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Effect of acclimation temperature on routine metabolic rate in triploid salmonids

Michelle E. Atkins, Tillmann J. Benfey *

Department of Biology, University of New Brunswick, P.O. Box 4400, Fredericton, New Brunswick, Canada E3B 5A3

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Abstract

The objective of this research was to determine whether triploid fish differ from diploids in their routine metabolic rates across a range of acclimation temperatures. Sibling diploids and triploids were acclimated to 12, 15 and 18 °C (Atlantic salmon; *Salmo salar*) and to 9, 12 and 15 °C (brook charr; *Salvelinus fontinalis*) prior to experimentation. Routine metabolic rates were then determined three times over a two-month period. Triploids of both species had higher metabolic rates than diploids at lower temperatures, and lower metabolic rates than diploids at higher temperatures, demonstrating that triploids have different (i.e., lower) thermal optima than diploids. This likely explains prior observations of high mortality of triploids at chronically elevated, but sub-lethal, rearing temperatures for sibling diploids.

Keywords: Cell size; Genome size; Metabolism; Temperature tolerance; Thermal optimum; Triploidy

1. Introduction

Triploidy can readily be induced in fishes through thermal or pressure treatment of eggs shortly after fertilization, thereby interfering with the completion of meiosis and causing retention of the second polar body (Ihssen et al., 1990; Pandian and Koteeswaran, 1998). The resultant triploids are sterile because meiosis is disrupted in triploid cells. Their sterility makes triploids of interest for aquaculture and fisheries management, as a tool for ensuring that flesh quality is maintained in production fish and that any fish which escape from fish farms cannot interbreed with wild fish or establish feral populations (Benfey, 2001). Although these are demonstrated outcomes of induced triploidy, there has been very little use of triploid populations in aquaculture because of perceived and real limitations in commercially relevant production traits (Benfey, 2006). The underlying causes of these triploid-based deficiencies are unknown, but likely reflect fundamental differences in genetics (heterozygosity and gene dosage) and physiology (cell size and number).

Triploid cells have larger nuclei than diploid cells, with a concomitant increase in cell volume in numerous cell types (Benfey, 1999). Cell numbers are generally reduced in direct proportion to cell size, resulting in normal organ and body size (Benfey, 1999). This relationship is especially well documented for erythrocytes and results in equivalent hematocrits for triploids and diploids (Benfey, 1999). However, although erythrocyte hemoglobin content is also elevated in triploids (due to larger cell size), the effects of triploidy on erythrocyte and total blood hemoglobin concentrations are unclear: some studies have found significant ploidy effects and others have not (Benfey, 1999). Differences in hemoglobin concentration will affect oxygen carrying capacity of the blood, and hence aerobic capacity. However, even in the absence of ploidy-related differences in blood oxygen carrying capacity, changes in cell size may affect rates of respiratory gas exchange across the cell membrane.

Although the cellular and tissue-level changes arising from induced triploidy presumably affect basic physiological processes, there has been no systematic evaluation of physiological responses to changing environments in triploids. For instance, although numerous studies have reported triploid salmonid fishes to have reduced tolerance to elevated rearing temperatures (Ojolick et al., 1995; Mercier et al., 2000; Altimiras et al.,

* Corresponding author. Tel.: +1 506 452 6293; fax: +1 506 453 3583.
E-mail address: benfey@unb.ca (T.J. Benfey).

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2002; Mercier et al., 2002; Hyndman et al., 2003), none have looked at the effects of acclimation temperature on basic physiological processes in sibling diploids and triploids. The aim of this study was to determine whether triploid fish differ from diploid siblings in their routine metabolic rates across a range of acclimation temperatures. Temperature has a profound effect on metabolism in fish: increasing temperature both reduces oxygen solubility and increases oxygen demand. Optimum temperature is dependent on previous thermal experience (Brett, 1979; Jobling, 1994), and is the temperature at which the organism maximizes metabolic efficiency for physiological functions such as growth, feeding and digestion. Thus, differences in metabolic rates at the same acclimation temperatures are indicative of differences in thermal optima.

2. Materials and methods

Diploid and triploid Atlantic salmon (*Salmo salar*) and brook charr (*Salvelinus fontinalis*) of both sexes were used, with triploids obtained by hydrostatic pressure treatment of eggs for 5 min at 65.5×10^3 kPa, applied 20 min after fertilization. The same egg lots were used for triploids and diploids within a species by first fertilizing the eggs and then removing a portion for pressure treatment. Thus diploids and triploids were siblings from the same families within a species. Salmon eggs originated from wild adults captured and spawned at the Fisheries and Oceans Canada (DFO) Mactaquac Biodiversity Facility (French Village, New Brunswick), where all salmon rearing and experimentation was done. Charr eggs were obtained from domesticated adults held in the aquaculture facilities of University of New Brunswick (Fredericton, New Brunswick), where all charr rearing and experimentation was done. The ploidy of individual fish was determined by measuring erythrocyte size (Benfey et al., 1984). Standard fish culture techniques (according to location and species) were used to rear the fish prior to experimentation. As salmon and charr were reared in different locations using different experimental protocols, no between-species comparisons are made in this paper.

Salmon were randomly sorted into duplicate 350 L circular fiberglass tanks as 1+ parr according to ploidy and temperature regime at a stocking density of 18–23 g/L, for a total of 12 tanks. All the tanks were set up in a single empty smolt production tank that was covered with a roof but exposed to natural daylight and photoperiod. Fish were fed to satiation twice daily and were maintained in these tanks for 1 month prior to temperature acclimation. Water temperature was then increased by 1 °C/day from ambient (10 °C) to 12, 15 or 18 °C using immersion heaters held in three separate head tanks. Temperature was controlled by computer (Controls and Equipment, Fredericton, New Brunswick) to ±1 °C of the desired temperature regime. Fish were allowed to acclimate to their experimental temperatures for 1 month prior to experimentation. Water flow was maintained at 1 L/kg fish/min. Rearing tanks were covered with clear plastic 3 days prior to experiments in order to prevent atmospheric gas exchange. Routine metabolic rate was determined from measurements of oxygen concentration in the out-flow of the sealed rearing tanks and in respective head tanks, using a PT4 multi-channel oxygen meter (Point Four Systems Inc., Vancouver, Canada). Oxygen concentration was measured every 15 min for a total of 96 readings per day. All tanks of the like temperature were used as flow through respirometers at the same time (i.e., 4 tanks at a time, representing duplicate diploid and triploid tanks at a given temperature). Respirometry trials began at 13:00 (±0.5 h) for all 3 rearing temperatures and all 3 trials, and lasted 72 h. However, only data from the last 24-hour period are presented as fish were most acclimated to the experimental set up at this time. Feeding to satiation was continued twice daily through sealed funnels, and tanks and probes were cleaned daily to prevent fouling and to ensure clean flow-though drainage. Respirometry trials occurred 3 times over a 2-month period, in October, November and December.

Charr were randomly sorted into duplicate 50 L plastic circular tanks according to ploidy and temperature regime at a stocking density of 23–28 g/L, again for a total of 12 tanks. The tanks were in an indoor facility where they were exposed to artificial lighting but with a seasonally adjusted photoperiod. Fish were allowed 1 week to habituate before acclimation to temperature regimes. Water temperature was changed from 12 °C (ambient) to 9 or 15 °C in 1 day. Head tank water temperature was maintained by immersion heater (±1.5 °C) or chiller (±0.5 °C) controlled by thermostat. Fish were fed to satiation twice daily and acclimated for 1 month prior to experimentation. Rearing tanks were used as flow through respirometry tanks by placing a PT4 multi-channel oxygen probe in the out-flow drainage pipe and comparing that value to that of the respective head tank. Oxygen concentration measurements were recorded every 15 min for 72 h but, as for the salmon, only data from the last 24 h are presented. Tanks of same temperature were sampled at the same time. Tanks were not covered as the difference between head tank oxygen concentration and that of a blank tank (see below) was less than 1.5%. To ensure that fish were not breaching the surface to obtain oxygen, fish were observed for 20 min per day for 3 consecutive days. It was determined that fish were not breaching the surface other than to feed. Feeding to satiation was continued twice daily during the experimental period, and tanks and probes were cleaned daily to prevent fouling and to ensure clean drainage. Respirometry trials were conducted in November, December and January.

Routine metabolic rate ($\dot{M}O_2$) was determined using the formula

$$\dot{M}O_2(\text{mg O}_2/\text{kg fish/h}) = \frac{(\Delta[O_2] \times Vw)_{\text{fishtank}} - (\text{BOD} \times Vw)_{\text{blanktank}}}{W}$$

where Δ [O_2] is the difference in dissolved oxygen concentration between the in-flow and out-flow water supplies (mg O_2/L), Vw is the flow rate (L/h), BOD is the biological oxygen demand (mg O_2/L) of a tank without fish and W is the total fish biomass. BOD was determined by measuring oxygen consumption against a "blank" tank that normally contained fish, but from which they had been removed, and was representative of normal