However, the hyacinths afford a protective cover for the surfacing forays of the animals thus making it safer for them than if they surfaced in open water. The shading effect of the hyacinths keeps the water in a temperature range in which the oxygen consumption rate of Pseudobranchus is relatively low and oxygen solubility of the water relatively high.

Pseudobranchus is well-adapted to the water hyacinth community of the prairie ponds. One important aspect of this adaptation is the possession of respiratory mechanisms which enable Pseudobranchus to survive severe fluctuations in the environment of its habitat.

SUMMARY

A study was made of the respiratory behavior and physiology of Pseudobranchius striatus axanthus in conjunction with an investigation of the effect of the water hyacinth Eichornia crassipes on the physical environment of the ponds in which Pseudobranchius lives.

A diurnal temperature differentiation in the ponds was measured in which the water under the hyacinth mats was up to 11°C cooler than the open water of the pond. The shading effect of the mats reduced the photosynthetic activity of the sub-hyacinth vegetation and thus the oxygen content was lower and the carbon dioxide content was higher in the water under the hyacinths than in the open water. The hyacinths also contributed organic debris which decreased the depth of the pond and perhaps added organic acids which lowered the pH of the water in addition to the higher carbon dioxide content.

The rate of oxygen consumption of Pseudobranchius was a linear function of temperature at 15°C to 25°C and showed a Q_{10} of 1.98 in that range. The rate of oxygen consumption of Pseudobranchius was lower than those rates known for other caudate amphibians of comparable size.

At 20°C the mean rate of oxygen consumption of five Pseudobranchus was $29.9 \text{ mm}^3/\text{gm/hr}$. This low rate is a reflection of the generally sluggish activity of the animal.

Pseudobranchus responded to high carbon dioxide and low oxygen tensions in water by periodically surfacing to take air into its buccopharyngeal cavity and lungs. At high oxygen tensions it could respire beneath the surface with constricted gills. At intermediate carbon dioxide and oxygen tensions Pseudobranchus respired aquatically with its gills expanded and rarely surfaced to gulp air. It was able to survive on lung respiration alone in oxygen-depleted water and individuals from which the gills had been removed could survive on cutaneous respiration alone in water of medium oxygen tensions.

The mean dimensions of 100 red blood cells of Pseudobranchus were 48.1×23.4 micra and the mean of 15 red blood cell counts was 86,000 cells per cubic millimeter of blood. These cell dimensions were considerably larger than those recorded from other caudate amphibians of similar body weights. Red blood cell size in Pseudobranchus was most nearly similar to the red blood cell size in Siren lacertina, a much larger animal than Pseudobranchus. The cell count was higher than those of many salamanders having cells of a smaller size than Pseudobranchus.

The mean hemoglobin value for eight adult Pseudobranchius was 6.8 grams per 100 cc of blood. The oxygen capacity of the blood was calculated to be 9.1 volumes per cent per 100 cc of blood. These values are in the ranges of most amphibians.

Pseudobranchius is adapted to meet its respiratory needs in the water hyacinth habitat by the utilization of its diverse respiratory mechanisms in different degrees as changes occur in the amounts of dissolved carbon dioxide and oxygen in its environment.

LITERATURE CITED

- Allee, W. C. and R. Oosting. 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zool.* 7:509-541.
- Angel, F. 1947. *Vie et moeurs des amphibians*. Paris: Payot-Paris. 317pp.
- Bishop, S. C. 1947. *Handbook of salamanders*. Ithaca: Comstock. xiv and 555pp.
- Brattstrom, B. H. 1963. A preliminary review of the thermal requirements of amphibians. *Ecology.* 44: 238-255.
- Brattstrom, B. H. and W. N. McFarland. 1955. Notes on the behavior of the salamander Taricha torosa, under the influence of carbon dioxide. *Behavior.* 7:317-320.
- Carr, A. F. 1940. A contribution to the herpetology of Florida. *Univ. of Fla. Biol. Sci. Series* 3:1-118.
- Chang, Y. T., J. M. Chen and T. Chen. 1943. Induced polycythemia in salamanders by cobalt, ascorbic acid, and other water-soluble vitamins. *Arch. Biochem.* 3:235-239.
- Claypole, E. J. 1896. Notes on the comparative histology of blood and muscle. *Proc. Amer. Micr. Soc.* 18:49-70.
- Cochran, D. M. 1961. *Living amphibians of the world*. Garden City: Doubleday. 199pp.
- Cochran, W. G. and G. M. Cox. 1957. *Experimental design*. New York: John Wiley and Sons. xiv and 611pp.
- Cohen, B. and A. H. Smith. 1919. The colorimetric determination of hemoglobin. A practical procedure. *J. Biol. Chem.* 39:489-496.

- Conant, R. 1958. A field guide to reptiles and amphibians. Boston: Houghton Mifflin Co. xv and 366pp.
- Czopek, J. 1962. Vascularization of respiratory surfaces in some caudata. *Copeia*. 1962:576-587.
- Dehadrai, P. V. 1962. Respiratory function of the swim-bladder of Notopterus (Lacepede). *Proc. Zool. Soc. London*. 139:341-357.
- Drastich, L. 1925. Veber das Leben der Salamandra-larven bei hohem und niedrigem Sauerstoffpartialdruck. *Z. vergl. Physiol.* 2:632-651.
- Etkin, W. 1934. The phenomena of anuran metamorphosis. II E. Oxygen consumption during normal metamorphosis. *Physiol. Zool.* VII:129-148.
- Evans, G. 1939. Factors influencing the oxygen consumption of several species of plethodontid salamanders in aerial and aquatic media. *Ecology* 20:74-95.
- Freeman, J. R. 1958. Burrowing in the salamanders Pseudobranchius striatus and Siren lacertina. *Herpetologica*. 14:130.
- _____. 1959. A record-size dwarf siren. *Herpetologica*. 15:16.
- Fuhrman, G. J. and F. A. Fuhrman. 1959. Oxygen consumption of animals and tissues as a function of temperature. *Jour. Gen. Physiol.* 42:715-721.
- Goin, C. J. 1941. The striped siren Pseudobranchius striatus. Masters thesis, Univ. of Florida.
- _____. 1942. A method for collecting the vertebrates associated with water hyacinths. *Copeia*. 1942:183-184.
- _____. 1943. The lower vertebrate fauna of the water hyacinth community of northern Florida. *Proc. Fla. Acad. Sci.* 6:143-153.
- _____. 1961. The growth and size of Siren lacertina. *Herpetologica*. 17:139.
- _____. and W. Auffenberg. 1955. The fossil salamanders of the family Sirenidae. *Bull. of Mus. of Comp. Zool.* 113 (7):497-514.

- Goin, C. J. and O. B. Goin. 1962. Introduction to herpetology. San Francisco: W. H. Freeman and Co. ix and 341pp.
- Harper, F. 1935. Records of amphibians in the southeastern states. Amer. Mid. Nat. 16 (3):275-310.
- Harris, J. P. 1953. Note on the blood of Necturus. Field and Laboratory. 21:147-148.
- Helff, O. M. 1927. The rate of oxygen consumption in five species of Amblystoma larvae. Jour. Exp. Zool. 49: 353-361.
- Hilton, W. A. 1952. The pulmonary respiratory system of salamanders. Herpetologica. 8:87-92.
- Hutchison, V. H. 1961. Critical thermal maxima in salamanders. Physiol. Zool. 34:92-115.
- Kitchen, H. and W. R. Pritchard. 1963. Physiology of blood. Department of Medicine. Univ. of Fla. Col. Med. 1963:109-115.
- Krogh, A. 1941. The comparative physiology of respiratory mechanisms. Philadelphia: University of Pennsylvania Press. 72pp.
- Luckhardt, A. B. and A. J. Carlson. 1920. Studies on the visceral sensory nervous system. II Lung automatism and lung reflexes in the salamanders (Necturus and axolotl). Amer. Jour. Physiol. 54:122-137.
- McCrady, E. 1954. A new species of Gyrinophilus (Plethodontidae) from Tennessee caves. Copeia. 1954:200-206.
- McCutcheon, F. H. and F. G. Hall. 1937. Hemoglobin in amphibia. Journ. Cell. Comp. Physiol. 9:191-197.
- Neill, W. T. 1951. A new subspecies of salamander, genus Pseudobranchius, from the Gulf Hammock region of Florida. Publi. Res. Div. Ross Allen's Rept. Inst. 1 (4):39-46.
- Noble, G. K. 1927. The value of life history data in the study of the evolution of the Amphibia. Ann. N. Y. Acad. Sci. 30:31-128.

- Noble, G. K. 1931. The biology of the Amphibia. New York: McGraw-Hill Book Co. XIII and 577pp.
- _____. and L. B. Richards. 1932. Experiments on the egg-laying of salamanders. Amer. Mus. Nov. 513:1-25.
- Oesting, R. B. 1934. A modified Van Slyke method for the determination of dissolved oxygen and total carbon dioxide in water. Physiol. Zool. 7:542-549.
- Prosser, C. L., D. W. Bishop, F. A. Brown, Jr., T. L. Jahn, and V. J. Wulff. 1950. Comparative animal physiology. Philadelphia: W. B. Saunders Co. ix and 888pp.
- Savage, R. M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. London. 122:467-514.
- _____. 1962. The ecology and life history of the common frog. New York: Hafner Publishing Co. vii and 221pp.
- Smith, H. M. 1925. Cell size and metabolic activity in amphibia. Biol. Bull. 48:347-378.
- Smith, Hobart. 1960. Evolution of chordate structure. New York: Holt Rinehart and Winston. xiv and 529pp.
- Stuart, L. C. 1951. The distributional implication of temperature tolerances and hemoglobin values in the toads, Bufo marinus and Bufo bocourti. Copeia. 1951: 220-229.
- Vernberg, F. J. 1952. The oxygen consumption of two species of salamanders at different seasons of the year. Physiol. Zool. 25:243-249.
- _____. 1955a. Correlation of physiological and behavior indexes of activity in the study of Plethodon cinereus (Green) and Plethodon glutinosus (Green). Amer. Mid. Nat. 54:382-393.
- _____. 1955b. Hematological studies on salamanders in relation to their ecology. Herpetologica. 11:129-133.
- Welch, P. S. 1948. Limnological methods. Philadelphia: The Blakiston Co. xviii and 381pp.

- Whitford, W. G. and V. H. Hutchison. 1963. Cutaneous and pulmonary gas exchange in the spotted salamander, Ambystoma maculatum. Biol. Bull. 124:344-354.
- Wilder, J. F. 1937. A correlation of results on oxygen consumption obtained by the Winkler method and by respirimeters, using the methods of Van Slyke. Physiol. Zool. 10:464-472.
- Willmer, E. N. 1934. Some observations of the respiration of certain tropical fresh-water fishes. J. Exper. Biol. 11:283-306.

BIOGRAPHICAL SKETCH

John Richardson Freeman was born August 24, 1927, at Murfreesboro, Tennessee. In May, 1945, he was graduated from Murfreesboro Central High School. From 1945 until 1946 he served in the United States Navy. He attended Middle Tennessee State College from 1946 until 1948 and in June, 1950, he received the degree of Bachelor of Arts from the University of North Carolina with a major in zoology. He taught in Spring City High School, Tennessee from 1950 until 1951. In June, 1953, he received the degree of Master of Arts from George Peabody College. From 1953 until 1955 he taught biology at Jacksonville Junior College. In 1955 he enrolled in the Graduate School of the University of Florida. He has worked as a graduate assistant in the Department of Biology and as a teaching assistant and half-time interim instructor in the Department of Biological Science. From September, 1959 until the present he has been an assistant professor in the biology department of the University of Chattanooga.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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