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A COMPARISON OF THE BLOOD OXYGEN CAPACITY IN SEMI-TERRESTRIAL AND AQUATIC FROGS

BY

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INTRODUCTION

Within the class Amphibia are found forms which exhibit varying degrees of transition from an aquatic to a terrestrial habitat. The oxygen carrying capacity of the blood is one of the factors which may influence these animals in their environmental distribution as well as it does their metabolic activity. It might be expected, therefore, that blood oxygen capacity values would be somewhat different in the various species of frogs. Considerable variation in blood oxygen capacity has been found in the other veterbrate groups(Prosser et. al. 1950; Hoar et. al. 1951; and Burke, 1953). It is also true that poikilothermic animals generally have lower blood oxygen capacities than homoiotherms.

Blood oxygen capacity may be measured quantitatively and is usually expressed in volumes per cent. In the present investigation this value is expressed as the number of milliliters of oxygen that combines with 100 ml. of blood when fully saturated with atmospheric oxygen and corrected to standard temperature and pressure.

Several reports regarding blood oxygen capacity in amphibians have been made previous to this investigation. Wolvekamp and Lodewijks (1934) found mean blood oxygen capacity values of 7.65 vol. % for nine aquatic frogs (Rana esculenta) and 12.05 vol. % for three semi-terrestrial ones (R. temporaria). McCutcheon (1936) reported a mean value of 10.21 vol. % for three adult bullfrogs (R. catesbeiana) and 7.80 vol. % for two tadpoles. The only other amphibian study revealed in available literature was by Scott (1931), who found that blood oxygen capacity in seven Congo eels (Amphiuma tridactyla) ranged from 2.5 to 8.4 with a mean value of 5.3 vol. %.

Blood oxygen capacities in other vertebrate groups are summarized by Prosser _et. al._ (1950). Mammalian studies are also summarized by Albritton (1952) and Burke (1953). Very recent studies have been made in white rats (Burke, 1957), fish (Burke and Woolcott, 1957), and turtles (Payne, 1957). These investigations were primarily concerned with the relation of blood oxygen capacity to body weight and to sex. Burke and Woolcott compared two species with approximately the same habitat, whereas Payne compared aquatic and terrestrial forms.

The present investigation is a comparison of the blood oxygen capacity in three species of frogs: a semi-terrestrial form, _R. pipiens_, the meadow frog; and two aquatic forms, _R. clamitans_, the green frog; and _R. catesbeiana_, the bullfrog. The habitats of these frogs are described by Wright and Wright (1949) as follows: _R. pipiens_ inhabits swampy marsh lands, upland backwaters, overflows, and ponds in the spring. In summer it is found in swamp lands, grassy woodlands, or in cultivated hay and grain fields. In winter it hibernates in pools or marshes. It has been found as far as 200 yards from any permanent body of water. _R. clamitans_ is strictly aquatic, living in large, deep ponds and resevoirs as well as in smaller ponds and pools. _R. catesbeiana_ is also strictly aquatic and inhabits millponds, hydraulic lakes, resevoirs, and kindred bodies of water.

The primary concern of this study was to compare blood oxygen capacity in the aforementioned three species of frogs in regard to the following parameters: body weight, habitat and sex. Two other parameters were studied as the investigation progressed: (1) hemoglobin concentrations were determined since the oxygen capacity of the blood is directly proportional to its hemoglobin content, and (2) erythrocyte counts were made to further substantiate blood oxygen capacity data.

METHODS AND MATERIALS

A total of 145 frogs, including 50 R. pipiens, 43 R. clamitans and 52 R. catesbeiana were used in this investigation. Specimens of R. clamitans and R. catesbeiana were collected in Chesterfield and Henrico Counties, Virginia from June until September, 1957. The frogs were captured by hand at night with the aid of a flashlight. R. clamitans were found along small creeks, drainage ditches and along the shoreline of small ponds. R. catesbeiana were found in several deep ponds, with the exception of several of the larger specimens which were obtained commercially from Minnesota.* All specimens of R. pipiens were obtained commercially from Wisconsin.**

In the laboratory the frogs were retained in deep sinks with a source of running tap water. Whenever possible, blood samples were taken within 48 hours after collection. Body weights were recorded to the nearest 0.1 of a gram, and standard length, (from tip of snout to cloacal aperture, as expressed by Wright and Wright, 1949) was recorded in millimeters for each frog.

Blood oxygen capacities were determined by the microgasometric syringe method described by Roughton and Scholander (1943) and modified by Grant (1947). Determinations were made on frogs as small as two grams, as only 39.3 cmm. of blood are required.

Blood samples were drawn directly into a heparinized pipette from an incision made in the ventricle, and transferred immediately to the syringe for oxygen analysis. Similar blood samples were taken from the same incision for erythrocyte counts and for hemoglobin concentration determinations. For

* J. R. Schettle Frog Farm, Stillwater, Minnesota
** The Lemberger Co., Oshkosh, Wisconsin

the latter procedure, the acid-hematin method was used employing the Fisher Electro-Hemometer (Kolmer and Boerner, 1945).

Erythrocyte counts were made in all of the specimens of R. pipiens and in several representative frogs of each of the other two species following the procedure as described by Kolmer and Boerner (1945). Each frog was sexed by gonadal examination (Noble, 1954).

Data were collected and analyzed statistically by the methods of Simpson and Roe (1939), and Arkin and Colton (1950). Tests of significance, employing the standard error of the difference between two means, were applied to the mean blood oxygen capacity values of the three species as well as to males and females within each species. Other statistical analyses were made on the relation of blood oxygen capacity to body weight, on hemoglobin concentrations, and on erythrocyte counts.

RESULTS

The relation of blood oxygen capacity in volumes per cent per gram of body weight to an increase in body weight is represented in Graphs 1, 2, and 3 for *R. pipiens*, *R. clamitans*, and *R. catesbeiana*, respectively. Other data pertinent to the investigation are presented in tabular form.

Data for 50 specimens of *R. pipiens*, 43 specimens of *R. clamitans*, and 52 specimens of *R. catesbeiana* are presented in Tables 1, 2, and 3, respectively. The data are arranged so that body weights are in increasing order. The frogs had a mean body weight of 33.1, 30.4, and 132.8 gm.; a mean standard length of 72.7, 68.9, and 98.1 mm.; a mean blood oxygen capacity of 10.3, 6.0, and 6.9 vol. %; and a mean hemoglobin concentration of 9.9, 6.5, and 6.5 gm. per 100 ml. of blood, respectively. Mean erythrocyte counts of 0.379×10^6, 0.320×10^6, and 0.280×10^6 per cmm. of blood were found for all specimens of *R. pipiens*, 15 specimens of *R. clamitans*, and 9 specimens of *R. catesbeiana*, respectively. The standard deviation and the standard error of the mean blood oxygen capacities were as follows: *R. pipiens*-1.68 and 0.16 vol. %; *R. clamitans*-1.95 and 0.32 vol. %; *R. catesbeiana*-2.31 and 0.34 vol. %.

In regard to the total sample as presented in Table 4, there was a significant difference between the mean blood oxygen capacities when one species was compared with another. When the mean blood oxygen capacity of *R. clamitans* was compared with that of *R. pipiens*, an arithmetic difference of 4.28 vol. % was found. When the mean blood oxygen capacities of *R. catesbeiana* and *R. pipiens* were compared, the arithmetic difference was 3.46 vol. %. These differences were significant as each is greater than three times the standard error of the difference between the means: ($3 \times 0.054 = 0.162$) and ($3 \times 0.052 = 0.156$). When *R. clamitans* was compared with *R. catesbeiana* a difference of only 0.82 vol. % was found between the mean blood oxygen capacities of these two aquatic forms. However, this difference was also found to be significant (3 x 0.067 = 0.201).

When treated statistically, it was found that the difference between mean blood oxygen capacities of males and females in R. clamitans and R. catesbeiana was significant as shown in Tables 3 and 4, respectively. Reference to Table 1 shows that this characteristic was not found in R. pipiens.

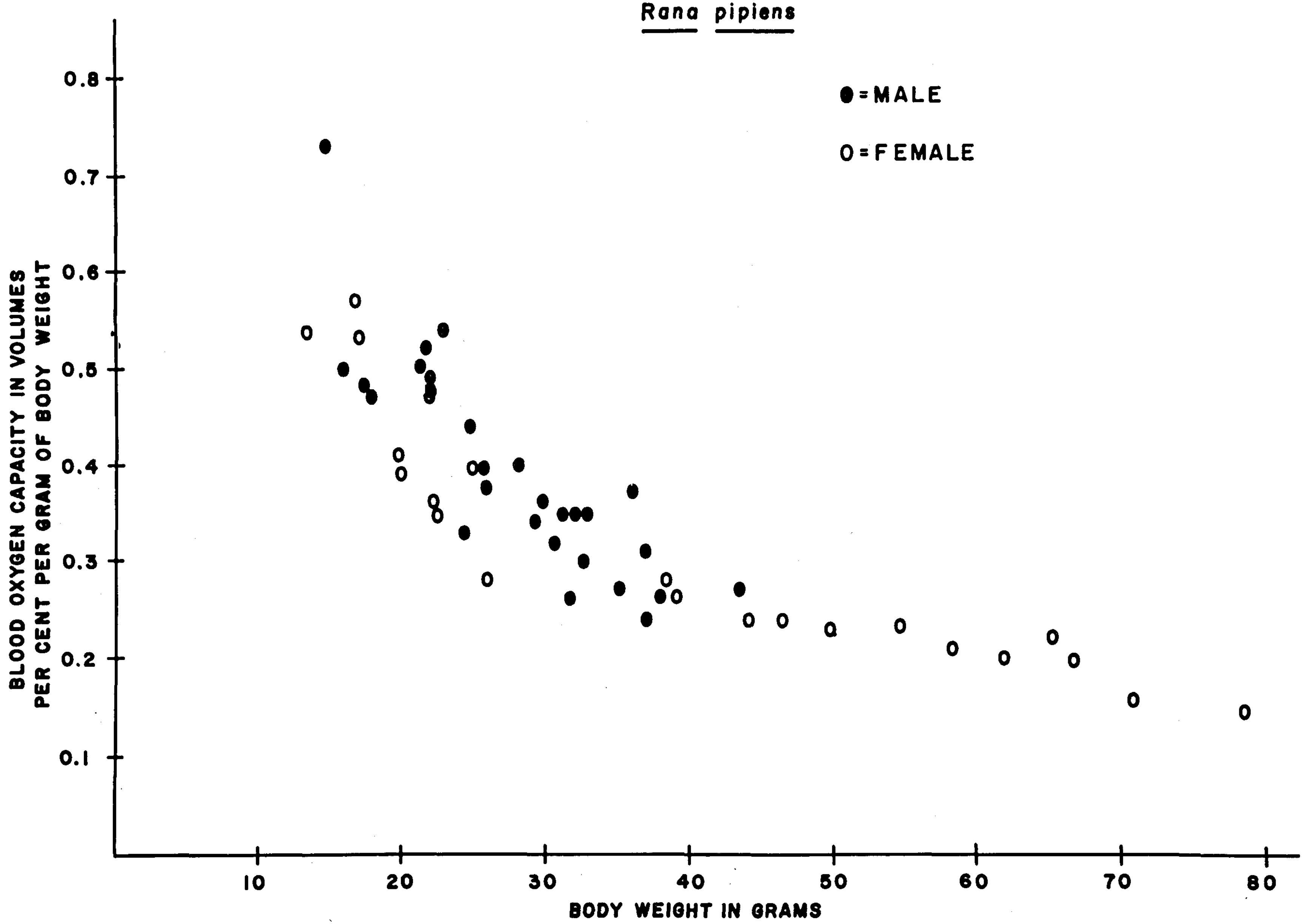
Table 5 presents blood oxygen capacity values from this investigation and from available literature for various amphibians.

GRAPH 1

The relation of blood oxygen capacity in volumes per cent per gram of body weight to an increase in body weight for *Rana pipiens*

GRAPH I

<u>Rana pipiens</u>

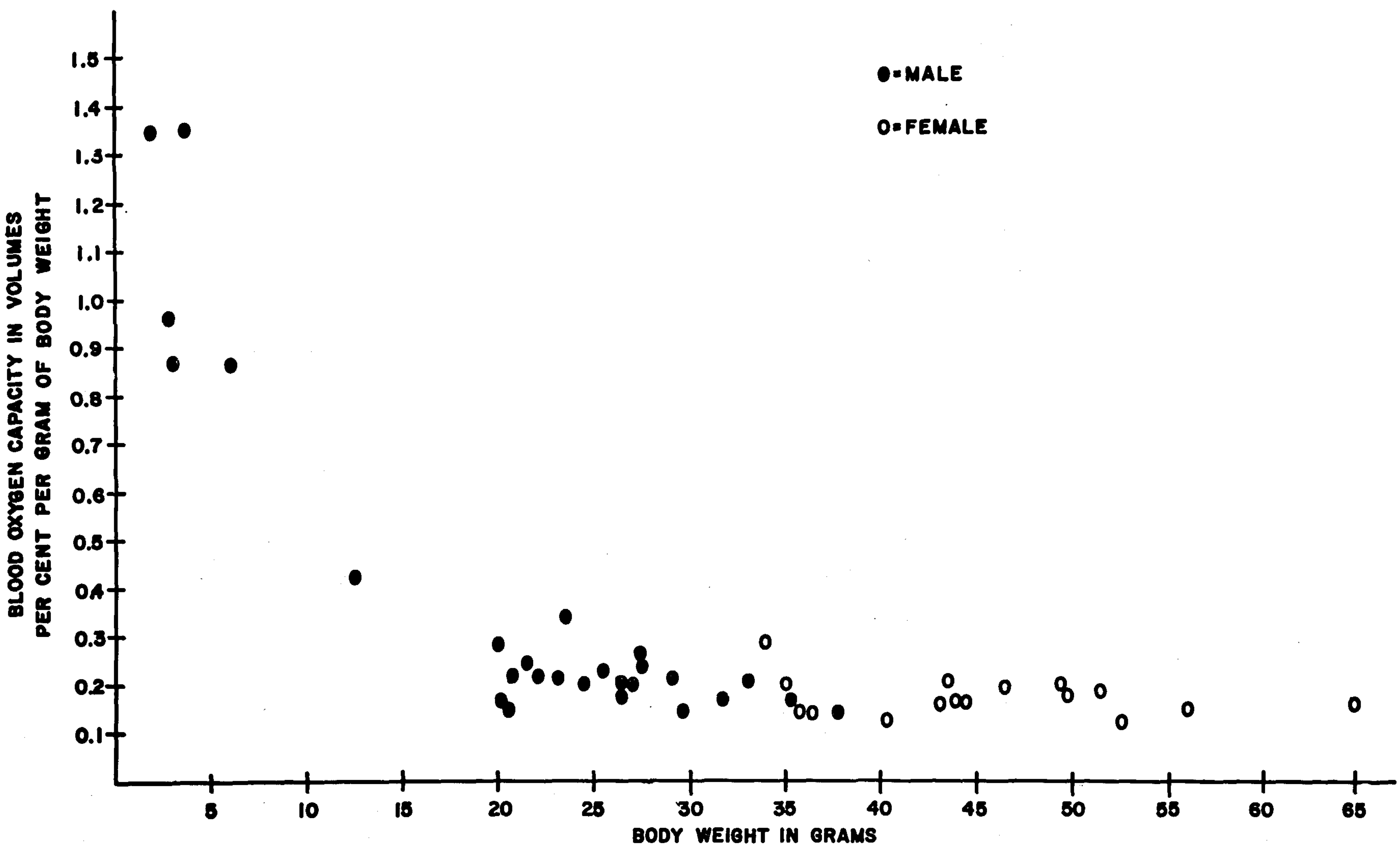


GRAPH 2

The relation of blood oxygen capacity in volumes per cent per gram of body weight to an increase in body weight for <u>Rana</u> <u>clamitans</u>

GRAPH 2

Rana clamitans

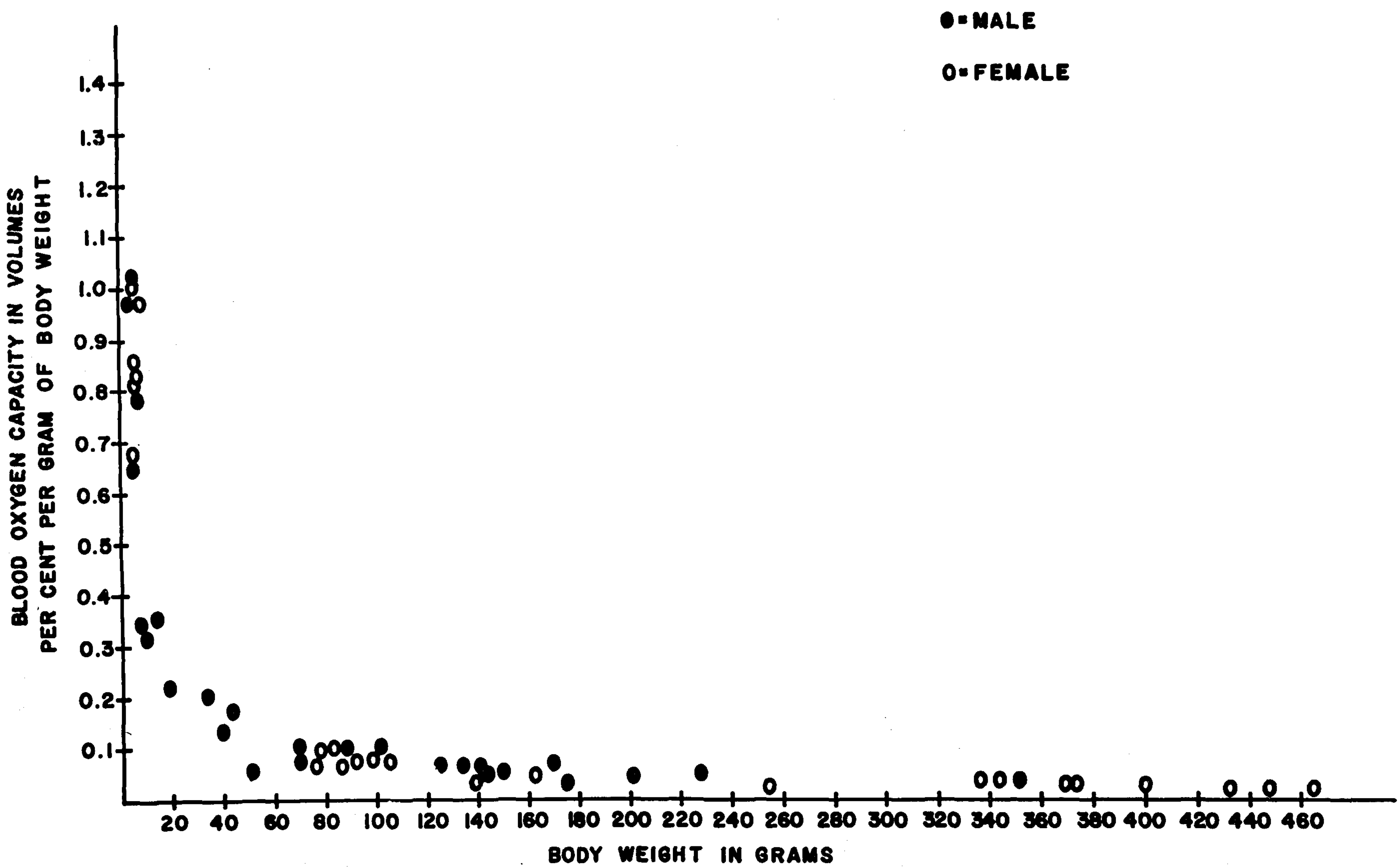


GRAPH 3

The relation of blood oxygen capacity in volumes per cent per gram of body weight to an increase in body weight for *Rana catesbeiana*

GRAPH 3

<u>Rana</u> <u>catesbeiana</u>



KEY TO ABBREVIATIONS

Table 1

Wt. in gm.	= Weight in grams
Lg. in mm.	= Length from tip of snout to cloacal aperture in millimeters
B.O.C. (Vol.%)	= Blood oxygen capacity in volumes per cent
Hb. in gm./ 100 ml. blood	= Hemoglobin concentration in grams per 100 ml. of blood
R.B.C. x 10^6	= Red blood cell count in million cells per cubic millimeter
Max.	= Maximum value in range
Min.	= Minimum value in range
S.D.	= Standard deviation of the mean blood oxygen capacity
S.E.m	= Standard error of the mean of the blood oxygen capacity
A.D.m	= Arithmetic difference of the mean blood oxygen capacity between males and females
S.E.d	= Standard error of the difference between the mean blood oxygen capacities of males and females

Table 1

Rana pipiens

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood	R.B.C. x 10^6
F	13.2	60	7.1	7.0	0.25
M	14.8	59	10.8	10.0	0.37
M	15.7	58	7.9	7.7	0.33
F	16.4	60	9.4	8.5	0.32
F	16.9	62	8.9	8.7	0.32
M	17.3	60	8.4	8.0	0.34
M	18.1	65	8.5	8.5	0.29
F	19.8	63	8.2	7.3	0.23
F	20.0	62	7.9	7.3	0.26
M	21.2	66	10.7	9.0	0.43
M	21.3	66	11.0	11.0	0.31
F	21.5	65	10.2	9.0	0.35
M	21.5	66	10.2	11.4	0.40
F	21.9	64	7.9	7.7	0.29
M	22.0	64	10.7	10.3	0.45
F	22.1	62	7.7	6.3	0.26
M	22.8	69	12.3	12.5	0.42
M	24.4	68	10.7	11.2	0.40
M	24.5	68	8.1	6.5	0.28
F	24.8	66	9.7	9.5	0.31
M	25.4	66	10.0	7.5	0.37
M	25.6	68	9.8	7.5	0.38
F	26.1	67	7.4	7.0	0.25
M	28.5	68	11.3	13.2	0.45
M	29.7	72	10.1	10.8	0.43
M	29.7	72	10.7	11.0	0.36
M	30.5	70	9.9	10.0	0.43
M	31.2	73	11.0	10.7	0.42
M	31.5	75	11.0	12.0	0.45
M	32.1	78	8.5	7.6	0.42
M	32.3	76	11.2	11.3	0.39
M	32.5	75	9.9	9.3	0.45
M	35.2	76	9.5	10.6	0.43
M	36.2	74	13.5	10.5	0.35
M	36.9	76	11.6	12.3	0.45
M	37.0	75	9.0	9.0	0.42
M	38.1	78	10.1	10.9	0.38
F	38.6	77	11.0	10.5	0.42
F	39.2	77	10.2	9.0	0.28
M	43.4	85	11.7	12.5	0.47
F	44.0	81	10.5	11.0	0.32
F	46.6	81	11.4	10.8	0.41

Table 1 (contimued)

Rana pipiens

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood	R.B.C. x 10^6
F	50.1	85	11.6	11.5	0.45
F	54.8	86	12.8	12.5	0.43
F	58.5	89	12.5	11.6	0.47
F	61.5	89	12.5	11.7	0.48
F	65.0	92	14.5	15.0	0.48
F	66.3	91	13.2	11.6	0.48
F	71.0	94	11.6	10.5	0.40
F	78.7	97	12.1	11.0	0.42
Female					
Mean	39.9	75.9	10.34	9.8	0.358
S.D.	-	-	2.10	-	-
S.E.m	-	-	0.20	-	-
Male					
Mean	27.8	70.2	10.29	10.1	0.395
S.D.	-	-	1.28	-	-
S.E.m	-	-	0.12	-	-
A.D.m	-	-	0.05	-	-
S.E.d	-	-	0.046	-	-
(S.E.d x 3)	-	-	0.138	-	-
Total Sample					
Mean	33.1	72.7	10.3	9.9	0.379
Max.	78.7	97	14.5	15.0	0.480
Min.	13.2	58	7.1	6.3	0.230
S.D.	-	-	1.68	1.94	0.075
S.E.m	-	-	0.16	-	-

KEY TO ABBREVIATIONS

Table 2

Wt. in gm.	= Weight in grams
Lg. in mm.	= Length from tip of snout to cloacal aperture in millimeters
B.O.C. (Vol.%)	= Blood oxygen capacity in volumes per cent
Hb. in gm./ 100 ml. blood	= Hemoglobin concentration in grams per 100 ml. of blood
Max.	= Maximum value in range
Min.	= Minimum value in range
S.D.	= Standard deviation of the mean blood oxygen capacity
S.E.m	= Standard error of the mean of the blood oxygen capacity
A.D.m	= Arithmetic difference of the mean blood oxygen capacity between males and females
S.E.d	= Standard error of the difference between the mean blood oxygen capacities of males and females

Table 2

Rana clamitans

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood
M	2.0	28	2.7	-
M	2.7	30	2.6	-
M	3.0	31	2.6	-
M	3.5	33	4.8	-
M	6.0	39	5.2	5.6
M	12.5	55	5.3	4.0
M	20.0	63	5.7	6.3
M	20.2	64	3.1	4.7
M	20.2	61	3.5	5.2
M	20.8	65	4.8	8.0
M	21.6	62	5.3	5.3
M	22.0	65	4.8	4.7
M	23.0	67	4.9	5.7
M	23.5	66	7.9	7.4
M	24.5	66	4.9	4.2
M	25.3	67	5.7	5.3
M	26.3	70	5.2	5.4
M	26.7	67	4.9	4.3
M	26.8	70	5.3	4.0
M	27.2	70	7.0	7.2
M	27.6	70	6.6	7.2
M	29.1	73	6.1	6.7
M	29.7	74	4.3	4.5
M	31.7	75	5.2	5.5
M	33.1	71	6.5	3.7
F	33.7	73	9.7	9.7
F	35.0	76	7.1	9.5
M	35.5	80	5.7	-
F	35.7	79	5.2	5.7
F	36.5	78	5.2	3.7
M	37.7	73	5.3	-
F	40.2	80	5.2	5.8
F	43.0	80	7.0	7.4
F	43.5	79	9.0	7.5
F	43.8	81	7.2	8.0
F	44.4	82	6.8	5.7
F	46.5	80	8.8	8.2
F	49.6	83	9.3	11.7
F	49.8	85	9.1	9.6
F	51.4	85	9.5	11.8
F	52.5	89	6.2	4.0

Table 2 (continued)

Rana clamitans

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood
F	56.1	88	8.2	7.7
F	65.5	92	10.6	10.5
Female				
Mean	45.4	81.9	7.77	7.9
S.D.	-	-	1.70	-
S.E.m	-	-	0.22	-
Male				
Mean	21.6	61.3	5.03	5.5
S.D.	-	-	1.27	-
S.E.m	-	-	0.25	-
A.D.m	-	-	2.74	-
S.E.d	-	-	0.067	-
(S.E.d x 3)	-	-	0.201	-
Total Sample				
Mean	30.4	68.9	6.0	6.5
Max.	65.5	92	10.6	11.8
Min.	2.0	28	2.6	3.7
S.D.	-	-	1.95	2.18
S.E.m	-	-	0.32	-

KEY TO ABBREVIATIONS

Table 3

Wt. in gm.	= Weight in grams
Lg. in mm.	= Length from tip of snout to cloacal aperture in millimeters
B.O.C. (Vol. %)	= Blood oxygen capacity in volumes per cent
Hb. in gm./ 100 ml. blood	= Hemoglobin concentration in grams per 100 ml. of blood
Max.	= Maximum value in range
Min.	= Minimum value in range
S.D.	= Standard deviation of the mean blood oxygen capacity
S.E.m	= Standard error of the mean of the blood oxygen capacity
A.D.m	= Arithmetic difference of the mean blood oxygen capacity between males and females
S.E.d	= Standard error of the difference between the mean blood oxygen capacities of males and females

Table 3

__Rana catesbeiana__

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood
M	4.0	36	3.9	-
F	4.1	34	4.0	3.5
M	4.2	38	5.2	5.2
F	5.0	38	5.3	3.5
F	6.0	38	4.0	4.0
F	6.0	40	4.8	4.6
F	6.0	41	4.9	2.8
F	6.2	41	5.3	3.7
M	6.7	42	5.3	4.5
M	6.9	41	4.4	5.7
F	7.3	40	4.9	7.2
M	8.2	45	2.6	5.0
M	8.3	40	2.8	-
M	13.0	53	4.6	5.3
M	19.8	62	4.3	5.3
M	34.4	79	7.4	5.3
M	40.0	80	5.2	6.0
M	43.5	76	7.9	-
M	53.5	86	2.7	4.7
M	70.0	93	6.9	7.9
M	71.6	98	5.2	7.3
F	76.0	97	5.6	7.7
F	79.2	95	7.2	6.0
F	83.0	98	8.0	5.5
M	86.0	94	5.2	5.4
F	86.2	97	6.3	4.6
F	91.0	96	7.3	6.3
F	96.5	105	8.1	6.3
M	100.0	103	9.0	8.3
F	104.5	115	7.8	7.0
M	126.0	111	8.1	7.9
M	133.7	116	8.7	7.0
F	138.9	114	4.9	6.0
M	139.0	119	9.0	7.2
M	140.0	116	8.0	6.5
M	142.9	128	6.1	7.0
M	149.5	130	7.0	6.1
F	163.4	126	8.9	7.3
M	170.0	119	11.2	8.0
M	176.5	133	5.3	5.5
M	200.0	124	9.0	6.5
M	228.0	126	10.8	7.6
F	254.3	136	7.4	7.6
F	338.5	152	10.8	9.8
M	344.4	159	10.9	11.5

Table 3 (continued)

Rana catesbeiana

Sex	Wt. in gm.	Lg. in mm.	B.O.C. (vol. %)	Hb. in gm./100 ml. blood
M	351.0	151	9.8	10.3
F	369.0	160	7.1	-
F	371.8	161	9.0	8.7
F	400.3	165	10.5	8.5
F	432.0	172	9.1	8.2
F	446.0	171	9.6	9.5
F	462.4	174	9.9	8.2
Female				
Mean	168.1	104.4	7.11	6.4
S.D.	-	-	2.05	-
S.E.m	-	-	0.29	-
Male				
Mean	102.5	92.8	6.66	6.7
S.D.	-	-	2.50	-
S.E.m	-	-	0.37	-
A.D.m	-	-	0.45	-
S.E.d	-	-	0.091	-
(S.E.d x 3)	-	-	0.273	-
Total Sample				
Mean	132.8	98.1	6.9	6.5
Max.	462.4	174	11.2	11.5
Min.	4.0	34	2.6	2.8
S.D.	-	-	2.31	1.94
S.E.m	-	-	0.34	-

KEY TO ABBREVIATIONS

Table 4

No.	= Number of specimens
Wt.m in gm.	= Mean weight in grams
B.O.C.m (Vol. %)	= Mean blood oxygen capacity in volumes per cent (Total Sample)
S.D.	= Standared deviation of the mean blood oxygen capacity
S.E.m	= Standard error of the mean of the blood oxygen capacity
A.D.m	= Arithmetic difference of the mean blood oxygen capacity between the two species compared
S.E.d	= Standard error of the difference between the mean blood oxygen capacities of the two species compared

Statistical Analysis of Data Showing Blood Oxygen Capacity Relations Between *Rana pipiens*, *Rana clamitans*, and *Rana catesbeiana* (Total Sample)

R. pipiens vs. *R. clamitans*

	No.	Wt.m in gm.	B.O.C.m (vol.%)	S.D.	S.E.m	S.E.d	A.D.m	(3 x S.E.d)
R. pipiens	50	33.1	10.3	1.68	0.16	0.054	4.28	0.162
R. clamitans	43	30.4	6.0	1.95	0.32			

R. pipiens vs. *R. catesbeiana*

	No.	Wt.m in gm.	B.O.C.m (vol.%)	S.D.	S.E.m	S.E.d	A.D.m	(3 x S.E.d)
R. pipiens	50	33.1	10.3	1.68	0.16	0.052	3.46	0.156
R. catesbeiana	52	132.8	6.9	2.31	0.34			

R. clamitans vs. *R. catesbeiana*

	No.	Wt.m in gm.	B.O.C.m (vol.%)	S.D.	S.E.m	S.E.d	A.D.m	(3 x S.E.d)
R. clamitans	43	30.4	6.0	1.95	0.32	0.067	0.82	0.201
R. catesbeiana	52	132.8	6.9	2.31	0.34			

KEY TO ABBREVIATIONS

Table 5

No.	=	Number of specimens
B.O.C.m (Vol.%)	=	Mean blood oxygen capacity in volumes per cent

Table 5

Blood Oxygen Capacity Values In Amphibia From The Present Investigation And From Available Literature

Species	No.	B.O.C.m (vol.%)	Method	Authority
Rana pipiens	50	10.3	Syringe	Leftwich
Rana clamitans	43	6.0	Syringe	Leftwich
Rana catesbeiana	52	6.9	Syringe	Leftwich
Rana catesbeiana	2(tadpoles) 3(adults)	7.80 10.21	Van Slyke Van Slyke	McCutcheon (1936)
Rana esculenta	9	7.65	Barcroft Microrespirometer	Wolvekamp & Lodewijks (1934)
Rana temporaria	3	12.05	Barcroft Microrespirometer	Wolvekamp & Lodewijks (1934)
Amphiuma tridactyla	7	5.3	Van Slyke	Scott (1931)

DISCUSSION OF RESULTS

Reference to Graphs 1, 2, and 3 shows that as the body weight of the frogs increases, the blood oxygen capacity per gram of body weight decreases. This relation was also found in mammals (Burke, 1957), in fish (Burke and Woolcott, 1957), and in turtles (Payne, 1957). Burke (1957) states that this characteristic in white rats, coupled with a decrease in blood volume per gram of body weight as body weight increases, may reflect a lowering of metabolism in larger rats. He based this assumption on a correlation of the metabolic study on shrews and mice by Pearson (1948) with blood volume studies on animals such as opossums and pigs (Burke, 1954). Burke and Woolcott (1957) suggest that this relation also exists in fish, since their study can be correlated with blood volume studies in fish by Martin (1950).

The present investigation indicates that this may very well be the situation in frogs also. Noble (1954) states that metabolism in frogs decreases with age and Conklin (1930) presents data which indicate that there is a tendency in frogs for blood volume per gram of body weight to decrease as body weight increases.

As shown in Tables 1, 2, and 3, there is a tendency for blood oxygen capacity to increase as body weight increases in each species. This characteristic has also been shown in white rats (Burke, 1957), in fish (Burke and Woolcott, 1957), and in turtles (Payne, 1957). There may be several factors responsible for this relation. First there is a tendency for the number of erythrocytes to increase as body weight increases. In relation to this, Burke (1957) presents a correlation of work of Reich and Dunning (1943) and Gardner (1947) which indicates an increase of more than 6,000,000 red blood cells per cmm. of blood from young to adult albino rats. Secondly, there is a tendency for hemoglobin concentration to increase as body weight increases. A third factor may be the oxygen affinity of the hemoglobin since Foreman (1954) has indicated a rough correlation between body weight and hemoglobin-oxygen affinity in different species of mammals. McCutcheon (1936) found in a study of R. catesbeiana, using three adult frogs, that the highest blood oxygen capacity value occurred in the medium-sized rather than the largest frog. He suggests that the maximum blood oxygen capacity occurs at the peak of sexual devlopment where the greatest demands on the respiratory pigment exist. It might be pointed out that the three blood oxygen capacity determinations by McCutcheon do not have population significance since Simpson and Roe (1939) state that at least seven animals are necessary for such a study. In 52 specimens of R. catesbeiana there was a tendency for blood oxygen capacity to increase as body weight increased.

Each individual blood oxygen capacity value shown in the tables was found to fall within its respective statistical range for a given population with the exception of values of 14.5 vol. % in R. pipiens and 10.6 vol. % in R. clamitans. Simpson and Roe (1939) state that this range is established when adding or subtracting two standard deviations to or from the mean of the population. The range will therefore include 95.5 per cent of the individuals. The limits of this range are as follows: R. pipiens (6.94 - 13.66 vol. %); R. clamitans (2.10 - 9.90 vol. %); R. catesbeiana (2.28 - 11.52 vol. %). Thus a valid sample of the populations was investigated since more than 95.5 per cent of the specimens fall within the given ranges.

As shown in the results, the semi-terrestrial R. pipiens had a mean blood oxygen capacity of 10.3 vol. % as compared with 6.0 and 6.9 vol. % in the aquatic R. clamitans and R. catesbeiana. There are several factors that may be responsible for the higher blood oxygen capacity in R. pipiens. First, R. pipiens had a mean hemoglobin concentration of 9.9 gm. per 100 ml. of blood as compared with 6.5 gm. found in both R. clamitans and R. catesbeiana. Secondly an erythrocyte count of 0.379×10^6 per cmm. of blood was found for R. pipiens as compared with 0.320×10^6 and 0.280×10^6 per cmm. of blood for R. clamitans and R. catesbeiana, respectively. This is in keeping with the blood study of amphibians by Heesen (1924), who found that aquatic forms tend to have a lower red blood cell count than terrestrial forms. The greater amount of hemoglobin coupled with the larger number of erythrocytes would allow an inherent mechanism to function so that R. pipiens could have a higher oxygen carrying capacity than the other two species.

Each of the individual hemoglobin and erythrocyte values included within the means discussed in the previous paragraph were found to fall within their respective statistical ranges with the exception of hemoglobin values of 15.0 in R. pipiens, 11.7 and 11.8 in R. clamitans, and 11.5 in R. catesbeiana, and one erythrocyte value of 0.42 in R. catesbeiana. The limits of these ranges are as follows: For hemoglobin concentration; R. pipiens (6.02 - 13.78 gm./100 ml. of blood); R. clamitans (2.14 - 10.86 gm./100 ml. of blood); R. catesbeiana (2.62 - 0.38 gm./100 ml. of blood)-For erythrocyte counts; R. pipiens ($0.229 \times 10^6 - 0.529 \times 10^6$ per cmm.), R. clamitans ($0.200 \times 10^6 - 0.440 \times 10^6$ per cmm.), R. catesbeiana ($0.180 \times 10^6 - 0.380 \times 10^6$ per cmm.).

The mean blood oxygen capacity of R. pipiens was 4.28 and 3.46 vol. % higher than the mean blood oxygen capacities of R. clamitans and R. catesbeiana, respectively. When these differences were treated statistically as shown in Table 4, it was found that each was greater than three standard errors of the difference between the means. Arkin and Colton (1950) state that such differences are significant and not due to chance. Both Baldwin (1949) and Redfield (1933) suggest that a terrestrial habitat is consistently associated with a higher blood oxygen capacity. Evidence is presented in the work of Wolvekamp and Lodewijks (1934), who found a mean blood oxygen capacity of 12.05 vol. % in three R. temprraria, the European grass frog, as compared with a mean blood oxygen capacity of 7.65 vol. % for nine R. esculenta, which are strictly aquatic frogs. It might be added however, that the mean value found in R. temporaria does not have population significance since only three frogs were used. Blood oxygen capacities in the study of Wolvekamp and Lodewijks were determined by the Barcroft microrespirometer. These findings may be correlated with the present investigation since Burke (1953) presents a literature survey which indicates that a difference of less than one volume per cent exists between the Barcroft and Van Slyke methods and between the Van Slyke and Roughton-Scholander methods.

It is suggested that the higher blood oxygen capacity in R. pipiens may be related to a higher metabolic activity associated with its terrestrial existance since Cronheim (1927) has shown in a metabolic study that the semi-terrestrial R. temporaria absorbed more oxygen than certain aquatic European frogs: R. esculenta, R. fusca, and R. mugiens. Also Helff (1927) showed that marked differences in oxygen consumption exist in several species of Ambystoma. Those species that consistently remained in an aquatic environment had a lower metabolism than those found more often on land. Also, as shown by McCutcheon and Hall (1937), that as amphibians move from an aquatic to a terrestrial habitat there is a marked shift to the right of the oxygen dissociation curve as the availability of oxygen becomes greater. Redfield, Coolidge, and Hurd (1926) point out that active species are likely to have hemoglobin which loads at high oxygen tensions, while sluggish species are likely to have hemoglobin which loads at low oxygen tensions. It is suggested, therefore, that R. pipiens is more active than R. clamitans and R. catesbeiana.

Noble (1931) states that R. pipiens has the widest disbribution of any American Rana. It would thus appear that the higher blood oxygen capacity in this frog, which would support a higher metabolism, is an important factor in the biological success of this species.

As shown in Table 4, an arithmetic difference of 0.42 vol. % exists between the mean blood oxygen capacities of R. clamitans and R. catesbeiana. Statistically, however, this difference was also found to be significant and not due to chance even though these frogs occupy approximately the same habitat.

No sexual dimorphism in regard to blood oxygen capacity was found in R. pipiens. This is in keeping with what has been found in other vertebrate groups: mammals, (Burke, 1957); fish, (Burke and Woolcott, 1957); and turtles, (Payne, 1957).

In R. clamitans and R. catesbeiana an apparent sexual dimorphism seemingly occurs in relation to their blood oxygen capacities. However, closer examination of the weight distributions of male and female frogs within these two species reveals an interesting difference. The mean weight of female R. clamitans exceeds the mean weight of males by 23.8 gm. Similarly, female R. catesbeiana outweigh males by 65.6 gm. Although the mean weight of females is only 12.1 gm. greater than the mean weight of male R. pipiens, this value was found to be significant with 95 per cent confidence, since a "t" value of 2.8 was found using the Student-t distribution test. However, for significant comparisons between males and females, it is necessary for mean weights to be approximately the same. Female R. clamitans and R. catesbeiana have also been found to have higher mean blood oxygen capacities than males as follows: R. clamitans - female (7.7 vol. %), male (5.0 vol. %); R. catesbeiana - female (7.1 vol. %), male (6.7 vol. %). It has already been shown that there is a tendency for blood oxygen capacity to increase as body weight increases.

It may be concluded, therefore, that while sexual dimorphism in regard to blood oxygen capacity does not exist in R. pipiens, no definite statement can be made as to the presence or absence of this feature in R. clamitans and R. catesbeiana because of the wider variation in body weights of male and female frogs. In the mammalian and fish studies referred to previously, this parameter was controlled by the use of male and female animals having approximately the same body weight.

It was found that a mean of 1.04. 0.98. and 1.07 cc. of oxygen combine with 1.0 gm. of hemoglobin in R. pipiens, R. clamitans, and R. catesbeiana, respectively. From these data a mean value of 1.03 was determined. With confirmation, this figure may be used as a constant in measuring blood oxygen capacity in Rana species when hemoglobin concentration is known. In humans, Bernhart and Skeggs (1943) report that 1.36 cc. of oxygen combine with 1.0 gm. of hemoglobin.

SUMMARY

1. The blood oxygen capacity of 145 frogs, including 50 *R. pipiens*, 43 *R. clamitans*, and 52 *R. catesbeiana*, was determined by the microgasometric syringe method.

2. Hemoglobin concentrations were determined by the acid-hematin method, employing the Fisher Electro-Hemometer.

3. Erythrocyte counts were made in all of the *R. pipiens*, in 15 *R. clamitans*, and in 9 *R. catesbeiana*.

4. *R. pipiens*, *R. clamitans*, and *R. catesbeiana* were found to have a mean body weight of 33.1, 30.4, and 132.8 gm.; a mean standard length of 72.7, 68.9, and 98.1 mm.; a mean blood oxygen capacity of 10.3, 6.0, and 6.9 vol. %; a mean hemoglobin concentration of 9.9, 6.5, and 6.5 gm. per 100 ml. of blood; and a mean erythrocyte count of 0.379×10^6, 0.320×10^6, and 0.280×10^6 per cmm. of blood, respectively.

5. The data indicate that as the body weight of the frogs increase, the blood oxygen capacity per gram of body weight decreases. This fact suggests a lowering of metabolism in larger frogs.

6. Results indicate that there is a tendency for blood oxygen capacity, hemoglobin concentration, as well as the number of erythrocytes to increase as body weight increases.

7. Statistically, it was shown that significant differences exist when the mean blood oxygen capacities of the strictly aquatic *R. clamitans* and *R. catesbeiana* were compared with the mean blood oxygen capacity of the semi-terrestrial *R. pipiens*. The higher blood oxygen capacity in *R. pipiens* may be an important factor in the biological success of this species.

8. A significant difference was also found between R. clamitans and R. catesbeiana in regard to their blood oxygen capacities.

9. Sexual dimorphism in regard to blood oxygen capacity was not found in R. pipiens. Wide differences in the body weight of male and female frogs in the other two species made the determination of this feature impossible.

10. In the Rana species studied, it was found that a mean of 1.03 cc. of oxygen combines with 1.0 gm. of hemoglobin.

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VITA

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