THE EFFECTS OF pH AND THREE HORMONES ON THE RATE OF DEVELOPMENT IN *RANA CLAMITANS* TADPOLES

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ABSTRACT

Analysis of the effects of pH and estradiol, testosterone, and progesterone on Rana clamitans tadpoles determined pH not to have any significant effect on development, while all three hormones increased development in terms of length as well as stage. Estradiol was particularly successful in development as one tadpole attained Stage XXII whereby it was then classified a frog.

One similarity throughout the experiment was the small length and overall size of the tadpoles, apparently due to the foreign food source. Also, the length had no relation to the stages attained, as the tail disappeared in the final stages of development.

INTRODUCTION

Sexual reproduction and development of amphibians integrates many internal as well as external conditions. The effects of weather, light, temperature, humidity, and rainfall are a few examples of external conditions studied to affect frogs (Savage, 1961).

pH is another important external condition that is frequently studied, especially with the increasing problem of acid rain. Oxides of sulfur, particularly SO_2 and SO_3, which form from coal emissions and automobile catalytic converters, are changed to sulfuric and sulfurous acid and fall to the ground, ending up in bodies of water directly or indirectly through groundwater systems. Not only does acid rain affect the survival of the inhabitants of a body of water, it also affects the development of subsequent generations.

A broad range of pHs are found in the bodies of water on the UNDERC property, making it an ideal location for this experiment. The extremities of this range are a pH of 3.5 in Tender Bog and a pH of 7.2 in Moccasin Lake as well as Peter Lake. Thus, with such a range, one may study the reasons why frogs and tadpoles are found in abundance in some bodies of water yet are absent in others.

Also, testosterone, progesterone, and estradiol have all been utilized in previous experiments to study their

effects on frogs; however, little research has been done studying the effects on tadpoles. Such research is needed, for the gonads in tadpoles have been demonstrated to produce significant quantities of steroid hormones such as the three in this experiment. However, since the tadpoles are not sexually mature, the function of the hormones is not known. Gonads of females tend to produce estradiol and progesterone, while those of males tend to produce testosterone. Also, females at adulthood tend to be significantly larger in size than males, posing the question of whether or not this is a direct result of the hormones.

The life cycle of Rana clamitans begins with a pectoral clasp by the male, subsequent egg release by the female, then external fertilization occurs as the male releases sperm. The embryo then starts its development, progressing through four general tadpole stages to a climactic metamorphosis into a frog. The primary source of nourishment for the tadpole is vegetable matter, though it will consume animal food. Following the final metamorphosis, the diet of the frog changes to earthworms, insects, and spiders (Savage, 121).

The goals of the proposed research are (1) to determine the effect of pH on the development of R. clamitans tadpoles, and (2) to determine the effect of the three hormones on development. Specific questions to be addressed include the correlation between the stage of development versus the length of the tadpole.

MATERIALS AND METHODS

FIELD SAMPLING

To conduct this experiment, a sufficient number of one species of tadpoles needed to be found on the Notre Dame Properties, which is located primarily in the upper peninsula of Michigan, with a small portion occurring in Vilas County of Wisconsin. An abundance of Rana clamitans was found in Bog Pot Lake, a shallow lake of pH around 5.2 with grasses growing on its shoreline. After confirmation of species identification using the Conant Field Guide (1975), four hundred R. clamitans were collected from the northwestern shore on June 14th, 1989. The tadpoles were initially at Stage VIII in their development (Fig. 1).

At this site, the weather conditions, shore vegetation, macrophyte types and densities, and air temperature were determined. The shore was grassy, with water lilies being the primary macrophytes. The air temperature was 28.0°C, and the sun was shining. A water sample wass then collected for analysis at the laboratory. The water temperature was determined to be 27.3°C, and the pH to be 6.56.

LABORATORY PROCEDURE

Fifty R. clamitans were placed into eight aquaria with conditions specified in Table 1. To enable this quantity of tadpoles to experience uniform media, the hormones used, β-Estradiol-3-Benzoate, progesterone, and testosterone propionate each were dissolved into one ml of 95% EtOH.

Then, 100 μl of each solution was added to seven liters of tap water in the respective twelve gallon tank. Tanks 1 and 4 were placed one foot away from the window, tanks 2 and 5 two feet away, tanks 3 and 6 three to four feet away, and tanks 7 and 8 were out of direct sunlight. The placement of the tanks had no significant effect on the lengths or stages of the tadpoles during their development. Also, to achieve the correct pH for each aquarium, a 5N solution of NaOH and a 5N solution of HCl were prepared and used in conjunction with a pH meter.

LABORATORY DATA

Every three days during the 38 day experiment, a tadpole of average length and developmental stage was selected from each tank. Under a binocular dissecting scope, the tadpole was then sized by using a ruler and measuring snout to the tip of its tail, and it was also staged, using the Kollros and Taylor (1946) article (Fig. 1).

The number that lived in each aquaria was also noted every third day, and macrophytes (primarily Potamogeton ampliofolius and Sparganium fluctuans) were taken from the shore of Tenderfoot Lake and added as a food source.

At the conclusion of the experiment, each individual tadpole (or frog, as the case may have been) was measured for length and staged.

RESULTS

EFFECTS OF pH

Two of the initial pH media proved to be fatal for the R. clamitans tadpoles (Fig.2). After changing the tanks of pH 4.0 and 5.3 to 6.3 and 7.0, respectively, the survival rate improved dramatically so that no other changes were needed. There was no significant pattern of survival over the broad range of pHs, but there was a significant 100% survival of tadpoles at pH 6.3.

In all the pH treatments utilized, the tadpoles had essentially the same rate of development, as seen in Figure 3. In fact, all treatments (including hormone treatments) had 75-100% of the tadpoles reach Stage XI or beyond. The percent reaching premetamorphic stages (Stage XI) or beyond (Fig. 1) showed no significant pattern with respect to increasing pH (Fig. 4). However, the lowest pH of 6.3 had the lowest percentage of tadpoles reaching Stage XI or beyond.

The mean lengths for all of the pH treatments were essentially equivalent (Fig. 5), except for the 8.2 pH treatment, which had tadpoles achieving only approximately 3/4 the length of tadpoles in the other pH media.

EFFECTS OF HORMONES

A sufficient number of tadpoles in each hormone treatment survived, enabling the initial concentrations to be used throughout the experiment.

Tadpoles in media of all three of the hormones studied reached the premetamorphic stages (Fig. 4), but only estradiol had any tadpoles that reached metamorphic stages of XVIII or beyond (Fig. 6). But the testosterone and progesterone treatments both had tadpoles that reached Stage XVI, and the control had tadpoles that reached Stage XIII. Tadpoles in estradiol also had the greatest mean developmental stage (Fig. 3), while those in testosterone and progesterone had essentially the same mean stage of XV.

The tadpoles in the testosterone medium had the greatest mean length of 2.65 cm (Fig. 5), while those in the estradiol medium had the smallest, which was almost half that of testosterone (1.95 cm).

DISCUSSION

The effect of the low pH treatment on development was very pronounced in respect to the survival of the tadpoles. Assuming the fatalities that occurred were due to the extreme acidity, one could hypothesize that survival would be the greatest in the treatment most closely imitating the natural habitat. Indeed, survival was the best (100%) at a pH of 6.3, the closest pH to the pH of 6.56 where the tadpoles were found. These data suggest that high pHs are better than low pHs for the survival and development of frogs.

These data also implicate the existence of micro-habitats within lakes. For example, the pH at the shore-line would be lower due to the warmer temperature from sunlight, while the pH in the middle of the lake would be greater. This would affect the tadpoles by restricting them to certain parts of the lake, where the macrophyte and algae populations would be.

The hormones in this experiment all had pronounced effects on the development of the tadpoles, the most striking being estradiol, which produced the total develop-ment to Stage XXII. These data indicate the potential of utilizing hormones for regulating development in stages as well as size.

The length data indicated extremely small lengths for the tadpoles. A possible hypothesis for these data was

the food source. Tenderfoot, where the food was collected, had different species and a much smaller algal population than Bog Pot. The primary feeding mechanism of tadpoles is scraping, and past research has confirmed their affinity for an algal food source often found on macrophytes (Kollros and Taylor, 1946). The final day of the experiment confirmed the small size of the developing tadpoles, as three-inch, fully developed R. clamitans were spotted in abundant numbers at Bog Pot. A future experiment might test the effect of the food source on development.

The error in this experiment was fairly large, mostly because the average tadpole from each aquarium was chosen every three days. Hence, the probability of choosing the same tadpole was low, as well as the fact that one tadpole had to represent often a large variance of stages. Also, the measuring of length was done on the live tadpoles in their specific media, and great difficulty ensued due to their rapid movements.

Future studies could further this experiment to determine if the various pHs, as well as the hormones, had an effect upon the sex of the frogs that would develop, and at what time the sexes are apparent. Greater numbers of tadpoles, as well as consistent numbers in each tank, would also help to increase the accuracy of these data.

Another possible study could address the question of whether the aquaria environment had an effect on the

development. One might address the confinement, the lack of mud or grasses, or the stress from overcrowding to determine what produced the small length of tadpoles and frogs.

pH was determined not to have a significant effect on development, while all three hormones had the effect of increasing development in terms of length as well as stage. The tadpoles remained a fairly small length and size, possibly because of the foreign food source. Length had no correlation to the stage attained.

ACKNOWLEDGEMENTS

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Table 1: Conditions of Aquaria

	pH	temp (°C)	other
Tank 1	4.0	14.0	
2	5.3	14.0	
3	7.4	14.0	
4	8.2	14.0	
5	9.0	14.0	
6	7.4	14.0	1 mg testosterone/liter H_2O
7	7.4	14.0	1 mg estradiol/liter H_2O
8	7.4	14.0	1 mg progesterone/liter H_2O
*9	6.3	14.0	
**10	7.0	14.0	

* used in replacement of 4.0 pH as of 6/17/89

** used in replacement of 5.3 pH as of 6/17/89

Figure 1: Developmental Stages of Rana pipiens according to Taylor and Kollros

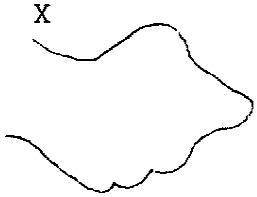
Limb Bud Stages

I II III IV V

Paddle Stages

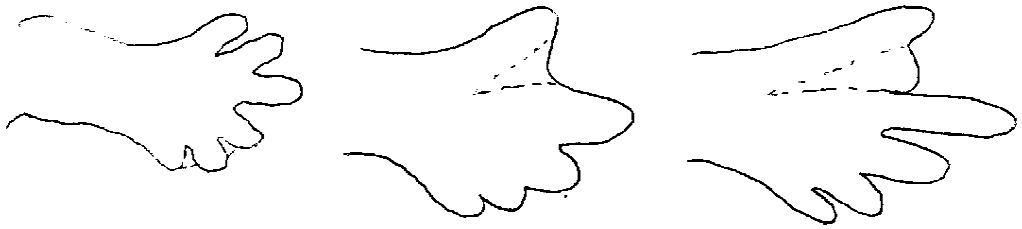
VI VII VIII IX

X



Premetamorphic Stages

XI XII XIII



XIV XV XVI

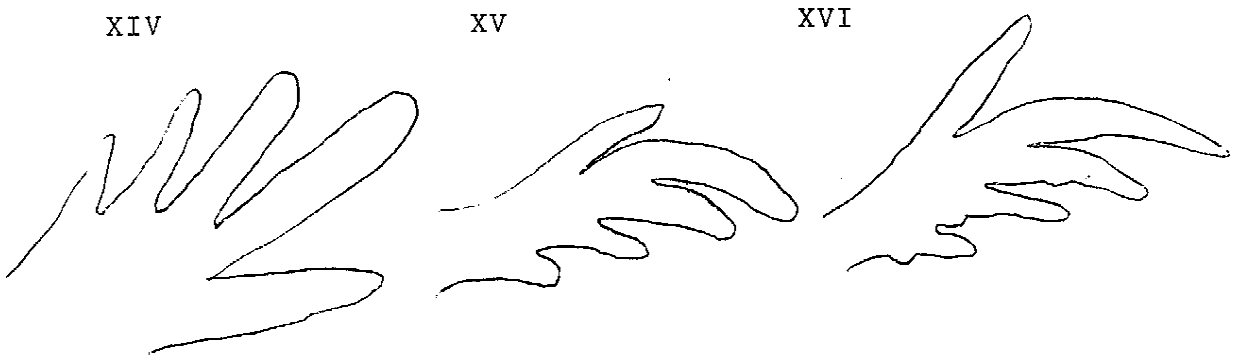
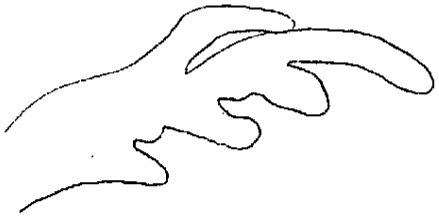


Figure 1: cont.

XVII



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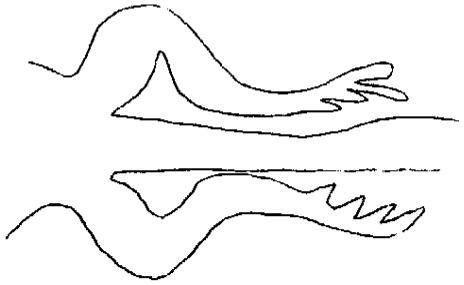
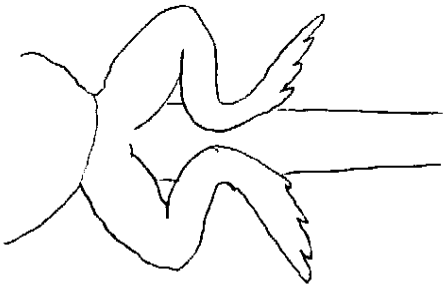


Metamorphic Stages

XVIII



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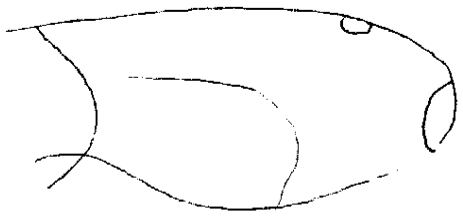
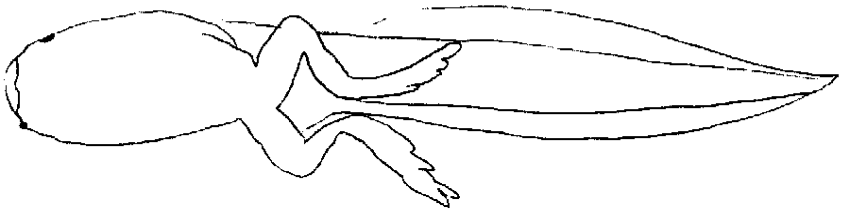


XX



XXI

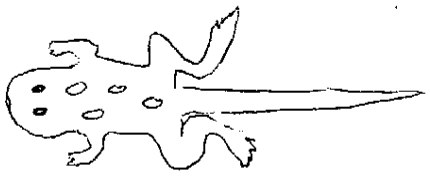


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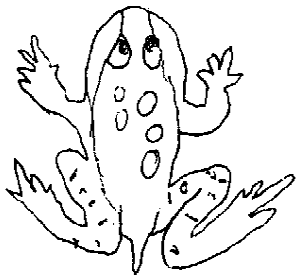
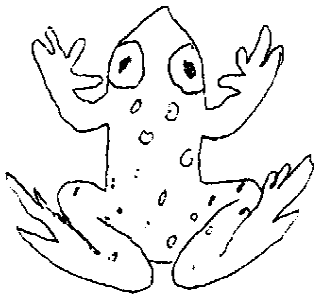
Figure 1: cont.

XXIII



XXIV

XXV



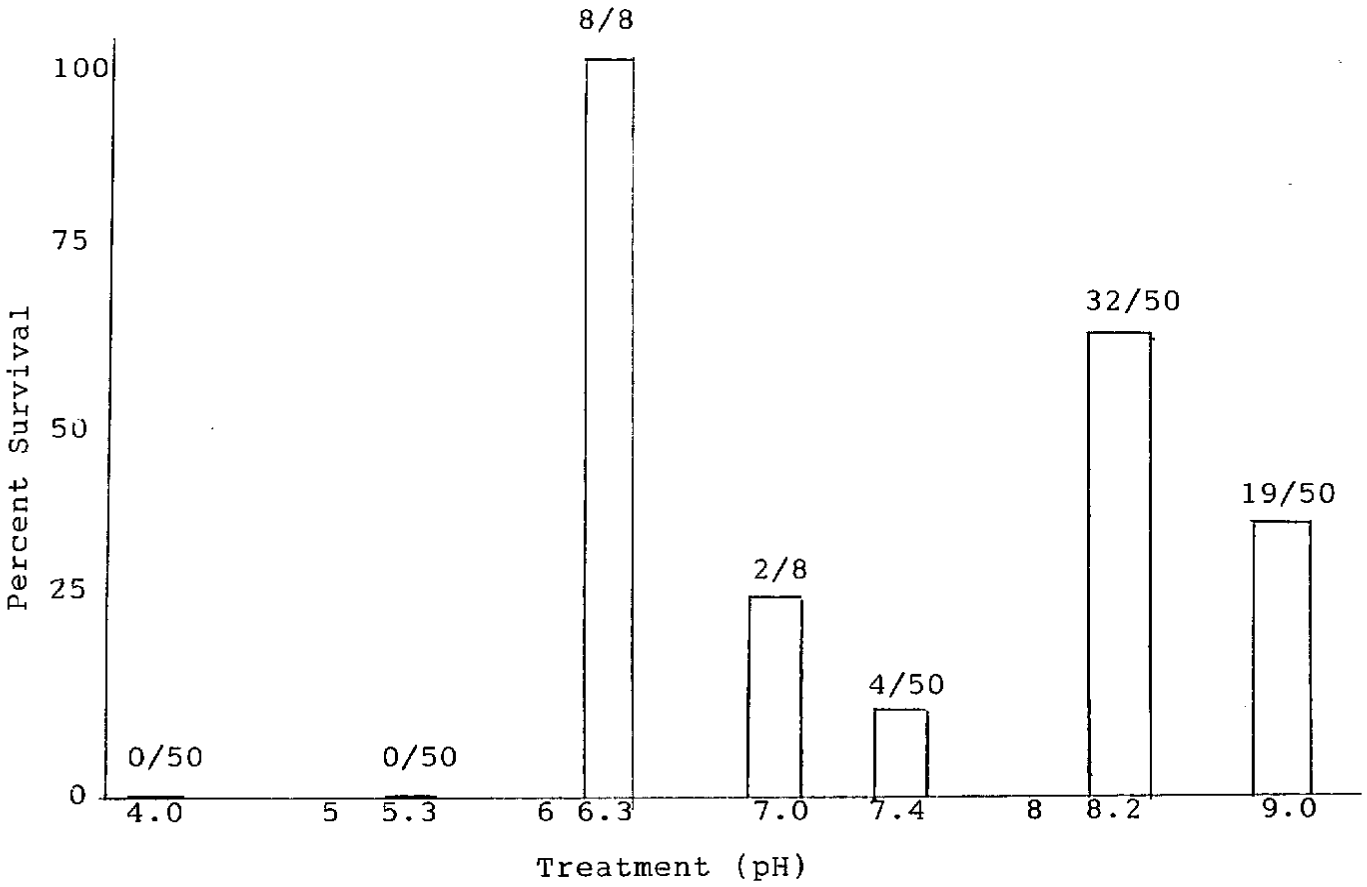


Figure 2: Percent survival of R. clamitans tadpoles in varying pHs. Statistical analysis using the Fisher's Exact Test (FET) showed the following comparisons to be significantly different from each other ($p<0.05$): 5.3 and 6.3; 6.3 and 7.4; and 7.4 and 8.2. The pH of 7.4 was the control for this experiment. The ratios given represent the number of living tadpoles over the total number of tadpoles initially present in each medium.

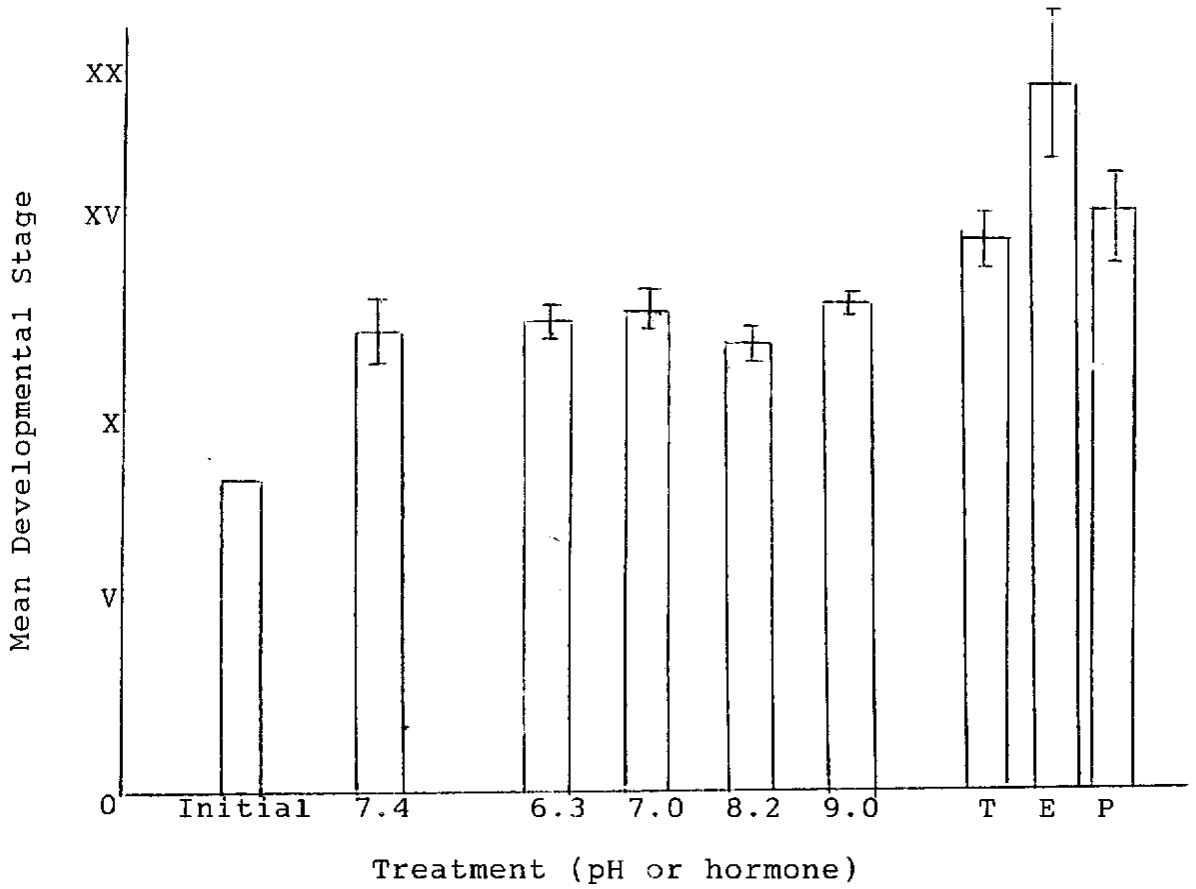


Figure 3: Mean stages of development found in R.clamitans tadpoles at the conclusion of the experiment (38 days). All values are means, per tank, ± S.E.M.

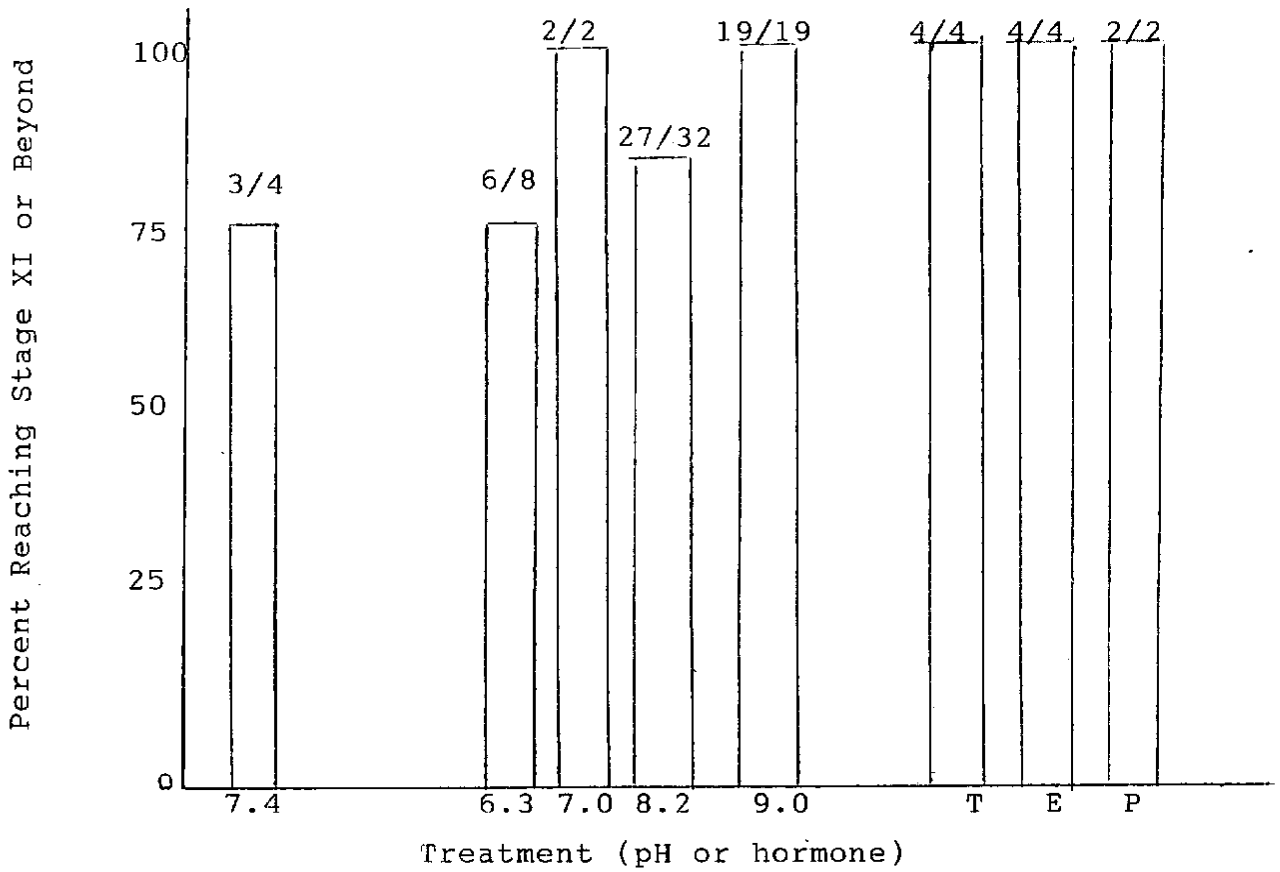


Figure 4: Percent of R. clamitans tadpoles reaching the premetamorphic stage XI or beyond in various treatments. Statistical analysis using the FET showed none of the data to be significantly different ($p<0.05$). Testosterone, estradiol, and progesterone are represented by T, E, and P, respectively.

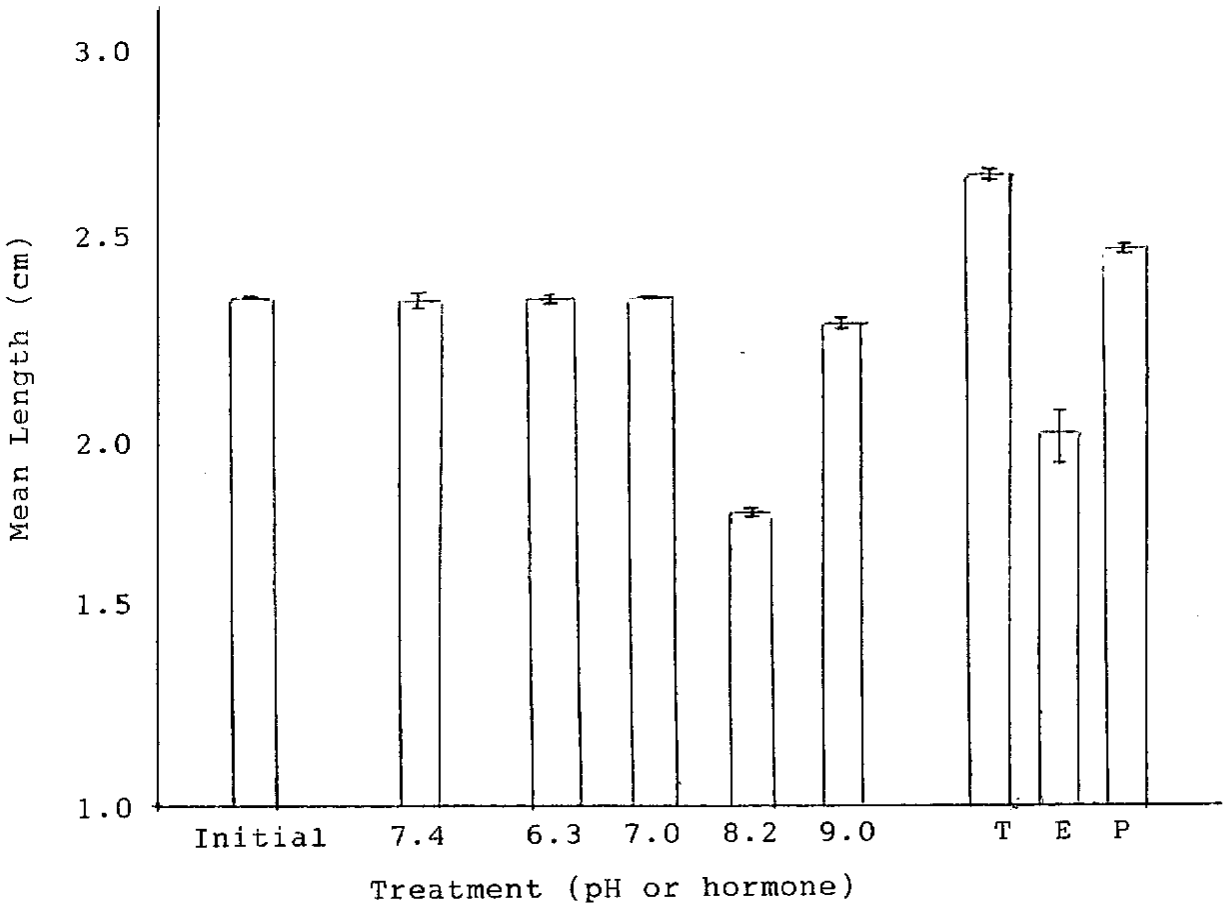


Figure 5: Mean lengths of R. clamitans in various treatments at the end of the experiment. Values are means, per tank, ± S.E.M.

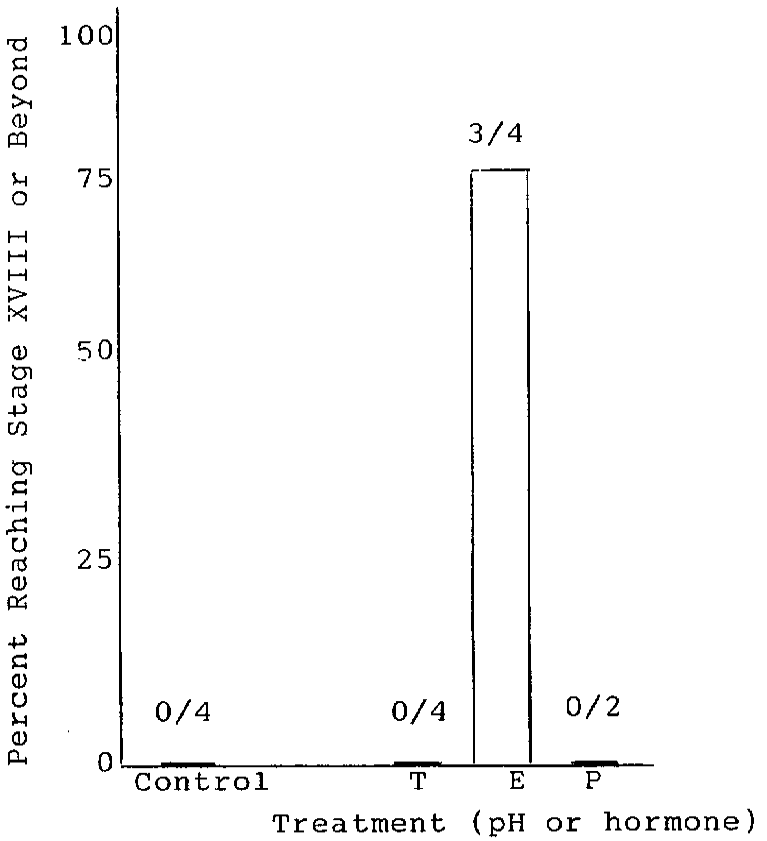


Figure 6: Percent of R. clamitans reaching the metamorphic stage XVIII or beyond in various treatments. Statistical analysis using the FET showed the following comparisons to be significantly different ($p<0.05$): control and E; T and E. A pH of 7.4 was present in all tanks.