A study of thyroid hormones in eggs and larvae of walleye, Stizostedion vitreum, and rainbow trout, Oncorhynchus mykiss

Jane Pires Hey
Iowa State University

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A study of thyroid hormones in eggs and larvae of walleye, *Stizostedion vitreum*, and rainbow trout, *Oncorhynchus mykiss*

Hey, Jane Pires, Ph.D.

Iowa State University, 1994
A study of thyroid hormones in eggs and larvae of walleye, *Stizostedion vitreum*, and rainbow trout, *Oncorhynchus mykiss*

by

Jane Pires Hey

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Iowa State University
Ames, Iowa

1994
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GENERAL INTRODUCTION

Organization of this dissertation

This thesis contains a general introduction with a literature review followed by two papers, 1 and 2, and concludes with a general summary. References cited in the general introduction and general summary will follow the text of the thesis. The papers will be submitted for publication to and are presented in the style format specified by the Canadian Journal of Zoology. The first paper was written by Jane Hey with editorial assistance from Dr. Eugenia Farrar. The second paper was prepared by Jane Hey and Dr. Eugenia Farrar in collaboration with graduate students and faculty in the department of animal ecology, Brian Bristow, Craig Stettner and Dr. Robert C. Summerfelt. All research involving thyroid hormone analysis by radioimmunoassay, sample preparation, extraction and larval fish immersion studies were performed by Jane Hey with assistance of Eugenia Farrar. For paper 2 most of the walleye procurement, culture and performance studies were conducted by Brian Bristow, Rick Bushman, and Craig Stettner.

Literature review and thesis research rationale

In endotherms, thyroid hormones have two regulatory functions: maintenance of basal metabolism (respiratory oxygen consumption) and morphogenesis (growth and development). While in ectotherms such as fish, regulation of oxidative metabolism or calorigenesis has been extremely difficult to demonstrate (Gorbman et al., 1983; Matty, 1985), morphogenetic regulation is clearly very important; however, the role of thyroid hormones in early development of fish is not well understood (Sullivan et al., 1987). Thyroid hormones are known to be essential for cerebellar neuron maturation and central nervous system development in mammals.
In birds, amphibians, and mammals, thyroid hormones are also known to have a role in embryonic development (reviewed by Frieden, 1961; Freeman, 1974; Schwartz, 1983; Porterfield and Hendrich, 1993). However, endocrine regulation of specific events in embryogenesis and larval development in lower vertebrates has not received much attention (Brown and Bern, 1989). In this introduction I will outline what is known about the possible role of thyroid hormone in early teleost development and present a plan for studying this problem in walleye and trout.

Thyroid hormones are composed of coupled iodinated tyrosine residues (Baulieu and Kelly, 1990). They are synthesized in thyroid follicles and bound to thyroglobulin but are released from this organic molecule before reaching the circulation. This hormone exists in two forms, T4 (thyroxine) or tetraiodothyronine and T3, triiodothyronine. T4 is considered to be the less active prohormone which is converted to the more active hormone, T3 (Pittman, 1979). According to the peripheral model of thyroid hormone regulation proposed by Eales (1985), the hypothalamic-hypophyseal axis participates by maintaining an adequate supply of prohormone, T4, which may be converted enzymatically to T3 by a specific deiodinase in peripheral tissues. Regulation of this system is proposed to be extrathyroidal.

Thyroid hormone receptors are nuclear T3 binding proteins (Oppenheimer et al., 1972). The receptor is a member of the steroid receptor supergene family and has been found through molecular cloning to be a product of the c-erb-A gene (Evans, 1988; Weinberger et al., 1986). According to Eales, (1985), these receptors have a higher affinity for T3 than for T4. In mammals, birds and amphibians two classes of binding sites (a and b) have been identified in brain, liver, kidney and
tailfin (Galton and Schaafsma, 1983; Forrest et al., 1991). Baker and Tata (1990) state that although in mammals the b form of thyroid hormone receptor is thought to be physiologically active they found that the a form mRNA is the predominant form in both larval and adult *Xenopus*. (Galton, 1992). In teleosts a single class of nuclear receptors has been identified in brain, liver and erythrocytes (Dasmahapatra et al., 1990, 1991; Sullivan et al., 1987). The thyroid sensitivity of a specific tissue depends on the number of hormone occupied receptors. The thyroid hormone - receptor complex initiates biological activity within the cell.

**Effects of thyroid hormones on teleost growth and development**

As shown by a variety of experiments over the past 50 years, fish respond to increases or depletion of thyroid hormones. Researchers have either supplemented thyroid hormones through immersion in T₄ or T₃ or ablated thyroid hormone activity chemically with antithyroid compounds, such as thiouracil or thiourea, or through radiothyroidectomy.

Thyroid hormones affect fish growth and development, appearance, survival, hatching and behavior. Responses to thyroid hormones often vary with the type of fish studied, dosage and experimental program. Thyroxine has been shown to accelerate larval growth and development and results in greater body weight, length and more adult form in milkfish, *Chanos chanos*, and tilapia, *Sarotherodon niloticus* L., (Nacario, 1983; Lam et al., 1985; Lam, 1985). However, Dales and Hoar (1954) report that thyroxine reduces growth rate and total length of chum salmon, *Oncorhynchus keta*, and Brown et al. (1988) found only a temporary increase in size and length of striped bass, *Morone saxatilis*, larvae.

At low concentrations T₄ has been shown to increase skeletal growth and head width of larval brown trout, *Salmo trutta* L., (Barrington and Rawdon, 1971).
However, at higher concentrations T₄ causes vertebral column abnormalities resulting in lordosis and scoliosis (Nacario, 1983). Radiothyroidectomy of larval platyfish, salmon and trout results in destruction of thyroid follicles, decreased bone, cartilage, muscle and skull growth in Chinook salmon, *Oncorhynchus tshawytscha*, and steelhead trout, *Salmo gairdneri*, (Norris, 1969). Matty et. al. (1982) reports that an injection of T₃ at physiological levels (0.05 and 2 µg/g body weight) increases protein and nucleic acid synthesis in liver and muscle of starving tilapia. In early development of fish, thyroxine increases the rate of growth of the blastoderm down over the yolk and causes epidermal thickening (Dales and Hoar, 1954; Nacario, 1983). Pectoral fin growth is enhanced in chum salmon by T₄ (Dales and Hoar, 1954). Although supplementation and antithyroid studies indicate that thyroid hormone promotes growth, these could be either direct thyroid hormone effects or indirect effects of thyroid hormone stimulation of growth hormone (GH) production. Without further experimentation using techniques such as thyroidectomy and hypophysectomy or blocking of growth hormone receptors, the possibility that thyroid hormone actions occur at least partially by stimulating the production of growth hormone cannot be eliminated. T₃ has been shown to trans-activate the growth hormone gene in rats (Glass et al., 1987). A recent review, Rodriguez-Arnao et al., (1993) states that thyroid hormones, growth hormone and insulin like growth factors are part of an interactive system. T₃ influences hypothalamic control of GH levels and it has been found to increase somatotroph numbers in vitro.

Thyroid hormone effects on hatching time and larval survival have also produced conflicting results. For example T₄ increased hatching of chum salmon (Dales and Hoar, 1954). An injection of 20 µg/g T₃ in prespawning female striped bass resulted in increased larval body size at one week substantially lower mortality
and enhanced swim bladder inflation (Brown et al., 1988). Thyroxine combined with salinities ranging from 1% to 10% sea water improved egg viability and hatchability in carp, Cyprinus carpio. It also promoted larval survival, growth and development (Lam and Sharma, 1985).

On the other hand T₃ and T₄ delayed hatching, hatching enzyme release and hatching success in tilapia (Reddy and Lam, 1991). Tagawa and Hirano (1991) found that an immersion of prespawning female medaka, Oryzias latipes, in 0.03% thiourea decreased their plasma thyroid hormone levels. After treatment, circulating T₄ and T₃ levels decreased to 20% of previous levels and there was a 75% reduction of thyroid hormones in their fertilized eggs. However, they found no significant difference in hatchability, time of hatching, or total length and weight or survival under starvation of the experimental and control groups of larvae. The authors point out that because these fish normally have a much higher survival rate than striped bass, medaka may have a much lower need for thyroid hormone.

Exogenous thyroid hormone caused increased silvering in developing fish (Lam et al., 1985; Dales and Hoar, 1954). Thiourea, an antithyroid agent, has been shown to reduce silvering (Dales and Hoar, 1954). Because silvering is caused by guanine deposition, this suggests that thyroid hormone increased purine nucleotide metabolism (Dales and Hoar, 1954). Increased activity and loss of negative phototaxis are responses by some larval fish to thyroid hormone treatments; these behavioral responses could be important for survival if they enable the developing fish to navigate and find food (Woodhead, 1966; Lam, 1985).

Thyroid hormone induces metamorphosis in flounder and accelerates dorsal fin ray reabsorption (Miwa and Inui, 1987). As in amphibian metamorphosis, the authors also found T₃ to be a much more potent inducer of development than T₄.
Role of thyroid hormone in teleost growth and development

According to Dickhoff and Darling (1983), all growth and development is influenced by activation of the thyroid hormone system which conveys the endocrine signal that the animal is in an appropriate state of fitness and nutrition to proceed forward with development. Eales (1979) views thyroid hormone as acting in a permissive or synergistic way but the system will be inactive when the organism is fasted or stressed. Specker (1988) considers thyroid hormones to be preadaptive in preparing the organism for transitions to a subsequent developmental stage. These transitions involve morphogenic and physiologic changes such as hatching, independent food getting and respiration by gills. These changes allow the animal to assume a new developmental form and often to move into a new environment.

Thyroid hormones may be necessary for assisting organisms to survive critical periods. Blaxter and others have recognized "critical periods" as times of massive morphogenic, behavioral or physiological changes which teleosts must complete in order to move on to the next developmental stage in their life cycle (Li and Mathias, 1982; Blaxter, 1988). Hatching, first feeding, respiratory transition, and swim-up are all possible critical periods in early teleost development. The significance and timing of these critical periods depends on the natural history of the organism in question.

Presence of hormones in eggs and early developmental stages

It was previously thought that early development events in embryogenesis were independent of endocrine regulatory control because hormones could not be produced by the developing embryo before organogenesis. However, in 1987, thyroid hormones were found to be present in unfertilized striped bass eggs (Brown et al., 1987) and since in chum salmon (Tagawa and Hirano, 1987), rabbitfish,
Siganus guttatus, (Ayson and Lam, 1993), medaka (Tagawa and Hirano, 1991),
tilapia, Oreochromis mossambicus, (Weber et al., 1992), and several additional
marine species (Tagawa et al., 1990). In other studies injection of prespawning
female striped bass with T3 (20 ng/g) resulted in a significant elevation of T3 in the
eggs and subsequent increases in growth, swim bladder inflation and survival of the
developing larvae (Brown et al., 1988). From this study they concluded that T3 can
be transferred from maternal circulation to developing oocytes and suggested that a
fully differentiated hypothalamic-adenohypophyseal-thyroid (HAT) axis is not a
prerequisite for larval responsiveness to thyroid hormones. Furthermore, other
hormones - testosterone, 11 ketotestosterone, 17α and 20β -
dihydroxyprogesterone, 17β - estradiol, IGF-1, insulin and cortisol - have been
reported in vertebrate eggs (Bern 1990, Feist et al., 1990, de Pablo and Roth, 1990,
development acknowledges that endocrine regulation of teleost development may
begin at fertilization through maternal hormones stored in the egg. Thereafter,
regulation of development gradually switches to endogenous sources as the
organism’s endocrine system becomes established.

Recent discoveries of thyroid hormones in teleost eggs and the demonstration
that supplementation of maternal thyroid hormones have significant effects on
growth and development have increased interest in the role of thyroid hormones in
embryonic and larval development. Most of this research has been limited to a few
marine or migratory species, and little is known about the presence and significance
of thyroid hormones in the larval development of freshwater fishes. Because fish
have such varied life histories, it is difficult to generalize about the pattern or
significance of thyroid hormones in early development at this time.
Oviparous fish may be our best models for studying developmental endocrinology in vertebrates because it is easier to study freeliving embryos in an aqueous medium (Dickhoff et al., 1990). Since early development is highly conserved in vertebrates, knowledge of maternal hormones in the eggs and their role in early development can serve as a way to dissect the fetal maternal endocrine relationships in other vertebrates including viviparous embryos.

**Rationale for a comparative study of thyroid hormones in teleost eggs and larvae.**

This research describes the content of thyroid hormones in eggs and larvae of two freshwater fish, walleye and rainbow trout, for several reasons.

- Walleye and trout are readily available and economically important species. Walleye and trout are raised in midwest hatcheries and stocked in lakes and streams for sport fishing. More than a billion walleye are stocked in North American lakes each year to sustain recreational fishing (Conover, 1986). There is a need for more successful walleye culturing techniques. Understanding thyroid hormone's role in regulating development should aid culture efforts.

- A comparative study of rainbow trout and walleye will enhance our understanding of the significance of thyroid hormone in early development of teleost fishes. These two species present significantly different life histories. Walleye have small eggs (1.60 mm), a short early developmental cycle (30 days), and a high incidence of larval mortality (Li and Mathias, 1982). Rainbow trout, on the other hand, have large eggs (5.5 mm), a long early developmental cycle (90 days), and a lower incidence of larval mortality in culture than walleye (Bromage and Cumaranatunga, 1988). Walleye broodstock are generally collected from nearby lakes and rivers and are considered wild. Rainbow trout have been widely cultured since 1874. Rainbow trout is reported to be an important farmed temperate fish with a world production of...
250,000 tons per annum (Bromage and Cumaranatunga, 1988). Several strains of rainbow trout (*irideus, kamloops and shasta*) exist; and they have undergone selective breeding for features that make them more amenable to hatchery culture such as late winter or early spring breeding.

- Walleye develop similarly to striped bass and should make a good model for understanding the potential role of thyroid hormone in gas bladder inflation and its contribution to larval survival. The gas bladders of walleye are physoclistous while those of trout are physostomous. The gas bladder or swim bladder of teleosts develops from the anterior region of the digestive tract and is connected to the gut via a pneumatic duct (Hoar, 1983). Walleye along with other physoclistous fish — striped bass, tilapia and sea bream — need to inflate their gas bladders by gulping air and forcing it through the pneumatic duct. The pneumatic duct is retained in physostomes but lost in adult physoclistes.

In nearly all of the previous studies of walleye larval culture, failure of the gas bladder to inflate has been the key factor in poor survival (Nickum, 1978; Barrows et al., 1988; Kindschi and MacConnell, 1989; Summerfelt, 1991). In walleye, gas bladder inflation occurs during the transition of the embryo from yolk sac respiration to gill respiration (McElman and Balon, 1979). Fry without an inflated gas bladder struggle to maintain position; eventually, the high energy cost of swimming and the difficulty in capturing food results in starvation. Failure of gas bladder inflation is also a major problem in the intensive culture of striped bass (Bulak and Heidinger, 1980; Cornacchia and Colt, 1984; Chapman et al., 1988).

Walleye and striped bass are advanced teleost fishes (Order Perciformes) with similar early life history patterns and presenting similar culture problems. Because thyroid hormone injections of prespawning female striped bass resulted in
increased larval growth rate and survivorship and percent of larvae with inflated gas bladders (Brown et al., 1988), increased thyroid hormone levels may have a similar effect on early walleye development.

• Comparing the patterns of hormonal change which accompany developmental events in rainbow trout and walleye should lead to a more comprehensive description and understanding of the roles of T₃ and T₄ in egg and larval development. Little is known about the presence and significance of thyroid hormones in the larval development of these economically important species.
PAPER 1:

COMPARISON OF THYROID HORMONE (T<sub>4</sub> and T<sub>3</sub>) CONCENTRATIONS IN EGGS AND EARLY LARVAL DEVELOPMENT OF WALLEYE, *Stizostedion vitreum*

AND RAINBOW TROUT, *Oncorhynchus mykiss*
Comparison of Thyroid Hormone (T\textsubscript{4} and T\textsubscript{3}) Concentrations in Eggs and Early Larval Development of Walleye, \textit{Stizostedion vitreum} and Rainbow Trout, \textit{Oncorhynchus mykiss}

Jane Hey\textsuperscript{1} and Eugenia Farrar
Department of Zoology and Genetics
Iowa State University, Ames, Iowa 50011

\textsuperscript{1}Present address: Morningside College, Sioux City, Iowa 51106, USA.
ABSTRACT

Many teleost eggs contain the thyroid hormones (THs) thyroxine (T4) and 3,5,3'-triiodothyronine (T3). These hormones are maternally deposited in the eggs and may regulate development prior to TH production by the larval thyroid. In some species elevating egg TH concentrations improves larval growth and survival especially during the larval critical period associated with onset of feeding. We compared TH levels of eggs, eyed eggs and larval walleye, Stizostedion vitreum, a small egged species having a high mortality critical period, and rainbow trout, Oncorhynchus mykiss, a species with larger eggs, slower development and no critical period. Samples of eggs and larvae were frozen for hormone determination or fixed for thyroid histology at intervals up to 14 and 49 days posthatch (walleye and trout). THs were extracted and their concentrations determined by radioimmunoassay. Both walleye and trout eggs contained T4 and T3. Trout egg TH content was greater than walleye, but TH concentrations were similar. Thyroid follicles were present in both species at 5 days posthatch and appeared before the walleye critical period. Thyroid hormone concentrations declined but stores were not depleted during early larval development of either species.
INTRODUCTION

Until recently early development of vertebrates was thought to take place without hormonal regulation because eggs and embryos lack endocrine glands and cannot synthesize most hormones. However, reports of thyroid hormones, T4 (thyroxine) and T3 (triiodothyronine) in eggs of coho and chum salmon, Oncorhynchus kisutch and O. keta, and striped bass, Morone saxatilis, have prompted interest in the role of these hormones in early teleost development (Brown et al., 1987; Leatherland et al., 1989; Kobuke et al., 1987 and Tagawa and Hirano, 1987). Thyroid hormones in larvae are thought to be from maternal sources and maternal contribution to the eggs may be important for regulating growth and development of fish before their own thyroid becomes functional. Several lines of investigation indicate that thyroid hormones are capable of influencing early development. Immersion of eggs or larvae in thyroxine affects the rate of hatching and larval growth in some fish (Nacario, 1983, Reddy and Lam, 1991). Brown et al., 1988 found that in striped bass, a fish that usually experiences high mortality in culture, injecting T3 into prespawning females would increase gas bladder inflation and survival.

Very little is known about thyroid hormone levels in the early life histories of commercially cultured freshwater fish found in the midwest such as walleye, Stizostedion vitreum or rainbow trout, Onchorynchus mykiss. Using these two species of fish allows us to compare thyroid hormone content patterns in fish with significantly different life histories and survival rates in intensive culture. Walleye are physoclistous, have small eggs (around 1.6 mm), a short early development period and a high incidence of larval mortality (Li and Mathias, 1982). Rainbow trout, on the other hand, are physostomous, have large eggs, 5.5 mm, a longer early
developmental cycle, and a lower incidence of larval mortality in culture than walleye (Bromage and Cumaranatunga, 1988). Comparative studies of egg and larval thyroid hormone patterns and thyroid ontogeny in these two fish and other species, such as striped bass, may be used to examine two hypotheses on the significance of thyroid hormone in the successful development of teleost eggs. Brown et al. (1989) states that species that hatch with competent thyroid follicles may be less dependent on maternal thyroid hormones than those such as striped bass with undeveloped follicles. Tagawa and Hirano (1991) observed that the eggs of fish with smaller yolk mass show lower survival than those of larger yolked eggs and hypothesize that these smaller egged fish are more dependent on thyroid hormone for survival.

Larval walleye, like striped bass, experience a high mortality following hatching, when thyroid hormones might be significant for survival. A critical period occurs during the rapid transition to independent feeding which is associated with morphological, nutritional and behavioral changes. Thyroid hormones may regulate key developmental events such as gas bladder inflation that occur during this time. Given that walleye have small eggs, they might have limited thyroid hormone stores which could be depleted before the larval follicles start functioning. By comparison, trout have large eggs which contain lots of yolk and they do not experience such a critical period following hatching. Thus rainbow trout would likely have more egg thyroid hormones and these hormones would be sufficient to support development until their thyroid follicles mature.

The objectives of this research are to determine whether thyroid hormones are present in walleye and trout eggs and compare the hormone concentrations and amount of variability in thyroid hormone concentrations between these fish; to describe the pattern of thyroid hormone \( \frac{T_3}{T_4} \) concentrations in developing walleye
and trout eggs and larvae; and to compare the levels and pattern of thyroid hormones in relation to key developmental events, such as first appearance of thyroid follicles, of walleye and rainbow trout.
MATERIALS AND METHODS

Animals. Eggs of walleye and Shasta strain of rainbow trout were provided by eight hatcheries located in Iowa, Kansas, Ohio, Minnesota, Wisconsin and North Dakota. Hatcheries supplying walleye included the Iowa Rathbun State Fish Hatchery, Iowa DNR in Moravia; the Spirit Lake State Fish Hatchery, Iowa DNR in Spirit Lake; Milford State Fish Hatchery, Kansas Department of Wildlife and Parks, Milford; Devil's Track Hatchery, Minnesota DNR, Cook County; Garrison Dam National Fish Hatchery, U.S. Fish and Wildlife Service, Riverdale, North Dakota; London State Fish Hatchery, Ohio DNR, London and Genoa National Fish Hatchery, U.S. Fish and Wildlife Service, Genoa, Wisconsin. Trout eggs were obtained from only one source, Manchester Fish Hatchery, Iowa DNR, Manchester.

Sample collection and storage. Fertilized eggs (day 1) and eyed eggs (170 DTU (1 Daily Temperature Unit = 1°C above 0°C for 24 hours) were collected from the hatchery and transported to Iowa State University in plastic bags or wrapped in cotton muslin and placed in trays in insulated coolers with a small amount of ice.

Eggs were counted, weighed, and measured and then frozen on dry ice and stored at -70°C for hormone analysis. Samples of eggs and larvae were fixed in 10% phosphate buffered formalin for 24 hours and then stored in 70% ethanol until they were sectioned. Histological sections of walleye and trout larva were prepared to examine thyroid follicle development. The tissue sections were stained with hematoxylin and eosin and cross-sectioned sectioned serially at 4 - 5 μm sections from just in front of the eye going posteriorly to include the lower jaw and head kidney. Longitudinal sections of walleye slides were also obtained from Dr. Tom Bell of Michigan State University. They were of larval walleye reared in the same culture environment as Iowa State University has used in the present study. The slides
were used to follow the progress of development and determine when the thyroid follicles are formed.

**Fish culture for developmental studies.** Four cohorts each of walleye and trout fertilized eggs from individual females were cultured for harvest in developmental studies. Walleye eggs were transferred to 3 gallon containers of dechlorinated tap water and were continuously aerated. Trout eggs were reared in horizontal flow-thru incubator trays supplied with charcoal filtered tap water until seven days after hatching when they were transferred to 10 gallon aquaria of dechlorinated tap water. Fish were cultured under a 10 hour photoperiod with temperatures ranging from 15 to 18°C for walleye and 12 to 14°C for trout. Samples were taken from these cultures at intervals and frozen for thyroid hormone analysis or preserved for histological analysis of thyroid follicle development. Both Rathbun and Manchester Hatcheries continued to rear a portion of our 4 cohorts of walleye and trout and we returned to these hatcheries for additional stock when our cultures got low.

**Extraction of thyroid hormones.** Thyroid hormones were extracted with methanol and chloroform using the method described by Tagawa and Hirano (1987) and modified by Weber et al. (1992). Samples weighing approximately 0.2 g (50 walleye or 2 trout eggs) were homogenized in cold extraction fluid (1 ml) containing 6-N-propylthiouracil (PTU) in methanol (99 parts) and 10 N NaOH (1 part). PTU is added to block deiodination (Brown et al., 1987). The homogenates were sonicated with an Artek Sonic Dismembranator at 71 kilocycles per second. Following sonication, $\text{I}^{125}$ T3 (1200 cpm) was added to each sample to determine extraction efficiency. Samples were vortexed, centrifuged, and the supernatant was extracted with chloroform and 2N NH$_4$OH. The aqueous phase was collected, combined with
the aqueous phases from two more extractions, and counted in a Packard 500 C gamma counter to determine extraction efficiency. Extraction efficiencies averaged 85%. The extracted samples were dried under vacuum and stored at -20°C.

_Radioimmunoassay of thyroid hormones._ Extracted hormones were assayed using a radioimmunoassay described by Brown and Eales (1977) and modified by Weber et al. (1992). Antibody-bound $^{125}$I labeled hormone was separated from free labeled hormone using 5 ml Sephadex G 25 (Sigma) minigel columns (Isolab Inc.). Extracts were suspended in 0.1N NaOH and 100 µl aliquots applied to the columns. Hormone standards, labeled hormones and hormone specific antisera were obtained from Endocrine Sciences Products. Hormone standards were prepared in 0.1N NaOH in concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5 and 10.0 ng/ml for both T3 and T4 assays, and 100 µl aliquots applied to the columns. $^{125}$I thryoxine or $^{125}$I T3 (3.2 ml = 10.7 mCi, specific activity 1200 m Ci/µg) in 50 µl 0.1N NaOH was added to each column. Antisera to T4 and T3 were reconstituted with deionized distilled water at dilutions of 1:16 and 1:33, and aliquots frozen at -70°C. For each assay aliquots were diluted to final concentrations of 1:10,000 and 1:8250 with phosphate buffer and 500 µl applied to each column. The RIA was performed in a 0.1 M sodium phosphate buffer containing 30 mM disodium EDTA, pH 7.4. Bound hormone was eluted from the columns with buffer after incubating overnight (T4) or 3 hour (T3) and the fractions were collected and counted in a gamma counter.

Samples and standards were run in triplicate. Quality control samples of pooled extract containing added T3 or T4 were run with each assay to determine intraassay and interassay variability. The walleye intraassay coefficients of variation (CVs) were 10.3% and 14.6% (N=3) for T4 and T3, while the interassay coefficients of variation were 3.5% and 8% (N=4) for T4 and T3 respectively. Trout intraassay
CVs were 8.8% for T4 and 9.5% for T3 (N=4). Trout interassay CVs were 13.2% and 6.1% for T4 and T3 (N=3). Assayzap software (Biosoft) was used to determine extract hormone concentrations and assay sensitivity. The assay routinely measured concentrations ranging from 0.1 ng/ml to 8 ng/ml for both T3 and T4. The mean values of 50% and 80% bound/bound zero were 2.0 and 0.4 ng/ml respectively for the T4 assays and 0.6 and 0.04 ng/ml for the T3 assays. Each extract was corrected for extraction efficiency in calculating the extract hormone concentration. Hormone concentration of the sample was expressed as ng/g wet weight. Total hormone content of the sample divided by the number of individuals per sample gave hormone content per individual.

Several types of validation studies were used to determine whether the RIA was precisely and specifically measuring thyroid hormone. The amount of cross reactivity between T4 antisera and T3 was 0.3% and the crossreactivity of T3 antisera for T4 was 0.4%. Dilution curves of reference standards and extract with standards added were parallel. Mean recovery of 6 concentrations of T4 standards added to walleye egg extract was 103 ± 6% and trout egg extract was 90 ± 5%. The recovery curves for the added hormones were linear and parallel to the recovery curves of the reference standards. Applying different volumes (50 to 200 μl) of either trout or walleye egg extract to the columns did not affect hormone recovery.

Data analysis. All data computations and statistical analysis were performed on a Macintosh L.C. computer using Statview and SuperAnova. Differences between groups with homogeneous variances, as determined by an F test (Wardlaw, 1987) or F_{Max} for several groups (Hartley, 1950), were determined by analysis of variance followed by Fisher's LSD test. If variances in the groups being compared were heterogeneous, Mann Whitney U tests were applied. Data are generally
expressed as mean ± SEM. Samples with hormone concentrations below the detectability limit (0.1 ng/ml) of the assay were eliminated from the statistical analysis.
RESULTS

Thyroid hormones, $T_4$ and $T_3$, were present in fertilized and unfertilized eggs of both walleye and trout. Fertilized walleye eggs from the Rathbun Fish Hatchery contained $1.46 \pm .13 \text{ ng/g } T_4$ and $4.70 \pm 1.58 \text{ ng/g } T_3$ ($n = 10$), and fertile rainbow trout eggs contained $2.79 \pm .26 \text{ ng/g } T_4$ and $5.65 \pm .32 \text{ ng/g } T_3$ ($n = 12$). Total hormone content of these eggs was $8 \pm 1 \text{ pg } T_4$ and $25 \pm 8 \text{ pg } T_3$ in walleye and $192 \pm 17 \text{ pg } T_4$ and $417 \pm 29 \text{ pg } T_3$ in trout. Fertilization did not affect thyroid hormone concentrations. Thyroid hormone concentrations of unfertilized and fertilized eggs were not significantly different when samples were taken from the same 3 females at spawning (Wisconsin hatchery, $1.12 \pm .28 \text{ ng/g } T_4$ fertile vs. $1.38 \pm .36$ for unfertile; $1.27 \pm .35 \text{ T}_3$ fertile vs. $1.40 \pm .28$ unfertile). Unfertilized eggs were difficult to obtain from the hatcheries because we had to be present at spawning, therefore, we chose to work mainly with fertilized eggs and developing embryos (eyed eggs) that could be transported with excellent viability at 160-200 DTU.

Variation in thyroid hormone concentration. Walleye eggs could be obtained from a single hatchery for only about 3 weeks of each year. To extend our research season we received eggs from a variety of hatcheries. When we compared thyroid hormone concentration of eyed walleye eggs, at 170 DTU from six hatcheries, IA - Spirit Lake, Kansas, Minnesota, North Dakota, Ohio and Wisconsin, significant differences were seen between hatcheries, Figure 1. For composite samples taken from many females fertilized by males from the six hatcheries studied in 1992, the mean $T_4$ concentration ranged from $9.30 \pm 2.20$ for Wisconsin to $0.48 \pm .07 \text{ ng/g}$ for Minnesota (see Figure 1). While three hatcheries Minnesota, Ohio and North Dakota, contained similar amounts of $T_4$, eggs from the Wisconsin hatchery contained significantly more $T_4$ in concentration and content than eggs from any of
the other walleye hatcheries (Mann Whitney U tests) in both 1991 and 1992. The mean T₃ concentrations in the 170 DTU eggs from the six hatcheries ranged from 1.52 ± .44 for Wisconsin to 0.70 ± .33 ng/g for Ohio in 1992 and were not significantly different from each other either year.

The difference in mean walleye egg T₄ concentration between the Wisconsin hatchery and the other five hatcheries may be due to environment, nutritional status, physiologic condition, age or genetics of the brood stock or other factors. On the other hand, the difference in T₄ concentration in eggs from different hatcheries may be due to greater variation in thyroid hormone content of eggs produced by individual females within a particular hatchery stock. Each of the hatchery stocks sampled represented a composite of fertilized eggs from approximately 25 females and 50 males. We decided to look more closely at the thyroid hormone content of eggs produced by individual females from two hatcheries, Genoa National Fish Hatchery, Genoa, Wisconsin and Rathbun Fish Hatchery, Moravia, Iowa.

Egg samples from individual walleye females from the Wisconsin hatchery were more variable in thyroid hormone concentration than samples from individual walleye females from Rathbun or Shasta rainbow trout from the Manchester hatchery, Table 1. The mean thyroid hormone concentrations in fertilized eggs from individual females from two walleye hatcheries were evaluated using 6 females from Wisconsin, 10 from Iowa and twelve females from the Manchester trout hatchery. The F test (Wardlaw, 1987) was used to compare variances associated with hatchery differences. The variability of T₄ concentration of fertile eggs from individual samples was significantly greater (P ≤ .01) among the females from the Wisconsin hatchery than either the walleye from the Rathbun hatchery or trout from the Manchester hatchery. T₄ concentrations in eggs of walleye from Wisconsin
ranged from 0.72 to 9.27 ng/g while T4 in eggs from Rathbun ranged from 0.68 to only 2.28 ng/g. Trout egg T4 ranged from 0.60 to 3.80 ng/g. The Wisconsin eggs sampled also showed greater heterogeneity for T3 concentration (range 0.72 to 117.5 ng/g) than the Rathbun walleye (1.43 to 15 ng/g) and the T3 concentrations from Rathbun walleye were more variable than the fertilized trout eggs from Manchester (P ≤ .01).

The variability of TH hormone concentrations in the fertilized eggs from individual females from the Wisconsin hatchery stock is reflected in both T4 and T3 concentrations found in eggs from Wisconsin hatchery stocks for two years, 1991 and 1992. With the exception of Wisconsin, T3 concentrations generally exceed T4 concentrations in eyed eggs for the six hatcheries sampled. However T4 concentrations in composite samples of 170 DTU eggs from the Wisconsin stock were significantly different and greatly exceeded their T3 concentrations. On the other hand when fertilized eggs from six individual females were assayed the mean T3 (20.8 ng/g) concentrations exceeded T4 (3.02) (Table 1) with T3 concentrations for eggs from individuals ranging from 0.72 to 117.5 ng/g. This is an example of how several eggs from individuals with very high T3 or T4 can drastically affect the hormone values for the population sampled. It appears that some individual females have extremely high levels of thyroid hormones. Mean hatchery concentrations the variability and number of females with extremely high concentration of hormone. Wisconsin is a population that demonstrates this higher concentration. and it may be provided by environmental diversity such as nutritional sources.

We conducted further validation studies to test for possible interference with the radioimmunoassay with the high T3 concentration samples using serial dilutions.
The dilution curve was parallel to the standard curve suggesting that interfering substances were not present.

**Thyroid hormones and development.** In 1992 fertilized eggs from four individual females from the Rathbun Fish Hatchery and four female trout from the Manchester Fish Hatchery were cultured separately and sampled at intervals to document thyroid hormone patterns and to establish the time of formation of thyroid follicles in each species.

Fertilized eggs of walleye were cultured for 27 days (400 DTU) at mean water temperatures of 16°C. Hatching began at 180 DTU (or day 13). By 235 DTU walleye larvae had developed eye pigmentation. Gas bladder inflation was noted at approximately 280 DTU and we estimate that at this time larvae entered the critical period, post larval stage 1 as described by Li and Mathias (1982). By 300 DTU the walleye had completely resorbed all yolk and the oil globule was decreasing in size. Development proceeded essentially as described by Li and Mathias (1982) and at a rate comparable to the fish cultured at the Rathbun Hatchery until the critical period when first feeding should take place. We did not feed the larvae because special culture techniques are required.

Thyroid hormone concentrations did not change significantly during the first 400 DTU of walleye development. In this early period mean $T_4$ varied from $1.3 \pm 0.2$ to $2.7 \pm 0.3$ ng/g and mean $T_3$ concentrations from $1.3$ to $2.1 \pm 0.8$ ng/g. High mortality of walleye prevented us from continuing to harvest larvae from more than one cohort through the next stage of interest, the critical period. The critical period, first conceptualized by Hjort in 1914, is described by Li and Mathias (1982) as a period of increased mortality that occurs during the transition from endogeneous to exogenous nutrition. Thyroid hormone concentrations appeared to decline at 400
DTU (14 days post hatch) but were not statistically significant due to the small sample. However, the mean represented hormone levels of a composite of two pooled samples of 200 larvae from a single female.

Shasta strain rainbow trout were cultured from fertilized eggs at mean water temperatures of 13°C for 70 days (900 DTU). By 260 DTU the embryos were eyed. Hatching began at 338 DTU (27 days). Yolk sac larva became heavily pigmented on the head and dorsal surface by 429 DTU. By 624 DTU the larva were developing iridescent green and pink colors. By day 50 (600 DTU) their yolk sacs were almost gone and they began feeding at about 720 DTU (30 days posthatch). Trout were fed trout chow and TetraMin. Development proceeded at the same rate as at the Manchester Hatchery (Dave Maroff, hatchery director, personal communication).

Both $T_4$ and $T_3$ concentrations declined during the 70 days from fertilized egg ($T_4$, 3.20 ± .32 ng/g; $T_3$, 5.95 ± .69 ng/g) to post yolk sac larvae ($T_4$, 1.03 ± .30 ng/g; $T_3$, 1.05 ± .09 ng/g), Figure 2. $T_4$ concentrations were significantly lower than fertilized eggs at 819 DTU and 900 DTU, 64 and 70 days. $T_3$ levels began to decline shortly after hatching at 429 DTU (4.75 ± 1.33 ng/g) and were significantly lower ($P > .05$) than the initial level in the fertilized eggs for the remaining five intervals sampled. $T_4$: $T_3$ ratios slowly increased during the 70 day study of trout larval development from 0.54 at fertilization to $\geq 1$, due to the greater decline in $T_3$ than $T_4$.

Thyroid follicles were detected at 5 days posthatch in both walleye (240 DTU) and in rainbow trout (390 DTU), Figure 3. Follicles were composed of a single layer of epithelial cells surrounding brightly stained vesiculated colloid. In both walleye and trout larvae the follicles were found just ventral to the anterior copula cartilage and usually near the bifurcation of the afferent branchial arteries from the ventral aorta. As larval development proceeded of both walleye and trout, follicles became
more numerous and were spread more extensively throughout the gill area, primarily between gill arches 1-2, and 2-3. Most follicles at days 5 to 9 posthatch were composed of squamous epithelial cells.
DISCUSSION

Walleye and rainbow trout may be added to the growing list of teleosts whose eggs have recently been found to contain thyroid hormones. These include: striped bass (Brown et al., 1987, 1988); several species of salmonids (Kobuke et al., 1987, Tagawa and Hirano, 1987, 1989; Greenblatt et al., 1989; Leatherland et al., 1989) and medaka, Oryzias latipes, (Tagawa and Hirano, 1991, Tagawa et al., 1990, Weber et al., 1992). Unfertilized, fertilized and eyed eggs of walleye all contained thyroid hormones, T4 and T3. For example, the mean T4 and T3 concentrations in fertilized eggs from individual females for two hatcheries in 1992 averaged 1.29 and 2.99 ng/g respectively. Shasta rainbow trout eggs also were found to contain both T4 and T3 averaging 2.79 and 5.66 ng/g. These concentrations fall well within the range of mean T4 and T3 values of 0.04 to 15 ng/g and 0.7 to 9.95 respectively reported for eggs from a wide variety of teleost species (Tagawa et al., 1990).

The meaning of species averages for egg thyroid hormone concentration is questionable, because of the great variability seen in walleye and several other species (Greenblatt et al, 1989. Tagawa and Hirano, 1990). When we compared the thyroid hormone concentrations for walleye in composite samples of eyed eggs from many females and males supplied by six hatcheries, the mean T4 concentrations varied between the hatcheries. T4 concentrations of eggs from the Wisconsin hatchery were at least tenfold greater than the other five hatcheries in two consecutive (1991 and 1992). On the other hand, eyed eggs from three hatcheries (Ohio, Minnesota and North Dakota) contained very similar amounts of T4. In the hatchery variability studies T3 was less variable than T4.

Walleye egg thyroid hormone concentrations varied among individual females especially those from the Wisconsin hatchery stocks. Occasionally a female walleye
produced eggs with $T_4$ or $T_3$ levels that were highly elevated over the mean for that hatchery stock. This occurred in walleye eggs from Wisconsin and Kansas in 1991 and Wisconsin in 1992. Greenblatt et al., 1989, also observed large variation in thyroid hormone levels between cohorts of both coho and chinook salmon, as well as individual variability within cohorts. Individual variation could be due to factors such as environmental conditions, nutritional status or genetics of the brood stock. The most variable egg thyroid hormone concentrations were from the Wisconsin stock collected from the Mississippi River near Genoa, Wisconsin. Such fish are likely to be more genetically heterogeneous and to experience more varied environmental and nutritional conditions during their spring migrations than are the six lake stocks we studied. The Ohio walleye stock is considered to be semidomesticated and might be more genetically uniform than the wild walleye stocks from other hatcheries. Eggs from the Ohio stock were uniformly low in thyroid hormones in both years of the study. The Shasta strain of rainbow trout are considered to be domesticated and their egg thyroid hormone concentrations were generally less variable than walleye.

The variability in egg thyroid hormones probably relates to maternal plasma hormone concentration and possibly to characteristics of yolk binding proteins. Circulating levels of maternal thyroid hormones must influence egg levels, because egg hormones are considered to be derived from maternal plasma hormones (Brown et al., 1987 and 1988; Weber et al., 1992). Many activities including feeding (Himlick et al., 1991) and acute physical stress of rainbow trout (Brown et al., 1978; Himlick and Eales, 1990) are known to increase adult plasma $T_4$ concentrations. Such changes in maternal plasma hormone levels, if they occurred at the time of hormone transfer, could translate into elevated egg thyroid hormones. Egg hormone
concentrations can be altered by artificially manipulating maternal plasma thyroid hormone levels (Brown et al., 1988; Tagawa and Hirano, 1991). The amount of yolk and its composition could also influence egg thyroid hormone content. Ninety six percent of salmonid thyroid hormones are in the yolk at hatching (Kobuke et al., 1987). It is probable that the hormones are stored bound to yolk proteins.

Greenblatt et al. (1989) cites unpublished work by Sullivan, Hara, Barnard and Dickhoff that both T4 and T3 specifically bind to vitellogenin. Only free hormone is active, so changes in amount or binding affinities of yolk binding proteins could be important to both egg hormone content and to active hormone availability in the larvae. Little is currently known about this.

T4 and T3 concentrations did not change significantly from fertilization to 14 days posthatch in developing walleye obtained from the Rathbun hatchery. There may be a decline at fourteen days post hatch, but the hormones were still detectable in larvae going through the critical period. Thyroid follicles were detected at 5 days posthatch, corresponding to the time when the larvae entered the critical period. The source of whole body thyroid hormones in larvae after five days post hatch is unclear because the functional status of the larval follicles is not known. However, the walleye thyroid hormone developmental pattern is different from striped bass, where a drop in maternal thyroid hormone occurs just prior to their period of high mortality and prior to formation of larval follicles (Brown et al., 1987, 1988). Boosting striped bass larval hormone concentrations by maternal T3 injection enhances their survival (Brown et al., 1988). This suggests that their egg thyroid hormones may be insufficient either in amount or in accessibility to larval tissues. Immersing walleye larvae in thyroid hormones during the critical period enhances their survival (Hey et al., paper 2 of this thesis). This could mean that even though walleye larvae have
detectable endogenous thyroid hormones during their critical period the amount of
hormone present is insufficient to achieve optimal survival or that the mechanisms
for making this stored hormone accessible to the tissues are deficient. As pointed
out by Leatherland and Lin (1989), supplying exogenous hormone by immersion
may enhance developmental processes by increasing the amount of hormone
directly accessible to larval tissues.

Trout eggs, as expected from their larger size and more plentiful yolk,
contained more total thyroid hormones than walleye but their thyroid hormone
concentrations were similar. These hormones were present throughout the 49 days
posthatch study period. While they gradually declined after hatching at no time were
the hormones depleted. In fact, rainbow trout thyroid follicles were present at five
days posthatch, about 19 days before complete yolk sac absorption. This is also
observed in the brown trout, *Salmo trutta*, whose follicles appear during the first week
posthatch (Barrington and Rawdon, 1971). The presence of follicles does not
indicate that they are functional, but they are a potential source of hormones during
the developmental period. In some species when larval follicles do become
functional a rise in hormone concentration is seen, but this did not occur in the 49
days after the rainbow trout hatched.

In conclusion both walleye and rainbow trout eggs have thyroid hormones.
Trout eggs contain more hormone than walleye due to their larger size. Species
values for egg hormone concentration probably mean very little since walleye egg
concentrations varied considerably between hatcheries and egg hormone
concentrations from riverine walleye varied between individual females. Both
walleye and trout had detectable thyroid hormones throughout the observed
developmental periods and both had thyroid follicles at five days posthatch. Further
study will be required to determine what levels of endogenous thyroid hormone stores and of free plasma hormones are required to maximize development in various teleost species.
ACKNOWLEDGEMENTS

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REFERENCES


Figure 1. Mean $T_4$ and $T_3$ Concentrations in Eyed Walleye Eggs from 6 Hatcheries. Each bar represents a mean of 2 pooled samples of 100 eggs each.
Table 1. Comparison of Variances of Mean T₄ and T₃ Concentrations in Fertilized Eggs from Individual Females from Two Walleye and One Trout Hatchery

<table>
<thead>
<tr>
<th>Hatchery</th>
<th>N</th>
<th>Mean ± Sd (ng/g)</th>
<th>C.V.¹</th>
<th>F test variance</th>
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<tr>
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<td></td>
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<tr>
<td><strong>T4</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rathbun walleye²</td>
<td>10</td>
<td>1.46 ± 0.41</td>
<td>28</td>
<td>Rathbun vs. Wisconsin</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P ≤ .01</td>
</tr>
<tr>
<td>Wisconsin walleye³</td>
<td>6</td>
<td>3.02 ± 3.32</td>
<td>110</td>
<td>Wisconsin vs. Manchester</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P ≤ .01</td>
</tr>
<tr>
<td>Manchester trout⁴</td>
<td>12</td>
<td>2.79 ± 0.91</td>
<td>32</td>
<td>Manchester vs. Rathbun</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P ≥ .05</td>
</tr>
<tr>
<td><strong>T3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rathbun walleye²</td>
<td>10</td>
<td>4.70 ± 5.01</td>
<td>107</td>
<td>Rathbun vs. Wisconsin</td>
</tr>
<tr>
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<td>P ≤ .01</td>
</tr>
<tr>
<td>Wisconsin walleye³</td>
<td>6</td>
<td>20.8 ± 47.4</td>
<td>227</td>
<td>Wisconsin vs. Manchester</td>
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<td>P ≤ .01</td>
</tr>
<tr>
<td>Manchester trout⁴</td>
<td>12</td>
<td>5.66 ± 1.11</td>
<td>20</td>
<td>Manchester vs. Rathbun</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>P ≤ .01</td>
</tr>
</tbody>
</table>

¹ C.V. = coefficient of variation = (Sd / mean) x 100
² Iowa DNR, Rathbun Fish Hatchery, Moravia, IA
³ Wisconsin, U.S. Fish and Wildlife, Genoa National Fish Hatchery, Genoa, WI
⁴ Iowa DNR, Manchester Fish Hatchery, Manchester, IA
Figure 2. Mean T₄ and T₃ Concentrations in Eggs and Developing Trout Larvae. Each bar represents a mean of four, two egg samples. * significantly different from fertilized eggs (0 DTU)
Figure 3a. Transverse section through the thyroid region of a walleye larvae at 5 days posthatch showing two follicles (f) beneath the anterior copula cartilage (c). The colloid is lightly stained and contains endocytotic vesicles. Hematoxylin and eosin. 400X

Figure 3b. Transverse section through the thyroid region of a rainbow trout larvae at 5 days posthatch showing several follicles (f) associated with blood vessels beneath the anterior copula cartilage (c). Little colloid is visible in these follicles. Hematoxylin and eosin. 400X
PAPER 2:

THYROID HORMONES AND THEIR INFLUENCES ON LARVAL PERFORMANCE

AND INCIDENCE OF CANNIBALISM IN WALLEYE, *Stizostedion vitreum*
Thyroid Hormones and Their Influences on Larval Performance and Incidence of Cannibalism in Walleye, *Stizostedion vitreum*

Jane Hey¹ and Eugenia Farrar
Department of Zoology and Genetics
and
Brian Bristow, Craig Stettner and Robert C. Summerfelt
Department of Animal Ecology
Iowa State University, Ames, Iowa 50011

¹Present address: Morningside College, Sioux City, Iowa 51106, USA.
Thyroid hormones, 3,5,3',5' - tetraiodothyronine (T₄) and 3,5,3' - triiodothyronine (or T₃) have been found in several species of teleost eggs and are potential regulators of development, larval growth and survival. In this study, thyroid hormone concentrations in walleye eyed eggs were determined and evaluated in relationship to several parameters of larval performance in culture. Walleye eggs contained T₄ and T₃ and their concentrations varied among stocks from different geographic regions. Egg T₃ concentrations correlated positively with gas bladder inflation rates and incidence of cannibalism in fry from six hatchery stocks. The concentration of T₃ in eggs correlated positively with larval size at hatching and egg T₄ concentration correlated positively with egg energy content. Increases in both incidence of cannibalism and survival (measured as the interval between hatching and estimated day to 50% mortality) were observed when fry were held in water containing between 0.01 and 0.1 mg/l (ppm) T₃ and T₄. A positive dose response between the concentration of T₄ and cannibalism was observed. T₃ was more effective in promoting survival and cannibalism than T₄. The findings of this study indicate that thyroid hormones are potentially important regulators of walleye development, and a full understanding of TH function and the maternal effects on TH concentration within eggs may help fish culturists maximize production.
INTRODUCTION

The thyroid hormones (TH), namely thyronine (3,5,3'-triiodo-L-thyronine, T₃), and thyroxine (T₄) have metabolic, structural, central nervous system, and behavior effects in vertebrates (Gorbman 1969). T₃ has been shown to be the more active form (Higgs et al. 1979). Furthermore, there is increasing evidence that thyroid hormone affects development and growth of larval fish (Brown et al. 1987). There is basic and practical interest in distinguishing between the contribution of maternal TH passed through the yolk and hormones derived from the early function of thyroid follicles present in the newly hatched larvae. Brown et al. (1987) reported presence of substantial amounts of T₃ and T₄ in the yolk of unfertilized ova of several fish species; they inferred that the TH were deposited there from maternal plasma. Brown et al. (1988) confirmed the transfer of T₃ from maternal circulation into the oocytes of striped bass, Morone saxatilis. They found a significant elevation of T₃ concentration in eggs from females that had received a large dose of intramuscularly injected T₃ shortly before spawning. The elevated T₃ levels in the eggs increased swimbladder inflation and survival rates in larval striped bass. Other studies have confirmed the maternal contribution of TH to eggs (Tagawa and Hirano 1991; Ayson and Lam 1993). Nicario (1983) reported that thyroid follicles were absent in yolk sac larvae of the tilapia, Sarotherodon niloticus, but present in the post-yolk sac fry.

Treatment of yolk-sac larvae of tilapia, Sarotherodon mosambicus, by immersion in 0.1 ppm solution of T₄ markedly accelerated their development and enhanced their survival (Lam 1980). Nicario (1983) reported increased growth in Sarotherodon niloticus exposed by immersion for 4 weeks in 0.1 ppm T₄, but exposure to 0.3 and 0.5 ppm caused abnormaly shaped pectoral fins, lordosis, and
scoliosis. Lam and Sharma (1985) also found that immersion of fertilized carp, *Cyprinus carpio*, embryos in T4 increased hatchability, larval survival, and growth.

The present experiments were designed to describe TH concentrations in fertilized eggs of several walleye, *Stizostedion vitreum*, stocks and to determine the relationship between the egg concentration of TH and energy density of the eggs, and the growth, survival, gas bladder development and incidence of cannibalism in larvae from these stocks. Because we observed a strong relationship between TH concentration in eggs and incidence of cannibalism, experiments were conducted to determine whether exposure of the eggs and fry by immersion in TH would affect survival and incidence of cannibalism.
MATERIALS AND METHODS

Fry performance evaluation

_Culture system._ Tanks with black sidewalls and screens and a rearing volume of about 157-L were used in all fry performance studies. Tap water was dechlorinated by passage through six pressurized, activated carbon tanks in series. In addition, sufficient sodium sulfite (4 mg/L per 1 mg/L chlorine) was added to neutralize residual chlorine and chloramines. The surface of the culture tank was sprayed with water to facilitate gas bladder inflation as recommended by Moore et al. (1994) and Barrows et al. (1993).

_Sources of Fish and Stocking Densities._ Six fish stocks were used in this study, four lake stocks from Iowa (IA), Minnesota (MN), Kansas (KS), and North Dakota (ND), one riverine stock from Wisconsin (WI), and a semi-domesticated stock from the London, Ohio State Fish Hatchery (OH). All stocks were received as eyed eggs and incubated at our facility for 3 to 5 days prior to their hatching. Each stock was cultured in three replicate tanks under as close to identical conditions as possible to reduce the environmental variance. Fry were stocked at 20/L (3,140 fry/tank). Fry numbers were estimated gravimetrically by draining the excess water from a 3 to 5 g sample of larvae in a beaker with a screened side wall of 710 μm (45% open area) Nitex™. The fry samples were weighed and counted. These results were then converted to g/1000 fry.

Fifty fry, collected randomly by passing a 100 μm mesh net through the water column 10 times at different depths and angles, were measured (total length) while they were hatching, when they were stocked into the individual tanks (19 to 28 DTU's post-hatch), and at 50, 150 and 250 daily temperature units (TU) °C.
Lighting was provided to each tank with one 150 watt projection flood lamp mounted 75 cm above the water surface (128 lx). Tanks were illuminated from 1530-1030 (19 h). The illumination period exceeded the feeding period (1530 to 0730 hrs) by three hours to facilitate tank cleaning and water sample collecting. Waste feed, dead fish, and fungus was siphoned from the tank bottom and the drain screens were cleaned daily.

Feeding. Feeding began when the fry were three days posthatch. Feed was provided from 1530 to 0730 hrs each day (16 hrs total). The fry were fed Kyowa Fry Feeds B-400 and B700. Fry were fed once per minute. Feeding rates were based on the estimated number of fish remaining in the tank. Feeding rates were adjusted for the estimated population every three days. Feed was dropped onto the water surface through a 7.6 cm ID PVC feeding ring which kept the water surface free of floating feed.

Fish sampling. Fry for measurements were collected randomly by passing a 100 μm mesh net through the water column 10 times at different depths and angles. At hatching, stocking, 50 and 150 TU posthatch, and upon completion of the experiment, 50 fish from each tank were examined for yolk sac length, oil globule diameter, gas bladder inflation, total length and acceptance of feed.

Cannibalism. The rates of cannibalism were estimated daily by observing and removing cannibals from each tank each day. The rate of cannibalism is the percentage of total mortality caused by cannibalism. Both the removed cannibal and its prey were counted as fish lost to cannibalism.

Energy content of eggs

Eyed-eggs from each of the six stocks were placed in pre-weighed stainless steel combustion capsules. The eggs were dried at 60°C to a constant weight (dry
weight). Energy contained in the eggs was determined by oxygen bomb calorimetry (Parr model 1341). The number of eggs used to determine the dry weight and energy content varied with egg availability (200 to 607 eggs per sample and three to five samples per stock).

**Thyroid hormone content of eggs**

Several samples of 50 eyed eggs from each stock, were weighed and frozen at -70°C for hormone analysis. Thyroid hormones were extracted using methanol and chloroform by the method described by Tagawa and Hirano (1987) and modified by Weber et al. (1992). Samples weighing approximately 0.2 g (50 eggs) were homogenized in cold extraction fluid (1 ml) containing 6-N-propylthiouracil (PTU) in methanol (99 parts) and 10 N NaOH (1 part). PTU is added to block deiodination (Brown et al., 1987). The homogenates were sonicated with an Artek Sonic Dismembranator at 71 kilocycles per second. Following sonication I$^{125}$ T$_3$ (1200 cpm) was added to each sample to determine extraction efficiency. Samples were vortexed, centrifuged, and the supernatant was extracted with chloroform and 2N NH$_4$OH. The aqueous phase was collected, combined with the aqueous phases from two more extractions, and counted in a Packard 500 C gamma counter to determine extraction efficiency. Extraction efficiencies averaged 85%. The extracted samples were dried under vacuum and stored at -70°C.

Extracted hormones were assayed using a radioimmunoassay described by Brown and Eales (1977) and modified by Weber et al. (1992). Antibody-bound I$^{125}$ labeled hormone was separated from free labeled hormone using 5 ml Sephadex G 25 (Sigma) minigel columns (Isolab Inc.). Extracts were suspended in 0.1N NaOH and 100 μl aliquots applied to the columns. Hormone standards, labeled hormones and hormone specific antisera were obtained from Endocrine Sciences Products.
Hormone standards were prepared in 0.1N NaOH at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5 and 10.0 ng/ml for both T3 and T4 assays, and 100 µl aliquots applied to the columns. I\(^{125}\) thyroxine or I\(^{125}\) T3 (3.2 ml = 10.7 mCi, specific activity 1200 m Ci/ng) in 50 ml 0.1N NaOH was added to each column. Antisera to T4 and T3 were reconstituted with deionized distilled water at dilutions of 1:16 and 1:33, and aliquots frozen at -70°C. For each assay aliquots were diluted to final concentrations of 1:10,000 and 1:8250 with phosphate buffer and 500 ml applied to each column. The RIA was performed in a 0.1 M sodium phosphate buffer containing 30 mM disodium EDTA, pH 7.4. Bound hormone was eluted from the columns with buffer after incubating overnight (T4) or 3 hour (T3) and the fractions were collected and counted in a gamma counter.

Samples and standards were run in triplicate. Quality control samples of extract containing added T3 or T4 were run with each assay. Assayzap software (Biosoft) was used to determine extract hormone concentrations and assay sensitivity. The assay routinely detected between 0.1 ng/ml and 8 ng/ml for both T3 and T4. Each extract was corrected for extraction efficiency in calculating the extract hormone concentration. Hormone concentration of the sample was expressed as ng/g wet weight. Total hormone content of the sample divided by the number of individuals per sample gave hormone content per individual. Several types of validation studies were used to determine whether the RIA was precisely and specifically measuring thyroid hormone. Dilution curves of reference standards and extract with standards added were parallel. Mean recovery of 6 concentrations of T4 standards added to walleye egg extract was 103 ± 6%. Applying different volumes (50 to 200 ul) of egg extract to the columns did not affect hormone recovery. The
amount of crossreactivity between T₄ antisera and T₃ was 0.3% and the crossreactivity of T₃ antisera with T₄ was 0.4%.

Effects of hormone immersion on larval survival and cannibalism.

The effects of thyroid hormone on larval walleye cannibalism and survival were studied by culturing hatchlings in thyroid hormones, T₃ and T₄, at concentrations of 0.01, 0.05 and 0.1 ppm. These doses were chosen based on studies showing that 0.1 ppm T₄ enhances growth and viability in mosambique tilapia, *Sarotherodon mossambicus*, (Lam, 1985) and *Sarotherodon niloticus*, (Nacario, 1983) without causing skeletal abnormalities. Because T₃ is considered to be about 10 times as effective as T₄ in promoting larval development (Chris Brown personal communication) we included lower (0.01 ppm) and intermediate doses (0.05 ppm).

Immersion experiments with T₃ were performed using all stocks, and T₄ immersion experiments were done with all but the Kansas and Ohio stocks. Fifty eyed eggs (170 DTU) were placed in small plastic aquaria containing two liters of dechlorinated tap water. Thyroid hormones were added to the tanks immediately prior to adding the eggs. Three aquaria were used for each hormone dose and for the no hormone controls. Aquaria were aerated and illuminated for 10 h/d. Water temperatures ranged from 15 to 18 °C during the several experiments. Daily water levels were monitored and maintained by adding the appropriate solutions. Walleye were not fed during the experiments which were continued until no new cannibals appeared (between 11 and 16 days).

Tanks were monitored daily for survival and cannibalism. Cannibals were recognized as fish that had prey in their mouths. Cannibals and dead larvae were counted and removed daily.
Data analysis

In the stock performance evaluation, correlation and regression analyses were completed to determine the significance of relationships between hormone level and performance. Relationships were considered significant at the 5% level (P ≤ 0.05). Tanks were the experimental units, and survival to the end of the experiment was determined by counting all fish remaining in the culture tank at the end of the experiment and expressing this number as a percentage of the initial stock. Viability was calculated as the percentage of the original number stocked which had an inflated gas bladder at the end of the experiment.

Survival in the immersion studies was determined by calculating the percentage of initial stock remaining each day. Data were corrected for cannibalism by subtracting the total number of cannibals in each aquarium from the initial stock. The daily thermal unit (degree days above 0°C) at which 50% larval survival occurred was predicted from regression analysis of survival curves. Fifty percent TUs of controls and hormone treated groups were compared by analysis of variance (ANOVA). When a significant difference between treatments was found, Fisher's protected LSD test was used to determine which groups were significantly different from each other.

In addition, comparisons among class means between the control tanks and all others combined (0 ppm ≠ all others) were performed to determine if the control differed from all other treatments (Snedecor, 1967).
RESULTS

Fry performance and TH content.

There were significant differences in thyroid hormone concentration of the eggs among stocks. The Wisconsin stock had the highest T4 concentration (9.27 ng/g), and the Minnesota stock had the lowest (0.48 ng/g) (Table 1). The T3 concentration of the eggs was more consistent between stocks than the T4, ranging from 0.7 to 1.54 ng/g.

Fry survival ranged from 1.5 to 17.2% (Table 1), and was not significantly correlated to either T3 or T4 content of the eggs (Table 2). Cannibalism was observed in all stocks between six and ten days after hatching. Observed cannibalism in the fry performance trials was not directly correlated with the T3 content of the eggs, however, when the Minnesota stock, a stock that consistently had a higher incidence of cannibalism than any other stock in the region (Bristow, 1993) was removed from the analysis, the relationship was significant (r=0.88, P=0.05), indicating that as T3 concentration increases, cannibalism increases (Figure 1).

The energy content of the eggs ranged from 5920 to 6749 cal/g. Energy content was correlated with T4 concentration (r=0.80, P=0.05), but there was no relationship between energy content and T3 (Table 1).

Effects of hormone immersion on cannibalism.

Cannibalism was observed as early as 7 days posthatch and it continued throughout the experiment. Immersion of hatchlings in both T3 and T4 had significant effects on incidence of cannibalism and survival (Table 3). Statistical contrasts between the controls (no TH added) and all other treatments (0.01, 0.05, and 0.1 ppm TH added) indicated that both T3 and T4 increased cannibalism and TU
to 50% mortality (Table 3). All concentrations of T₃ caused significant increases in cannibalism and survival. (Table 3 and Figure 2). Even the lowest dose, 0.01 ppm, was above the apparent T₃ threshold. T₃ was more effective in promoting survival than T₄. Only the highest concentration of T₄ (0.1 ppm) significantly increased cannibalism and survival. Regression analysis of the T₄ dose response curves suggested that T₄ concentrations between 0.01 and 0.10 ppm is associated with incidence of cannibalism (R²=0.79) and larval survival (R²=0.87).
DISCUSSION

Thyroid hormones have been reported in the eggs of many species of fish including coho, *Oncorhynchus kisutch*, and chum salmon, *O. keta*, striped bass, tilapia, *Oreochromis mossambicus*, and medaka, *Oryzias latipes*, (Brown et al., 1988; Greenblatt et al. 1989; Kobuke et al. 1987; Leatherland et al. 1989; Tagawa and Hirano 1991; Weber et al. 1992). Thyroid hormone concentrations in our studies of walleye eggs from six separate hatchery stocks varied from 9.27 to 0.48 ng/g for T₄ and from 1.54 to 0.7 ng/g for T₃. The source of variability in egg thyroid hormone concentrations may be due to differences in genetics, environmental conditions or maternal nutrition as well as to differences in eggs from different females (Hey and Farrar, unpublished results). Although thyroid hormones are acknowledged to have important roles in vertebrate development, their functions and required levels in the development of fish eggs and larvae remain largely unknown and the questions: "Does the amount of variation in egg thyroid hormone within a species have significance?" and "Can stock differences in thyroid hormone content be used as indicators of probable survival during early development?" need to be addressed.

Thyroid hormones are composed of coupled iodinated tyrosine residues and exist in two forms, T₄ (thyroxine) or tetraiodothyronine and T₃, triiodothyronine. Thyroxine is considered to be the less active prohormone which is enzymatically converted to the more active hormone, T₃. Higgs et al. (1979) found that T₃, but not T₄, can be administered in the diet of coho salmon to enhance growth and improve food conversion. Our results support the concept that in fish, T₃ is the more active thyroid hormone since immersion in T₃ was more effective than immersion in T₄ in
promoting cannibalism and survival and T₃ concentration of eggs from different stocks correlated with percent cannibalism in fry performance evaluation.

Walleye egg thyroid hormone concentration from various stocks correlated with several performance parameters in intensive culture. Triiodothyronine concentration correlated positively with both gas bladder inflation and cannibalism. Egg T₃ content correlated positively with larval size at hatch. Thyroxine concentrations correlated positively with energy content of the egg. Several other correlations are also potentially important as well, even though their probability levels were higher than 0.05. Triiodothyronine concentration also correlated positively with oil globule diameter (p=0.17), egg weight (p=0.12), survival (p=0.2) and viability (p=0.19) but not with growth rate.

Is thyroid hormone content an important predictor of larval survival or performance? Our results demonstrate that naturally occurring TH concentrations in walleye eggs can predict several parameters of larval performance. Previous experiments that raised or lowered the TH content of eggs by manipulating maternal hormone levels produced contradictory results. Medaka development, hatchability, body weight, length and survival under starvation are not affected by reducing TH content in eggs by 90% (Tagawa and Hirano, 1991). On the other hand, elevating striped bass egg thyroid hormone content can result in a ten fold increase in gas bladder inflation rates and larval survival (Brown et al., 1988). Egg thyroid hormones may play different developmental roles in fish according to their life histories and survival rates. For example, walleye and striped bass are closely related and are difficult to culture. Medaka, on the other hand, may have fewer larval survival problems, require less TH and be more independent of egg TH.
Our immersion studies demonstrated thyroid hormone enhancement of two apparently opposing processes - larval walleye survival and cannibalism. In our immersion studies survival of fasting larvae was extended in the hormone treated groups even in the presence of increased cannibalism. The effects of thyroid hormone immersion on larval survival have been observed previously (Lam, 1980; Lam and Sharma, 1985), but the cannibalism effects have not been reported.

In this study, thyroid hormone immersion increased the cannibalism rate in fasting larval walleye 8-10 fold. T₃ was more effective than T₄ in stimulating cannibalism and acted in a dose related manner while T₄ did not. Cannibalistic behavior of walleye larvae has been described previously (Cuff, 1977, 1980) but has not been related to TH. Sibling or "cohort cannibalism" begins with the onset of feeding and is most prevalent in the postlarval stages (Li and Mathias, 1982). Cannibalistic behaviors are of two types: tail attacks and trunk attacks. Both contribute significantly to larval mortality (Loadman et al., 1986). Our cannibalism rates are conservative estimates because only tail attacks were counted.

Several factors that increase cannibalism rates include decreased food density (Loadman, et al., 1986), starvation (Li and Mathias, 1982), light (Loadman et al., 1986), aggregation of larvae in the dark (Loadman et al., 1986) and increased larval density (Li and Mathias, 1982). Our study suggests that stock differences and thyroid hormone content of walleye eggs also influence cannibalism rates. Stock differences in walleye cannibalism rates could be due to genetic and or environmental influences, but further research will be required to determine the involvement of these influences. A genetic component of cannibalism is seen in the Sonoran top minnow, *Poeciliopsis occidentalis occidentalis* (Meffe, 1984).
Cannibalism has not previously been shown to be stimulated by thyroid hormones. It has been suggested as a factor reducing cannibalism in striped bass (Brown et al. 1988). Our data are consistent with the idea that thyroid hormone may regulate developmental processes that enhance both survival and cannibalism. Such processes could involve promotion of central nervous system development as seen in mammals (Nicholson and Altman, 1972), stimulation of larval swimming activity as has been suggested for tilapia, *Sarotherodon mossambicus* (Lam, 1980), appetite stimulation, enhanced stress tolerance or improved use of energy stores.

Cannibalism causes major losses in walleye intensive culture systems in spite of efforts to optimize culture conditions. We have presented data which shows that other components beside food availability and larval density, such as thyroid hormones and stock differences, may contribute to cannibalism. Maternal blood levels of thyroid hormone at the time of spawning, and consequently egg thyroid hormone content, may link diet and environmental factors to larval performance. Thyroid hormones are potentially important regulators of developmental processes that may lead to both cannibalism and survivorship in walleye and are potentially important contributors to their successful culture.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Performance, egg thyroid hormone content, and energy content of several stocks of cultured walleye fry.

<table>
<thead>
<tr>
<th>Performance variable</th>
<th>Stock</th>
<th>OH</th>
<th>KS</th>
<th>IA</th>
<th>WI</th>
<th>MN</th>
<th>ND</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td></td>
<td>3.3a</td>
<td>1.5a</td>
<td>7.2b</td>
<td>9.1b</td>
<td>0.1c</td>
<td>17.2d</td>
<td>0.01</td>
</tr>
<tr>
<td>GBI (%)</td>
<td></td>
<td>92.0a</td>
<td>100.0c</td>
<td>98.7bc</td>
<td>100.0c</td>
<td>100.0c</td>
<td>96.7ab</td>
<td>0.01</td>
</tr>
<tr>
<td>Viability (%)</td>
<td></td>
<td>3.1a</td>
<td>1.5a</td>
<td>7.1b</td>
<td>9.1b</td>
<td>0.1c</td>
<td>16.6d</td>
<td>0.01</td>
</tr>
<tr>
<td>Cannibalism (%)</td>
<td></td>
<td>3.4a</td>
<td>5.4abc</td>
<td>4.7ab</td>
<td>11.4cd</td>
<td>17.6d</td>
<td>10.5bcd</td>
<td>0.01</td>
</tr>
<tr>
<td>Growth (mm/100TU)</td>
<td></td>
<td>1.7a</td>
<td>2.0b</td>
<td>2.7cd</td>
<td>2.6c</td>
<td>2.6c</td>
<td>2.8d</td>
<td>0.01</td>
</tr>
<tr>
<td>T₃ (ng/g)</td>
<td></td>
<td>0.7a</td>
<td>1.3a</td>
<td>1.0a</td>
<td>1.5a</td>
<td>1.0a</td>
<td>1.5a</td>
<td>0.75</td>
</tr>
<tr>
<td>T₄ (ng/g)</td>
<td></td>
<td>0.53a</td>
<td>0.72a</td>
<td>0.95a</td>
<td>9.27b</td>
<td>0.48a</td>
<td>0.58a</td>
<td>0.01</td>
</tr>
<tr>
<td>Energy (cal/g of eggs)</td>
<td></td>
<td>6749a</td>
<td>6588ab</td>
<td>6354bc</td>
<td>5920d</td>
<td>6243c</td>
<td>6519ab</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values in the same row with letters in common are not significantly different.
Table 2. Correlation coefficients and P-values of selected larval walleye stock performance values and T<sub>4</sub> and T<sub>3</sub> thyroid hormone concentrations of the eggs (ng/g) of these stocks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.21</td>
<td>0.68</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.61</td>
<td>0.20</td>
</tr>
<tr>
<td>GBI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.34</td>
<td>0.51</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td>Cannibalism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.21</td>
<td>0.69</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.29</td>
<td>0.57</td>
</tr>
<tr>
<td>Excluding MN stock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.66</td>
<td>0.22</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.88</td>
<td>0.05*</td>
</tr>
<tr>
<td>Viability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.23</td>
<td>0.65</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.62</td>
<td>0.19</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.23</td>
<td>0.66</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.52</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Table 3. The effects of thyroid hormone immersion on cannibalism and TU to 50% mortality of walleye fry in small aquaria. The ANOVA P values test for a significant difference between treatments, and the contrast values represent a significant difference between the control and all other treatments.

<table>
<thead>
<tr>
<th>TH concentration (ppm)</th>
<th>Cannibalism (%)</th>
<th>TU to 50% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T₃</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0(control)</td>
<td>0.5a</td>
<td>193a</td>
</tr>
<tr>
<td>0.01</td>
<td>4.2b</td>
<td>425b</td>
</tr>
<tr>
<td>0.05</td>
<td>5.0b</td>
<td>369b</td>
</tr>
<tr>
<td>0.10</td>
<td>5.6b</td>
<td>342b</td>
</tr>
<tr>
<td>P values</td>
<td>0.0005</td>
<td>0.0415</td>
</tr>
<tr>
<td>Contrast</td>
<td>0.0001</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

| **T₄**                 |                 |                     |
| 0(control)             | 0.8a            | 139a                |
| 0.01                   | 1.7a            | 162a                |
| 0.05                   | 1.5a            | 162a                |
| 0.10                   | 7.3b            | 209b                |
| P values               | 0.0001          | 0.0006              |
| Contrast               | 0.0038          | 0.0278              |

Values in the same column with letters in common are not significantly different.
Figure 1. Egg T₃ concentrations and the percent of cannibals in several stocks of cultured walleye fry from Ohio, Iowa, Kansas, Minnesota, Wisconsin and North Dakota. (Each point represents the mean of 2 pools of 100 eggs each and the percent of total mortality caused by cannibalism throughout the culture period.)

* Minnesota (a stock notorious for high cannibalism) was excluded from the regression.
Figures 2. Effects of immersing larval walleye in increasing T₃ and T₄ concentrations on cannibalism and survival. (Each point is the mean of 4 T₄ and 6 T₃ experiments with triplicate tanks of 50 larvae each).
GENERAL SUMMARY

According to Gorbman et al. (1983) thyroid hormones have been credited with a longer list and more diverse array of functions ranging from morphologic, to physiologic and metabolic than any other hormone, however they have only recently been implicated in vertebrate development. Embryos without thyroid glands have been presumed to develop independently of thyroid hormones. With the discovery by Brown et al. (1987) that various teleost eggs have thyroid hormones in their yolk these hormones became recognized as potential regulators of early development as well. Thyroid hormones have also been found in chicken eggs (Sechman and Bobek, 1988) and amphibian eggs (Niinuma et al., 1991; Weber et al., 1994). There is growing evidence that thyroid hormones can cross the human placenta via plasma binding to LDLs and LDL and HDL receptors in placental cell membranes (Benvenga and Robbins, 1993). Since human embryonic thyroid follicles don't begin functioning until the third month (Arey, 1960), early embryonic development may rely on maternal sources of thyroid hormone (Porterfield and Hendrich, 1993). The precise developmental roles of thyroid hormone throughout this early developmental period are not known for any vertebrate.

In some teleost species endogenous thyroid hormones may be required for larval growth and survival. The best evidence for this is in the work of Brown et al. (1988) who boosted thyroid hormone content of striped bass eggs through maternal injection, stimulating growth and survival. Normal non-injected striped bass eggs became depleted of thyroid hormones prior to development of their embryonic thyroid gland. The hormone depletion occurred during the larval critical period coinciding with high mortality. Boosting the thyroid hormone content of the egg prevented this thyroid hormone depletion and resulted in larger larvae and enhanced
survival. In this thesis we reasoned that walleye because of their small eggs and similar life history and culture problems to striped bass, might also be depleted of thyroid hormone before their thyroid follicles begin functioning. We compared hormone content of eggs and larvae of this small egged species with rainbow trout which have a larger egg and more extended early development period.

Both walleye and trout eggs contained thyroid hormones, T₄ and T₃. Trout eggs contained more total hormone but this was a consequence of their larger size since hormone concentrations were similar in the two species. Egg T₃ concentrations were generally higher than T₄ in walleye and trout. These findings provide another exception to statements by Tagawa et al. (1990) that T₄ concentrations are greater than T₃ in most freshwater fish eggs. Weber et al. (1992) found that in the tilapia (*Oreochromis mossambicus*) more T₃ than T₄ is accumulated in the oocyte and that storage patterns were not directly coupled to serum hormone levels. Preferential storage of T₃ could be of physiologic significance to the embryo since this is the more active form of the hormone and embryos may lack deiodionases capable of converting T₄ to T₃.

We found striking differences in walleye egg T₄ concentration variability. The subject of variability has not been addressed in the literature; very few studies of thyroid hormones in teleost eggs have included multiple stocks or egg sources. We found variation between hatchery stocks and between egg samples from different individual females from those stocks. Trout egg thyroid hormone concentrations from individual females were much less variable than from individual walleye. The source of this variability could be genetic, environmental and or nutritional since walleye stocks represented a wider range of habitats and more genetic diversity than the domesticated rainbow trout stock.
Thyroid hormones were detectable throughout all stages observed: eggs, eyed eggs and larval walleye up to fourteen days posthatch and larval trout up to 49 days posthatch. Thyroid follicles appeared by five days posthatch in both species. We hypothesized that trout, having larger eggs would have more initial thyroid hormone and would not experience hormone depletion prior to thyroid follicle development and this appears to be the case. We also hypothesized that walleye, having smaller eggs and a life cycle similar to the striped bass, could experience thyroid hormone depletion prior to the appearance of functional thyroid follicles. This hormone depletion would occur during the larval critical period and could contribute to larval mortality. However, walleye thyroid follicles appeared prior to the critical period and even though we have no evidence that they were functional, thyroid hormone stores were evidently not depleted half-way through the larval critical period.

We used two approaches for studying the roles of thyroid hormones in walleye development. First we looked for correlations between thyroid hormone concentrations in walleye eggs and parameters of larval performance. Second we immersed hatchlings in thyroid hormones and measured their survivorship. Both types of studies independently implicated thyroid hormones in promoting larval cannibalism. We found a positive correlation between T₃ concentration in eggs from walleye from various hatchery stocks and larval cannibalism but no correlation with gas bladder inflation, growth rate, viability or survival. T₃ was more effective than T₄ in the immersion studies, but both stimulated cannibalism and larval survival in a dose related manner. Cannibalism is an aquaculture problem for both walleye and striped bass. Brown et al. (1988) speculated that in striped bass thyroid hormone reduces cannibalism and thus promotes larval survival. However, our research
shows that in walleye thyroid hormone promotes cannibalism and increases survival. There may be some processes common to both cannibalism and survival that are regulated by thyroid hormone. Whatever these processes are, they probably assist with the transition to independent feeding. Such processes could include nervous system maturation, stimulation of appetite, enhanced feeding behaviors and maturation of the gastrointestinal tract.

Walleye and striped bass continue to be attractive models for further study of the role of thyroid hormones in larval teleost development because they experience a high mortality critical period as physiological, morphological and behavioral changes associated with the successful transition from egg to fry occur. As models these larva can be used to define minimal requirements for thyroid homones and their roles in regulating changes during the transition. This could be accomplished by creating eggs with minimal thyroid hormone content through maternal injection of thyroid hormone inhibitors and observing their development and survival during the critical period. Such experiments would address the question of whether yolk hormone is actually required to optimize development and, if so, how much hormone is required. Furthermore, such experiments might begin to identify the specific structural, physiologic or metabolic changes that require thyroid hormone.
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