Critical partial pressures of oxygen causing precocious hatching in Coregonus lavaretus and C. albula embryos

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Abstract

Embryos of whitefish (Coregonus lavaretus) and vendace (C. albula) were exposed to various hypoxic conditions at constant temperatures of 8°C and 11°C at the developmental stages of “eye movement visible” and “first embryos hatched”.

Eggs exposed to hypoxia responded with precocious hatching and the response depended on the degree of hypoxia, test temperature, and developmental phase. The calculated critical partial pressures of oxygen (pO₂) causing precocious hatching at 8°C were 40 mm Hg (3.0 ppm dissolved oxygen concentration—DO) for whitefish and 28 mm Hg (2.1 ppm DO) for vendace embryos. The sensitivity of embryos to hypoxic stress increased rapidly as development progressed. Eventually, the critical pO₂ for vendace eggs increased to 81 mm Hg (6.0 ppm DO) at the stage of “first embryos hatched”.

Higher temperatures caused stronger response of embryos to hypoxia: exposure of whitefish embryos for 60 min to pO₂ of 3 mm Hg (0.2 ppm DO) at 8°C resulted in hatching of 43% of eggs, whereas at 11°C, hatching increased to 95% (at the same oxygen concentration).

Adequate DO concentrations must be provided in incubation to prevent early hatching and increased mortality. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Critical partial pressure; Oxygen; Coregonus lavaretus

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1. Introduction

In natural hatching of teleosts, the dissolution of the chorion is brought about by the action of a proteolytic hatching enzyme (chorionase), secreted by hatching gland cells (HGCs) of the embryo (Yamagami, 1981; Schoots, 1982). Enzymatic digestion of the zona radiata interna is followed by rupturing of the zona radiata externa due to movements by the embryo (Yamamoto and Yamagami, 1975).

Hatching is not closely connected with a particular stage of embryonic development (DiMichele and Taylor, 1981). Some environmental factors, such as temperature, salinity or pH, strongly influence timing and course of the hatching process. One of the most important factors that triggers chorionase secretion is an inadequate supply of oxygen to the embryo. Delivery of oxygen to developing embryos is achieved both by diffusion and by entry of oxygen dissolved in the water into the egg as a result of the osmotic gradient (Daykin, 1965; O’Brien et al., 1978; Alderdice et al., 1979, 1984).

In the final stages of egg incubation, oxygen consumption of the well-developed and active embryo reaches its maximum (Braum, 1973; Kioerboe and Moehlenberg, 1987; Rombough, 1988). Any environmental factor limiting dissolved oxygen availability to the pre-hatching embryo as well as presence of the chorion imposes a stress, which often results in precocious hatching. Finally, a precociously hatched embryo escapes from the gas exchange limitations imposed by the egg capsule (Alderidge et al., 1958).

This paper reports the experimental exposure of the eggs of vendace (Coregonus albula) and whitefish (C. lavaretus) to hypoxic conditions. The response of embryos was assessed by their precocious hatching, correlated with critical partial pressures of oxygen causing precocious hatching, differences in time of egg exposure, and the stage of embryonic development at the time of exposure.

2. Materials and methods

Eyed eggs of vendace and whitefish were obtained from a commercial hatchery, transported to the laboratory and acclimated for a week to two constant test temperatures: 8°C and 11°C. Eggs were incubated in small (100 ml) incubation jars supplied with air-saturated water at a rate adjusted to cause a slight movement of eggs.

Experimental exposures of eggs to hypoxia were carried out in the test chambers, which were glass columns 1 cm in diameter and 12 ml capacity. Water of desired oxygen concentration, prepared by bubbling nitrogen through the water column (Bardega et al., 1992), flowed to three test chambers through glass pipes submerged in a thermoregulated water bath. Water flow (4, 8, 9, 11 or 15 ml min⁻¹) was adjusted according to experimental needs, and flow stability was controlled by peristaltic pump. Three control chambers received air-saturated water.

Dissolved oxygen concentration (DO) was measured (to 0.03 ppm) using the modified Winkler method. Water samples for measurement of dissolved oxygen concentrations were taken from the outflow of the storage bottle and from the outflow of each test chamber.
Table 1

Description of *C. albula* developmental stages used for the determination of embryonic development, and time in days (from fertilisation) required for *C. albula* embryos to reach successive developmental stages when incubated in constant temperatures after Luczynski and Kirklewska, 1984

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Stage description</th>
<th>Mean incubation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>DS 9</td>
<td>Erythroblast circulation visible</td>
<td>73</td>
</tr>
<tr>
<td>DS 10</td>
<td>Stellate chromatophores along entire</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>length of body, except head</td>
<td></td>
</tr>
<tr>
<td>DS 11</td>
<td>Stellate chromatophore on head</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>between auditory vesicles</td>
<td></td>
</tr>
<tr>
<td>DS 12</td>
<td>Pectoral fin movement visible</td>
<td>117</td>
</tr>
<tr>
<td>DS 13</td>
<td>Eye movement</td>
<td>130</td>
</tr>
<tr>
<td>DS 14</td>
<td>10% embryos hatched</td>
<td>163</td>
</tr>
<tr>
<td>DS 15</td>
<td>50% embryos hatched</td>
<td>171</td>
</tr>
<tr>
<td>DS 16</td>
<td>90% embryos hatched</td>
<td>178</td>
</tr>
</tbody>
</table>

Samples of 100 eggs were placed in each of three test and three control chambers. All eggs were checked individually under a low-power stereo-microscope to choose embryos of the same developmental stage with no visible malformations (Table 1). The eggs were exposed for 15, 30, 45, 60 or 90 min, after which they were removed from the chambers, divided into two lots of 50 individuals each, and placed into separate incubation jars. The divided eggs were kept for 5 h after the exposure, and hatching embryos and dead eggs were removed and counted every 30 min. One batch of eggs was used for one exposure. As some control embryos also hatched precociously in response

Fig. 1. Percentage of hatched whitefish (*C. lavaretus*) embryos at the developmental stage of "eye movement visible" 5 h after exposure to hypoxia for 15 to 60 min in 11°C (water flow rate 5 ml min⁻¹).
to handling (Fig. 1), the percentage of precociously hatched embryos in response to the hypoxia stress was calculated according to the formula:

\[ P = E - C \]

where: \( P \) — percent of precociously hatched embryos in response to the hypoxia, \( E \) — percent of embryos hatched in the experimental groups, \( C \) — percent of embryos hatched in the respective control groups.

Regression equations were calculated to estimate the critical partial pressures of oxygen causing precocious hatching.

3. Results

After exposure to normoxia or hypoxia, all embryos were alive and showed no recognizable malformations. Whitefish and vendace embryos subjected to low partial pressures of oxygen (\( pO_2 \)) hatched precociously and the rate of hatching depended on the degree of hypoxia. Most embryos hatching in response to hypoxia emerged between 60 and 120 min after their exposure (almost all responding embryos hatched within 5 h) (Fig. 1).

The percentage of precociously hatched embryos increased with prolonged exposure to hypoxia (Fig. 1). The rate of precocious hatching of whitefish embryos at the developmental stage of “eye movement visible” was highest (\( \bar{x} = 42\% \)) in eggs exposed to 7 mm Hg \( pO_2 \) (0.5 ppm DO). There was practically no hatching (\( \bar{x} = 2.5\% \)) in eggs exposed to 40 mm Hg \( pO_2 \) (3.0 ppm DO). Fig. 2 shows that the critical partial pressures of oxygen (\( C_{pO_2} \)) causing precocious hatching in whitefish embryos at “eye movement” stage exposed for 60 min to different \( pO_2 \) at 8.0°C, was about 40 mm Hg (3.0 ppm DO).

Vendace embryos at the “eye movement” stage hatched precociously (\( \bar{x} = 43\% \)) after exposure to 3 mm Hg \( pO_2 \) (0.2 ppm DO), and the reaction ceased at 27 mm Hg \( pO_2 \) (2.0 ppm DO; \( \bar{x} = 2\% \) hatched). The \( C_{pO_2} \) for vendace embryos was estimated as about 28 mm Hg (2.1 ppm DO, Fig. 2).

The sensitivity of vendace embryos to low \( pO_2 \) at the later stage of development (“first embryos hatched”) was greatly increased compared to embryos at the “eye movement” stage. The rate of precocious hatch averaged 84\% at 7 mm Hg \( pO_2 \) (0.5 ppm DO) and most of the embryos emerged within 60 min after exposure. The response ceased at 74 mm Hg \( pO_2 \) (5.5 DO; \( \bar{x} = 9\% \) hatched), and the \( C_{pO_2} \) was estimated at 81 mm Hg (6.0 ppm DO; Fig. 2).

Increased mortality was observed in vendace embryos exposed to hypoxia for 60 min and was even higher after 90 min of exposure. Closer examination of the eggs showed that the embryos responded by secretion of the hatching enzyme, because the \( zona radiata interna \) was completely digested. However, after prolonged hypoxia the embryos were too weak to move and they could not disrupt the remaining \( zona radiata externa \). Finally, the embryos were dying within the thin and delicate residual layer of egg.
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Fig. 2. Percentage of precociously hatched embryos of whitefish (*C. lavaretus*) and vendace (*C. albula*) at different developmental stages in response to 60-min exposure to various hypoxic conditions in water temperature 8°C. Points represent observed values corrected for handling (% of embryos hatched in experimental group – % of hatched controls). Lines are plotted according to the respective regression analysis equations: *C. lavaretus* DS 13: \[ y = 42.468 - 1.0718x \], \( r^2 = 0.8446 \); *C. albula* DS 13: \[ y = 46.569 - 1.6935x \], \( r^2 = 0.8818 \); *C. albula* DS 14: \[ y = 82.571 - 1.0221x \], \( r^2 = 0.9045 \).

envelope which adjusted its shape to the embryo’s body thereby causing an irregular outline characteristic for such eggs.

4. Discussion

Developing eggs act as an ‘oxygen sink’, so that even at high flow velocities oxygen concentration at the egg surface may be much less than that of the surrounding water (Daykin, 1965). Oxygen level decreases through the egg envelope and perivitelline fluid towards the embryo’s tissues (Berezovsky et al., 1979), although fin and body movements of the developing embryo aid in circulating the perivitelline fluid, reducing the steepness of the oxygen gradient across the perivitelline fluid. The \( pO_2 \) gradient across the egg membrane increases in the course of embryonic development and is positively correlated with the weight of the embryo and the rate of its metabolism (Silver et al., 1963). The limiting values of \( pO_2 \) and the velocity of surrounding water are not constant, but are related to each other as well as to the oxygen uptake (which in turn is temperature dependent), and to the egg diameter (Daykin, 1965).

In the final stages of development, teleost embryos already possess mature hatching gland cells (HGCs) containing hatching proenzyme (Iuchi and Yamagami, 1976; Yamamoto et al., 1979). The number of hatching gland cells (HGCs) in coregonid embryos
increases from the beginning of developmental stage (DS) 9 (eyed egg stage) to DS 11 (stellate chromatophores on head) (Luczynski and Kirklewieska, 1984) and reaches a maximum lasting until hatching (DS 15) (Luczynski and Ostaszewska, 1991). However, the amount of hatching proenzyme stored within HGCs increases constantly up to hatching time. Thus, maturation of the hatching gland apparatus involves two parallel processes: increase in the number of HGCs to a certain level and continuous increase of the amount of chorionase (Luczynski and Ostaszewska, 1991). With chorionase stored in mature HGCs, coregonid embryos are capable of escaping from their egg envelope long before natural hatching time (Luczynski and Kolman, 1987).

Oxygen uptake by well-developed and active embryos reaches its maximum just before hatching (Braum, 1973; Kioerboe and Moehlenberg, 1987; Rombough, 1988). At this time, even temporary exposure to hypoxia causes precocious hatching. As more developed embryos possess larger amounts of the hatching enzyme, their hatching process was more effective, and the percentage of embryos hatched in response to hypoxia stress was higher than that in the less developed eggs (Fig. 2).

Salmonid embryos (also at final stages of development) tolerate wide fluctuations in dissolved oxygen concentration. However, below certain levels of \( pO_2 \) they suffer from insufficient oxygen supply and tend to hatch prematurely (Alderdice et al., 1958). The lowest \( pO_2 \) supporting satisfactory oxygen supply to developing embryos can be defined as the critical partial pressure of oxygen (\( CpO_2 \)) below which embryonic metabolism is disturbed to such an extent that secretion of chorionase ensues and is followed by precocious hatching. It was evident that the \( CpO_2 \) increased with developmental advancement (Fig. 2) because of increased oxygen requirements of larger and more active embryos (Braum, 1973) and was accompanied by a more efficient mechanism for chorionase secretion (DiMichele and Taylor, 1981; Oppen-Berntsen et al., 1990).

The effect of lowered \( pO_2 \) level was more pronounced at higher temperatures because the rate of embryonic metabolism is temperature dependent (Hamor and Garside, 1976; Gehrke, 1988). Increasing the temperature from 8°C to 11°C doubled precocious hatching of whitefish embryos at the developmental stage of “eye movement” (DS 13) exposed to the same hypoxia stress after both 30- and 60-min exposures (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>30-min exposure</th>
<th>60-min exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8°C</td>
<td>11°C</td>
</tr>
<tr>
<td>Water flow (ml min(^{-1}))</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Partial pressure of oxygen in control water (mm Hg)</td>
<td>167.5</td>
<td>no data</td>
</tr>
<tr>
<td>Partial pressure of oxygen in test water (mm Hg)</td>
<td>2.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Dissolved oxygen concentration in control water (ppm)</td>
<td>12.5</td>
<td>no data</td>
</tr>
<tr>
<td>Dissolved oxygen concentration in test water (ppm)</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Precocious hatch in control group (%)</td>
<td>0</td>
<td>no data</td>
</tr>
<tr>
<td>Precocious hatch in test group (%)</td>
<td>2</td>
<td>45</td>
</tr>
</tbody>
</table>
Precocious hatching of embryos in response to hypoxic conditions should be considered as an extreme reaction, enabling embryos to escape from unfavourable oxygen conditions. However, at a certain range of low $pO_2$, embryos still do not hatch, even though they are suffering from insufficient oxygen supply. Observations of a bradycardia reaction revealed that at 8°C the limiting role of oxygen occurs at a $CpO_2$ as high as 54 mm Hg (4.0 ppm DO) for whitefish eggs and 62 mm Hg (4.6 ppm DO) for vendace (Czerkies, unpublished data).

Extended exposure to hypoxia disturbed the course of hatching in vendace embryos; they secreted chorionase but were not capable of rupturing the remaining layer of the indigestible zona radiata externa. This has never been observed in whitefish eggs.

The critical partial pressures causing precocious hatching, found in this study, should be considered as the lowest tolerable limits of oxygen concentration in incubation water during the final stages of development of Coregoninae embryos.

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