The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion

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Growth was measured in dechorionated and chorionated embryos of rainbow trout *Oncorhynchus mykiss* exposed to three oxygen treatments: hypoxia, normoxia and hyperoxia (nominally 5, 10 and 15 mg l^{-1}). Dechorionated embryos grew faster than chorionated ones in all three oxygen treatments. Similar patterns, however, were found in both dechorionated and chorionated groups; embryos exposed to hypoxia grew less than normoxic and hyperoxic exposed embryos. Moreover, there was no significant interaction between the effect of oxygen levels and presence or absence of the chorion on growth after hatching. These results suggest that the effect of oxygen on growth was not affected by the presence of the chorion.   © 2005 The Fisheries Society of the British Isles

Key words: dechorionated; diffusion; dissolved oxygen; hyperoxia; hypoxia.

INTRODUCTION

Temperature, oxygen and egg size are factors that affect growth in fishes during the period between fertilization and hatch (Blaxter, 1969; Kamler, 1992). Rainbow trout *Oncorhynchus mykiss* (Walbaum) prefer to spawn in areas of streams with gravel and cobble to areas with silt or clay and areas that were shallower and had higher water velocities (Workman *et al.*, 2004). Rainbow trout embryos require different amounts of oxygen during development with the lowest requirement a few days post-fertilization (*c.* 2 mg l^{-1}), gradually increasing to 8·9 mg l^{-1} at 9° C during the alevin stage (Rombough, 1988*a*). Not much is known about the relation of these requirements to what is actually experienced in the wild because there are few measurements of oxygen levels within the gravel beds of natural redds.

Despite limited data, it is known that oxygen levels vary considerably in natural salmonid redds. For example, oxygen levels range between 2·9 and 10·9 mg l^{-1} in chum salmon *Oncorhynchus keta* (Walbaum) (Peterson & Quinn, 1996), from 1·4 to 12·3 mg l^{-1} in anadromous brown trout *Salmo trutta* L. (Ingendahl, 2001) and from 2·6 to 9·25 mg l^{-1} in steelhead trout (*O. mykiss*)

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redds (Coble, 1961). Low dissolved oxygen does alter survival under certain circumstances and therefore may limit growth of developing embryos in the field.

In fish embryos, gas exchange occurs primarily across the skin because the gills, although evident early in development, do not become functional until well after hatching (Rombough, 1992; Wells & Pinder, 1996). The chorion or egg capsule is an acellular layer made up of proteins and glycoproteins, and one of its functions is to protect the developing embryo from external environmental conditions (Cotelli *et al.*, 1988). The controversy regarding the role of the chorion as a barrier to the diffusion of oxygen has been reviewed by Rombough (1988*b*). More recent arguments provide indirect or circumstantial evidence rather than being direct tests of the chorion acting as a barrier to oxygen. For example, Matschak *et al.* (1995) suggested that dechorionated embryos had a greater increase in body length than chorionated embryos because the chorion was a physical barrier. They did not, however, test the effect of oxygen level in their studies. The purpose of the present study was to test whether the inhibiting effect of hypoxia on growth was related to the presence of the chorion. Pre-hatch rainbow trout embryos with and without chorions were exposed to either hypoxia, normoxia or hyperoxia for 11 days. Embryonic dry mass was measured on days 25, 27, 30, 33 and 36 days post-fertilization (dpf) and compared between groups and treatments.

MATERIALS AND METHODS

ANIMALS

Rainbow trout embryos were purchased from Rainbow Springs (Thamesford, Ontario, Canada) on the day they were fertilized and were held in continuous-flow incubation trays with mesh bottoms supplied with local well water ($10°$ C, pH $7·9$; water hardness 411 mg l^{-1} as $CaCO_3$; Ca^{2+}, $2·6$ mmol l^{-1}; Cl^-, $1·5$ mmol l^{-1}; Mg^{2+}, $1·5$ mmol l^{-1}; K^+, $0·06$ mmol l^{-1}; Na^+, $1·1$ mmol l^{-1}) in the Hagen Aqualab, University of Guelph, Guelph, Ontario, Canada. Incubation trays were protected from the light during the entire experiment. Hatching took place between 30 and 35 days dpf. Embryos for the experiment were obtained from three females.

EXPERIMENTAL PROTOCOL

Pre-hatch embryos (25 dpf) were dechorionated by hand using forceps under a binocular microscope. A second group of embryos (25 dpf) was left intact and served as the control group.

Dechorionated and intact (chorionated) embryos were placed in three different treatments: normoxia (nominally 10 mg l^{-1}), hypoxia (5 mg l^{-1}) and hyperoxia (15 mg l^{-1}). Hypoxic water was produced by adding nitrogen which displaced oxygen; hyperoxic water was produced by adding oxygen. The total gas pressure was measured in all three oxygen treatments and it varied between 100 and 102%; fish were not exposed to nitrogen supersaturation. Oxygen levels were measured with an oxygen meter (YSI, Model 55) every 12 h over the duration of the experiment.

An initial embryo sample was taken at 25 dpf and then six groups of five embryos ($n = 6$) were removed from each treatment at 27, 30, 33 and 36 dpf. Embryos were anaesthetized using MS-222 ($0·15$ g l^{-1}) and the embryonic body was separated from the yolk by hand using forceps. The embryonic body was oven-dried ($80°$ C) to constant mass.

STATISTICAL ANALYSIS

ANOVA was used to test for the effect of the presence or absence of the chorion on growth for each oxygen treatment, and to test for an interaction between oxygen concentration and presence or absence of a chorion at 33 and 36 dpf. ANCOVA was used to test for the influence of dissolved oxygen on growth in chorionated embryos, and separately for dechorionated ones. When significant differences were found, a Tukey test was used to identify the differences ($P < 0.05$). Statistical analyses were performed using the Minitab Version 12.1 software package. Results are presented as means $\pm$ CI.

RESULTS

Oxygen concentrations in the water were relatively constant over the duration of the experiment [Fig. 1(a)]. Hatching took place between 30 and 35 dpf and the embryos in the hypoxic group started to hatch 1 day before the normoxic and hyperoxic embryos [Fig. 1(b)]. Mortality was low; <4% in all six groups and unrelated to oxygen concentration.
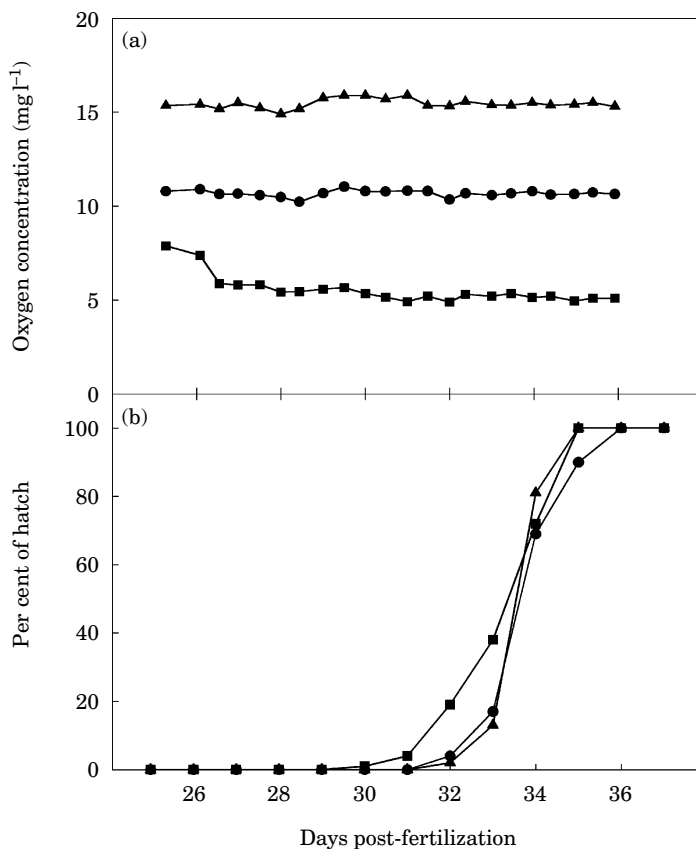

FIG. 1. (a) The oxygen concentrations for the three treatments: hypoxia (■) (*c.* 5 mg l^{-1}), normoxia (●) (*c.* 10·5 mg l^{-1}), and hyperoxia (▲) (*c.* 15 mg l^{-1}) during the duration of the exposure experiment. (b) Time of hatching of the chorionated embryos exposed to hypoxia (■), normoxia (●) and hyperoxia (▲).

In all six groups, body mass increased over the 11 day period. In each of the three oxygen treatments the dechorionated embryos increased in mass more than the chorionated ones (ANOVA, $n = 6$ per treatment, $P < 0.0001$). In the hypoxic group, the dechorionated embryos weighed 35% more than chorionated controls at 36 dpf and in the normoxic and hyperoxic groups the dechorionated embryos weighed 60% more than chorionated controls.

There was no difference in mass between embryos exposed to normoxia and hyperoxia in either chorionated embryos [Tukey, $P = 0.37$; Fig. 2(a)] or dechorionated embryos [Tukey, $P = 0.99$; Fig. 2(b)]. The mass of the hypoxic group, however, was significantly less than the normoxic and hyperoxic groups in both chorionated [Tukey, $P < 0.0001$; Fig. 2(a)] and dechorionated embryos [Tukey, $P < 0.0001$; Fig. 2(b)]. In chorionated embryos, the hypoxic group weighed 31% less than the normoxic or hyperoxic groups at 36 dpf; in dechorionated
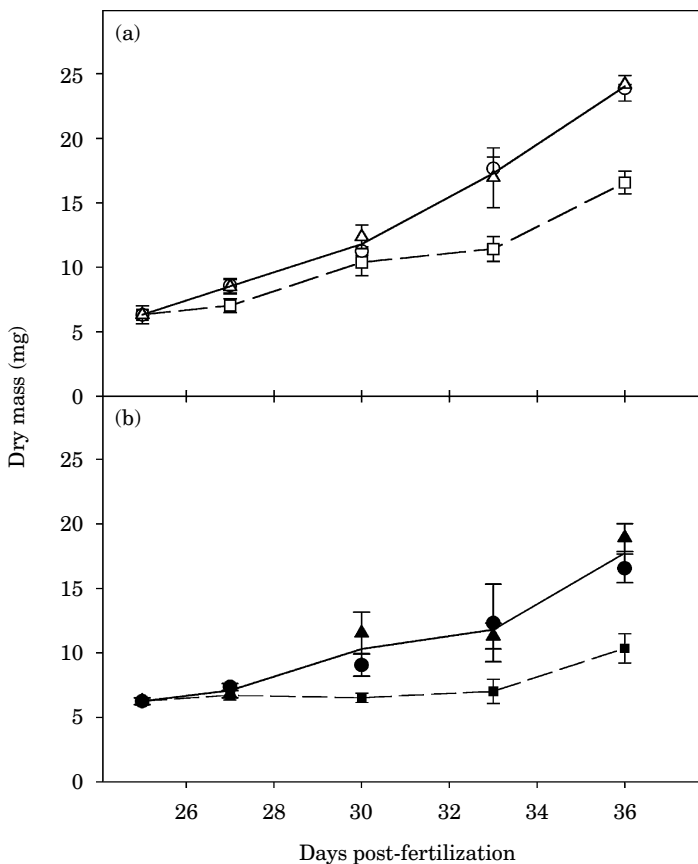

FIG. 2. Means ± CI of pooled dry mass of five embryos ($n = 6$) exposed to three different oxygen treatments: hypoxia (■ □), normoxia (● ○) and hyperoxia (▲ △) of (a) dechorionated (□, ○, △) and (b) chorionated (■, ●, ▲) embryos. There was no significant difference between hyperoxia and normoxia in both (a) chorionated (Tukey, $P = 0.37$) and (b) dechorionated (Tukey, $P = 0.99$) embryos, therefore the average (—) values between the two are given. Growth of the hypoxic group was significantly lower than both normoxic and hyperoxic exposed groups (Tukey, $P < 0.0001$) in chorionated as well as dechorionated embryos.

embryos, the hypoxic group weighed 42% less than the normoxic or hyperoxic groups.

The effect of oxygen concentration was virtually the same for chorionated and dechorionated embryos (Fig. 3). That is, the interaction of oxygen concentration × dechorionated/chorionated was not significant at either 33 dpf (ANOVA, $F_{2,30}$, $P = 0.65$) or at 36 dpf (ANOVA, $F_{2,30}$, $P = 0.08$).

DISCUSSION

Mortality was low and not related to oxygen concentration in the present experiment perhaps because the lowest level of oxygen (5 mg l^{-1}, 49% saturation) was above the critical oxygen level for these embryos (Rombough, 1988*a*). Hypoxia induced early, as well as prolonged hatching, while there was no difference in hatch timing between normoxia and hyperoxia-exposed
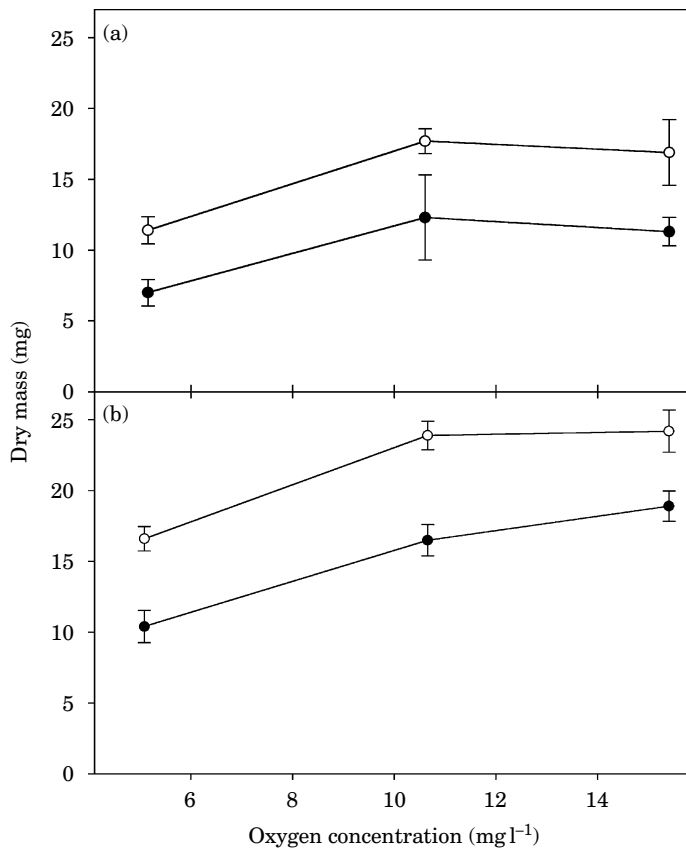

FIG. 3. Means ± CI of pooled dry mass of five embryos ($n = 6$) exposed to three different oxygen treatments: hypoxia, normoxia, and hyperoxia of dechorionated (○) and chorionated (●) at (a) 33 and (b) 36 days post-fertilization. The interaction between oxygen concentration and presence or absence of the chorion was not significant at either 33 dpf (ANOVA, $P = 0.65$) or 36 dpf (ANOVA, $P = 0.08$).

embryos. Previous studies have reported earlier hatching in fish embryos exposed to hypoxia (Latham & Just, 1989; Oppen-Berntsen *et al.*, 1990; Helvik & Walther, 1993), and delayed hatching in fish embryos exposed to hyperoxia (DiMichelle & Taylor, 1980; Latham & Just, 1989). The oxygen concentrations in studies showing delayed hatching in hyperoxia were 28 mg l^{-1} (DiMichelle & Taylor, 1980) and 28·7 mg l^{-1} (Latham & Just, 1989), much higher than the 15 mg l^{-1} used in the present experiment.

Dechorionated embryos grew faster than chorionated ones in all three oxygen treatments, indicating that the presence of the chorion altered growth rates. Similar results have been found in Atlantic salmon *Salmo salar* L. (Matschak *et al.*, 1995). Matschak *et al.* (1995) showed that dechorionated embryos grew longer and also had a greater white muscle cross sectional area than chorionated ones. There are three probable explanations for this effect. First, the difference between chorionated and dechorionated embryos could be due to a loss of factors that inhibit growth, such as hormones and growth factors (Matschak *et al.*, 1995, 1998), or reduction of ammonia that may accumulate inside the chorion during development (Wright *et al.*, 1995). There are, however, conflicting reports on the effect of ammonia on growth in fishes. In sole *Solea solea* (L.) and turbot *Scophthalmus maximus* (L.), ammonia as low as 8 μmol l^{-1} inhibited growth (Alderson, 1979; Person-Le Ruyet *et al.*, 1997), whereas in rainbow trout a 14 month exposure to 70 μmol l^{-1} resulted in an increase in growth (Linton *et al.*, 1998). Second, the chorion could inhibit growth by restricting spontaneous movements of the embryos. Dechorionated rainbow trout embryos move more than chorionated ones (M. Ninness, E.D. Stevens & P.A. Wright, unpubl. data). This increased movement over time could produce an exercise effect that stimulates growth. Third, the chorion might limit oxygen diffusion from bulk water to the embryo.

This third possibility was tested in the present experiment by comparing the effect of oxygen concentration separately in chorionated and dechorionated embryos. In both dechorionated and chorionated embryos, there was no significant difference in mass between the normoxic and hyperoxic groups, although these were both significantly different from the hypoxic group. This indicates a retardation of growth in hypoxic conditions, probably not due to the presence of a chorion because the same pattern was observed for dechorionated and chorionated embryos. The conclusion that the chorion is not a barrier to oxygen was supported by the fact that consistent results were found at both post-hatch sampling times (33 and 36 dpf); the effect of oxygen concentration was the same on chorionated embryos as on dechorionated embryos. Other studies also have reported that hypoxia slowed growth (Silver *et al.*, 1963; Hamor & Garside, 1977). The mechanism involved in slower growth in hypoxia involves a series of molecular, biochemical and physiological responses, such as metabolic depression, channelling energy to essential metabolic processes, and down-regulation of genes involved in protein synthesis and locomotion (Wu, 2002). These responses can manifest themselves in growth reduction. Gracey *et al.* (2001) found that in the goby *Gillichthys mirabilis* Cooper some genes involved in the suppression of cell growth were induced by hypoxia.

Embryos exposed to hypoxia grew less than those exposed to normoxia and hyperoxia in both dechorionated and chorionated embryos; therefore, in well irrigated embryos the effect of oxygen was independent of the presence of the

chorion at oxygen concentrations >5 mg l^{-1}. The chorion might act as a barrier to oxygen diffusion at oxygen tensions below that tested in the study or in slow moving water. The observation that the chorion was not a diffusion barrier at these levels of hypoxia does not preclude the possibility that there may have been subtle developmental changes that were not measured. Yet to be tested are the two other possible explanations for the observations that dechorionated embryos grow faster than chorionated embryos. The faster growth could be related to absence of growth inhibition factors that are retained by the chorion or could be related to the increased activity of dechorionated embryos.

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