# Metamorphic Stages in *Ambystoma mexicanum*

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## Abstract

Although the induction of metamorphosis in *Ambystoma mexicanum* has been studied, no information is available on the recognition of metamorphic stages in this species. The aim of our study was to define the stages of metamorphosis in *A. mexicanum* using morphometric criteria. Metamorphosis was induced with an injection i.p. of l-thyroxine ($T_4$; 2.5 µg/g). Metamorphosis is a gradual process. All the animals survived, and metamorphosis was completed in 24 ± 1 days. Four metamorphic stages were defined using as principal criteria the reduction in the tail height (considering the ratios lower/upper tail fin and upper tail fin/total tail height) and the reduction in gill length. Body weight and dorsal ridge length were recorded too. *A. mexicanum* is used in many neuroendocrinological and biochemical studies in which the knowledge of the stages of metamorphosis may be necessary for the interpretation of results.*

## Introduction

The axolotl, *Ambystoma mexicanum*, is a neotenic amphibian with no external evidence of metamorphosis. The animals become sexually mature and breed in a larval form (Duellman and Trueb, 1986). However, *A. mexicanum*, like other neotenic species, undergoes metamorphic changes if it is treated with thyroid hormones [thyroxine ($T_4$), triiodothyronine ($T_3$)] or thyrotropin hormone (TSH) (Kuhn and Jacobs, 1989).

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Although the induction of metamorphosis in *A. mexicanum* has been studied (Kuhn and Jacobs, 1989), little information is available about the recognition of metamorphic stages in this species (Marx, 1935).

Metamorphic stages have been documented clearly in anurans (Bentley, 1976; Gosner, 1960; Taylor and Kollros, 1946), but to our knowledge only one study of urodele amphibians exists that provides quantitative criteria for metamorphosis. This study is of *A. tigrinum*, a urodele which spontaneously metamorphoses. In A. tigrinum metamorphosis generally begins at a different time in each animal. This situation limits the use of *A. tigrinum* for studies in which it is necessary to know the start time of metamorphosis.

The neotenic species susceptible to metamorphosis provide an alternative for those studies which require control of the onset of metamorphosis. A useful species may be *A. mexicanum*, one which furthermore is already extensively used in neuroendocrine and biochemical studies.

In the present work we define four metamorphic stages in *A. mexicanum* following the general criteria suggested by Norman for *A. tigrinum* (Norman, 1985).

## Material and Methods

**Biological Material.** The axolotls [*A. mexicanum* (n = 30), 1 year old, 20 ± 2 cm long and weighing 60 ± 5 gr] were obtained from our colony (Cano-Martínez et al., 1990). The animals were kept in 50x20x20 cm glass containers and maintained in a room with a light-dark cycle of 12:12, starting the light at 7 a.m. Each container held five animals in 15 liters of 20% modified Holtfreter solution, prepared with water without chloride at a temperature of 18-20°C. During metamorphosis, 1/3 of the solution was changed each day, and the solution level was lowered gradually until metamorphosis was complete. The animals were fed little fish (*ad libitum*) and small pieces of beef (individually with forceps) three times per week.

**Induction of Metamorphosis.** The experiments were carried out in the months of July and August, 1992. Handling during the experiment and measurements were done by same person, using the same instrument, at the same hour of the day (noon to 1 p.m.), and using the same morphological criteria. Each

## Table 1. Measurement Criteria

| Parameters | Measurement Procedure |
|---|---|
| Dorsal Ridge Length (mm) | obtained by subtracting the distance from mouth to the origin point of the dorsal ridge from the total body length; the measurement was made on the back dorsal center. |
| Gill Length (mm) | Length of the central gill on each sides: the distance from the ventral angle of the gill base to the distal tip of the ventral gill epithelium (without the gill filaments). The measurement was made with the gill spread out along the animal´s back. |
| Body Weight (g) | The total weight in grams. The water excess was eliminated with absorbent paper in each measurement. |
| Tail Height<br>Lower tail fin height<br>Upper tail fin height (mm) | These three parameters were measured 1 cm anteroposterior of the cloaca (See Figure 1). |

The measurement procedures were based in general on Norman's criteria with some modifications (Norman, 1985).

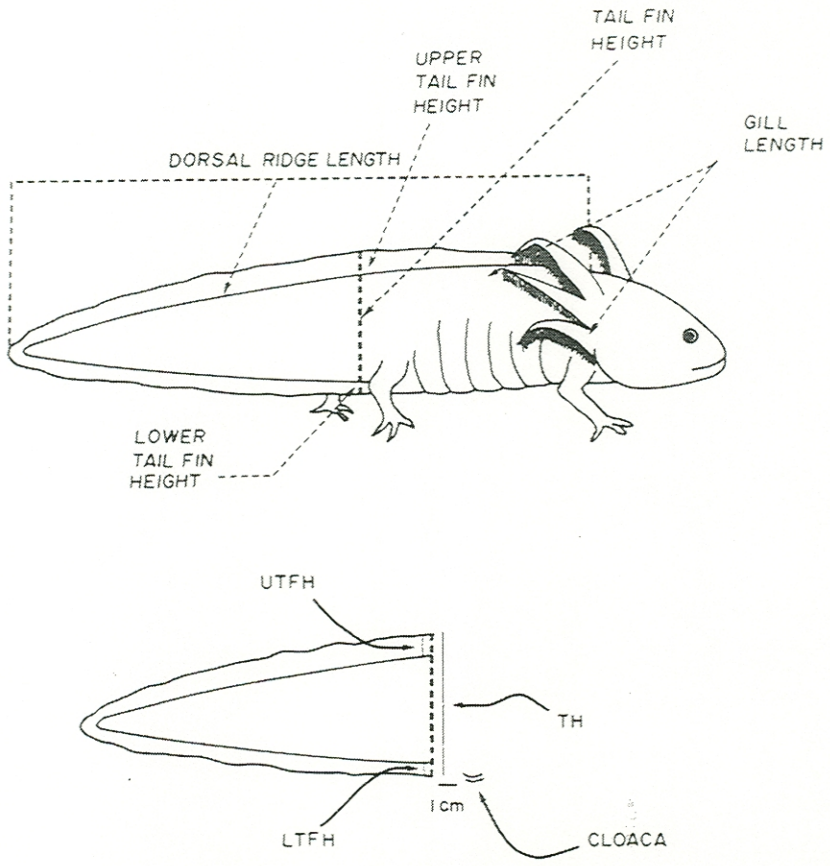


Figure 1. Axolotl body representation showing the points for measurement of morphometric parameters . TH = tail height; UTFH = upper tail fin height; LTFH = lower tail fin height.

**Table 2. Values of the measured parameters in each metamorphic stage for *Ambystoma mexicanum*.**

| Parameter Stage | DRL % | TH % | LTFH % | UTFH % | LTFH/UTFH X 100 | LTFH | UTFH/TH X 100 | GL % |
|---|---|---|---|---|---|---|---|---|
| 0 | 100 | 100 | 100 | 100 | 40 or greater | ——— | ——— | 100 |
| I | 83±3* | 83±4* | 25±2* | 75±1* | 25 to 10 | ——— | ——— | 85±5* |
| II | 62±1* | 69±3* | 0.0 | 58±1* | ——— | 0.0 | ——— | 49±3* |
| III | 52±2* | 65±6 | ——— | 27±2* | ——— | ——— | 25 to 10 | 24±2* |
| IV | 21±3* | 54±5* | ——— | 1.1±0.5* | ——— | ——— | ——— | 1±0.3* |

The values represent the mean ± SE (n=24). The comparison was by Student's "t" test.
* = P<0.001; DRL = Dorsal Ridge Length; TH = Tail Height; LTFH = Lower Tail Fin Height; UTFH = Upper Tail Fin Height; GL = Gill Length.

animal was identified by a colored cotton thread placed just behind the gills. The experimental animals (n = 24) received one i.p. injection of l-thyroxine ($T_4$; 2.5 µg/g) in 150 µl of amphibian Ringer solution; the dosage was determined previously (Vargas-González et al., 1990). The control group (n = 6) received an equivalent volume of Ringer solution.

**Metamorphic Parameters.** In each animal the following parameters were measured each day: body weight in grams, total body length, central gill length (GL) on each side, dorsal ridge length (DRL), tail height (TH), and lower and upper tail fin height (LTFH and UTFH), in millimeters. The last three parameters were measured one centimeter anteroposterior to the cloaca (Figure 1). The ratios LTFH/UTFH and UTFH/total tail height were estimated every day; the percent of reduction in the other parameters was calculated too. The measurement procedures are summarized in Table 1.

## Results

The animals with hormonal stimulation presented metamorphic changes; four metamorphic stages were described and metamorphosis was completed in 24 ± 1 days. The control group did not present metamorphic signs; however we observed a slight body weight reduction (Figure 2). In the experimental group, our results show a correlation between the metamorphic stages and the time of the beginning of each stage.

In all experimental animals the reduction in body weight, dorsal ridge length, gill length, tail height, and lower and upper tail fin height was clear. (Figures 2 and 3).

The parameters' values for each stage are shown in Table 2 and a comparison with *A. tigrinum* metamorphic stages is shown in Table 3.

## Discussion

In the present study we have demonstrated that metamorphosis in *A. mexicanum* is a gradual process with a duration of 24 ± 1 days if it is induced with only one injection of $T_4$ (2.5 µg/g). Under these conditions four metamorphic stages were defined by morphometric criteria. We observed that during induced metamorphosis with this dosage it is possible to use the length of the gills as a morphological standard for staging metamorphosis, as well as the lower and upper fin regression. We found that variability in the response by the animals with induced metamorphosis can be reduced considerably if the dosage of thyroid hormone is adjusted to body weight and if the reference point for the fin height measurements is not dependent on the subjective decision by the researcher about the "tail's highest point," but is instead tied to an invariable anatomical reference point such as the cloaca.

The results of our study are in agreement with Norman's study. Time cannot be used as a basis for predicting the onset and staging of metamorphosis in wild species, even under controlled laboratory conditions. However, in neotenic species such as *A. mexicanum* bred in laboratory conditions, the onset of meta-

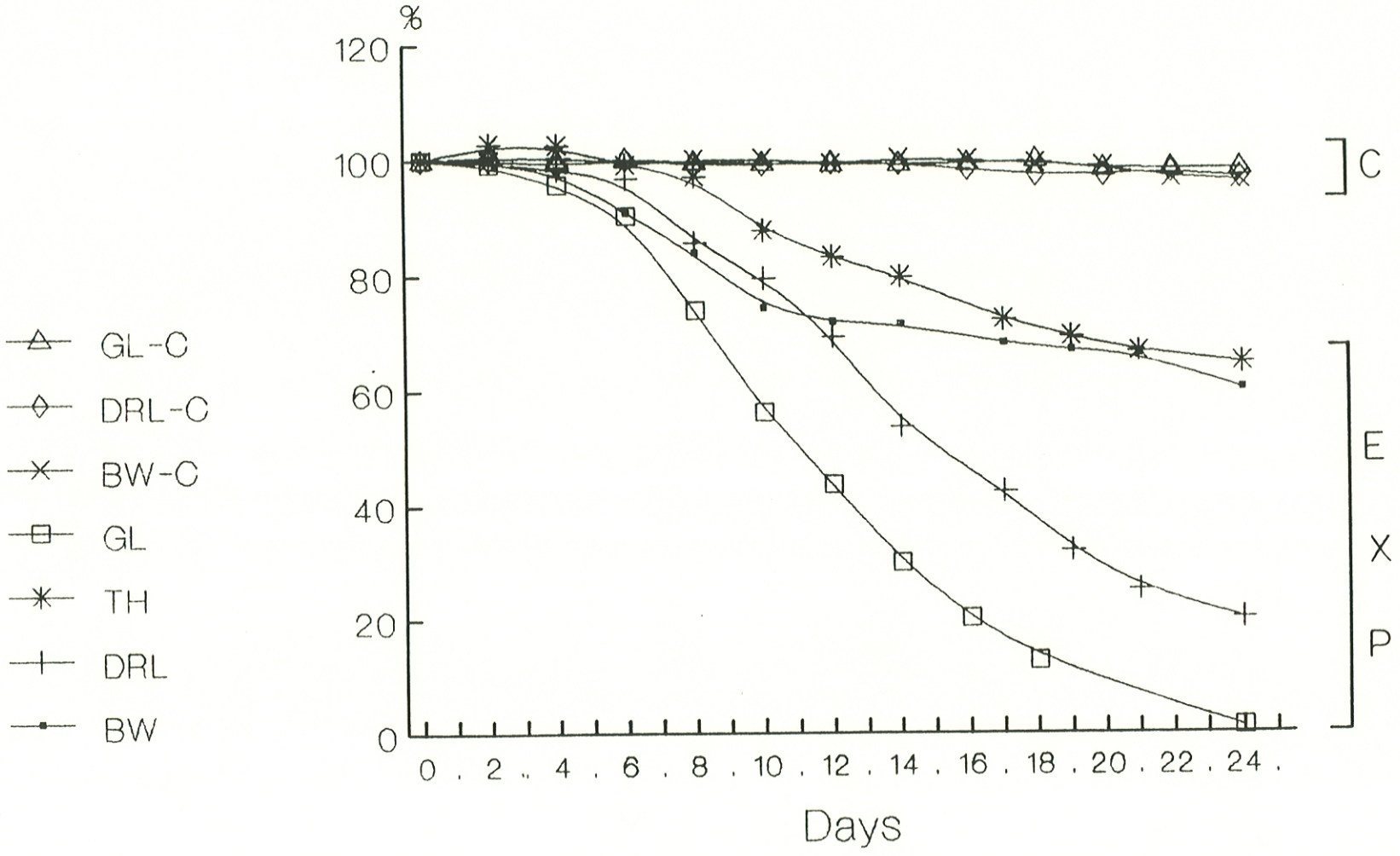


Figure 2. Time of metamorphosis. Comparison between control and experimental groups. A little reduction was observed in the parameters of the control group, but the difference is not significant. Each value represents the average ± SEM (experimental, n = 24, and control n = 6). Error bars are not drawn in the figure for clarity. C = control; EXP = experimental; GL = gill length; DRL = dorsal ridge length; BW = body weight; TH = tail height.

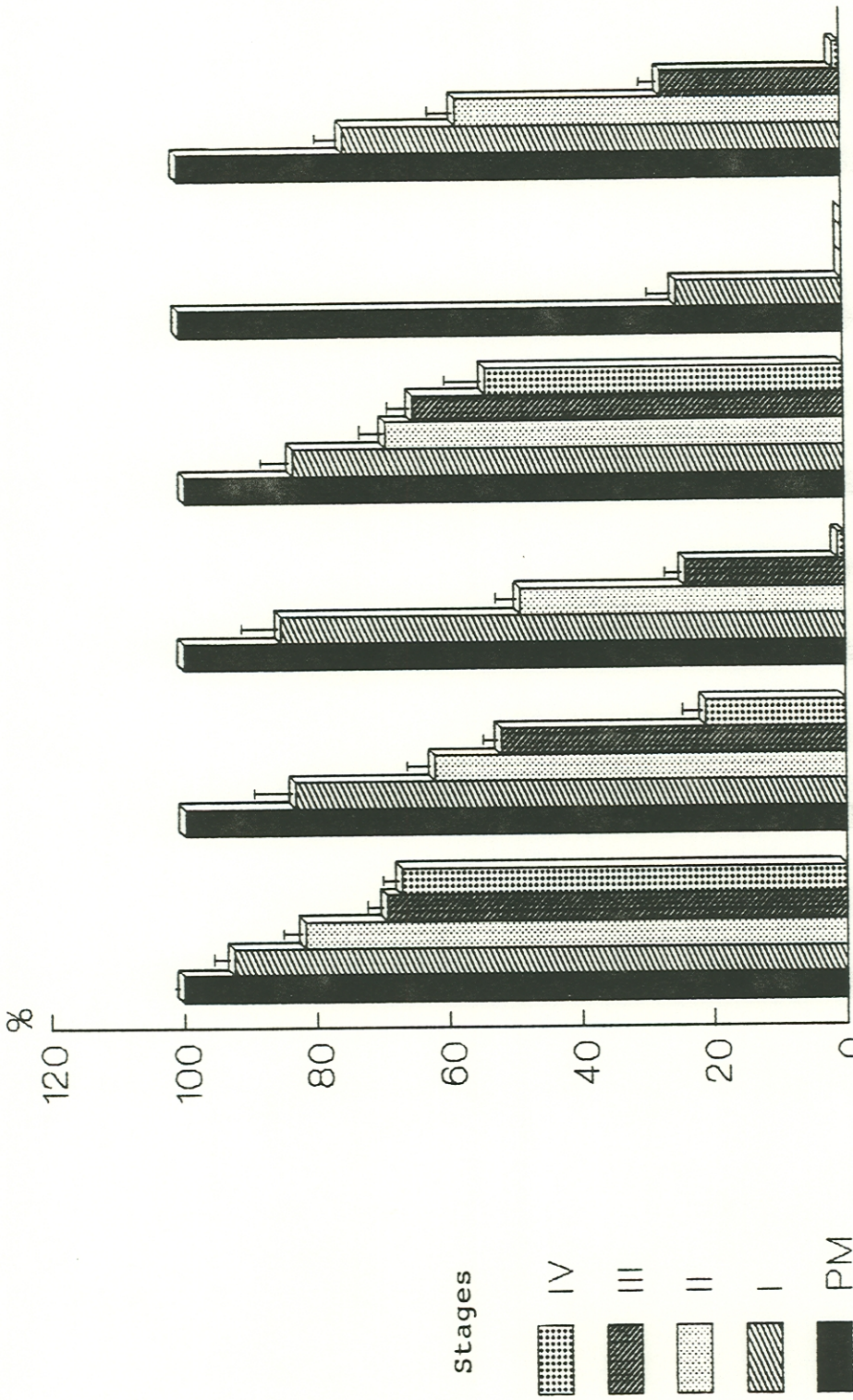


Figure 3. Morphometric parameters and stages of metamorphosis. Each bar is the average ± SEM (n = 24). * = P < 0.001. The significance was estimated by Student's "t" test.
PM = premetamorphosis; BW = body weight; DRL = dorsal ridge length, GL = gill length, TH = tail height; LTFH = lower tail fin height; UTFH = upper tail fin height.

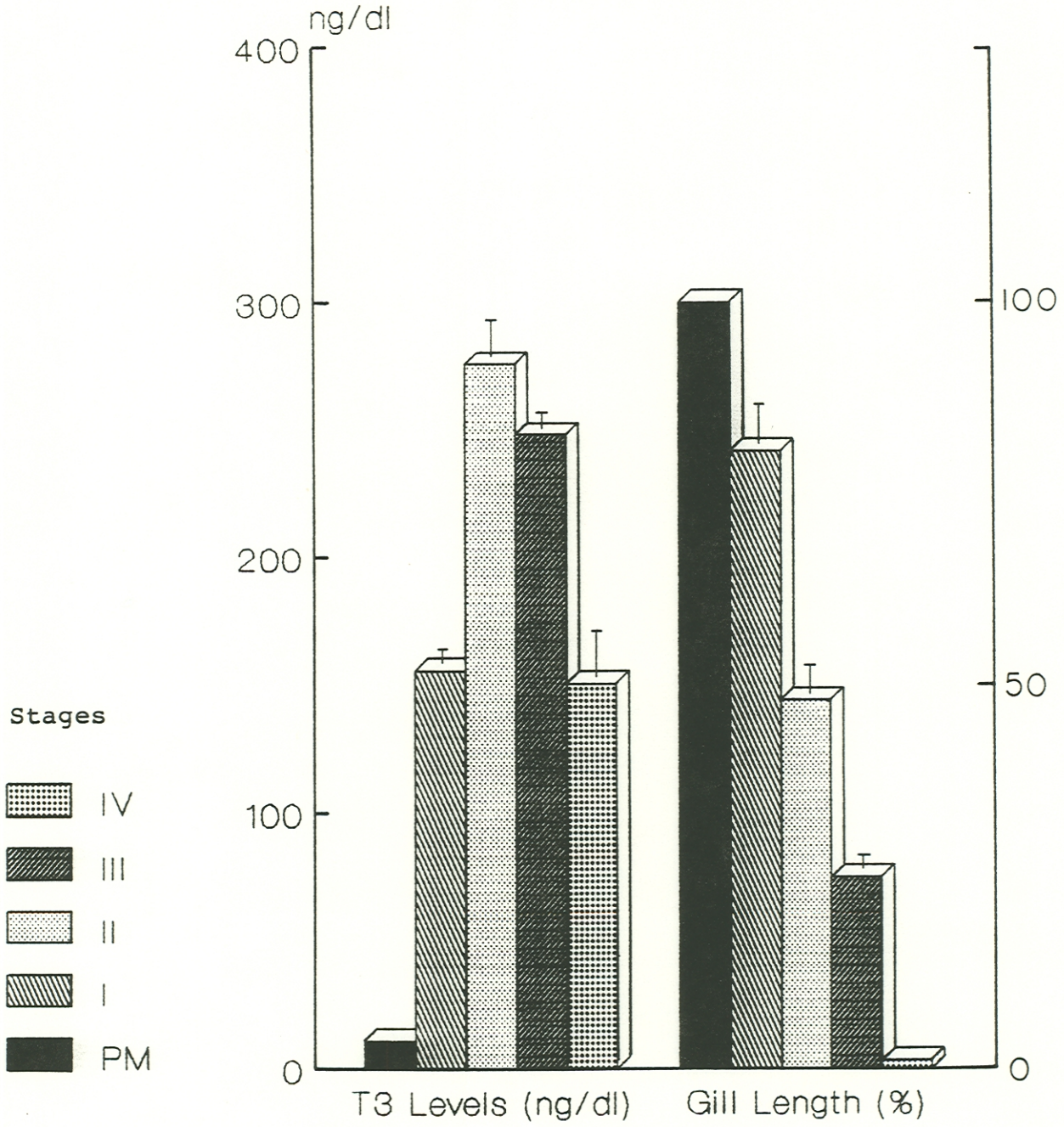


Figure 4. Changes in $T_3$ serum levels in relation to gill length reduction during metamorphosis $T_4$-induced metamorphosis in *A. mexicanum*. Each bar is the average ± SEM. * = $P < 0.001$. The significance was estimated by Student's "t" test. On the right side is the scale for gill reduction in percent. PM = premetamorphosis.

**Table 3. Metamorphic Stages in *Ambystoma mexicanum*. A comparison with metamorphic stages in *Ambystoma tigrinum*.**

| | *Ambystoma tigrinum** | | *Ambystoma mexicanum* |
|---|---|---|---|
| | | **Premetamorphic** | Neotenic: lower/upper tail fin ratios of 40% and greater. Gill length = 100%. |
| I | Larval: lower/upper tail fin ratios of 40% and greater. | I | Lower/upper tail fin heights X 100 = 25 to 10. Gill length = 85 to 75%. |
| II | Lower/upper tail fin heights X 100 = 25. | | |
| III | Lower/upper tail fin heights X 100 = 10. | | |
| IV | Lower tail fin height = 0. | II | Lower tail fin height = 0. Gill length = 45 to 50%. |
| V | Upper/total tail height x 100 = 25 to 10. | III | Upper/total tail height X 100 = 25 to 10. Gill length = 25 to 20% |
| VI | Upper/total tail height X 100 = 10. | | |
| VII | Gill length = 0. | IV | Gill length = 0 to 2%. |

* According to Norman (1985) and Carr and Norris (1988). We included the values of the gill length for *A. mexicanum*. Norman's stages II and III correspond to stage I in *A. mexicanum*. Norman's stages V and VI correspond to stage III in *A. mexicanum*.

morphosis can be determined by the researcher. We observed a correlation between the metamorphic stage and the time of the beginning of each stage in the experimental population; however, this time varied within a range of one day (Figure 2).

In practice it is difficult for all animals of an experimental population to have the same exact value for the lower to upper fin height ratios; this situation introduces uncertainties which are unfavorable for physiological studies.

We suggest that it is necessary to include some physiological correlate to metamorphosis for staging metamorphosis, such as the circulating thyroid hormone levels at each stage. In fact, we reported in another study the $T_3$ serum levels at the different metamorphic stages in *A. mexicanum* (Cano-Martínez et al., 1994). The $T_3$ serum levels were detectable in the animals with $T_4$ administration. A hormone increase at stages I and II followed by a decrease at stages III and IV with a trend to return to neotenic values was observed (Figure 4) just as occurred during metamorphosis in other amphibians (Bentley, 1976; Kuhn and Jacobs, 1989; Larras-Regard et al., 1981).

Just as Norman's criteria have been used for *A. tigrinum*, we consider that our study can be used as a basis for staging metamorphosis in *A. mexicanum* and other urodeles.

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## References

Bentley, P. 1976. *Comparative Vertebrate Endocrinology*. Cambridge University Press, London.

Cano-Martínez, A., Asai ,M., Valverde-Rodríguez, C., and Aceves, C. 1994. Diencephalon and pituitary enkephalins

levels in *Ambystoma mexicanum* at various stages of thyroxine-induced metamorphosis. Submitted to Comp. Biochem. Physiol. January, 1994.

Cano-Martínez, A., Vargas-González, A., Matamoros-Trejo, G., Sandoval, A., Patiño, C., Avila, M.E., and Asai, M. 1990. Estudio sobre la formación de una colonia de *Ambystoma mexicanum* en condiciones de laboratorio. XXXIII Congreso Nacional de Ciencias Fisiológicas, México, C203.

Carr, J. and Norris, D. 1988. Interrenal activity during metamorphosis of tiger salamander *Ambystoma tigrinum*. Gen. Comp. Endocrinol. **6**:492-500.

Duellman, W. and Trueb, L. 1986. *Biology of Amphibians*. McGraw-Hill Book Company, New York.

Gosner, K., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**:183-190.

Kuhn E. and Jacobs F. 1989. Metamorphosis. In *Developmental Biology of the Axolotl*. Armstrong, J. and Malacinski, G., eds. Oxford University Press, New York.

Larras-Regard, E., Taurog, E. and Dorris, M. 1981. Plasma $T_4$ and $T_3$ levels in *Ambystoma tigrinum* at various stages of metamorphosis. Gen. Comp. Endocrinol. **43**:443-450.

Marx, L. 1935. Bendingungen fur die metamorphose des axolotls. Ergeb. Biol. **11**:244-234.

Norman, F. 1985. A practical method for staging metamorphosis in the tiger salamander *Ambystoma tigrinum*. Anat. Record **211**:102-109.

Taylor, A. and Kollros, J. 1946. Stages in normal development of *Rana pipiens* larvae. Anat. Record **94**:7-23.

Vargas-González, A., Cano-Martínez, A., Matamoros-Trejo, G. and Asai, M. 1990. Metamorfosis inducida con tetraiodotironina ($T_4$) en *Ambystoma mexicanum*. Determinación de la dosis óptima mediante criterios morfométricos. XXXIII Congreso Nacional de Ciencias Fisiológicas, México, C50.