Maternal Thyroid and Glucocorticoid Hormone Interactions in Larval Fish Development, and Their Applications in Aquaculture

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Maternal Thyroid and Glucocorticoid Hormone Interactions in Larval Fish Development, and Their Applications in Aquaculture

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5Teens4Oceans, Boulder, Colorado, USA

Thyroid hormones (THs) have long been known to have regulatory roles in the differentiation and maturation of vertebrate embryos, beginning with the knowledge that hormones of maternal origin are essential for human fetal central nervous and respiratory system development. Precise measurements of circulating THs led to insights into their critically important actions throughout vertebrate growth and development, initially with amphibian metamorphosis and including embryogenesis in fishes. Thyroid cues for larval fish differentiation are enhanced by glucocorticoid hormones, which promote deiodinase activity and thereby increase the generation of triiodothyronine (T3) from the less bioactive thyroxin (T4). Glucocorticoids also induce the expression of thyroid hormone receptors in some vertebrates. Maternally derived thyroid hormones and cortisol are deposited in fish egg yolk and accelerate larval organ system differentiation until larvae become capable of endogenous endocrine function. Increases in the T3/T4 ratio during larval development may reflect the regulatory importance of maternal thyroid hormones. Experimental applications of individual hormones have produced mixed results, but treatments with combinations of thyroid and corticoid hormones consistently promote larval fish development and improve survival rates. The developmental and survival benefits of maternal endocrine provisioning are increased in viviparous fishes, in which maternal/larval chemical contact is prolonged. Treatments with exogenous thyroid and corticoid hormones consistently promote development and reduce mortality rates in larval fishes, with potential hatchery-scale applications in aquaculture.

Keywords  thyroid, larvae, embryo, maternal, cortisol

INTRODUCTION

Thyroid hormones (THs) are recognized not only as the main determinant of metabolic rate in homeotherms, but also as primary signals for the regulation of diverse growth and developmental and maturational processes throughout the vertebrates. Our initial understanding of the actions of THs was based almost entirely on human pathology and physiology, and the familiar and devastating pathological effects of acute thyroid deficiencies in cases of goiter and cretinism. A comparative perspective emerged with the technology for precise quantification of circulating hormones, and critically important morphogenic roles of THs among the vertebrates became apparent. These morphogenic actions evolved in fishes and they are essential to successful embryogenesis.

The first clear link between thyroid function and metamorphosis was reported by Gudernatsch (1912), who described a simple bioassay in which feeding various...
mammalian thyroid gland tissues to *Rana* sp. tadpoles produced “extremely small (pigmy-) frogs.” Thyroid-dependent developmental actions extend well beyond the regulation of amphibian metamorphosis and a role in cretinism, and most morphogenic actions of thyroid hormones are far less dramatic than Gudernatch’s (1912) “maturational” effects. Milder transitions, e.g., larval metamorphoses among a majority of fishes, are also controlled by THs (reviewed by Power et al., 2001), as are a range of developmental and growth effects among vertebrates. The maturational actions of THs include the promotion of growth and development by way of hypertrophy and hyperplasia as well as the reorganization of tissues resulting in differentiation. Many of the actions of THs are closely associated with reproduction and embryogenesis.

Serious risks to the offspring of hypothyroid mothers are recognized, and hormone replacement treatment is customary (Lebeau and Mandel, 2006). The human maternal endocrine contribution is viewed as “critical for proper in utero brain development” (Howedshell, 2002). Routine screening of pregnant human subjects for adequate thyroid function began in the early 1970s (Dussault, 1999). Maternal thyroid activity is enhanced during human embryogenesis, partly in response to a thyrotropic action of *Human Chorionic Gonadotropin* (HCG) during pregnancy (Fantz et al., 1999). Human fetal central nervous system (CNS; Haddow et al., 1999) and respiratory tract (e.g., see Rajatapiti et al., 2005) differentiation are among processes in human development that are directly dependent on maternal thyroid contributions. Thyroid hormone receptors (THRs) enable early human embryos to respond to maternal thyroid hormones before fetal thyroid hormone production has begun (Patel et al., 2011). Incidental elevations of circulating maternal THs in pregnant sheep result in changes of the texture and color of wool in the resultant lambs (Kenyon et al., 2004). Elevated maternal circulating levels of thyroxin (T4) during human pregnancy provide vital support to healthy human embryo differentiation before endogenous fetal hormone production begins, and a suite of placental deiodinases ensures fetal provision of essential THs by even mildly hypothyroidal mothers, as well as protection of the fetus against exposure to excessive quantities (Burrow et al., 1994). The practice of clinical hormone replacement therapy for premature infants also underscores the early developmental importance of maternal endocrine contributions. The effectiveness of treatment of premature infants with T3 or triiodothyronine (T3) alone was challenged by various investigators who noted that thyroid hormone applications did not consistently improve survival or correct problems associated with respiratory distress syndrome (see Osborn, 2001; Farwell, 2008). This may have been at least partly a result of the absence of permissive actions imparted by glucocorticoids; the combined effectiveness of thyroid and corticoid hormones administered together is well established (Smith and Sabry, 1983). This combination of treatments is routinely used to promote lung maturation and surfactant production in preterm babies (see Ballard, 1980).

**THYROID HORMONE SYNERGISM WITH CORTISOL IN FISH DEVELOPMENT**

A possible significance of THs in early fish growth and development was implied by the acute responsiveness of very young fish to antithyroid drugs and hormone supplementation treatments (reviewed by Youson, 1988; Brown and Bern, 1989; Dickhoff et al., 1990). The sensitivity of newly hatched fish to THs was reported by Lam and others who found that T4 treatment accelerated development and larval metamorphosis, and increased survival rates (Lam, 1980; Lam, 1985; Lam and Sharma, 1985). Also, in a role similar in many ways to the endocrine regulation of anuran metamorphosis, thyroid signals initiate some developmental changes later than the larval-to-juvenile metamorphosis, such as the transition of upright-swimming larval flatfishes into asymmetrical juveniles beginning after the completion of yolk absorption (Tagawa et al., 1991; Inui et al., 1994). By the time of metamorphosis in flatfishes, maternal hormones stored in yolk have been fully depleted and the larval pituitary/thyroid axis has attained functionality (De Jesus et al., 1991). Metamorphic climax among the flatfishes bears strong similarities to the events seen in amphibian metamorphosis (see Miwa et al., 1988; Tagawa et al., 1991). The regulation of smolting in salmonids is also controlled in part by thyroid system signals (see Robertson, 1949; Miwa and Inui, 1985). Early perceptions of the role of THs in development have been confounded by a history of contradictory reactions to exogenous thyroid compounds; some cases of aberrant development are associated with higher hormone doses (see Table 1) and other negative responses remain unexplained.

Physiological effects of cortisol on thyroid-mediated processes in fishes are more than additive, or synergistic (Ban, 2005). A synergistic action of cortisol and THs has been identified in flatfish metamorphosis (De Jesus et al., 1990; De Jesus et al., 1991), and a close interaction of the two endocrine systems is also seen in the promotion of amphibian metamorphosis (Krug et al., 1983). An emerging view of glucocorticoid signaling is that it not only enables important vertebrate organ systems to respond to THs through synergistic actions, as in amphibians, but that surging glucocorticoid secretions facilitate major transitions within a life cycle, serving a preparatory and synchronizing role in organisms approaching a major change to the next life history stage (Wada, 2008).

In some cases, cortisol treatment results in increases in thyroid hormone activity by increasing the generation of T3 (Redding et al., 1991). Thyroxin is generally viewed as a thyroid system *prohormone* (Kühn et al., 1994), in which thyroid-mediated endocrine activity is determined largely by the action of peripheral deiodinase enzymes. Peripheral outer ring deiodination converts T4 into 3,5,3′-triiodothyronine, or T3, which
is an order of magnitude more biologically potent than T4 (Orozco and Valverde, 2005). Cortisol promotes the conversion of T4 into T3 by activating outer ring deiodinase activity (Vijayan et al., 1988; Arjona et al., 2011), and increasing the T3/T4 ratio is one mechanism by which cortisol promotes thyroid-mediated processes. Ontogenetic patterns in developing fishes that reflect this relationship can be seen in the golden sea bream, Sparus aurata; increasing cortisol levels are accompanied by a sharp increase in the ratio of T3/T4 late in larval development, resulting in significant physiological and morphological changes (Szisch et al., 2005). Concurrent elevations of T3, T4 and cortisol also precede the larval-to-juvenile transition in the silver sea bream, S. sarba (Deane and Woo, 2003). Whole body cortisol content increases as

<table>
<thead>
<tr>
<th>Species</th>
<th>Hormone treatment, dose actions reported</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chum Salmon</td>
<td>Immersion T4, 0.08–0.4 ppm</td>
<td>Dales and Hoar, 1954</td>
</tr>
<tr>
<td>(Oncorhynchus keta)</td>
<td>Accelerated but disproportionate growth and pigmentation</td>
<td></td>
</tr>
<tr>
<td>Chum Salmon</td>
<td>Immersion T4, 1 ppm</td>
<td>Honma and Murakawa, 1955</td>
</tr>
<tr>
<td>(Oncorhynchus keta)</td>
<td>Reduced weight, physical deformities</td>
<td></td>
</tr>
<tr>
<td>Chum Salmon</td>
<td>Immersion T4, 0.08 ppm</td>
<td>Ali, 1961</td>
</tr>
<tr>
<td>(Oncorhynchus keta)</td>
<td>Early hatch</td>
<td></td>
</tr>
<tr>
<td>Brown trout</td>
<td>Immersion T4, 1.0–10 ppm</td>
<td>Woodhead, 1966</td>
</tr>
<tr>
<td>(Salmo trutta)</td>
<td>Rapid differentiation, morphological abnormalities</td>
<td></td>
</tr>
<tr>
<td>Mozambique tilapia</td>
<td>Immersion T4, 0.1 ppm</td>
<td>Lam, 1980</td>
</tr>
<tr>
<td>(Sarotherodon mossambicus)</td>
<td>Accelerated development and % survival</td>
<td></td>
</tr>
<tr>
<td>Mozambique tilapia</td>
<td>Immersion T4, 0.1–0.5 ppm</td>
<td>Nacario, 1983</td>
</tr>
<tr>
<td>(Sarotherodon mossambicus)</td>
<td>Accelerated growth, % deformities at higher T4 doses</td>
<td></td>
</tr>
<tr>
<td>Olive flounder</td>
<td>Immersion T4, 0.01–0.1 ppm</td>
<td>Inui and Miwa, 1985</td>
</tr>
<tr>
<td>(Paralichthys olivaceus)</td>
<td>Induced metamorphosis</td>
<td></td>
</tr>
<tr>
<td>Common carp</td>
<td>Immersion T4, 0.01, 0.05, 0.1 ppm</td>
<td>Lam and Inui, 1985</td>
</tr>
<tr>
<td>(Cyprinus carpio)</td>
<td>Improved hatch rate, % survival</td>
<td></td>
</tr>
<tr>
<td>Milkfish</td>
<td>Immersion T4, 0.5 ppm</td>
<td>Lam et al., 1985</td>
</tr>
<tr>
<td>(Chanos chanos)</td>
<td>Accelerated development</td>
<td></td>
</tr>
<tr>
<td>Olive flounder</td>
<td>Immersion T4, 0.01–0.1 ppm</td>
<td>Miwa and Inui, 1987</td>
</tr>
<tr>
<td>(Paralichthys olivaceus)</td>
<td>Induced metamorphosis</td>
<td></td>
</tr>
<tr>
<td>Striped bass</td>
<td>Maternal injection T3 20 μg/gbw</td>
<td>Brown et al., 1988a,b</td>
</tr>
<tr>
<td>(Morone saxatilis)</td>
<td>Improved growth, % swimbladder inflation, % survival</td>
<td></td>
</tr>
<tr>
<td>Goldfish</td>
<td>Immersion T3, 0.01–0.02 ppm, T4, 0.01–0.1 ppm</td>
<td>Reddy and Lam, 1992a</td>
</tr>
<tr>
<td>(Carassius auratus)</td>
<td>Faster fin differentiation, growth and development</td>
<td></td>
</tr>
<tr>
<td>Mozambique tilapia</td>
<td>Immersion T3, 0.01 ppm, T4, 0.05 ppm</td>
<td>Reddy and Lam, 1992b</td>
</tr>
<tr>
<td>(Oreochromis mossambicus)</td>
<td>Fin differentiation, deformities at highest dose</td>
<td></td>
</tr>
<tr>
<td>Rabbitfish</td>
<td>Maternal T3 injection, 1,10,100 μg/gbw</td>
<td>Ayson and Lam, 1993</td>
</tr>
<tr>
<td>(Siganus guttatus)</td>
<td>Improved growth, % survival</td>
<td></td>
</tr>
<tr>
<td>Brown trout</td>
<td>Maternal T3 injection, 20 mg/kg</td>
<td>Mylonas et al., 1994</td>
</tr>
<tr>
<td>(Salmo trutta)</td>
<td>% skeletal abnormalities</td>
<td></td>
</tr>
<tr>
<td>Striped bass</td>
<td>Immersion T3, 0.05–0.1 ppm</td>
<td>Huang et al., 1996</td>
</tr>
<tr>
<td>(Morone saxatilis)</td>
<td>At higher dose; % growth, % survival</td>
<td></td>
</tr>
<tr>
<td>Zebrafish</td>
<td>Immersion T3, 5 nM (.003 ppm) T4 30 nM (.02 ppm)</td>
<td>Brown, 1997</td>
</tr>
<tr>
<td>(Danio rerio)</td>
<td>Accelerated fin development</td>
<td></td>
</tr>
<tr>
<td>Amberjack</td>
<td>Maternal injection T3, 20 mg/kgbw</td>
<td>Tachihara et al., 1997</td>
</tr>
<tr>
<td>(Seriola lalandi)</td>
<td>Survival more than doubled</td>
<td></td>
</tr>
<tr>
<td>Korean rockfish</td>
<td>Maternal injection T3, 20 mg/kgbw</td>
<td>Kang and Chang, 2004</td>
</tr>
<tr>
<td>(Sebastes schlegeli)</td>
<td>Increased growth, % survival</td>
<td></td>
</tr>
<tr>
<td>Goldfish</td>
<td>Feeding T3, 1.25–6.25 ppm</td>
<td>Swain and Sahoo, 2003</td>
</tr>
<tr>
<td>(Carassius auratus)</td>
<td>Increased growth, disease resistance</td>
<td></td>
</tr>
<tr>
<td>Pirarucu</td>
<td>Maternal T3 injection, 0.01, 0.05, 0.1, 0.5, 1 ppm, % survival-reduced cannibalism</td>
<td>Landines et al., 2010</td>
</tr>
<tr>
<td>(Brycon orbignyanus)</td>
<td>% survival</td>
<td></td>
</tr>
<tr>
<td>Zebrafish</td>
<td>Immersion T3, 5 nM = .003 ppm</td>
<td>Walpita et al., 2007</td>
</tr>
<tr>
<td>(Danio rerio)</td>
<td>Accelerated pigmentation and % hatching</td>
<td></td>
</tr>
<tr>
<td>Matrinxá</td>
<td>Maternal injection T3, 10–20 mg/kg</td>
<td>Urbinati et al., 2008</td>
</tr>
<tr>
<td>(Brycon amazonicus)</td>
<td>Immersion, 0, 0.01, 0.05, 0.1 mg/L</td>
<td></td>
</tr>
<tr>
<td>Nile tilapia</td>
<td>Maternal injection T3, 1 or 10 μg/g</td>
<td>Khalil et al., 2011</td>
</tr>
<tr>
<td>(Oreochromis niloticus)</td>
<td>Increased growth, rapid development, % survival</td>
<td></td>
</tr>
</tbody>
</table>
numerous species of larval fishes approach the transition into juvenile forms, possibly to coordinate various events (Wada, 2008), in some cases by synergizing with thyroid hormones (Hwang et al., 1992; Tanaka et al., 1995). Sudden increases in T3/T4 ratios coincident with transitions from the larval to juvenile stage have been observed (e.g., see Nayak et al., 2001; McComb et al., 2005), which may or may not reflect glucocorticoid synergism in thyroid-driven developmental actions.

The acute responsiveness of very young larval fishes to THs seemed paradoxical or artifactual for a number of years, since those cases often revealed hormone-sensitivity in animals that had not yet developed functional pituitary/thyroid axes (Mommsen and Walsh, 1988). Hormone radioimmunoassays of yolk extracts revealed the presence of ample amounts of T4 and T3 in unfertilized fish eggs (Kobuke et al., 1987; Brown et al., 1987; Tagawa and Hirano, 1987). Maternal deposition of hormones in yolk raises the possibility of maternal/larval endocrine communication in early development in oviparous fishes (Mommsen and Walsh, 1988). Thyroid hormones of maternal origin have been found in unfertilized eggs consistently among oviparous vertebrates, suggesting maternal provisioning and a mechanism of delivering regulatory signals to developing embryos long before the onset of endogenous thyroid competence. Circulating THs in pre-spawning female rabbitfish (Siganus guttatus) accumulate in oocytes during vitellogenesis, resulting in improved larval performance and survival (Ayson and Lam, 1993). Developmental research using zebrafish (Danio rerio) as a model confirms thyroid stimulation of various processes of fin, scale, and pigment differentiation in this species (Brown, 1997). Goldfish respond in similar ways to exogenous THs, with improvements in fin, skin, and scale development, growth, and resistance to challenges with pathogens (Reddy and Lam, 1992a; Shinobu and Mugiya, 1995; Swain and Sahoo, 2003). Other maternal hormones, especially small steroid molecules such as cortisol are also found in the yolk of unfertilized fish eggs (reviewed by Tagawa, 1996; Brooks et al., 1997) and may be necessary for permissive or other effects. Interactive and sometimes synergistic effects of thyroid and corticoid hormones in larval fish development (Table 2) are consistent with the concept of combinations of maternal signals interacting to coordinate and promote developmental processes in young fishes, increasing the likelihood of larval survival. Maternally derived cortisol and testosterone deposited in yolk interact in the regulation of growth and development in larval damselfishes (McCormick, 1999). Combined treatments with supplemental TH and cortisol consistently accelerate digestive system ontogeny (Kim and Brown, 2000; Nayak et al., 2000a) and swimbladder function, resulting in reduced rates of mortality of larval fishes (Kim and Brown, 1997; Sri Prudi et al., 2002; Nayak et al., 2003).

Extensive evidence implicates THs in the regulation of various aspects of growth (see reviews by Donaldson et al., 1979; Power et al., 2001). A majority of the studies mentioned above indicate that exogenous THs stimulate or accelerate development and result in enhanced survival in larval fishes. Some species are unresponsive or have very limited responses to alterations of thyroid hormone levels alone (Tagawa and Hirano, 1990; Witt et al., 2009) or show detrimental effects of exogenous THs (see Table 1; Higgs et al., 1982; Huang et al., 1996). Hormone interactions (e.g., low levels of permissive compounds), species differences and dose-dependency may account for some inconsistencies in the responses of larvae to exogenous THs. Among species differences, untoward effects of exogenous THs are often seen in fishes with large eggs, high survival rates, and presumably ample supplies of maternal THs, such as the salmonids (see Mylonas et al., 1994). Overstimulation of skin, bone, and scale development by higher doses of THs in post-larval stages may partially explain their capacity to induce deformities (Karg, 2007). Some deformities may be caused by the promotion by exogenous hormones that regulate developmental processes when the timing of exposure or sensitivity is skewed or when ample amounts of required materials are unavailable (Brown et al., 2010). Similarly, treatment of larval tilapia with cortisol alone promotes growth and development inconsistently and in a dose-related fashion (Mathiyalagan et al., 1996). Under some conditions, treatment with exogenous cortisol alone improves the survival rate of larval Asian seabass, Lates calcarifer, by undetermined mechanisms (Sampath-Kumar et al., 1993).

**THE PITUITARY/THYROID AXIS AND MOLECULAR MECHANISMS IN DEVELOPMENT**

The presence of THs and cortisol has been reported in fish at all developmental stages beginning in unfertilized eggs (see Brooks et al., 1997; Szisch et al., 2005), and stimulation by endocrine compounds during embryogenesis and organogenesis appears to be of vital importance. Ontogenetic studies of endocrine functions suggest that the maternal endocrine contributions to offspring sustain fish through the earliest of developmental stages, beginning with fertilization, before developing embryos have acquired endocrine competence. One apparent cascade of actions in oviparous fishes begins with maternal deposition of cortisol and THs in egg yolk, resulting in the promotion of deiodinase activity and THR expression by cortisol, and induction by THs of growth factors in developing embryos leading to accelerated differentiation. The central nervous system, digestive tract, skin, fins, and swimbladder appear to be targets for endocrine stimulation of development. Although some authors conclude that the physiological significance of maternally derived cortisol in fish eggs is uncertain, it appears likely that permissive or synergistic actions of corticoids in thyroid-regulated processes may be of adaptive value (Brooks et al., 1997). Very early contributions of maternal THs to larval differentiation and survival are confirmed by the presence of thyroid hormone receptors (THR) long before endogenous thyroid hormone synthesis begins; several isoforms are expressed from the very first...
stages of development in zebrafish (Darras et al., 2011). Genes encoding the TRα1 thyroid hormone receptor (TR) begin expression in zebrafish zygotes about 3 hr post-fertilization (Essner et al., 1997), suggesting that hormone responsiveness is an urgent embryonic need. The detection of THR long before the onset of larval thyroid function in zebrafish has led to some doubt of the physiological role of hormone-receptor endocrine signaling in early development (Essner et al., 1997; discussed by Power et al., 2001), but zebrafish embryos have (Walpita et al., 2007) and are capable of responding to THs of maternal origin (Darras et al., 2011).

Unfertilized zebrafish eggs contain maternally derived THR transcripts, followed by early zygotic transcription of TR and TR mRNA (Liu and Chan, 2002; Walpita et al., 2007). Maternal provisioning of egg yolk in the zebrafish also includes cortisol, and experimental interference with the response of zebrafish embryos to glucocorticoids leads to reduced growth, impaired organogenesis, and increases in the rate of mortality (Nesan, 2013). The possible interdependence of cortisol and THs in larval fish development is supported by the maternal provisioning of both groups of hormones in egg yolk and their synergistic actions when applied together experimentally (Table 2). Significant cross-talk between interrenal and thyroid systems may be characteristic of early teleost development, with synergistic promotion by glucocorticoids of the expression of THR and the stimulation of peripheral deiodinases (De Nayer et al., 1987; Terrien and Prunet, 2013). These two mechanisms are at least partly responsible for the synergy of corticosteroids with TH during tadpole metamorphosis (Bonett et al., 2010).

The evolutionary implications of the sensitivity of larval fishes to maternal hormones are profound. Subtle effects of early exposure to maternal hormones on the dynamics of developmental processes include not only improved survival, but also altered trajectories of developmental patterns, with disproportionately large impacts throughout later developmental stages and substantial evolutionary consequences (Bernardo, 1996). Beneficial effects of maternal investment on development and consequently on larval survival (Brown et al., 1988a,b; Chambers and Legget, 1996) can have a considerable impact on year-class size through the attainment of reproductive capability (Leggett and Deblois, 1994). Early thyroid-driven events among teleosts led one group of authors to conclude that “even a slight change in activity of the thyroid axis during ontogeny can be the cause of wide morphological variability... and may underlie explosive diversification of the species” (Shikil et al., 2010, p. 369).

It is not known whether patterns of maternal signaling may influence behavior in a lasting manner, comparable to the apparent effects on physiology and morphology, although some adaptive behavioral effects of maternal cortisol signals to young fishes and other vertebrates have been described. Female sticklebacks (Gasterosteus aculeatus) responded to pre-spawning exposure to simulated threats of predation by producing enlarged eggs with elevated cortisol content (Geissing et al., 2010). Enlarged, cortisol-enriched eggs produced fry that showed potentially adaptive physiological and behavioral effects, interpreted by these authors as a maternal signal imparting improved fitness to fry by pre-adapting offspring to a sub-optimal environment (Geissing et al., 2010). Exposure of gravid damselfish, Pomacentrus amboinensis, to competitors or predators caused elevations in plasma cortisol, with potentially adaptive influences on larval size and quality (McCormick, 1998). Maternal stress in an oviparous lizard, Lactea vivipara, increases the corticosteroid content of eggs. Experimental increases of egg content of corticosterone in this species result in altered, apparently adaptive patterns of predator-avoidance behavior in the resultant offspring (Uller and Olsson, 2006).

Insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) are mitogenic factors, which play important roles in growth and development in vertebrates (Jones and Clemmons, 1995).

### Table 2  Effects of combined thyroid hormone (T₃ or T₄)/cortisol (F) treatment on larval fish development

<table>
<thead>
<tr>
<th>Hormones, dose species</th>
<th>Actions reported</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese flounder</td>
<td>T₃ or T₄ 0.001–1.0 + F 0.1 ppm</td>
<td>De Jesus et al., 1990</td>
</tr>
<tr>
<td>(Paralichthys olivaceus)</td>
<td>Early fin ray resorption</td>
<td></td>
</tr>
<tr>
<td>Pacific threadfin, or moi</td>
<td>T₃ 2.6 ppm + F 0.1 ppm</td>
<td>Brown and Kim, 1995</td>
</tr>
<tr>
<td>(Polydactylus sexfilis)</td>
<td>Early larval gut function</td>
<td></td>
</tr>
<tr>
<td>Pacific threadfin, or moi</td>
<td>T₃ 2.6 ppm + F 0.1 ppm</td>
<td>Kim and Brown, 1997</td>
</tr>
<tr>
<td>(Polydactylus sexfilis)</td>
<td>Earlier gut formation, ↑ % survival, ↓ deformities</td>
<td>Kim and Brown, 2000</td>
</tr>
<tr>
<td>Pacific threadfin, or moi</td>
<td>T₃ 2.6 ppm + F 0.1 ppm</td>
<td></td>
</tr>
<tr>
<td>(Polydactylus sexfilis)</td>
<td>Enhanced &amp; earlier digestive enzyme expression</td>
<td></td>
</tr>
<tr>
<td>Indian carp</td>
<td>T₄ 2.6 ppm + F 0.1 ppm</td>
<td>Nayak et al., 2000a</td>
</tr>
<tr>
<td>(Catla catla Ham.)</td>
<td>Earlier yolk absorption, gut &amp; swimbladder formation</td>
<td></td>
</tr>
<tr>
<td>Indian catfish</td>
<td>T₄ 2.6 ppm + F 0.1 ppm</td>
<td>Nayak et al., 2000b</td>
</tr>
<tr>
<td>(Heteropneustes fossilis)</td>
<td>Early larval gut absorption &amp; gut formation, ↑ % survival</td>
<td>Sri Pudju et al., 2002</td>
</tr>
<tr>
<td>Sand goby</td>
<td>T₃ 2 ppm + F 1 ppm</td>
<td></td>
</tr>
<tr>
<td>(Oxyeleotris marmorata Blkr.)</td>
<td>Improved swimbladder formation, ↑ % survival</td>
<td></td>
</tr>
<tr>
<td>Indian catfish</td>
<td>T₄ 0.05 ppm + F 0.3 ppm</td>
<td>Nayak et al., 2003</td>
</tr>
<tr>
<td>(Heteropneustes fossilis)</td>
<td>↑ % hatch, early yolk abs., ↓ deformities, ↑ weight, ↑ % survival</td>
<td></td>
</tr>
</tbody>
</table>
including teleosts (Wood et al., 2005). The presence of IGFs and their receptors has been demonstrated during early development of fish (Greene and Chen, 1997, 1999). Although lacking direct evidence at present, there exists a possibility that some cortisol and thyroid hormone mediated developmental actions may be transduced by IGF signals, at least partly, to regulate embryogenesis in teleosts.

Both IGF-1 and IGF-2 are expressed in the unfertilized eggs and embryos of gilthead seabream (Funkenstein et al., 1996) and rainbow trout (Greene and Chen, 1997), whereas only IGF-2 mRNA was detected in rabbitfish unfertilized eggs and embryos during the cleavage stage, and IGF-1 mRNA was first detected in the larvae soon after hatching (Ayson et al., 2002). These results suggest that the roles of IGF-2 in fish embryogenesis may be crucial and conserved as in mammals (Dechiara et al., 1990). Cortisol suppression of IGF-1 expression seems to be conserved in teleosts, but in hepatocytes of coho salmon (Pierce et al., 2010) and tilapia (Pierce et al., 2011) cortisol was shown to up-regulate IGF-2 mRNA levels, either directly or via enhanced growth hormone (GH) response. In rainbow trout embryos reared from cortisol-enriched oocytes, however, IGF-1 expression was significantly enhanced from the zygote to 21-days post-fertilization (dpf) embryos and IGF-2 was enhanced in the 21-dpf embryos (Li et al., 2010). These results suggest that the regulation of IGF expression by cortisol may be different in fish embryos from that in adults, and cortisol may act via IGF-1 and/or IGF-2 signals to regulate embryogenesis.

Insulin-like growth factors (IGF) expression is promoted by thyroid hormones in fish. Hepatic IGF-1 mRNA expression was stimulated either by T3 in tilapia (Schmid et al., 2003) and zebrafish (Wang and Zhang, 2011) or by T4 in sea bream (Leung et al., 2008). Tissue remodeling during metamorphosis in the Atlantic halibut (Hippoglossus hippoglossus) is mediated by endocrine changes involving both THs and IGF-1 (Hildahl et al., 2008). However, the basal and GH-stimulated IGF-2 expression was suppressed by T3 in salmon hepatocytes (Pierce et al., 2010). The above studies in fish adults suggested that hepatic IGF-1 and IGF-2 are inversely and differentially regulated by cortisol and THs. However, in fish embryos, the regulation of IGF-1 and IGF-2 by cortisol and particularly by THs is poorly understood, and worthy of further study.

RELATEDNESS OF THYROID AND REPRODUCTIVE ENDOCRINE SYSTEMS

The connection between thyroid signaling and embry competence does not appear to be coincidental. It has been suggested that the regulation of reproduction and thyroid function are evolutionarily intertwined (reviewed by Cyr and Eales, 1996). Several lines of evidence point to the interrelatedness of these two endocrine systems, starting with chemical and functional similarities in adenohypophysial compounds in fish. The alpha sub-units of hypophysial glycoproteins thyrotropin (TSH) and leuteotropic hormone (LH) are identical, with considerable overlap and systemic interaction in their actions (see Norris, 2007); both come from histologically indistinguishable pituitary basophil cells and function by way of cAMP-dependent second messenger systems. Differing functional activities among this related family of glycoprotein hormones are conferred by distinct beta subunits, encoded by different genes than the shared alpha subunits (Szukulinski et al., 2002). Prevailing opinions are that that these regulatory hormones arose from a series of gene duplications, with considerable conservation of structural and biochemical characteristics (Wallis, 2010). The elaboration of hypophysial glycoproteins (TSH, LH, FSH) appears to have begun with an ancestral hormone in the cyclostomes, probably with gonadotropic function (Kawachi and Sower, 2006).

A consistent functional link has persisted between cyclic changes in thyroid and gonadal activities in modern fishes. Although fishes exhibit innumerable reproductive strategies, a pattern of increased thyroid hormone activity during early to peak oogenesis is consistently seen among diverse female fishes (Cyr and Eales, 1996).

Transfer of maternally derived corticoid hormones to fish eggs reportedly has a mix of beneficial and harmful effects on embryogenesis. It is possible that adaptive acceleration of organogenesis contingent on transient endocrine effects including thyroid/corticoid synergism may give way to damaging effects of relatively higher or more prolonged, dose-dependent maternal signals during longer-term stress. Prolonged elevations of cortisol in rainbow trout (Oncorhynchus mykiss) under chronic stress resulted in compromised larval fitness (Campbell et al., 1992, 1994).

MATERNAL HORMONES IN ELASMOBRANCH REPRODUCTION, AND THE EVOLUTION OF VIVIPARITY

The reproductive strategies of elasmobranchs (sharks, skates, and rays) are subject to tremendous diversity, with oviparity being the most ancestral mode and the one found in all skates and a minority of sharks. Viviparity is the predominant mode; live birth is found in 55% of chondrichthyan fishes and in 70% of shark species (245–270 species). There are 270 viviparous ray species, which include stingrays, guitarfish, electric rays, and the sawfish. In contrast, only 2–3% of bony fish are viviparous. Viviparity has evolved from oviparity on as many as 18 separate occasions (Wourms and Lombardi, 1992).

The evolution of viviparity in elasmobranchs has resulted in lecithotrophic and matrotrrophic modes. Yolk sac viviparity involves retention of eggs inside the female until fully developed with no maternal chemical contribution beyond yolk. This is the most widespread mode in elasmobranchs and confers adaptive advantages over the vulnerabilities inherent in egg deposition. There are matrotrrophic modes including histotrophy, in which yolk stores are supplemented with maternal
proteins and lipid-rich histotroph secreted directly from the uterus, and oophagy in which embryos continually ingest unfertilized eggs. The most highly derived form of matrotrophic viviparity is the placental association in which embryonic yolksacs eventually become depleted and differentiate into the fetal portion of placentas, providing direct humoral exchange with the maternal circulation (Wourms and Demske, 1993). Prolonged exposure to yolk followed by direct humoral exchange via placenta enables plasticity in maternal contributions of development-promoting hormones (Hamlett, 1993).

Progesterone has been identified as a potential driver as it inhibits myometrial contractions preventing egg laying while also inhibiting vitellogenin synthesis necessary for yolk production driving placental evolution (Callard et al., 1992). Thyroid hormone roles in elasmobranch reproduction and early development have been studied in a limited number of sharks and rays. Thyroxin levels have been correlated with reproductive status in the Atlantic stingray, Dasyatis sabina and the placental viviparous bonnethead shark, Sphyrna tiburo (Volkoff et al., 1999; Gash, 2000). Levels of T₄ and T₃ follow a pattern of variation in the maternal serum and yolk of S. tiburo during pre-ovulatory, ovulatory, and early gestation stages, with highest TH levels in serum and yolk occurring concurrently during the gravid state nearing parturition. Embryonic S. tiburo show indications of endogenous TH synthesis later, as yolk stores approach depletion (McComb et al., 2005). Significant increases were seen in T₃/T₄ ratios later in pregnancy. It is possible that early endogenous TH synthesis could be supplemented by absorption of maternal TH diffusing into the yolk as a later component of maternal endocrine provisioning. Levels of corticosterone were determined in the freshwater stingray, Potamotrygon cf. histrix (Brinn et al., 2012) and in a separate study levels of T₄, T₃, and corticosterone were determined in the serum and yolk of several freshwater ray species. A nearly nine-fold increase was found in the glucocorticoid concentration of uterine fluids that bathed the developing embryos as compared to yolk and maternal serum levels, suggesting that these maternal fluids provide a means of continuous and modulated transfer of development promoting hormones. These fluids also contained T₃ and T₄ presumably of maternal origin (M. McComb-Kobza, Teens4Oceans, unpublished data).

Continuous access to maternal endocrine signals imparts profound developmental and survival advantages. This, together with prolonged nourishment and physical protection appears to be a strong suite of drivers behind the repeated evolution of viviparity as a reproductive strategy.

AQUACULTURE APPLICATIONS OF THYROID AND CORTICOID HORMONES

Increasing recognition of the importance of hormones for growth and differentiation of embryos was indentified as a central theme of “the new endocrinology” (Bern, 1990, 1992). Overlapping roles of hormones and growth factors are critically important in early embryogenesis, especially with the knowledge that maternally derived regulatory compounds are transferred to and stored in yolk and active from the earliest stages of development (de Pablo and Roth, 1990). Maternal compounds are supplemented by de novo embryonic mRNAs beginning in extremely early developmental stages, possibly directing tissue remodeling and differentiation processes as paracrine/autocrine factors (Sanders and Harvey, 2008).

Aquaculture management is an effort to optimize aquatic animal development, growth, survival, and reproduction under artificial conditions. Aquaculture as a production system has inherent biological advantages and disadvantages in comparison with wild fisheries; hatchery production often imposes artificially high densities and simplified nutrition parameters to which some species respond more favorably than others. The agrarian approach to fish relies on enhanced survival and accelerated growth, as compared with the survival of an orders-of-magnitude smaller percentage of wild fishes to reproductive age. Nutrition can be controlled and predation and environmental contamination can be regulated or eliminated in aquaculture settings. The reproductive strategy of the culture subject is a major source of variation in the selection and development of culture methods. Freshwater fishes generally produce larger eggs with a higher probability of hatching and survival (by about tenfold; Rao, 2003). Among marine species that produce large numbers of tiny pelagic eggs, with smaller yolk reserves and less maternal investment per individual egg (often described as r-selected) the larvae are consequently small and fragile and rearing them in the hatchery is technically challenging. Most of the species exhibiting increased survival in response to exogenous hormones fit in this category and could be characterized as pelagic spawners that produce large numbers of small, delicate larvae that would normally be subject to high rates of mortality. It is noteworthy that early feeding is among the most problematic of larval rearing issues among these fishes, and that the GI tract, fins, and swimbladder are repeatedly observed to be among targets of exogenous TH (see Tables 1 and 2). Demersal spawning involves a smaller number of larger eggs, and with a higher probability of survival—a reproductive strategy that has been viewed as a preliminary form of parental care (Duarte and Alcaraz, 1989).

The substitution of formulated feeds for natural diets can have endocrine consequences; e.g., Atlantic halibut (Hippoglossus hippoglossus) larvae fed Artemia have a hypothyroid status compared with larvae that eat wild zooplankton; Artemia has relatively poorer iodine content, resulting in elevated frequencies of incomplete and problematic halibut metamorphoses (Solbakken et al., 2002). Artemia enriched with iodine impart thyroid-mediated advantages to developing fish (Hawkyard et al., 2011). Further complicating the endocrine implications of aquaculture feeds is the confirmation that Artemia synthesizes and store both T₃ and T₄ (see Hawkyard et al., 2011). Fishmeal-based aquaculture diets also contain T₄,
among other hormones (Feist and Schreck, 1990). Dietary ingestion of T4—including the consumption of Artemia—can induce negative feedback, leading to reduced TSH secretion, resulting in both reduced thyroid hormone production and decreased deiodination of T4 into T3 (Conti et al., 1978).

The use of thyroid and glucocorticoid hormones to promote survival in larval fishes for food production is subject to stringent and somewhat complicated regulation in the USA; both Food and Drug Administration (FDA) approval and Environmental Protection Agency (EPA) compliance are required (Johnson and Bosworth, 2012). Hormone use in food production arouses popular concerns, in part because hormone applications are popularly associated with patterns of misuse and with anabolic and secondary sex disfigurement. Public perceptions of hormone use in the food industry are often poorly distinguished from heavy and at times abusive antibiotic and steroid use in the meat industries, or with the production of genetically modified organisms. Because consumer-oriented publications strongly encourage the public to avoid meat products of animals exposed to antibiotics, negative associations with hormone use have accelerated (e.g., see Bohne and Halloran, 2012). The Food and Agriculture Organization of the United Nations concludes that the general public’s acceptance of aquaculture products is affected by perceptions of chemical hazards that can be far out of proportion to real health or environmental risks (FAO, 1997).

In addition to a resistance by popular opinion, chemical use by aquaculture farms is also under stringent governmental regulation because, relative to meat industries, it is a relatively new practice and the potential for contamination of surrounding bodies of water is more significant (FAO, 1997). Producers must closely monitor hormone levels in fish and the surrounding environment in order comply with United States regulations, but this can be particularly challenging for imported aquaculture products, or for domestic producers that lack the means to do the requisite testing (FAO, 1997).

A typical approach of fish culturists wishing to use endocrine methods to reduce mortality in larviculture in the USA is to seek a limited-term exception, an Investigational New Animal Drug or INAD exemption. Direct applications of hormones in food production are prohibited, even though the chance of residual thyroid hormones from larval treatments is negligible. Thyroid hormones deposited in egg yolk disappear as yolk is consumed, and before endogenous production begins (e.g., see Greenblatt et al., 1989). Exogenously applied THs are metabolized shortly after hatching (e.g., see Tachihara et al., 1997). Material Safety Data Sheet (MSDS) hazard ratings are lower for Thyroxin, rated at zero for each of the four hazard categories, than those published for Vitamin C, for which two numerically-ranked categories of hazards are listed.

Some aquaculture-related efforts have been made to enhance maternal hormone synthesis and transfer of development-promoting hormones by environmental and dietary manipulations to promote endogenous production, with encouraging results (see Witt, 2008; Witt et al., 2009). A more direct approach is the maternal injection of exogenous hormones; a cost-effective method that is favored where it can be used because a single, simple procedure can improve the egg quality of an entire cohort (Tagawa, 1996). For practical reasons, many advances involving applications of TH in larval fish culture have occurred in countries other than the United States. In Japan, maternal T3 injections increased the survival of larval amberjack, Seriola lalandi, by four-fold, with production-scale application in an aquaculture center (Tachihara et al., 1997). Maternal T3 injections of the viviparous Korean rockfish Sebastes schlegeli, improved growth and survival sufficiently to provide “a distinct advantage on the enhancement of seedling productivity…” (Kang and Chang, 2004, p. 652). The larval development of numerous cultured fish species has been accelerated or improved by thyroid hormone supplementation (reviewed by Tagawa, 1996; see Table 1), and many of the beneficial effects can be seen in desirable but difficult-to-culture marine species that produce small eggs and altricial larvae.

The larvae of several freshwater fishes of South America with strong aquaculture potential have also responded favorably to experimental thyroid supplementation, including the pintado, Pseudoplatystoma coruscans and the Fortunata Salminimus maxillosus (Landines Parra, 2003), the matrixa, Brycon amazonicus (Urbinati et al., 2008; Leonardo et al., 2013), and the piracanjuba, Brycon orbignyanus (Landines et al., 2010).

The matrixa, Brycon amazonicus, and the piracanjuba, Brycon orbignyanus, are omnivorous fishes that present low survival rates and intense cannibalism during the earlier stages of development in captivity (Senhorini et al., 1998). Supplementation of T3 was tested in both species either by maternal injection or egg immersion. A study comparing the larval performance of matrixa after maternal T3 injection or egg immersion of T3 showed that both routes of T3 administration stimulated the early growth of larvae of this species, but earlier effects were documented when broodstock females were injected. Larvae from T3-injected females had increased length after hatching and higher weight from 24 h after hatching, while the length of larvae produced from T3-immersed eggs exceeded the control values from 6 days and weight from 8 days (Urbinati et al., 2008). More recently, Leonardo et al. (2013) demonstrated that newly-hatched larvae were significantly heavier when eggs were immersed in T3 and this effect persisted until 12 days post-hatch when larvae were transferred to earthen tanks in the routine of the farming of this fish. Larval survival increased following the hormone treatments, from 26.5% in the control group to approximately 40% in groups treated with T3. Cannibalism was not affected by the treatments. The higher weight of T3-supplemented larvae at hatching may have contributed to the improvement in the survival rate. Previously, Urbinati et al. (2003) showed that maternally administered T3 significantly improved the survival of matrixa, probably as a result of reduced cannibalism. Treated larvae grew more than control specimens up to 6 days post-hatch and had similar weight at 9 days but at 12–18 days...
the control group showed higher growth compared to the treated group. Heterogeneous growth in control larvae may have resulted in an increase in the rate of cannibalism, which accelerates growth and could account for the lower survival rate (21.8%) compared with the hormone-treated group (61.6%).

Triiodothyronine (T3) has also been demonstrated to promote organogenesis in developing matrinx as seen in accelerated muscle hypertrophy in larvae developing from eggs exposed to the hormone. Those larvae presented larger transverse section area of the muscle fibers than untreated control larvae at 54 hr after hatching (Vasques, 2003). Moreover, T3 also promoted the development of components of the larval digestive system. Larvae from T3-treated groups showed the digestive lumen partially at 18 hr after hatching and, at 30 hr, it was observed that the mucus layers of the stomachs of treated larvae were more highly organized than those of control larvae. At 54 hr, in treated larvae the number of villosities in the mucus layer was higher than those of the control larvae, and they also presented vacuolus cells among those forming the stomach mucus layer. Only liver tissue of larvae from the T3 treatments had cells organized in parallel strings.

The supplementation of the matrinx larval diet with tyrosine, precursor for the biosynthesis of THs, also showed positive results in the early larval development of this species (Hoshiba, 2007). Dietary tyrosine enrichment (5.39 g/100 g diet—2.4 times the amount of the commercial diet) promoted higher growth in terms of both weight and length, reduced cannibalism and enhanced larval survival.

In the piracanjuba, which has recently been listed as an endangered species (Fundação Biodiversitas, 2003), T3 increased the survival to 15 days (Landines et al., 2010), without affecting growth rate. In another study (Dr. Leonardo and E. Urbinati, Universidade Estadual Paulista, personal communication), T3 immersion quadrupled the hatch rate compared with controls (to 858,890 from 204,020 respectively) and hormone-supplementation also produced significant increases in larval length and weight.

Thirty six hours after hatching larvae were transferred to aquaria and monitored up to the 9th day of rearing. The weight and length difference remained along the rearing period and survival was similar among larvae from all treatments. Two carnivorous fish species were also investigated in the same laboratory, the dourado, <i>Salminus maxillosus</i>, and the pintado, <i>Pseudoplatystoma coruscans</i> (Landines Parra, 2003). In both species, fertilized eggs immersed in T3 solutions did not show significant changes in larval growth, but had dramatic improvements in rates of survival (21% for dourado and 23% for pintado).

**CONCLUDING REMARKS**

Maternal TH supplementation alone or in combination with cortisol can confer distinct developmental advantages to larvae, and has the potential to dramatically enhance fingerling survival and hatchery production rates (Tables 1 and 2). This appears to reflect an evolutionary mechanism in which maternally derived endocrine signals stimulate embryogenesis and thereby convey survival value through the critical time of first-feeding, and it has potential applicability as a means of improving production in the mass culture of fishes with large numbers of small, delicate larvae. Perceived and perhaps legitimate hazards of thyroid and glucocorticoid hormone applications in aquaculture are the subject of legal and public opinion based concerns.

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