Oxygen and carbon dioxide transport during sustained exercise in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*)

Nicholas J. Bernier, Colin J. Brauner, John W. Heath, and David J. Randall

Abstract: To better understand the respiratory physiology of triploid fish, we conducted an analysis of O_2 and CO_2 transport in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*) swimming at 0.4 body lengths (BL)·s^{-1}, at 2.0 BL·s^{-1}, and at the critical swimming velocity (Ucrit). While O_2 consumption rates (MO_2), MO_2 max, and Ucrit did not differ between ploidies, triploids had a smaller increase in MO_2 over the course of the swimming trial and lower arterial O_2 content (C_aO_2) values than diploids. Relative to diploids, triploids swimming at Ucrit had a reduced Hb–O_2 saturation, lower red blood cell (RBC) pH, but similar O_2 partial pressures (P_aO_2) and methemoglobin values. Overall, triploids and diploids did not differ in C_aCO_2, P_aCO_2, arterial pH, or lactate at any of the swimming speeds. Taken together, triploidy does not appear to impair CO_2 transport or acid–base balance during sustained exercise in chinook salmon. In contrast, our results show that triploids have a smaller O_2 carrying capacity than diploids. While triploids may be able to compensate for their reduced aerobic capacity under the current exercise regime, we suggest that the effects of triploidy on O_2 transport may contribute to the inferior performance of triploid salmon when reared under suboptimal conditions.

Résumé : Afin de mieux comprendre la physiologie respiratoire des poissons triploïdes, nous avons analysé le transport d'O_2 et de CO_2 chez des saumons quinnat (*Oncorhinchus tshawytscha*) diploïdes et triploïdes qui nagent à une vitesse égale à 0,4 leur longueur corporelle (BL)·s^{-1}, à 2,0 BL·s^{-1} et à la vitesse critique de nage (Ucrit). Bien que les taux de consommation d'O_2 (MO_2), MO_2 max et Ucrit ne varient pas entre les deux groupes, les poissons triploïdes subissent une augmentation plus faible de MO_2 et un contenu artériel d'O_2 (C_aO_2) moins élevé que les diploïdes au cours des essais de nage. Par comparaison avec les poissons diploïdes, les triploïdes nageant à Ucrit ont une saturation réduite d'Hb–O_2 et un pH des érythrocytes (RBC) plus bas, mais des pressions partielles d'O_2 (P_aO_2) et des concentrations de méthémoglobine semblables. À toutes les vitesses de nage, les deux groupes n'affichent généralement pas de différences de C_aCO_2, de P_aCO_2, de pH artériel, ni de lactate. Dans l'ensemble, la triploïdie ne semble pas détériorer le transport de CO_2, ni l'équilibre acide–base durant l'exercice soutenu chez le saumon quinnat. En revanche, les poissons triploïdes ont une une capacité de transport de l'O_2 plus faible que les diploïdes. Bien que les poissons puissent compenser pour leur capacité aérobie réduite dans le programme d'exercice actuel, nous pensons que les effets de la triploïdie sur le transport d'O_2 peut contribuer à la performance inférieure des saumons triploïdes élevés dans des conditions suboptimales.

[Traduit par la Rédaction]

Introduction

Developed as a technique to control sexual development in fish in the late 1970s, triploidy can be induced to effectively produce sterile salmonids (Donaldson and Hunter 1982; Thorgaard 1983). In addition to the economic advantages of chromosome set manipulation for maintaining the flesh quality of immature fish, the sterility of triploids may offer an effective means of reducing the genetic impact of cultured fish on wild populations. Aside from having larger but fewer cells and impaired gametogenesis, triploids are remarkably similar to diploids at the whole-animal level (see Benfey 1999 for review). However, despite the similarities between triploids and diploids and their potential advantages in culture, the production of triploid fish has not gained acceptance as a standard practice in the salmonid aquaculture industry (Withler et al. 1998; Benfey 2001). In general, the performance of triploids is inferior to diploids when reared under suboptimal environmental conditions, and the cause for this physiological difference is not understood (Benfey 1999).

Although the aerobic (Small and Randall 1989; Stillwell and Benfey 1996, 1997) and anaerobic (Hyndman et al. 2003*a*) capacities of diploid and triploid salmonids appear to be similar when assessed at cool temperatures, several studies have shown that triploids have reduced survival rates

Received 25 September 2003. Accepted 6 April 2004. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 24 November 2004.
J17763

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 doi: 10.1139/F04-110

when reared or exercised at elevated temperatures (Ojolick et al. 1995; Altimiras et al. 2002; Hyndman et al. 2003*b*). Therefore, while subtle physiological differences between diploids and triploids may be without consequence when metabolic conditions are favourable, they may compromise the physiological performance of triploids when O_2 availability is low and energetic demands are high. Whether these physiological differences are due to a reduction in the O_2 carrying capacity of triploids is not clear. While some studies have found that triploid salmonids have a lower blood hemoglobin (Hb) concentration than diploids (Benfey and Sutterlin 1984*a*; Small and Randall 1989; Sadler et al. 2000), others have observed no difference (Stillwell and Benfey 1996; Benfey and Biron 2000; Hyndman et al. 2003*b*). Similarly, although Graham et al. (1985) found that the amount of O_2 carried by a given amount of Hb is lower in triploids than in diploids, Sadler et al. (2000) found no difference between ploidies in blood O_2 affinity. Still, relative to diploids, there is circumstantial evidence suggesting that the O_2 delivery system of triploid fish may have a reduced capacity. For example, in rainbow trout (*Oncorhynchus mykiss*) infected with bacterial gill disease, triploids experience higher mortality rates than diploids when exposed to hypoxic conditions (Yamamoto and Iida 1994). Similarly, during exercise at high temperatures, triploid brown trout (*Salmo trutta*) appear to have a reduced factorial metabolic scope (Altimiras et al. 2002), and triploid brook trout (*Salvelinus fontinalis*) take longer than diploids to recover from the metabolic disturbances of exhaustive exercise (Hyndman et al. 2003*b*).

In salmonids, the properties of Hb ensure that O_2 uptake and CO_2 excretion at rest are tightly coupled in the red blood cells (RBCs) (Brauner and Randall 1996, 1998; Randall 1998). In brief, the protons produced during Hb oxygenation are the major source of protons for erythrocytic bicarbonate dehydration (Jensen 1989), an essential step for CO_2 excretion in teleosts (Perry et al. 1982). Therefore, any constraints on O_2 uptake imposed by the larger RBC of triploids also have the potential of interfering with CO_2 excretion and vice versa.

To date, neither the pattern of CO_2 excretion nor the O_2 carrying capacity of exercising triploid fish has been examined. Therefore, as a means of improving our basic understanding of the respiratory physiology of triploid fish, we conducted an analysis of O_2 and CO_2 transport during aerobic swimming and at maximal critical swimming velocity (Ucrit) in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*).

Materials and methods

Experimental fish

Mixed-sex diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*) were obtained from Yellow Island Aquaculture Ltd. (Campbell River, British Columbia, Canada) and held outdoors at the Department of Fisheries and Oceans (West Vancouver, British Columbia, Canada) in separate 200-L fiberglass tanks for at least 1 month prior to experimentation. Fish were fed to satiation with a commercial salmon food and maintained in 29 ppt flow-through seawater at 9 °C. Triploidy was induced by heat shock (Benfey and Sutterlin 1984*c*), and the ploidy of fish was ascertained using propidium iodide flow cytometry (Allen 1983). Diploid fish had a mean body wet weight of 280.5 ± 9.3 g and mean fork length of 27.8 ± 0.3 cm (N = 11). The mean body wet weight and fork length of the triploid fish was 267.9 ± 8.3 g and 27.4 ± 0.4 cm (N = 9), respectively.

Surgical procedure

Salmon were anaesthetized in a 1:10 000 solution of tricaine methanesulphonate (MS-222) in seawater, buffered with $NaHCO_3$ to pH 7.5, and bubbled with air. The anaesthetized fish were transferred to an operating table, where the gills were continually irrigated with a more dilute solution of buffered and aerated MS-222 (1:30 000). The dorsal aorta was cannulated chronically with polyethylene tubing (Clay Adams PE 50, Becton Dickinson, Sparks, Maryland, USA) using the technique of Soivio et al. (1975). Following surgery, weight, fork length, maximum height, and width were recorded, and the fish were allowed to recover for 24 h in separate flow-through opaque acrylic boxes. The cannulae were filled and flushed with heparanized (10 units·mL^{-1} ammonium heparin; Sigma, Missouri, USA) teleost saline solution. The cannulated fish were transferred to a 39.2-L Brett-type swim tube respirometer (Brett 1964) the night before swimming experiments. During this acclimation period, water velocity in the swim tunnel was maintained at 11 cm·s^{-1} (approximately 0.4 body lengths (BL)·s^{-1}), and an overflow of water from the respirometer prevented build up of metabolic waste products.

Experimental protocol

At the start of each swimming trial, the swim tunnel was sealed and the rate of O_2 depletion was recorded over a 6-min interval for calculation of O_2 consumption rate (MO_2). Once resting MO_2 had been assessed, swimming speed was gradually elevated to 1 BL·s^{-1} over a 10-min period and maintained at this velocity for 30 min. The swimming speed was then gradually increased by 0.5 BL·s^{-1} at 30-min intervals until the fish could no longer maintain a given velocity. Thirty minutes is sufficient time for blood–gas and acid–base parameters to stabilize following a change in water velocity (Kiceniuk and Jones 1977; Thomas et al. 1987). MO_2 was determined at each new speed unless the fish fatigued in the first 15-min of the interval. Between estimates of MO_2, the respirometer was flushed with fresh seawater to restore O_2 levels. The maximum swimming speed and time to fatigue were recorded for the calculation of the maximal Ucrit and corrected for the blocking effect of the maximal cross-sectional area of the fish (Beamish 1978).

In each swimming trial, we withdrew three blood samples from the dorsal aorta at set swimming speeds: one 0.5-mL sample at the acclimation velocity and at 2 BL·s^{-1} and one 0.7-mL sample at Ucrit. At each sampling time, we replaced the blood sample by an equivalent volume of heparinized teleost saline. Aliquots of whole blood were taken for measurement of arterial blood O_2 content (C_aO_2), hemoglobin concentration ([Hb]), and red blood cell count (RBCC). In addition, arterial blood partial pressure of O_2 (P_aO_2), red cell pH (pH_i), and methemoglobin concentration ([MetHb]) were assessed at Ucrit. Whole blood was also collected in heparinized capillary tubes. These tubes were sealed, centri-

Table 1. Hematocrit (Hct), hemoglobin (Hb), red blood cell count (RBCC), mean cellular hemoglobin concentration (MCHC), mean cellular hemoglobin content (MCH), and mean RBC volume (MCV) of diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*) swimming either at 0.4 body lengths (BL)·s^{-1}or 2.0 BL·s^{-1} or at maximal critical swimming velocity (Ucrit).

	Diploid			Triploid		
Parameter	0.4 BL·s^{-1}	2.0 BL·s^{-1}	Ucrit	0.4 BL·s^{-1}	2.0 BL·s^{-1}	Ucrit
Hct (%)	22.8±1.3a	20.0±1.2b	20.4±1.4b	20.7±1.2a	18.9±1.4b	18.7±1.4b
Hb (g·dL^{-1})	9.1±0.6a	7.9±0.5b	8.2±0.6b	8.1±0.5a	7.6±0.6b	7.1±0.6b
RBCC ($\times10^6$·mm^{-3})	0.94±0.06a	0.83±0.06b	0.83±0.07b	0.61±0.07a*	0.55±0.07b*	0.52±0.07b*
MCHC (g·mL^{-1})	0.400±0.010	0.398±0.011	0.402±0.010	0.398±0.003	0.399±0.009	0.394±0.012
MCH (pg)	97.7±4.5	97.5±4.8	102.9±10.3	138.5±13.6*	145.7±15.1*	150.0±21.0*
MCV (fL)	24.9±1.0	24.4±0.9	24.9±1.8	35.5±3.4*	36.6±3.9*	37.0±4.4*

Note: Values are means ± standard error; $N = 11$ for diploids and $N = 9$ for triploids. Values that do not share a common letter within a given ploidy are significantly different from each other as determined by one-way repeated measures analysis of variance and pairwise Tukey's multiple comparison test ($p < 0.05$).

*Significantly different from "diploid" at given swimming speed as determined by Student's t test ($p < 0.05$).

fuged at 13 400*g* for 5 min, and used to determine hematocrit (Hct) and arterial CO_2 content (C_aCO_2) from the plasma fraction. The remaining blood was spun down as above and plasma removed for measurement of arterial plasma pH (pH_a) and later measurement of plasma lactate concentration. The plasma aliquot for determination of lactate was deproteinized with ice-cold 0.6 N perchloric acid, spun down as above, and the supernatant was frozen in liquid nitrogen and stored at –80 °C for later analysis.

Analytical techniques and calculations

The PO_2 of the water in the swim tube respirometer was continuously measured with a thermostatted PO_2 electrode (model E5046; Radiometer, Copenhagen, Denmark) and displayed on a PHM71 acid–base analyzer (Radiometer). The electrode was calibrated daily with air-saturated water. The analog output from the PHM71 meter was sampled at 1 Hz using the data acquisition system Labtech Notebook (Measurement Computing Corporation, Middleboro, Massachusetts, USA). MO_2 was calculated from the change in water PO_2 over the duration that the respirometer was sealed, taking into account the solubility of O_2 in seawater at 9 °C (Boutilier et al. 1984), the volume of the swim tunnel, and the volume occupied by the fish. For a given fish, the ratio of maximum to acclimation MO_2 within a swimming trial was used to determine the fold increase in MO_2. Measurements of P_aO_2 and C_aO_2 were made using thermostatted Radiometer PO_2 electrodes with PHM71 acid–base analysers. C_aO_2 was measured using the method described by Tucker (1967). C_aCO_2 was measured with a gas chromatograph (model III; Carle Instruments, Anaheim, California, USA) following the methods of Boutilier et al. (1985). The arterial partial pressure of CO_2 (P_aCO_2) and the plasma bicarbonate (HCO_3^-) concentration were calculated from C_aCO_2 and pH_a by rearrangement of the Henderson–Hasselbalch equation, with values of plasma pK and CO_2 solubility coefficients from Boutilier et al. (1984). pH_a and pH_i were measured using a thermostatted Radiometer G297/G2 glass capillary electrode with a PHM71 acid–base analyser. pH_i was measured according to the freeze–thaw method of Zeidler and Kim (1977). Whole-blood lactate concentration was measured spectrophotometrically using an NAD^+-linked enzymatic procedure (826-UV, Sigma). RBCC were performed on a Coulter Counter® model TAII (Coulter Electronics Ltd., Luton, England), and the accuracy of the counts was verified on six random samples using an improved Neubauer hemocytometer (Hesser 1960). Blood [Hb] was determined spectrophotometrically using a total Hb assay kit (525-A, Sigma). The mean cellular [Hb] (MCHC) was calculated from [Hb]/Hct, the mean cellular Hb content (MCH) from ([Hb]/RBCC)×10, and the mean RBC volume (MCV) from Hct/RBCC. Methemoglobin was determined using the method of Barlett et al. (1987). The percent saturation of Hb with O_2 (SO_2) was calculated as

$$\frac{C_aO_2 - (P_aO_2 \times \alpha O_2)}{[\text{Hb}] \times 4} \times 100$$

where C_aO_2 and Hb are in µmol·L^{-1} and αO_2, the solubility of O_2 at 9 °C and 29 ppt, is in µmol·L^{-1}·$mmHg^{-1}$ (1 mmHg (0 °C) = 133.322 Pa) (Boutilier et al. 1984).

Statistics

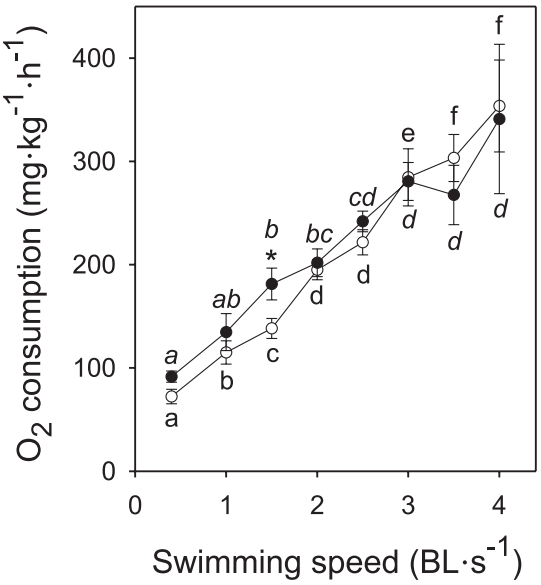
All data are presented as means ± standard error. Differences between swimming speeds within a given ploidy were assessed by one-way repeated measures analysis of variance (ANOVA) followed by pairwise Tukey's multiple comparison test. Differences between ploidies at a given swimming speed were determined by Student's t test. The significance level for all statistical tests was $p < 0.05$.

Results

Throughout the exercise protocol, there was no difference between ploidies in Hct, Hb, and MCHC (Table 1). In contrast, the RBCC of triploids were significantly lower, and the MCH and MCV were significantly higher than those of diploids. In the diploid and triploid fish, the Hct, Hb, and RBCC at both 2 BL·s^{-1} and Ucrit were significantly lower than the control values obtained at the acclimation swimming speed of 0.4 BL·s^{-1}. Blood samples analyzed on the Neubauer hemocytometer and the Coulter Counter® gave equivalent RBCCs, the two methods differing by a maximum of 6%.

In diploid and triploid fish, MO_2 increased linearly with swimming speed, and with the exception of the 1.5 BL·s^{-1} values, MO_2 did not differ between ploidies throughout the exercise protocol (Fig. 1). Similarly, diploids and triploids

Fig. 1. Oxygen consumption as a function of swimming speed in diploid (open circle) and triploid (solid circle) chinook salmon (*Oncorhynchus tshawytscha*). Swimming speed is in body lengths (BL)·s^{-1}. Values that do not share a common letter within a given ploidy (italicized letters are used for the triploid group) are significantly different from each other as determined by one-way repeated measures analysis of variance and pairwise Tukey's multiple comparison test ($p < 0.05$). The asterisk (*) denotes a significant difference between ploidies at a given swimming speed as determined by Student's t test ($p < 0.05$). Values are means ± standard error; N values decrease with increasing swimming speed and range from 6–11 for diploids and 5–9 for triploids.



did not differ significantly in Ucrit or MO_2 max (Table 2). However, whereas 100% of the diploids maintained a swimming velocity of 3.5 BL·s^{-1} for 30 min, only 56% of the triploids did not fatigue at this swimming speed. Moreover, over the course of the swim performance test, the average increase in MO_2 was significantly higher in diploids than in triploids (Table 2). Also, while swimming speed did not affect C_aO_2 within a given ploidy, O_2 carrying capacity was significantly lower in triploids than in diploids at all sampling times (Fig. 2). At Ucrit, triploids also had significantly lower Hb–O_2 saturation and RBC pH than diploids, but similar P_aO_2 and methemoblobin values (Table 2).

With regards to CO_2 transport and acid–base balance, diploids and triploids did not differ in CO_2 content, P_aCO_2, plasma pH, or plasma lactate at the acclimation swimming speed, during sustained exercise, or at Ucrit (Figs. 3, 4). In both ploidies, an increase in swimming velocity had no significant effect on CO_2 content (Fig. 3*a*) but resulted in significant changes in P_aCO_2 (Fig. 3*b*), plasma pH (Fig. 4*a*), and plasma lactate (Fig. 4*b*). Relative to the values obtained at 0.4 BL·s^{-1} and 2.0 BL·s^{-1}, exercise at the critical swimming velocity increased both P_aCO_2 and plasma lactate, but decreased plasma pH. The overall changes in acid–base balance observed during the exercise regime are shown in the pH / HCO_3^- plots of Fig. 5. In both ploidies, while changes in acid–base balance between the acclimation swimming speed and 2.0 BL·s^{-1} followed the blood buffer line, Ucrit was associated with a marked acidosis without an increase in plasma HCO_3^-.

Table 2. Maximal critical swimming velocity (Ucrit), O_2 consumption rate (MO_2) at the acclimation (acc) swimming speed, maximum MO_2, fold increase in MO_2 throughout the swim performance test, arterial O_2 partial pressure (P_aO_2), percent saturation of hemoglobin with O_2 (SO_2), percent methemoglobin (MetHb), and red blood cell intracellular pH (RBC pH_i) in diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*).

Parameter	Diploid	Triploid
Ucrit (BL·s^{-1})	3.92±0.12	3.60±0.22
MO_2 acc (mg·kg^{-1}·h^{-1})	72.3±6.7	87.7±7.0
MO_2 max (mg·kg^{-1}·h^{-1})	364.9±26.7	311.1±35.1
MO_2 fold increase	5.21±0.29	3.60±0.35*
P_aO_2 at Ucrit (mmHg)	95.3±5.9	94.3±6.9
SO_2 at Ucrit (%)	81.5±4.6	62.2±4.4*
MetHb at Ucrit (%)	3.2±0.9	1.8±0.5
RBC pH_i at Ucrit	7.49±0.01	7.42±0.02*

Note: Values are means ± standard error; $N = 11$ for diploids and $N = 9$ for triploids. BL, body length; 1 mmHg (0 °C) = 133.322 Pa.
*Significantly different from diploid for given parameter as determined by Student's t test ($p < 0.05$).

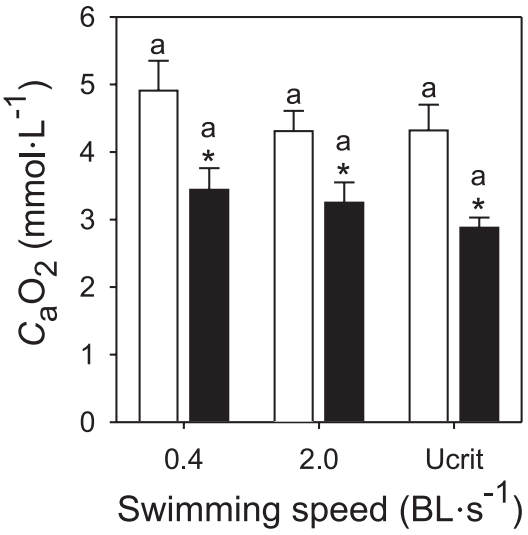
Discussion

While confirming that triploid and diploid salmonids have similar Ucrit and O_2 consumption rates, results from this study also clearly show that triploid chinook salmon have a reduced aerobic capacity relative to diploid fish from the same stock. In contrast, we found no evidence that triploidy affects CO_2 excretion or blood acid–base status either during aerobic swimming or at Ucrit.

Triploid chinook salmon, in agreement with previous hematological assessments of other salmonid species (e.g., Benfey 1999; Benfey and Biron 2000; Sadler et al. 2000), are characterized by having fewer but larger erythrocytes, a higher erythrocyte Hb content, and a similar Hct to diploids. Although some studies have observed that triploids have lower total blood [Hb] and (or) mean cellular [Hb] than diploids (Benfey and Sutterlin 1984*a*; Small and Randall 1989; Sadler et al. 2000), overall the effects of triploidy on these hematological parameters are equivocal (see Benfey 1999), and we observed no difference in either value between ploidies in this study. Similarly, while the blood sampling protocol significantly reduced Hct and total blood [Hb] over the course of the swimming trial, the magnitude of these changes did not differ between ploidies and were relatively small. Therefore, based solely on total blood [Hb], we expected diploid and triploid fish in this study to have equivalent maximal O_2 carrying capacities and aerobic scope.

As previously observed in coho salmon (*Oncorhynchus kisutch*; Small and Randall 1989) and brook trout (Stillwell and Benfey 1997), we found no difference between the swimming performance of triploid and diploid chinook salmon. Similarly, in accordance with several studies investigating the aerobic capacity of triploid salmonids (Benfey and Sutterlin 1984*b*; Oliva-Teles and Kaushik 1990; Yamamoto and Iida 1994), we observed similar MO_2 values

Fig. 2. Arterial O_2 content (C_aO_2) of diploid (open bar) and triploid (solid bar) chinook salmon (*Oncorhynchus tshawytscha*) swimming either at 0.4 body lengths (BL)·s^{-1} or 2.0 BL·s^{-1} or at maximal critical swimming velocity (Ucrit). Values that do not share a common letter within a given ploidy are significantly different from each other as determined by one-way repeated measures analysis of variance and pairwise Tukey's multiple comparison test ($p < 0.05$). An asterisk (*) denotes a significant difference between ploidies at given swimming speed as determined by Student's t test ($p < 0.05$). Values are means ± standard error; $N = 11$ for diploids and $N = 9$ for triploids.



for triploid and diploid chinook salmon during aerobic swimming and at Ucrit. In contrast, throughout the swimming trial, whereas the increase in metabolic rate and the C_aO_2 and SO_2 values in diploids were consistent with the data reported for other salmonids (Kiceniuk and Jones 1977; Brett and Groves 1979; Brauner et al. 2000*b*), triploids were characterized by significantly lower values. Overall, diploids had a 45% greater increase in MO_2 than did triploids over the course of the swim performance test, SO_2 at Ucrit was 24% less in triploids than in diploids, and on average the C_aO_2 of triploids was approximately 30% lower than that of diploids at all sampling times. Thus, despite having similar Ucrit and MO_2, our results clearly suggest that the aerobic capacity of triploid chinook salmon is more limited than their diploid counterpart.

Although the Ucrit test is traditionally used as an indicator of aerobic capacity in fish (Brett 1964; Hammer 1995), our results suggest that the critical swimming velocity test is not particularly sensitive to the arterial O_2 carrying capacity of the blood. This is consistent with the results of Brauner et al. (1993), who observed that Ucrit in chinook salmon is virtually independent of functional [Hb] until the latter is reduced to less than 51% of control level. Similarly, in rainbow trout that have an Hct within the normal range, increasing C_aO_2 has a very limited impact on Ucrit (Gallaugher et al. 1992, 1995). Overall, the relationship between Ucrit and C_aO_2 in fish may be weak because it can be influenced by several compensatory physiological adjustments (Brauner et al. 1993). For example, during exercise fish may compensate for a reduction in O_2 carrying capacity by increasing arterial–venous O_2 content, cardiac output, and ventilation volume and frequency (Kiceniuk and Jones 1977) by redistributing blood from the gut towards the aerobic red muscles (Thorarensen et al. 1993) or by recruiting the anaerobic white muscle fibers (Jones 1982). Whether the Ucrit value and the MO_2 of triploids in this study mask the recruitment of these or other compensatory mechanisms is not clear. While Altimiras et al. (2002) found no abnormalities in the cardiac performance of triploid brown trout swimming at Ucrit, a detailed study comparing the cardiovascular performance of swimming triploid and diploid salmonids from the same stock has yet to be performed. Similarly, while resting ventilation frequency may (King and Lee 1993) or may not (Stillwell and Benfey 1996) be higher in triploid than diploid salmonids, a comparison between ploidies during exercise of ventilation frequency and more importantly, ventilatory stroke volume (Jones and Randall 1978), has not been performed. In contrast, there is no evidence that triploids utilize anaerobic pathways to a greater extent than diploids following exhaustive exercise (Hyndman et al. 2003*a*, 2003*b*), and the plasma lactate values in this study suggest equal recruitment of anaerobic metabolism at Ucrit between ploidies. However, the extent to which triploids utilize anaerobic energy stores and clear lactate from tissues during sustained swimming and Ucrit remain to be characterized.

Our observation of in vivo triploid chinook salmon having a reduced C_aO_2 relative to diploids concurs with the in vitro results of Graham et al. (1985), who found a reduced Hb–O_2 loading ratio in Atlantic salmon blood. Although only measured at Ucrit, the lower erythrocytic pH of triploids will reduce the affinity of blood for O_2 (Bohr effect) and its maximal O_2 carrying capacity (Root effect). While the difference in erythrocytic pH between triploid and diploid chinook salmon at Ucrit is modest, in salmonids small changes in pH_i can have large effects on the O_2 carrying capacity of the blood (Pelster and Weber 1990; Brauner and Randall 1996). In Atlantic salmon at least, there appears to be no differences in Hb–O_2 affinity or in the magnitude of the Bohr and Root effects between diploid and triploid blood (Graham et al. 1985; Sadler et al. 2000). Similarly, we found no evidence that MetHb contributes to the reduced O_2 carrying capacity of triploids. At Ucrit, both ploidies had MetHb levels that were on par with previously reported values for resting chinook salmon (Brauner et al. 1993). Also, while RBC organic phosphates are powerful modulators of Hb–O_2 affinity in fish (Nikinmaa 1990), analysis of diploid and triploid Atlantic salmon erythrocytes has revealed no difference in organic phosphate concentrations (Graham et al. 1985; Sadler et al. 2000). Therefore, whereas many factors determine the amount of O_2 carried by a given amount of Hb in fish, few appear to contribute to the reduced O_2 carrying capacity of triploids. While the precise mechanism remains to be determined, our data suggest that differences in erythrocyte pH may be a contributing factor. Interestingly, triploid Atlantic salmon (King and Lee 1993) and brook trout (Benfey 1999) have a higher incidence of erythrocyte abnormalities than diploids, including cells with bisected nuclei and pinched cells. Whether chinook salmon blood also contains a higher proportion of deformed erythrocytes and whether these abnormal RBCs can transport O_2 is not known.

Fig. 3. (*a*) Arterial CO_2 content (C_aCO_2) and (*b*) partial pressure (P_aCO_2) of diploid (open bar) and triploid (solid bar) chinook salmon (*Oncorhynchus tshawytscha*) swimming at either 0.4 body lengths (BL)·s^{-1} or 2.0 BL·s^{-1} or at maximal critical swimming velocity (Ucrit). Values that do not share a common letter within a given ploidy are significantly different from each other as determined by one-way repeated measures analysis of variance and pairwise Tukey's multiple comparison test ($p < 0.05$). Values are means ± standard error; $N = 11$ for diploids and $N = 9$ for triploids.

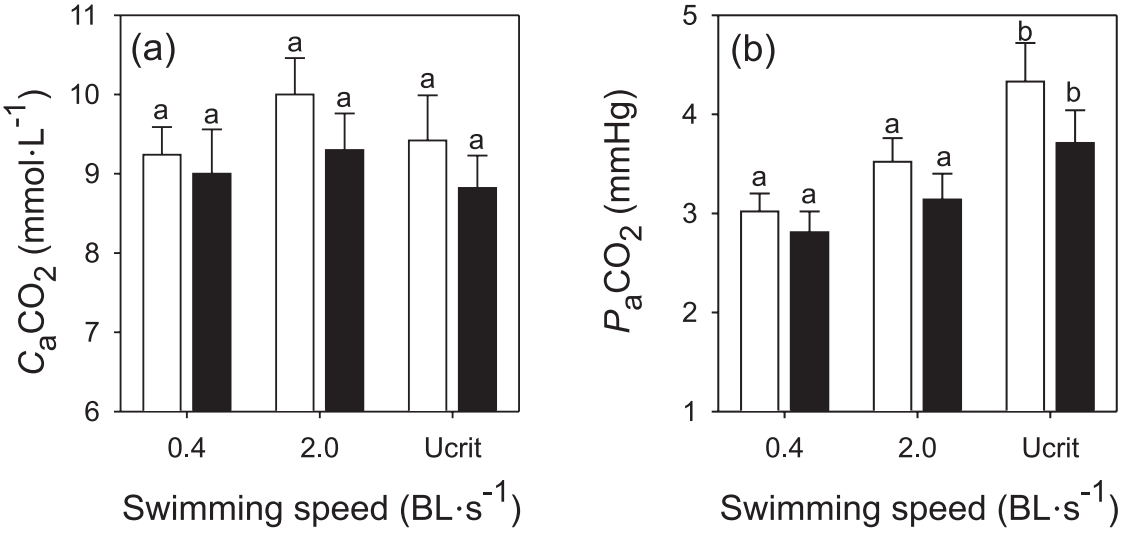
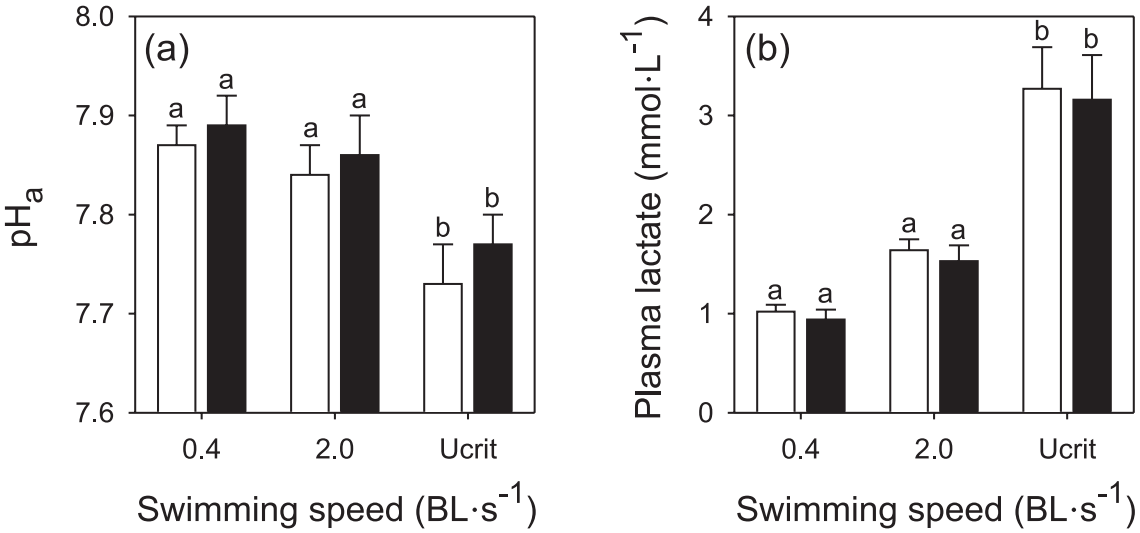


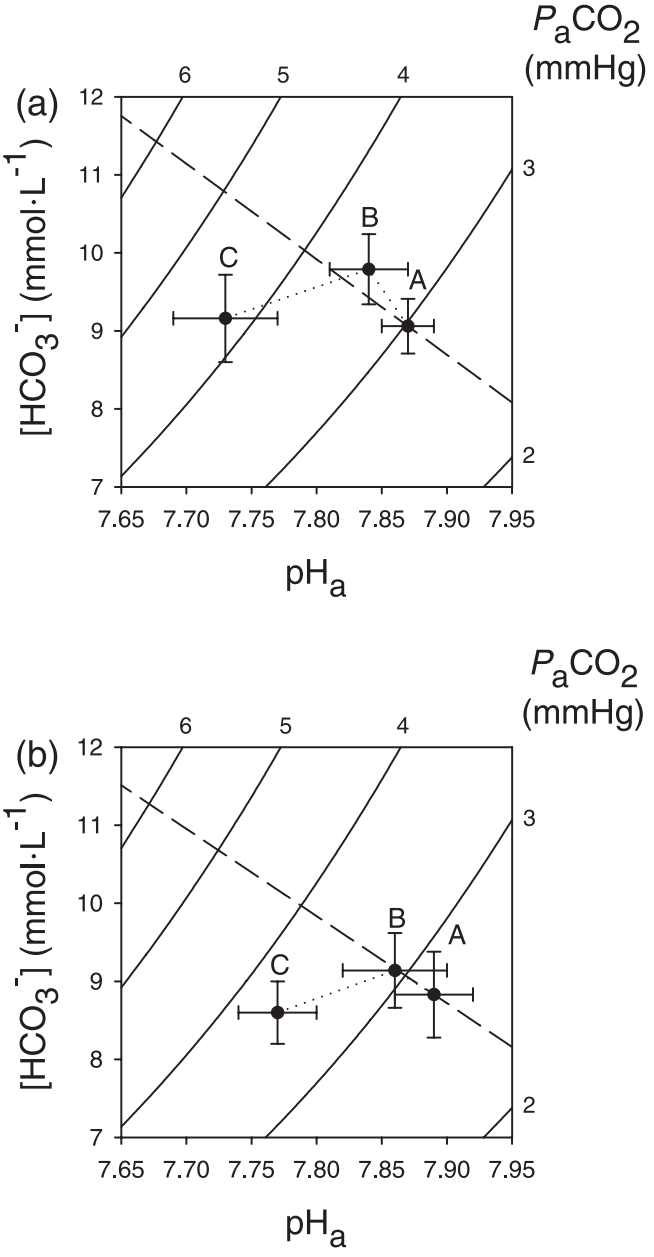
Fig. 4. (*a*) Arterial pH (pH_a) and (*b*) plasma lactate of diploid (open bar) and triploid (solid bar) chinook salmon (*Oncorhynchus tshawytscha*) swimming at either 0.4 body lengths (BL)·s^{-1} or 2.0 BL·s^{-1} or at maximal critical swimming velocity (Ucrit). Values that do not share a common letter within a given ploidy are significantly different from each other as determined by one-way repeated measures analysis of variance and pairwise Tukey's multiple comparison test ($p < 0.05$). Values are means ± standard error; $N = 11$ for diploids and $N = 9$ for triploids.



Available evidence also suggests that diffusion limitations to branchial O_2 transfer may contribute to the reduced aerobic capacity of triploid salmonids. For brook trout swimming at low speed, triploids take up less O_2 than diploids per opercular cycle (Stillwell and Benfey 1996). Moreover, although their findings have yet to be corroborated in other salmonid species, Sadler et al. (2001) showed that triploid Atlantic salmon have a significantly smaller gill surface area than diploids. The large increase in O_2 uptake during exercise in salmonids is achieved in part via a significant increase in the percentage of gill lamellae that are perfused (Randall and Daxboeck 1984). With a reduced gill surface area, the respiratory apparatus in triploids may have a smaller capacity than in diploids and, as shown in this study, a limited ability to increase gas exchange during exercise or other conditions that require an increase in O_2 uptake.

While several studies have compared the aerobic capacity of triploid and diploid salmonids (see above for discussion), to our knowledge this is the first study to report on CO_2 excretion in triploids. CO_2 excretion in fish depends primarily upon the dehydration of HCO_3^- by carbonic anhydrase within RBCs prior to gill blood entry (Brauner and Randall

Fig. 5. A pH_a/HCO_3^- plot of changes in blood acid–base status of diploid (*a*) and triploid (*b*) chinook salmon (*Oncorhynchus tshawytscha*) swimming at either 0.4 body lengths (BL)·s^{-1} (A) or 2.0 BL·s^{-1} (B) or at maximal critical swimming velocity (Ucrit; C). The isobars are for the arterial partial pressure of CO_2 (P_aCO_2) at 9 °C. The buffer lines (dotted lines) were calculated from the regression equation for β as a function of hemoglobin concentration ([Hb]) derived by Wood et al. (1982) and a [Hb] of 9.1 g·dL^{-1} for diploids and 8.1 g·dL^{-1} for triploids (the mean values measured at 0.4 BL·s^{-1}). Values are means ± standard error; N = 11 for diploids and N = 9 for triploids.



1996) and at rest the rate-limiting step for CO_2 excretion is the rate of HCO_3^- – Cl^- exchange at the surface of red cells (Perry et al. 1982). During exercise, CO_2 excretion may also be restricted by gill diffusion limitations (Thomas et al. 1987; Brauner et al. 2000*a*) and, as venous Hb–O_2 content falls, by the availability of erythrocytic Bohr protons (Brauner et al. 2000*b*). Therefore, in triploids, any effect of increased cell size on erythrocytic HCO_3^- – Cl^- exchange or on the availability of protons for HCO_3^- dehydration could impair CO_2 excretion. Similarly, as discussed above, a reduction in gill surface area in triploids (Sadler et al. 2001) could limit CO_2 excretion during exercise. However, we observed no difference in C_aCO_2, P_aCO_2, or in arterial plasma HCO_3^- levels between triploid and diploid chinook salmon at any swimming speed. Moreover, throughout the exercise protocol, diploids and triploids were characterized by similar changes in C_aCO_2, P_aCO_2, and plasma HCO_3^-. In both ploidies, there was no significant change in CO_2 transport between the acclimation swimming speed and 2.0 BL·s^{-1}, and as previously observed in diploid rainbow trout (Thomas et al. 1987), a significant increase in P_aCO_2 at Ucrit. Therefore, despite their larger size, reduced Hb–O_2 content, and slightly lower pH, triploid RBCs appear to play a similar role in CO_2 excretion as their diploid counterpart.

Triploid and diploid chinook salmon also experienced similar acid–base disturbances throughout the swim performance test. As previously observed in rainbow trout during aerobic swimming (Thomas et al. 1987; Brauner et al. 2000*a*), relative to the acid–base status of the fish swimming at the acclimation speed, the pH_a/HCO_3^- plots show that both diploid and triploid fish swimming at 2.0 BL·s^{-1} experienced a small respiratory acidosis. At Ucrit, in contrast, both ploidies experienced a marked respiratory and metabolic acidosis. The significant increase in P_aCO_2 at Ucrit was accompanied by the recruitment of anaerobic metabolism as indicated by the arterial acidosis and the significant increase in plasma lactate. These results are consistent with the observations of Jones (1982) and Burgetz et al. (1998), who estimated that salmonids gradually recruit anaerobic white muscle fibers once their swimming speed surpasses 70%–80% of Ucrit.

In summary, CO_2 transport and acid–base balance in chinook salmon either during aerobic swimming or at Ucrit do not appear to differ from that of their diploid counterpart. Moreover, as previously observed in other salmonid species (Small and Randall 1989; Stillwell and Benfey 1996; Benfey 1999), based solely on Ucrit and O_2 consumption rates, it appears that aerobic capacity may not differ between triploids and diploids. However, a more in-depth examination of O_2 transport parameters clearly shows that triploid chinook salmon have a lower O_2 carrying capacity than diploids. Larger cells mean longer diffusion distances, but this does not appear to be limiting exercise, as triploid and diploid fish can achieve the same swimming speeds. For similar reasons, blood O_2 content also does not seem to be limiting swimming speed in these animals. Thus O_2 transport may not be limiting swimming speed in these fish. What then does limit swimming speed? Perhaps some intrinsic property of the muscle, rather than O_2 delivery, determines swimming speed in these fish under favorable environmental conditions. Therefore, while the specific mechanisms are mostly unknown (however see Mercier et al. 2002), our results suggest that triploids are able to compensate for their reduced aerobic capacity under the current exercise regime. Although such compensatory measures may mask limitations in the aerobic capacity of triploids under favourable thermal regimes, the effects of triploidy on O_2 transport might contrib-

ute to the inferior physiological performance of triploid salmonids reared under suboptimal conditions.

Acknowledgements

We thank Dr. Paul J. Harrison, Department of Botany, The University of British Columbia, for the use of a Coulter Counter in the determination of red blood cell counts. This study was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to D.J.R.

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