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# THE EFFECTS OF PHOTOPERIOD, TEMPERATURE, AND THYROXIN ON THE TRANSFORMATION OF LARVAL *Ambystoma annulatum*

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## Introduction

The salamander, *Ambystoma annulatum* Cope, is endemic to the Ozarkian highlands (Connant 1958). Partially due to this limited range, little information concerning the biology of this species is available (Trapp 1956), and no information dealing with the development of the larvae has been previously published. The purpose of this investigation was to elucidate some of the ecological factors which initiate and regulate transformation of *A. annulatum* larvae.

*A. annulatum* exhibits a pattern of reproduction typical of Ambystomids. Adults migrate in mass during heavy rain to specific breeding ponds. The breeding migration of *A. annulatum* takes place in late September or October. The males arrive at the breeding ponds several hours before the females. After reaching the ponds, the males engage in an elaborate courtship with the females and then deposit sperm packets (spermatophores) on the floor of the pond. The females pick up the spermatophores with the cloaceal lips and then deposit 50-250 eggs in a large mass. Fertilization occurs as the eggs pass through the cloaca. The egg mass is then attached to the pond substratum. The entire process is completed within two or three days. The adults then leave the pond in favor of the mesic forest floor. Gilled larvae emerge from the eggs in 30 to 40 days depending on temperature. The larvae remain in the ponds for approximately nine months and then transform into terrestrial adults and leave the pond.

The process of transformation involves several major morphological changes. The larvae lose their gills and begin to utilize lungs. The blood vessels of the head and neck region become rearranged as does the skeletal and integermentary systems. Transformation involves an almost complete rearrangement of the salamander. Three factors, temperature, photoperiod and thyroxin, were investigated in order to determine how they affected the rate at which *A. annulatum* larvae transform.

## MATERIALS AND METHODS

### THYROXIN

A total of 560 larval *A. annulatum,* representing all size classes, were treated with seven different concentrations of thyroxin (thyroid hormone). The larvae were segregated, according to size, to reduce cannibalism and maintained in standard culture dishes containing 400 ml of natural spring water. Five larvae were placed in each dish. The larvae were kept at 24°C and a 24 hour dark photoperiod. The water was changed every two days after which thyroxin (in a basic solution) was added. This insured that the larvae were maintained in a known thyroxin solution at all times. Food was offered on alternate days. Earthworms were used for food but the aggressive larvae would eat almost any type of meat offered. This method has been used to transform tadpoles and is described in detail by Tata (1956). Controls were treated exactly as experimentals with the exception that no thyroxin was given. This procedure was used to determine if the hormone thyroxin did stimulate transformation of *A. annulatum* larvae and if all size classes of the larvae were uniformly effected by the hormone.

To determine if the halogen component of thyroxin could stimulate transformation, iodide (NaI) was given to 25 larvae. Iodine was introduced to the water in the same concentration that the halogen was present in the thyroxin concentrations.

## TEMPERATURE

Temperature is known to affect transformation of amphibians (Noble 1931). Artificially induced transformations, using thyroxin, of 270 *A. annulatum* larvae were carried out at three different temperatures (5, 20, 30°C) in a 24 hour dark photoperiod. Three concentrations of thyroxin were used (0.5, and 10.0 u/100 ml water). The larvae were transformed by the techniques previously described.

## PHOTOPERIOD

The effects of photoperiod on the transformation of amphibian larvae are unknown. A total of 160 larval *A. annulatum* were artificially transformed (5.0 u thyroxin/100 ml water) at four different photoperiods. These transformations were conducted at 24°C. The photoperiods selected were: 24 hours dark and 24 hours light, 12 hours light and 6 hours dark, 12 hours dark and 6 hours light.

## CONTROLS

All experimentals were accompanied with controls. The controls were placed in standard culture dishes with 400 ml spring water but no thyroxin was added. The controls were subjected to the same environmental variables as the experimentals.

## RECOGNITION OF TRANSFORMATION

The process of transformation was not accompanied with an increase in total length or snout-vent length. Rapid skin shedding and reduction of the tail fin at its most anterior point were the first indications that the larvae was undergoing transformation. As transformation continued, the resorption of the tail fin progressed posteriorly, after approximately one half of the tail fin was resorbed the gills began to disappear, the head became narrow due to rearrangement of the gill arches, and the skin color became darker. The first indication of resorption of the tail fin was considered to be the beginning of transformation. Transformation was considered complete when the gills were reduced to stubs and the salamander could survive out of water.

## Results

### THYROXIN

Thyroxin stimulates transformation of *A. annulatum* larvae (Table 1). Within a given size class the rate of transformation was increased by increasing the amount of thyroxin administered. The controls did not exhibit any indications of transformation.

### TEMPERATURE

Temperature affects the rate of transformation of *A. annulatum* larvae (Table 2). Increasing the water temperature decreased the time required for transformation to begin and reduced the time required for complete transformation. Smaller larvae responded differently to temperature than did larger larvae (Table 2).

### PHOTOPERIOD

Manipulation of photoperiods had no significant (99% confidence level) effect on the rate of larval *A. annulatum* transformation (Table 3). In natural populations transformation can be correlated with photoperiodicity but this is probably not due to the photoperiod per se, but merely reflects the correlation of photoperiod to environmental temperature and the physiological maturation of the larvae.

## Discussion

The thyroid hormone, thyroxin, initiates and regulates the rate of transformation of *A. annulatum* larvae. An increase in the amount of thyroxin reduced the time required for transformation but increasing the thyroxin two fold resulted in a time decrease of only one or two days. Doubling the thyroxin concentration did not result in a time decrease of 50%. This suggests that the larvae require a given amount of thyroxin to begin transformation and the addition of more hormone above this threshold has a relatively minor effect. Larvae of the same size class responded in the same manner to a constant thyroxin concentration. Larvae of different size classes responded differently to the same thyroxin concentrations. Snyder (1956) demonstrated that larval *A. gracile* of different year classes respond differently to identical thyroxin concentrations but found no difference within larvae of the same size class.

Two postulations may be presented, each of which would explain the varying reaction of different size classes to identical thyroxin concentrations but none of which can be supported by experimental data. One possibility is that the amphibian tissue will not respond to thyroxin until it has attained a certain degree of development. This physiological aging would require time, and the smaller larvae would have not had sufficient time to complete this developmental process. The tissue of the smaller larvae thus would be less sensitive to thyroxin than the older tissue. The second explanation is that the thyroxin artificially administered complemented and possibly stimulated the normal production of thyroxin by the larval thyroid gland. The larger larvae would presumably have a larger and more active thyroid and so a positive feedback reaction involving the thyroxin and thyroid would thus be greater. If a positive feedback mechanism is involved it would necessarily be of short duration. A long term positive feedback reaction would eventually result in death. The administration of thyroxin did not alter the sequence of morphological changes involved in *A. annulatum* transformation as it does in *Rana.*

The addition of iodide (NaI) did not stimulate transformation in any size class of larvae. This suggests that the halogen component of thyroxin is not a major limiting factor in the transformation of *A. annulatum* larvae. Iodide is known to stimulate transformation of salamander larvae (Dundee 1957) but this apparently takes place only when excessively high concentrations of iodide are given. This study shows that the administration of iodide in the same concentrations that it was present in thyroxin solutions does not stimulate transformation in *A. annulatum* larvae.

Temperature did effect the rate of transformation of *A. annulatum* larvae (Table 2). An increase in temperature decreases the time required for transformation to be initiated. This relationship between transformation and temperature is probably true for all poikilotherms (Noble 1931). Maher and Levendahl (1959) suggested that the sensitivity of poikilotherm tissue to thyroxin may vary with temperature. This investigation tends to support the theory of Maher and Levendahl (1959) but does not rule out the possibility that the actual production of thyroxin and not the sensitivity of tissue to thyroxin is affected by temperature.

Photoperiod affects tissue maturation and differentiation in many animals and plants. However, the photoperiod appears to have no effect on the process of larval *A. annulatum* transformation (Table 3).

## Summary

The hormone thyroxin initiates the process of transformation and regulates the rate at which this process proceeds in larval *A. annulatum.* Although iodide is essential in the biosynthesis of

thyroxin, it does not appear to be the limiting factor in the synthesis and secretion of thyroxin. Larvae of different size classes respond differently to the administration of thyroxin. This may be due to increased sensitivity of the older tissue to thyroxin or a positive feedback between thyroxin and the thyroid gland. There was a direct relationship between temperature and the rate of transformation but photoperiod did not have an effect on transformation.

**Table I. The relation of size and thyroxin concentration to time of transformation of *A. annulatum* larvae**

| Snout-Vent Length Size (mm) | Thyroxin (u/100 ml) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.25 | | 0.5 | | 1.0 | | 2.5 | | 5.0 | | 7.5 | | 10.0 | |
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| 5-10 | 22 | 8 | 21 | 6 | 18 | 7 | 17 | 3 | 4 | 3 | 8 | 2 | 6 | 2 |
| 11-20 | 20 | 5 | 16 | 6 | 18 | 5 | 17 | 3 | 4 | 3 | 7 | 1 | 6 | 2 |
| 21-30 | 14 | 4 | 16 | 4 | 14 | 3 | 17 | 2 | 4 | 2 | 4 | 2 | 4 | 2 |
| 30-42 | 18 | 4 | 13 | 3 | 16 | 2 | 14 | 1 | 9 | 1 | 7 | 1 | 6 | 1 |

A-days to begin transformation; B-days to complete transformation - 20 larvae of each size class were subjected to each thyroxin concentration.

**Table 2. The effect of temperature on the rate in days of transformation of *A. annulatum* larvae**

| Snout-Vent Length (mm) | Temperature (c) | Thyroxin (u/100 ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | | | 5.0 | | | 10.0 | | |
| | | 5 | 20 | 30 | 5 | 20 | 30 | 5 | 20 | 30 |
| 15 | | -[a] | 20[b] | 14 | - | 6 | 5 | 14 | 6 | 5 |
| 30 | | - | 19 | 14 | 18 | 5 | 5 | 15 | 4 | 5 |
| 40 | | - | 19 | 14 | 20 | 5 | 5 | 14 | 4 | 4 |

[a]did not begin transformation after thirty days; [b]days required to begin transformation 10 larvae were tested at each temperature and concentration.

**Table 3. The effects of photoperiods on the rate of transformation of *A. annulatum* larvae in a 5.0 u/100 ml thyroxin solution maintained at 24°C**

| Snout-Vent Length (mm) | Photoperiod in Hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 dark | | 24 light | | 12 dark - 6 light | | 12 light - 6 dark | |
| | A | B | A | B | A | B | A | B |
| 5-10 | 7 | 3 | 5 | 2 | 6 | 2 | 5 | 1 |
| 11-20 | 4 | 1 | 4 | 3 | 5 | 3 | 4 | 2 |
| 21-30 | 5 | 1 | 4 | 1 | 4 | 1 | 5 | 3 |
| 31-44 | 3 | 2 | 4 | 2 | 5 | 1 | 4 | 2 |

A-days to begin transformation; B-days to complete transformation. 10 larvae of each size class were transformed at each photoperiod.

## LITERATURE CITED

Connant, R. 1958. A Field Guide to Reptiles and Amphibians of Eastern North America. Houghton Miffin Co. Boston, 366p.

Dundee, H. A. 1957. Partial Metamorphosis induced in *Typhlomolge Rathbuni.* Coperia 1957:52-53.

Maher, M. J. and B. H. Levendahl. 1959. The Effects of the Thyroid Gland on the Oxidative Metabolism of the Lizard *Anobis Carolinensis.* J. Exper. Zool. 140:169-189.

Noble, G. K. 1931. The Biology of the Amphibian. McGraw Hill. N. Y. 577p.

Synder, R. C. 1956. Comparative features of the life history of *Ambystoma Gracile* (Baird) from populations at high and low altitudes. Copeia 1956:41-50.

Tata, J. R. 1965. Thalidomide and induced Amphibian Metamorphosis. Nature 204: 939-940.

Trapp, Mary M. 1956. Range and Natural History of the ringed salamander *Ambystoma annulatum* (Cope). Southwestern Nat. 1:78-82.