Comparative Biochemistry and Physiology, Part A 213 (2017) 28–35

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

# Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins

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ARTICLE INFO

*Keywords:*
Atlantic salmon
Hypoxia
Temperature
Acclimation
Metabolic compensation

ABSTRACT

Hypoxia is common in aquatic environments and has substantial effects on development, metabolism and survival of aquatic organisms. To understand the physiological effects of hypoxia and its dependence on temperature, metabolic rate ($\dot{M}_{O_2}$) and cardiorespiratory function were studied in response to acute hypoxia (21 → 5 kPa) at different measurement temperatures ($T_a$; 4, 8 and 12 °C) in *Salmo salar* alevins that were incubated under normoxic conditions ($P_{O_2}$ = 21 kPa) or following hypoxic acclimation ($P_{O_2}$ = 10 kPa) as well as two different temperatures (4 °C or 8 °C). Hypoxic acclimation lead to a developmental delay manifested through slower yolk absorption. The general response to acute hypoxia was metabolic depression (~60%). Hypoxia acclimated alevins had higher $\dot{M}_{O_2}$s when measured in normoxia than alevins acclimated to normoxia. $\dot{M}_{O_2}$s were elevated to the same degree (~30% per 4 °C change) irrespective of $T_a$. Under severe, acute hypoxia (~5 kPa) and irrespective of $T_a$ or acclimation, $\dot{M}_{O_2}$s were similar between most groups. This suggests that despite different acclimation regimes, $O_2$ transport was limited to the same degree. While cardiorespiratory function (heart-, ventilation rate) was unchanged in response to acute hypoxia after normoxic acclimation, hypoxic acclimation led to cardiorespiratory changes predominantly in severe hypoxia, indicating earlier onset and plasticity of cardiorespiratory control mechanisms. Although $\dot{M}_{O_2}$ in normoxia was higher after hypoxic acclimation, at the respective acclimation $P_{O_2}$, $\dot{M}_{O_2}$ was similar in normoxia and hypoxia acclimated alevins. This is indicative of metabolic compensation to an intrinsic $\dot{M}_{O_2}$ at the acclimation condition in hypoxia-acclimated alevins after re-exposure to normoxia.

## 1. Introduction

Hypometabolism has been described as a widespread response to hypoxia throughout the animal kingdom (Mortola et al., 2012; Pelster, 2003; Richards, 2009), and chronic hypoxia has been shown to delay developmental rates in fish (Bagatto, 2005; Ciuhandu et al., 2005; Hamor and Garside, 1976; Shumway et al., 1964). This has generally been regarded as an adaptive response to reduce the $O_2$ demand (Miller et al., 2008) causing reduced ATP consumption when $O_2$ dependent ATP production cannot be sustained (Richards, 2010). In contrast, the physiological response to chronic hypoxia may be regarded as maladaptive/pathological, as exemplified by reduced performance of fish during development (Widmer et al., 2006). While adult fish generally have been shown to have the capacity to maintain metabolism through anaerobic pathways, the compensatory anaerobic capacity of salmonid embryos and larvae appeared to be small (Gnaiger et al., 1981; Ninness et al., 2006). Similarly, hypoxia reared zebrafish during early development (*Danio rerio*) did not show differences in lactate concentrations to normoxia reared individuals (Barrionuevo et al., 2010).

To meet the $O_2$ demand in acute hypoxia, adult salmonids decreased heart rate ($f_H$, bradycardia) while cardiac output ($\dot{Q}$) was maintained through an increase in cardiac stroke volume (Holeton, 1971; Holeton and Randall, 1967; Randall, 1982; Farrell, 1991; Gamperl and Driedzic, 2009). In contrast to this, salmonid larvae have been reported to either show elevated heart rates (tachycardia) or lack a response to acute hypoxia (Holeton, 1971; McDonald and McMahon, 1977). The absence of a bradycardic response to acute hypoxia could have originated from the lack of cardio-inhibitory control at this developmental stage or an uncoupling of metabolic rate ($\dot{M}_{O_2}$) and $f_H$. A more recent study provided evidence that cardio-inhibitory control is initiated just after hatching in rainbow trout (*Oncorhynchus mykiss*, Miller et al., 2011). In addition, they found that chronic hypoxia elicits bradycardia before

*Abbreviations:* ATP, adenosine triphosphate; bpm, beats per minute; $\beta_{O_2}$, solubility of oxygen; $f_H$, heart rate; $f_V$, ventilation rate; Hb, hemoglobin; kPa, kilopascal; $\dot{M}_{O_2}$, metabolic rate; $P_{O_2}$, partial pressure of oxygen; $T_a$, ambient temperature; $Q_{10}$, the temperature coefficient (the factor by which the rate of a reaction increases for every 10 °C rise in temperature)

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http://dx.doi.org/10.1016/j.cbpa.2017.08.011
Received 10 April 2017; Received in revised form 16 August 2017; Accepted 23 August 2017

hatching, elevates the adrenergic tone and delays the onset of cholinergic control.

In early zebrafish embryos (a hypoxia tolerant, tropical species), $\dot{M}_{O_2}$ decreased without altering $f_H$ under acute hypoxia, suggesting substantial resistance of the heart to hypoxia (Barrionuevo and Burggren, 1999). In contrast, zebrafish larvae (1 day after hatching) exhibited increased $f_H$ and $\dot{M}_{O_2}$ when raised in chronic hypoxia (10 kPa, Jacob et al., 2002) and more severe hypoxia (3 kPa) abated $f_H$ and developmental rate (Bagatto, 2005). In the latter study, the onset of the cardiac adrenergic response was advanced in animals that were developmentally delayed under chronic hypoxia, compared with normoxia.

In fish, developmental plasticity of the gills (gill size, gene expression) in response to hypoxia and ionic disturbance has also been described (Craig et al., 2007; Crispo and Chapman, 2010). This suggests that hypoxia may elicit structural improvements to favor $O_2$ delivery, hence permitting to some degree maintenance of $\dot{M}_{O_2}$, or to protect against a severe drop in $\dot{M}_{O_2}$ should more severe hypoxia be encountered. Although structural changes may mitigate some of the adverse effects of hypoxia, functional ventilatory adjustments may be critical to meet the $O_2$ demand. In most adult teleost fish for example, exposure to hypoxia elicited hyperventilation through an increase in ventilation frequency ($f_V$, Dunn and Hochachka, 1986; Perry et al., 2009; Smith and Davie, 1984). The hyperventilatory response to hypoxia in zebrafish appeared to develop well before 14 days post fertilisation (dpf), suggesting the presence of an $O_2$ sensing mechanism before complete gill development (Jacob et al., 2002; Jonz and Nurse, 2005). In larval fish, $O_2$ chemoreception occurred in gill neuroepithelial cells (NECs) that were involved in the hypoxic ventilatory response (Burleson and Milsom, 1993; Burleson and Smatresk, 1990; Jonz and Nurse, 2006). In zebrafish, NECs were present shortly after fertilisation (5 dpf) and at this stage appeared sensitive to a hypoxic stimulus (Jonz and Nurse, 2006). However, in zebrafish the development of hypoxic ventilatory control was not altered by a chronic hypoxic pre-exposure (Vulesevic and Perry, 2006).

The interplay between hypoxia, temperature and other environmental factors on performance at different life stages in ectotherms has received significant attention over recent years (Burggren et al., 2016; Motani and Wainwright, 2015; Munday et al., 2012; Peck and Moyano, 2016; Pörtner and Farrell, 2008). The rate of development in a range of taxa, which depended on the $\dot{M}_{O_2}$, was therefore also dependent on temperature and increased proportionally with increasing temperature (Gillooly and Dodson, 2000).

In adult fish, cardiorespiratory function also increased with temperature to meet the elevated $O_2$ demand, however a tight coupling between cardiac activity and metabolic requirements at early developmental stages, where the nervous control is not full established, is debated (Pelster, 1999). During embryonic and larval development of salmonids, $\dot{M}_{O_2}$ and $f_H$ increased proportionally with temperature while $\dot{Q}$ remained unchanged (Mirkovic and Rombough, 1998; Rombough, 1988a), suggesting that $f_H$ can be used as a predictor of $\dot{M}_{O_2}$ (Klinkhardt et al., 1987; Benfey and Bennett, 2009). This assumption was somewhat surprising as cutaneous $O_2$ uptake has been thought to predominate at this developmental stage and $O_2$ convection was not considered critical for $O_2$ delivery to the tissues (Burggren, 2004).

Taken together, it has become clearer that the timeframe around hatching in fish is a very dynamic, transitory stage, where the preconditions for the mature cardiorespiratory function is formed, including the physiological response to critical environmental changes such as in temperature or $O_2$ levels. Despite our improved knowledge, a more comprehensive, multifactorial approach to elucidate the interplay of environmental factors on cardiorespiratory function during early ontogeny has been lacking. Therefore, by taking an integrative approach in this study, *S. salar* eggs were incubated at 4 °C and 8 °C and the effects of acute, progressive (21 kPa → 5 kPa) and chronic hypoxia (10 kPa for 15 days immediately post-hatching) exposure and ambient temperature (4, 8, 12 °C) on $\dot{M}_{O_2}$ and cardiorespiratory function ($f_H$ and $f_V$) in newly hatched Atlantic salmon yolk-sac alevins (life stage after hatching, until yolk absorption) were investigated.

It was hypothesised that acute hypoxia (by aggravating adequate $O_2$ supply to meet the demand) would result in a decrease in metabolism, reflected in alterations to $f_H$ and $f_V$, since the preconditions for $O_2$ sensing and chronotropic cardiorespiratory reflexes appear to be functional at early larval stages of fish. Secondly, it was hypothesised that chronic hypoxia will result in a lower $O_2$ demand, compared to normoxic conditions, due to delayed developmental rates and that the responses to hypoxia (either acute or chronic) will be amplified at both higher experimental temperatures and incubation temperatures due to the $Q_{10}$ driven, increased demand for $O_2$.

## 2. Experimental protocol

### 2.1. Fish husbandry and maintenance

*Salmo salar* eyed eggs from a single commercial mass spawning event were collected from the SALTAS Wayatinah Hatchery (Tasmania, Australia) where they had been incubated in continuous-flow incubation trays at 8 °C (standard hatchery temperature) and 4 °C (standard temperature to delay embryonic development for late input smolt and production backup). Eggs acclimated to 8 °C were collected at 312 degree days (39 days at 8 °C post fertilisation) and eggs acclimated to 4 °C at 380 degree days (95 days at 4 °C post fertilisation). In the laboratory they were kept separately similar to hatchery conditions and maintained in well aerated spring water in the dark with a constant temperature set to 8 °C and 4 °C ( ± 1 °C differential, the actual temperatures were 4.4 °C ± 0.07 °C and 8.2 °C ± 0.03 °C), respectively. Immediately after hatching, alevins were separated into two treatment groups for each incubation temperature. One group was kept under normoxic conditions while the other was exposed to chronic hypoxia (10 kPa) for 15 days by bubbling an air/ nitrogen mixture into the covered holding tray using a high precision gas mixer (GF-3/MP, gas mixing flowmeter, Cameron Instruments, Texas, USA).

### 2.2. Treatments and measurements

After the 15-day acclimation period, $\dot{M}_{O_2}$ in normoxia and in response to progressive hypoxia (21 → 5 kPa) was measured in alevins at experimental temperatures of 4, 8 and 12 °C (*Series 1*). At each temperature, the sample size was $n = 40$ for normoxic alevins and $n = 20$ for hypoxia-acclimated alevins. The resulting treatment groups were denoted: $4/8_{norm}$, $8/8_{norm}$, $12/8_{norm}$, $4/4_{norm}$, $8/4_{norm}$, $12/4_{norm}$ and $4/8_{hypo}$, $8/8_{hypo}$, $12/8_{hypo}$, $4/4_{hypo}$, $8/4_{hypo}$ and $12/4_{hypo}$ where the numerator and denominator represent measurement ($T_a$) and incubation temperature respectively and the subscript indicates normoxic (norm) or hypoxic (hypo) acclimation.

In addition, cardiorespiratory responses ($\dot{M}_{O_2}$, $f_H$ and $f_V$) of eight experimental groups ($4/8_{norm}$, $8/8_{norm}$, $4/4_{norm}$, $8/4_{norm}$, $4/8_{hypo}$, $8/8_{hypo}$, $4/4_{hypo}$, $8/4_{hypo}$) to a stepwise challenge of hypoxia (21 → 15 → 10 → 5 kPa) were measured (*Series 2*). Here, sample sizes were $n = 8$ for normoxic animals and $n = 6$ for hypoxia-acclimated groups. These latter experiments were repeated in a control group at 8 °C where the $P_{O_2}$ was kept at normoxic levels and served as a control to preclude temporal artefacts on the parameters measured. $\dot{M}_{O_2}$ here refers to mass-specific $\dot{M}_{O_2}$ (μmol $O_2$ $min^{-1}$ $g^{-1}$) which was calculated based on yolk-free, wet body mass.

Embryos and yolk-sac alevins were killed after an experiment, weighed using a microbalance (Mettler Toledo PR1203, accuracy: 0.001 g), and then the embryo (with yolk-sac) was removed from the egg capsule with micro-operating scissors. Total wet body mass in embryos and yolk-sac alevins was weighed, then the yolk-sac was separated from the alevin body with micro-operating scissors to measure wet yolk-free body and wet yolk mass.