

## The Effect of Thyroxine on the Growth and Development of *Xenopus laevis* Tadpoles

### *Introduction*

Hormones, the basic components of the endocrine system, are crucial in the regulation of vital cellular responses. They are receptor specific, and though there are a great variety of hormones, each serves a particular function in an organism. One such hormone, thyroxine (T4) – a derivative of tyrosine with four iodine groups – has been established to affect growth and rate of development (Mechanism 2005, Yen 2001). Human disorders linked to T4 levels in the body have enabled scientists to better understand the exact function of this hormone as it relates to cell maturation. A condition known as hyperthyroidism in which the body produces more T4 than normal is characterized by heightened metabolic activity and formation of goiters while its opposing condition, hypothyroidism, in which T4 levels are less than normal, is characterized by more depressed metabolism and may even lead to mental retardation in earlier stages of development (Brown 1994).

Produced and secreted by the thyroid gland of the throat, T4 has been shown to affect growth in a variety of tissues in a variety of organisms. Thyroidectomized rats treated with T4 by injection clearly exhibited accelerated growth in terms of weight and length over controls (Scow 1959), and similarly, dwarf mice, whose T4 levels were below normal, outgrew control mice of the same stunted condition when provided an outside source of T4 (Bates and Holder 1988), implicating T4 as a key player in cell proliferation and muscle mass buildup. The effect T4 has on growth and development is also consistent with data on metamorphosis. Exogenously administered T4 was demonstrated not only to increase size but also to speed up metamorphosis in an aquatic species (Solbakken et. al 1999). T4's ability to promote cell maturation, visualized in epidermal skin differentiation in tadpoles, can also be used to show the growth and developmental promoting effects of T4 (Wright 1973).

### *Hypothesis*

It has been reasonably established that thyroxine is an important factor in determining the growth and development of certain cells and tissues whether administered endogenously or exogenously. Since the presence or excess of T4 appears to stimulate more rapid growth and

maturation than absence or insufficiency, it would seem that higher dosages of T4 will induce faster rates of development. In the present study, it is predicted that increasing the concentration of exogenous T4 will accelerate growth in *Xenopus laevis* tadpoles up until cellular thyroxine receptors become saturated, producing a logarithmic curve.

*Methods*

One hundred *Xenopus laevis* tadpoles were purchased through a mail order catalog although not all were used. Four glass fingerbowls were used to separate the four sample groups, each group containing ten tadpoles by random selection. Thyroxine solutions of 0.00 ppm, 0.48 ppm, 0.64 ppm, and 0.96 ppm were prepared in separate fingerbowls by serial dilution using the pond water provided with the shipping order as the diluting liquid medium. Before immersing the tadpoles in the thyroxine solutions, initial length and weight measurements were recorded. The fingerbowls were stored in the lab room and kept at room temperature. Dead tadpoles were removed and discarded on a daily basis, and live tadpoles were fed four to five small pellets of tadpole food purchased from a local pet store every other day. Length and weight measurements of live specimens were also acquired every other day and averaged.

*Results*

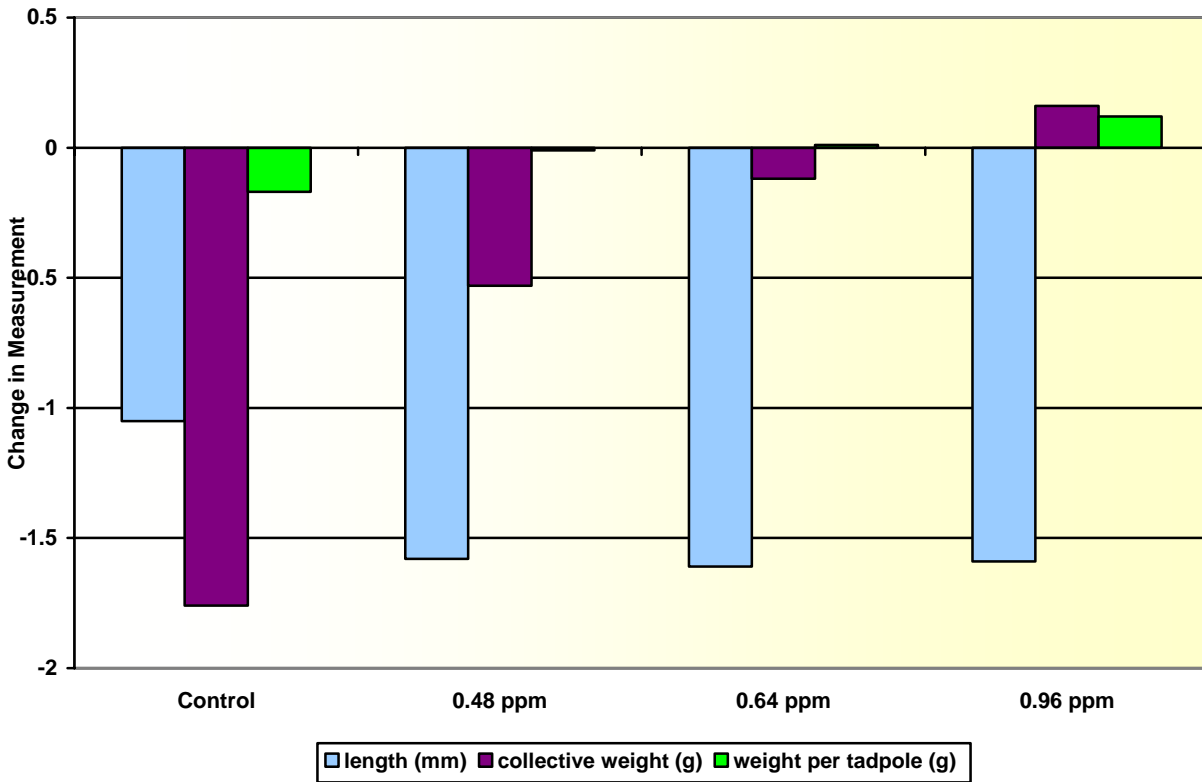
Mortality was prevalent in all groups (table 1), making it difficult to collect much usable data. All the tadpoles did not survive longer than a week, and only two sets of data (Day 1 and Day 3) were obtained before termination of the experiment. When the tadpoles first arrived, they looked lively and healthy, but after a couple of days in the fingerbowls, they became lethargic and did not appear to eat any of the food though supplied with a sufficient amount.

**Table 1.** Percentage of tadpole deaths in each of the samples. Control tadpoles died before thyroxine treated groups. Of the three variable groups, tadpoles immersed in the highest concentration, 0.96 ppm, showed the greatest mortality.

<b>Mortality Rate (% of total)</b>				
	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Day 4</i>
<i>0.00 ppm</i>	0	60	100	100
<i>0.48 ppm</i>	0	20	50	100
<i>0.64 ppm</i>	0	10	30	100
<i>0.96 ppm</i>	0	30	70	100

With respect to growth, all tadpoles shortened, more so with higher concentrations of thyroxine. Although control group tadpoles were longer than their thyroxine treated

counterparts, the greatest and only positive change in weight was observed in the 0.96 ppm thyroxine treated tadpoles. Essentially no weight per tadpole fluctuations occurred at intermediate thyroxine levels while control tadpoles appeared to lose weight (figure 1).



**Figure 1.** Effect of thyroxine on length and weight of tadpoles. Direct measurements of the tadpoles could not be compared, so instead the changes in length (blue), collective weight (purple), and weight per tadpole (green) were plotted. Tadpoles seemed to shorten and lose weight in all groups, but tadpoles in the highest concentration did show weight gain.

### *Interpretation*

In the present study, it is inconclusive whether the data supports the hypothesis since only two days worth of data was collected. It is thus impossible to suggest any solid trend associated with the thyroxine treatment, given that two points cannot produce a logarithmic curve as was predicted. However, from the data that was collected, there were small changes in length and weight that could be of some significance. The greater shortening (i.e. more negative value) of thyroxine treated tadpoles (figure 1) over control tadpoles may signify accelerated rates of metamorphosis. Because thyroxine appears to affect metamorphic rates in fish (Solbakken et. al 1999), it would be reasonable to conclude that an exogenous source of thyroxine would produce

a similar effect in tadpoles. Since tadpole metamorphosis is characterized by the shortening of the tail and because measurements were taken from the top of the head to the tip of the tail, perhaps the decrease in length was in direct correlation with tadpoles being farther along in metamorphosis. The greater weight gain of the highest concentrated thyroxine treated tadpoles (figure 1) would also be consistent with a faster rate of development. This is not to say that weight gain was due to tail recession, for this is merely cell redistribution. A more sound explanation for the observed change in weight would be that the excess of thyroxine stimulated and accelerated growth and cell proliferation of the entire organism, causing the 0.96 ppm group to be the heaviest. This relates back to thyroxine's role in enhancing protein synthesis (Bates and Holder 1988, Scow 1959).

It was also inconclusive if thyroxine played any role in mortality rates. All of the control tadpoles died before any other treatment group (table 1), which might suggest that the added concentrations of thyroxine may have provided tadpoles with more ATP energy to handle and cope with the stressful change to a closed system, more so than tadpoles that were not enhanced in this way. In other words, faster development may have made thyroxine treated tadpoles more resilient against the elements working against them. However, of the three thyroxine treated groups, the highest concentration at 0.96 ppm showed the poorest survival rate (table 1), which may suggest that too much thyroxine may have had a detrimental effect as well when administered in a closed system. As is known, thyroxine speeds up metabolism (Mechanism 2005, Yen 2001), which in turn increases oxygen consumption and possibly boosts waste excretion. Thus, in the closed system, where the water was not replenished and the tadpoles had a limited water supply, it might be possible that oxygen was depleted at a faster rate and that toxic ammonia levels built up in greater quantities in the higher concentrated thyroxine solution, thereby forcing the tadpoles to compete for available resources. With little oxygen to power ATP production and with toxins saturating the aqueous solutions, all the tadpoles died in a very short time span.

Another highly probable factor in premature tadpole mortality was the improper care of already stressed creatures. Not only were the tadpoles removed from their natural environments into overcrowded conditions, but measuring techniques that required movement between many different containers may have proved too stressful for the tadpoles, especially in the sensitive stages of early development. Furthermore, the aforementioned combination of thyroxine treated

water, ammonia buildup, and oxygen depletion probably induced stress on the cellular level. Under these proposed stresses, the tadpoles did not eat the provided food, and in hopes that they would eat, a small amount of food was left in the bowls, which might have inadvertently polluted the water. To better this experiment, it is advisable to raise an initial group of tadpoles prior to thyroxine treatment if given enough time, as a means to not only become familiar with tadpole care beforehand but also to maintain optimum living conditions for tadpoles when they must be exposed to variable circumstances.

#### Works Cited

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