**Influence of Elemental Iodine and Thiourea on Metamorphosis of Philautus sp.**

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**ABSTRACT**

The developmental stages of frog species are usually influenced by the presence or absence of naturally occurring acceleratory or inhibitory agents. The embryonic development of *Philautus* sp. has been studied in its natural habitat, followed by laboratory observation. The study concentrates on the determination of the influence of thyroid related hormones on the metamorphosis of *Philautus* sp., especially the acceleratory action of iodine and inhibitory action of thiourea. The series of iodine concentrations as 0.5, 1.0, 1.5 and 2.0 ppm and thiourea concentrations as 5.0 and 10.0 ppm were maintained with a control. The rate of metamorphosis was found to show a gradual increase from its lower to higher concentration in case of iodine. The highest 2.0 ppm iodine culture tadpoles, completed metamorphosis on the 24th day, while controls metamorphosed completely on 46th day, since iodine feeding. On the other, in the case of thiourea, there was a gradual inhibition of the metamorphosis rate from its lower to higher concentrations.

**Keywords**: Philautus, Iodine, Thiourea, Metamorphosis

**INTRODUCTION**

The radical changes that convert a larva into an adult constitute metamorphosis. The progressive metamorphosis of amphibian tadpoles involves the gradual limits from aquatic to terrestrial mode of life. The significant morphological change involves Keratinization of skin, Herbivorous to carnivorous feeding habit, the total regression of tail and appearance of limbs. The regression of the tail is brought about by apoptosis, and it occurs in four stages. First, protein synthesis decreases in the striated muscle cells of the tail[1]. Next, there is an increase in concentrations of digestive enzymes within the cells. Concentrations of lysosomal proteases, RNase, DNase, collagenase, phosphatase, and glycosidases all rise in the epidermis, notochord, and nerve cord cells [2]. Cell death is probably caused by the release a metalloproteinase inhibitor (TIMP) which is added to the tail and prevents tail regression [3]. As the tadpole grow it develops a pair of hind limb, followed by a pair of forelimbs. The gills of the tadpole gradually close over as the animal develops lungs and becomes a mouth breather, as such its mouth becomes wider [4]. All the diverse changes in amphibian metamorphosis are brought about by the diffusion of the hormones, thyroxine (T₄) and tri-iodothyronine (T₃) from the thyroid during metamorphosis. Of these, T₃ is the active hormone. The release of T₃ is under the control of the hypothalamic portion of the brain. Action of T₄ is more organ specific than tissue specific. The importance of the thyroid gland and thyroxine on metamorphosis is proved by the following experiments. When the frog tadpoles were fed with dried and powdered thyroid glands of sheep, they metamorphosed precociously [5]. When a thyroid gland is removed from young tadpoles, they fail to metamorphose [6]. The thyroidless tadpoles continue to grow and attain much greater size and form “Giants”. The maximum length of a normal tadpole is about 60 mm. But a thyroidectomised tadpole grow to a length of about 123 mm. When a thyroid-less tadpole is fed with dried thyroid gland, it proceeds to metamorphosis. Similarly a thyroid-lesstadpole can be stimulated to undergo metamorphosis by rearing the tadpole in water containing powdered thyroid gland. The urodele *Amblistomananceanum* will not undergo metamorphosis under normal condition. But it may be induced to metamorphose by thyroid treatment [7]. A hypothalamic–pituitary–thyroid axis controls the production of TH, at the onset of metamorphosis[8]. The response to thyroid hormones is specific to the region of the body. During metamorphosis T₃ and T₄ increase in concentration, thereby causing the tadpoles to become frogs [6]. In amphibian larvae, thyroid hormone is the primary morphogen controlling metamorphosis [9]. A role for CRF (Corticotropin-Releasing Factor) as a TRF has been shown in larvae of several amphibian species, and CRF can accelerate metamorphosis (*Rana catesbeiana* and *Spea hammondii*); [10]. Rana perezi, [11]; *Bufo arenarum*, [12]; and *Ambystoma tigrinum*; [13]. Recent work has shown that CRF can activate locomotion, and it functions as a potent anorectic agent in both tadpoles and juvenile frogs (*Bufo*;[14]; *Xenopus laevis*[15, 16, 17, 18, 19]).

*Philautussp.* was found in streams or terrestrial habitat, restricted in vegetations surrounding ponds. All clutches of eggs were put either under the leaves or inside the coiled leaves, directly above water, thus protected from the sun. Number of eggs in one clutch is variable. Breeding female will lay all of
her eggs, clumped into a single clutch during amphilimixis. Eggs are unpigmented, pale yellow in colour, coated with sticky jelly substances. Fertilized eggs will hatch after 16 days. Tadpoles will emerge and slide to water below the leaves. Tadpoles will stay in stage 25 for quite some time (48 days the farthest) growing its body before moving to the next stage. After about two month tadpoles will be in stage 26 - 38 where hind limb develops. In the next 20 days forelimb will emerge. It takes about three months for an egg to become a fully metamorphosed frog. [20]. In this investigation, we have undergone feeding experiments for the metamorphosing Philautus Sp. with different concentrations of both iodine and thiourea.

MATERIALS AND METHODS

Animals and study area
The animals used for this experiment were tadpoles of Philautus sp hatched from the same egg mass collected from a small temporary pond from Mattampuram, Thrissur, Kerala, India. The collected species are brought to laboratory and identified by a Zoologist at University of Madras, Chennai, India.

Larval Feeding
The larvae subsist and increase in size by utilization of yolk present in the body cells. Hence, they were not fed initially, for some time period. After the larvae developed to a length of 2 cm, they were fed with food which consisted entirely of algae gathered from the same habitat. The environmental factors such as temperature, pH, aeration, light and water supply were kept uniform. Each culture consisted of ten larvae in 2 L of water collected from their natural habitat. The water was changed periodically to prevent fouling.

Chemicals
Chemicals, Iodine anhydrous, and 1,3-Dimethyl-2-Thiourea, were purchased from Sigma (St. Louis, MO, USA).

Iodine Feeding
The concentrations were prepared and fed according to the method of [21]. Five different groups of tadpoles were tested to study the accelerating effect of iodine: a control, 0.0, 0.5, 1.0, 1.5 and 2.0 ppm (parts per million) iodine groups. The tadpoles were maintained in the required concentrations of iodine and tested every day to see the rate at which it develops in comparison to other groups.

Thiourea feeding
The concentrations were prepared and fed according to the method of [21]. Three different groups of tadpoles were tested to study the inhibitory effect of thiourea: a control, 10 and 5 ppm thiourea groups. The tadpoles were maintained in the required concentrations of thiourea and tested every day to see the rate at which it develops in comparison to others.

Morphological examination
The whole body length and tail length of each tadpole were measured (cm) every day and the average length of the body and tail were measured for each culture. The morphological changes such as limb formation and pigmentation were also noticed regularly.

Statistical Analysis
Test samples were carried out independently in triplicates, data was expressed as the mean ± standard deviation (SD) and the results were processed using Excel 2003 (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION
The normal embryonic development of Philautus sp. was studied in the field. The tadpoles measuring around 2 cm in length and showing no indication of limb buds, which were utilizing yolk since their emergence from the egg jelly, were fed with iodine and thiourea. They completely metamorphose into adult after 46 days. Based on this, the following experiments were conducted to study the effect of iodine and thiourea on the metamorphosis of Philautus sp. As the tail shrinks and the hind limb develops, the tadpole will show a gradual behavioral change owing to its aquatic to terrestrial mode of life. The tadpoles swim gradually as the tail starts shrinking. The animal move away from water towards the termination of metamorphosis. They become sluggish in water after it completes around 70% of its metamorphic stages.

No change was observed during the first 48 hours in the cultures. After 48 hours, on microscopic examination, 1.5 ppm and 2.0 ppm iodine group showed considerable fraying of the delicate tail fin and extravagation of blood from the capillary vessels. No mortality was seen in any cultures. All the iodine fed animals were more deeply pigmented than the controls. The control, 0.0, 0.5 and 1.0 ppm iodine group showed practically no tail shrinkage, whereas it was shown clearly in 1.5 ppm and 2.0 ppm iodine group. The tadpoles in stronger solutions developed epithelial hind limb buds. (Fig.1).
faster was the metamorphic rate. Iodine, even in such minute quantities present naturally in control showed its effect. Iodine feeding was abandoned after 50 days from the date of first iodine feeding except for 2.0 ppm iodine culture, for which it was terminated on the 24th day since metamorphosis was completed. None of the control tadpoles had completed metamorphosis. Three tadpoles of 1.5 ppm iodine culture metamorphosed by 32nd day. Two larvae of 1.0 ppm culture metamorphosed by 36th and 37th day respectively. Remaining tadpoles failed to metamorphose and consequently died. When the tadpoles were fed with still higher concentrations of iodine (2.5 and 3.0 ppm), massive death of tadpoles within 48 hours resulted.

On 8th day of iodine administration, a bulging of hind limb nodes was observed in 1.5 and 2.0 ppm iodine group on microscopic examination. The same protrusion of hind limb was seen in 0.5 and 1.0 ppm group on 12th and 11th day respectively. While control showed a microscopic hind limb development on 17th day. But, on 17th day, 2.0 ppm group showed larger toed hind limbs with signs for the development of fore limbs. On 23rd day 2.0 ppm iodine group showed full development of limbs. In comparison, limb development was complete for 0.5, 1.0 and 1.5 ppm iodine groups on 29th, 28th and 25th day respectively (Fig. 1). Control group developed limbs on 42nd day.

For 2.0 ppm iodine group, the significant shrinkage of tail started on 12th day after iodine administration. For 0.5, 1.0 and 1.5 ppm iodine groups, it started on 22nd, 18th and 16th day respectively. In case of control group, the tail regression was significant by 29th day. The full adsorption of tail was seen in 2.0 ppm group on 28th day. While, it was noticed for 0.5, 1.0, 1.5 ppm iodine groups and control groups on 38th, 35th, 32nd and 29th day respectively (Fig.2 and 3).

The pigmentation rate also varies as the metamorphosis proceeds. As the metamorphic rate increases the pigmentation rate also increased. (Table 1 and 2).

**Table 1. Pigmentation observed during the study of metamorphosis of Philautus sp. upon Iodine feeding.**

| Day of development | Control | 0.5 ppm | 1.0 ppm | 1.5 ppm | 2.0 ppm |
|--------------------|---------|---------|---------|---------|---------|
| 2                  | -       | -       | -       | -       | -       |
| 8                  | -       | -       | -       | -       | +       |
| 12                 | -       | -       | -       | -       | ++      |
| 18                 | -       | -       | -       | +       | +++     |
| 24                 | -       | -       | -       | ++      | ++++    |
| 25                 | -       | -       | +       | +++     | ++++    |
| 28                 | -       | +       | ++      | +++     | +++++   |
| 29                 | +       | ++      | +++     | +++     | ++++++  |
| 32                 | ++      | +++     | ++++    | +++++   | +++++++ |

-No Pigmentation, +Initiation of pigmentation, ++Pigmentation was found to spread on the whole body, +++Pigmentation towards the hindlimbs, ++++Pigmentation towards forelimbs and head, +++++Pigmentation was delicate towards the shrinking tail, ++++++Pigmentation intense, +++++++Pigmentation was highly prominent with melanin formation, ++++++++Fully pigmented

**Table 2. Pigmentation observed during the study of metamorphosis of Philautussp. upon Thiourea feeding:**

| Day of development | Control | 5 ppm thiourea | 10 ppm Thiourea |
|--------------------|---------|----------------|-----------------|
| 2                  | -       | -              | -               |
| 8                  | -       | -              | -               |
| 12                 | -       | -              | -               |
| 18                 | -       | -              | -               |
| 24                 | -       | -              | -               |
| 25                 | -       | -              | -               |
| 28                 | -       | +              | ++              |
| 29                 | +       | ++             | +++             |
| 32                 | ++      | +++            | ++++            |
Upon feeding, Inorganic iodine and its compounds; iodoform and potassium iodide greatly accelerate metamorphosis of tadpoles. With very high iodine percentage, metabolism is stimulated to such an extent that the animal emaciate rapidly and die early before there is time for cellular differentiation. In comparatively lower iodine percentage, the size of animals is roughly inversely proportional to the percentage of iodine administered. But a close association of differentiation with pigmy size is not characteristic of thyroid feeding as such. It is safe to assume that an absolutely iodine free diet fed to frog larvae reared in iodine free water would inhibit metamorphosis indefinitely. Moreover, when the frog larvae restricted to certain diets (e.g. Thymus gland) apparently fail to undergo metamorphosis at the proper time, or not at all, the cause of this delayed metamorphosis is lack of sufficient iodine [5]. Overdose with iodine or thyroid extract leads to decrease in size and great emaciation of the animals [21]. The iodine in minimum doses stimulates growth, though not to any great degree, and in large doses it leads to cessation of growth and tissue disintegration or absorption. Iodine feeding has little effect on pigmentation. If fed in sufficiently strong concentrations, the animals appear darker than the controls.

- No Pigmentation, + Initiation of pigmentation, ++ Pigmentation was found to spread on the whole body.

Fig 2. Variations in body length (cm) of Philautus sp. during iodine feeding

Fig 3. Variations in tail length (cm) of Philautussp. during iodine feeding

In thiourea groups, none showed limb formation till the abandoning of experiment on 50th day after iodine feeding in both 5.0 and 10.0 ppm concentrations. While control group developed limbs on 42nd day. The tail regression was very slow for thiourea fed tadpoles compared to iodine fed groups. A retrogressive regression of tail was seen in control group. In 5.0 and 10 ppm thiourea group the regression was so insignificant on 18th day while it was significant in control group. On 25th day, 5.0 ppm group showed shrinkage and similar was shown by 10 ppm group on 29th day. (Fig. 4 and 5).
or tadpoles with reduced pigmentation were obtained. Of these tyrosinase inhibitors, thiourea, unpigmented tadpoles activity. In our experiment, when larvae were treated with one formation in frog embryos is the inhibition of tyrosinase in vitro, it is assumed that their role in preventing melanin demonstrated that thiourea derivatives inhibit tyrosine activity. Since it has been concentrations on metamorphosis of comparative analysis of the influence of iodine and thiourea implements its endocrinological influence to bring out a keeping the amphibians – a connecting link between aquatic and terrestrial mode of life - as a biological model. It also amphibians. Gen and Comp. Endo, (1992) 87:6–13.

CONCLUSION
Elemental iodine administered to Philatus sp. tadpoles in sufficient amount brings about rapid metamorphosis. None can complete metamorphosis without the influence of iodine at least in minute quantities present normally in the environment. Amphibian larvae show no response to iodine or thyroid stimulation during their early stages of development, acquiring an ability to respond only after they have reached a specific fairly advanced stage of development, differing fairly widely in different species. There is an orderly succession of steps in metamorphosis due to differences in the threshold of response in different tissues. Thiourea, a thyroid inhibiting agent, decreased the rate of metamorphosis. Thiourea, derivatives inhibit tyrosine activity in vitro and prevents melanin formation in frog embryos.

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