

AN ABSTRACT OF THE THESIS OF

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Title: THE RESPIRATORY ECOLOGY OF THE ROUGH-SKINNED
NEWT TARICHA GRANULOSA (SKILTON)

Abstract approved Signature redacted for privacy.
Dr. Robert M. Storm

An investigation was undertaken to determine if respiratory changes might occur in conjunction with migrations of the rough-skinned newt, Taricha granulosa (Skilton), in and out of ponds in the Willamette Valley. A field study was carried on from December 1964 through October 1965 to investigate certain physical and chemical parameters imposed on T. granulosa in relation to respiration. A laboratory study of Taricha in the summer and winter was conducted to uncover any changes in oxygen uptake by the various respiratory surfaces.

The investigations found that the animals had a higher respiratory rate in winter than in summer. When they left the ponds in late summer the percent of pulmonary respiration increased and the percent of cutaneous respiration decreased. When the animals again took up their aquatic habitat in the winter cutaneous respiration assumed a dominant role.

Oxygen uptake through the pulmonary system appeared to increase substantially with temperature in the range 10° to 25° C, while cutaneous oxygen uptake appeared to increase slightly in the same range. Carbon dioxide was released primarily by the skin in both summer and winter.

Temperature coefficients were generally lower than values predicted by van't Hoff's rule which may be an adaptation to Taricha's exposure to wide fluctuations in temperature.

The exponential value of b in the equation $M = aW^b$ indicated metabolism (M) increased by the $2/3$ power of weight (W). This relationship did not appear to change significantly from summer to winter, that is, from terrestrial phase to aquatic phase, even though the predominant route of oxygen uptake changed from lungs to skin.

The buccopharyngeal membrane appeared to have a respiratory function in T. granulosa. Animals maintained underwater with their lungs and skin eliminated from respiration were shown to consume measurable amounts of oxygen. The atmospheric respiratory potentials of the buccopharyngeal membrane and lungs were found to be of the same order of magnitude, except at 25° C.

The flexibility of shifting to alternate respiratory surfaces for oxygen uptake is believed to be a factor in the ability of Taricha granulosa to survive and to successfully exploit new habitats.

The Respiratory Ecology of the Rough-skinned Newt
Taricha granulosa (Skilton)

by

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THE RESPIRATORY ECOLOGY OF THE ROUGH-SKINNED
NEWT TARICHA GRANULOSA (SKILTON)

INTRODUCTION

In their evolution the amphibians were able to exploit the terrestrial environment by improvements in the respiratory ability of their aquatic ancestors. Some amphibia are completely independent of ponds and streams, but most of them still return to the aquatic environment to reproduce. Because of the necessity to accommodate both terrestrial and aquatic environments, amphibians may at one time or another utilize gills, lungs, and skin for respiratory gas exchange. In addition to these surfaces, the buccopharyngeal membrane is claimed to have a respiratory function by some authors (Whitford and Hutchison, 1965), while others ascribe a purely olfactory role to this membrane (Vos, 1926; Matthes, 1927; Elkan, 1955).

The rough-skinned newt, Taricha granulosa (Skilton), undergoes seasonal morphological changes as it migrates in and out of ponds. The possibility of metabolic changes that might accompany these migrations has not been investigated in this salamander. Although laboratory experiments under controlled conditions are desirable in understanding the metabolism of animals, these experiments are often left unrelated to the ecology of the animal. The biology of Taricha granulosa (Triturus=Taricha) has been studied by

Pimentel (1952), but this general study did not follow the respiratory activity of this species in depth. The present study was undertaken to elaborate the respiratory metabolism of adult Taricha granulosa, and to relate this metabolism to the animal in its environment.

A field study was carried on from December 1964 to October 1965 to determine the physical and chemical parameters imposed on Taricha granulosa in relation to respiration. This study preceded a laboratory investigation directed toward finding the roles of the various respiratory mechanisms used by T. granulosa. The limnological conditions of four artificial ponds located in the Willamette Valley were investigated. The ponds were located approximately ten miles north of Corvallis, Oregon near the west bank of Soap Creek (Figure 1). Although the ponds were built for fisheries research, Taricha granulosa has invaded them to varying degrees.

Respiration is a convenient metabolic process to measure, and may serve as an index to metabolic changes resulting from movement from terrestrial to aquatic environments and vice versa. A laboratory study of summer and winter Taricha was conducted to uncover any changes in oxygen uptake by the various respiratory surfaces. Of primary importance in this environmental shift are the relative roles of skin and pulmonary respiration. Krogh (1904) carried out the first quantitative study of pulmonary and cutaneous respiration in amphibians using Rana esculenta and R. fusca. Krogh

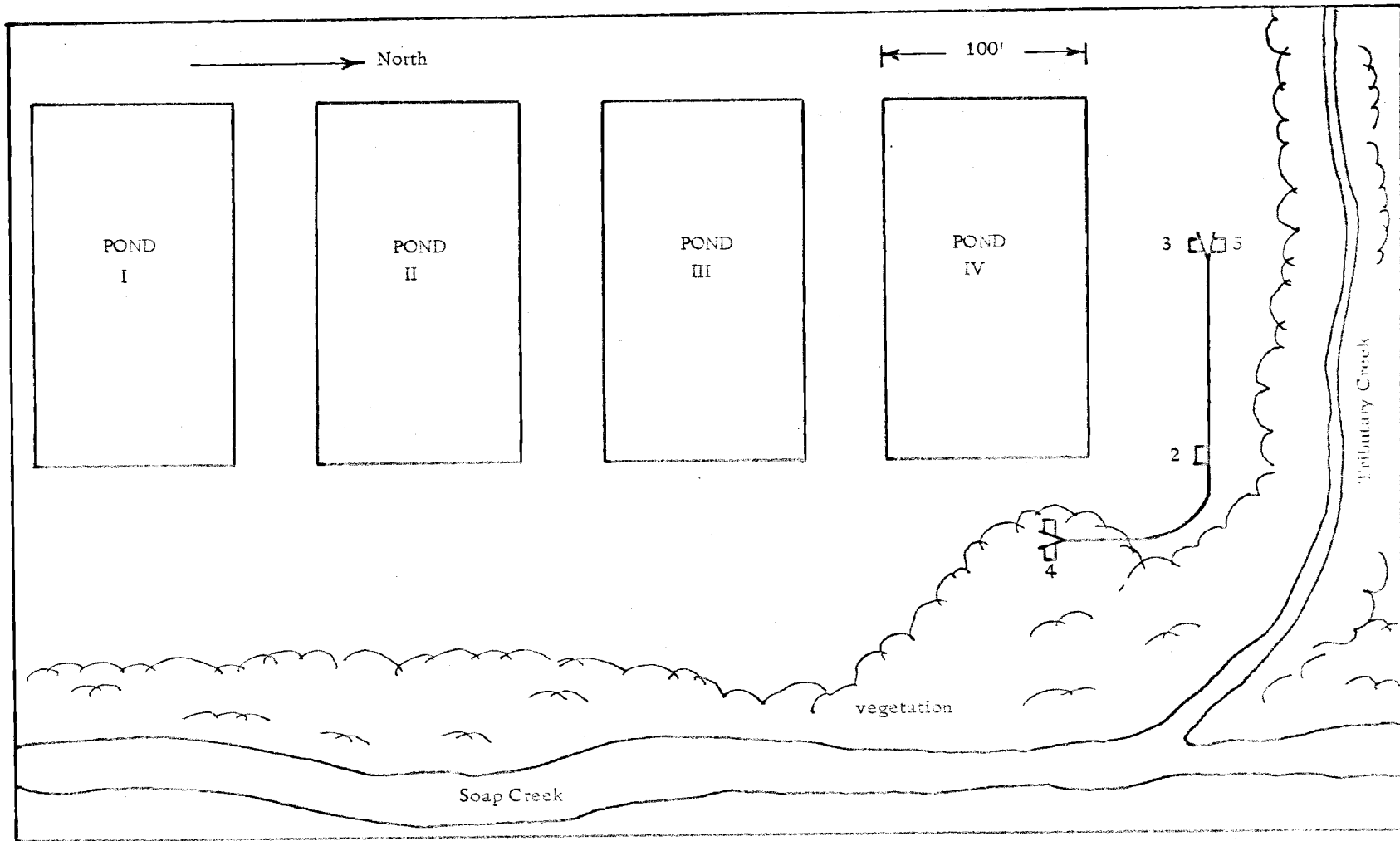


Figure 1. Map of Soap Creek Ponds study area.

found that carbon dioxide was released predominantly through the skin, while oxygen was taken up chiefly by the lungs. He also found that the oxygen uptake by the skin remained relatively constant throughout the year, while oxygen uptake by the lungs was greatest during the spring and dropped below cutaneous uptake in the fall and winter. Dolk and Postma (1927) using Rana temporaria substantiated Krogh's findings.

Lapicque and Petetin (1910) found that cutaneous respiration in the lungless salamander, Euproctus montanus, may be more important than lung and/or buccopharyngeal respiration. They were able to show that when an E. montanus was submerged in vaseline with its head free it quickly died, but could live when the buccopharyngeal respiration was eliminated by placing the head in vaseline. Their study did not solve the problem of the relative importance of cutaneous and buccopharyngeal respiration. The relative roles of cutaneous and pulmonary respiration in salamanders were not investigated thoroughly until the studies by Whitford and Hutchison (1963, 1965). In spite of their excellent quantitative results for cutaneous and pulmonary respiration, a technique to measure the relative oxygen uptake by the buccopharyngeal membrane and the lung was not developed. However, by indirect evidence they were able to propose a respiratory role for the buccopharyngeal membrane. The present study attempts to further the understanding of the lung and buccopharyngeal

respiration.

Oxygen uptake in animals increases as an exponential function of body weight. There is a considerable literature demonstrating the relationship of body weight to oxygen consumption. For comprehensive reviews the reader is directed to Brody (1945, Chapter 13), Kleiber (1947), Zeuthen (1953), and Scholander et al. (1953).

In an animal where a seasonal shift of oxygen uptake with respect to respiratory surfaces may occur, as in Taricha granulosa, the possibility of a shift in the exponential function appeared to need clarification. The question is, does a shift in the avenue of oxygen entry alter the basic relationship of weight to oxygen? The analysis of data in the present study was directed towards finding the relationship of metabolism and animal weight in Taricha granulosa.

METHODS AND MATERIALS

Field Methods

The four Soap Creek Ponds investigated in this study were approximately 100 feet in width, but varied in length from about 160 feet (Pond I) to 250 feet (Pond II). The maximum depths are located in the east end, and are approximately seven feet in all ponds when full. The ponds were excavated in 1958, and have been used continuously since then for farm pond fish production studies. The Soap Creek Ponds have been subjected to various enrichment programs since 1959 to evaluate the effects of artificial fertilizers on plankton and benthos organisms as well as standing crops of fish. Table 1 shows the 1965 program for the application of fertilizer.

Table 1. Monthly nutrient applications in Soap Creek Ponds, March through October, 1965.

Pond	Pounds of fertilizer applied			
	Urea	PO ₄	P ₂ O ₅	Steer Manure
1	25	38	---	---
2	40	---	60	---
3	30	42	---	142
4	31	47	---	---

Monthly population estimates were started on May 1, 1965, when Taricha were seined from Soap Creek Ponds II, III, and IV for marking. Pond I had too few animals to make a valid population estimate using marked animals. A 200 foot by 10 foot, one inch-stretch-mesh seine was used to capture salamanders in the ponds. The captured animals were marked by toe clip according to pond of capture and released to the same pond. Additional seining and markings were carried out at 30 day intervals until the ponds were drained in the autumn of 1965.

Monthly limnological data collected from the study ponds included water temperature (surface and bottom), dissolved oxygen (surface and bottom), pH, and water depth changes. Related meteorological data were also recorded.

The multiple recapture technique (Schnabel, 1938) was used to determine monthly changes in salamander population in the ponds.

Assumptions included in the Schnabel method are:

1. Animals retain their toe clips through the period of study.
2. Marked animals can be readily detected.
3. Marked animals will be randomly distributed.
4. Both marked and unmarked animals are equally likely to be captured.
5. Natural mortality in both marked and unmarked animals is the same.

6. The population is unaffected by:

a. immigration

b. emigration

The first five above listed assumptions were deemed reliable, but the sixth assumption could lead to error, since Taricha granulosa has been known to migrate. In order to evaluate this variable, the data for the marks at large (m) in Table 2 were corrected for marked animals emigrating out of the ponds. This was done by following the changes in the percent of marked animals in each sample from month to month.

A polyethylene fence 1.5 feet high and 200 feet long was erected between Pond IV and Soap Creek. The fence crossed the most likely route to and from the ponds, i. e., the shortest distance to the vegetative cover bordering nearby Soap Creek. The animals meeting the fence in their migration were forced to turn right or left along the fence. Eventually they dropped into one of the topless five-gallon cans buried in the earth along the fence (Numbers 1-5, Figure 1). The cans were so arranged that direction of movement could be determined. Captured animals were checked for toe clip marks, their sex determined, and then released on the opposite side of the fence where they were caught.

A variety of observations in the field were made to learn the respiratory behavior of Taricha granulosa. These included underwater

observations in the ponds with an aqualung, underwater movies of swimming animals, and careful observations of the animals surfacing for respiration.

Laboratory Methods

The laboratory study was divided into two parts, summer and winter. Accordingly, summer animals were captured during August and September 1965, and winter animals during December 1965. The summer animals were captured in traps on land in the vicinity of Soap Creek, and represent the terrestrial phase of Taricha respiration. The winter animals were captured in Pond IV, and represent the aquatic phase of Taricha respiration. Male animals were used exclusively in the metabolism studies to eliminate any sex differences.

The words respiration, metabolism, oxygen consumption and oxygen uptake will be defined as oxygen uptake per organism per hour. The terms rate of oxygen consumption, rate of oxygen uptake, respiratory rate, and metabolic rate will be defined as oxygen uptake per gram per hour.

The animals were acclimated a minimum of two weeks in constant temperature rooms before being subjected to experimental procedures. These procedures were grouped in two general categories: first, simultaneous measurement of atmospheric cutaneous

and pulmonary oxygen consumption at four temperatures (10°, 15°, 20°, 25°C); second, measurements aquatic respiration rate at the four temperatures listed above.

In order to simultaneously measure the cutaneous and pulmonary atmospheric respiration a four-chambered respirometer was constructed similar to one developed by Whitford and Hutchison (1963). In this apparatus cutaneous and pulmonary respirations were measured simultaneously and separately in the two front chambers, while the two rear chambers served as thermobarometers (Figure 2). A salamander being studied would be placed in chamber A, with his head held in a hole between chambers A and C by a mask made from 0.5 inch Tygon tubing (Figure 3). Thus, cutaneous respiration was measured by a manometer between chambers A and B, and pulmonary respiration by a manometer between chambers C and D.

A Tygon tubing mask, constructed to avoid interfering with buccal movements, was sutured to the head of an animal at least 24 hours prior to use. Animals being tested were securely held in place on a hardware cloth platform by two pieces of rubber tubing.

The respirometer, which was constructed of 0.25-inch acrylic plastic, was fitted with a plastic lid containing a series of stopcocks opening into each chamber. Both the cutaneous and pulmonary chambers were fitted with syringes filled with oxygen. The oxygen was injected into the chambers to compensate for oxygen consumed

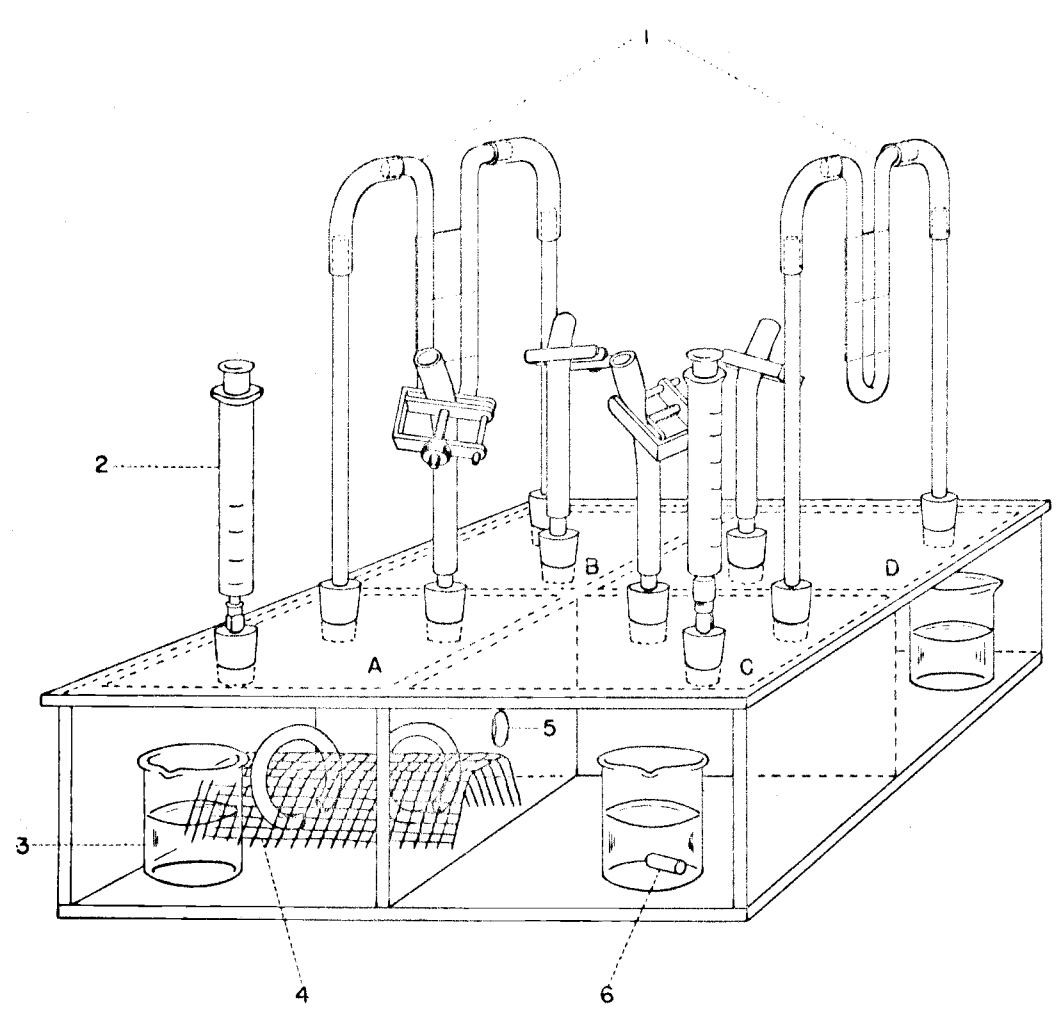


Figure 2. Double Scholander respirometer for simultaneous measurement of cutaneous and pulmonary respiration in salamanders. 1, manometers; 2, oxygen syringe; 3, barium hydroxide beakers; 4, wire platform for animals; 5, hole for animal mask; 6, bar magnet. A and C are respiratory chambers; B and D are thermobarometers.

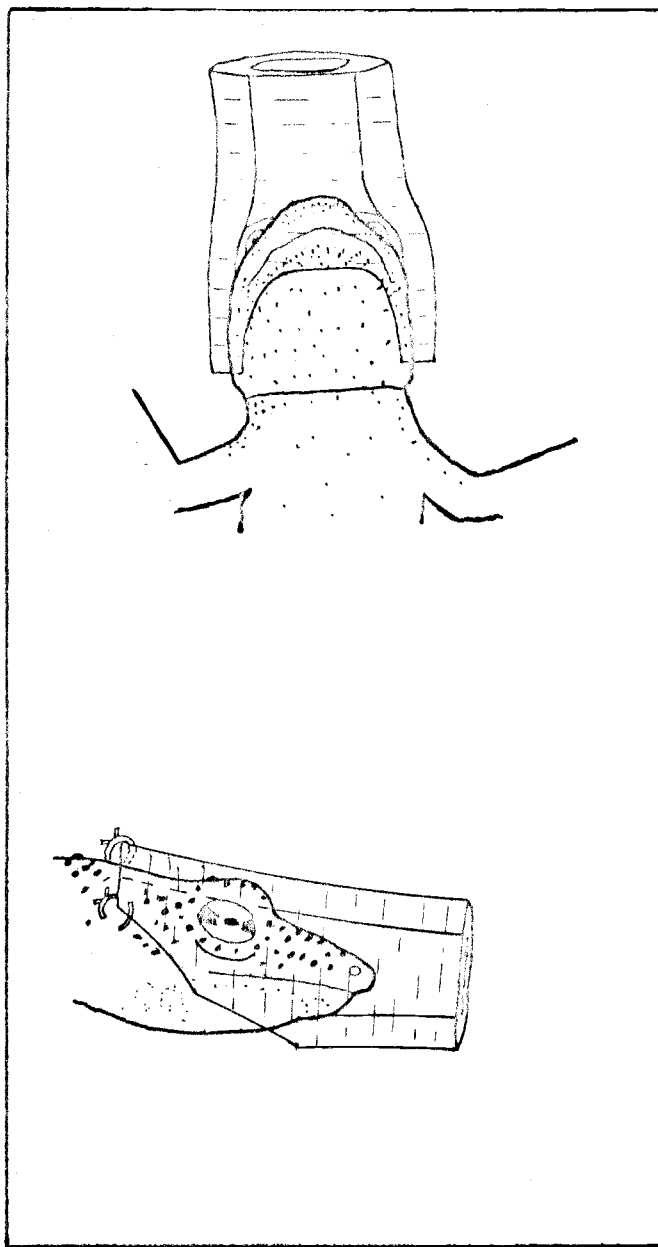


Figure 3. Plastic masks used in respirometer studies.

by the animal.

With an animal in place on the hardware cloth platform, the masked head of the animal was fitted into a hole between chambers A and C. Beakers containing 10 ml of barium hydroxide were placed in each chamber to absorb carbon dioxide. Plastic-coated magnetic bars in the barium hydroxide stirred the solution to insure effective absorption of carbon dioxide by breaking the barium carbonate film which formed on the surface.

When the lid was placed on the respirometer and sealed with vaseline, the whole apparatus was submerged underwater maintained at the experimental temperature. After the animal became accustomed to the apparatus, the syringes were filled with oxygen, and the stopcocks were closed. The standard metabolism was measured for a period of five hours. Oxygen consumption was read directly from the graduated syringes.

At the end of a set of experiments, the four beakers of barium hydroxide were removed from the chambers and titrated with sulfuric acid to determine the carbon dioxide produced. The beakers of barium hydroxide in the thermobarometers (chambers B and D) served as controls, since each beaker absorbed carbon dioxide at a similar rate prior to an experimental run, during the experiment, and during the time required for titration. To determine the actual amount of carbon dioxide released by an animal, the amount of carbon

dioxide absorbed in the thermobarometers was subtracted from the amounts of carbon dioxide absorbed in the cutaneous and pulmonary chambers.

Tidal volume was measured by connecting an animal's mask to a graduated manometer. Later the volume of air (measured by a microliter syringe) required to move the manometer column a distance equal to that moved by the breathing animal was taken as the tidal volume.

In order to test the oxygen consumed by Taricha granulosa underwater, the Winkler method for determining dissolved oxygen was used. The experimental animals were placed in a 500 ml flask filled with water in which an aereator was diffusing bubbles of air. The animals were kept in the flasks for 30 minutes before the flask was corked and the actual experiment begun. In addition to five flasks containing animals, two additional flasks containing water only were maintained as controls. Just before an experimental series, the dissolved oxygen (D. O.) in one flask was determined for later reference. The animals were maintained at an experimental temperature by the constant room temperature apparatus. The number of buccopharyngeal pumps per minute were periodically recorded.

At the end of 30 minutes the corks were removed, the animals released, and the water tested for residual dissolved oxygen. The D. O. in the remaining control flask was taken as the oxygen removal

by organisms in the water other than T. granulosa. Using this correction, and by subtracting the ending D. O. from the beginning D. O. in each of the five flasks, the amount of D. O. used by each of the Taricha could be determined.

The role of the mouth membrane in respiration was tested in another series of experiments. The cutaneous respiration was eliminated by placing a masked T. granulosa in a test tube filled with vaseline, but which permitted the head to extend out of the test tube. Since this would allow both lung and mouth respiration, the animal (in its test tube) was suspended underwater, thereby eliminating the lung respiration. The water was held at 20° C and was saturated with dissolved oxygen by an aereator. A magnetic stirrer circulated the water so that the animal had constant access to the dissolved oxygen. A check was made on the traumatic effect of the test tube and underwater immersion by placing a masked animal in a tube open at the caudal end, thus permitting water to pass along the body. Another check was made to determine how long T. granulosa could survive being completely immersed in a test tube full of vaseline.

RESULTS

Population Estimates

The population estimates for Ponds II, III, and IV show that Pond IV had more T. granulosa than any of the other ponds (Table 2). Examination of Pond I by means of seine and aqualung swims indicated the population there never exceeded 100 animals (Figure 4). When Pond I was drained on October 11, 1965, only ten T. granulosa were observed. In Pond II the population estimates, based on the Schnabel Method, indicate an increase in numbers from 517 on June 29 to 640 on August 2, 1965 (Figure 5). This increase, although modest, may indicate salamanders were still entering the pond from the surrounding land, or possibly from other nearby ponds further removed towards the west. The population decreased to 455 in early September, and 234 animals remained when the pond was drained completely on December 8, 1965.

The peak population in Pond III was estimated to be 1224 in late May, then decreased steadily to 711 in early September (Figure 6). Only 180 animals were counted when Pond III was drained on November 22, 1965.

The May 29 estimate of 3911 T. granulosa in Pond IV was the highest number recorded in any pond (Figure 7). The estimate decreased to 2153 on July 1, rose to 2465 on August 2, and decreased

Table 2. Population estimates of Soap Creek Ponds based on the Schnabel Method.

Date	Unmarked Captures u	Marked Recaptures r	Marked and Released	Marks at Large	Corrected Marks at Large m _l	m _l u	$P = \frac{m_l u}{r}$ P	P+ Removals Total Estimate
Pond II								
5/ 1/65			119					
5/29/65	287	66	101	119	119	34,153	517	517
6/29/65	299	110	41	220	208	62,192	565	565
8/ 2/65	315	128	0	261	244	76,860	600	640
9/ 4/65	155	60	0	261	176	27,280	455	455
Pond III								
5/ 1/65			143					
5/29/65	522	61	103	143	143	74,646	1,224	1,224
6/29/65	339	84	54	246	225	76,275	908	933
8/ 2/65	560	176	0	300	274	153,440	872	872
9/ 4/65	306	74	0	300	172	52,632	711	711
Pond IV								
5/ 1/65			110					
5/29/65	567	18	121	110	110	62,370	3,465	3,911
7/ 1/65	367	45	0	279	264	96,888	2,153	2,153
8/ 2/65	607	65	0	279	264	160,248	2,465	2,465
9/ 4/65	346	40	0	279	239	82,694	2,067	2,067

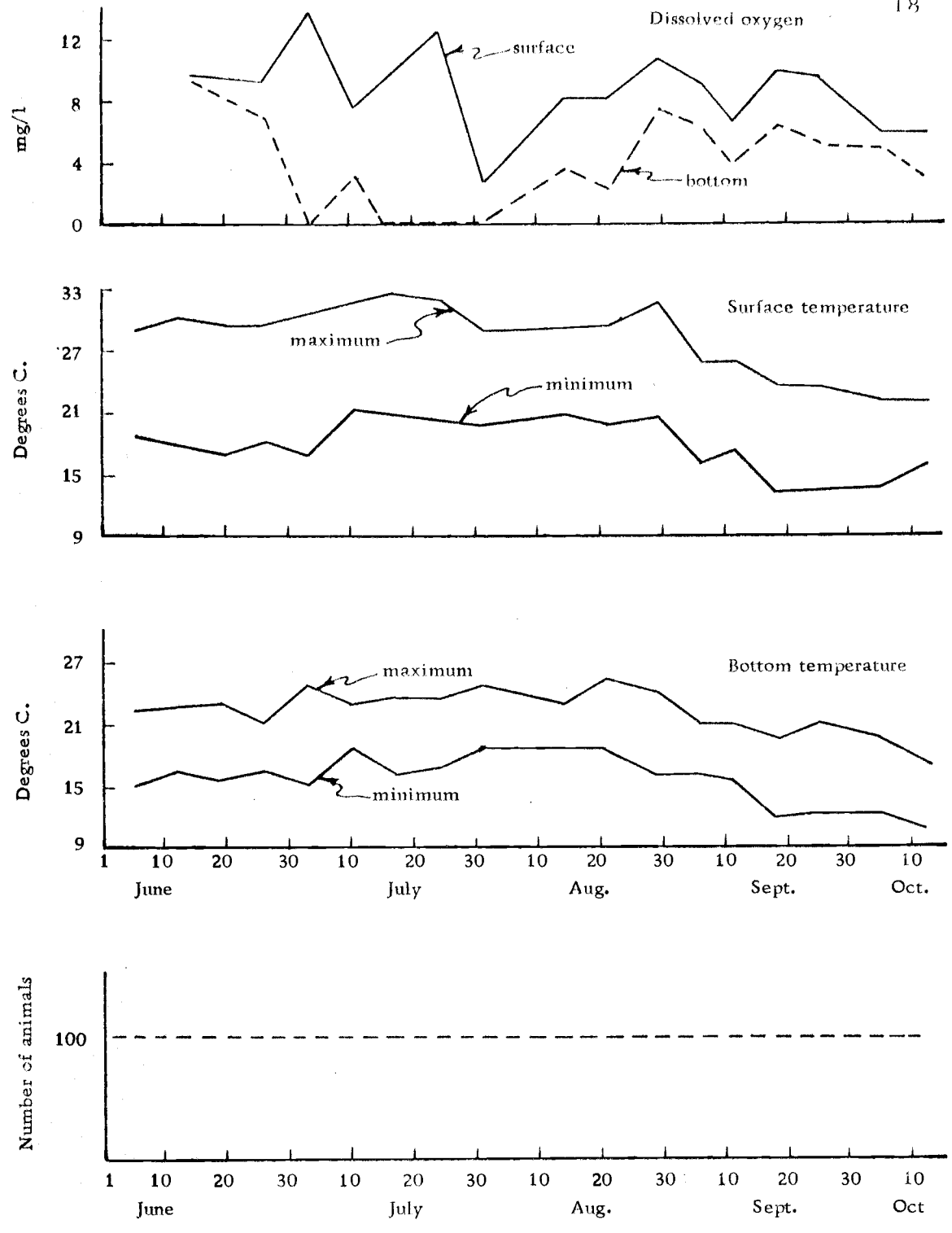


Figure 4. Population estimate, pond temperatures and dissolved oxygen in Pond I, June through October 1965.

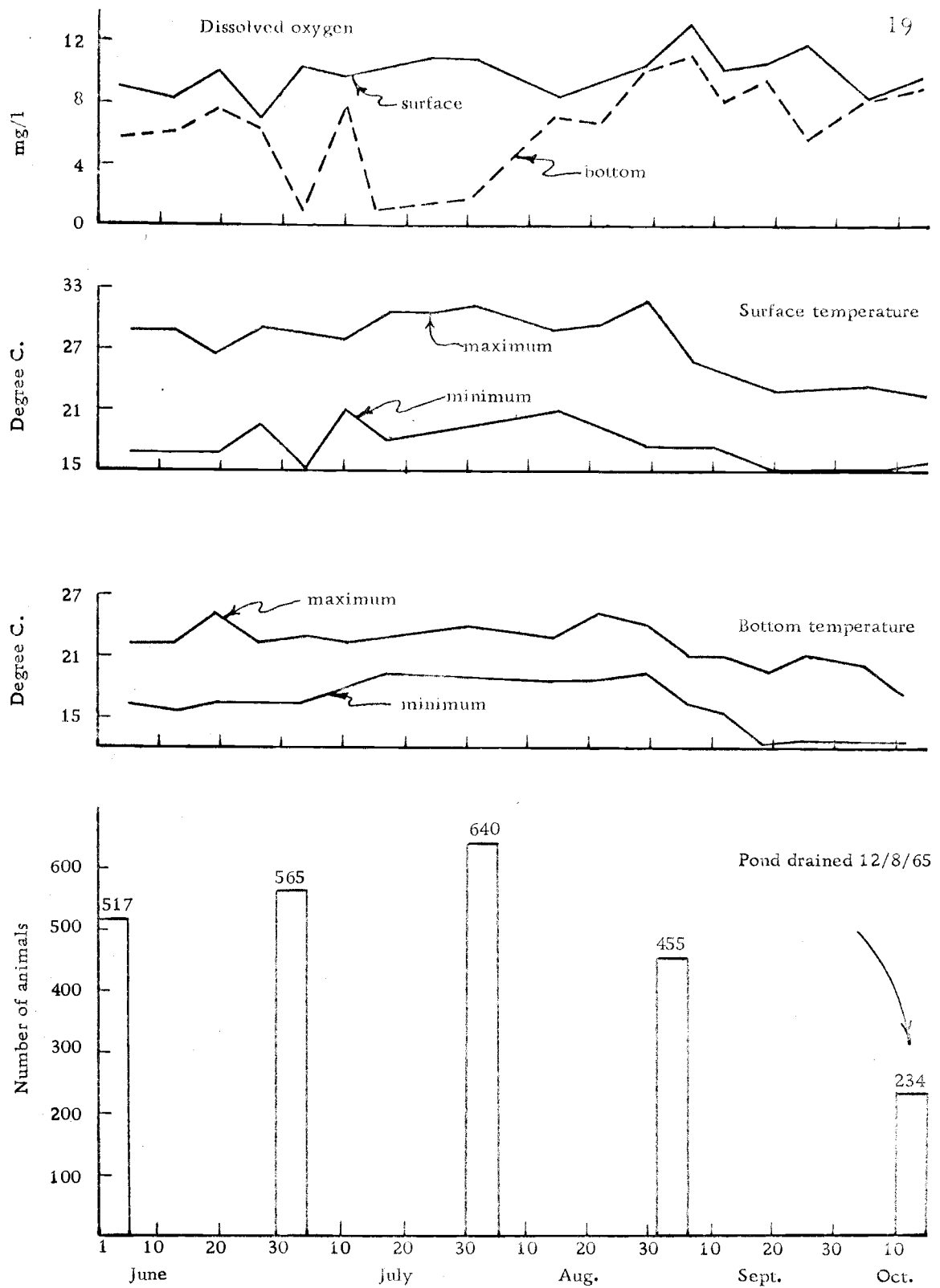


Figure 5. Population estimates, pond temperatures and dissolved oxygen in Pond II, June through October 1965.

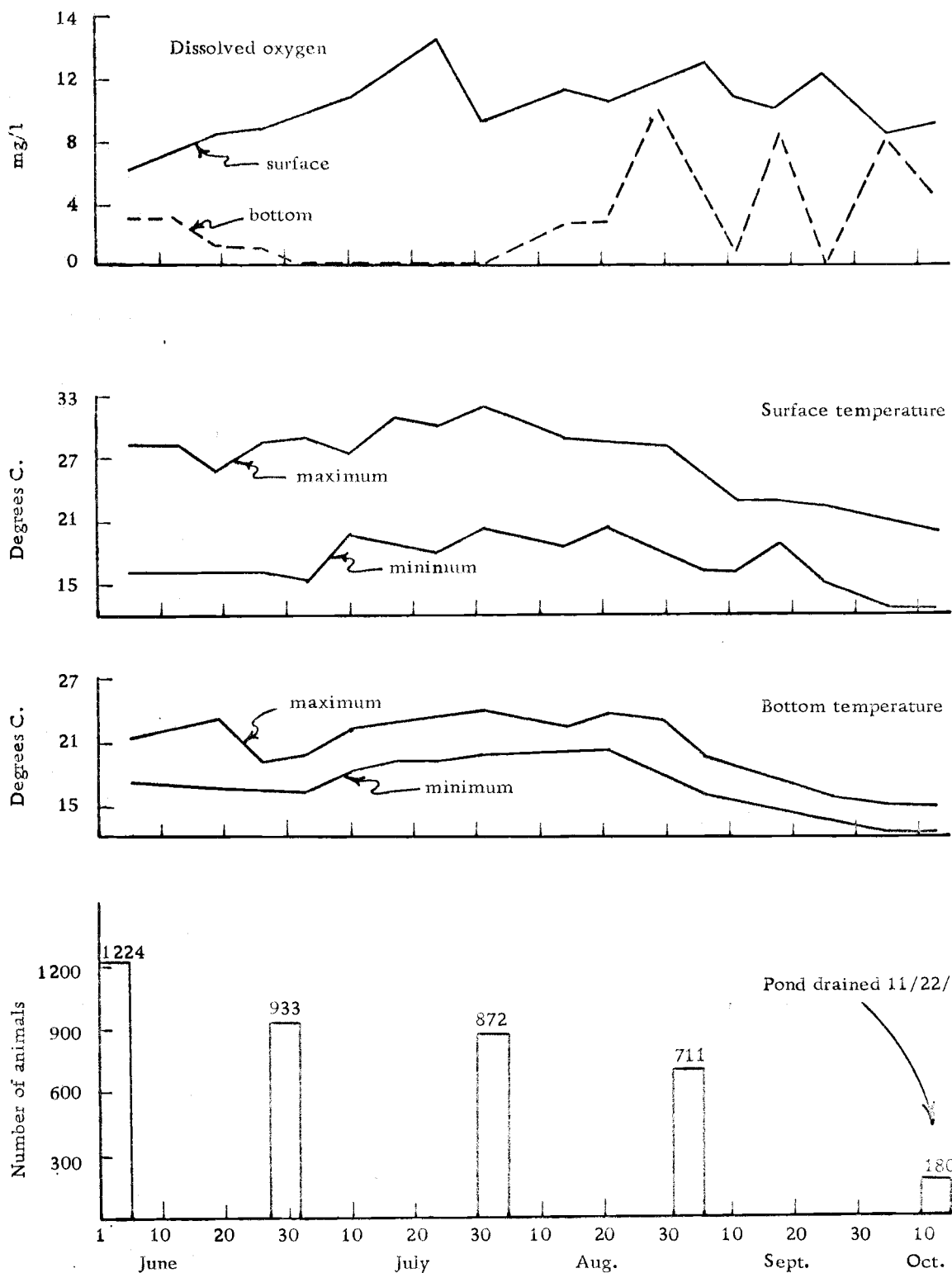


Figure 6. Population estimates, pond temperatures and dissolved oxygen in Pond III, June through October 1965.

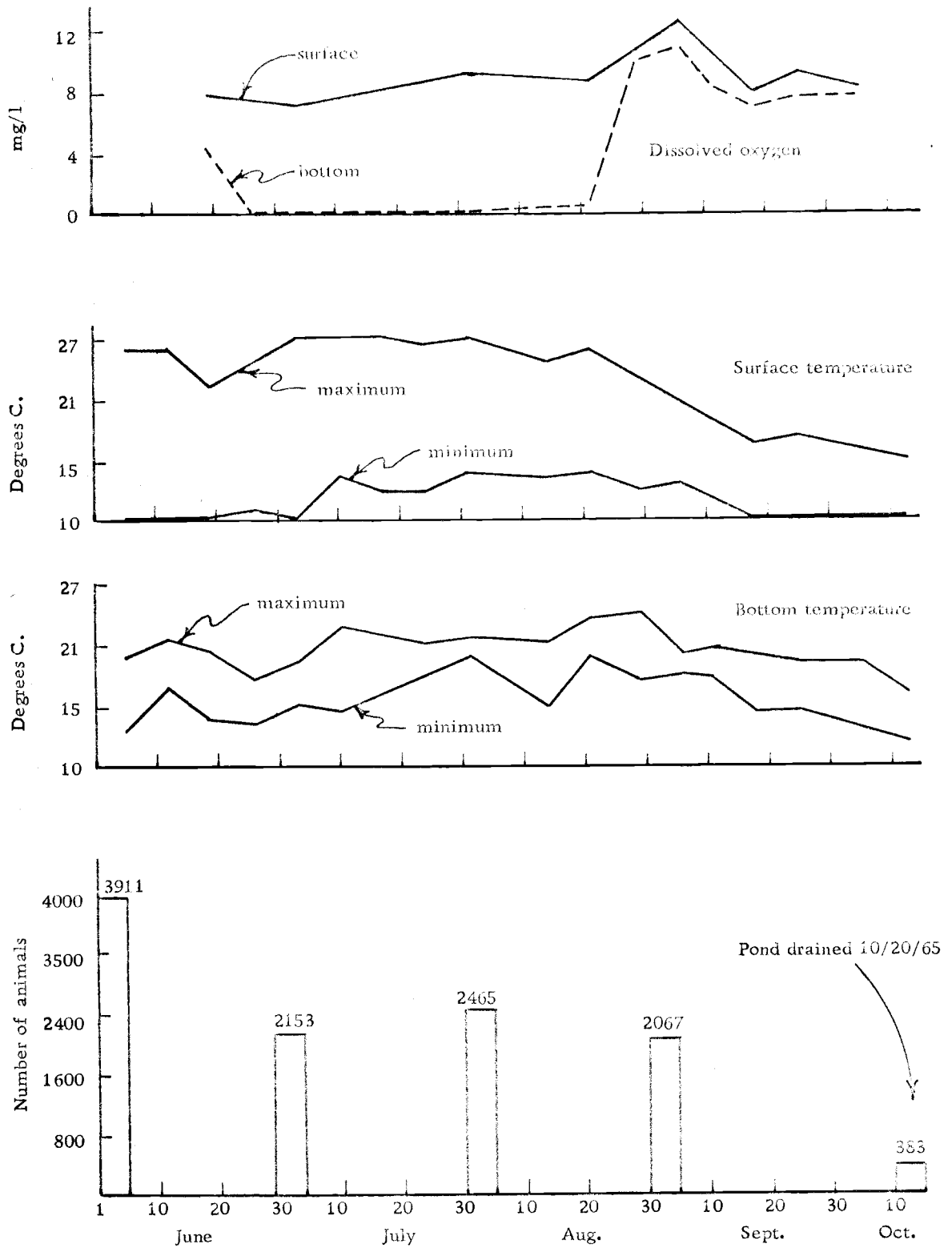


Figure 7. Population estimates, pond temperatures and dissolved oxygen in Pond IV, June through October 1965.

again to 2067 on September 4, 1965. When the pond was drained on October 20, 1965, only 383 animals were present.

Intercept Trapping

Animals were first caught in the traps between Pond IV and Soap Creek on July 7, 1965 (Figure 8). No measurable precipitation fell in the area from June 15 until a trace of rain fell on July 11, 1965. The urge to leave may have been so strong in a few individuals that they left Pond IV during the nights of July 6th and 8th. The first measurable rainfall following the dry period was on July 20 and 21, and this coincided with an increased number of animals in the traps. The next traces of rainfall on August 2nd and 3rd coincided with traps captures of 5 and 51 animals respectively. Thereafter, increased numbers of Taricha were captured as cooler weather and occasional rains occurred beginning on August 13, 1965 (Figure 9).

Rain began falling at 2030 hours on the night of August 18, and continued through most of August 19, 1965. At 0830 hours on August 19, 104 animals were in the traps. Between 0830 and 1230, 30 animals were caught, and between 1230 and 2030 hours 25 animals were caught. Thus it appears T. granulosa show a preference for nocturnal migration, but they will travel during the daylight if the vegetation is wet. A large male T. granulosa was discovered in the woods east of Pond III during the morning of August 19, 1965. The animal was

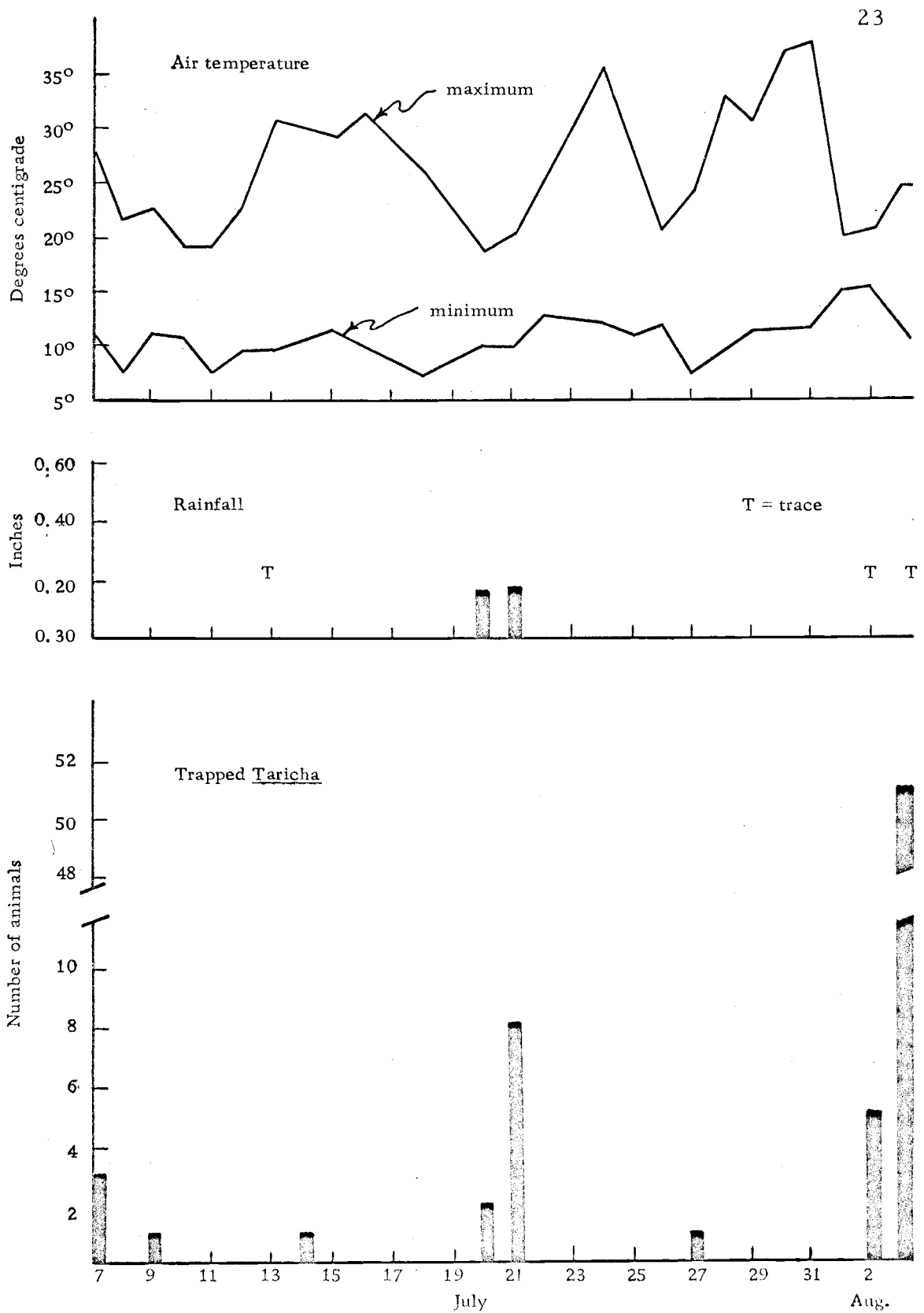


Figure 8. Trapped T. granulosa with associated rainfall and air temperatures, July 7 through August 3, 1965.

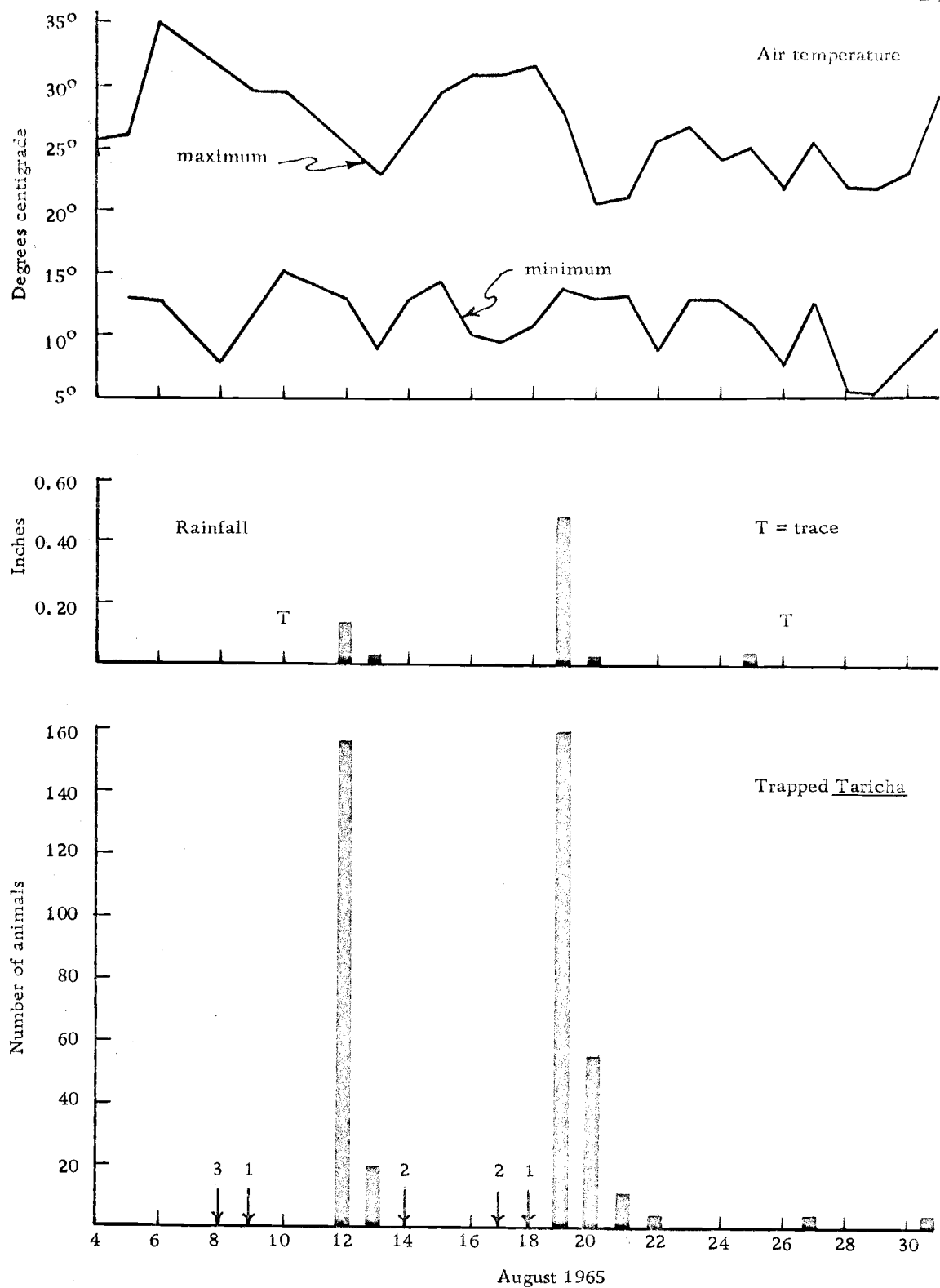


Figure 9. Trapped T. granulosa with associated rainfall and air temperatures, August 4 through 31, 1965.

about 40 feet from the creek moving east at about five feet-per-minute. Entering the creek, he paused in the shallow edge five seconds, and then swam rapidly downstream. On the other side of the creek ten animals were found at varying distances of 2 to 50 feet away from the water.

During the month of August 470 T. granulosa were captured leaving Pond IV (Figure 10). The number of migrants leaving the pond remained high through November 1965, but decreased to less than 20 per month during December 1965, January and February 1966. A few T. granulosa males entered Pond IV early in November 1965. The number increased steadily through February, then decreased slightly in March 1966. Entering females were first taken in January 1966, and continued to be captured through the winter months (Figure 10). Captured males were always greater in number than females. Males also predominated in the samples removed from Pond IV by seine in May, August, and September 1965 (Table 3). In Ponds II and III the males and females were caught in more equal numbers. A total of 1085 emigrants and 273 immigrants were captured in the traps. Marked animals comprised 4.2 percent of emigrating salamanders caught in the traps, of which six animals came from Pond I, one from Pond II, 14 from Pond III, and 24 from Pond IV. Marked animals comprised 2.2 percent of the salamanders caught immigrating, of which two animals came from Pond I, one

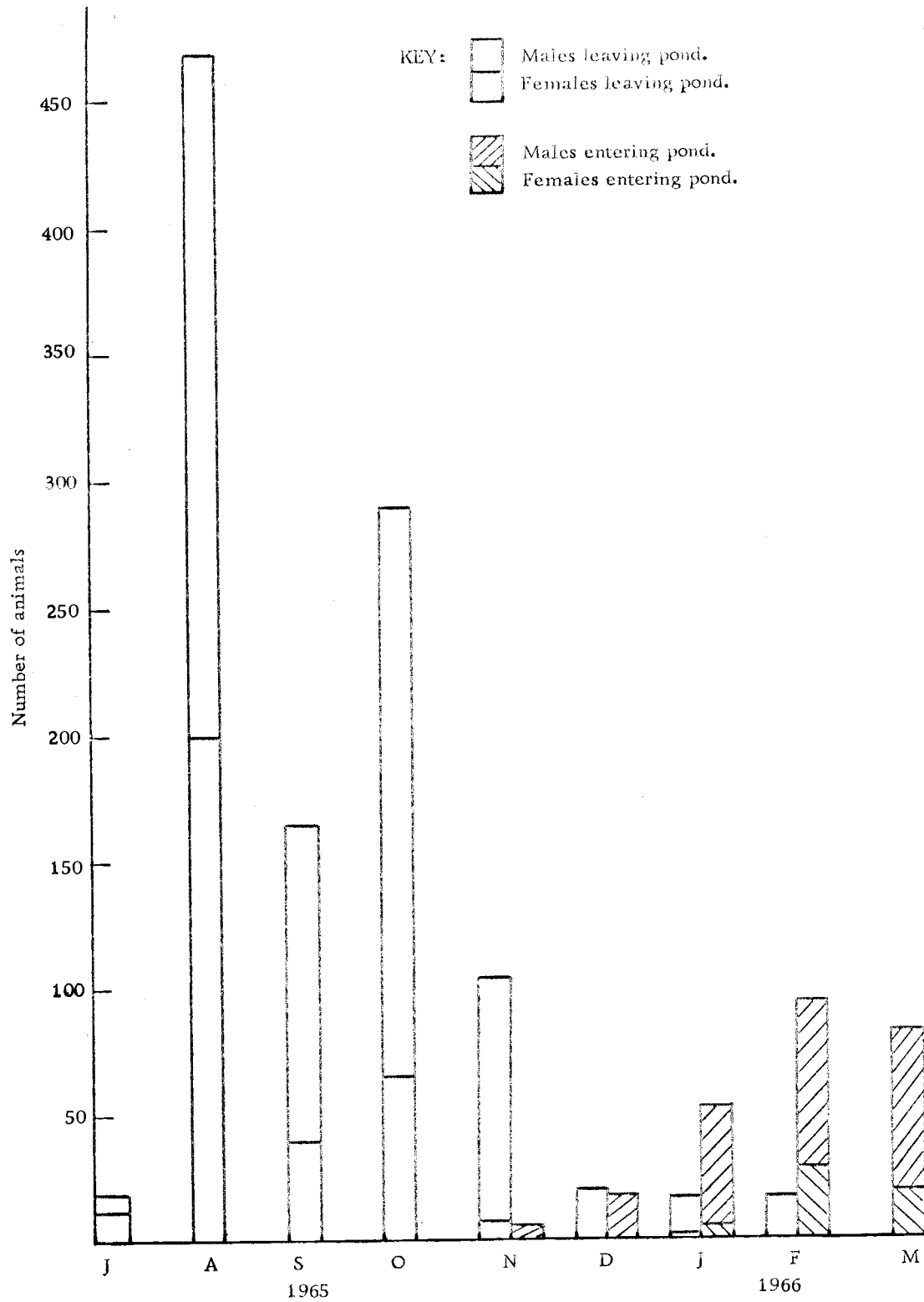


Figure 10. Number of *T. granulosa* trapped migrating in and out of pond IV, July 1965 through March 1966.

from Pond II, two from Pond III, and one from Pond IV.

Table 3. Sex ratios for Taricha granulosa seined in Ponds II, III, and IV.

Date	Pond II		Pond III		Pond IV	
	Males	Females	Males	Females	Males	Females
5/ 1/65	33	101	53	70	93	17
5/29/65	54	47	54	49	65	56
8/ 2/65	145	170	190	370	390	217
9/ 4/65	73	82	107	197	214	132

Dissolved Oxygen

During the period from December 1, 1964 through May 1965 the dissolved oxygen (D. O.) values ranged from a low of 6.4 mg/l on the bottom to 13.4 mg/l on the surface. Oxygen levels between bottom and surface were often only slightly different. With the development of plankton blooms in May, bottom-surface D. O. differentials developed.

The additional data presented in Figures 4 through 7 were selected from the limnological study to point out certain pond conditions preceeding and during the migration of T. granulosa from Soap Creek Ponds. The information contained in these four graphs cover the period from June 1, 1965 to October 12, 1965. The surface D. O. in Pond I varied from 2.8 to 13.7 mg/l during the period under investigation (Figure 4). The bottom D. O. varied from 0 to 9.3 mg/l during the same period. The surface D. O. in Pond II varied from

7.4 to 13.1 mg/l in this period, while on the bottom of the pond the D. O. varied from 1.1 to 11.6 mg/l (Figure 5). During most of this four and one-half month period the bottom D. O. in Pond II was higher than the other ponds. It was noted that T. granulosa in Pond II surfaced for breathing much less than salamanders in Ponds III and IV, perhaps utilizing cutaneous respiration. Underwater observations using aqualungs indicated that Pond II Taricha were feeding and swimming on or near the bottom of the pond. The D. O. at the surface of Pond III varied from 6.1 to 12.4 mg/l. The bottom D. O. ranged from 0.5 to 10.0 mg/l (Figure 6). The D. O. at the surface of Pond IV varied from 7.4 to 12.4 mg/l (Figure 7). The bottom D. O. varied from 0.4 to 10.6 mg/l. During late June, all of July and early August the bottom D. O. remained close to 0.5 mg/l. Taricha granulosa were evident in the cold water near the bottom, but they were more frequently observed in the mid-depths. They surfaced to gulp air much more frequently than those in Ponds II and III. The sharp rise in bottom D. O. in Pond IV followed the rainfalls of August 19, 20, 25, and 26. This period also produced considerable numbers of animals in the traps outside of Pond IV.

Temperature

The water temperatures were less divergent through the winter months than during the summer months. The lowest temperature of

2°C in December 1964 occurred when the air temperature was -11°C and the water froze with three-quarters of an inch of ice on the surface for three days. Taricha were unable to surface during this period. The summer maximum and minimum temperatures in the ponds varied considerably, especially at the surface. The surface maximums reached as low as 13° and as high as 33°C. When the water was warm at the surface the animals remained four to six feet below the surface where the temperature ranged from 15° to 20°C.

The summer maximums on the bottom of the ponds reached 25° in Ponds I and II, and 24°C in the other ponds. The summer minimum on the bottom of the ponds was 11° in Pond I and 12°C in the other ponds. Many Taricha crawled on the bottom feeding at all summer temperatures. The highest temperature on the bottom of the ponds from December 1964 through May 1965 was 20°C. The minimum temperatures in Pond IV were lower than the other ponds during the summer months. At other times of the year Pond IV maximum and minimum bottom temperatures were similar or a few degrees higher than those in Ponds I, II, and III.

From the data presented in Figures 8 and 9 it can be noted that the air temperatures ranged from 7.5° to 35°C during July and August 1965. However, it appeared animals emigrating in appreciable numbers encountered temperatures ranging from 10° to 23°C.

Hydrogen-ion Concentration

During the course of the field study the pH of Pond I varied from 6.8 to 10.2, Pond II values ranged from 6.7 to 8.9, Pond III values varied from 6.5 to 9.3, and Pond IV values ranged from 6.5 to 7.8. The high values occurred during the daytime in the surface water during photosynthetic activity.

The ponds were drained beginning on October 11, 1965 and systematic recording of physical and chemical data were terminated.

Respiratory Rates

The measurement of metabolism by means of oxygen consumption and carbon dioxide production indicate a higher metabolic rate in winter than in summer (Figure 11). The average summer terrestrial oxygen consumption rate varied from 38.4 $\mu\text{l/gm/hr}$ at 10° to 95.0 $\mu\text{l/gm/hr}$ at 25°C, while the winter terrestrial values were 48.9 to 139.6 $\mu\text{l/gm/hr}$ at the respective temperatures. The summer terrestrial rate of carbon dioxide production varied from 32.1 $\mu\text{l/gm/hr}$ at 10° to 71.4 $\mu\text{l/gm/hr}$ at 25°C, while the winter values ranged from 36.4 to 90.5 $\mu\text{l/gm/hr}$ at the same temperatures.

Figure 11 also shows that the winter aquatic rate of oxygen consumption was higher than in summer. During winter the oxygen consumption rate increased from 56.6 $\mu\text{l/gm/hr}$ at 10° to

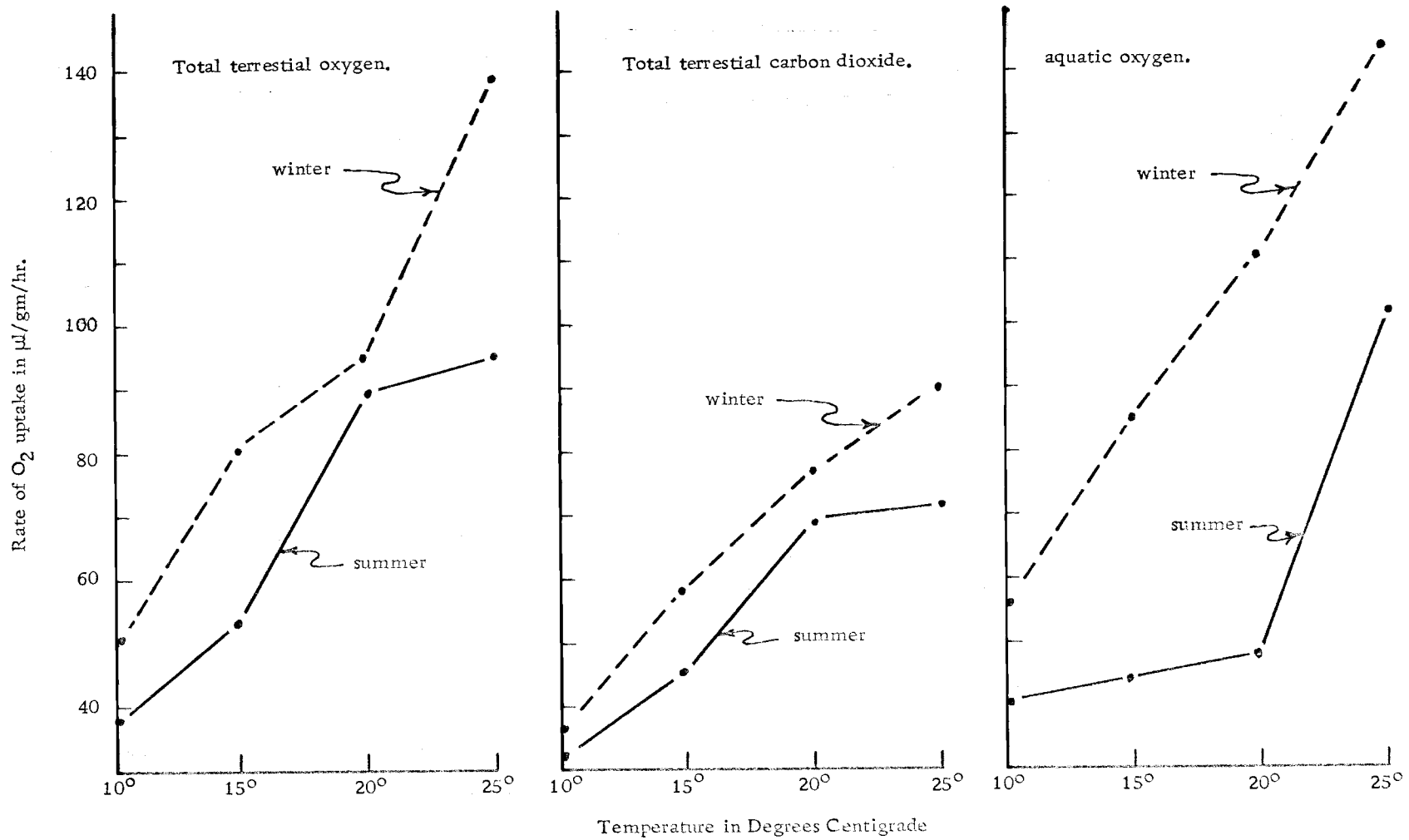


Figure 11. Respiratory rates in *T. granulosa*. Each point represents five animals.

145.4 $\mu\text{l/gm/hr}$ at 25°C, while during the summer the rate increased from 40.8 to 101.5 $\mu\text{l/gm/hr}$ at the respective temperatures.

Examination of Figure 11 and Table 4 shows that during the summer the pulmonary rate of oxygen uptake increased almost linearly from 21.4 $\mu\text{l/gm/hr}$ at 10°C to 67.0 $\mu\text{l/gm/hr}$ at 25°C. The large standard deviation at 25°C may indicate the animals had difficulty with respiration at that temperature. The skin appeared to have a lesser role in oxygen uptake during the summer, since more oxygen entered via the skin in winter, except at 25°C, where the cutaneous rate of 61.1 $\mu\text{l/gm/hr}$ is exceeded by the pulmonary rate of 77.6 $\mu\text{l/gm/hr}$.

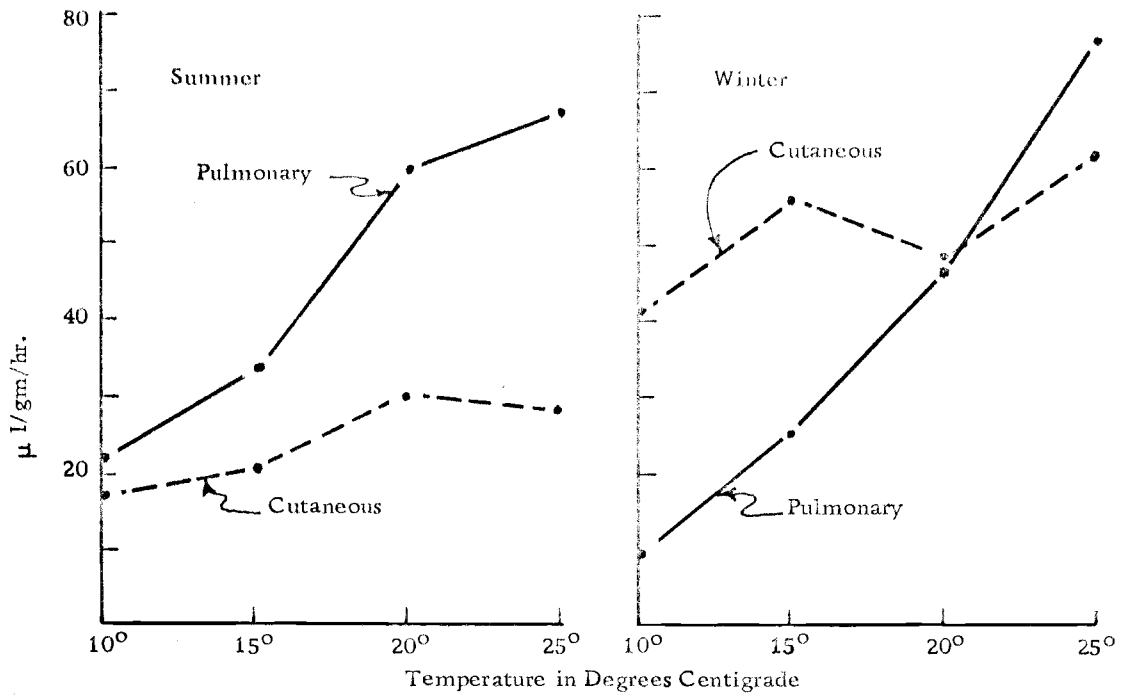
Table 4. Summary of pulmonary, cutaneous, and total rates of oxygen uptake in summer and winter animals. Each number is an average of five animals. The standard deviations are in parentheses.

Temp °C	Respiration			Q_{10} Values
	Pulmonary $\mu\text{l/gm/hr}$	Cutaneous $\mu\text{l/gm/hr}$	Total $\mu\text{l/gm/hr}$	
Summer				
10°	21.4 (2.4)	17.1 (1.7)	38.5	
15°	33.1 (6.2)	20.5 (2.2)	53.6	10°-20° = 2.35
20°	59.5 (7.7)	30.7 (7.8)	90.2	
25°	67.0(16.4)	28.0 (3.8)	95.0	15°-25° = 1.77
Winter				
10°	8.6 (1.8)	40.4 (2.5)	49.0	
15°	25.4 (2.2)	55.7 (3.7)	81.1	10°-20° = 1.94
20°	46.6 (1.6)	48.5 (3.9)	95.1	
25°	77.7 (9.8)	61.9(12.7)	139.6	15°-25° = 1.72

In contrast to the seasonal juxtaposition of cutaneous and pulmonary rates of oxygen uptake, the carbon dioxide release is carried out mainly by the skin in both summer and winter (Figure 12). During the winter the 20°C temperature produced almost equal respiratory rates, with 46.6 $\mu\text{l/gm/hr}$ entering via the skin, and 48.5 $\mu\text{l/gm/hr}$ via the pulmonary route (Figure 12). Only at 25°C did winter animals take up more oxygen via the pulmonary route compared to the skin.

The ratio of pulmonary to cutaneous respiration rises linearly from 1.26 (10°C) to 2.39 (25°C) during the summer (Figure 13a). The role of pulmonary respiration during the winter is indicated in Figure 13b, where the ratio varies from 0.21 (10°C) to 1.26 (25°C). The percent of pulmonary respiration increased linearly from 55.7 percent (10°C) to 70.5 percent (25°C) in the summer animals (Figure 13c). The percent of cutaneous respiration correspondingly decreased in summer animals under the same conditions. During the winter the percent of pulmonary respiration is subordinate to cutaneous respiration, except at 25°C, where pulmonary respiration accounts for 55.7 percent and cutaneous respiration for 44.3 percent (Figure 13d).

Taricha granulosa continuously moves air in and out of the oral cavity through the nostrils with a shallow buccopharyngeal pumping action. The shallow pumping action is periodically interrupted by a



Rate of Carbon Dioxide Production

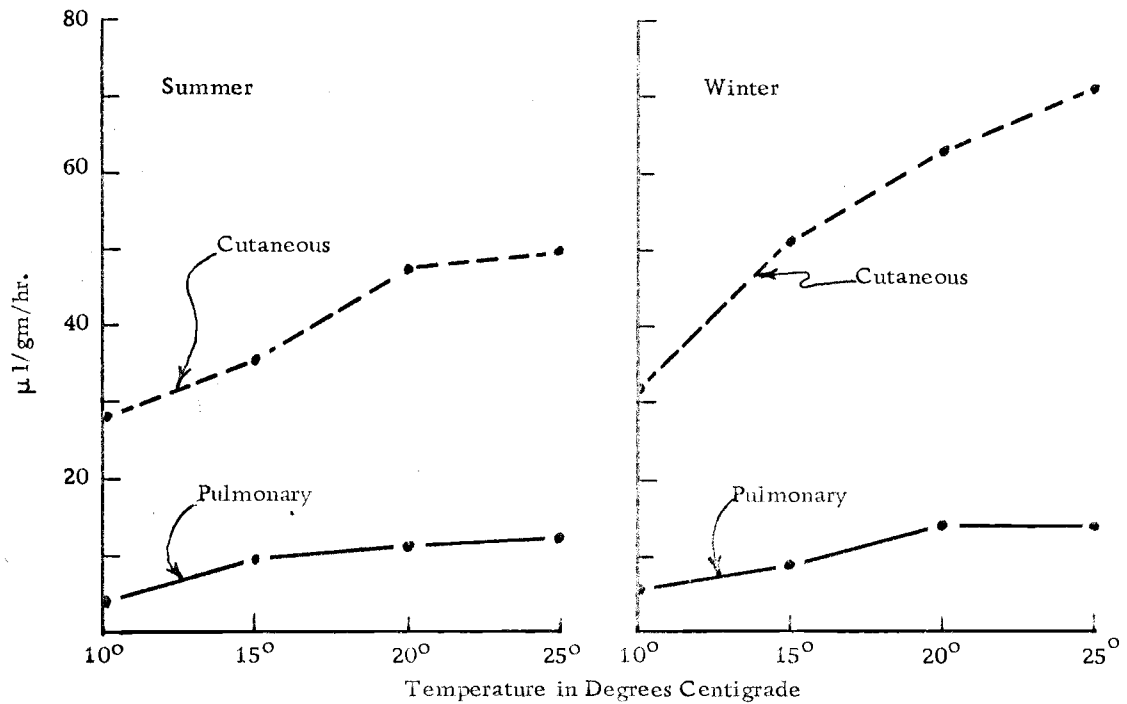


Figure 12. Simultaneous measurement of rates of oxygen and carbon dioxide respiration. Each point represents the average of five *Taricha*.

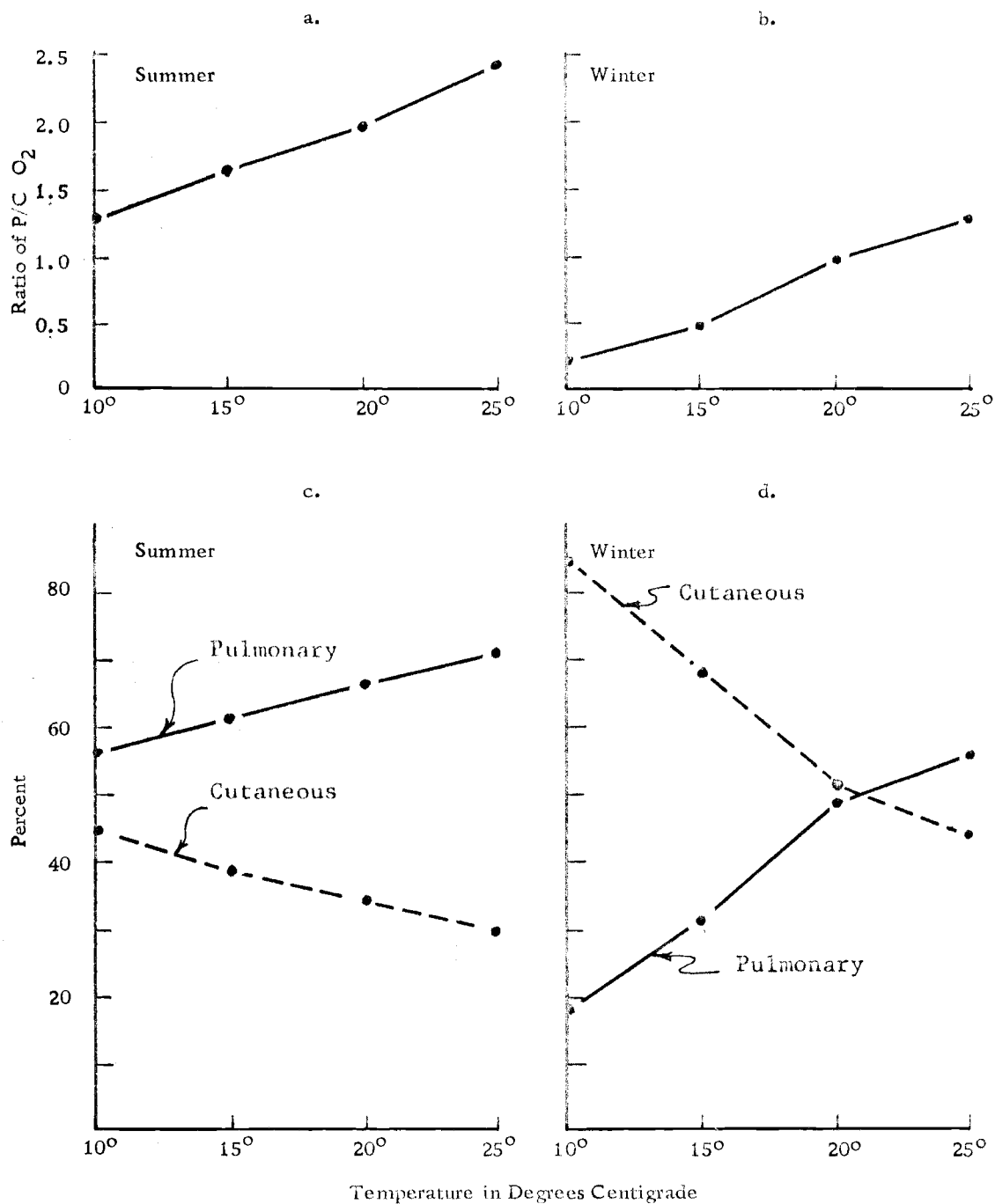


Figure 13. Relative changes in pulmonary and cutaneous respiration with season and temperature. Upper graphs show ratio of pulmonary rate to cutaneous rate. Lower graphs show percent of pulmonary and cutaneous oxygen rates. Each point represents five animals.

deep lowering of the buccopharyngeal region, and upon closure of the nostrils, the oral cavity floor rises and thus pushes air into the lungs. A typical sequence is illustrated in Figure 14a, and the relationship of inspiration to expiration is shown in Figure 14b.

Temperature had a noticeable effect on the shallow buccopharyngeal pumping rate, where air is merely taken in and out of the nostrils without going to the lungs (Figure 15). The average pumping rate at 10°C was 103 per minute, rising to 141 per minute at 15°C, and 157 per minute at 20°C. Then the rate decreased to 134 per minute at 25°C, being perhaps a reflection of stress in T. granulosa at this warm temperature. The number of deep buccopharyngeal depressions whereby air is forced into the lungs remained relatively stable, ranging from 0.74 per minute at 10°C to 4.1 per minute at 25°C. Deep inspirations were usually followed by rapid shallow inspirations. However, at 25°C the animals smooth pumping rate was interrupted by open mouth "yawning", and they appeared to be in some distress. Underwater pumping of water in and out of the nostrils increased from 11.0 per minute at 10°C to 22.0 per minute at 25°C (Figure 15). The volume of air moved by the buccal pumping also increased with temperature. The average volume of air directed into the lungs increased from 100 μ l per pump at 10°C to 500 μ l per pump at 25°C (Figure 16). The shallow buccal pumping volume increased gradually from 11 μ l per pump at 10°C to 49 μ l per pump

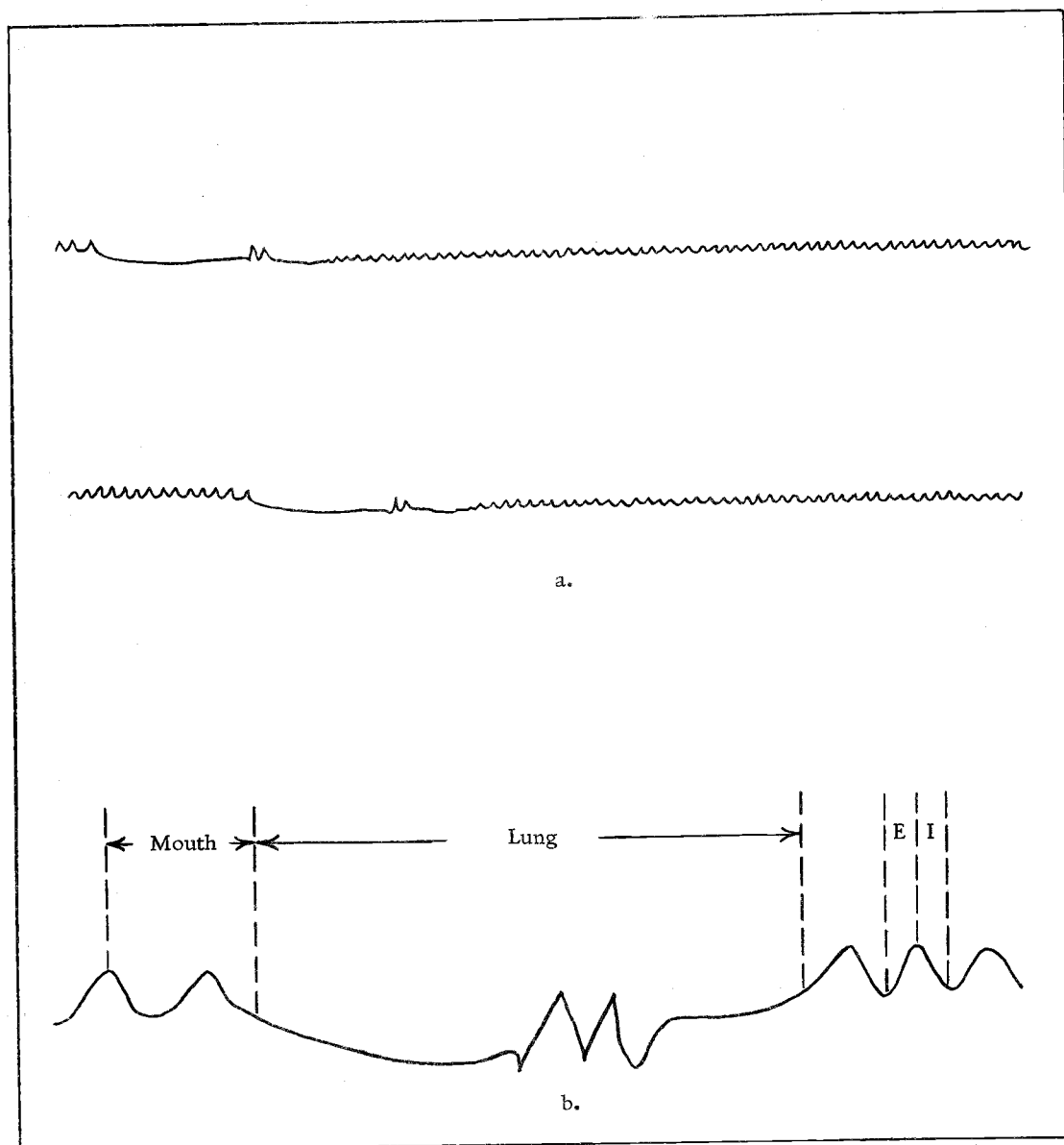


Figure 14. Buccopharyngeal oscillations in *T. granulosa*. Kymograph record is shown in part a. Illustration of mouth and lung oscillations is shown in part b. E refers to exhalation, I refers to inhalation.

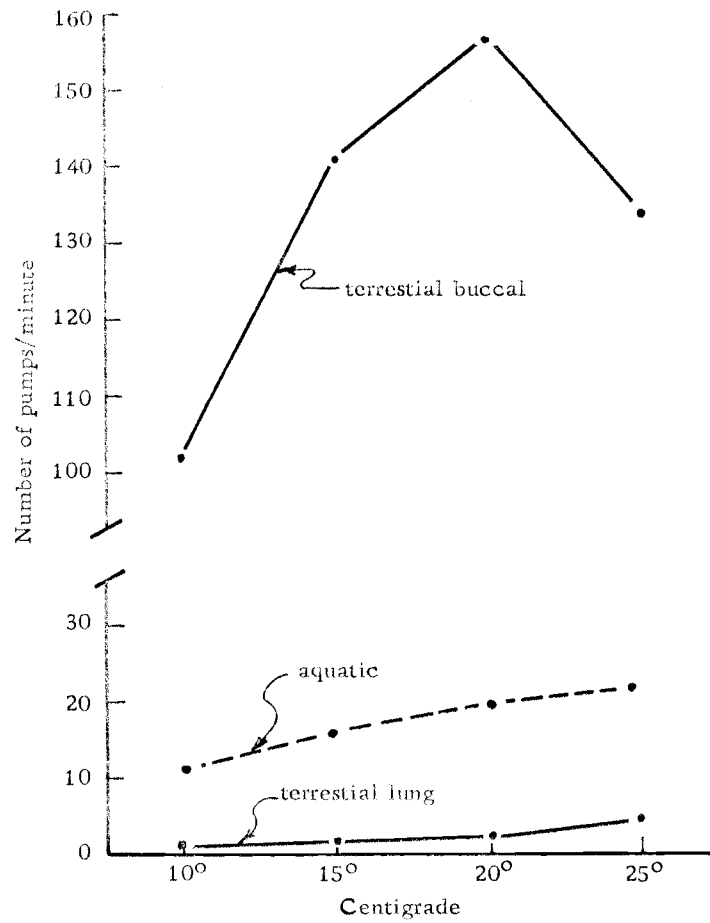


Figure 15. Average pumping rates for buccal and lung respiration. Each point represents 19 animals.

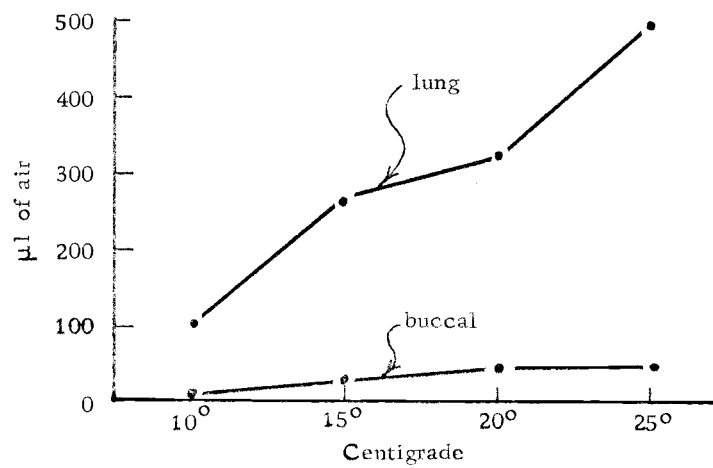


Figure 16. Average terrestrial tidal volumes. Each point represents 10 animals.

at 25°C.

Temperature Coefficients

The temperature coefficients (Q_{10}) were higher in the summer animals, yielding a value of 2.35 between 10° and 20°C, and 1.77 between 15° and 25°C (Table 4). In contrast, the Q_{10} for winter animals was 1.94 between 10° and 20°C, dropping to 1.72 between 15° and 25°C.

Metabolism and Body Weight

Metabolism does not increase directly with body weight, but rather varies to an exponential power of weight. The equation $M = aW^b$ has been used to describe the relation between oxygen consumption and body weight in a wide variety of animals. In this equation the letters represent the following:

M = oxygen consumption in $\mu\text{l/hr}$.

W = body weight in grams.

b = exponential power of increase in oxygen consumption with weight.

a = constant for a given experimental temperature.

In order to find the value of b for Taricha granulosa, regression lines were fitted to the data by the method of least squares.

Calculations by this method yield values for both a and b of the

equation. Calculated values for b in summer animals were 0.70, 0.76, 0.65, and 0.52 at the four experimental temperatures (Table 5). The four values for b in winter animals were 0.68, 0.70, 0.62, and 0.56 at the four experimental temperatures. The average of all eight values of b was 0.65. In order to compare slopes of the various values of b , the data were plotted on log-log graph paper (Figure 17). The values of a in the equation $M = aW^b$ determines the intercept of the various lines with the ordinate axis.

In order to test the possibility that metabolic rate in Taricha granulosa is proportional to $W^{2/3}$, the following null hypothesis was tested: "The experimental value of b is not significantly different from the surface law value B , where B is 0.67" (Table 6). Of the eight values of b tested, only the 0.56 value (25°C winter) was significantly different from 0.67 at the 95 percent confidence level. Thus, the hypothesis that b is not significantly different from B cannot be rejected, except at 25°C winter where animals may have been experiencing an abnormal temperature for that season.

In order to test the possibility that metabolism varied by season, the respiration of a 13.5 gram Taricha granulosa was compared, summer versus winter, at the four experimental temperatures. The weight chosen 13.5 grams, is the average weight of the 40 experimental animals used in the metabolic rate study. The calculated values of a and b were used in the equation $\log M = b \log W + \log a$,

Table 5. The relation of oxygen consumption to body weight in *Taricha granulosa* at four environmental temperatures. The values of a and b were calculated by the least squares method. S_b is the standard error of the estimate.

10°		15°		20°		25°	
Wt. gms.	O ₂ μl/hr	Wt. gms.	O ₂ μl/hr	Wt. gms.	O ₂ μl/hr	Wt. gms.	O ₂ μl/hr
<u>Summer</u>							
14.2	610	11.2	500	15.1	1160	13.5	780
11.6	440	13.1	600	15.0	1260	9.6	1120
14.4	620	11.2	740	8.9	1010	14.4	1400
15.5	500	14.4	780	9.7	840	13.6	1340
16.1	580	12.1	700	16.3	1470	15.1	1580

b = 0.70		b = 0.76		b = 0.65		b = 0.52	
S _b = 0.047		S _b = 0.052		S _b = 0.123		S _b = 0.095	
a = 62.7 μl/hr		a = 96.9 μl/hr		a = 215.5 μl/hr		a = 316.0 μl/hr	
<u>Winter</u>							
11.5	600	10.0	850	12.4	1210	13.5	1900
12.6	660	10.7	890	13.1	1240	11.6	1720
14.1	700	13.7	1100	14.5	1340	13.9	1910
14.5	710	13.3	1140	13.6	1350	14.0	1900
13.2	550	14.6	1040	14.3	1310	14.1	1920

b = 0.68		b = 0.70		b = 0.62		b = 0.56	
S _b = 0.038		S _b = 0.070		S _b = 0.027		S _b = 0.038	
a = 111.3 μl/hr		a = 170.0 μl/hr		a = 260.0 μl/hr		a = 435.2 μl/hr	

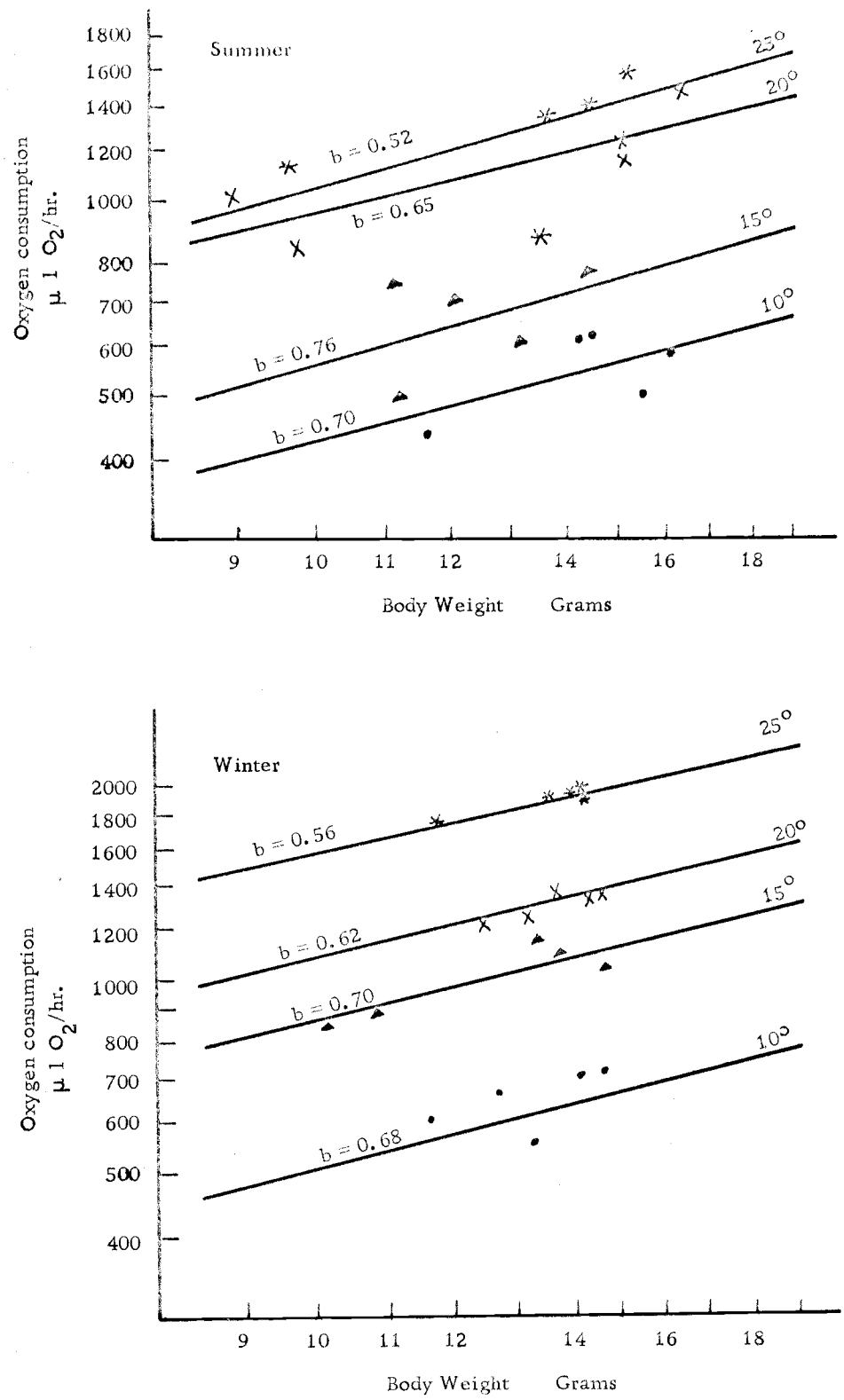


Figure 17. The relation between oxygen consumption and body weight in *T. granulosa* at four environmental temperatures, plotted on a double-logarithmic grid. The regression lines were fitted by the method of least squares with five points determining each line.

Table 6. Null hypothesis analysis of b in the equation $M = aW^b$. The exponent b is the ratio of the percentage change in M (metabolism) to the corresponding change in W (weight).

OBJECT: To show that the exponent b with respect to metabolism in Taricha granulosa is not significantly different from the surface law value of 0.67.

1. HYPOTHESIS: The experimental value of b is not significantly different from the surface law value, B , where B is 0.67.
2. STATISTIC: $t = \frac{b - B}{S_b}$

b = calculated exponent value
 B = surface law exponent value
 S_b = standard deviation of b
3. CONFIDENCE LEVEL: 95 percent.
4. DEGREES OF FREEDOM: Assuming the observations are from a normal population, and that the hypothesis is true, the distribution of t is $(n-2)$. Thus, the degrees of freedom are $(5 - 2) = 3$.
5. CRITICAL REGION: $t_{.95} > 2.35$ (one tailed rejection)
6. REJECTION POINT: Reject the hypothesis if t is greater than 2.35.

SUMMARY OF CALCULATIONS

Summer Data Temp. °C.	Calculated b value	S_b	Calculated t value	Accept or Reject H
10°	0.70	0.047	0.64	Accept
15°	0.76	0.052	1.73	Accept
20°	0.65	0.123	0.16	Accept
25°	0.52	0.095	1.58	Accept
Winter Data				
10°	0.68	0.038	0.26	Accept
15°	0.70	0.070	0.43	Accept
20°	0.62	0.027	1.85	Accept
25°	0.56	0.038	2.89	Reject

where W equalled 13.5 grams (Table 7). The difference in oxygen consumption between summer and winter was calculated in percent. These data show that oxygen consumption increased in winter at all four temperatures. The percent increase was substantial in all cases, except at 20°C , where the 5.0 percent is within the possibility of experimental error.

To show that the four experimental temperatures caused significant increases in the metabolic rate, an analysis of variance was made for each of ten sets of data. The null hypothesis, "There is no difference between the means of oxygen consumption rates at the four experimental temperatures (10° , 15° , 20° , and 25°C)", was analyzed statistically by the F test (Table 8). The results of this analysis (Table 9) show that the hypothesis should be rejected in all ten sets of data at the 99 percent confidence level. This means that unless a 1 in 100 chance error has occurred in each set of data, there is a significant difference in the metabolic rates at 10° , 15° , 20° , and 25°C for:

1. Summer pulmonary oxygen consumption.
2. Winter pulmonary oxygen consumption.
3. Summer cutaneous oxygen consumption.
4. Winter cutaneous oxygen consumption.
5. Summer pulmonary carbon dioxide production.
6. Winter pulmonary carbon dioxide production.

Table 7. Statistical analysis of seasonal variation in metabolism.

OBJECT: To show the extent of seasonal metabolic change by using a 13.5 gram Taricha granulosa to compare summer versus winter metabolism at four temperatures. For each calculation the following formula was used:

$$\log M = b \log W + \log a$$

Calculations	M calculated $\mu\text{l O}_2/\text{hr}$
10°C	
Winter $\log M = (0.68)(1.1303) + 2.0466 = 2.8152 =$	653.3
Summer $\log M = (0.70)(1.1303) + 1.9296 = 2.7208 =$	525.8
Difference	127.5
Sum	1179.1
Percent Difference	10.8
15°C	
Winter $\log M = (0.70)(1.1303) + 2.2300 = 3.0212 =$	1050.0
Summer $\log M = (0.76)(1.1303) + 1.9863 = 2.8453 =$	700.4
Difference	349.6
Sum	1750.4
Percent Difference	20.0
20°C	
Winter $\log M = (0.62)(1.1303) + 2.4145 = 3.1153 =$	1304.4
Summer $\log M = (0.65)(1.1303) + 2.3334 = 3.0681 =$	1179.5
Difference	124.9
Sum	2483.9
Percent Difference	5.0
25°C	
Winter $\log M = (0.56)(1.1303) + 2.6388 = 3.2718 =$	1870.0
Summer $\log M = (0.52)(1.1303) + 2.5004 = 3.0881 =$	1227.5
Difference	642.5
Sum	3097.5
Percent Difference	20.7

Table 8. Statistical analysis of oxygen consumption rates utilizing the analysis of variance or F test.

OBJECT: To be able to reject the hypothesis, and thus show that the four experimental temperatures produce significantly different rates of oxygen consumption.

1. **HYPOTHESIS:** There is no difference between the means of the rate of oxygen uptake (u) at the four experimental temperatures 10° , 15° , 20° , and 25°C . In statistical notation $u_1 = u_2 = u_3 = u_4$.

(Note: The above hypothesis was used 10 times with similar columns of data).

2. **STATISTIC:** F test:

$$F = \frac{\text{mean square of group means}}{\text{mean square of individuals}}$$

3. **CONFIDENCE LEVEL:** 99 percent
4. **DEGREES OF FREEDOM:** Assuming the observations are from a normal population, with

$$\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$$

(homogeneous variance), and that the hypothesis is true, the distribution of F is $F(k-1, n_1-k)$. Thus the degrees of freedom are (4-1, 20-4) or (3, 16).

5. **CRITICAL REGION:** $F_{.99}(3, 16) > 5.29$
6. **REJECTION POINT:** Reject the hypothesis if F is greater than 5.29.
-

Table 9. Analysis of variance data for the rates of oxygen uptake in T. granulosa.

Respiratory Region	Season	Ave. Respiratory Rate and Standard Deviation at four temperatures				F Value	D. F.
		10°C	15°C	20°C	25°C		
<u>Scholander Apparatus</u>							
<u>Oxygen</u>							
Pulmonary O ₂	Summer	21.4	33.2	59.5	67.0	19.98	3, 16
		2.4	6.2	7.7	16.4		
Pulmonary O ₂	Winter	8.6	25.4	46.6	77.7	132.33	3, 16
		1.8	2.2	1.6	9.8		
Cutaneous O ₂	Summer	17.1	20.5	30.7	28.0	7.09	3, 16
		1.7	3.5	7.8	3.8		
Cutaneous O ₂	Winter	40.4	55.7	48.5	61.9	7.04	3, 16
		2.5	3.7	3.9	12.7		
<u>Carbon Dioxide</u>							
Pulmonary CO ₂	Summer	4.0	10.0	21.8	22.1	28.57	3, 16
		0.8	1.7	5.6	3.2		
Pulmonary CO ₂	Winter	5.4	8.8	14.1	14.2	34.11	3, 16
		1.1	1.2	1.2	2.2		
Cutaneous CO ₂	Summer	28.3	35.5	47.7	49.1	59.07	3, 16
		2.1	1.1	3.9	2.5		
Cutaneous CO ₂	Winter	31.0	50.6	63.2	76.3	162.93	3, 16
		2.7	3.4	3.0	2.9		
<u>Winkler Method</u>							
Aquatic O ₂	Summer	40.8	44.1	48.0	101.5	33.77	3, 16
		4.2	7.9	4.2	17.1		
Aquatic O ₂	Winter	56.6	84.8	111.1	145.4	37.37	3, 16
		3.4	18.9	14.6	5.8		

7. Summer cutaneous carbon dioxide production.
8. Winter cutaneous carbon dioxide production.
9. Summer aquatic oxygen consumption.
10. Winter aquatic oxygen consumption.

Surface Respiration

In approaching the surface to take air into the pulmonary system, underwater moving pictures showed that T. granulosa swam rapidly to the water-air interface (Figure 18a). The animal exhaled (Figure 18b), and then air was taken in via the nostrils with a snap of the head, followed by a quick submergence. As it starts descending, the nostrils are closed, and the inspired air is pushed into the lungs (Figure 18c). As the animal continued to descend, air was sometimes expelled out the nostrils forming bubbles in the water (Figure 18d). Taricha were sometimes observed to exhale a bubble several inches below the surface and return to the bottom without coming to the surface. Careful observation of their surfacing frequency at 14°C revealed an average of 13.1 minutes between inhalations at the surface. The maximum submerged period under these conditions was 55 minutes.

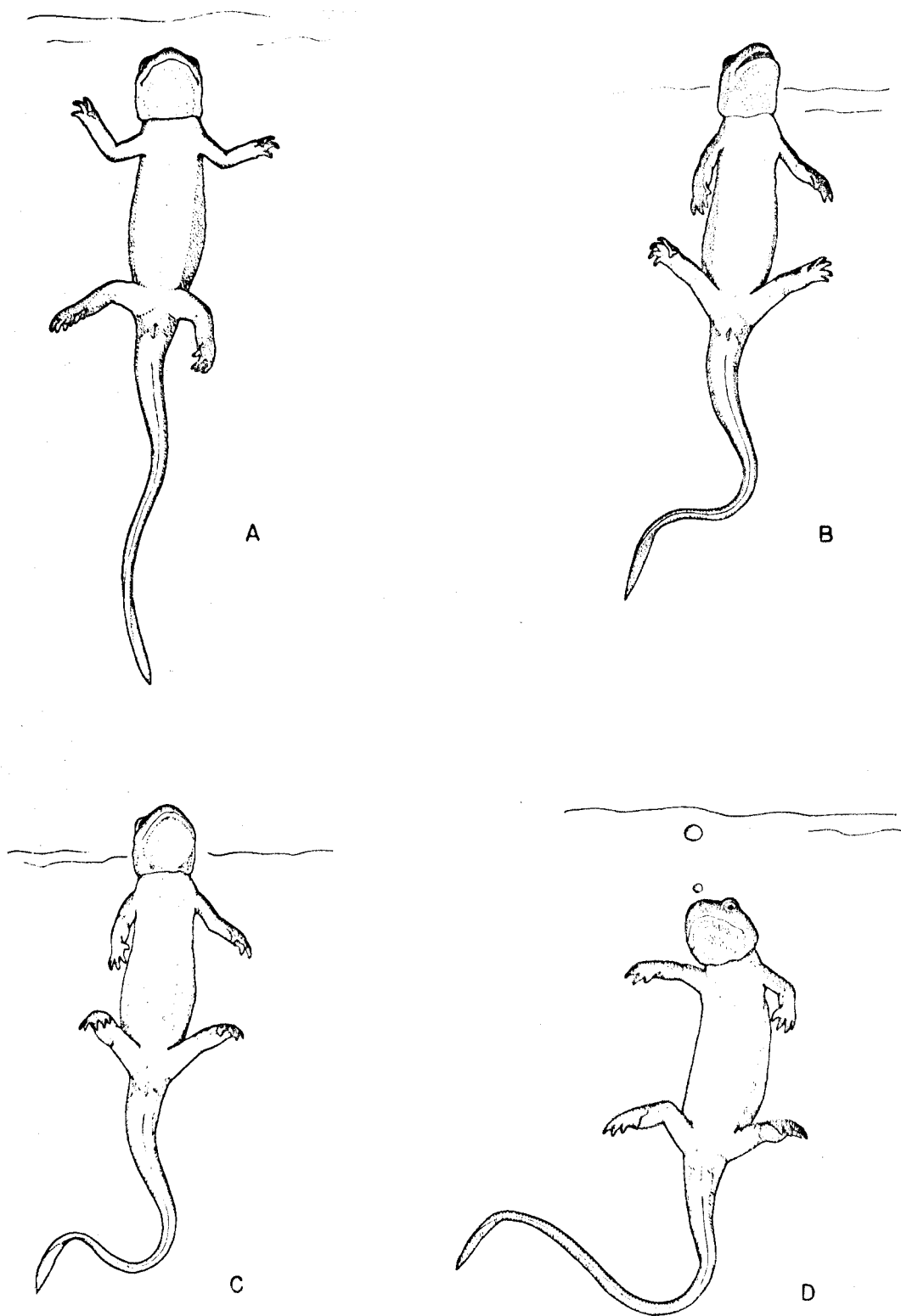


Figure 18. Swimming movements of *T. granulosa* approaching the surface of a pond for pulmonary respiration.

Underwater Respiration

When Taricha granulosa is underwater it makes buccopharyngeal pumping motions at reduced rates, taking water in and out of the oral cavity via the nostrils. The average number of pumps per minute varied from 11.0 at 10° to 16.5 at 15° to 19.8 at 20° to 22.0 at 25° C (Figure 16). In an experiment designed to test the exclusive use of mouth membranes for respiration, animals were fitted with a mask and then encased in a test tube containing vaseline. The skin was eliminated from respiration by the vaseline, and the lungs were eliminated by keeping the animal underwater at a constant temperature of 20° C. The longest time any of four experimental animals survived in this experiment was 4.5 hours, while the average was 4.1 hours. A check on the traumatic effect of being masked and enclosed in a tube underwater indicates an animal can survive ten hours in the apparatus. Another animal completely immersed in vaseline died in one hour and 25 minutes. To investigate this approach further, another series of experiments were started to measure the utilization of mouth membranes for oxygen uptake. The animals were first permitted to use both cutaneous and buccopharyngeal respiration to determine the total aquatic respiration. After resting each animal was put in a vaseline filled tube with only the head exposed to the water and the oxygen consumption was again measured. Based on five animals at each temperature, the average

aquatic buccopharyngeal oxygen consumption rates varied from 4.0 $\mu\text{l/gm/hr}$ at 15° to 7.0 $\mu\text{l/gm/hr}$ at 20° to 12.0 $\mu\text{l/gm/hr}$ at 25°C. The buccal pumping rate increased approximately two times the normal aquatic pumping rate. When released after the 60 minute test period the salamanders took 10 to 15 deep lung inspirations. By subtracting the buccopharyngeal oxygen consumption from the previously measured combined buccopharyngeal-cutaneous oxygen consumption one can find the amount of cutaneous oxygen uptake. The average cutaneous oxygen consumption rates ranged from 55.0 $\mu\text{l/gm/hr}$ at 15° to 59.0 $\mu\text{l/gm/hr}$ at 20° to 79.0 $\mu\text{l/gm/hr}$ at 25°C. (Figure 19). No data are available at 10°C because the level of buccopharyngeal respiration proved to be too low to be reliable.

Respiratory Quotients

The average respiratory quotients of experimental animals were calculated from both summer and winter data. The respiratory quotients for summer animals decreased from 0.84 at 10° to 0.75 at 25°C. In winter the respiratory quotient was 0.74 at 10° and 15°, increased to 0.81 at 20°, and then decreased to 0.65 at 25°C. Acclimating animals were not fed prior to the experiments and were forced to rely on existing reserves stored in the body.

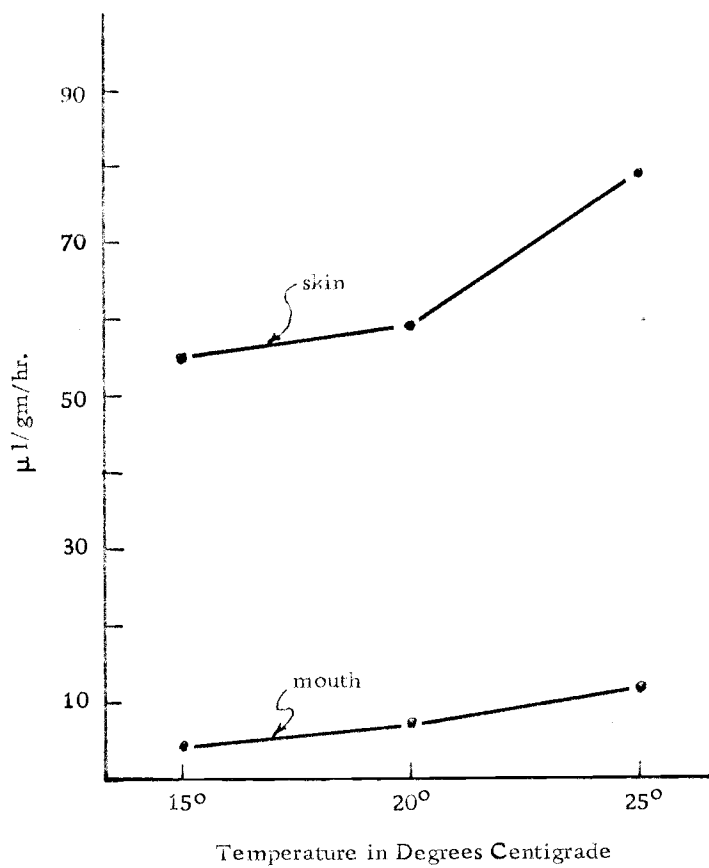


Figure 19. Rate of oxygen uptake in submerged *T. granulosa*. Each point represents the average of five animals.

DISCUSSION

Pond Ecology

The abundance of food in the Soap Creek ponds was probably responsible for the initial immigration of Taricha granulosa following fertilization of the new ponds in 1959. Since then the ponds have supported a heavy population of algae, zooplankton, aquatic invertebrates, and aquatic vertebrates, including Taricha granulosa. It appears from earlier studies that the heavy applications of nitrogen and phosphorous contributed to the success of the larger populations in Pond IV. According to MacIntyre and Bond (1962) the effects of fertilization were best reflected by the benthos communities. They believed the mechanism involved a dense "bloom" of Micractinium, which after death, furnished the necessary organic food for larval midges. Although this mechanism occurred in Ponds II, III, and IV, it built up more rapidly in Pond IV. Pond I was not fertilized.

Isaac and Bond (1963) report that when Ponds II, III, and IV were drained in the fall of 1961, the numbers of Taricha granulosa in the ponds were 752, 1630, and 6375 respectively. They report stomach contents contained primarily aquatic insect larva and that Taricha were competing with pond fishes for food. Their study shows Pond IV produced 34 pounds of fish per acre and 245 pounds

of T. granulosa per acre.

In the present study marked recoveries indicated some animals chose to leave and enter the pond complex via Pond IV, thus introducing a transient population into the pond. The fact that Pond IV was closest to the vegetative cover bordering the ponds probably made it the most desirable migration route for the salamanders.

Goodwin (1967) made a study of the effect of fertilizer and steer manure on the standing crop of fish and related organisms in the food web of Soap Creek Ponds. He reports heavy plankton blooms followed by large populations of benthos organisms. Tubifex and Chironomus comprised the bulk of the weight of benthos animals in his study. Tubificids were found only once in the stomachs of largemouth bass (Micropterus salmoides), while chironomids were found in all the bass examined. In the present investigation chironomids were also found in most of the T. granulosa stomachs. The T. granulosa in the Soap Creek Ponds were the fattest of any observed in Oregon and Washington. Occasionally their stomachs contained young bluegills (Lepomis macrochirus), and largemouth bass (Micropterus salmoides). Three dead catfish (Ictalurus catus) were found with a single T. granulosa in each of their stomachs, apparently poisoned by the salamander's granular glands. Brodie (1967) concludes that of the animals he tested, only garter snakes, Thamnophis sirtalis, are able to eat adult T. granulosa with immunity to

their toxin.

The bottom D. O. 's in Ponds III and IV approached 0 mg/l in July and early August due to the decomposition of planktonic organisms. This coincided with the increased migratory activity in Taricha, but whether there is any correlation between these two factors is not apparent from this study. The D. O. 's near the bottom of Pond II were higher than those in the other ponds. Although this permitted Pond II animals to expose themselves less at the surface for respiration, this apparently was not an important factor since Pond II contained fewer animals than Ponds III and IV. In all ponds, while the bottom D. O. 's were near 0 mg/l, and supersaturation due to photosynthesis occurred near the surface, Taricha continued to take air into mouth and lungs from the atmosphere.

In addition to the factors already mentioned contributing to large numbers of T. granulosa in Pond IV, it appeared that the cooler bottom temperatures may also have been a factor. This cool water condition was brought about by heavy photosynthesis near the surface, and also by turbid water in the deeper parts of the pond. There appeared to be little mixing of bottom and surface waters, and temperature differences between surface and bottom minimums reached as much as 11°C on a number of days. The surface maximum temperatures exceeded 30°C on a number of days, and it would appear from the metabolic studies that this would be an undesirable

sustained temperature for T. granulosa. The respirometer studies show that even at 25°C summer animals have a regression line slope of 0.52, indicating their metabolism is out of proportion to their weight. In the water Taricha granulosa seemed to favor temperatures of 15° to 20°C, which were available during the spring, summer, and fall through November 1965. However, December through March 1965 presented temperatures less than 15°C.

Hydrogen-ion concentrations in the ponds did not appear to be an important factor in the Taricha granulosa habitat. Pimentel (1952) concluded strongly alkaline or acid bodies of water are probably shunned, but that this salamander seemed at home in the relatively low pH of Oregon bogs.

Migratory Activity

Other studies (Livezey, 1943; Pimentel, 1952; and Storm, 1948) have shown that the first male newts usually arrive at the breeding ponds about one month earlier than the females. This is confirmed in the present study, where males first returned in late November, and females did not arrive until mid-January. Based on population intercept studies it appeared that Taricha granulosa began migrating out of Soap Creek Ponds to aestivate in July 1965. Yet undiscovered causes appear to bring these animals up to an emigrating threshold during the month of July. The occurrence of rainfall, even in trace

amounts, promotes the emigration of animals. They prefer darkness for migration, but if sufficient moisture is available to prevent desiccation, they will migrate during the daylight hours. The preferable air temperature for migratory activity ranged from 10°C to 23°C.

Summer-winter Variations in Respiration

Taricha granulosa undergoes certain anatomical and physiological changes with its migrations. In going from water to land these include roughness of skin, reduction of epithelial mucus, and a fatter tail. Returning to the water, the animals have a smoother skin, greater amounts of epithelial mucus, and a more compressed tail. Males have a greatly swollen cloaca characteristic of the nuptial stage. In addition to these changes, the results of the present study show that their metabolic rate was higher in winter than in summer. The lower metabolic rates in summer may indicate the metabolism of T. granulosa is declining prior to aestivation, while higher metabolic rates in winter may reflect the onset of the reproductive activities.

During the summer the pulmonary system took up a greater percent of oxygen than the cutaneous system (Figure 13). In view of the roughening of the skin in terrestrial animals one might expect such a decrease in cutaneous oxygen uptake during the summer. Livezy

(1943) found terrestrial newts crawling into cracks and crevices in the ground and under large logs. Stebbins (1954) found terrestrial T. granulosa under logs, bark, boards, and other surface objects. It is possible these confining terrestrial habitats would restrict cutaneous respiration and emphasizes the necessity for increased pulmonary respiration during the terrestrial phase.

According to Whitford and Hutchison (1966) there is an apparent correlation between tidal volume and the importance of pulmonary respiration in ambystomatids. In Ambystoma tigrinum and A. talpoideum, which obtain approximately 54 percent of their oxygen via the pulmonary surfaces, there is a greater tidal volume per unit of weight ($\bar{x} = 0.03$ cc/g) than in A. maculatum, A. macrodactylum, and A. opacum ($\bar{x} = 0.01$ cc/g) in which the pulmonary surfaces account for only 34 percent of the total oxygen uptake. The present study indicated the percent of pulmonary oxygen uptake shifts from summer to winter, and also the tidal volume increased with temperature. The use of an average tidal volume by Whitford and Hutchison for only one temperature at one time of year may be too simple an approach and may need further study.

During the winter, when T. granulosa either live in ponds or on land exposed to considerable rain, the percent of cutaneous respiration is higher than in summer. This increased moisture may facilitate the cutaneous oxygen uptake. Only at the unseasonably high

experimental temperature of 25°C did the pulmonary oxygen uptake exceed the cutaneous rate. The skin appeared unable to rapidly accommodate substantial changes in metabolism due to temperature changes. Pimentel (1952) reported aquatic Taricha granulosa possess a thinner stratum corneum with a thickened stratum granulosum and sub-epithelial connective tissue, plus a striking increase in vascularity. All these seasonal integumentary changes would facilitate cutaneous respiration.

Whitford and Hutchison (1965) propose that increased blood flow through the capillaries and decreased affinity of hemoglobin for oxygen (at temperatures above 15°C) acting in opposites are partly responsible for the relatively constant skin oxygen consumption in frogs, and that this might also apply to salamanders. Also, they point out that the extent to which blood is oxygenated in the lungs will determine the oxygen gradient between the cutaneous capillaries and the surrounding air. Increase in pulmonary tidal volumes and breathing rates could result in a higher pulmonary oxygen level in the venous return from the lung, which would result in a lower concentration gradient between skin capillaries and the air. They may contribute to the maintenance of relatively constant skin oxygen consumption at higher temperatures.

The data in the present study show that the summer atmospheric cutaneous rate of oxygen consumption in Taricha granulosa

reached a peak at 20°C and declined at 25°C (Figure 12). It is possible that T. granulosa is adapted to an optimum temperature of 20°C, permitting success in exploiting warm terrestrial environments when emigrating from ponds. In Ambystoma maculatum the cutaneous oxygen consumption peaked at 15°C and then declined at 25°C and 30°C (Whitford and Hutchison, 1963). The pulmonary rate increased linearly with increased temperature in both species. However, Taricha granulosa appears better adapted to utilize cutaneous respiration in warm environments than A. maculatum.

The respirometer studies show that there was a significant increase in metabolism for each 5°C increase in temperature. All the respiratory systems, whether tested in summer or winter, or whether atmospheric or aquatic respiration, responded with a significant increase. This would appear to indicate Taricha granulosa metabolism is sensitive to external temperature changes in the range of 10°C to 25°C, and that respiration is a reliable indicator of this metabolism.

In both summer and winter, as metabolism rose with increased temperature, the pulmonary apparatus accommodated the largest part of the increase. Uptake of oxygen through the pulmonary system (lungs and buccopharyngeal membrane) appeared to increase with temperature.

Temperature Coefficients

The summer Q_{10} value of 2.35 in the 10° to 20°C range is within the order of magnitude predicted by van't Hoff's rule, while the 15° to 25° value of 1.77 is slightly lower. For the same temperature ranges the winter animals produced Q_{10} values of 1.94 and 1.72 respectively, both of which are less than predicted by van't Hoff's rule. These low Q_{10} values may have adaptive significance for Taricha granulosa. That is, going out on land in the summer where the temperature fluctuates more than in the ponds, their metabolism is confined to a manageable increase. As Rao and Bullock (1954) point out, it is natural to look for lower temperature coefficients in animals subject to wide temperature fluctuations, and their temperature coefficients would be lower than predicted by van't Hoff's rule. Other exceptions to this rule are found in papers by Dawson and Bartholomew (1956), Roberts (1957), and Tashian and Ray (1957).

Respiratory Quotients

While finding certain respiratory quotient values does not prove metabolism of carbohydrate, protein, or fat, they may serve as an index of the process occurring. It would appear that under these experimental conditions Taricha granulosa are metabolising

stored fat.

Exponential Increase of Metabolism with Weight

There has been considerable variation in the literature concerning the exponent b in the equation metabolic rate = aW^b . The data compiled in Table 10 from various sources, indicates the approximate value of the exponent b for different vertebrate groups. Some of the experimental values are close to the "surface law" value of 0.67, and others are as low as 0.47 and as high as 0.86.

Table 10. The approximate value of b in the equation $M = aW^b$ for various vertebrate groups.

Group	b^*	Source
Fish	0.67	Rubner 1924
Frogs		
<u>R. esculenta</u>	0.67	Rubner 1924
Tropical anurans	0.83-0.86	Tashian and Ray 1957
Reptiles		
Reptiles	0.67	Benedict 1932
Uta	0.47-0.64	Dawson and Bartholomew 1956
Sceloporus	0.54-0.68	Dawson and Bartholomew 1956
Homeotherms	0.73-0.75	Brody 1945 Kleiber 1947

* B is the ratio of the percentage change in metabolism to the corresponding change in weight.

The values of the exponent b obtained in this study do not appear to change significantly from summer to winter (Figure 18). The average summer value of b is 0.656, with a standard deviation 0.088, while the average winter value is 0.640, with a standard deviation of 0.055. It appears this exponent has a stable value in Taricha granulosa even though the relative oxygen uptake by pulmonary and cutaneous respiration did change from summer to winter.

In small homeotherms the metabolic rate is higher than in large animals, heat being lost from the external body surface. The rate of cooling of a body is proportional to its surface area, and heat production must likewise be proportional to the surface, since in homeotherms heat production must equal heat loss. Thus surface, or heat loss, or heat production, or oxygen consumption, Y , is proportional to the square of the linear size, or to the $2/3$ power of volume, or to the $2/3$ power of weight, W . The equation $Y = aW^{2/3}$ summarizes these relationships. This is the so called "surface law" first developed by Sarrus and Rameaux in 1837, cited by Brody (1945). Since poikilotherms also exhibit size-dependent rate functions, and since they do not maintain a constant body temperature, one must consider other size-dependent factors which may determine the metabolic rate differences between large and small animals.

The data in the present study show that metabolic increase in

T. granulosa is not significantly different from $W^{2/3}$ at the 95 percent confidence level. The factors which cause energy metabolism of a poikilotherm to vary by a fractional power of body weight are not yet fully understood. Since their body temperature does not depend on heat production, in theory the surface law would not appear valid.

The most likely cause of metabolism increasing by $W^{2/3}$ in T. granulosa may be a basic growth pattern. Krebs (1950) concluded a decrease occurs in the oxygen consumption rate with body size because cells with lower respiration, like those of connective tissue, are bound to become more preponderant in the larger species. Martin and Fuhrman (1955) report that because of different relative weight of various organs, the oxygen consumption of different tissues need not be expected to change with the same exponent of body weight as total metabolism. Thus, in metazoans, with tissues of low metabolic rates such as bone, fat, and connective tissues, metabolism does not increase with weight directly, but metabolic rate declines with increased body size. Zeuthen (1953) points out in a classic paper that, for reasons inherent in living matter, the metabolism always grows less than the body. Organisms were always in danger of outgrowing their supplying mechanisms. Davison (1955) states that if the metabolic performance of the animal is conditioned by reaction systems localized at cell surfaces, then one might expect

similar surface-dependent activities in homeotherms and poikilotherms alike. In the genus Rana he proposes buccopharyngeal frequencies (representing central nervous system activity) multiplied by the muscle cell surface as a possible mechanism of control of in vivo respiration. Stated another way, in the equation $M = aW^b$, buccopharyngeal frequency may be substituted for a , and muscle cell surface for b . This approach may be valid for T. granulosa if both pumping rate and buccopharyngeal volume changes are taken into account. Taricha granulosa is an example of a vertebrate that has developed larger size with a regression coefficient (b) of approximately 0.65, but with an intercept value (a) higher than protozoa, but not so high as homeotherms.

Role of Buccopharyngeal Respiration

Matthes (1927), Vos (1926), Elkan (1955), and Foxon (1964) concluded that the buccal pumping of amphibians was olfactory in function. Noble (1925) assumed they were primarily respiratory in function. Czopek (1962) attributes buccal floor pulsations to olfactory rather than respiratory function on the basis of cytological evidence. However, he cautions that conclusions derived exclusively from morphological findings must be accepted with prudence until they are supported by physiological investigations. Physiological evidence supporting Czopek's view has not yet appeared. Whitford

and Hutchison (1963) presented evidence that the buccopharyngeal oscillations in amphibians play an important role in respiration. The same authors (1965) show that in plethodontids at 15°C, 15 to 24 percent of total oxygen consumption was through the buccopharyngeal mucosa. They further state, "Assuming the buccopharyngeal mucosa plays a similar role in both lunged and lungless forms, then 30 to 50 percent of the pulmonary oxygen uptake of lunged forms would be accounted for by the buccopharyngeal cavity." In order to evaluate this assumption, one needs to know the respiratory potential of the various respiratory organs, since lunged forms such as T. granulosa may use lungs to a much greater extent than buccopharyngeal membranes. Czopek's study (1962) shows that the surface capillaries in the mouth cavity of adult T. granulosa account for approximately three percent of the respiratory capillaries, while the skin and lungs contain approximately 59 and 38 percent respectively. By multiplying the percent of capillaries times the number of buccal pumps per minute times the mouth volume for both buccopharyngeal and lung breathing, one can arrive at the respiratory potential of the mouth and lung. The data in Table 11 show that the respiratory potential of both mouth and lung are of the same order of magnitude at 10°, 15°, and 20°C. However, the lung has a slightly greater potential than the mouth at 15° and 20°, and a vastly greater potential at 25°C. Thus it appears that T. granulosa, a lunged salamander,

Table 11. Comparison of respiratory potential of mouth and lungs utilizing atmospheric and aquatic respiration.

Temp. °C	A Average number of pumps/min.	B Mouth volume μl	C Czopek's capillary factor*	D Dissolved oxygen correction	Index of respiratory potential
<u>Atmospheric Respiration</u>					<u>A x B x C</u>
<u>Mouth</u>					
10°	103	11	3%		34.0
15°	141	30	3		126.9
20°	157	45	3		211.9
25°	134	49	3		197.0
<u>Lung</u>					
10°	0.74	100	38		28.1
15°	1.32	270	38		135.4
20°	2.0	320	38		243.2
25°	4.1	500	38		779.0
<u>Aquatic Respiration</u>					<u>A x B x C x D</u>
<u>Mouth</u>					
10°	11.0	11	3	1/18.6	0.20
15°	16.5	30	3	1/21	0.70
20°	19.8	45	3	1/22.8	1.20
25°	22.0	49	3	1/25	1.30

*Percent of total respiratory capillaries.

might be able to utilize mouth membranes for 30 to 50 percent of atmospheric oxygen uptake at 10°, 15°, 20°C. This lends support to the assumption by Whitford and Hutchison mentioned above. In the present study, by experimentally eliminating cutaneous and pulmonary respiration, it was shown that T. granulosa will use buccopharyngeal membranes for underwater respiration. The respiratory potential of the mouth for removal of dissolved oxygen from water is considerably below the atmospheric potential. The aquatic values range from 0.2 at 10°C to 1.3 at 25°C (Table 11). These calculations are supported by data that show animals were unable to survive underwater more than 4.5 hours using mouth membranes exclusively. Similar experiments above water in the atmospheric environment were not attempted due to the lack of a suitable technique to separate mouth and lung oxygen uptake.

As pointed out by Whitford and Hutchison (1965), obtaining sufficient oxygen at higher temperatures may be an important factor in determining optimum temperature for a species, and thus their distribution. Lunged forms such as T. granulosa and Ambystoma tigrinum with efficient buccal pumping mechanisms are found in areas where ambient temperatures during active periods may exceed 20°C, whereas those with less efficient buccal pumping, for example Desmognathus, Gyrinophilis, and Pseudotriton, are found in microhabitats characterized by relatively lower temperatures.

It appears from the present study that T. granulosa are quite flexible in their ability to change their methods of oxygen uptake. Not only does a change occur from summer to winter, but increased metabolism at higher temperatures is accommodated by the pulmonary apparatus, which may include some buccopharyngeal respiration. The increase of pulmonary respiration during the summer terrestrial phase is primarily caused by their movement onto land with higher temperatures and resulting desiccation of the skin. The separation of atmospheric pulmonary respiration into two components (lung and buccopharyngeal) has not yet been achieved. However, T. granulosa can use buccopharyngeal membranes to remove dissolved oxygen from water, and it appears likely that they might also be able to use the buccopharyngeal membranes for atmospheric respiration. Whitford and Hutchison (1965) attribute an atmospheric rate of oxygen uptake of $5.1 \mu\text{l}/\text{gm}/\text{hr}$ solely by buccal mucosa in T. granulosa at 5°C , since no deep inspirations were observed at that temperature. The fact that T. granulosa have one of the lowest erythrocyte counts of amphibians (Hutchison and Szarski, 1965) would appear to be a limiting factor in metabolism. Apparently this is overcome to some extent by the flexibility of their respiratory mechanisms.

Concluding Statement

It is interesting to speculate on the annual emigration of T. granulosa, especially from ponds with such abundant food in August and September as the Soap Creek Ponds. The maximum bottom temperatures in Ponds II and III exceeded 24°C several times, while Pond IV remained several degrees cooler. Perhaps this served to attract the salamanders into Pond IV. Increased population density along with other undiscovered factors may have raised some of these salamanders to a migratory threshold. Some Taricha granulosa appear to have a fundamental drive to leave ponds in the summer. It appeared their respiratory mechanisms are adaptable to accommodate this emigration from water in summer, and are capable of reversing the process as they return to the ponds in winter. Perhaps other metabolic processes also change to accommodate their change in environment.

Taricha granulosa, the most aquatic of our Pacific coast newts, spend part of the year on land. In spite of leaving the relative safety of ponds and exposing themselves to the hazards of terrestrial life, they are a very successful species. A large part of this success may be due to the few predators that can overcome their potent granular gland toxin (Brodie, 1967). This leaves them relatively free to feed, reproduce, migrate, and to carry on other

activities important to survival. From the data in the current study it appeared that the ability of these animals to shift oxygen uptake from skin to lungs to buccopharyngeal membrane as they move from water to land also is a contributory factor in their success as a species.

CONCLUSIONS

From the preceding data, one may draw the following conclusions:

1. All other things being equal, Taricha granulosa prefer a pond with cool water during the summer. In addition, they prefer a pond with a nearby migration route with vegetative cover.
2. In emigrating from a pond, Taricha granulosa prefer to migrate at night, but if sufficient moisture is available to prevent dessication, they will migrate during the daylight hours.
3. Taricha granulosa undergo a change in metabolism from summer to winter. This is indicated by a respiratory rate which is higher in winter than summer.
4. When Taricha granulosa left the Soap Creek Ponds to live a terrestrial existence, the percent of pulmonary respiration increased and the percent of cutaneous respiration decreased.
5. When Taricha granulosa returned to the Soap Creek Ponds to live an aquatic existence, the percent of pulmonary respiration decreased and the percent of cutaneous respiration increased.
6. Oxygen uptake through the pulmonary system appeared to increase substantially with temperature. Oxygen uptake via the skin appeared to increase slightly with temperature.
7. Carbon dioxide was released primarily by cutaneous

respiration both in summer and winter.

8. Temperature coefficients were generally lower than values predicted by van't Hoff's rule. This may be an adaptation to confine metabolism to a manageable increase when T. granulosa move to the terrestrial environment with more variable temperatures.

9. The exponential value of b in the equation $M = aW^b$ appeared to approximate 0.65. Changes in the percent of oxygen uptake from the pulmonary to cutaneous respiratory surface did not significantly alter the 0.65 value.

10. The buccopharyngeal membrane appeared to have a respiratory function in Taricha granulosa. Animals maintained underwater with their lungs and skin eliminated from respiration were shown to consume measurable amounts of oxygen. The atmospheric respiratory potentials of the buccopharyngeal membrane and lungs are of the same order of magnitude, except at 25°C where lung potential is four times higher.

11. The flexibility of shifting to alternate respiratory surfaces for oxygen uptake may be a factor in the ability of Taricha granulosa to survive and to successfully exploit new habitats.

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