Oxygen concentration in the water boundary layer next to rainbow trout (*Oncorhynchus mykiss*) embryos is influenced by hypoxia exposure time, metabolic rate, and water flow

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Abstract: The objective of this study was to examine the influence of hypoxia exposure time, metabolic rate, and water flow rate on the O_2 concentration in the boundary layer outside and inside the chorion of rainbow trout (*Oncorhynchus mykiss*) embryos. Oxygen consumption, growth, and dissolved O_2 in the boundary layer were measured at 15, 22, and 29 days post fertilization (dpf) from embryos reared in normoxia (O_2 concentration at 100% saturation) and chronic hypoxia (>24 h, 50% saturation) and exposed to acute hypoxia (30 min, 50% saturation). Chronic and acute hypoxia exposure decreased the dissolved O_2 in the boundary layer to the same extent at 15 and 22 dpf; however, at 29 dpf, O_2 levels were significantly lower in acute relative to chronic hypoxia. At 29 dpf, O_2 uptake per individual was significantly lower in embryos exposed to chronic relative to acute hypoxia. In addition, mass-specific O_2 uptake in chronic hypoxia-exposed embryos was ~40% less than that of controls but the same as that of acutely exposed embryos. This correlates with reduced growth in embryos exposed to chronic hypoxia. We conclude that boundary layer O_2 is lower after 30 min of hypoxia compared with 2 weeks of hypoxia simply because embryos exposed to chronic hypoxia grow slower and consume less O_2.

Résumé : Notre étude examine l'influence de la durée de l'exposition à l'hypoxie, du taux métabolique et du taux d'écoulement de l'eau sur la concentration d'oxygène dans la couche limite à l'extérieur et à l'intérieur du chorion des embryons de truites (*Oncorhynchus mykiss*). Nous avons mesuré la consommation d'oxygène, la croissance et la concentration d'oxygène dissous (DO) dans la couche limite 15, 22 et 29 jours après la fécondation chez des embryons élevés en normoxie ([O_2] à 100 % de saturation) ou en hypoxie chronique (>24 h, 50 % de saturation) ou alors exposés à une hypoxie aiguë (30 min, 50 % de saturation). Les hypoxies chroniques et aiguës causent une diminution de DO dans la couche limite de même importance 15 et 22 jours après la fécondation; cependant, 29 jours après la fécondation, les concentrations d'oxygène sont significativement plus basses lors d'une hypoxie aiguë que d'une hypoxie chronique. Vingt-neuf jours après la fécondation, l'absorption d'oxygène par individu est significativement plus faible chez les individus exposés à une hypoxie chronique plutôt qu'à une hypoxie aiguë. De plus, l'absorption d'oxygène spécifique à la masse chez les embryons exposés à une hypoxie chronique est ~40 % inférieure à celle des témoins, mais semblable à celle des embryons ayant subi une exposition aiguë. Cela s'explique par la croissance réduite des embryons exposés à une hypoxie chronique. Nous concluons que la concentration d'oxygène dans la couche limite est plus basse après 30 min d'hypoxie qu'après 2 semaines, parce que les embryons exposés à l'hypoxie chronique croissent plus lentement et consomment moins d'oxygène.

[Traduit par la Rédaction]

Introduction

Rainbow trout (*Oncorhynchus mykiss*) are relatively sensitive to hypoxia exposure (Gesser 1977). This sensitivity reaches a peak during early ontogeny, just before hatching (Rombough 1988). Understanding the ability of the developing embryos to adjust to hypoxic conditions is important for predicting the success of emerging rainbow trout populations. The physiological responses of salmonids to hypoxia are dependent on the species, age, and intensity of the hypoxia exposure (Jensen et al. 1993). Furthermore, responses to acute hypoxia may be different from those in chronic hypoxia. Several studies have focused on metabolic responses of salmonid adults to hypoxia (e.g., Dunn and Hochachka 1986; Boutilier et al. 1988; Zhou et al. 2000), but few studies are available on embryonic stages.

Previous studies have demonstrated that rainbow trout embryo survival within the redd is dramatically influenced by water O_2 levels. Youngson et al. (2004) reported that there was 83% mortality of rainbow trout embryos in redds where mean dissolved O_2 (DO) was at 28% saturation. Also, survival was significantly related to the velocity of ground-

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water in redds (Sowden and Power 1985). An assessment of a rainbow trout spawning redd found that DO concentrations ranged from ~23% to ~80% saturation (Coble 1961) and that the intergranular flow rate of water through salmonid redds was also variable from 2.7 to 4.5 mL·min^{-1}, typically averaging ~3 mL·min^{-1} (Zimmermann and Lapointe 2005). Coble (1961) has also suggested that DO concentrations and water flow rates directly impact the intragravel water of a rainbow trout redd. Therefore, in addition to hypoxia resulting from variability in DO, variability in water flow rate can also limit the delivery of O_2 to embryos.

Prehatch development represents an extremely sensitive period in trout, as organogenesis occurs between 4 days post fertilization (dpf) and hatching (Balon 1975). Any disturbances to this may result in malformation and defects that will ultimately impact the fitness of adults (Shang and Wu 2004). Specifically, the growth of embryos can be open to considerable ontogenic modification in response to habitat conditions. For instance, low DO concentrations have been shown to affect the rate of development of chum salmon (*Oncorhynchus keta*; Alderdice et al. 1958), lake trout (*Salvelinus namaycush*; Garside 1959), and Chinook salmon (*Oncorhynchus tshawytscha*; Silver et al. 1963) embryos. Furthermore, during periods of low O_2 availability, it is essential that fish embryos maintain respiratory homeostasis and sufficient gas exchange required for the increase in growth, development, and O_2 consumption (M_{O_2}) associated with embryogenesis. It is known that rainbow trout adults show a metabolic depression after 24 h in mild hypoxia (~50% O_2 saturation) (Boutilier et al. 1988). Also, nonsalmonid embryos such as zebrafish (*Danio rerio*) decrease M_{O_2} in response to severe (~30% O_2 saturation) acute (20 min) hypoxia (Barrionuevo and Burggren 1999). The ability of trout embryos to depress M_{O_2} has not been well studied. Thus, it is necessary to determine how ontogeny, with its periods of variable sensitivity, influences physiological tolerances and responses to short- and long-term mild hypoxia in the trout embryos.

The boundary layer consists of an O_2-depleted region of stagnant water owing to O_2 consumption by the embryos, creating a significant O_2 gradient (Pinder and Feder 1990). The O_2 boundary layer around developing embryos depends on both the supply of and the demand for O_2. Ciuhandu et al. (2007) showed that increased O_2 demand in later stages of trout embryogenesis decreased O_2 levels in the boundary layer, but they did not directly measure O_2 consumption. A decrease in O_2 supply (e.g., hypoxia) may further reduce the amount of O_2 in the boundary layer available to the embryos. Ciuhandu et al. (2007) found a decrease in DO and the DO gradient in the boundary layer of rainbow trout embryos in acute hypoxia (30 min, 35% saturation). However, in the Ciuhandu et al. (2007) study, DO inside the chorion (perivitelline fluid) was not measured, nor was the influence of prolonged hypoxia exposure investigated.

In the present study, we considered the impact of a mild hypoxia (50% saturation) that may be frequently encountered by salmonid embryos in their natural environment. We initiated chronic hypoxia exposure at the eyed stage (14 dpf) because it correlates with the onset of simple circulation (Rombough 1997), a juncture in embryonic development coinciding with a gradual increase in convective O_2 transport and its altering effects on diffusion properties and critical DO levels (Alderdice et al. 1958). For the purpose of this study, chronic hypoxia is defined as the condition in which the embryos are maintained in water with an O_2 concentration at 50% saturation for a period greater than 24 h.

There were four hypotheses addressed in this study. The first hypothesis was that the distribution of DO inside the chorion and the boundary layer O_2 gradient would be influenced by environmental DO. We predicted that the DO levels inside the chorion and in the boundary layer would be lower when exposed to acute and chronic hypoxia at three sampling times during the course of development. This was tested by measuring the DO profile inside the chorion and in the boundary layer at 15–16, 22–23, and 29–30 dpf in normoxia (100% O_2 saturation) and acute (30 min) and chronic hypoxia. The second hypothesis was that hypoxia would depress O_2 uptake. We predicted that acute and chronic hypoxia exposure would reduce M_{O_2} below the control rate in normoxia during late embryonic development but not earlier because of the low O_2 requirements of embryos early in development. To test this, M_{O_2} was measured at the same sampling times as above and with the same O_2 treatments. The third hypothesis was that hypoxia influences the growth of rainbow trout embryos throughout development. We predicted that there would be a decrease in growth in response to hypoxia in chronic but not in acute exposure at all sampling times, with the most impact on growth late in development because of high O_2 requirements of embryos late in development. To test this, the embryonic dry mass of embryos was determined at the same sampling times and with the same O_2 treatments as above. The fourth hypothesis was that water flow rate would affect the DO inside the chorion and in the boundary layer outside the chorion. We predicted that a decrease in water flow rate would lower the DO concentration inside the chorion and in the boundary layer outside the chorion. To test this, DO levels in the boundary layer and inside the chorion of 28 dpf embryos were measured at water flow rates of 3 and 6 mL·min^{-1}.

Materials and methods

Experimental animals

Rainbow trout embryos were obtained from Rainbow Springs Trout Farm in Thamesford, Ontario, on the day of fertilization and transported to the Hagen Aqualab, University of Guelph (Guelph, Ontario). Embryos were held in mesh-bottom Heath trays (length 62.9 cm × width 52.1 cm × depth 6 cm) throughout the incubation period shielded from light with a continuous flow (4 mL·min^{-1}) of local well water (10 °C, 10 mg O_2·L^{-1}, pH 7.9, water hardness 411 mg·L^{-1} as $CaCO_3$, ion concentrations (mmol·L^{-1}): 2.6 Ca^{2+}, 1.5 Cl^-, 1.5 Mg^{2+}, 0.06 K^+, and 1.1 Na^+). Separate batches of embryos were used for series I and series II, II, and IV. For each batch, eggs were obtained from three different females and these were mixed together with sperm from multiple males. The resultant embryos were randomly divided into the treatment and control groups. Therefore, there should be no differences between treatment and control in embryo quality. Hatching occurred between 29 and 32 dpf and there was no difference between treatment and control groups in the time to hatch.

Experimental protocols

There were four series of experiments conducted: series I, DO in the boundary layer during development and hypoxia; series II, O_2 consumption (M_{O_2}) during development and hypoxia; series III, growth rate and hypoxia; and series IV, DO in the boundary layer with changes in water flow rate. In series I, II, and III, measurements were taken after acute (30 min) and chronic hypoxia exposure. Embryos at the eyed stage (14 dpf) were randomly divided into two groups and chronically reared at either normoxia (100% O_2 saturation) or hypoxia. Equilibrating the input of N_2 gas into a header tank (~50 L) established hypoxic water flowing in the Heath tray for the duration of the chronic hypoxia exposure. DO levels were monitored periodically and were always between 95% and 100% for the normoxic group and between 48% and 52% for the hypoxic group. For series I, II, and III, embryos were sampled at the following times: 15–16 dpf (eyed stage), 22–23 dpf, and 29–30 dpf (just before hatching). For simplicity, these times have been designated as 15, 22, and 29 dpf in subsequent referrals.

Series I: O_2 gradient with sampling time and hypoxia

Oxygen microelectrodes were used to measure DO profiles in the boundary layer and inside the chorion of rainbow trout embryos as described by Ciuhandu et al. (2007). In brief, a syringe with a 28-gauge needle was attached to a micromanipulator (Brinkmann, Germany) and used to pierce the chorion. Once penetrated, a very small amount of food colouring was introduced to mark the location of the puncture. The needle was then replaced with an O_2 electrode (model OX25; Unisense A/S, Aarhus, Denmark) connected to a picoammeter (model PA2000; Unisense A/S). DO was measured ~100–200 μm inside the chorion in the perivitelline fluid. As the penetration of the chorion could have disturbed the boundary layer inside the chorion, only one measurement was taken inside the chorion (Ciuhandu et al. 2007), and for consistency, this was completed immediately after the chorion was pierced at the same location and distance from the chorion for each embryo. For each embryo, DO was also measured at the outside surface of the chorion and at 100 μm increments away from the surface of the chorion up to 1000 μm to accurately demonstrate the size of the boundary layer.

The DO concentration in the boundary layer was measured before the chorion was pierced to prevent disturbances to the boundary layer. These measurements were taken in a Plexiglas chamber (length 7.5 cm × width 3.3 cm × depth 2 cm) supplied with flowing well water at 10 °C. The water flow rate was controlled using a variable-flow peristaltic pump (model 3385; Fisher Scientific, Pittsburgh, Pennsylvania). Calibrated ratios of air and N_2 were mixed using a Wösthoff pump (Calibrated Instruments Inc., Ardsley, New York) and bubbled into a glass reservoir with a water jacket maintained at 10 °C by a circulating water bath (Isotemp 3016; Fisher Scientific). The O_2 microelectrode was used to measure DO profiles in the boundary layer of embryos at the three sampling times in embryos exposed to acute hypoxia, chronic hypoxia, and normoxia. All measurements were on separate embryos (n = 6 for each sampling time). The O_2 gradient was calculated as the O_2 concentration in the free stream minus the O_2 concentration in the perivitelline fluid. Water flow rate during measurements was 4 mL·min^{-1}. Measurements were taken 30 min after the embryo was placed in the sample chamber to allow adequate time for the boundary layer to form.

Series II: M_{O_2} with development and hypoxia

Closed respirometry was used to measure M_{O_2} in normoxia, acute hypoxia, and chronic hypoxia at three sampling times (as above). Embryos were placed in water-jacketed (10 °C) glass respirometers (for setup details, see Ninness et al. 2006). For each respirometer, three embryos were used at 15 dpf and two embryos were used at 22 and 29 dpf (n = 8 at each sampling time). The chambers were filled with autoclaved well water: 100% O_2 saturated (normoxia) and 50% O_2 saturated (acute or chronic hypoxia). DO levels in the respirometers were monitored continuously for 1.5 h after an initial 30 min chamber acclimation period. The water O_2 content did not fall below 70% saturated in normoxia or below 30% saturated in hypoxia during this time period. Measurements were made using automatic, temperature-compensated DO sensors (Clark-type electrodes) with built-in thermistor and amplifier connected to a LabPro serial interface and recorded on a computer using LoggerPro software (version 3.4.2; Vernier Software and Technology, Beaverton, Oregon). The O_2 probes were calibrated daily. Preliminary trials were conducted without embryos in the respirometers for 2 h to determine background O_2 uptake or sensor O_2 uptake (negligible). M_{O_2} was calculated using a formula previously described by Ninness et al. (2006). M_{O_2} is presented per individual (nanomoles O_2 per minute per individual) and mass (micromoles O_2 per minute per gram dry mass).

Series III: growth rate and hypoxia

To measure and compare growth rate in embryos exposed to normoxia and acute and chronic hypoxia, embryos from series II were dechorionated and the embryonic proper was separated from the yolk sac by hand using fine forceps. The embryonic proper was oven-dried (~96 h at 50 °C) to a constant mass (milligrams). The embryonic body dry mass was pooled for each treatment and divided by the number of embryos used in each treatment to determine dry mass per individual (n = 8 at each sampling time).

Series IV: O_2 gradient with changes in water flow rate

The O_2 microelectrode was used to measure DO profiles in the boundary layer as described above. DO measurements were taken at 28 dpf in normoxic water on seven embryos at two different flow rates: 3 and 6 mL·min^{-1}. Over this range of flow rates, the linear relationship between water velocity and flow rate in the sample chamber was represented by the following equation: velocity (cm·s^{-1}) = –0.078 + 0.040 × flow (mL·min^{-1}). Velocity was measured by timing the movement of dye in the free stream near the embryo in the sample chamber. Separate embryos were used for all measurements. This experiment was only completed on embryos under normoxic conditions, as it was not possible to maintain 50% O_2 saturation in the water if the flow through the system was varied from 4 mL·min^{-1}. In addition, these experiments were not completed at a flow rate of zero, as there

has to be water flow through the experimental setup for temperature and O_2 levels to be maintained.

Statistical analyses

A general linear model analysis of variance (GLM-ANOVA) was performed to test for differences between normoxia and acute and chronic hypoxia boundary layer DO curves (pairwise) outside the chorion at each sampling time (series I). The same procedure was performed to test for differences between the boundary layer DO curves (series IV) outside the chorion at the two flow rates (3 and 6 mL·min^{-1}). Differences between DO values inside and at the surface of the chorion were analyzed using paired Student's *t* tests (series IV). A one-way ANOVA was performed to analyze differences in DO under the chorion between normoxia and acute and chronic hypoxia for each sampling time (series I) and between the two water flow rates (series IV). Differences in O_2 gradients (series I) were tested with a two-way ANOVA (treatment, sampling time, and interaction). The effect of acute and chronic hypoxia on M_{O_2} (series II) was tested with a three-way nested ANOVA (treatment, sampling time, respirometer, and interaction). Differences in growth owing to acute and chronic hypoxia exposure (series III) were analyzed using a two-way ANOVA (treatment, sampling time, and interaction).

All statistical analyses were performed using Minitab version 15. In all analyses, Tukey tests were used to identify the differences when significant differences were found ($p < 0.05$). Results are presented as means ± SE of the mean.

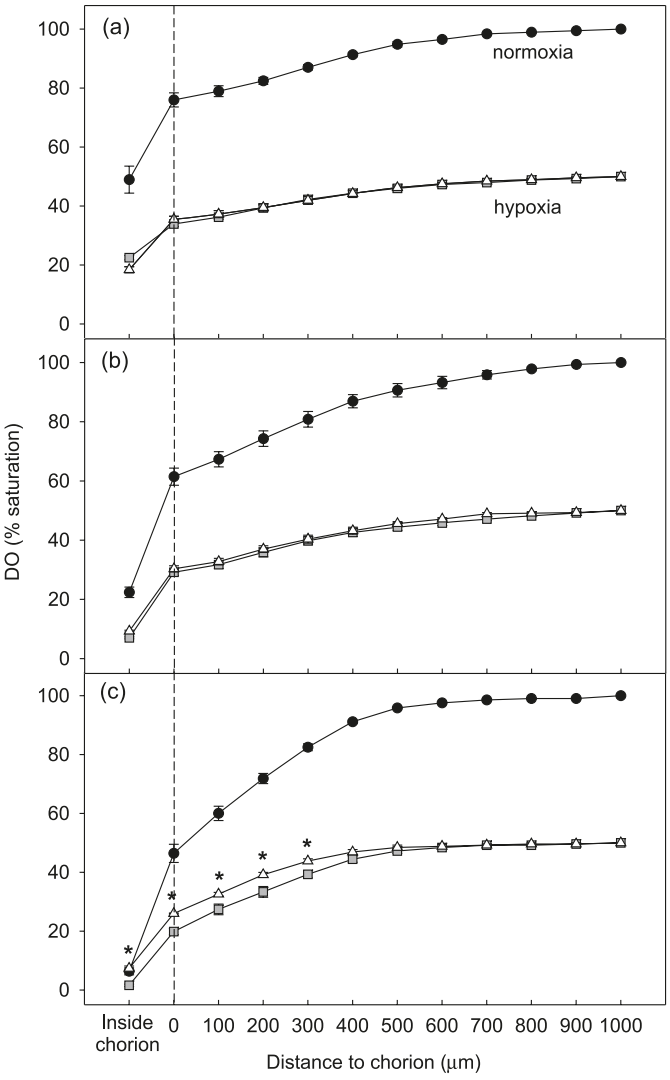
Results

Series I: O_2 gradient with sampling time and hypoxia

Oxygen concentration gradually decreased in a nonlinear fashion from the free stream to the chorion (Fig. 1). At 15, 22, and 29 dpf, the DO in the boundary layer curve was significantly decreased with acute and chronic hypoxia compared with that under normoxic conditions (Fig. 1). There was no significant difference between DO in the boundary layer curve in acute and chronic hypoxia at 15 dpf (Fig. 1*a*) and 22 dpf (Fig. 1*b*). On the contrary, at 29 dpf, the DO in the boundary layer curve in chronic hypoxia was significantly higher than that of acute hypoxia (Fig. 1*c*). Overall, the boundary layer effect on the distribution of DO (treatment and distance to chorion interaction) was the same for the two hypoxia exposures at 15 dpf (Fig. 1*a*) and 22 dpf (Fig. 1*b*) but different at 29 dpf (Fig. 1*c*). There were significant changes in the O_2 gradients with sampling time and treatment (Fig. 2). The O_2 gradient increased with sampling time in normoxia and acute hypoxia but only between 15 and 22 dpf in chronic hypoxia. The O_2 gradient was significantly less in acute and chronic hypoxia than in normoxia at each sampling time. At 29 dpf, the O_2 gradient in chronic hypoxia was significantly lower than that in acute hypoxia (Fig. 2).

At 15 and 22 dpf, DO values inside the chorion (~20% and ~8% saturation, respectively) for both acute and chronic hypoxia did not differ from one another significantly, but both DO values were significantly lower relative to DO values inside the chorion in normoxia (Figs. 1*a* and 1*b*). At 29 dpf, the DO inside the chorion in normoxia (~6% saturation) was significantly higher than DO inside the chorion in acute hypoxia (~2% saturation) (Fig. 1*c*). Also, DO inside the chorion during chronic hypoxia exposure was on average 78% higher compared with that in acute hypoxia but did not significantly differ from DO inside the chorion in normoxia (Fig. 1*c*).

Fig. 1. Boundary layer dissolved oxygen (DO) profile of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid circles), acute (shaded squares), and chronic hypoxia (open triangles) (50% saturation, 10 °C) at three developmental times: (*a*) 15, (*b*) 22, and (*c*) 29 days post fertilization (dpf). Water flow rate was set to 4 mL·min^{-1}. The boundary layer curve was decreased with hypoxia at 15 and 22 dpf, but at 29 dpf, the DO curve in chronic hypoxia was significantly higher than that of acute hypoxia. An asterisk indicates a significant difference in DO between embryos chronically exposed to hypoxia and those acutely exposed to hypoxia ($p < 0.05$). The vertical broken line indicates the chorion surface. Values are means ± SE of the mean ($n = 6$).



Series II: O_2 consumption with sampling time and hypoxia

Sampling time had a statistically significant effect on M_{O_2}

Fig. 2. Boundary layer gradient of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid circles), acute (shaded squares), and chronic hypoxia (open triangles) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). The gradient (% O_2 saturation) was calculated from boundary layer curves in Fig. 1 as the difference between the DO in the free stream and the DO in the perivitelline fluid. Values are means ± SE of the mean. The O_2 gradient was less in hypoxia than in normoxia at each developmental time. At 29 dpf, the O_2 gradient in chronic hypoxia was significantly lower than that in acute hypoxia.

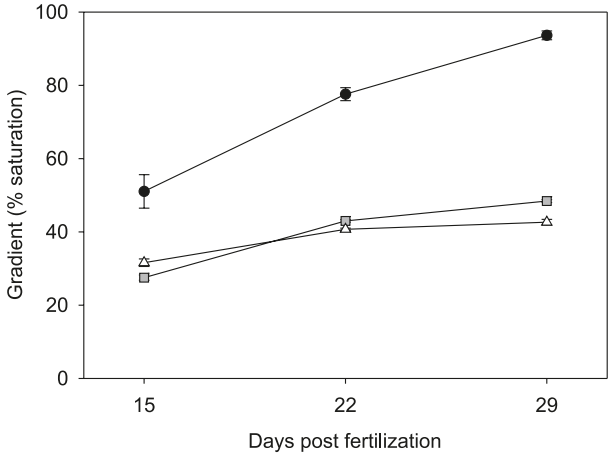
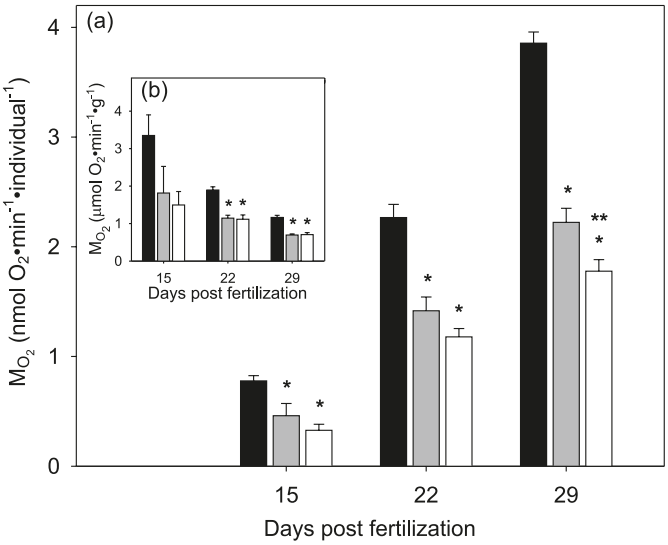


Fig. 3. Oxygen consumption of rainbow trout (*Oncorhynchus mykiss*) embryos (*a*) per individual and (*b*) mass-specific in normoxia (solid bars), acute (shaded bars), and chronic hypoxia (open bars) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). A single asterisk indicates a significant difference from normoxia, and double asterisks indicate a significant difference from acute hypoxia ($p < 0.05$). Values are means ± SE of the mean ($n = 8$).



per individual (Fig. 3*a*). In normoxia, M_{O_2} per individual increased fivefold between 15 and 29 dpf. Similarly, M_{O_2} per individual in acute and chronic hypoxia significantly increased during development but overall remained lower than normoxic values.

Treatment had a significant effect on M_{O_2} per individual (Fig. 3*a*), and this effect was dependent on development. Hypoxic conditions (acute and chronic) significantly decreased M_{O_2} per individual at all sampling times. At 29 dpf, M_{O_2} per individual in chronic hypoxia was 54% lower relative to normoxia and 20% lower relative to acute hypoxia (Fig. 3*a*). There was a significant decrease in mass-specific M_{O_2} in embryos exposed to hypoxia relative to normoxia at 22 and 29 dpf but not at 15 dpf (Fig. 3*b*).

There is no significant source of variation in M_{O_2} produced among different respirometers within each O_2 treatment.

Series III: growth rate and hypoxia

Overall, growth increased throughout embryonic development for each treatment (Fig. 4). In addition, hypoxic conditions did not have an effect on growth at 15 and 22 dpf (Fig. 4). However, at 29 dpf, the embryonic body dry mass of embryos reared in chronic hypoxia was 24% lower than of those reared in normoxia.

Series IV: O_2 gradient with changes in water flow rate

Under normoxic conditions, a decrease in water flow rate from 6 to 3 mL·min^{-1} resulted in a statistically significant lower boundary layer DO curve outside the chorion (Fig. 5). There was a statistically significant difference between the DO inside the chorion and at the chorion surface for both flow rates. The DO inside the chorion at 3 mL·min^{-1} was significantly lower by 56% compared with the DO inside the chorion at 6 mL·min^{-1}.

Discussion

Before the onset of convective O_2 transport, the aerobic requirements of embryos are met solely through O_2 diffusion (Krogh 1941). Water convection past the respiratory surface is not initiated until several days after hatching (Rombough 1988), but a rudimentary circulatory system is in place at the eyed stage (~14 dpf, 10 °C; Vernier 1969). We show here that body mass and O_2 uptake increased by 12- and 5-fold, respectively, over this 2-week period between 15 and 29 dpf, in agreement with previous reports (Rombough 1986). Thus, as the demand for O_2 increases multifold, changes in the supply of O_2 (i.e., hypoxia) should result in a larger impact on O_2 levels in the microenvironment next to older compared with younger embryos (hypothesis 1). Indeed, our results show that the DO inside the chorion (perivitelline fluid) becomes progressively lower during both acute and chronic hypoxia from 15 dpf (~20% saturation) to 22 dpf (~8% saturation) and both values are lower than control values. At 29 dpf, however, we were surprised to see that the DO inside the chorion of embryos in chronic hypoxia was about the same as control values (~6% saturation), significantly higher than respective values in embryos exposed to only 30 min of hypoxia (~2% saturation). Interestingly, the M_{O_2} of embryos chronically exposed to hypoxia was ~54% lower that of the control embryos at day 29. The fact that the DO inside the chorion of both of these groups was the same at this time indicates that the lower M_{O_2} is measurably affecting DO concentrations.

We predicted that acute and chronic hypoxia exposure would depress O_2 uptake during late embryonic develop-

Fig. 4. Individual dry mass of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid bars), acute (shaded bars), and chronic hypoxia (open bars) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). A single asterisk indicates a significant difference from normoxia, and double asterisks indicate a significant difference from acute hypoxia ($p < 0.05$). Values are means ± SE of the mean ($n = 8$).

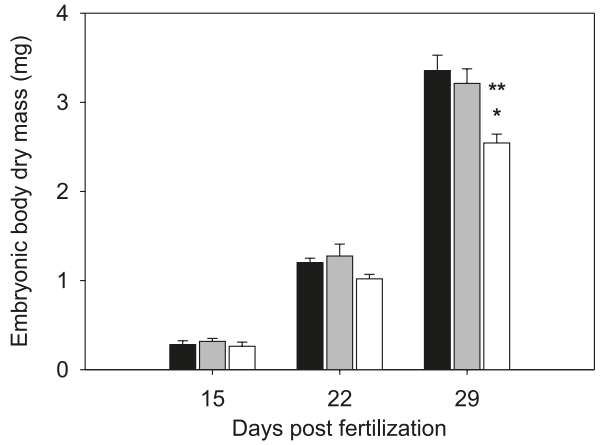
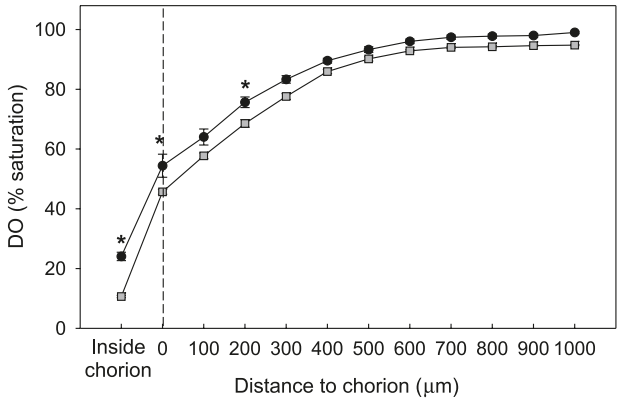


Fig. 5. DO concentrations of the boundary layer in rainbow trout (*Oncorhynchus mykiss*) embryos 28 days post fertilization (dpf) exposed to two water flow rates in normoxia: 3 (shaded squares) and 6 mL·min^{-1} (solid circles) (10 °C). The boundary layer curve at 3 mL·min^{-1} was significantly lower than at 6 mL·min^{-1} ($F_{[1,150]}$, $p < 0.01$). The vertical broken line indicates the chorion surface. An asterisk indicates a significant difference in DO between embryos exposed to 3 and 6 mL·min^{-1} ($p < 0.05$). Values are means ± SE of the mean ($n = 7$).



ment but not earlier because of the low O_2 requirements of the younger embryos (hypothesis 2). The data do not fully support this hypothesis because M_{O_2} per individual was depressed in all embryos, regardless of developmental stage. Furthermore, in 29 dpf embryos, M_{O_2} per individual in chronic hypoxia was significantly lower than comparable values for the acute hypoxia group. This demonstrates that O_2 demands were lower in the embryos exposed to chronic hypoxia compared with acute hypoxia, and therefore, DO inside the chorion was higher in chronic relative to acute hypoxia. The higher DO inside the chorion resulted in the observed decrease in the O_2 gradient in chronic hypoxia. Is the lower M_{O_2} in the chronic hypoxia group due to a deeper metabolic depression or to mass differences between embryos? The results show that chronic hypoxia depressed growth rate and the 29 dpf embryos attained a smaller mass relative to the other two groups, normoxia and acute hypoxia. Thus, the mass-specific M_{O_2} values for the chronic hypoxia group are not different from those for the acute hypoxia group. We can conclude, therefore, that O_2 levels in the fluid surrounding the 29 dpf embryos were higher after 2 weeks compared with 30 min of mild hypoxia because embryos exposed to chronic hypoxia grow slower and consume less oxygen. A previous study by Ninness et al. (2006) found a small increase in lactate in the tissues of hypoxia-exposed rainbow trout embryos just before hatch. Therefore, it is possible that embryos could increase their reliance on anaerobic metabolism during hypoxic stress, but the small change in lactate levels indicates that the overall anaerobic capacity is low and could only play a very minor role at these early life stages.

Two interesting findings that emerge from the 22 and 29 dpf data sets are that mass-specific M_{O_2} (*i*) is depressed by no more or less than 40% and (*ii*) decreases within 30 min of hypoxia exposure and not any further after 1 or 2 weeks of hypoxia. These results indicate that trout embryos are O_2 conformers, at least when exposed to 50% O_2 saturation between the eyed stage and hatching, similar to Atlantic salmon (*Salmo salar*) embryos (Hamor and Garside 1979) and the classic principles of Fry (1957). The extent of metabolic depression may be dependent on the magnitude of the hypoxia; however, to our knowledge, there are no reports in the literature of M_{O_2} at O_2 levels less than 50% saturation. Ciuhandu et al. (2007) found that DO levels in the boundary layer outside the chorion in 30 dpf trout embryos exposed to 35% saturation increased between 30 min and 8 h of exposure, presumably because of a progressive metabolic depression.

The 40% decrease in metabolic rate in hypoxia in the present study would help to balance the O_2 demand to the reduced O_2 supply but still enable the embryo to grow (body mass increased by 10-fold between 15 and 29 dpf). Growth retardation and delayed development in salmonid embryos in response to hypoxic water are well established in the literature (e.g., Shumway et al. 1964; Hamor and Garside 1976; Ciuhandu et al. 2005). In the present study, we evaluated growth by measuring the dry mass of the embryos. As we did not "stage" or examine the morphology of the individual embryos, it is not possible to say how hypoxia exposure affected the development of these embryos. It is obvious, however, that the hypoxia exposure did negatively influence the growth of the embryos. It is also not known if the embryos that displayed a delay in growth and (or) development would be able to "catch up" if they were then exposed to normoxic water.

We hypothesized that water flow rate would affect the DO inside the chorion and in the boundary layer outside the chorion. Indeed, a decrease in water flow rate lowered the DO concentration inside the chorion and in the boundary layer to a magnitude similar to that of mild hypoxia. Furthermore, hypoxia and water flow rate may become additive, further decreasing DO. A reduction in water flow rate decreases the rate at which the boundary layer is renewed and results in low DO levels in the boundary layer (Ciuhandu et al. 2007). Also, the gradient of O_2 depletion from the free

stream to the chorion and the significant drop in DO between the chorion surface and perivitelline fluid are evidence that the boundary layer and the chorion are limiting O_2 uptake (Pinder and Feder 1990; Ciuhandu et al. 2007).

Although flow rates are difficult to measure in natural salmonid redds, Zimmermann and Lapointe (2005) determined that in some redds, water flow is relatively slow, varying between 2.7 and 4.5 $mL \cdot min^{-1}$. In the present study, slow flow rates resulted in unfavourable DO gradients, demonstrating the importance of water exchange even in highly saturated water. In the spawning redd, the embryos are covered with substrate for protection and the DO and velocity of the water are related to substrate size and permeability (Peters 1962).

By initiating the hypoxia exposure at the eyed stage, we studied the ability of animals to deal with low O_2 levels with a functioning circulation system. If we had started hypoxia prior to this time, there would likely have been differences between treatment groups in circulatory system development and effectiveness. These factors would further complicate the interpretation of our data. In addition, it is also expected that an earlier exposure of the embryos to hypoxia would also have had a greater effect on the metabolic processes of these animals. Future studies will build on the current study and characterize how hypoxia exposure, at an earlier developmental time, alters embryo O_2 consumption.

In conclusion, the O_2 concentration of the perivitelline fluid of rainbow trout embryos is significantly decreased by exposure to mild hypoxia. We showed for the first time that rainbow trout embryos exhibit a decrease in M_{O_2} during mild acute and chronic hypoxia within 2 weeks of hatching. This indicates that rainbow trout embryos are O_2 conformers and are capable of responding to acute hypoxia early in development by maintaining a balance in their aerobic energy. However, when O_2 supply becomes limiting at accelerated phases of embryonic growth, mass is reduced accordingly. The results also suggest that low water flow rates produce unfavourable boundary layer conditions and ultimately create a hypoxic microenvironment. Overall, the differences in responses to chronic or acute hypoxia exposure may reflect the fact that thresholds for physiological responses are defined by developmental stage and duration of the stimulus.

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