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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

OXYGEN UPTAKE DURING SPONTANEOUS AND INDUCED METAMORPHOSIS IN TADPOLES OF *PHYLLOBATES SUBPUNCTATUS*

A DISSERTATION

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BY

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OXYGEN UPTAKE DURING SPONTANEOUS AND INDUCED METAMORPHOSIS

IN TADPOLES OF PHYLLOBATES SUBPUNCTATUS

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OXYGEN UPTAKE DURING SPONTANEOUS AND INDUCED METAMORPHOSIS IN TADPOLES OF PHYLLOBATES SUBPUNCTATUS

CHAPTER I

INTRODUCTION

During spontaneous amphibian metamorphosis, respiratory rate has been assumed to increase (Frieden, 1961; 1963; Bennet and Frieden, 1962). However, there is no clear-cut experimental evidence to support this assumption. Fletcher and Myant (1959) found no increase in oxygen consumption before or during metamorphosis in naturally transforming tadpoles (Xenopus laevis and Rana temporaria). Whether the data were calculated per unit wet or dry weight, oxygen uptake remained constant. The somewhat inconclusive results of Lewis and Frieden (1959) for Bufo americanus show no increase in metabolic rate during spontaneous metamorphosis. Earlier reports (Wills, 1936; Witschi, 1956) show a small rise in oxygen uptake at the end of metamorphosis, but the information is not complete enough for any certain conclusions. In no case are the data for larvae compared with those for adults, and only Fletcher and Myant (1959) give results per unit dry weight.

The assumption that oxygen uptake must increase during spontaneous metamorphosis has received indirect support from studies of metabolic rate during induced metamorphosis. Lewis and Frieden (1959) reported significant increases after injection of thyroxine (T_4) and

triiodothyronine (T_3) in Rana grylio and R. catesbeiana, and after immersion of Bufo americanus tadpoles in T_3. Similarly, Mehes and Berde (1947) reported increased oxygen consumption after tadpoles were immersed in T_4. In contrast, Fletcher and Myant (1959) indicated a decrease in QO_2 after treatment of Xenopus laevis and Rana temporaria tadpoles.

However, changes which may be observed in respiratory rate during induced metamorphosis cannot necessarily be assumed to occur during spontaneous metamorphosis. The "morphological and biochemical responses in induced metamorphosis are not always identical with the slower, apparently more ordered changes of spontaneous metamorphosis" (Frieden, 1961). Unless the conditions of the experiment are rigidly controlled (see Chapter IV, Discussion) there are significant differences in the sequence with which morphological changes take place, and in the concentrations of a variety of enzymes (Kubler and Frieden, 1964).

Respiration studies of amphibia are plagued by certain problems associated with the changing structure of the animal from aquatic larva to land living adult. Methods suitable for one may not be for the other (Frieden, 1961). During metamorphic climax the animal's mouth and digestive tract are passing through changes which make feeding impossible. The normal decrease in body weight which occurs at this time, as well as the weight decreases caused by hormone treatment in induced metamorphosis, must be taken into account when calculating oxygen consumption data (Etkin, 1934; Lewis and Frieden, 1959). Unless a careful regimen of increasing concentrations of hormone is followed (Kollros, 1961) metamorphic changes do not occur in proper sequence and assignment of stages becomes difficult. This disruption of the normal sequence of metamorphic

change is most pronounced in young tadpoles (Taylor and Kollros, 1946, stage X and under). If metamorphosis is induced in tadpoles which have not reached sufficient size to have adequate reserves to carry them over the non-feeding period, they quickly become moribund (Lewis and Frieden, 1959), and respiration data are meaningless for comparison with animals undergoing spontaneous metamorphosis. An unfortunate oversight in some studies is inadequate staging of the animals, making it impossible to compare one set of data with another. Still another problem arises when some tadpoles are fed and others are not. A standard practice in short term (5-7 days) studies of induced metamorphosis has been to withhold food from the animals during the course of the experiment regardless of whether or not they are still at feeding stages. Data from such experiments are not directly comparable to those from studies of spontaneous metamorphosis in which the tadpoles feed until the beginning of metamorphic climax.

The base line of activity against which oxygen uptake is being measured must also be explicitly stated. Etkin (1964) regrets that "modern studies use Warburg techniques involving the shaking of the animals instead of methods permitting a closer approach to basal conditions as stressed in earlier studies." The point is well taken, but "more constant results are frequently obtained by measuring metabolism at a fixed level of forced activity" (Prosser, 1961).

In a developing tadpole, weight increases until just before metamorphic climax, then begins to decrease about the time the animal stops eating. Tadpole weight also decreases with the hormone treatment used to induce metamorphosis. Oxygen uptake data calculated on the basis

of wet weight may not always reflect such weight changes. Therefore, data should be reported in more than one way for each experiment, e. g., oxygen uptake per unit dry weight and uptake per animal as well as the conventional oxygen uptake per unit wet weight (Etkin, 1934, 1964; Frieden, 1961).

Most studies of respiration during induced metamorphosis are not concerned with reproducing the normal sequence of metamorphic changes with gradually increasing amounts of hormone (Kollros, 1961; Bennett and Frieden, 1962). A single, relatively large dose of hormone is usually given. When metamorphic changes do not occur in the usual sequence, normal stages may be inadequate to express the appropriate changes (Bennett and Frieden, 1962). The most frequently used criteria for measuring morphological changes which are not in normal sequence are percent decrease in tail length or tail width, and the various length ratios of hind leg to tail, tail to body, and hind leg to body. Unfortunately, to measure any of these accurately requires either anesthesizing or killing the tadpoles. If anesthesized, another variable is introduced, and tadpoles can be killed for such measurements only if there is a most abundant supply.

Any study intended to monitor oxygen uptake during metamorphosis should include data from both larvae and adults taken while the animals are at a given level of activity, and data calculated against some standard other than wet weight (e. g., individual weight, dry weight, percent nitrogen). Larval stages should be clearly defined. Obviously, some of these criteria are easier to meet than others. When working with intact, unanesthesized animals it is virtually impossible to assure that all

were then reared to metamorphosis in the laboratory. The animals were kept in 8-inch finger bowls, the number per bowl depending upon individual tadpole size--from 100 per bowl at stage I, to 10 at stages X through XXV. Tadpoles survived best when kept in boiled tapwater at 15°C and fed a diet of boiled lettuce and Pablum. Except during the time oxygen uptake was being measured the tadpoles always had food available until they reached stage XX.

Field-collected tadpoles were obtained from a small stream in the bottom of the same ravine from which the adults had been collected. In nature it was observed that a considerable quantity of soil is normally ingested while feeding. This made freshly collected tadpoles appear to weigh considerably more than those of similar stages kept in the laboratory. To reduce this difference, field-collected animals were fed only Pablum for one day, then changed to the regular diet. After a maximum of three days white feces were observed, indicating that extraneous material had passed through the digestive tract. All other conditions were the same as for laboratory-raised tadpoles.

Leptodactylus podicipinus

Tadpoles were collected from a stream near the town of Fusugasuga, Colombia (elevation 5000 feet; mean annual temperature 20°C), and maintained at 20°C. Rearing conditions (diet, media and space) were the same as for the Phyllobates tadpoles.

Weights

Dessication is quickly fatal to larvae, so all tadpoles were weighed in water as follows: the prepared Warburg flask (without KOH)

at 10 minute intervals. In all cases data are expressed as µl O_2 per hour per gram wet weight, per gram dry weight, or per animal.

Shaking rate was constant at 80 per minute.

Partial Removal of Tail

Tadpoles in stages XI to XIII were used in those experiments involving removal of part of the tail. The tail was cut transversely to remove at least one half of its total length. Such a cut removes more than one half of the total fin surface, but less than half of the tail musculature (Fig. 9). All tadpoles in these experiments were kept in boiled tapwater which was changed daily, and were fed boiled lettuce and Pablum.

Induced Metamorphosis

As far as possible, tadpoles in early prometamorphosis (see Table 1) were selected for the studies on induced metamorphosis. However, the number of tadpoles of the desired stages which were available at any one time was limited, so some earlier or later stages were sometimes included, but none younger than stage X were used.

In the immersion experiments, tadpoles were maintained in a quantity of solution equivalent to 50 ml per tadpole during the whole period of the experiment. Solutions were changed daily.

In those experiments involving injection, tadpoles were injected intraperitoneally using a 27 gauge needle with 0.05 ml of amphibian Ringer's solution containing the amount of drug indicated in the data tables. Controls were injected with an equal volume of amphibian Ringer's.

In each of the four series of experiments (immersion and injection of T_4 and T_3), one experimental group (minimum of 20 animals) was

kept until metamorphosis was complete and the animals were observed to eat, and one group used to determine dry weight.

Hormones for induced metamorphosis (3'5-3 triiodothyronine Na and l-thyroxine) from Nutritional Biochemicals Corporation.

CHAPTER III

RESULTS

Life History and Morphological Changes

The genus Phyllobates does not occur in the United States and has not previously been used for studies in which staging of the tadpoles was necessary. Therefore, to make these data comparable to information from the most commonly used form in the United States, the staging terminology of Rana pipiens was followed as closely as possible. As outlined in Table I (modified from Etkin, 1964), premetamorphosis includes all stages to XII during which there is considerable growth and little external morphological change. During prometamorphosis the tadpoles continue to grow, but there is significant differential development of parts, especially hind legs. Metamorphic climax refers to the last five stages during which the tadpoles rapidly complete their transformation from aquatic to land living animals.

In popular terminology Phyllobates is closer to a "toad" than it is to a "frog". It is ground dwelling, largely diurnal, and unless desiccated the adults rarely enter water, although they live in an extremely moist habitat. Eggs are laid in damp cavities in the ground. When the larvae hatch they are carried on the backs of males to water where larval development takes place. This attachment to the male was observed to

TABLE I

Metamorphic Periods of Phyllobates

Period	Stages Included	Gross Morphological Characteristics
Premetamorphosis	Hatching through stage XII	Stage I. Carried by male; non-feeding; yolk. Stage II. Transition to feeding; weight increase slight. Stages III-XII. Increase in size; very slight development of hind legs.
Prometamorphosis	XIII through XX	Stage XVI. Maximum size; hind legs reach almost maximum development. Stage XX. Forelegs emerge; feeding stops.
Metamorphic climax	XXI through XXV	Completion of all metamorphic changes.

last as long as one week. Except that embryological development takes place on land, there is nothing unusual in the life cycle.

The eggs are large (2.5 mm diameter) and yolky, and less than 20 are laid at one time. In a two year period six clutches of eggs were obtained from the field. These were at various stages of development, the youngest in early gastrula. All such eggs were kept in the laboratory on moist filter paper, and the tadpoles placed in boiled tapwater as they hatched. Young from eggs were then maintained in the same way as other Phyllobates tadpoles. All eggs in these clutches were fertile, only 2 out of the total of 71 eggs failed to hatch, and all that did hatch completed metamorphosis. There was no evidence that attachment to the male is necessary for normal development.

Rana pipiens hatches at embryological stage 18 and begins feeding at stage 25 (Shumway, 1940). However, Phyllobates does not hatch until it has reached a degree of development equivalent to R. pipiens embryonic stage 25, and does not feed during larval stage I. For this genus stage I has been arbitrarily assigned as the time during which the tadpoles are normally attached to the male's back. Histological examination of tadpoles in stages I through III showed that they are abundantly supplied with yolk during stage I, this yolk supply is exhausted during stage II, but the animals do not begin to feed regularly until stage III. In the laboratory a small percent (less than 5%) of the tadpoles evidently never feed, becoming emaciated and dying within a week. Similar tadpoles were encountered in the field, but such animals were never used for respiration studies.

The Taylor and Kollros (1946) stages for Rana pipiens were used

to provide specific reference points for oxygen uptake. The early premetamorphic stages of R. pipiens are based primarily on degree of development of the hind limb buds, and those of metamorphic climax on mouth and tail changes. These morphological characteristics are quite similar in Phyllobates, so the Taylor-Kollros descriptions can be used to define stages I through X and stages XX through XXV (descriptions of these stages in Appendix I).

However, the intermediate stages of R. pipiens (XI through XIX) are based on development of digital tubercles which are absent in the genus Phyllobates. A system for staging this group was devised based on development and degree of angulation of the hind limbs, and development of the forelimbs (Fig. 1). Because this system did not require that the animals be examined individually under a microscope (see legend, Fig. 1), it allowed rapid separation into discrete groups. The only disadvantage was that in preserved tadpoles the hind limbs tend to straighten out, destroying the characteristic limb positions. However, the usefulness of the system was not affected for the work reported here which was concerned only with living animals.

The same staging system was used for all Phyllobates tadpoles, whether laboratory-raised or field-collected, and including those treated with thyroid hormones. Field collected tadpoles tended to weigh slightly more than laboratory-raised ones of equivalent stages, but weight alone does not affect the staging. For induced metamorphosis the concentrations of hormone used were small and the tadpoles were past stage X so that metamorphic changes occurred in the usual order, and staging of these animals was not difficult.

LEGEND, FIGURE I

Intermediate Metamorphic Stages of *Phyllobates subpunctatus* tadpoles

Stage X. Margin of the foot paddle indented between all five toes. Hind limbs not visible when animal viewed from above.

Stage XI. Hind limbs visible as very small projections when animal viewed from above.

Stage XII. Hind limbs visible as distinctly triangular projections when the animal is viewed from above.

Stage XIII. Hind limbs are sufficiently developed so that a space (arrow in illustration) is visible between knee and tail musculature. Foot held against tail musculature.

Stage XIV. Foot at about 45° angle to tail.

Stage XV. Leg and foot held at right angles to tail. Body smoothly rounded.

Stage XVI. Arm bulge (indentation just below middle of body, arrow in illustration) just beginning. Knees extend almost as far as edge of body.

Stage XVII. Arm bulge conspicuous.

Stage XVIII. Arms visible beneath skin; strong indentation below elbows. As in the corresponding Taylor-Kollros stage, the cloacal tail piece, present in the preceeding stages, has disappeared.

Stage XIX. Arm movement visible when animal is prodded.

Stage XX. One or both forelimbs have protruded.

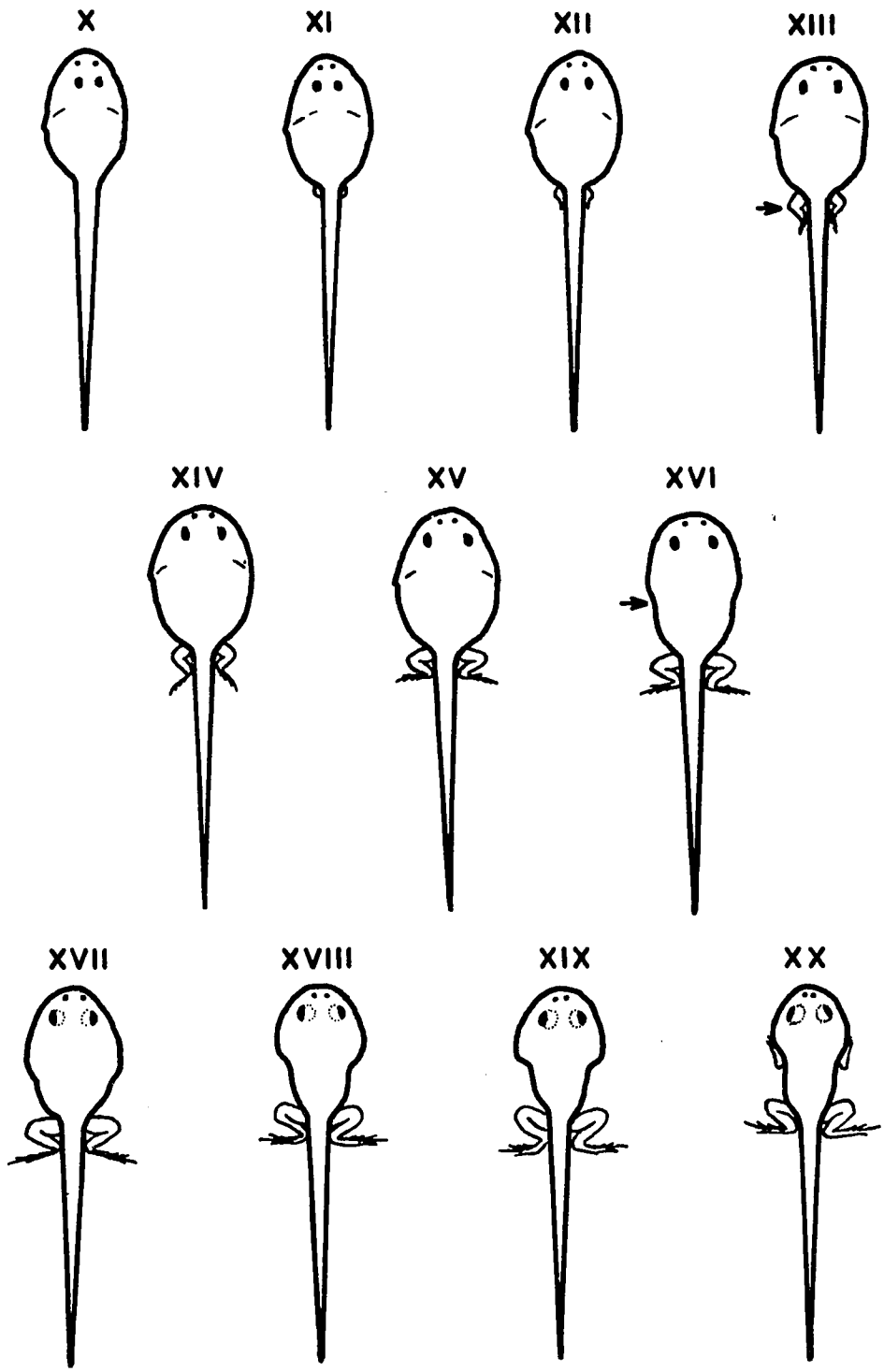

Figure 1

Intermediate stages of Phyllobates subpunctatus tadpoles

Under laboratory conditions, tadpoles removed from the backs of males (stage I) reach complete metamorphosis in four to five months, averaging about 140 days (Fig. 2). For a "normal" tadpole the rate of change from one stage to the next is slow during premetamorphosis, increases during prometamorphosis and slows again at the end of metamorphic climax. The most rapid rate of change occurs at the end of prometamorphosis and beginning of metamorphic climax.

A plot of weight change during larval development (Fig. 3) shows an increase to a maximum weight (in this case at stage XVI), followed by a decrease as the animal begins to utilize stored reserves and stops eating at stage XX.

Dry weight determinations (Fig. 4) showed that the percentage of water in the tadpoles decreased during development. In premetamorphic stages dry weight was fairly constant at 6-7% of total weight, increased to 10% by the end of prometamorphosis, and increased rapidly to 14% for newly transformed froglets. Variation in the percentage of dry weight of the tadpoles was never more than 2%. Dry weight of adults ranged from 20 to 25% of the wet weight.

Oxygen Uptake During Spontaneous Metamorphosis

The rate of oxygen uptake per gram wet weight at each stage in development is shown in Fig. 5. The shaded areas indicate one standard deviation above and below the arithmetic mean. From the earliest larval stage to complete metamorphosis there is a significant decrease in oxygen consumption per gram wet weight, amounting to over 50% from stage I to stage XXV.

Oxygen uptake determinations based only on wet weight make no allowances for possible changes in the percentage of water in the tissues. From the dry weight determinations it is evident that the percentage of water decreases significantly in the course of development, and that this decrease is more pronounced in the stages immediately preceeding the end of metamorphosis. When data for oxygen consumption are plotted as a function of dry weight (Fig. 6), the slope of the curve is greatly increased, showing even more clearly the decrease in oxygen uptake as metamorphosis proceeds. When calculated on the basis of dry weight, the total decrease in oxygen consumption from stage I to stage XXV is 80%.

A third type of plot is frequently used for this kind of information--oxygen uptake per animal per unit time. This is useful for short term experiments where animal weight does not vary significantly. However, in any developing tadpole, weight increases during the first part of development, then decreases during the later stages, particularly when the animal stops eating. The oxygen uptake per animal (Fig. 7) follows the weight curve closely, increasing as weight increases, and decreasing as weight decreases. There is a straight line relationship between oxygen consumption per unit time and wet weight (Fig. 8).

As a check on whether or not the observed decrease in oxygen uptake during advancing metamorphosis might be a peculiarity of this species, some measurements were made on the quite unrelated form, Leptodactylus podicipinus. It was impossible to obtain oxygen uptake information covering the whole larval period of this species because only a few tadpoles were available at widely different times of year. For this reason the animals could not be satisfactorily staged, and none were

sacrificed for dry weight determinations. Because no stages could be assigned, the abcissa of Fig. 9 is individual tadpole weight, which is shown as increasing to 0.5 g, then decreasing. The figure of 0.5 g was chosen because at this weight those animals with well developed hind legs are clearly separated from those in which the hind legs have not yet become prominent. The increasing then decreasing weight scale was used because it best represents the events of metamorphosis in lieu of proper stages. Despite the small number of measurements, the pattern of decreasing oxygen consumption during metamorphosis is even more striking than in Phyllobates.

Partial Removal of the Tail

There was no significant difference in oxygen uptake between control animals and those with part of the tail removed (Fig. 10). These experiments were of brief duration and the animals did not change more than one stage. Comparison with Fig. 3 shows that oxygen uptake of the Phyllobates tadpoles remained within the limits of two standard deviations of the original control group for the appropriate stages.

Oxygen Uptake During Induced Metamorphosis

Precocious metamorphosis was induced with T_4 and T_3 using both immersion and injection methods for each substance. In all cases a scarcity of tadpoles imposed severe limits on the number of experiments which could be done. There were never enough of any one stage to complete a series (3-4 tadpoles in each of 9 flasks). Since earlier determinations of oxygen consumption during spontaneous metamorphosis were essentially "controls" for this work, a larger number of experimental

than control animals was always selected.

In all cases data are expressed four ways; 1) oxygen consumption per gram wet weight per stage, regardless of day of treatment, 2) oxygen consumption per gram wet weight per day of treatment, regardless of stage, 3) oxygen consumption per animal per stage, and 4) oxygen consumption per animal per day of treatment.

Metamorphosis was accelerated by use of these substances as shown in Tables 2 and 3. In most cases the experimental tadpoles passed through at least twice as many stages during the course of the experiments as did the controls.

Both laboratory-raised and field-collected tadpoles were immersed in a solution of 10^{-7} M l-thyroxine. There was no difference in response between these two groups, nor was there a difference between thyroxine-immersed and control groups. When plotted by stage (Fig. 11A) the oxygen uptake in all groups was within two standard deviations of the normal oxygen consumption for the same stages during spontaneous metamorphosis (Fig. 5). (In Figs. 11 through 18 the shaded areas indicate the limits of 1 standard deviation above and below the mean for oxygen consumption during spontaneous metamorphosis as shown in Fig. 5). When oxygen uptake per gram wet weight is plotted against days of treatment (Fig. 11B) there is no significant change during the experimental period. As in spontaneous metamorphosis, oxygen consumption per animal is a function of weight, whether calculated by stage (Fig. 12A) or by days of treatment (Fig. 12B).

Thyroxine injected at the lower concentration (10^{-5} M) did not induce metamorphic change, but the higher concentration (10^{-3} M) induced

TABLE 2

Morphological Effects of T_4 Immersion and Injection on Phyllobates subpunctatus Tadpoles

	T_4 immersion 1:1,000,000			T_4 injection 1 x 10^{-5} M			T_4 injection 1 x 10^{-3} M		
A	B[1]	C	D	B[2]	C	D	B[3]	C	D
Control X	1	+15	6				2	+12	4
Expmtl. X	10	-30	20						
Control XI							1	+8	6
Expmtl. XI	9	-24	12				9	-31	16
Control XII				1	+4	3	3	+3	4
Expmtl. XII				2	0	4	8	-30	8
Control XIII	2	+5	4	1	+5	3	2	-2	5
Expmtl. XIII	9	-33	8	1	+7	4	8	-30	13
Control XIV	4	-19	4	1	+2	3	5	-15	5
Expmtl. XIV	8	-20	10	2	+1	4	9	-35	15
Control XV	4	-16	4				4	-17	6
Expmtl. XV	7	-31	6				6	-35	6
Control XVI	4	-19	6				3	-14	3
Expmtl. XVI	7	-36	12				7	-32	12
Control XVII	4	-23	6				3	-17	3
Expmtl. XVII	7	-39	8				6	-31	14
Control XVIII	4	-30	4						
Expmtl. XVIII	6	-31	6				5	-31	4
Control XIX	3	-31	3						
Expmtl. XIX	6	-30	4						
Control XX	3	-31	6						

A. Initial stage
B. Number of stages changed
   1. in 15 days
   2. in 7 days
   3. in 10 days
C. Percent weight change
D. Number of tadpoles

more rapid metamorphosis (Table 2) than did T_4 immersion. However, during the course of the experiment there was no significant increase in oxygen uptake. Oxygen consumption data plotted by tadpole stage (Fig. 13A) show moderately good agreement with the standards (Fig. 5), although stages XXI and XXII are somewhat high. When plotted as days after treatment (Fig. 13B) both experimental and control animals increase during the course of the experiment (see Chapter IV, Discussion). Data plotted as oxygen uptake per animal per stage (Fig. 14A) or per day of treatment (Fig. 14B) show changes in oxygen uptake associated with weight changes similar to those observed in the original untreated controls.

Oxygen uptake during T_3 immersion (10^{-7} M) does not indicate that this substance has a calorigenic effect on these tadpoles under these conditions. When data are plotted by stage (Fig. 15A) there is a fairly good agreement with standard oxygen uptake for the stages covered. Stage XII is low, but still within the limits of 1 standard deviation from the mean for normal tadpoles (Fig. 5). When data are plotted by time after immersion (Fig. 15B) there is a slight (11%) increase in oxygen uptake by day 7. Data plotted as uptake per animal per stage (Fig. 16A) or per animal per day of treatment (Fig. 16B) show the decreasing oxygen consumption associated with decreasing weight.

The only treatment which caused a significant increase in oxygen uptake was injection of T_3 (2×10^{-4} M). This increase does not show up when data are calculated per stage (Fig. 17A), but it is evident when plotted as time after injection (Fig. 17B). By day 5, oxygen uptake of experimental animals is 25% higher than that of the controls. As in previous experiments, uptake per animal per day of treatment (Fig. 18A) and

TABLE 3

Morphological Effects of T_3 Immersion and Injection on Phyllobates subpunctatus Tadpoles

	T_3 immersion 1×10^{-7} M			T_3 injection 2×10^{-4} M		
A	B[1]	C	D	B[2]	C	D
Control X				1	+10	4
Expmtl. X				12	-45	8
Control XI				3	+6	6
Expmtl. XI				11	-48	10
Control XII	2	+5	6	3	+5	6
Expmtl. XII	8	-47	18	10	-44	16
Control XIII	2	-6	8	6	-6	8
Expmtl. XIII	9	-46	12	9	-47	12
Control XIV	2	-16	6	5	-17	8
Expmtl. XIV	8	-47	10	8	-38	20
Control XV	4	-20	6			
Expmtl. XV	7	-45	15	7	-25	8
Control XVI	4	-30	6			
Expmtl. XVI	7	-47	9			

A. Initial stage
B. Number of stages changed
   1. in 10 days
   2. in 11 days
C. Percent weight change
D. Number of tadpoles

uptake per animal per stage (Fig. 18B) show decreasing oxygen consumption as weight decreases.

CHAPTER IV

DISCUSSION

In Phyllobates subpunctatus the wet weight of individual tadpoles increases from the time of hatching until the onset of metamorphosis, then decreases, especially during the non-feeding period of metamorphic climax (Fig. 3). Dry weight remains fairly constant during premetamorphosis, then increases with the greatest increase during metamorphic climax (Fig. 4). Similar patterns of wet and dry weight change have been reported for a variety of other species of tadpoles, and it is likely that these changes are characteristic of all apoda which do not have aberrant life histories (Brown, 1964).

Oxygen uptake per animal increases then decreases (Fig. 7), as individual wet weight increases then decreases (Fig. 3). Comparison of these two rates shows that there is a straight line relationship between them (Fig. 8). Thus oxygen uptake per animal in this species is a direct function of individual tadpole weight. This is in agreement with the generalization that total oxygen consumption of larger animals is greater than that of smaller ones.

Throughout the entire larval period oxygen uptake per gram of animal decreases from the time of hatching until metamorphic climax. This decrease is evident when oxygen consumption is considered per unit wet weight (Fig. 5) or per unit dry weight (Fig. 6). However, the percentage

of dry weight per gram more than doubles during larval life (from 6 to 14%). An increase in total serum protein has been reported for a number of species (Frieden, et al., 1957), as have increases in total nitrogen of a few specific tissues. Although the available information is sometimes conflicting, the phenomenon of increasing nitrogen during larval life is probably of general occurance in typical apoda larvae (Bennett and Frieden, 1959). If Phyllobates subpunctatus is typical in this respect, part of the increase in dry weight can be ascribed to an increase in body proteins.

Because oxygen uptake per gram is decreasing during larval life, a portion of this protein must be structural--i.e., not metabolically active. There are also increases in the amounts of non-protein structural material such as bone salts (Brown, 1964) which could account for some of the increase in dry weight. As the relative amount of such metabolically inactive substances increases, the oxygen consumption per gram tissue (measured as whole animal oxygen uptake) will decrease. The generalization that a gram of tissue from a small animal has a higher metabolic rate than a gram of tissue from a larger animal is true during the period when the tadpoles are increasing in size (premetamorphosis and early prometamorphosis), but not during the period when individual wet weight is decreasing (late prometamorphosis and climax). During these later periods oxygen uptake per gram wet or dry weight, or per animal, decreases as the individual tadpole weight decreases because of the disproportionately more rapid formation of metabolically inactive substances.

A similar decrease in oxygen consumption during larval development was observed in Leptodactylus podicipinus. Although it was not possible

to obtain information covering the whole larval period, the decrease in oxygen uptake per gram wet weight during that part of metamorphosis covered is even greater than in Phyllobates.

One of the reasons which has been used to support the assumption that oxygen consumption increases toward the end of metamorphosis is that such an increase would be of adaptive value to the terrestrial frog. Presumably the more active frog requires more oxygen than the less active aquatic tadpole. But any assumed relationship between oxygen uptake and environment is dangerous in the case of amphibia which are so imperfectly terrestrial. If we consider normal activity under natural conditions, most frogs are remarkably sedentary. In contrast, tadpoles are in almost constant motion. This difference could be related to the amount of food necessary to meet the energy requirements of the animal and the efficiency with which that food is digested. The bulk of the diet in these tadpoles was lettuce. In nature, the principle food for the adults is a small beetle (determined by examination of the feces). Assuming complete oxidation, lettuce provides 1.5 calories per gram and a hypothetical insect (average of several values from Spector, 1956) about 8.5 calories per gram. Fresh lettuce is about 95% water; the hypothetical insect is about 70% water. Thus a carnivorous frog may have to expend more energy to obtain a meal, but once obtained the caloric intake per unit volume is considerably greater than that of the vegetarian tadpole. Examination of the feces of both laboratory-raised and field-collected tadpoles showed that much of the food passes through the digestive tract in an essentially unaltered condition--i.e., cells and even large fragments are often quite intact. This is in contrast to frog feces in which only a few of the harder parts of insect

exoskeletons can be recognized. Perhaps the question is less one of changing environment than of a change from inefficient utilization of a low energy foodstuff (low energy in terms of the food value per unit volume) to efficient utilization of a high energy one.

The sharp decrease in QO_2 at stage II (Fig. 5 and Fig. 6) may be species specific in Phyllobates, and related to this early part of the life history. By definition stage I in this genus is the time during which the tadpoles are normally attached to the male's back. The observation that larvae can develop normally without attachment to the male indicates that the male functions only as a carrier from land to water. During stage I the larvae are utilizing yolk stored in the intestines. Oxygen consumption, highest during stage I, drops sharply during stage II as the animals become free-swimming, but are not yet feeding. Since yolk reserves are exhausted during stage II, this may be considered a period of fasting or semi-starvation. Oxygen consumption increases during stages III and IV as the animals begin feeding. but is not as great as during stage I. If the composition of yolk in amphibian eggs is similar to that of chicken eggs, the proportion of fat is considerably greater in yolk than in green vegetable material. The reverse proportions are true for carbohydrates. The caloric value of each unit of oxygen utilized is slightly less for fats than for carbohydrates. Therefore, to meet similar caloric requirements, more oxygen will be required for a high fat diet than for one proportionately higher in carbohydrates. It is possible that these early premetamorphic changes in oxygen uptake may be correlated with dietary changes. A more definite correlation could be established with information about RQ and nitrogenous end products (Brown, 1964).

During the remainder of premetamorphosis, and all of prometamorphosis, oxygen uptake per gram wet weight decreases only slightly. Dry weight increases during these periods, so oxygen uptake per gram dry weight decreases (Fig. 6). Other than development of the hind limbs in premetamorphosis, the animal is not changing except in size. As the name of the period implies, this is a time of preparation for the varied and rapid changes of climax. The absence of marked change in rate of oxygen utilization would be expected in the absence of marked physiological change.

An overall decrease in oxygen uptake was observed during metamorphic climax. The animals stop eating at stage XX, and depend upon stored reserves until the end of metamorphosis, so that climax is a fasting period. Since the animal is changing from strictly herbivorous to "carnivorous" in the sense of feeding upon its own reserves, the drop in oxygen consumption at the beginning of climax may be associated with this transition in type of food utilized. Current reviews indicate that the greatest number of both anabolic and catabolic processes are taking place during metamorphic climax. Structures in which tissue build-up predominates would include the developing limbs, eyelids and tongue, while tissue breakdown would occur in loss of the tail and gills. Simultaneous anabolism and catabolism are occurring in the alterations of the skin and pigments, in the liver, pancreas and intestine. Associated with these largely morphological changes are changes in virtually every enzyme system so far studied. (Literature reviewed in: Brown, 1964; Etkin, 1964; Bennett and Frieden, 1962). It is hardly to be expected that these transformations would not affect respiratory rate, quite likely some having a depressing and others a stimulating effect. It has been proposed that alternate metabolic pathways may be used

by developing tadpoles, especially during the critical period of metamorphic climax (Bennett and Frieden, 1962). However, measurement of whole animal oxygen consumption provides information only of the total respiration. To properly interpret the particular pattern of oxygen uptake observed during metamorphic climax (a decrease at stage XIX, followed by a rise and subsequent fall) would necessitate detailed information on the effect of each physiological change on oxygen consumption. At present, adequate information is not available for any amphibian species. Perhaps a clearer picture will emerge from study of specific fractions of the animal (Lewis and Frieden, 1959).

One such study of a particular tissue was that of Barch (1953) who established that there is a two-fold increase in metabolic rate of Rana pipiens skin during both normal and induced metamorphosis. This relates to the long-established idea that adult amphibian skin is an important respiratory organ. One might then hypothesize that the decreased oxygen uptake observed during metamorphic climax is related to the decrease in surface area of the animal which results from loss of the tail. To test this idea, a short series of experiments were performed on both Phyllobates subpunctatus and Leptodactylus podicipinus in which more than half of the tail was removed (Fig. 10). Tadpoles in mid-prometamorphosis were used for these experiments because oxygen consumption per gram wet weight is relatively constant at this time (Fig. 5).

An approximation of the surface area changes following this operation can be calculated by assuming that the body of the animal is a sphere with an arbitrary diameter of 1, and the tail a parallepiped whose width is equal to the diameter of the body and whose length is twice that of the

body. Using these numbers, the surface of the body is 3.1 and of the tail 4, for a total surface area of about 7. Removing half the tail would then be equivalent to removing 28% of the total surface area. The actual weight loss in the experimental animals following tail removal accounts for about 20% of the Leptodactylus total weight and about 5% of the Phyllobates total weight. Relating these weight changes to the arbitrary numbers for surface area, it is evident that if surface area of the animal plays a significant role in oxygen uptake, then after partial tail removal, QO_2 should decrease by at least 8% in Leptodactylus and 23% in Phyllobates. No change in QO_2 was found in either species (Fig. 10). Evidently surface area (as measured by skin area) cannot alone account for the decreased oxygen uptake observed during metamorphic climax.

Because removing the tail does not remove any organs or tissues of major metabolic significance, and in no way affects cellular oxygen utilization by the remaining tissues, the equivalent rates of oxygen uptake in tail-less and control animals might have been predicted. These results suggest an alternative hypothesis. It is possible that at the stages measured, the skin is not the principle breathing organ. If it were, some increase in fin circulation would be expected. The fin section of the tail in Phyllobates is very lightly pigmented, and although small increases in circulation would not be evident, any marked change would have been apparent by reddening of this almost colorless tissue. No color changes were observed, and the tails were carefully examined each day for evidence of regeneration. The major site of respiratory exchange is probably the gills, the skin being only a supplementary means of gas exchange during larval life.

For many years it has been known that thyroid hormones are necessary for amphibian metamorphosis. Direct measurement of circulating thyroxine in body fluids is difficult, but a variety of indirect methods have provided abundant evidence of changing thyroid activity during metamorphosis (Etkin, 1935, 1964; Dent and Hunt, 1952; Hunt and Dent, 1957; Kaye, 1961; Kollros, 1961; Saxen et al., 1957; Saxen, 1958; Steinmetz, 1954). The preceeding authors present clearcut evidence that thyroid activity in the variety of apoda larvae investigated is low during premetamorphosis, increases to a peak in late prometamorphosis, and then decreases during climax. If data can be compared between species, a similar pattern of thyroid activity may be assumed to occur in Phyllobates. The rate at which identifiable morphological change occurs (Fig. 2) parallels the changing levels of thyroid activity found in other species. However. the rate of oxygen uptake does not follow this pattern. Comparison of Fig. 2 and Fig. 5 shows that QO_2 decreases during the period of most rapid morphological change, which is the time that thyroid activity has been shown to be greatest in other species.

The high level of circulating thyroxine in late prometamorphosis and early climax, combined with the calorigenic effect of thyroxine in mammals has been used to support the assumption that larval respiratory rate must increase toward the end of metamorphosis (Frieden, 1961). However, relatively new work on mammals has indicated that "the effects of thyroxine on oxidative metabolism are secondary to its (stimulating) effects on protein synthesis" (Michels, et al., 1963). If a similar situation prevails in amphibia, it provides a rationale for the extremely high level of hormone necessary for completion of metamorphosis.

In summary, the respiratory activity of Phyllobates during spontaneous metamorphosis is as follows:

Premetamorphosis: Total decrease of about 30% in oxygen uptake per gram wet weight; 40% decrease per gram dry weight.

Prometamorphosis: No change in oxygen uptake per gram wet weight; 20% decrease per gram dry weight.

Metamorphic climax: Overall decrease of almost 30% in oxygen uptake per gram wet weight; 20% decrease per gram dry weight.

The tacit assumption by some authors that tadpole respiration increases toward the end of metamorphosis has received partial support from the increased respiratory rate observed in induced metamorphosis (Lewis and Frieden, 1959). Since respiratory rate in Phyllobates tadpoles drops during spontaneous metamorphosis, the question remains whether or not these tadpoles are capable of a calorigenic response to thyroid secretions. Although a variety of analogs have been demonstrated in vivo they are present only under special conditions or not at all in body fluids, and their action is qualitatively different from that of the thyronines found in the blood (Barker and Lewis, 1956; Frieden and Westmark, 1961).

Of the naturally occurring thyronines, T_3 has at various times been reported to have an activity of from 2 to 200 times that of T_4 (Dolphin and Frieden, 1955; Fraser, 1956; Kollros, 1958; Money et al., 1958). In an elegantly simple analysis, Frieden and Westmark (1962) showed that much of this difference (as well as the anamolous activity of other analogs) was due to the method of administration. As they point out, most studies have used response of the animal to immersion in a test solution, a procedure which is subject to a number of potential errors--relative rate of

penetration of the molecule, instability of the molecule and adsorption on glass in dilute solutions. Frieden and Westmark conclude that activity after injection gives a more valid measure of activity than do immersion tests. Injected T_3 is more active than T_4, but specifically how much more active depends upon what is being measured and in what animal. Even when considering induced amphibian metamorphosis, the value may differ depending upon the criterion used to measure degree of metamorphic change.

Despite the acknowledged drawbacks of the immersion technique, it has long been a standard method for inducing metamorphosis, so in the present study the respiratory activity of T_4 and T_3 was tested by immersion as well as by injection. These experiments were designed to test the effect of a specific concentration of the hormone on oxygen uptake. Therefore, a single dose was administered. Specific doses were chosen to coincide as closely as possible with already published data (Etkin, 1935; Fraser, 1956; Kollros, 1956, 1961; Lewis and Frieden, 1959).

To ascertain whether any observed changes in respiratory rate might be due to abnormal development, especially abnormalities severe enough to prevent eventual metamorphosis, one experimental group in each series was kept past stage XXV until they were observed to eat. Developmental rate was increased in all experimental animals, but oxygen consumption differed from the spontaneously metamorphosising controls only in the group injected with T_3. These animals were still alive two months after complete metamorphosis. This does not imply that metamorphic changes occurred in completely normal sequence in tadpoles in which induction was begun at relatively early stages (of necessity, a few as young as stages X-XI were used), but no abnormality prevented eventual development as a normal froglet.

Despite evidence that thyroidization of some species of tadpoles causes increased oxygen uptake, no increase was found in Phyllobates. When QO_2 after immersion or injection of T_4 is related to stage or to day of treatment, a normal pattern is observed (Fig. 11 and Fig. 13). Figure 13B appears to show an increase in oxygen consumption after injection of T_4, but both experimental and control rates increase. The experiment was repeated three times with similar results. Of necessity, field-collected tadpoles were used in this test. Control values for day zero were low in each case, and it is assumed that the tadpoles were not in prime condition when collected.

Comparison of oxygen uptake per animal with stage or day of treatment (Fig. 12 and Fig. 14) shows the same direct relationship between oxygen consumption and tadpole weight found in the spontaneously metamorphosizing animals.

Injected T_3 was the only experimental condition in which there was a significant increase in oxygen uptake. As previously reported by Lewis and Frieden (1959) for Rana grylio tadpoles, the maximum response occurred on the fifth day after treatment. Quantitatively the response was considerably less in Phyllobates (25% increase) than in Rana grylio (70% increase). This may be a species difference. Also, in Frieden's study the oxygen consumption of both experimental and control animals dropped markedly after the fifth day, perhaps "due to the effects of prolonged non-feeding". In the present experiments the animals were fed, and oxygen uptake of experimental tadpoles had dropped only to the control level by the eleventh day.

Unlike the results of Lewis and Frieden (1959), when oxygen uptake per animal is related to stage or to day of treatment the results are essentially those found in spontaneously transforming tadpoles. Oxygen uptake is related most directly to weight, not to the experimental variable.

A number of reasons could be proposed to explain the difference between these results and those of some other workers. Perhaps the most obvious is species difference. Most work in the United States has been done on a variety of ranas and bufos and on Xenopus laevis. Phyllobates belongs to the family Brachycephalidae, which is fairly closely related to the Bufonidae, but has no close relationship to the Ranidae or Pipidae (Noble, 1931).

Tissue dehydration (calculated on the basis of percent decrease in wet weight) was only around 30% after T_3 treatment of Phyllobates. Lewis and Frieden (1959) reported decreases of 40 to 50% in Rana. This may be the result of feeding in Phyllobates vs non-feeding in Rana. However, the same percent dehydration was observed in Phyllobates tadpoles in which metamorphosis was induced as late as stage XVIII. Such animals stop eating within a day or so after treatment begins, so ingested food can hardly be a factor in maintaining body water.

The carefully controlled experiments of Etkin (1935) and Kollros (1961) showed that a concentration of only 200 ppb T_4 is sufficient to cause complete metamorphosis in thyroidectomized tadpoles. The tissue level of circulating hormone is probably of the same order. Yet the concentrations used to demonstrate calorigenic response are of the order of 1000 ppb--five times higher than those which evidently occur naturally in the tadpole. It would appear that the calorigenic response in induced

CHAPTER V

SUMMARY AND CONCLUSIONS

Oxygen uptake per gram wet or dry weight decreases during spontaneous metamorphosis in Phyllobates subpunctatus tadpoles. The total decrease in oxygen consumption from stage I to stage XXV was 60% when determined on the basis of wet weight and 80% when determined on the basis of dry weight. A similar pattern of decreasing oxygen uptake during metamorphosis was observed in Leptodactylus podicipinus tadpoles. The specific decrease in Phyllobates at stage II is probably species specific, and related to a non-feeding period at stage II. In agreement with the reports of other workers, dry weight of these animals increased during the whole of metamorphosis, with the most pronounced increase during metamorphic climax. An increase in total nitrogen and inorganic substances such as bone salts has been reported to occur during this period in other tadpoles. The decrease in oxygen consumption during climax in this tadpole may partly be the result of increase in the proportion of metabolically inactive substances.

Comparison of individual tadpole weight and oxygen consumption per animal shows that oxygen uptake per animal in Phyllobates is a direct function of individual tadpole size.

Oxygen uptake was measured in mid-prometamorphic Phyllobates and Leptodactylus tadpoles in which part of the tail had been removed. Relating surface area changes to weight changes caused by this operation, oxygen

uptake should have decreased from 8 to 20%. No difference was found between control and experimental animals, indicating that some factor other than decreasing surface area is probably responsible for the decreased oxygen consumption which occurs during the later part of metamorphosis.

During induced metamorphosis, Phyllobates tadpoles showed no calorigenic response to immersion in thyroxine or triiodothyronine, or to injection of thyroxine. A small increase (25%) in respiratory rate occurred after triiodothyronine injection. The concentrations used to induce metamorphosis were not strong enough to produce abnormal development, but did significantly increase the rate of development. The concentration of hormone required to produce calorigenic effects is greater by at least five times than that reported by other workers to be normally present in the tissues at any time during metamorphosis. It would appear that the increased oxygen uptake observed during induced metamorphosis is an artificial response.

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APPENDIX I

Descriptions of Larval Stages of Phyllobates subpunctatus

Except for references to webs (absent in the genus Phyllobates) the following stages II through X are quoted directly from Taylor and Kollros (1946), and stages XX through XXV are adapted from the same source. Illustrations for these stages will be found in the Taylor and Kollros reference.

Premetamorphic stages

Stage I. The stage during which the tadpole is carried by the male. Labial teeth are present, but the animals do not feed. The whole ventral surface of the body forms a sucker, which disappears by stage II.

Stage II. The height of the limb bud elevation (length of the limb bud) is equal to one-half of its diameter.

Stage III. The length of the limb bud is equal to its diameter.

Stage IV. The length of the limb is equal to one and one-half times its diameter.

Stage V. The length of the limb bud is twice its diameter. The distal half of the bud is bent ventrad. There is no flattening of the tip.

Stage VI. The distal end of the limb bud is flattened medio-laterally to form the foot paddle. There are no interdigital indentations of the paddle margin.

Stage VII. The fourth and fifth toe prominences are separated by a slight indentation of the margin of the foot paddle.

Stage VIII. The margin of the foot paddle is indented between toes 5-4 and 4-3.

Stage IX. The margin of the foot paddle is indented between toes 5-4, 4-3, and 3-2.

Metamorphic climax

Stage XXI. The angle of the mouth has reached a point midway between the nostril and the anterior margin of the eye.

Stage XXII. The angle of the mouth has reached the level of the middle of the eye. The tail is shorter than the extended hind limb.

Stage XXIII. The angle of the mouth has reached the level of the posterior margin of the eyeball. The tail is about equal to the length of the femur.

Stage XXIV. The angle of the mouth reaches well behind the posterior margin of the eyeball. A stub of dark tissue is all that remains of the tail. At this stage the young will pursue small insects.

Stage XXV. The tail is completely gone, and no dark tissue remains.

APPENDIX 2

Data for Figures 2 - 4

Phyllobates subpunctatus tadpoles

1	2	3	4	5	6	7
Stage	Total number of animals	Number of measure-ments	Mean age in days	Weight in grams mean	range	Per cent dry weight
I	215	10	1	.02	.01-.02	6
II	289	9	5	.02	.02-.03	6
III	325	21	12	.03	.03-.05	6
IV	123	12	23	.06	.03-.08	6
V	155	17	38	.10	.09-.19	*6
VI	129	16	52	.12	.09-.19	6
VII	159	19	66	.14	.10-.33	*6.2
VIII	133	20	78	.15	.10-.33	*6.5
IX	62	10	86	.16	.11-.23	*6.7
X	56	14	94	.21	.18-.23	7
XI	56	13	100	.24	.20-.27	*7
XII	78	20	105	.25	.20-.33	*7
XIII	69	20	109	.25	.20-.29	7
XIV	53	18	113	.28	.20-.32	*7.5
XV	50	16	116	.29	.20-.33	8
XVI	42	15	119	.31	.21-.38	*8.5
XVII	45	15	122	.27	.19-.35	9
XVIII	24	9	124	.27	.23-.34	*9.3
XIX	28	10	126	.26	.20-.31	*9.6
XX	36	13	128	.24	.17-.34	10
XXI	59	18	129	.19	.17-.25	*10.5
XXII	45	15	131	.19	.15-.22	11
XXIII	47	15	133	.19	.12-.29	*11.5
XXIV	45	12	135	.16	.10-.20	12
XXV	46	10	140	.13	.09-.18	14
Adults	200	200				20

* = interpolated values

APPENDIX 3

Data for Figures 5 - 8

Phyllobates subpunctatus tadpoles

1	8	9	10	11
Stage	Mean µl O_2 per animal per hour	Mean ul O_2 per gram wet weight per hour	Standard deviation of column 9	µl O_2 per gram dry weight per hour
I	2.9	152	11.3	2530
II	2.2	105	8.1	1751
III	4.1	113	23.6	1880
IV	9.7	135	14.4	2252
V	12.8	124	21.8	2074
VI	14.5	132	16.3	2202
VII	14.9	126	13.4	2038
VIII	16.3	125	16.0	1924
IX	18.3	126	13.9	1866
X	21.8	108	18.5	1544
XI	23.7	114	11.9	1624
XII	27.6	108	10.5	1540
XIII	28.6	106	13.2	1512
XIV	30.1	105	13.9	1400
XV	32.9	105	11.5	1310
XVI	34.2	107	12.6	1254
XVII	29.7	105	13.4	1172
XVIII	30.3	106	13.1	1138
XIX	30.4	107	13.5	1112
XX	29.9	97	19.4	970
XXI	21.5	80	10.9	760
XXII	20.4	86	17.4	780
XXIII	17.7	92	14.5	798
XXIV	14.6	93	13.3	778
XXV	10.0	70	13.0	502
Adults				350

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APPENDIX 5

Data for figure 10

A. *Phyllobates subpunctatus*

Days after tail removal	µl O_2/gram wet weight/hour			
	Control		Experimental	
	Mean	Range	Mean	Range
1	98	85-106	107	76-129
2	117	96-136	113	90-129
3	110	101-120	113	101-118
5	91	80-101	97	91-117

B. *Leptodactylus podicipinus*

Days after tail removal	µl O_2/gram wet weight/hour			
	Control		Experimental	
	Mean	Range	Mean	Range
-2	117	94-150	117	94-150
0	114	76-134	149	138-165
2	115	115	117	117
4	162	147-172	162	144-171
6	139	99-199	139	107-155

APPENDIX 6A

Data for Figures 10 - 11

T_4 immersion of Phyllobates subpunctatus tadpoles

Stage	No. mmts.		µl O_2/g wet wt/hour			
			Exp.		Control	
	Exp.	Conts.	Mean	Range	Mean	Range
X	17	3	108	104-110	108	104-110
XI	8	3	108	104-110	112	102-116
XII	8	4	110	106-114	112	100-116
XIII	10	5	106	90-112	100	98-103
XIV	11	5	109	104-114	99	96-102
XV	14	4	102	96-120	104	101-108
XVI	11	4	102	80-114	106	104-110
XVII	14	6	107	90-116	107	99-110
XVIII	14	6	109	103-113	107	103-116
XIX	20	6	104	90-109	103	95-106
XX	17	8	92	75-104	89	84-97
XXI	13	6	83	79-101	83	78-88
XXII	12	3	86	84-88	96	93-104
XXIII	6	2	94	92-96	100	96-104
XXIV	4	0	90	88-94	-	-
Day of treatment						
0	26	26	100	84-110	100	84-110
1	26	-	98	87-107	-	-
2	-	12	-	-	100	85-115
3	24	-	98	79-110	-	-
6	24	-	100	81-111	-	-
7	-	12	-	-	98	90-106
8	24	-	99	81-116	-	-
9	-	12	-	-	97	82-108
10	24	-	104	92-120	-	-
13	24	-	93	85-104	-	-
15	-	12	-	-	86	77-102

APPENDIX 6B

Data for Figures 10 - 11

T_4 immersion of Phyllobates subpunctatus tadpoles

Stage	µl O_2/animal/hour		Mean weight	
	Exp.	Control	Exp.	Control
X	19.0	19.8	.36	.35
XI	21.1	20.1	.38	.35
XII	20.3	19.9	.37	.36
XIII	20.1	20.7	.37	.37
XIV	20.8	22.1	.40	.40
XV	23.2	25.6	.42	.39
XVI	22.8	27.3	.44	.41
XVII	24.0	30.1	.43	.41
XVIII	24.8	24.1	.44	.41
XIX	22.7	20.6	.37	.38
XX	18.6	22.7	.34	.38
XXI	14.5	17.6	.33	.36
XXII	13.5	18.9	.31	.35
XXIII	12.4	15.2	.28	.32
XXIV	11.1	-	.26	-
Day of treatment				
0	22.3	22.3	.47	.45
1	22.2	-	.47	-
2	-	21.5	-	.44
3	20.6	-	.42	-
6	19.8	-	.37	-
7	-	10.1	-	.40
8	20.1	-	.34	-
9	-	17.3	-	.39
10	19.6	-	.32	-
13	17.3	-	.29	-
15	-	15.5	-	.35

APPENDIX 7A

Data for Figures 12 - 13

T_4 injection

Phyllobates subpunctatus tadpoles

Stage	No. mmts.		µl O_2/g wet wt/hour			
			Exp.		Control	
	Exp.	Conts.	Mean	Range	Mean	Range
XI	5	4	110	106-114	109	102-115
XII	0	8	-	-	115	111-117
XIII	11	8	108	90-124	97	87-118
XIV	10	10	101	86-118	96	90-103
XV	8	9	108	90-118	102	90-110
XVI	7	6	99	96-101	96	82-103
XVII	10	0	112	108-118	-	-
XVIII	10	0	113	109-117	-	-
XIX	11	0	110	91-123	-	-
XX	6	9	103	87-123	102	98-106
XXI	4	0	107	90-120	-	-
XXII	4	0	105	86-138	-	-
Day of treatment						
0	21	21	87	70-111	87	87-115
2	6	3	96	90-114	100	82-109
3	18	9	99	88-118	100	96-110
5	12	6	113	86-123	113	90-117
6	12	6	112	88-118	-	-
7	18	9	116	90-138	114	90-118

APPENDIX 7B

Data for Figures 12 - 13

T_4 injection

Phyllobates subpunctatus tadpoles

Stage	µl O_2/animal/hour		Mean Weight	
	Exp.	Control	Exp.	Control
XI	21.6	22.1	.33	.33
XII	-	23.5	-	.32
XIII	23.6	24.0	.31	.33
XIV	24.2	26.7	.33	.34
XV	24.3	28.2	.29	.35
XVI	28.0	30.3	.36	.38
XVII	24.7	-	.32	-
XVIII	24.0	-	.30	-
XIX	23.6	-	.28	-
XX	22.0	26.8	.24	.30
XXI	20.1	-	.21	-
XXII	17.5	-	.20	-
Day of treatment				
0	23.7	23.9	.37	.37
2	22.4	24.2	.32	.35
3	21.4	22.1	.31	.35
5	21.9	20.3	.28	.33
6	18.3	-	.27	-
7	17.0	19.5	.20	.32

APPENDIX 8A

Data for Figures 14 - 15

T_3 immersion

Phyllobates subpunctatus tadpoles

Stage	No. mmts.		µl O_2/g wet wt/hour			
			Exp.		Control	
	Exp.	Conts.	Mean	Range	Mean	Range
XII	10	7	96	93-98	97	94-99
XIII	6	8	98	88-102	100	93-105
XIV	16	10	96	79-106	101	94-113
XV	10	11	100	97-103	101	81-110
XVI	20	9	106	92-129	110	103-117
XVII	8	-	104	94-124	-	-
XVIII	12	-	111	99-123	-	-
XIX	16	-	114	99-124	-	-
XX	10	-	110	96-129	-	-
XXI	-	-	-	-	-	-
XXII	10	-	102	98-105	-	-
XXIII	8	-	105	101-117	-	-
Day of Treatment						
0	18	18	99	93-119	99	93-119
1	18	-	94	79-105	-	-
2	18	-	100	83-113	-	-
3	18	-	104	82-121	-	-
4	18	9	109	99-124	102	100-113
7	18	9	117	96-123	104	94-112
10	18	9	108	91-124	99	81-117

APPENDIX 8B

Data for Figures 14 - 15

T_3 immersion

Phyllobates subpunctatus tadpoles

Stage	µl O_2/animal/hour Exp.	Control	Mean Weight Exp.	Control
XII	25.5	29.2	.39	.36
XIII	20.6	25.6	.35	.36
XIV	20.3	24.9	.34	.37
XV	23.4	25.8	.36	.40
XVI	27.9	22.7	.40	.41
XVII	28.5	-	.34	-
XVIII	22.1	-	.22	-
XIX	15.6	-	.21	-
XX	18.6	-	.25	-
XXI	-	-	-	-
XXII	15.5	-	.21	-
XXIII	11.3	-	.20	-
Day of treatment				
0	30.8	30.8	.45	.45
1	22.6	-	.35	-
2	19.1	-	.34	-
3	24.0	-	.35	-
4	22.7	31.0	.29	.39
7	19.1	31.0	.24	.39
10	12.5	30.8	.20	.36

APPENDIX 9A

Data for Figures 16 - 17

T_3 injection

Phyllobates subpunctatus tadpoles

Stage	No. mmts.		µl O_2/g wet wt/hour			
			Exp.		Control	
	Exp.	Cont's.	Mean	Range	Mean	Range
X	4	-	100	83-117	-	-
XI	6	11	110	70-124	104	70-114
XII	6	-	103	88-121	-	-
XIII	12	12	97	81-116	106	79-120
XIV	8	-	104	-	-	-
XV	14	14	100	92-115	97	89-111
XVI	11	-	104	77-113	-	-
XVII	15	-	97	90-110	-	-
XVIII	6	-	112	86-119	-	-
XIX	11	8	107	95-124	111	94-126
XX	-	-	-	-	-	-
XXI	-	-	-	-	-	-
XXII	15	-	86	71-111	-	-
Day of treatment						
0	18	18	79	70-116	79	70-114
1	18	-	86	71-115	-	-
3	12	9	-	-	85	79-126
4	18	-	109	83-124	-	-
5	18	-	120	86-124	-	-
6	18	-	115	88-121	-	-
7	-	9	-	-	97	94-120
11	12	9	97	86-111	98	89-111

APPENDIX 9B

Data for Figures 16 - 17

T_3 injection

Phyllobates subpunctatus tadpoles

Stage	µl O_2/animal/hour Exp.	Control	Mean Weight Exp.	Control
X	22.7	-	.45	-
XI	21.5	22.3	.40	.42
XII	22.8	-	.39	-
XIII	19.2	24.7	.47	.45
XIV	18.0	-	.48	-
XV	19.1	25.8	.44	.46
XVI	21.6	-	.42	-
XVII	22.0	-	.41	-
XVIII	22.0	-	.34	-
XIX	18.9	19.9	.31	.40
XX	-	-	-	-
XXI	-	-	-	-
XXII	13.5	-	.29	-
Day of treatment				
0	19.4	20.2	.50	.49
1	19.6	-	.45	-
3	-	21.7	-	.47
4	20.7	-	.41	-
5	22.0	-	.37	-
6	19.8	-	.35	-
7	-	18.9	-	.46
11	13.3	16.7	.28	.45

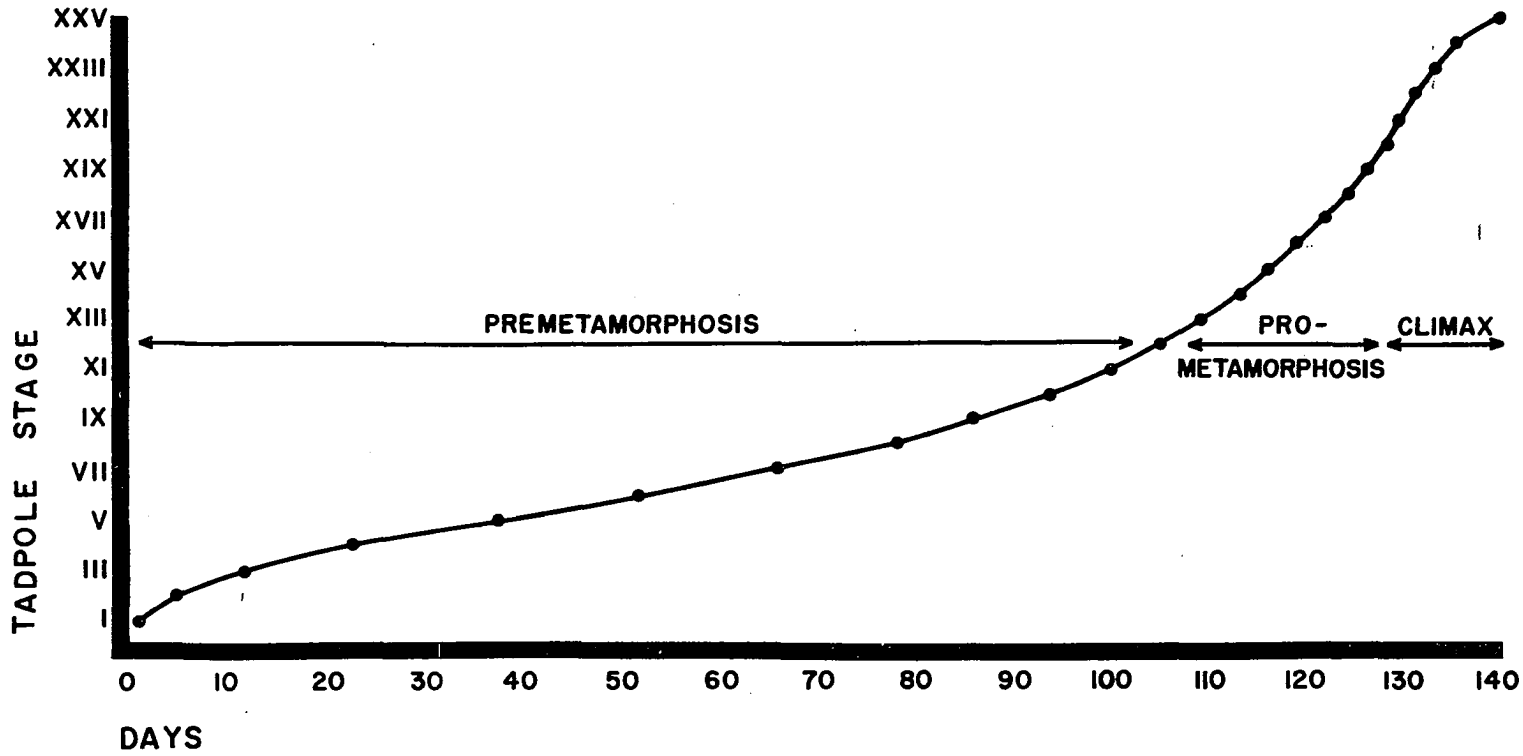


Figure 2

Larval development in Phyllobates subpunctatus.

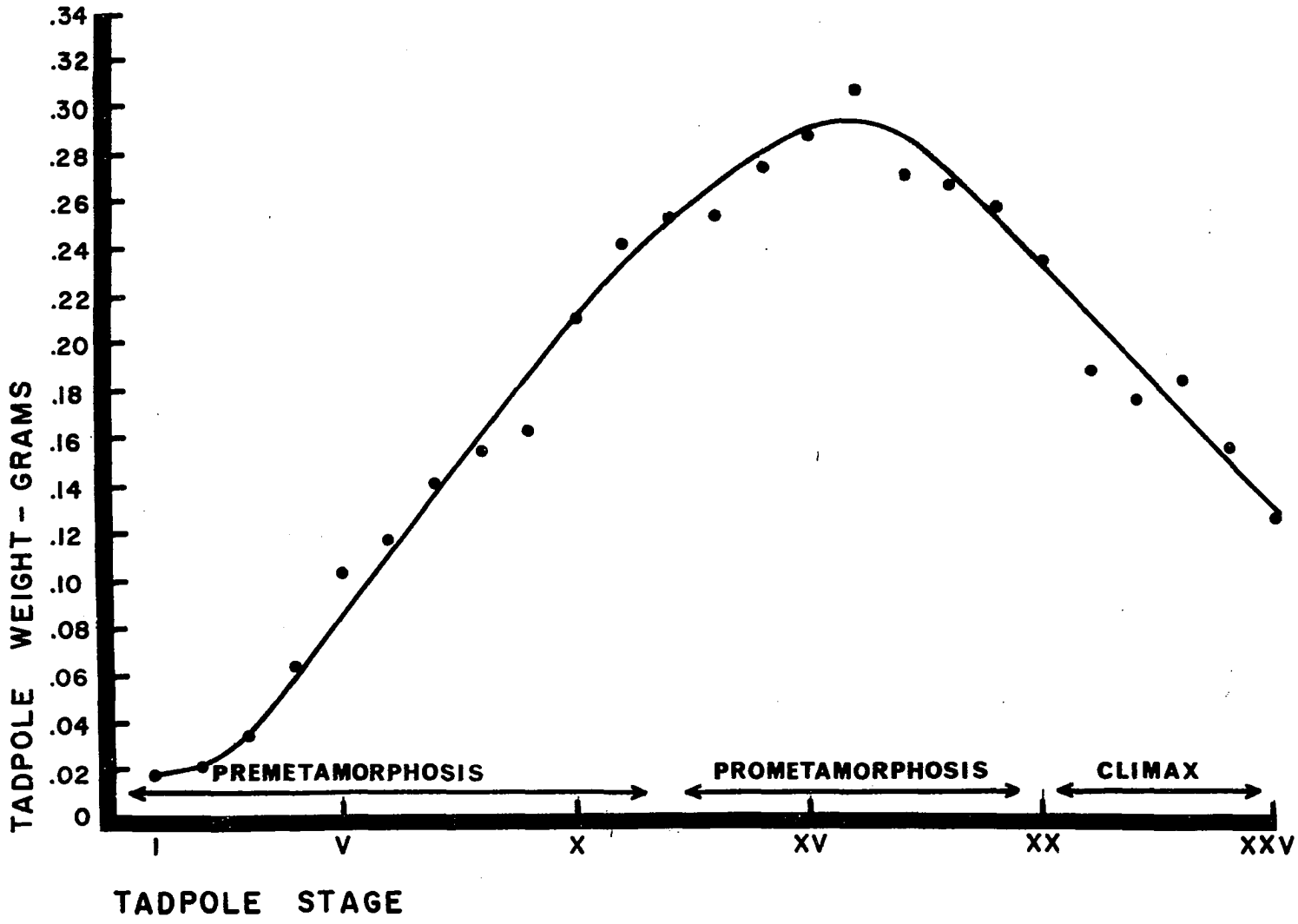


Figure 3

Mean weight of individual Phyllobates subpunctatus larvae.

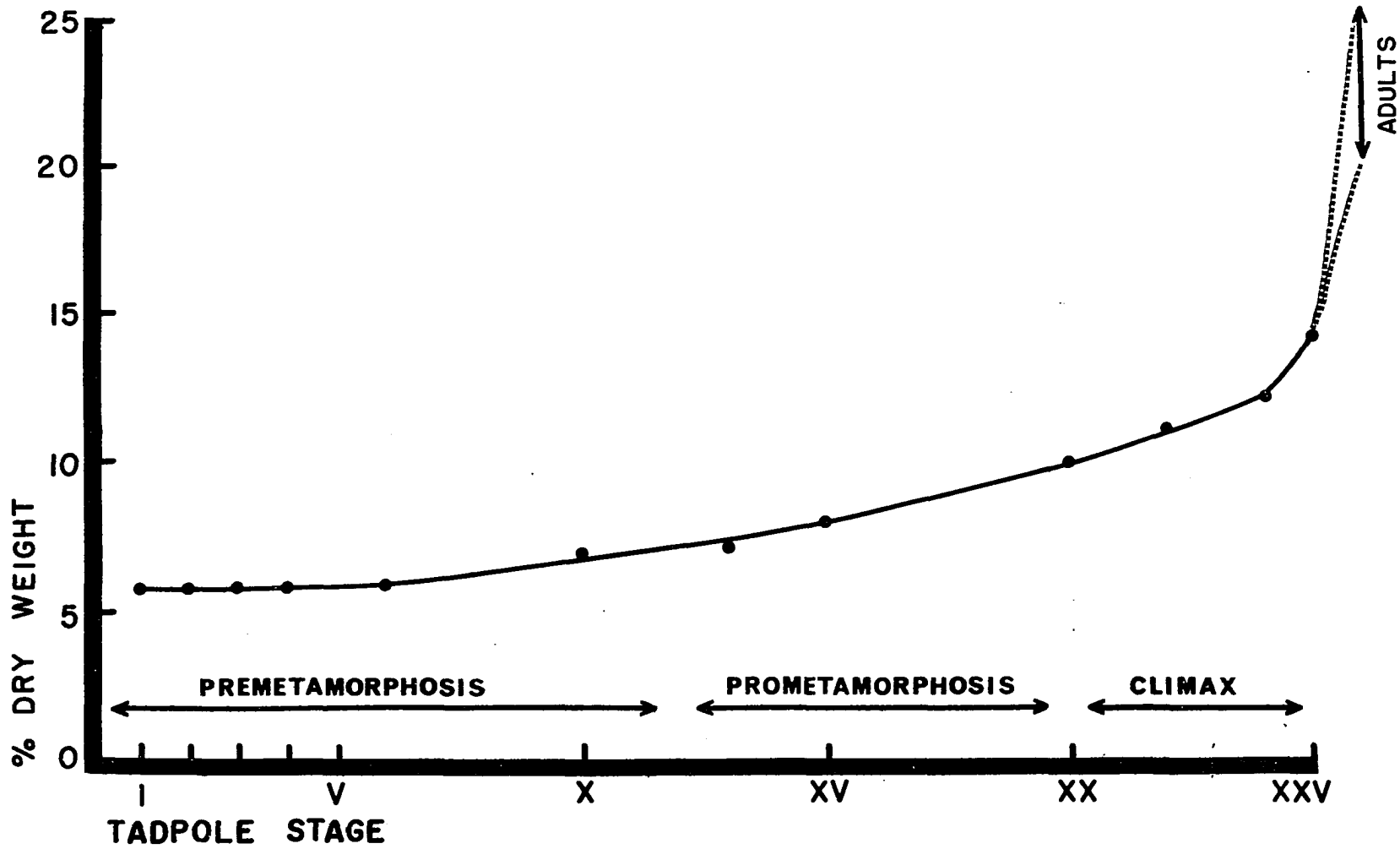


Figure 4

Mean dry weight changes in Phyllobates subpunctatus.

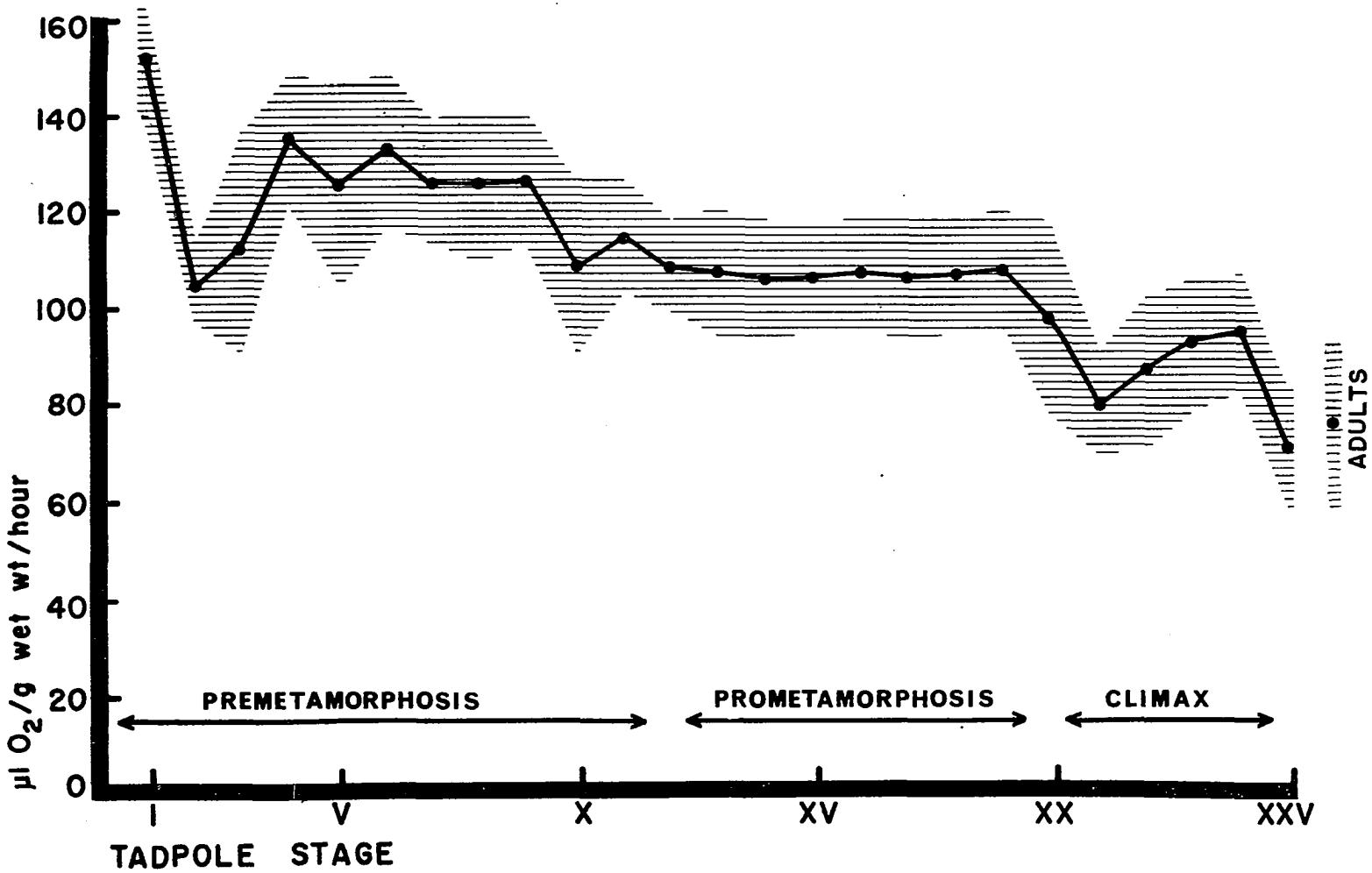


Figure 5

Oxygen uptake in Phyllobates subpunctatus.
Shaded area indicates one standard deviation above and below the mean.

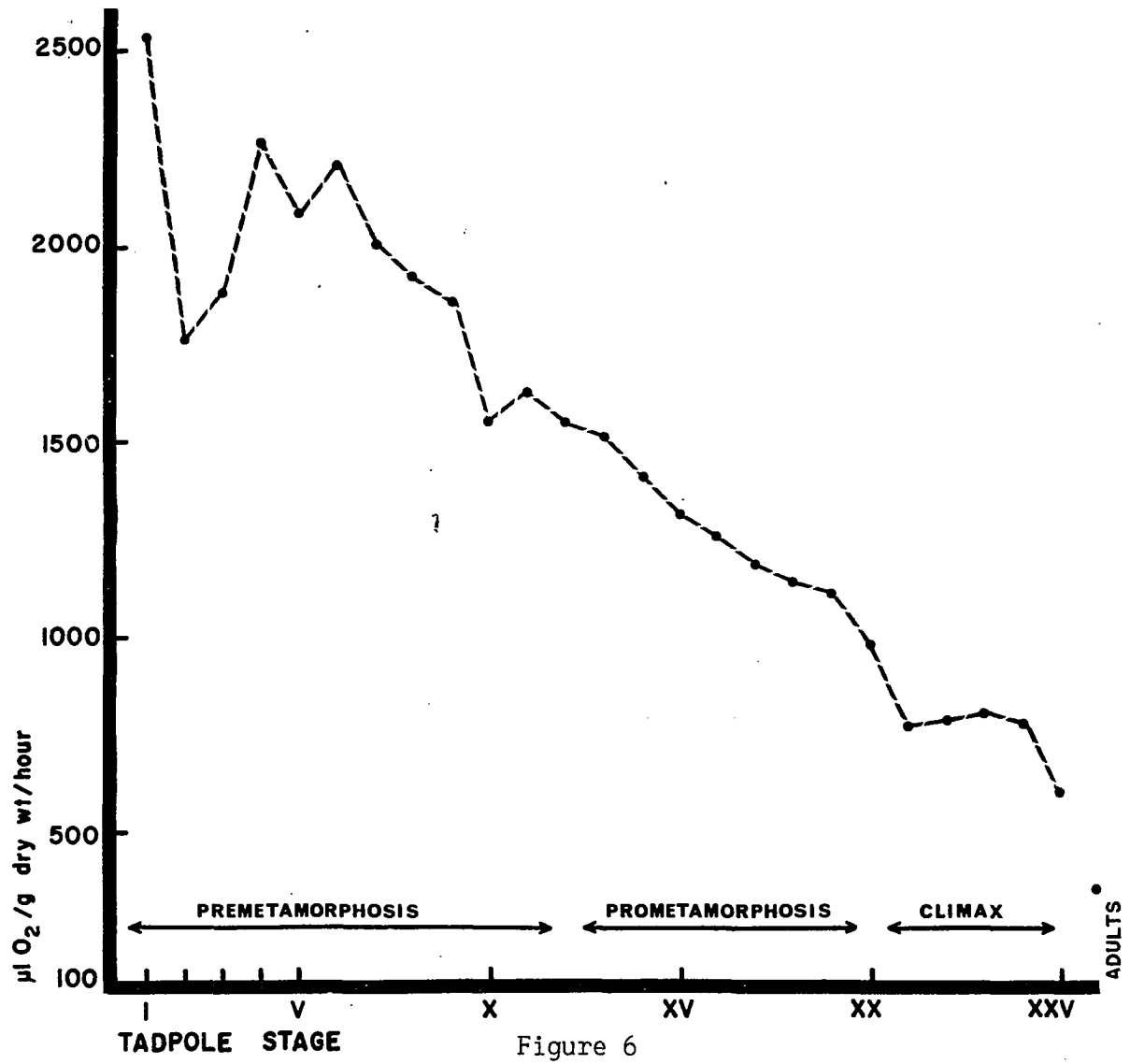


Figure 6

Oxygen uptake in Phyllobates subpunctatus.

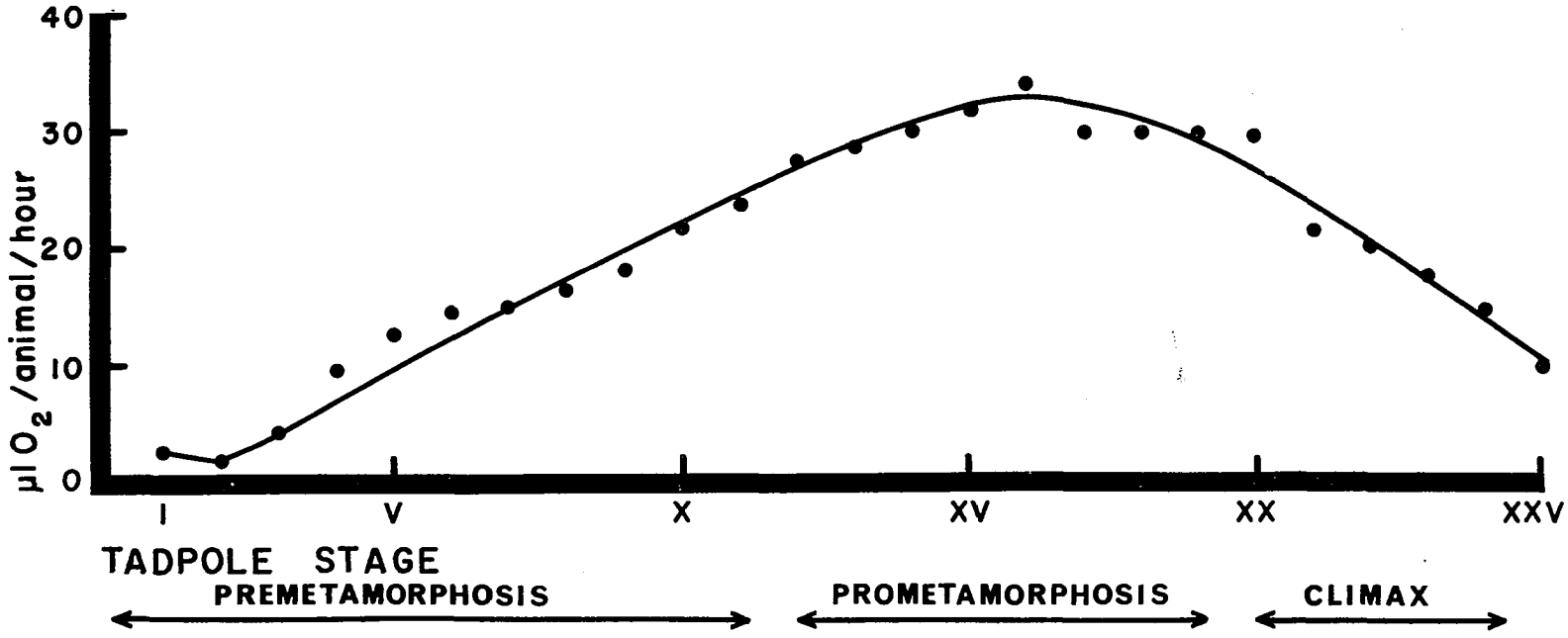


Figure 7

Mean oxygen uptake per animal in Phyllobates subpunctatus larvae.

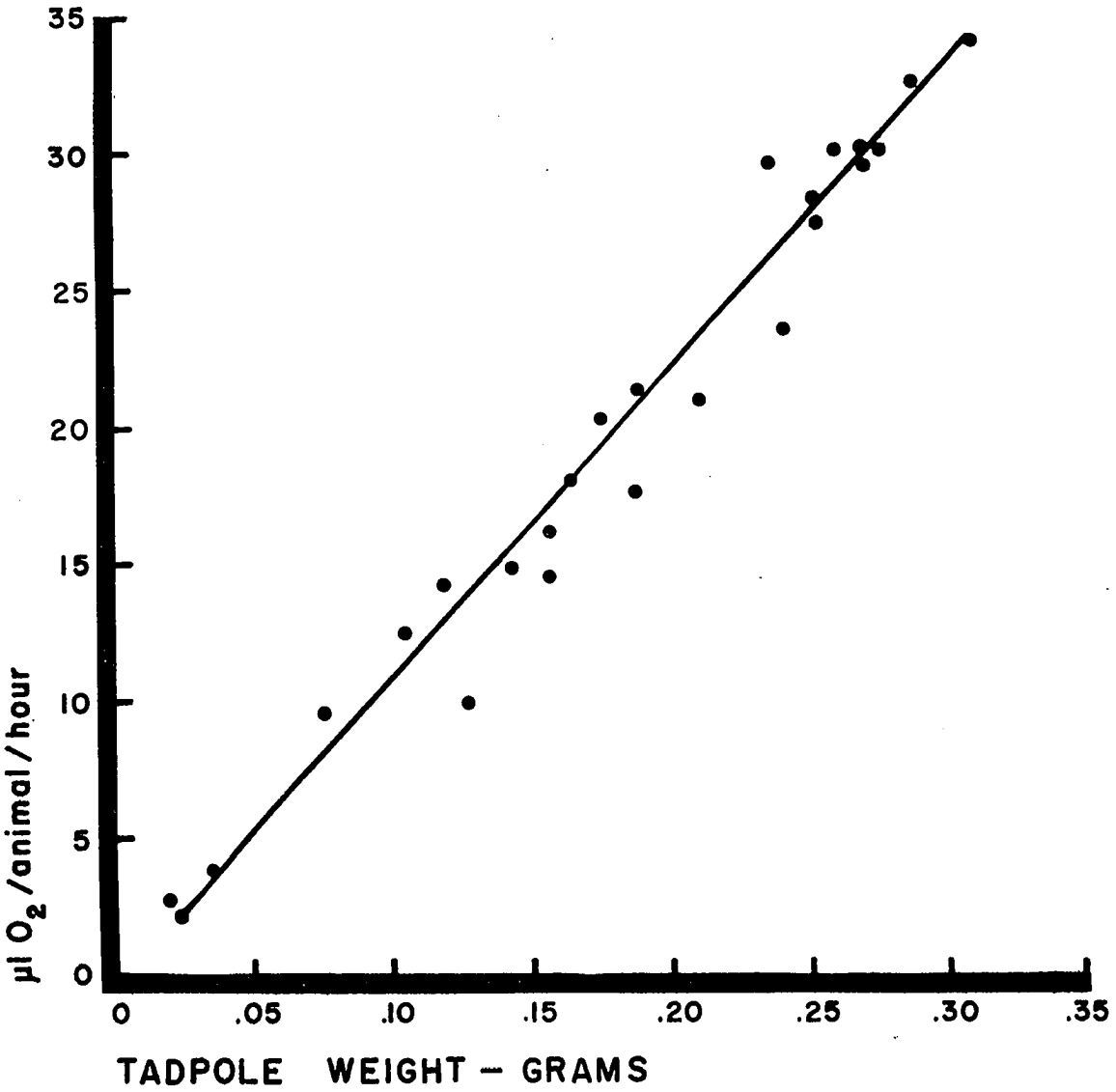


Figure 8

Oxygen uptake per animal in Phyllobates subpunctatus larvae plotted as a function of individual tadpole weight.

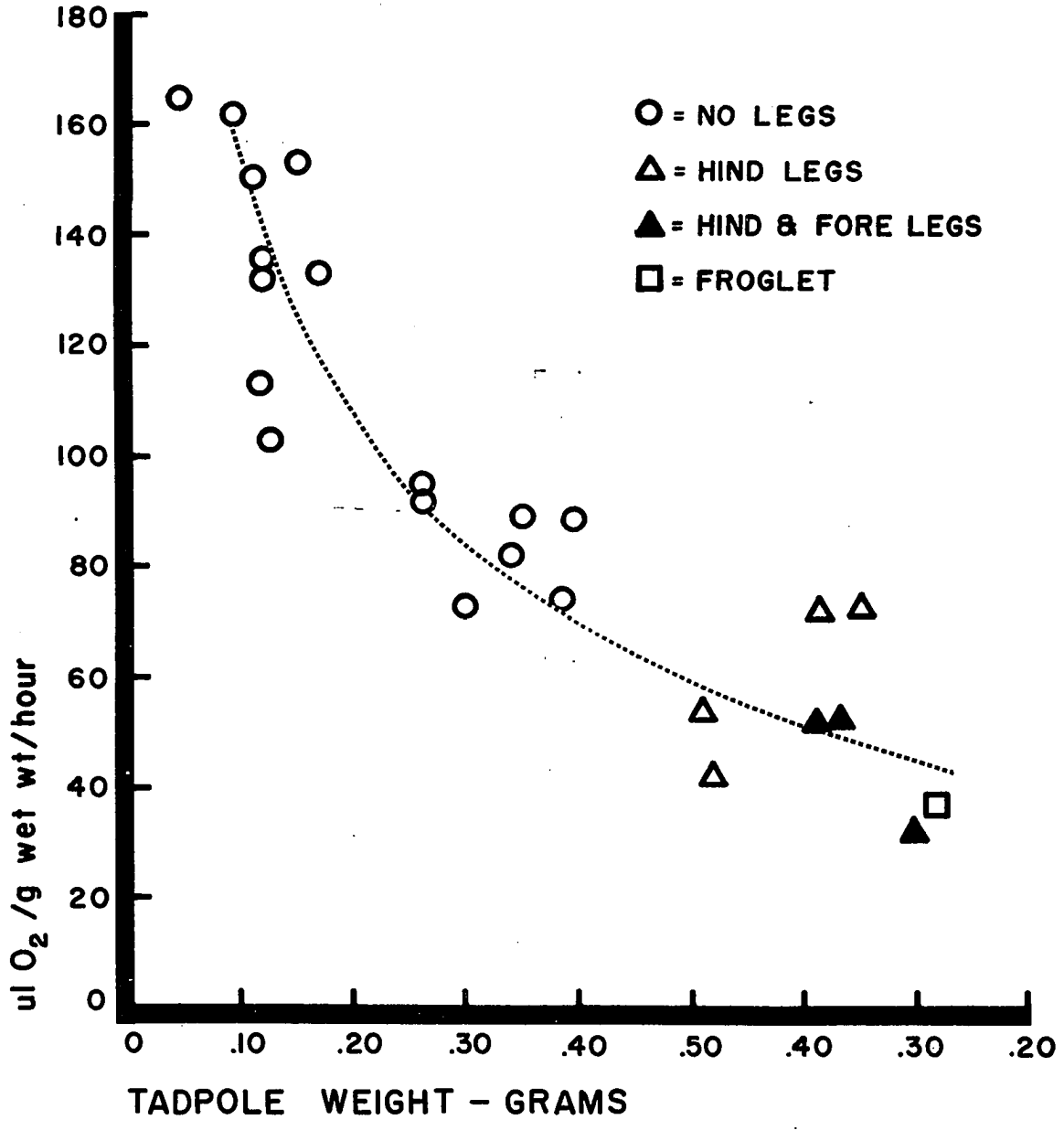


Figure 9

Oxygen uptake in Leptodactylus podicipinus larvae.
See text for explanation of abscissa.

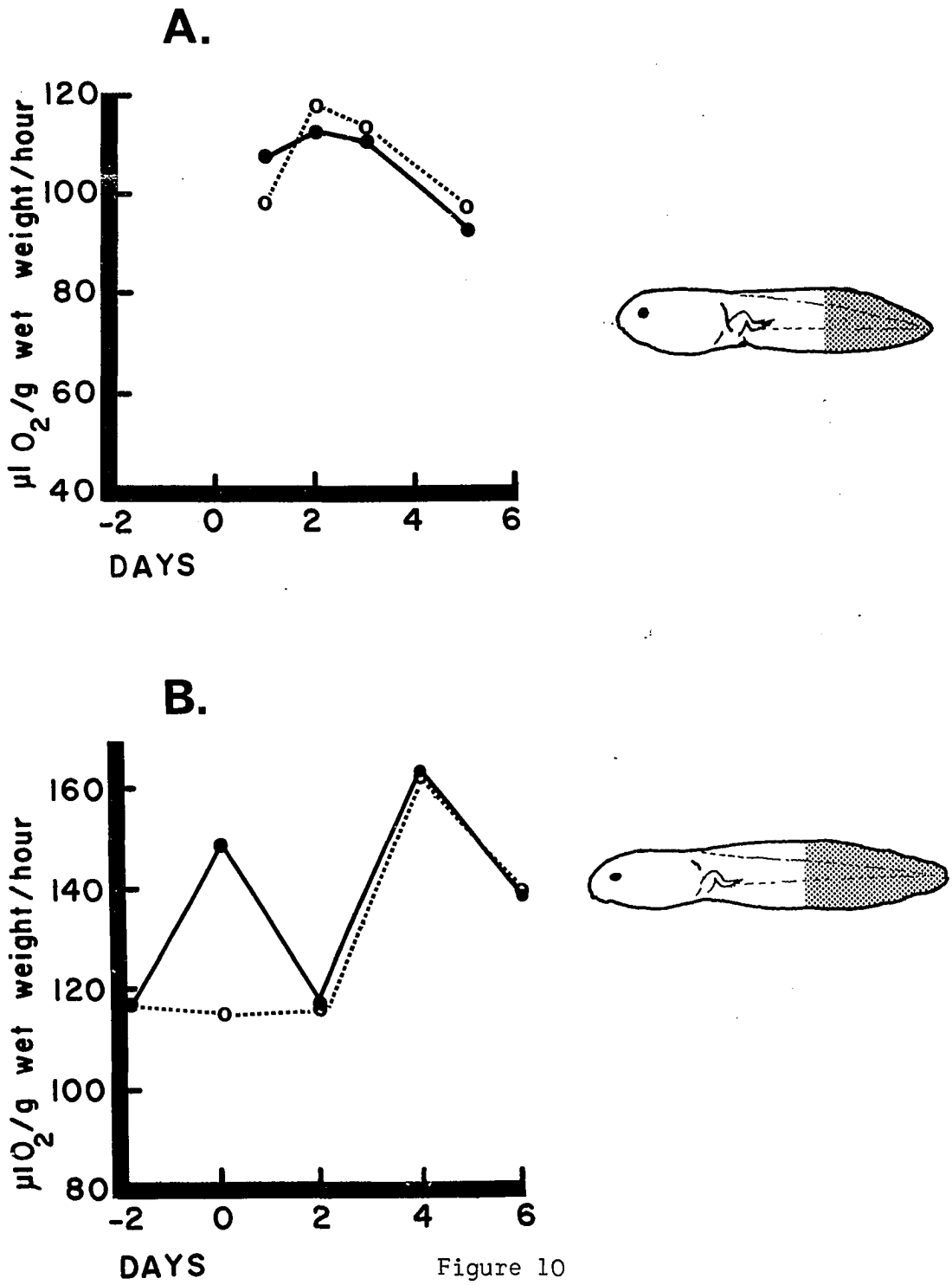
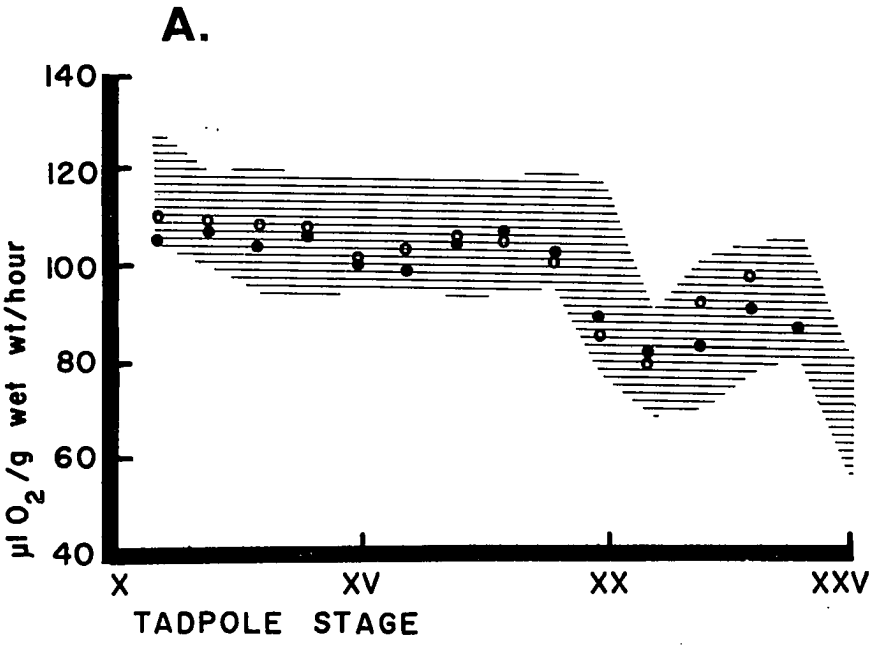


Figure 10

Mean oxygen uptake in tadpoles with part of tail removed at 0 days.
A. Phyllobates subpunctatus **B. Leptodactylus podicipinus**
Shaded area of diagrams to right indicate amount of tail removed.



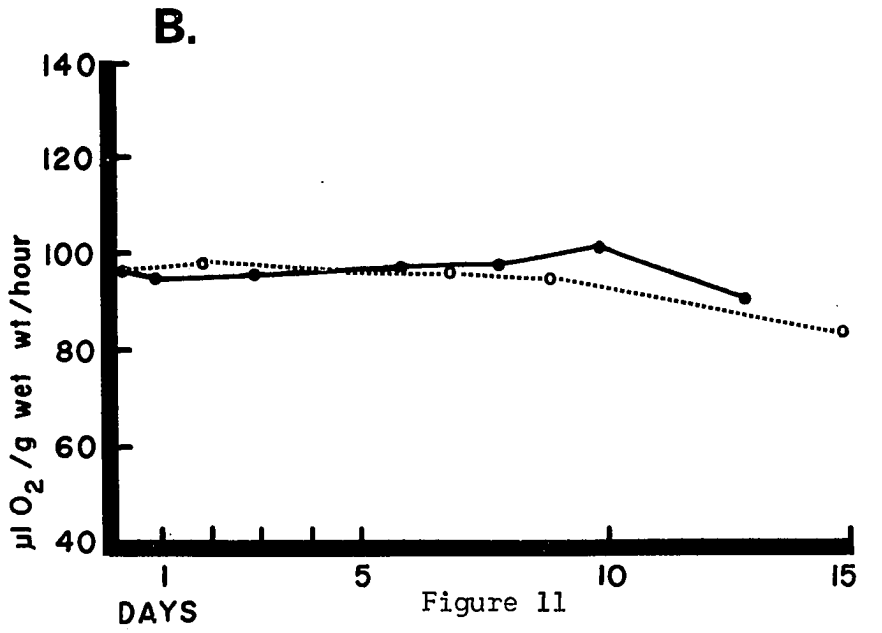


Figure 11

Mean oxygen uptake in Phyllobates subpunctatus tadpoles immersed in 1 part per million thyroxine (● = experimental; o = control). A. Changes per stage. Shaded area indicates the limits of one standard deviation above and below the mean of each stage originally shown in figure 5. B. Changes per day of treatment.

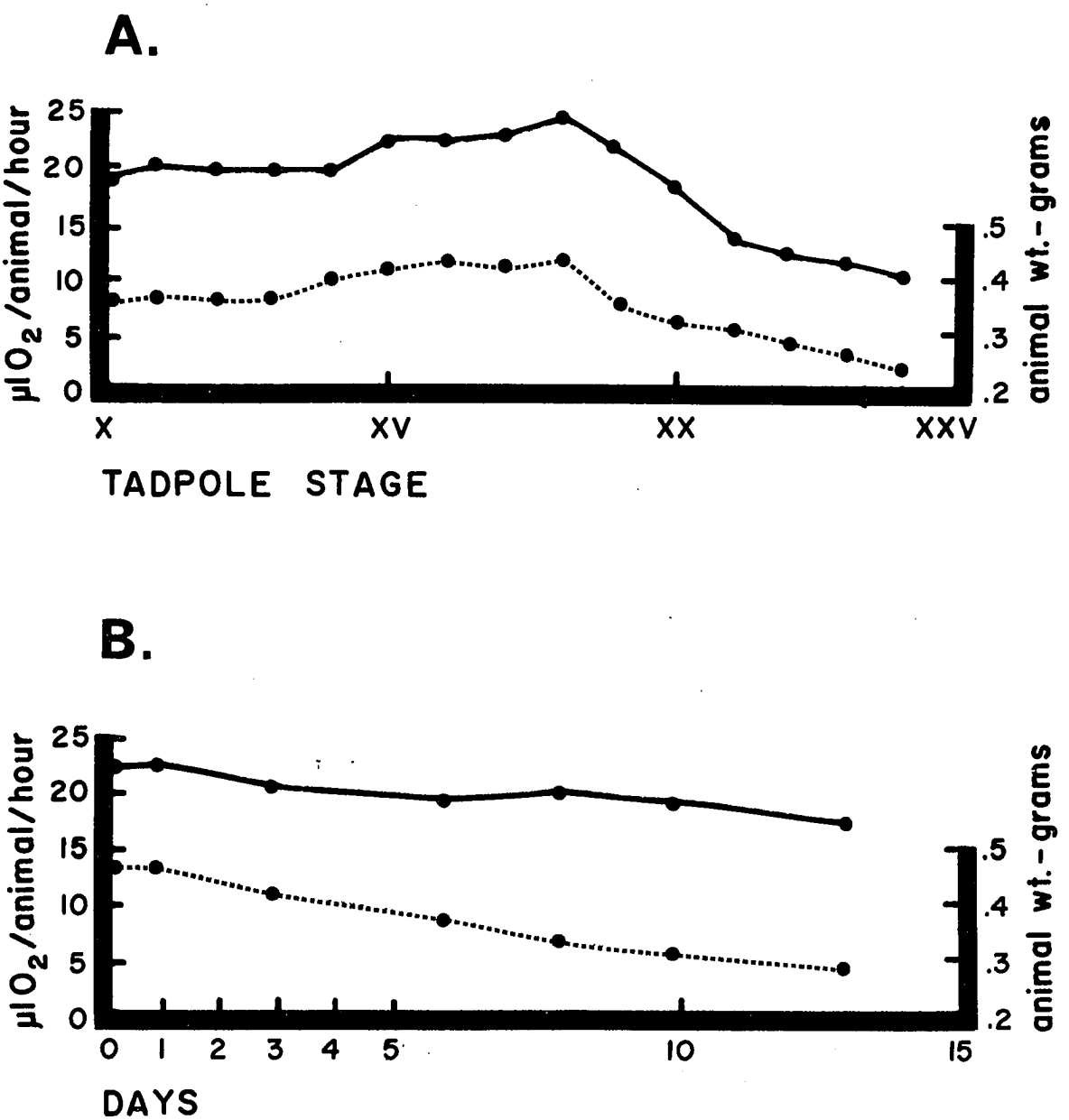
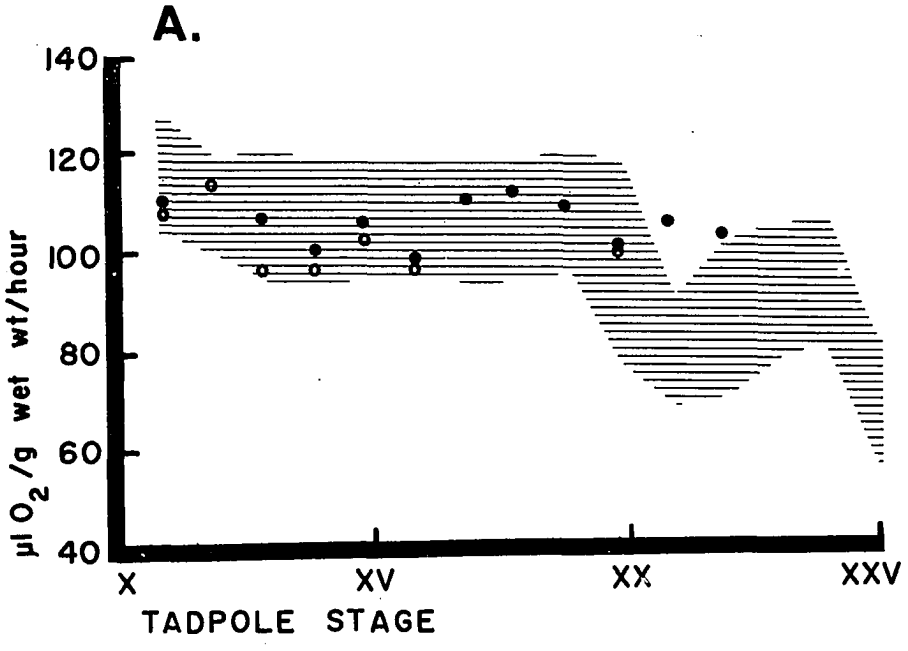


Figure 12

Mean oxygen uptake per animal (solid line) and individual weight (dotted line) in Phyllobates subpunctatus tadpoles immersed in 1 part per million thyroxine. A. Changes per stage. B. Changes per day of treatment.



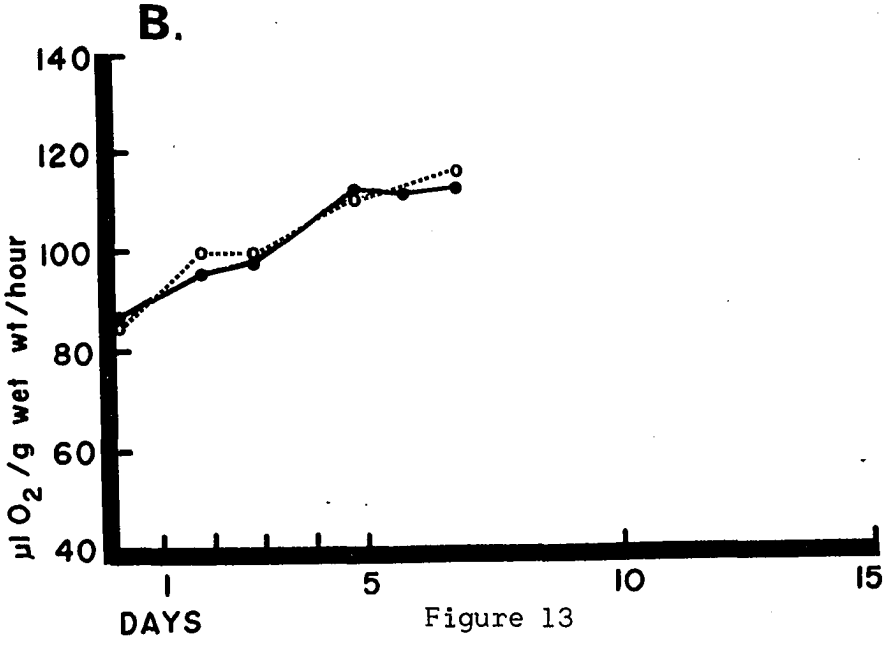
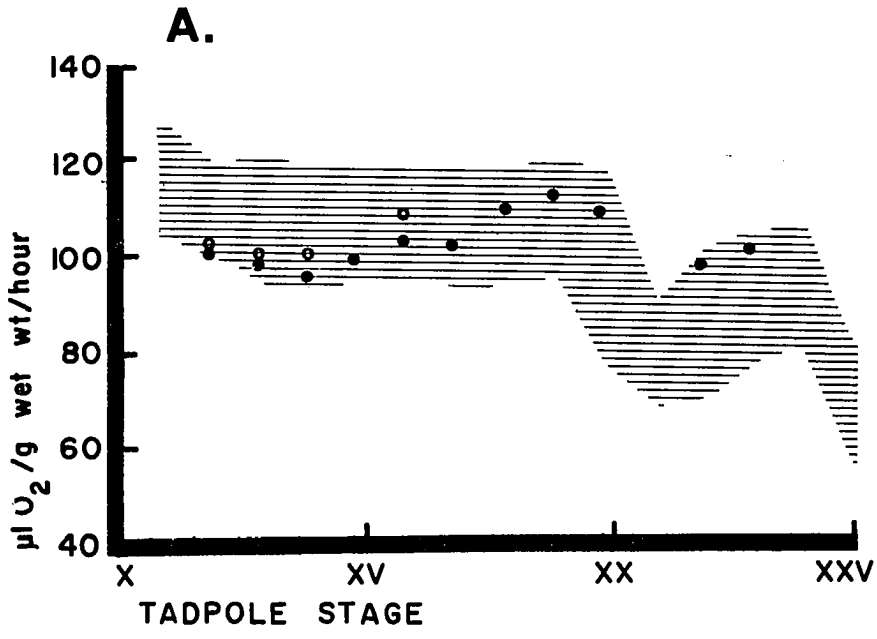


Figure 13

Mean oxygen uptake in Phyllobates subpunctatus tadpoles injected with 0.05 ml of 1×10^{-3} M thyroxine (• = experimental; o = control). A. Changes per stage. Shaded area as in figure 11. B. Changes per day of treatment.



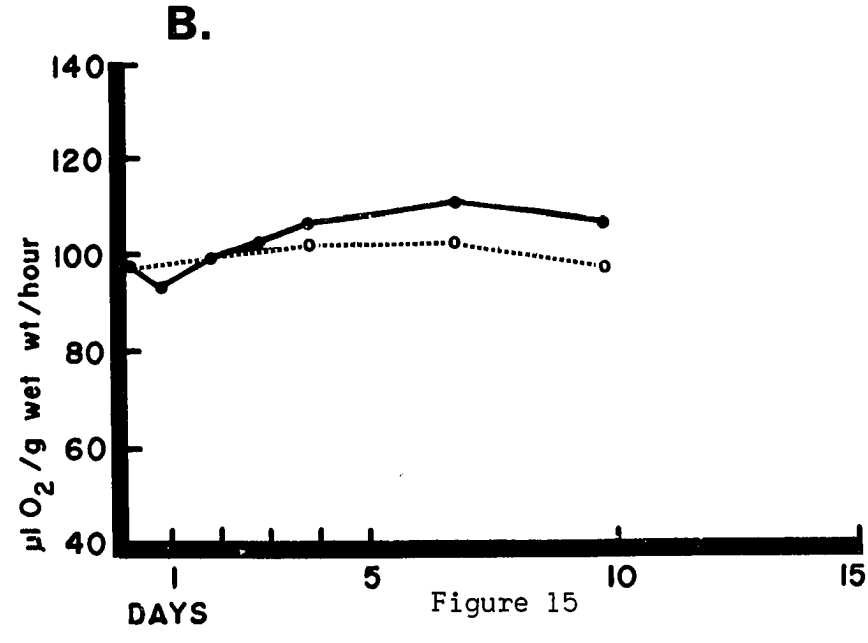


Figure 15

Mean oxygen uptake in Phyllobates subpunctatus tadpoles immersed in 1×10^{-7} M triiodothyronine (● = experimental; o = control). A. Changes per stage. Shaded area as in figure 11. B. Changes per day of treatment.

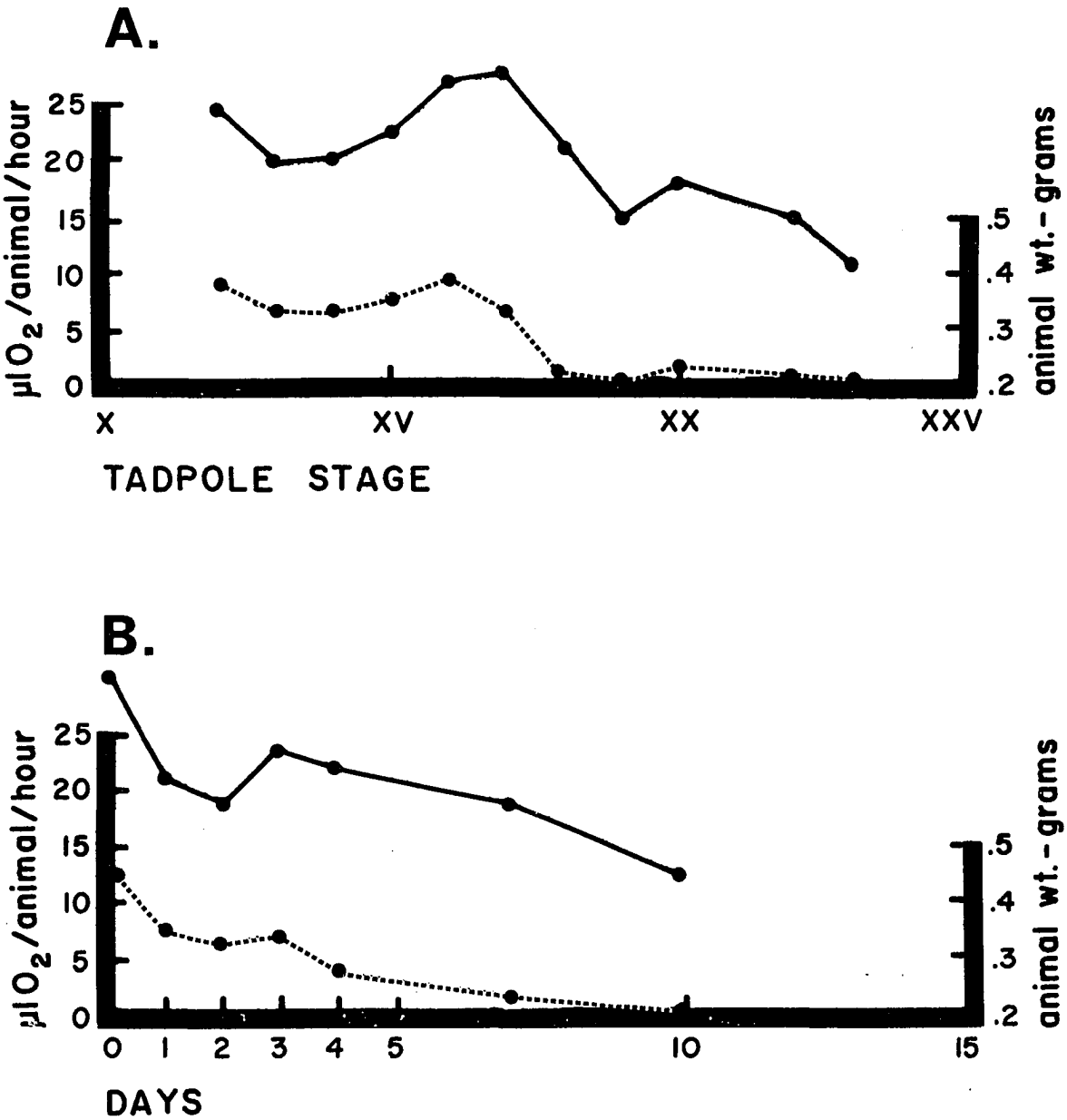


Figure 16

Mean oxygen uptake per animal (solid line) and individual weight (dotted line) in Phyllobates subpunctatus tadpoles immersed in 1×10^{-7} M triiodothyronine. A. Changes per stage. B. Changes per day of treatment.

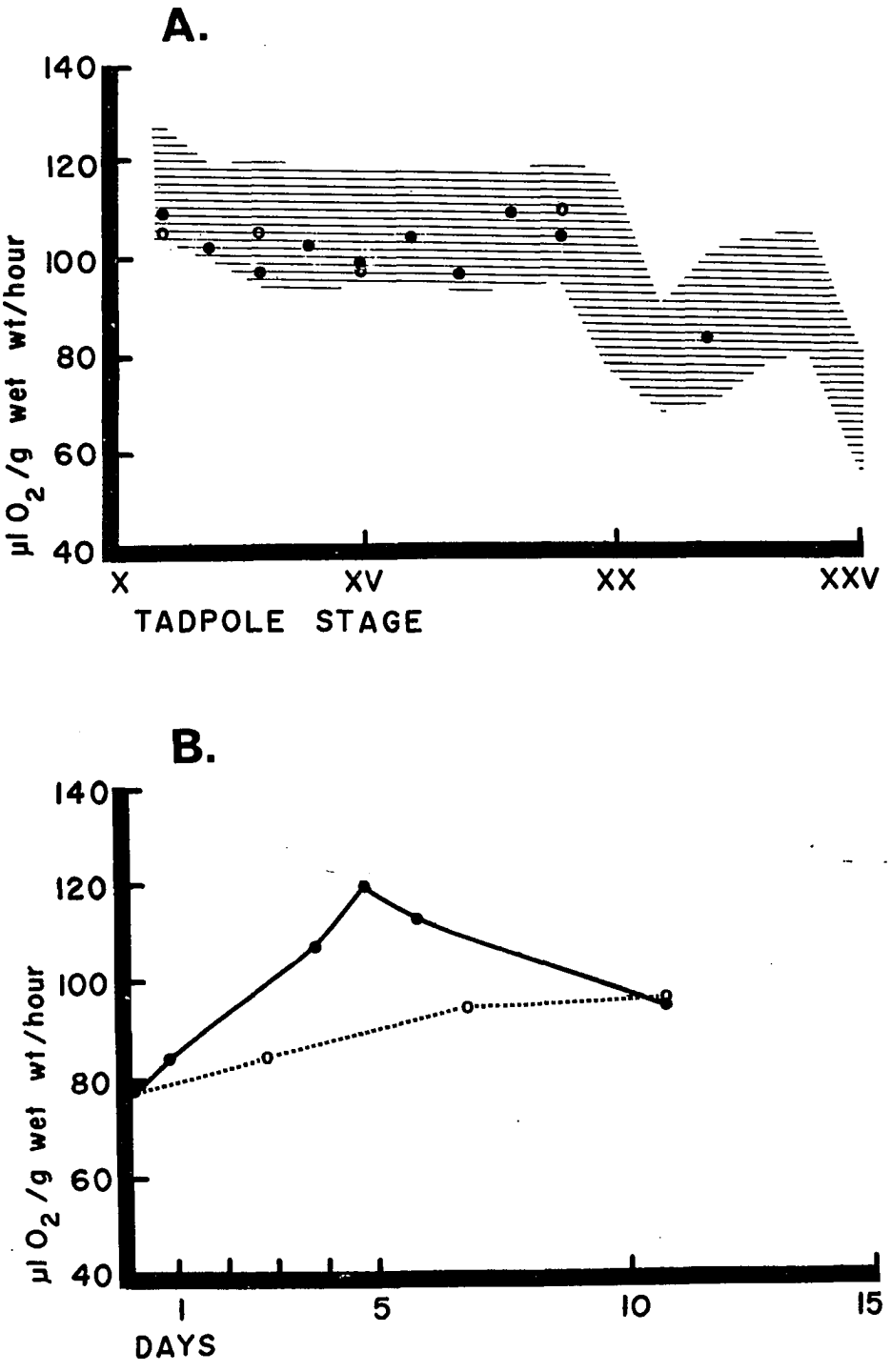


Figure 17

Mean oxygen uptake in Phyllobates subpunctatus tadpoles injected with 0.05 ml of 2 x 10^{-4} M triiodothyronine (● = experimental; o = control). A. Changes per stage. Shaded area as in figure 11. B. Changes per day of treatment.

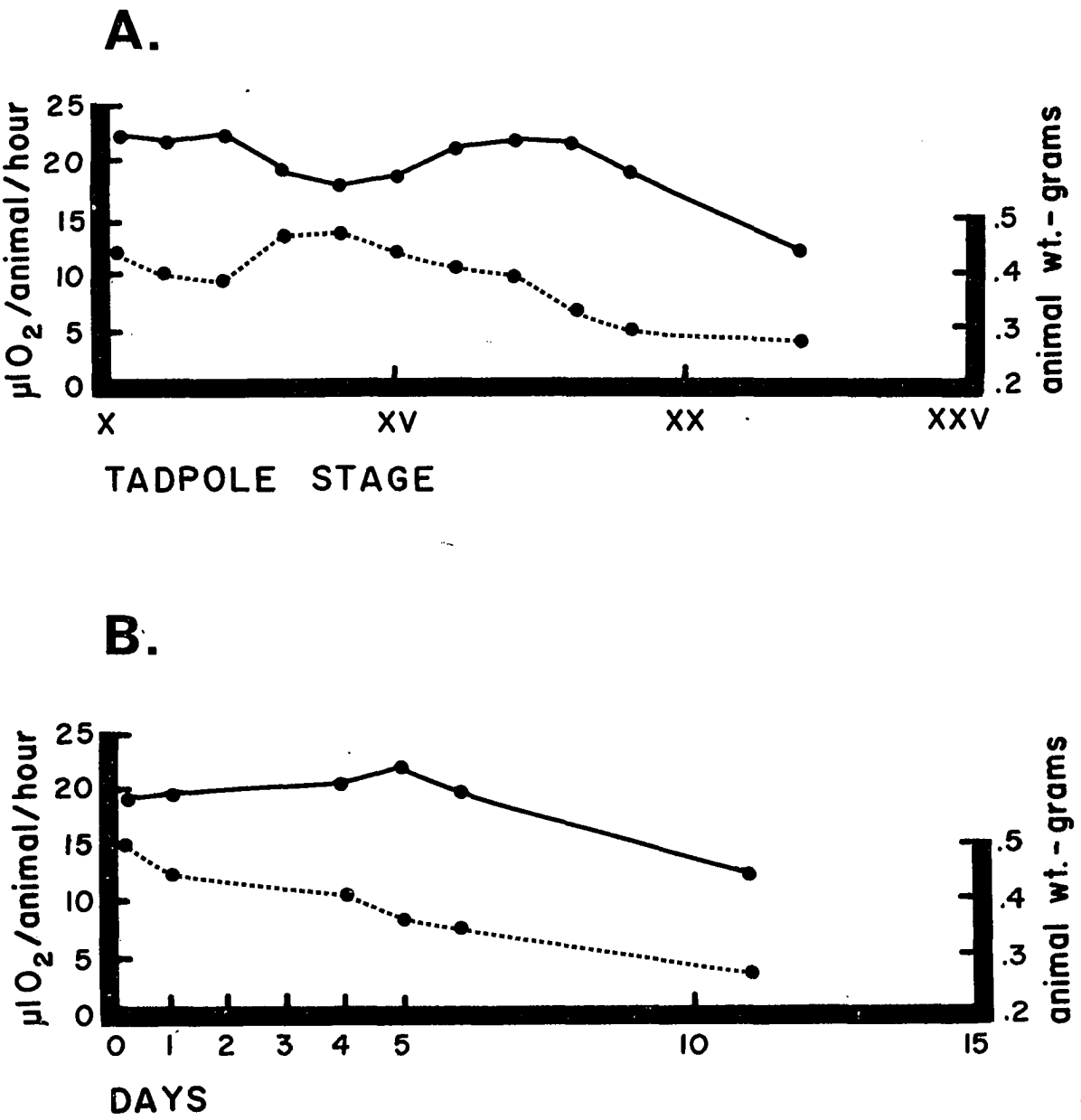


Figure 18

Mean oxygen uptake per animal (solid line) and individual weight (dotted line) in Phyllobates subpunctatus tadpoles injected with 0.05 ml of 2 x 10^{-4} M triiodothyronine. A. Changes per stage. B. Changes per day of treatment.