Chronic Hypoxia Exposure of Trout Embryos Alters Swimming Performance and Cardiac Gene Expression in Larvae

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Accepted 6/20/2013; Electronically Published 8/14/2013

ABSTRACT

Hypoxia exposure during embryonic development of rainbow trout causes developmental delay and bradycardia and alters the ontogeny of cardiac regulatory control mechanisms. The purpose of this study was to characterize how hypoxia exposure from the day of fertilization until stage 34 (57 d postfertilization) affects the aerobic fitness and growth of the hatched fish at multiple stages. In addition, we characterized the expression of gene transcripts for seven troponin I (TnI) isoforms to examine the effect of hypoxia treatment on cardiac muscle development. Results demonstrate that the critical swimming speed of the hypoxia-exposed fish was significantly less than that of the control group at stage 35 and the fry stage. Growth was reduced in the hypoxia-treated fish between stages 35 and 37, as was the relative lipid content at stage 37. Finally, six TnI isoforms were found in all hearts. One of these isoforms, *RTcTnI*, decreased in abundance between stage 35 and the fry stage, but hypoxia-exposed fish had higher levels than did controls at the fry stage. The abundance of $AScTnI_2$ was significantly lower in hypoxia-exposed fry fish than in controls. These results indicate that chronic hypoxia exposure during embryonic development has long-term consequences on aerobic fitness, growth, and cardiac gene expression following hatch.

Introduction

Embryonic development of salmonid fish occurs slowly in cold temperate waters. While the natural conditions in salmonid redds are typically oxygen rich, dissolved oxygen (DO) levels as low as 19% have been reported (Youngson et al. 2004). With eutrophication becoming increasingly common, environmental hypoxia in both marine and freshwater ecosystems may become more of a standard stressor for natural fish populations (Boulekbache 1981; Kennish 2002; Shang and Wu 2004; Diaz and Rosenberg 2008). Characterizing how embryonic exposure to such environmental conditions influences the fitness of the hatched animals is critical to predicting the long-term survival of natural populations.

Laboratory studies have demonstrated that hypoxia exposure of teleost embryos has multiple consequences on the physiology of the developing fish. For example, we have demonstrated that chronic exposure of rainbow trout (*Oncorhynchus mykiss*) embryos to 30% DO causes a reduction in metabolic rate, bradycardia, a delay in the onset of cardiac cholinergic control, an increase in the response of the heart to adrenergic stimulation, and developmental delay (Miller et al. 2008, 2011). Studies of other teleost species have also found that hypoxia exposure during embryogenesis causes developmental delay (Shumway et al. 1964; Spicer and Burggren 2003; Bagatto 2005; Kajimura et al. 2005). In addition, Schewerte et al. (2003) have demonstrated that hypoxia exposure of zebrafish (*Danio rerio*) during embryonic development causes a 50% reduction in the vascularization of the gut. Together these studies indicate that hypoxia exposure impacts morphology, the maturation of critical physiological control systems, and, potentially, the ability of the animal to obtain nutrients from the diet. While it is not known how these ontogenic effects of embryonic hypoxia exposure affect the future fitness of the fish, a recent study in zebrafish supports lasting physiological consequences resulting from an altered environment during embryogenesis (Scott and Johnston 2012). In this study, zebrafish embryos that were reared at different temperatures until hatch showed differences in adult swimming performance, skeletal muscle composition, and gene expression patterns (Scott and Johnston 2012).

The purpose of this study was to determine how hypoxia exposure during embryogenesis impacts posthatch growth and performance when returned to normoxic conditions. We hypothesized that hypoxia exposure during ontogeny would reduce the aerobic capacity and growth of the hatched fish and also impair normal cardiac development. To test this hypothesis, we exposed rainbow trout embryos to chronic hypoxia from fertilization until the hatched fish were able to surface feed (swim-up/stage 34) and then returned them to normoxic conditions. We measured aerobic capacity with a critical swim speed (U_{crit}) test, body composition at several developmental stages, and quantified changes in cardiac gene expression of seven troponin I (TnI) isoforms as a molecular marker of cardiac development.

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Physiological and Biochemical Zoology 86(5):567–575. 2013. 1522-2152/2013/8605-3011$15.00. DOI: 10.1086/672012

Material and Methods

Experimental Animals and Hypoxia Exposure

Rainbow trout eggs were fertilized at Rainbow Springs Trout Farm (Thamesford, ON) and transported to the Hagen Aqualab at the University of Guelph (Guelph, ON) the same day. Embryos were divided into two groups and placed in separate heath trays in an environmentally controlled aquatic recirculating system unit. Photoperiod was 12L : 12D, and water temperature was maintained at 11° ± 1°C. Hypoxia exposure was carried out exactly as previously described (Miller et al. 2011). Briefly, inflow water was directed to a header tank and nitrogen bubbled in at a constant rate such that outflow water to the heath tray was maintained at a DO level of 34% ± 0.6%. DO levels in the water were routinely monitored using a Luminescent dissolved oxygen meter (model HQ40d-LDO101; Hach, Loveland, CO). The control embryo heath tray was fed water directly from the recirculating system and maintained in normoxic conditions (100% O_2 saturation). DO levels in the water were recorded daily until hypoxia exposure ceased at 57 d postfertilization (dpf) when ~50% of sac fry in the hypoxia group were noted to be swimming at the surface (stage 34; Vernier 1969). The hypoxia and control groups were then transferred to separate 2,000-L flow-through tanks receiving normoxic water and were fed daily with a standard Corey growth starter diet (Corey Feed Mills, Alma, ON). The University of Guelph's Animal Care Committee approved care and use of all experimental animals, as per the principles of the Canadian Council for Animal Care.

Animal Staging

Fish were staged using the morphological criteria of Vernier (1969), including parr mark definition, shape of caudal and pelvic fins, length of rays, and state of yolk absorption. In order to compensate for the developmental delay caused by hypoxia exposure, all experiments and sampling were performed on stage-matched rather than age-matched fish. This corresponded to a 5-d difference between normoxic and hypoxic animals. Sampling and swim trials (described below) were completed in the control group at 65, 85, and 105 dpf. These times corresponded to developmental stages 35, 37, and what we are calling the "fry stage." There is no enumerated stage in the Vernier protocol for this developmental stage. The hypoxia group was matched to the control group for the fry stage using gross external morphology including fin coloration and dorsal pigmentation marks (spotting). Sampling and swim trials for the hypoxia group were completed at the same developmental stages, but these occurred at 70, 90, and 110 dpf.

Swimming Performance

In order to compare the aerobic capacity of the normoxia-raised and hypoxia-raised fish, U_{crit} of the hatched fish was measured using a swim tunnel (Loligo Systems, Denmark) following established methods (McClelland et al. 2006). The swim tunnel was calibrated with a pitot tube and manometer across the range of velocities used, and water temperature was maintained at 12° ± 0.5°C for all experiments. Prior to the swim test, fish were held in the swim chamber for 1 h and water velocity was maintained at 0.5 body lengths per second (BL s^{-1}). Following this acclimation period, water velocity was increased by 0.5 BL s^{-1} every 5 min until the fish were unable to hold their position in the water column. At this point the water velocity was noted and the fish were removed and immediately killed by pithing. Average U_{crit} for each group was determined by the equation

$$U_{crit} = V_1 + \frac{t_i}{t_{ii}} \times V_2,$$

where V_1 is the maximum velocity attained (cm s^{-1}), V_2 is the velocity increment (cm s^{-1}), t_i is the total time elapsed at exhaustion (min), and t_{ii} is the time increment (min; Plaut 2001). The sample size for each swim treatment was 20 individuals.

Tissue Sampling and Lipid Analysis

Immediately following death, the standard length of each fish was measured using a Zeiss stereomicroscope. The hearts were then removed, rinsed in ice-cold physiological saline (94 mM NaCl, 24 mM $NaCO_3$, 5 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 0.7 mM $CaCl_2$, pH 7.6), flash-frozen, and then stored at −80°C until RNA extraction. The body of each animal, minus the heart, was dried in an oven to a constant weight at 65°C. It should be noted here that all embryos at stage 35 had an obvious external yolk sac. This was not removed for weight determination or lipid analysis. The weight of total body lipids from each group was determined using the Folch method (Folch et al. 1957). In brief, pulverized samples were weighed and then saturated with 20 parts 2 : 1 chloroform : methanol to 1 part tissue. Tissue from one fish was sealed in an envelope constructed from Whatman 100 filter paper. Each envelope was incubated in the solvent for 20 min while being agitated on an orbital shaker. Following incubation, each sample was washed with chloroform and then oven-dried overnight before reweighing. The change in weight represents the weight of the total body lipid. The relative lipid content for each animal was also calculated by dividing the weight of the extracted lipids by the total dry weight. The sample size was 20 individuals per group.

Quantitative Real-Time Polymerase Chain Reaction

The abundances of seven TnI transcript isoforms (gene, GenBank accession no.: *AScTnI*$_1$, U84394; *AScTnI*$_2$, BT057580; *ASfsTnI*, BT047312; *ASssTnI*$_2$, BT057890; *ASssTnI*$_3$, BT049066; *RTcTnI*, BT057537; *RTudTnI*, NM_001185028) in the hearts of the hatched fish in each group were quantified at stage 35 and the fry stage (n = 3–4, 5 pooled hearts/n). Total RNA was extracted from homogenized tissue using Trizol (Life Technologies, Grand Island, NY) according to the manufacturer's instructions and quantified using a Nanodrop 8000

(ThermoFisher Scientific, Ottawa, ON). One microgram of total RNA was treated with DNase I (Life Technologies) and used to synthesize cDNA with a high-capacity cDNA synthesis kit (Life Technologies) following the manufacturer's instructions. Duplicate cDNA reactions in which the MultiScribe reverse transcriptase enzyme was omitted were included for 10% of total samples, chosen randomly, to verify the efficacy of the DNase treatment. Transcript abundances were measured in duplicate reactions on a StepOne Plus (Life Technologies) using default cycling conditions and a dissociation cycle. Each 15-μL reaction contained 1 × Power SYBR Green Master Mix (Life Technologies), 200 nM each gene-specific primer (Alderman et al. 2012), and 1 : 15 vol : vol cDNA. All reactions generated a single-peaked dissociation curve at the predicted amplicon melting temperature. The mRNA abundance of each isoform was quantified by fitting the threshold cycle to the antilog of standard curves prepared from serially diluted cDNA. Isoform transcript abundance was normalized to the mRNA abundance of *elongation factor 1α* (*EF1α*; GenBank accession no. AF498320; forward primer GGGCAAGGGCTCTTTCAAGT; reverse primer CGCAATCAGCCTGAGAGGT), *18s ribosomal RNA* (*18s*; GenBank accession no. AF309412; forward primer GACCCAACACGGGAAACCT; reverse primer GCCGGAGTCTCGTTCGTTATC), and *acidic ribosomal phosphoprotein* (ARP; GenBank accession no. AY685220; forward primer TGAAAATCATCCAATTGCTGGA; reverse primer CGCCGACAATGAAACATTTG). All non–reverse transcribed control samples failed to amplify.

Statistics

Data were analyzed using two-way ANOVA followed by Holm-Sidak tests for multiple comparisons in order to detect age and hypoxia differences in critical swimming speed, dry mass, lipid content, standard length, and mRNA abundance. Additionally, to assess the relative abundances of the TnI isoforms in the hearts of stage 35 and fry fish, a one-way ANOVA was performed on the control data for each developmental stage. Any data set that failed to conform to the assumption of normality was log transformed prior to analysis. All data are presented as mean ± SEM ($P < 0.05$).

Results

Swim Trials

There was a significant interaction between the effects of age and hypoxia exposure on absolute swimming speed (U_{crit} [cm s^{-1}]; $P < 0.001$). Post hoc multiple comparisons analysis showed a significant increase in absolute U_{crit} of the hypoxia group with each subsequent stage measured (35, 37, and fry), while absolute U_{crit} in the control group increased only between stage 37 and the fry stage ($P < 0.01$). At stages 35 and 37, the absolute U_{crit} of the hypoxia group was significantly lower than that of the control group ($P < 0.05$), but there was no difference between treatments by the fry stage (fig. 1*A*). When the U_{crit} values were standardized to BL (s^{-1}), there was no interaction between the effects of age and hypoxia on swimming performance. Post hoc analysis of main effects revealed an overall decrease in standardized U_{crit} of the hypoxia group relative to controls and that fry fish were faster than either stage 35 fish or stage 37 fish ($P < 0.001$; fig. 1*B*).

Growth

There was a significant interaction between the effects of age and hypoxia on both length and mass ($P < 0.01$). Within the control group, the average standard length increased 14% be-

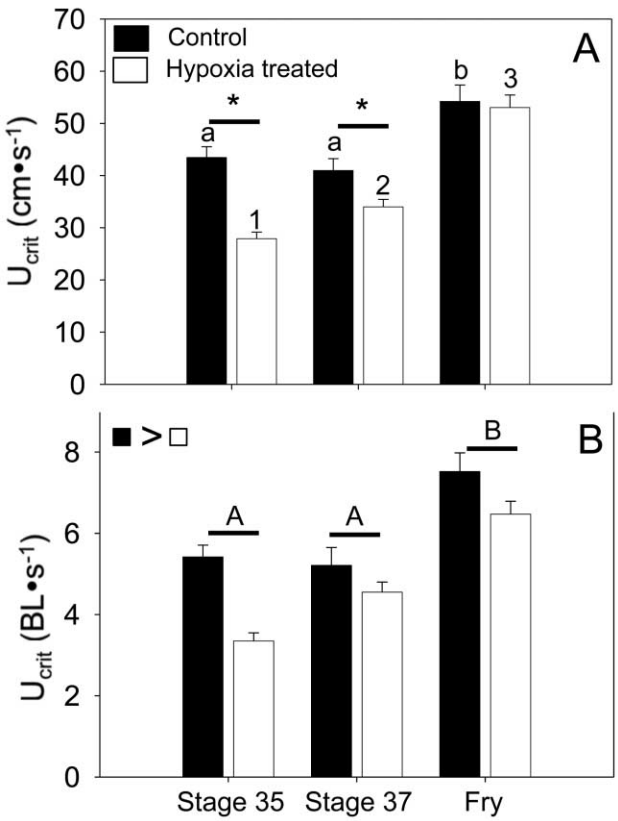

Figure 1. Chronic hypoxia exposure during embryonic development impairs the critical swimming speed (U_{crit}) of the hatched fish. U_{crit} is plotted as the absolute swimming speed (*A*; cm s^{-1}) and standardized swimming speed (*B*) in body lengths per second (BL s^{-1}). Statistical differences, calculated by two-way ANOVA, are indicated on each panel ($P < 0.05$). A significant interaction exists in the effects of age and hypoxia on absolute swimming speed ($P < 0.001$) but not standardized U_{crit}. In *A*, lowercase letters indicate differences in the control group between stages, numbers indicate differences in the hypoxia group between stages, and an asterisk indicates an effect of hypoxia exposure at the stage indicated ($P < 0.05$). In *B*, overall differences between normoxia and hypoxia groups are indicated in the upper left corner, and uppercase letters indicate an overall effect of age on U_{crit}.

tween stages 35 and 37 and 27% between stage 37 and the fry stage ($P < 0.001$; fig. 2*A*). Within the hypoxia group, the average standard length did not change between stages 35 and 37 but increased 64% between stage 37 and the fry stage ($P < 0.001$; fig. 2*A*). Comparing the hypoxia and control groups at each developmental stage revealed no difference at stage 35, but the standard length of the control group was significantly greater than that of the hypoxia group by stage 37 ($P < 0.001$). The difference in length was reversed at the fry stage, with the hypoxia group being significantly longer than the control group ($P < 0.001$; fig. 2*A*). The average dry mass of the control group increased 84% between stages 35 and 37 and 188% between stage 37 and the fry stage ($P < 0.001$; fig. 2*B*). The average dry mass of the hypoxia group did not change between stages 35 and 37 but increased 278% between stage 37 and the fry stage ($P < 0.001$; fig. 2*B*). The dry mass of the hypoxia group was significantly greater than that of the control group at the fry stage ($P < 0.001$; fig. 2*B*).

Lipid Content

There was a significant interaction between the effects of treatment and age on whole-body lipid content, whether expressed as lipid mass or percent dry mass ($P < 0.05$). Whole-body lipid mass increased with each stage in the normoxia group ($P < 0.001$), whereas there was no difference between stages 35 and 37 in the hypoxia group (fig. 2*C*). At stage 35 the whole-body lipid content of the hypoxia group was greater than that of the control group, while at stage 37 the whole-body lipid content of the hypoxia group was less than that of the control group ($P < 0.05$). There was no difference between the hypoxia group and the control group at the fry stage (fig. 2*C*). When expressed

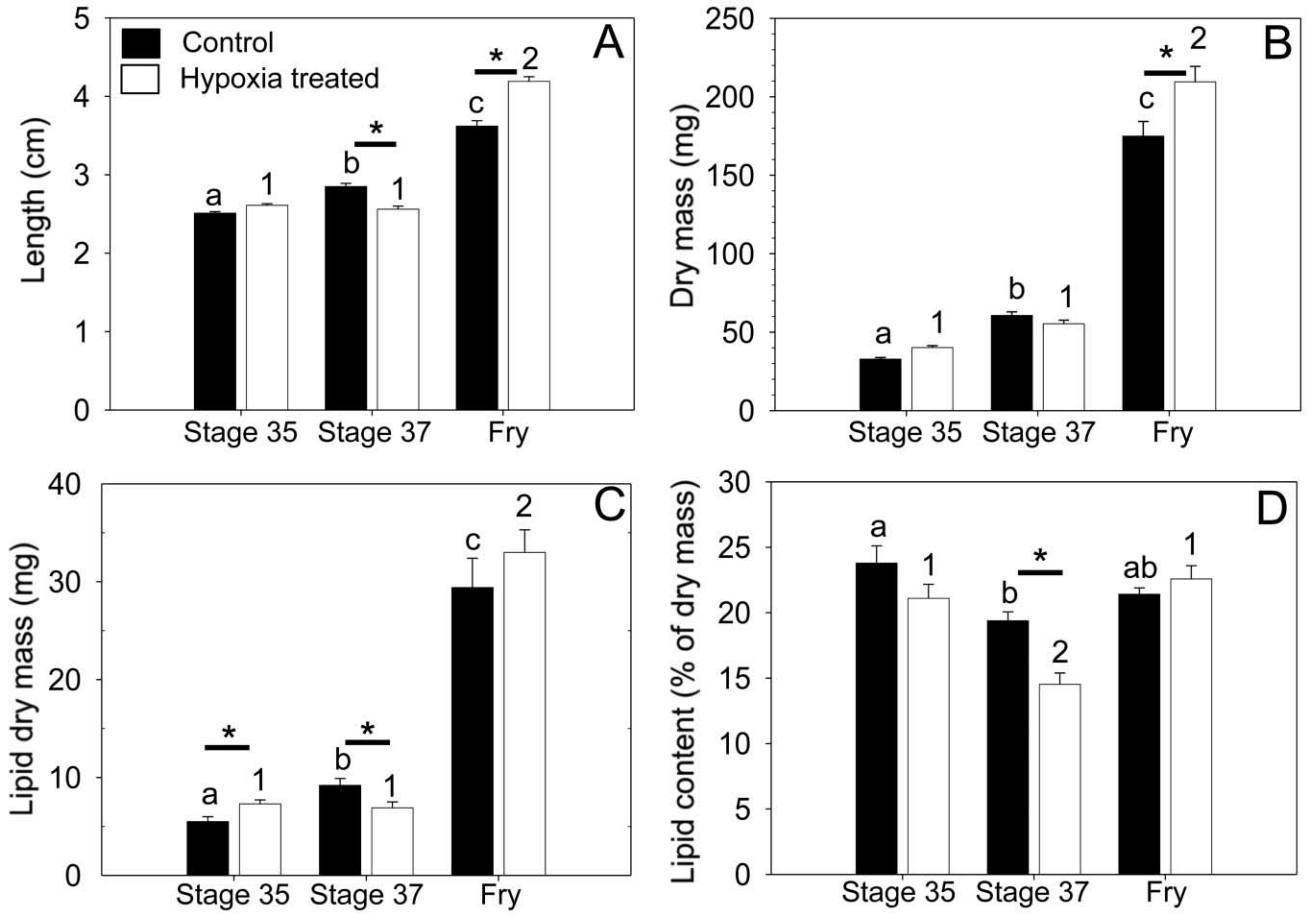

Figure 2. Chronic hypoxia exposure during embryonic development impairs growth and fat deposition in the hatched fry fish. *A*, Standard length of the hypoxia group and the control group at stages 35 and 37 and the fry stage. *B*, Whole-body dry mass of the hypoxia group and the control group at stages 35 and 37 and the fry stage. *C*, Lipid dry mass of the hypoxia group and the control group at stages 35 and 37 and the fry stage. *D*, Lipid content expressed as percent total body mass of the hypoxia group and the control group at stages 35 and 37 and the fry stage. Statistical differences, calculated by two-way ANOVA, are indicated on each panel ($P < 0.05$). Letters indicate differences in the control group between stages. Numbers indicate differences in the hypoxia group between stages. An asterisk denotes an effect of hypoxia exposure at the stage indicated ($P < 0.05$). Significant interactions exist in the effects of age and hypoxia on length, mass, lipid dry mass, and percent lipid content ($P < 0.05$).

as a percentage of body weight, there was no difference in the lipid content between the hypoxia and control groups at stage 35 (fig. 2*D*). The percent lipid content did decrease in each group between stages 35 and 37 ($P < 0.01$; fig. 2*D*). Percent lipid also increased significantly between stage 37 and the fry stage in the hypoxia group ($P < 0.001$; fig. 2*D*). In addition, the percent lipid content was significantly higher in the control group than in the hypoxia group at stage 37 but not at stage 35 or the fry stage ($P < 0.01$; fig. 2*D*).

Gene Expression

Assessing experimental effects on gene expression across multiple developmental stages is often compounded by natural ontogenic shifts in transcript abundance (McCurley and Callard 2008). Therefore, the transcript abundances of three housekeeping genes (*EF1a*, *18s*, *arp*) were quantified and analyzed for development and hypoxia effects. *EF1a* abundance was significantly affected by both age and hypoxia exposure and was therefore not used for normalization of the TnI isoforms. The abundance of *18s* mRNA was significantly higher in fry fish compared to stage 35 fish but was unaffected by hypoxia (fig. 3*A*); conversely, the abundance of *arp* mRNA was significantly higher in hypoxia-exposed fish relative to controls but unaffected by age (fig. 3*B*). Given that *18s* and *arp* were oppositely affected by the experimental variables, TnI isoform expression was normalized separately to each housekeeping genes, and only statistical differences identified with both normalized data sets were considered valid. However, only two statistical comparisons were significant with one housekeeping gene and not the other; all other comparisons were identical with both housekeeping genes.

Six of the seven TnI gene transcripts were detected in hearts of trout larvae (*RTudTnI* did not amplify). Listed in decreasing order, the relative abundance of each isoform in the hearts of the control group at stage 35 was $RTcTnI = ASfsTnI = AScTnI_2 > AScTnI_1 = ASssTnI_3 = ASssTnI_2$. In fry fish, the order in the hearts of the control group was $AScTnI_2 = AScTnI_1 = ASfsTnI = ASssTnI_2 = ASssTnI_3 > RTcTnI$. Of these six TnI isoforms, two were significantly affected by the experimental variables, regardless of normalization gene; however, significant interactions in the effects of age and hypoxia on mRNA abundance were also observed ($P < 0.05$). *RTcTnI* mRNA abundance decreased in both control and hypoxia-treated animals between stage 35 and the fry stage, and fry fish from the hypoxia group had significantly higher levels of this transcript than did control fry fish ($P < 0.05$; fig. 3*C*). $AScTnI_2$ mRNA abundance was significantly lower in fry fish from the hypoxia group compared to control fry fish ($P < 0.05$; fig. 3*D*).

Discussion

Results of this study lend support to a growing body of literature showing that environmental conditions during early development have considerable and lasting influence on subsequent growth, development, and fitness. We have shown that hypoxia exposure during embryonic development of trout impacts the future aerobic capacity, growth, and cardiac gene expression in the hatched fish. These results demonstrate that the metabolic depression and developmental delay caused by hypoxia exposure during embryogenesis have a significant and lasting effect on the fitness of the hatched fish even when returned to normoxic conditions. Taken together, these findings may carry important ecological implications. In a natural environment, fish that developed in hypoxic waters would be at a potential disadvantage to individuals that developed in normoxia due to reduced fitness.

The Effect of Hypoxia Exposure on Swimming Performance

The swimming performance of a fish, quantified by the U_{crit} test, is an established estimate of fish fitness (reviewed in Plaut 2001). As such, the lower U_{crit}, expressed as absolute speed, of the hypoxia group at stages 35 and 37 indicates that fish are not able to maintain as high a sustained swimming speed as the control group. In addition, the significantly lower standardized U_{crit} of the hypoxia group supports an overall decrease in swimming ability in the hypoxia group. A likely explanation for this observation is that the cardiorespiratory system of hypoxia-reared fish is underdeveloped. In mice, relative hypoxia (8% vs. 21%) during embryonic development slows the maturation of the heart, causes ventricular dilation, and reduces the ventricular mass (Ream et al. 2008). While we did not explicitly measure cardiac development in this study, our earlier work demonstrating that hypoxia exposure causes a delayed onset of cholinergic control and an increase in the response of the heart to adrenergic stimulation indicates that cardiac development is affected by hypoxia exposure (Miller et al. 2011). In this previous study we also demonstrated that embryos chronically exposed to hypoxia have lower heart rates (20%) at stages 26 and 29 relative to those of normoxic controls (Miller et al. 2011). This period of development is equal to 14 d. Reduced cardiac function for such a relatively long period has the potential to impact angiogenesis throughout the body. Recent work using zebrafish demonstrated that blood flow during embryogenesis is essential to the formation of the vasculature and that a reduction impedes angiogenesis (Gray et al. 2007; Nicoli et al. 2010). A delay in the development of the heart and circulatory system caused by hypoxia exposure would explain the lower swimming performance of the hypoxia group. The increase in standardized U_{crit} between stage 37 and the fry stage indicates that the aerobic capacity is increasing. This is a significant finding because standardized U_{crit} typically decreases as length increases (Plaut 2001). Work by McClelland et al. (2006) also demonstrated that swim training increases the absolute U_{crit} of zebrafish but that the standardized U_{crit} was not affected due to the concomitant increase in length. The increase in fitness found in this study with development is likely a result of the swimming ability of the fish increasing at a greater rate than the rate of growth, indicating that the fitness of all larvae

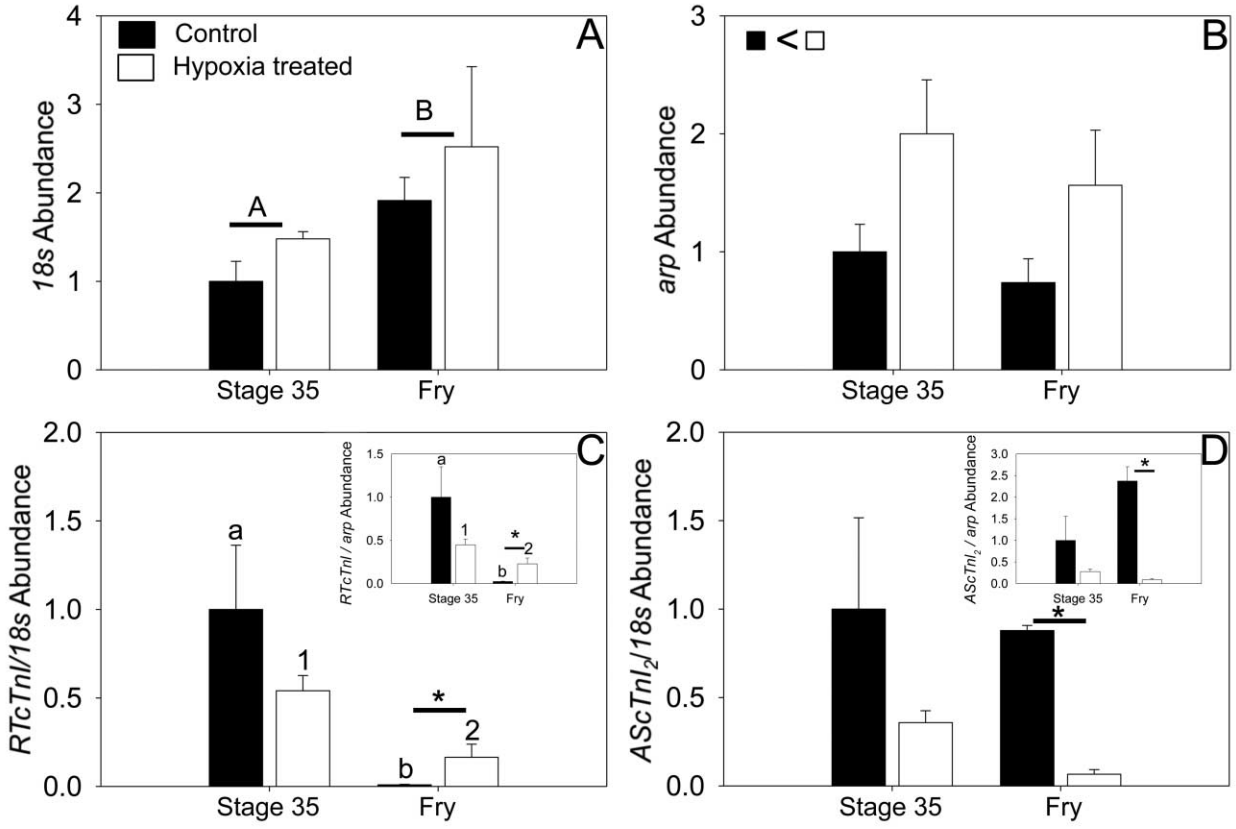

Figure 3. Influence of developmental stage and hypoxia exposure during ontogeny on the expression of *18s ribosomal RNA* (*18s*), *acidic ribosomal phosphoprotein* (*arp*), *rainbow trout cardiac troponin I* (*RTcTnI*), and *Atlantic salmon cardiac troponin I 1* (*AScTnI*$_1$) in trout heart. *A*, Abundance of the housekeeping gene *18s* in the control and hypoxia groups at stage 35 and the fry stage. *B*, Abundance of the housekeeping gene *arp* in the control and hypoxia groups at stage 35 and the fry stage. *C*, *RTcTnI* expression standardized using the housekeeping gene *18s*. Inset shows *RTcTnI* standardized using *arp* as the housekeeping gene. *D*, *AScTnI*$_1$ standardized using the housekeeping gene *18s*. Inset shows *AScTnI*$_1$ standardized using *arp* as the housekeeping gene. The expression of each transcript is relative to the amount of that transcript in the control group at stage 35. This value is set to 1 in each panel. Statistical differences, calculated by two-way ANOVA, are indicated on each panel. Significant interactions exist between the effects of age and hypoxia on the abundances of both *RTcTnI* and *AScTnI*$_1$ ($P < 0.01$) but not for *18s* or *arp*. In *A* and *B*, uppercase letters indicate an overall effect of age on gene expression, and an overall difference between the normoxia and hypoxia groups is indicated in the upper left corner. In *C* and *D*, letters indicate differences between stages for the control group, numbers indicate differences between stages for the hypoxia group, and an asterisk indicates an effect of hypoxia on gene expression within the developmental stage ($P < 0.05$).

is accelerating with development. Finally, the lower standardized U_{crit} of the hypoxia group at all stages, compared to controls, indicates that the fitness of these fish is still impaired despite having been maintained in normoxic conditions for up to 53 d. This indicates the sustained effect that hypoxia exposure during embryonic development has on the swimming capacity of the hatched fry fish.

The Effect of Hypoxia Exposure on Growth

As in previous studies, hypoxia exposure was found to cause a developmental delay. This delay is due in part to impairment of anabolic and catabolic pathways. Both processes require oxygen; thus, hypoxic conditions limit growth by impairing the rate of tissue building (Boulekbache 1981; Pelletier et al. 1994). As expected, the control group grew between each developmental stage as measured by dry mass and length. In contrast, the hypoxia-treated group failed to increase in either dry mass or length between stages 35 and 37, suggesting that these animals were not as effective as the controls at utilizing nutrients from their diet. The hypoxia group was observed to actively feed at 57 dpf, as did the controls, so nutrient availability was likely not a contributing factor to reduced growth. One possible explanation for the observed difference in growth is that the gut of the hypoxia-treated animals was underdeveloped compared to that of the controls and therefore was not as effective at absorbing nutrients. The digestive tract in fish continues to differentiate throughout larval development in both capacity and complexity (Stroband and Dabrowski 1981; Rombout et al. 1984; Watanabe 1984; Dabrowski 1986). If hypoxia exposure

during embryonic development also impedes gut maturation, such a reduced capability would not be surprising. Hypoxia exposure of zebrafish during embryonic development impairs vascularization of the gut (Schwerte et al. 2003), and such a reduced blood supply could limit both the rate of nutrient absorption and gut maturation.

The greater increase in weight (278% vs. 188%) and length (64% vs. 27%) of the hypoxia group compared to the control group between stage 37 and the fry stage indicates that the growth rate was accelerated in the hypoxia group. This may represent a compensatory response. Such a compensatory acceleration in growth rate has been previously reported in zebrafish embryos after 36 h of chronic hypoxia exposure following fertilization (Kamei et al. 2011).

The higher lipid content in the hypoxia group compared to controls at stage 35 is likely due to a higher lipid content in the yolk sack still present in this stage. We have previously demonstrated that chronic hypoxia exposure of trout embryos causes a reduction in metabolic rate (Miller et al. 2008). This would act to conserve the metabolic fuels present in the yolk. The reduction in lipid content, expressed as a percentage of dry mass, between stages 35 and 37 in the control group is due to the rate of lipid deposition being slower than the gain of lean body mass. In the hypoxia group there was no change in fat content between stages 35 and 37 but a small, not significant, increase in dry mass. This also resulted in a significant decrease in lipid content. The lack of change in lipid mass in the hypoxia group between stages 35 and 37 and the significantly lower relative lipid content compared to controls at stage 37 indicates that the hypoxia group is not as effective at incorporating dietary lipids once the yolk has disappeared. This could be due to high rates of lipid oxidation or to an inability to absorb lipid from the diet. This could also indicate, as did the lack of growth between stages 35 and 37, that the gut of the hypoxia fish is underdeveloped compared to that of the control fish. The increase in the mass of lipid and in the relative amount of lipid between stage 37 and the fry stage in both groups indicates that the animals are becoming more effective at obtaining lipid from their diet.

Cardiac Gene Expression

In healthy adult mammals, each striated muscle type expresses a single unique TnI. We recently reported that the fast skeletal, slow skeletal, and cardiac muscles of adult trout contain gene transcripts for seven unique TnI isoforms and each muscle type expresses the protein products of at least three of these (Alderman et al. 2012). This previous study also found that isoform-specific expression changes occurred in all three muscle types following cold acclimation (Alderman et al. 2012). Similarly, a recent study by Scott and Johnston (2012) found that thermal acclimation altered the mRNA levels of three TnI isoforms in the skeletal muscle of adult zebrafish, and this change correlated with a difference in aerobic fitness (Scott and Johnston 2012). In this study we observed ontogenic and hypoxia-induced changes in specific TnI isoforms. In all of these studies, the transcription of TnI isoforms was affected by environmental factors that are likely to affect contractile function. Cold acclimation affected the transcript abundance of four TnI isoforms including $AScTnI_2$ (Alderman et al. 2012), and hypoxia exposure in this study modified transcript abundance of both *RTcTnI* and $AScTnI_2$ in fry fish hearts. Combined, these studies support the physiological relevance of the maintenance of seven unique TnI isoforms in the trout genome. We hypothesize that modulation of TnI isoforms allows fish striated muscles to optimize contractile function under varying physiological conditions. In support of this hypothesis, previous work in rodents and humans has demonstrated that the neonatal heart expresses the slow skeletal TnI isoform and is replaced by cardiac TnI following birth (Murphy et al. 1991; Gorza et al. 1993; Sasse et al. 1993). It is thought that the presence of the slow skeletal isoform helps protect cardiac function from low pH in utero (Westfall and Metzger 2007). In addition, the replacement of native cardiac TnI with an alternative isoform can alter the Ca^{2+} activation of the cardiac troponin complex (Kirkpatrick et al. 2011) and alter the response of cardiac muscle to a change in cellar pH (Westfall et al. 2002) and to phosphorylation by protein kinase A (PKA; Fentzke et al. 1999). (PKA is stimulated following adrenergic stimulation and is responsible for the associated changes in myocyte contractility.) Manipulation of the TnI isoform in the muscle can therefore have a significant influence on cardiac contractile function. Together these studies indicate that TnI content of fish muscle is highly plastic and that such a change in expression has the potential to alter contractile function.

Conclusions and Perspective

The results of this study demonstrate that the physiological and developmental changes caused by hypoxia exposure during embryonic development have a carryover effect on the fitness of the hatched fish. There also appears to be a long-term impact on the pattern of gene expression in the heart. The reduced aerobic fitness of the hypoxia group persists to the fry stage, which was measured 53 d after the fish had been transferred to normoxia. The lower growth rate and lipid content of the hypoxia group compared to that of the controls indicate that these animals also have a reduced ability to obtain nutrients from their diet. The catch-up in weight, length, and lipid deposition by the fry stage indicates that these fish are able to compensate for the initial slow growth rate if given enough time. However, in the natural environment, where newly hatched fry fish must compete for limited resources and avoid predation, the combined effects of reduced aerobic fitness and impaired ability to obtain nutrients from the diet are likely to significantly impact survival. Further work is therefore required to examine the long-term consequences of hypoxia exposure

of salmonid fish during embryogenesis on the fitness of natural fish populations.

Acknowledgments

We would like to thank Jordan M. Klaiman for editorial and technical assistance. This work was supported by an NSERC Discovery Grant to T.E.G.

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