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# Temperature and ration effects on components of the IGF system and growth performance of rainbow trout (*Oncorhynchus mykiss*) during the transition from late stage embryos to early stage juveniles

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**Abstract**

The study investigated the effects of incubation temperature, and the size of ration fed to the transitional embryo/juvenile stage of rainbow trout (*Oncorhynchus mykiss*) on growth, liver and gastrointestinal (GI) tract IGF-1 content, and the expression of insulin-like growth factor-related genes (IGF-1, IGF-2, IGF-RIa, and IGF-RIb) by the liver and GI tract. Embryos were reared from zygote to "swim-up" at either 8.5 °C ($E_{8.5}$) or 6.0 °C ($E_{6.0}$); at "swim-up" (51-days post-fertilization [dpf] and 72-dpf for the $E_{8.5}$ and $E_{6.0}$ groups, respectively), the embryos were transferred to grow-up tanks supplied with water at 8.5 °C. Late stage embryos (LSEs) at the same developmental stage from the two temperature treatment groups (64-dpf and 86-dpf for the $E_{8.5}$ and $E_{6.0}$ groups, respectively) were fed with salmonid starter diet at levels of 5.0%, 2.0%, and 0.5% of live body mass per day. Embryos were sampled just prior to first feeding (PFEs), and before complete absorption of the yolk [late stage embryos (LSEs)], and early stage juveniles (ESJs) were sampled after yolk sac absorption when they were fully reliant on exogenous sources of food. The early incubation temperature and ration levels had significant affects on mortality (with lower mortalities in the $E_{6.0}$ group) and growth performance of the fish; dry body mass values for fish fed the 5.0% ration were significantly lower in the $E_{6.0}$ group of LSEs and ESJs compared with the respective treatment in the $E_{8.5}$ group; a similar pattern was seen for total body length, although this was only significant for the LSEs. Whole embryo IGF-1 content was significantly lower in the $E_{6.0}$ group compared with the $E_{8.5}$ group of PFEs, and hepatic IGF-1 content was significantly lower in the $E_{6.0}$ group fed the maintenance ration (0.5%) compared with the $E_{8.5}$ fed a similar ration; restricted ration significantly elevated hepatic IGF-1 content in the LSE stage for both temperature treatment groups. GI tract IGF-1 levels were considerably lower than in liver tissue, and there were no differences among treatment groups. Ration size-related differences were found for the expression of genes encoding for hepatic IGF-1, IGF-2, and IGF-RIb, and GI tract IGF-1, and IGF-2. Rearing temperature-related differences were also found for genes encoding for GI tract IGF-1, IGF-RIa, and IGF-RIb. The results of the study showed that the early rearing temperature of the embryos affected subsequent growth, and hepatic and GI tract gene expression by the LSEs and ESJs. As was the case for tissue IGF-1 content, with some exceptions, a restricted ration significantly elevated the expression of the targeted genes indicative of an important metabolic-regulating role for the IGF system during this transitional developmental phase. In addition, the higher abundance of IGF-2 mRNA compared with IGF-1 mRNA, and the higher abundance of IGF-RIa, relative to IGF-RIb, suggests that these two genes may also play a regulatory role during this transitional developmental phase.


*Keywords:* Rainbow trout; Incubation temperature; Ration levels; Transitional feeding period; Late stage embryos (LSEs); Early stage juveniles (ESJs); IGF-1; IGF-2; IGF-RIa; IGF-RIb

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## 1. Introduction

All aspects of development and reproductive behaviour are affected by environmental temperature, and its related variables, such as day length, and food availability. In particular, the growth and development of ectothermic animals is very temperature sensitive; at low temperatures, metabolic rates, feeding rates, and growth rates are suppressed; elevated temperatures tend to cause an increase in growth up to an optimum point above which thermal stress occurs (Baum et al., 2005). However, the factors that determine the developmental and growth rates of fish, other than the quantity and quality of diets, are currently not well understood, particularly those factors involved in energy partitioning and energy conservation events. Some components of the endocrine system are known to regulate, directly or indirectly, different aspects of growth in fish; thyroid hormones, glucocorticoids, growth hormone (GH), insulin-like growth factor-1 (IGF-1), pancreatic hormones, and gastrointestinal tract hormones all appear to play essential roles (Mommsen et al., 1999; Mommsen, 2001; Power et al., 2001). Specifically, the hormones of the somatotropic (ST) axis, GH and IGF-1, and the hypothalamic hormones that regulate GH secretion, play key integrative roles in regulating metabolic events during episodes of food deprivation or dietary restriction.

GH, acting through its receptors, stimulates hepatocytes to synthesize and release IGF-1 (Rosenfeld and Robert, 1999; Biga et al., 2004). In turn, plasma IGF-1 exerts chronic and acute negative feedback control over the secretion of GH from the pituitary gland (Blaise et al., 1995; Fruchtman et al., 2000; Cameron et al., 2005, 2007). In some fish species, food deprivation is accompanied by a lowering of plasma IGF-1 concentration (Pierce et al., 2005; Fox et al., 2006; Gabillard et al., 2006; Cameron et al., 2007), possibly because of the down-regulation of hepatic GH receptors (GH-Rs) (Fukada et al., 2004; Pedroso et al., 2006), a subsequent reduction in the transcription of IGF-1 mRNA (Pedroso et al., 2006), and reduced hepatic IGF-1 synthesis. Because of the negative feedback control of GH secretion by IGF-1, the decline in plasma IGF-1 concentrations sometimes results in an increase in GH gene expression (Small et al., 2002; Pedroso et al., 2006; Ayson et al., 2007), which may explain the increase in plasma GH concentration sometimes found in food-deprived fish (Farbridge and Leatherland, 1992; Duan, 1998; Fox et al., 2006; Gabillard et al., 2006). This pattern of elevated plasma GH concentrations and lowered plasma IGF-1 levels in food-deprived fish is not always evident, however (Holloway et al., 1994; Pottinger et al., 2003; Uchida et al., 2003; Frantzen et al., 2004; Small, 2005; Cameron et al., 2007).

Although most studies have focused on IGF-1, IGF-2 may also be involved in growth and energy partitioning in fish (Duan, 1998; Gabillard et al., 2006; Ayson et al., 2007; Li et al., 2007). Gabillard et al. (2006) found hepatic and muscle IGF-2 mRNA to be more abundant than IGF-1 mRNA in juvenile rainbow trout during food-deprivation and during the re-feeding period. In addition, Peterson et al. (2004) found IGF-2 mRNA to be higher in the faster growing strains of channel catfish (*Ictalurus punctatus*), and Ayson et al. (2007) found changes in IGF-2 gene expression in food-deprived and re-fed rabbitfish (*Siganus guttatus*) that suggested a role for the hormone in growth regulation. Although both forms of IGF appear to respond to changes in the animal's nutritional status and ambient temperature, the biological relevance of the IGF responses is currently poorly understood; however, the cumulative evidence from several studies does suggest a link between changes in IGF homeostasis, physiological responses to food-deprivation, and the responses to changes in ambient temperature in fish (Gabillard et al., 2003, 2006; Peterson et al., 2004; Davis and Peterson, 2006; Fox et al., 2006; Cameron et al., 2007).

Although most studies to date have focused on the roles of the GH and IGF produced by the pituitary gland and liver, respectively, it is important to remember that the ST axis is not the only source of GH and IGF. GH and IGF-1 gene expression (and probably also hormone synthesis) occurs locally in tissues other than the pituitary gland (GH) and liver (IGF-1); these locally produced hormones probably play as yet unknown autocrine or paracrine regulatory roles; they appear to be particularly important in aspects of early development of fish (Greene and Chen, 1999; Gabillard et al., 2003, 2006; Li et al., 2006, 2007; Raine et al., 2007).

In teleostean fish, the late stage embryos (LSEs) undergo behavioural and physiological changes that enable the early stage juvenile (ESJ) fish to commence feeding, and ultimately rely entirely on exogenous food. This stage of development is associated with marked physiological changes in the gastrointestinal (GI) tract and liver that facilitate the digestion of exogenous food, the absorption of nutrients, and the processing of the absorbed nutrients; the transitional phase is characterized by an increase in mortality rates in salmonid species (Kamler, 1992), and delays in first feeding are commonly associated with high mortalities and reduced feeding rates (and hence reduced growth) when food was made available (Peña and Dumas, 2005). Relatively little is known about the physiological events that occur during this key adaptive transitional period. Changes in GI tract thyroid hormone (TH) content and TH receptor (TR), GH and GH-R gene expression in response to delayed feeding, and restricted feeding have been found in rainbow trout at this stage of development (Raine et al., 2005, 2007); however, the specific roles of TH and GH during this developmental phase have yet to be determined. The present study was undertaken to investigate whether the nutritional state of the transitional embryo-to-juvenile animal affects the function of the IGF system, specifically tissue IGF-1 levels, and the expression of genes encoding for IGF, and their receptors. Because of the size of the pre-feeding "swim-up" embryos (PFEs), gene expression in that group was measured using whole