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Negligible differences in metabolism and thermal tolerance between diploid and triploid Atlantic salmon (*Salmo salar* L.)

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Summary statement

High temperatures are thought to physiologically compromise triploid animals due to their enlarged cells. Here, we show that triploid juvenile Atlantic salmon achieve similar metabolism and acute thermal tolerance as their diploid counterparts.

Abstract

The mechanisms that underlie thermal tolerance in aquatic ectotherms remain unresolved. Triploid fish have been reported to exhibit lower thermal tolerance than diploids, offering a potential model organism to better understand the physiological drivers of thermal tolerance. Here, we compared triploid and diploid juvenile Atlantic salmon (*Salmo salar*) in freshwater to investigate the proposed link between aerobic capacity and thermal tolerance. We measured specific growth rates (SGR) and resting (aerobic) metabolic rates (RMR) in freshwater at 3, 7 and 9 weeks of acclimation to either 10, 14 or 18°C. Additionally, maximum metabolic rates (MMR) were measured at 3 and 7 weeks of acclimation, and critical thermal maxima (CT_{max}) were measured at 9 weeks. Mass, SGR, and RMR differed between ploidies across all temperatures at the beginning of the acclimation period, but all three metrics converged between ploidies by week 7. Aerobic scope (MMR – RMR) remained consistent across ploidies, acclimation temperatures, and time. At 9 weeks, CT_{max} was independent of ploidy, but correlated positively with acclimation temperature despite the similar aerobic scope between acclimation groups. Our findings suggest that acute thermal tolerance is not modulated by aerobic scope, and the altered genome of triploid Atlantic salmon does not translate to reduced thermal tolerance of juvenile fish in freshwater.

Introduction

Triploid fish have been proposed as useful experimental models to understand the mechanisms underlying environmental tolerance because their altered genome may influence tolerance levels under challenging conditions (Maxime, 2008). Indeed, triploid brown trout (*Salmo trutta*, Linnaeus) exhibited higher mortality rates than diploids when exposed to a high temperature challenge (18°C) for 3 weeks, and triploid mortalities reached 50% after 12 weeks at this temperature (Altimiras et al., 2002). Moreover, triploid rainbow trout (*Oncorhynchus mykiss*, Walbaum) suffered immediate mortalities when exposed to 21°C, and a total mortality of 69% was recorded after 3 weeks compared with 39% in diploid conspecifics (Ojolick et al., 1995).

Such observations raise the possibility of using triploids to elucidate the physiological mechanisms underlying temperature tolerance in fish. Triploid fish are typically produced by subjecting eggs to one or more pressure shocks within the first hour or two following fertilisation, resulting in the retention rather than extrusion of a polar body that gives rise to a third chromosome (Teskeredžić et al., 1993). Triploids compensate for this extra genetic material by having larger but fewer cells than their diploid counterparts, resulting in the two ploidies achieving similar sizes as adults (Swarup, 1959; Small and Benfey, 1987). Growth rates of triploids, however, have been inconsistent across studies and have been the same, greater than, or less than their diploid counterparts (Galbreath et al., 1994; McGeachy et al., 1995; McCarthy et al., 1996).

Furthermore, triploids are thought to have similar haematocrit (packed erythrocyte volume) as diploids but lower haemoglobin concentration, subsequently reducing blood oxygen carrying capacity (Benfey and Sutterlin, 1984a; Benfey et al., 1984; Graham et al., 1985). Blood oxygen transport of triploids may be further reduced due to the smaller surface area to volume ratio of the enlarged erythrocytes, which could inhibit oxygen diffusion dynamics (Sadler et al., 2000). In light of the proposed link between oxygen transport capacity and thermal performance, a logical extension is that inferior oxygen transport capacity in triploids drives their reportedly lower thermal tolerance (Pörtner and Knust, 2007). Having said that, the role of oxygen transport capacity in governing thermal tolerance is debated and requires additional investigation using different model organisms (Clark et al., 2013; Brijs et al., 2015; Ern et al., 2016).

Despite the apparent reduction in oxygen carrying capacity of triploid blood, contrasting results exist regarding their aerobic metabolic rates at both high and low acclimation temperatures. Higher routine metabolism has been reported in triploid Atlantic salmon (*Salmo salar*, Linnaeus) at an acclimation temperature of 12°C, but lower routine metabolism at a higher temperature (18°C) was considered to be indicative of lower thermal tolerance in triploids (Atkins and Benfey, 2008). In contrast, no differences in routine metabolic rate were found between diploid and triploid Atlantic salmon at 15°C, nor between diploid and triploid rainbow trout at 19°C (Benfey and Sutterlin, 1984b; Oliva-Teles and Kaushik, 1990). While maximum metabolic rate (MMR) has received less attention, a study on Atlantic salmon reported no differences in MMR between ploidies at 15°C (Lijalad and Powell, 2009). Scant data and disparate findings point to a requirement for more comprehensive investigations to determine whether aerobic capacity may be causally linked with acute and/or chronic thermal tolerance across ploidies (Benfey et al., 1997; Galbreath et al., 2006).

Here, we investigated acute and chronic thermal tolerance of diploid and triploid juvenile Atlantic salmon with an aim to clarify some of the conflicting findings reported previously. Specifically, our objectives were first to compare the growth performance, resting metabolic rate (RMR), MMR and aerobic scope at three acclimation temperatures (10, 14, and 18°C), and then to quantify routine metabolic rate during assessments of critical thermal maxima (CT_{max}) in the different acclimation groups. We tested the hypothesis that triploids exhibit lower chronic and acute thermal tolerance than diploids, and that these differences are at least partly explained by lower aerobic capacity of triploids.

Materials and Methods

Animal husbandry

Diploid and triploid Atlantic salmon (n=35 and n=40, respectively) were sourced from the Salmon Enterprises of Tasmania Pty Ltd (SALTAS) freshwater hatchery in Wayatinah, Tasmania (water temperature ~9°C) and transported to Hobart, Tasmania. All experiments were conducted at the Aquaculture Physiology laboratory at CSIRO under the animal ethics permit A0013794. Both groups were from the 2015 commercial spawning and had been incubated at ~8°C. The fish were separated by ploidy into two x 200 L tanks that were supplied by a freshwater recirculating system held at 10°C. After 7 days of recovery from transport, the fish were individually weighed (mean ± s.e.m.; diploids: 46.63 ± 2.31 g, triploids: 68.75 ± 2.08 g) and their fork lengths measured (diploids: 163.41 ± 2.95 mm, triploids: 188.63 ± 2.13 mm). Additionally, the fish were tagged intraperitoneally with a passive integrated transponder (PIT) to track individual performance and tagged with coloured elastomer in the adipose fin to visually discern ploidy (yellow for diploids and green for triploids) before being returned to their respective tanks for three days to recover from tagging. Thereafter, mixed groups of diploids and triploids (n=5-8 per ploidy) were assigned to six 68 L tanks (n=11-13 per tank) with recirculating freshwater and maintained at 10°C for three weeks.

Water temperature was subsequently raised to 14°C (to represent current-day summer sea temperatures of 14-15°C in southeast Tasmania) in two tanks and to 18°C (forecast of regional sea temperatures under a business-as-usual emissions scenario) in another two tanks (2°C d^{-1}) using 600 W titanium heaters with a digital Nema thermostat (Aquasonic, Wauchope, NSW, Australia). The remaining two tanks were maintained at 10°C to represent current-day winter temperatures. For each temperature, the heaters were placed in a common sump supplying the two tanks and the water maintained at ± 0.5°C of the desired temperature. Water was changed periodically to maintain water quality (ammonia: <0.7 mg L^{-1}; nitrite: <0.2 mg L^{-1}; nitrate: <20 mg

L^{-1}). The fish were fed to satiation daily and were allowed to acclimate for three weeks to their respective temperatures prior to the experiments commencing. Dissolved oxygen was maintained above 85% air saturation and the lighting regime was kept at 10 h light and 14 h dark. During the ~4 weeks following tagging, there were 2 mortalities in the 10°C acclimation group (2 diploids), 2 in the 14°C acclimation group (1 diploid, 1 triploid) and 1 in the 18°C acclimation group (1 triploid). We attribute these mortalities to transport/tagging effects rather than to temperature.

Respirometry

Oxygen consumption rates of individual fish were measured in six intermittently-closed respirometers (2.8 L) following previously-described methods (Clark et al., 2013). The respirometers were submerged in a 160 L temperature-controlled water bath that was maintained at >90% air saturation with compressed air. The temperature of the water bath was adjusted as necessary to match the acclimation temperature of the fish involved in the respirometry trial. Each respirometer was intermittently flushed every 15 min by a pump at 0.5 L min^{-1} to replenish the oxygen levels. Water was continuously mixed by a submersible pump (0.1 L min^{-1}) within a closed recirculation loop. Dissolved oxygen concentration was measured in each respirometer by fibre optic oxygen probes (FireSting O_2, Pyroscience, Aachen, Germany) sealed in the recirculation loop and recorded at 5 s intervals using an eight-channel PowerLab/8sp and LabChart 7 Pro software (ADInstruments Pty Ltd, Bella Vista, New South Wales, Australia).

Resting and maximum oxygen consumption rates were measured at three and seven weeks after the acclimation temperatures had been established. Fish from an acclimation temperature treatment were fasted for 24 hours and then dip-netted out of their holding tanks during mid-morning, anaesthetised with 0.02 mL L^{-1} Aqui-S (50% active isoeugenol) and their mass, length, and PIT tag number recorded. The fish were then placed in individual respirometers at their respective acclimation temperature and resting metabolic rate (RMR) was determined from oxygen consumption measurements (see below) during the 17-20 hour overnight recovery. The following morning, the fish were removed from the chambers and individually exercised in a 40 L round swim tank. Water temperature in the swim tank was maintained using an Aquasonic heater and Nema thermostat, and dissolved oxygen levels were maintained above 95% air saturation through aeration. A chase protocol was used to elevate oxygen consumption to the maximum level (Clark et al., 2013; Norin and Clark, 2016; Killen et al., 2017). Briefly, each fish was individually chased by hand for 2 min before being immediately placed back in the respirometer to measure the maximum oxygen consumption rate as a proxy for maximum metabolic rate (MMR). All fish became exhausted by 2 min of vigorous chasing in a preliminary

set of experiments (n=6). Exhaustion was recorded when the fish no longer swam away when being chased and tapped on the tail. Oxygen consumption measurements continued for 30 min to ensure the maximum rate of decline in oxygen concentration was captured. The fish were returned to their respective holding tanks after the respirometry protocol. Notably, a pump failure impacted one of the 14°C acclimation tanks between week 3 and week 7, essentially halving the sample size at this acclimation temperature and resulting in n=6 diploids and n=8 triploids being measured at 14°C thereafter.

Critical thermal maxima

At nine weeks of acclimation, fish were placed in respirometers, as described above, to measure RMR, but a critical thermal maximum (CT_{max}) protocol rather than an exercise protocol was conducted the following morning. The temperature of the water bath containing the respirometers was increased stepwise by 2°C every 75 min (ramped increase over 15 min and held stable for 60 min to ensure stable oxygen measurements) and routine metabolic rate was measured over two 15-min periods during each 60 min interval once temperature had plateaued (similar to Brijs et al. (2015)). The protocol was ceased when the fish displayed loss of equilibrium (LOE), which was defined as the inability to right themselves after 10 s. The time and temperature at LOE were recorded and each fish was immediately euthanized via anaesthetic overdose. Blood was sampled from the caudal vasculature in a 1 ml heparinised syringe, transferred to a 2 ml Eppendorf tube, and immediately placed on ice until processing (< 1 h).

Dissections and ploidy verification

Following LOE and blood sampling, the fish were dissected and the ventricle, liver, and spleen masses were recorded (Ohaus Scout Pro Portable Electronic Balance, Parsippany, NJ, USA). A sample (2 µL) of blood was smeared on a glass slide, dried, and stained in Dif Quik (Sigma Aldrich, Castle Hill, NSW, Australia) for ploidy verification. The blood smears were examined using light microscopy (Leitz Wetzler, Germany) and photographed using a Leica DFC310 FX microscope camera connected to a PC with Leica Application Suite Version 4.0.0 software (Leica Microsystems Limited, Switzerland). The photographs were processed using ImageJ 1.48v (Wayne Rasband, National Institutes of Health, USA). The nucleus major axis was measured for at least 50 randomly chosen erythrocytes per individual and triploids and diploids were statistically separated through cluster analysis on the mean nuclear length (Benfey et al., 1984).

Data analyses

Specific growth rate (SGR) was calculated using Eq. 1:

$$SGR = [(\ln(M_f) - \ln(M_i)) \times t^{-1}] \times 100 \quad (1)$$

where M_f and M_i are the final and initial masses, respectively, of an individual and t is the elapsed time between mass measurements in days.

Oxygen consumption rates (mg O_2 kg^{-1} h^{-1}) were calculated using slopes derived from linear regressions between oxygen concentration and time during each sealed event in the chamber and accounting for the volume of the respirometer as in Eq. 2:

$$\dot{M}O_2 = \frac{\frac{\Delta O_2}{\Delta t}(P_B - P_V) \times \beta_{O_2} \times Vol \times 0.2093}{M_b} \quad (2)$$

where ΔO_2 is the change in oxygen concentration within the respirometer over the change in time in hours (Δt), P_B is the barometric pressure in kPa, P_V is the water vapour pressure (kPa, Antoine equation), β_{O2} is the calculated oxygen capacitance of freshwater at the acclimation temperature (mg L^{-1} kPa^{-1}; Dejours 1981), Vol is the volume of the respirometer minus that of the fish (assuming 1 kg wet mass = 1 L) in L, 0.2093 is the fractional concentration of oxygen in well-aerated water, and M_b is body mass (kg). Note that most of the statistical analyses were conducted using oxygen consumption rate in mg O_2 h^{-1} and body mass was included as a covariate (details below).

Resting metabolic rate (RMR) was determined as the mean of the lowest 10% of oxygen consumption values throughout the measuring period (17-20 hours), excluding outliers (values ± 2 s.d. from the mean (Norin et al., 2014)). Maximum metabolic rate (MMR) was calculated from the slope immediately after the exhaustive chase protocol, which was always found to be the highest. Absolute aerobic scope was calculated by subtracting RMR from MMR, while factorial aerobic scope was calculated by dividing MMR by RMR.

Critical thermal maximum (CT_{max}) was calculated by modifying the critical swimming speed equation from Brett (1964) into Eq. 3:

$$CT_{max} = T_f + \left(\frac{t_f}{t_i} \times T_i\right) \quad (3)$$

where T_f is the highest temperature the fish endured for the full time period, t_f is the time the fish lasted at its final temperature step, t_i is the prescribed time for each temperature (i.e. 60 min), and T_i is the incremental temperature increase (i.e. 2°C).

Temperature coefficients (Q_{10}) were calculated to quantify the influence of acclimation temperature on the metabolism of diploids and triploids using mean RMR values in Eq. 4:

$$Q_{10}=\left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2-T_1}} \quad (4)$$

where R_2 and R_1 are the mean RMR values that correspond to two acclimation temperatures (T_2 and T_1). Q_{10} values were calculated between 10 and 18°C for each ploidy at each acclimation time point (3, 7 and 9 weeks).

All data are presented as mean ± 95% confidence intervals in figures and text. Organ weights were analysed using ANCOVAs (see below) and presented as body mass adjusted means from the ANCOVA outputs.

Statistical analyses

Mass and SGR data were analysed using a series of two-way ANOVAs and ANCOVAs, respectively. The two factors (ploidy and acclimation temperature) were analysed within each time point and alpha for significance was set at 0.05 / 3 tests = 0.017. ANCOVAs for SGR used the initial mass as the covariate. Differences in metabolic rates were analysed using general linear mixed models, testing metabolic rate (mg O_2 h^{-1}) against sampling time point, ploidy, and acclimation temperature with mass as a covariate and accounting for repeated measures on an individual. Results are reported from the repeated measures analyses using F tests (type III Wald F tests with Kenward-Roger degrees of freedom approximation). If applicable, post hoc tests for pairwise comparisons using Bonferroni corrections were utilised to investigate the differences between ploidies within time points and the differences across time points within ploidy. CT_{max} data were analysed using a two-way ANOVA because there was no time point variable. Measurements of routine metabolic rate (mg O_2 kg^{-1} h^{-1}) during the CT_{max} protocol were averaged for each 60 min interval and then analysed with a linear mixed effects model to test the effect of temperature (covariate) increase on metabolic rate between ploidies (factor) with individual as a random factor to control for repeated measures. Organ weights were analysed using ANCOVAs testing the absolute values against ploidy and acclimation temperature with mass as a covariate. Significance was accepted at $p<0.05$ unless otherwise indicated (for multiple tests) and Bonferroni corrected post hoc tests were conducted on covariate-adjusted means where applicable. All analyses were conducted using R Studio (Version 1.0.136) with R packages *car* (Fox and Weisberg, 2011), *nlme* (Pinheiro et al., 2016), and *lsmeans* (Lenth, 2016).

Results

Ploidy verification

The erythrocyte nuclei measurements confirmed that the ploidy of all fish was correctly classified. In this study, the major axis of the nucleus in triploids was 1.48 times longer than that of diploids, which compares favourably with a value of 1.26 times longer reported in Benfey et al. (1984).

Survival, mass and growth

There were no natural mortalities in either ploidy at the 10 or 14°C acclimation temperatures once the experiment commenced. There were two triploid mortalities at 18°C between weeks 7 and 9, although the low mortality rate makes it difficult to attribute these deaths to a lower chronic thermal tolerance of triploids.

Triploid mass was ~1.5 times greater than diploids at week 0 across all acclimation temperatures (Fig. 1A, 10°C: $F_{1,18}$=9.01, p=0.008; 14°C: $F_{1,21}$=37.58, p<0.001; 18°C: $F_{1,23}$=11.91, p=0.002). Triploids and diploids acclimated to 10 and 18°C were similar sizes at week 3 and remained similar at subsequent time points. On the other hand, triploid fish acclimated to 14°C continued to be larger than diploids at week 3 ($F_{1,21}$=6.93, p=0.016) but the masses of the ploidies converged by week 7. The temporal dynamics in fish mass across the ploidies and acclimation temperatures were consistent with SGR of the different groups (Fig. 1B).

Relative ventricle, liver, and spleen masses were similar between diploids and triploids across all acclimation temperatures. Not surprisingly, gonad mass was significantly greater in diploids than in the inherently sterile triploids at all acclimation temperatures (Table 1, 10°C: $F_{1,17}$=54.32, p<0.001; 14°C: $F_{1,6}$=17.68, p=0.006; 18°C: $F_{1,13}$=56.77, p<0.001).

Metabolic rates

As expected, there was a general increase in RMR with acclimation temperature from ~55 to ~125 mg kg^{-1} h^{-1} in both ploidies (Q_{10} between 10 and 18°C (week 3, week 7, and week 9, respectively) = 1.75, 1.75 and 2.31 for diploids; 1.87, 1.73 and 1.99 for triploids). Counter to our expectation that RMR would decrease as fish progressively acclimated to the higher temperatures, there were some significant increases in RMR between week 3 and week 7 (~75 to ~115 mg kg^{-1} h^{-1}) in both ploidies at 14°C (diploids: $F_{2,79}$=10.96, p=0.004; triploids: $F_{2,79}$=34.87, p<0.001) and in triploids at 18°C (~96 to ~120 mg kg^{-1} h^{-1}: $F_{2,79}$=21.61, p<0.001; Fig. 2A).

On average, RMR was 15% higher in diploids compared with triploids across all acclimation temperatures during the week 3 measurements (Fig. 2A, 10°C: $F_{1,62}$=10.20, p=0.002; 14°C: $F_{1,62}$=7.84, p=0.007; 18°C: $F_{1,62}$=12.72, p=0.001). Diploids also had a higher RMR at 10°C during the week 7

measurements (10°C: $F_{1,62}$=6.39, p=0.014), but RMR was similar between ploidies at 14 and 18°C. There were no differences in RMR between diploids and triploids during the week 9 measurements.

Absolute aerobic scope and MMR were generally stable across temperatures (~400 mg kg^{-1} h^{-1} and ~500 mg kg^{-1} h^{-1}, respectively) for both ploidies (Figs. 2B, C), while there was a tendency for a ~25% decline in factorial aerobic scope across the range of acclimation temperatures (Fig. 2D). Within ploidies, there were small but significant increases in MMR between weeks 3 and 7 at 14 and 18°C, and an increase in absolute aerobic scope between weeks 3 and 7 at 18°C (Figs. 2B, C; MMR: 14°C: diploids: $F_{1,40}$=4.63, p=0.038; triploids: $F_{1,40}$=7.85, p=0.008; 18°C: diploids: $F_{1,40}$=17.04, p<0.001; triploids: $F_{1,40}$=10.83, p=0.002; aerobic scope at 18°C: diploids: $F_{1,40}$=15.85, p<0.01; triploids: $F_{1,40}$=4.46, p=0.041).

Between ploidies, MMR and absolute aerobic scope were similar at all acclimation temperatures within both measuring time points (weeks 3 and 7) (Figs. 2B, C). Factorial aerobic scope, however, was higher in the triploids compared with diploids within the 10°C acclimation group at week 3 (Fig. 2D, $F_{1,18}$=11.29, p=0.047) and within the 14°C acclimation group at week 7 ($F_{1,11}$=6.62, p=0.026). There was some evidence of factorial aerobic scope decreasing between weeks 3 and 7 in triploids at 10°C (Fig. 2D, 10°C: $F_{1,13.4}$=11.45, p=0.001).

Critical thermal maxima

CT_{max} did not differ between ploidies (Fig. 3A, $F_{1,51}$=1.76, p=0.190), but increased with acclimation temperature from ~26 to ~29°C ($F_{2,51}$=66.27, p<0.001). Thus, there was generally a ~0.4°C improvement in CT_{max} for every 1°C increase in acclimation temperature. Within diploids, CT_{max} was similar between fish acclimated to 10 and 14°C, but higher for fish acclimated to 18°C ($p_{10\text{-}18°C}$<0.001, $p_{14\text{-}18°C}$<0.001). Thermal effects were more consistent for triploids, whereby CT_{max} increased significantly between the 10 and 14°C acclimation groups and between the 14 and 18°C acclimation groups ($p_{10\text{-}14°C}$=0.016, $p_{14\text{-}18°C}$=0.001).

Consistent with the findings for RMR described above, diploids had a higher routine metabolic rate than triploids during the CT_{max} protocol when commencing at the acclimation temperatures of 14 and 18°C. In contrast, there were no differences in routine metabolic rate between the ploidy groups acclimated to 10°C (Fig. 3B, 10°C: $F_{1,20}$=0.002, p=0.969; 14°C: $F_{1,11}$=5.115, p=0.045; 18°C: $F_{1,20}$=6.676, p=0.018).

Discussion

In contrast with our initial hypothesis, triploids performed similarly to their diploid counterparts when held chronically at three acclimation temperatures and during the CT_{max} challenge. Our finding of similar thermal performance between ploidies is consistent with some previously published studies on Atlantic salmon, although there is some evidence for species differences (Ojolick et al., 1995; McGeachy et al., 1995); (O'Flynn, 1997; Altimiras et al., 2002). Ploidy differences in the present study were observed for mass, SGR, and RMR, but not for MMR and aerobic scope (Figs. 1, 2). We discuss below how these parameters may interact to result in subtle but important differences between ploidies.

Growth and metabolism

As expected, RMR increased with acclimation temperature in both ploidies, a trend that is well documented for fishes (Fry, 1971; Clarke and Johnston, 1999; Gillooly et al., 2001). Interestingly, there was a difference between the ploidies for RMR within an acclimation temperature in the first periods of the study (weeks 3 and 7) but not at the end (week 9). This is similar to the temporal trends in mass and SGR (Figs. 1, 2A), indicating there may be some common mechanisms between the measured parameters. Indeed, food consumption, growth and RMR are thought to be intrinsically linked (Metcalfe et al., 1995; Pedersen, 1997; Yamamoto et al., 1998; Norin et al., 2016; Van Leeuwen et al., 2012).

The lower RMR of triploids indicates a lower maintenance cost per unit body mass, which should theoretically translate to higher growth rates for a given energy intake assuming equivalent assimilation efficiencies. However, diploids rather than triploids displayed a higher SGR in the earlier weeks of the experiment when the RMR of triploids was lower. It is possible that these counter-intuitive findings are a consequence of fish size, as growth rates are known to be negatively related to body size in fish (Iwama, 1996). Indeed, when diploids caught up to triploids in mass by week 7, SGR and RMR also converged, supporting the idea that it was fish size rather than ploidy that drove the differences in the earlier weeks of the experiment.

Nevertheless, it is also possible that behavioural differences between the ploidies played some role in the counter-intuitive findings. Specifically, diploid salmonids have been reported to out-perform their triploid counterparts when raised in mixed-ploidy populations, resulting in improved growth of diploids (Carter et al., 1994; Galbreath et al., 1994). This potential behavioural difference between ploidies may explain why investigations into growth between triploids and their diploid conspecifics have been largely inconclusive, whereby triploid Atlantic salmon have been reported to grow faster, slower, and similarly to their diploid counterparts (Galbreath et al., 1994; McGeachy et al., 1995; McCarthy et al., 1996). That is, the disparate findings may be largely attributed to husbandry practices such as keeping the ploidies separate versus mixing them in a common tank (Galbreath et al., 1994; Maxime, 2008). For example, Atlantic salmon triploids grew

at a faster rate and had a higher mass when kept in a separate tank than diploids, whereas diploids out-performed the triploids when the two groups were mixed (Galbreath et al., 1994). In instances where diploids out-performed triploids in mixed tanks, it has been attributed to a more aggressive nature of diploids (Galbreath et al., 1994). In this context, intraspecific competition and dominance status has been correlated with standard metabolic rate, such that individuals with an inherently higher standard metabolic rate (as with the diploids in this study) are stimulated to eat more food and thus be more competitive/aggressive (Metcalfe et al., 1995; Cutts et al., 1998; Norin et al., 2016). In any event, similar growth rates have also been observed between ploidies of Atlantic salmon and coho salmon (*Oncorhynchus kisutch*, Walbaum) when the ploidies are mixed in tanks, highlighting that further controlled experiments are required on this topic to tease apart the roles of ploidy, body size, RMR and behaviour (Johnson et al., 1986; Carter et al., 1994).

Acute thermal tolerance and aerobic capacity

Ploidy did not influence the CT_{max} of Atlantic salmon in this study, despite triploids from the 14 and 18°C acclimated groups maintaining a slightly lower routine metabolic rate during the temperature ramp (Fig. 3). The lower routine metabolic rate is consistent with the lower RMR observed for triploids in the earlier weeks of acclimation (Fig. 2A). Despite the common observations that triploids suffer higher mortality at elevated temperatures, few studies have found ploidy differences within CT_{max} protocols with a variety of heating rates (e.g. 2°C h^{-1}, 15°C h^{-1}, 2°C d^{-1}) and species (e.g. rainbow trout, brook charr, brook trout) (Benfey et al., 1997; Galbreath et al., 2006; Maxime, 2008; Scott, 2012; Scott et al., 2015). However, the CT_{max} experiments conducted here and in previous studies used juvenile fish, and it is known that smaller individuals can be more thermally tolerant than their adult conspecifics (Clark et al., 2012; Messmer et al., 2016; Clark et al., 2017). Furthermore, adult salmonids face other energetically costly physiological processes such as sexual maturation and ion regulation in a hypertonic environment upon their movement from fresh to salt water during their later life stages (Bœuf and Payan, 2001). While the enlarged erythrocytes of triploids do not seem to impair oxygen transport capacity, they could hinder other critical functions like ion regulation. Therefore, it is possible that experimental results from juvenile triploids in freshwater may not be directly applicable to adult triploids in marine environments.

Another consideration is that this study investigated metabolism and CT_{max} exclusively under normoxic conditions, whereas salmon in wild or cultured environments may be subjected to periods of hypoxia. In this context, upper thermal limits have been proposed to be oxygen-limited when in normoxic environments (Pörtner and Knust, 2007). Nevertheless, CT_{max} of red drum (*Sciaenops ocellatus*) and marine lumpfish (*Cyclopterus lumpus*) was found to be independent of oxygen availability over a wide range of oxygen tensions (Ern et al., 2016). Indeed, CT_{max} was not affected by oxygen availability until close to (lumpfish) or below (red drum) the species-specific critical oxygen tensions (P_{crit}) despite significant decreases in aerobic

scope (72 and 89% reductions, respectively) (Ern et al., 2016). Given that it would be rare for Atlantic salmon in natural or cultured environments to experience oxygen levels below their P_{crit} (~35% air saturation for the size used here; (Barnes et al., 2011)), our findings should be applicable across the range of oxygen levels Atlantic salmon typically experience throughout their lifecycle.

Interestingly, a larger relative ventricle mass has been correlated with higher temperature tolerance in fish (Ozolina et al., 2016). It has been suggested that a deterioration in cardiovascular performance may be causal to the upper thermal tolerance limits of fishes (Lannig et al., 2004; Clark et al., 2008; Farrell, 2009). Indeed, enhancing oxygen availability to the heart of European perch has been shown to play a role in maintaining stroke volume at critically high temperatures. Nevertheless, a concurrent decline in heart rate was likely to reflect direct temperature effects rather than an oxygen limitation (Ekström et al., 2016). Since ventricle mass is positively correlated with stroke volume (Graham and Farrell, 1989), and we did not see any differences in ventricle mass between ploidies in the present study (Table 1), we suggest that stroke volume was similar between triploids and diploids at the three acclimation temperatures. As such, we did not find any evidence that enhanced cardiovascular performance played any role in increasing CT_{max} with acclimation temperature (Fig. 3). Contrasting results for relative ventricle mass between ploidies have been reported, ranging from significantly heavier in Atlantic salmon triploids, to similar among ploidies for rainbow trout and Atlantic salmon (Verhille et al., 2013; Fraser et al., 2013; Fraser et al., 2015). The heart is a highly plastic organ, so the contrasting observations could be due to environmental conditions such as rearing temperature, salinity, and natural versus controlled environmental challenges (Fraser et al., 2015).

Values of CT_{max} increased by approximately 0.4°C with every 1°C increase in acclimation temperature in both triploids and diploids, which is consistent with the changes in CT_{max} observed with acclimation temperature in previous studies of salmonids (Lutterschmidt and Hutchison, 1997; Currie et al., 1998). Nevertheless, aerobic scope remained stable across acclimation temperatures in both ploidies, suggesting that increased aerobic capacity was not responsible for the increase in CT_{max} of the fish acclimated to the higher temperatures (Fig. 2C). This contrasts with the idea of oxygen- and capacity-limited thermal tolerance (OCLTT), which assumes that temperature-dependent performance and thermal tolerance are governed by aerobic scope (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). The lack of linkage between aerobic scope and CT_{max} adds to a growing database indicating that thermally-dependent fitness is primarily driven by factors other than oxygen supply capacity (Clark et al., 2013; Lefevre, 2016; Sandblom et al., 2016).

Conclusions and future directions

We have shown that the thermal tolerance of juvenile Atlantic salmon in freshwater is similar between diploids and triploids, and it does not appear to be influenced by aerobic capacity. We have also shown that differential growth rates between ploidies can emerge when ploidies are mixed within the same tanks.

Based on this and previous studies, we recommend that future efforts to understand reported differences in thermal tolerance between ploidies should utilise large size ranges, freshwater vs. saltwater, and mixed-ploidy vs. single-ploidy tank arrangements. Moreover, investigations should examine different physiological attributes in concert with the oxygen transport cascade. For example, Sambraus et al. (2017) found lower ion (Cl^-, Na^+, and K^+) concentrations and higher glucose levels in blood plasma of seawater-acclimated triploid compared with diploid post-smolt Atlantic salmon at warm temperatures, suggesting lower physiological tolerance in triploids. Therefore, osmotic challenges (parr-smolt transformation, spawning migration of adults) could be investigated in conjunction with thermal stress to illuminate any role of ion regulation in determining differential ploidy survival at high temperatures. Such multi-stressor occurrences also exist during different phases of salmon aquaculture (e.g. freshwater influx in coastal aquaculture facilities), where triploids are increasingly favoured due to the advantages gained by their inherent sterility (Benfey, 1999; Benfey, 2001). The findings and suggestions we have highlighted here should pave the way for future studies to further ascertain the extent to which triploids represent a useful model for elucidating the mechanisms underlying environmental tolerance in fish.

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Competing interests

The authors have no competing interests to declare.

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Table 1: Body mass adjusted means (from ANCOVA) for the organ masses of diploid and triploid Atlantic salmon acclimated to three different temperatures. Values are presented as a percentage of body mass and are mean ± 95% confidence intervals. (*) denotes significant differences between ploidies within a temperature.

Measurement	Acclimation temperature (°C)					
	10		14		18	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
Ventricle (%)	0.077 ± 0.007	0.076 ± 0.007	0.092 ± 0.012	0.091 ± 0.012	0.069 ± 0.006	0.070 ± 0.005
Liver (%)	0.858 ± 0.110	0.943 ± 0.110	1.054 ± 0.221	1.053 ± 0.204	0.636 ± 0.101	0.788 ± 0.093
Spleen (%)	0.084 ± 0.018	0.101 ± 0.019	0.184 ± 0.068	0.093 ± 0.049	0.073 ± 0.018	0.069 ± 0.017
Gonad (%)	0.193 ± 0.023*	0.075 ± 0.023	0.217 ± 0.042*	0.093 ± 0.059	0.239 ± 0.034*	0.046 ± 0.044

Figures

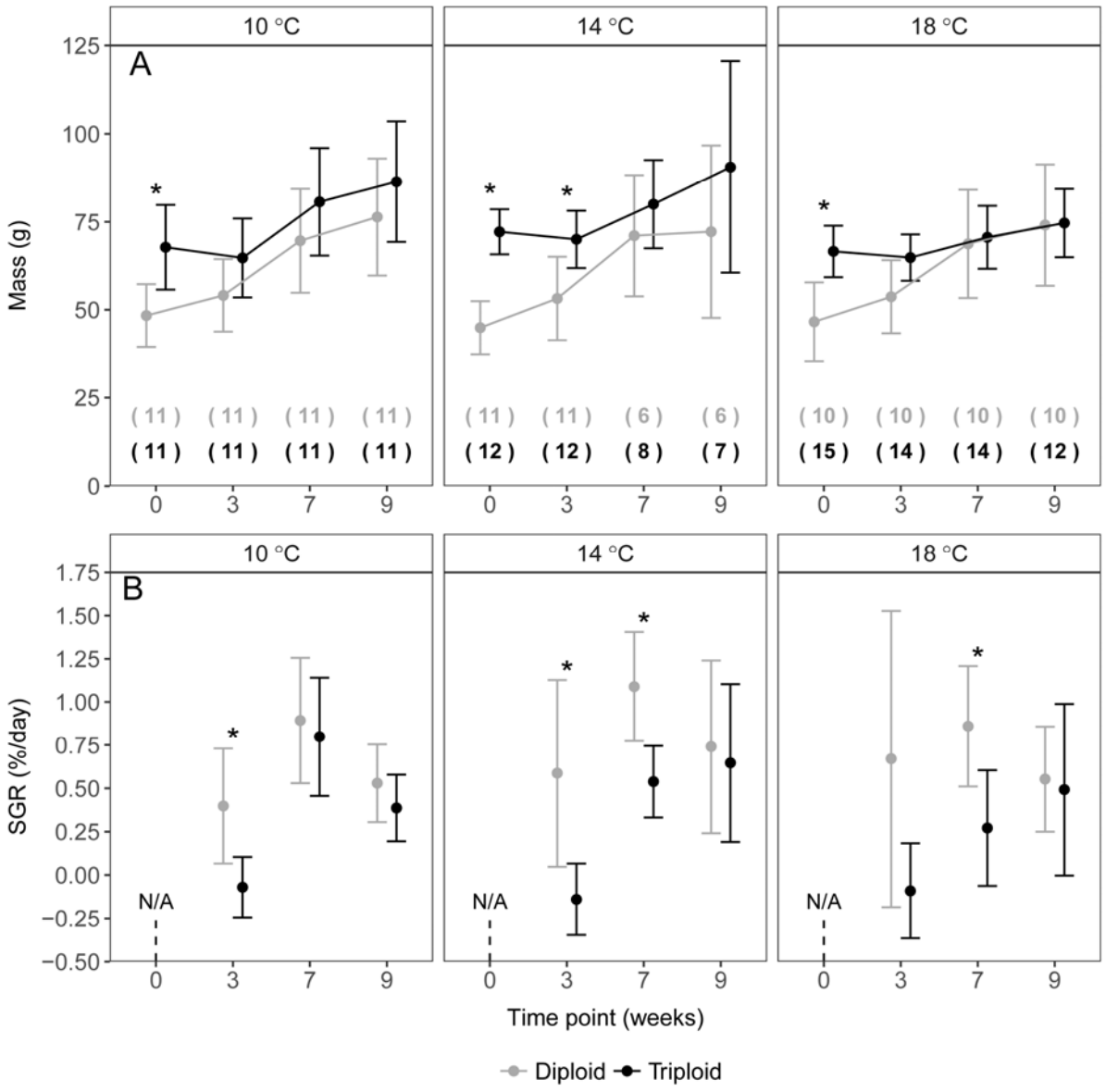

Figure 1: (A) Mass and (B) specific growth rate (SGR) for diploid (grey) and triploid (black) Atlantic salmon during 9 weeks of temperature acclimation to 10, 14, and 18°C. Samples sizes are in parentheses in (A). All values are means ± 95% confidence intervals and positioned side by side to reduce overlap for clarity. (*) demarcates significance between ploidies based on ANOVAs (mass) and ANCOVAs (SGR) with alpha reduction for multiple testing (see Methods). Note that in (B), values represent SGR between time points (e.g. from 0 to 3 weeks) and therefore could not be calculated for week 0 (N/A).

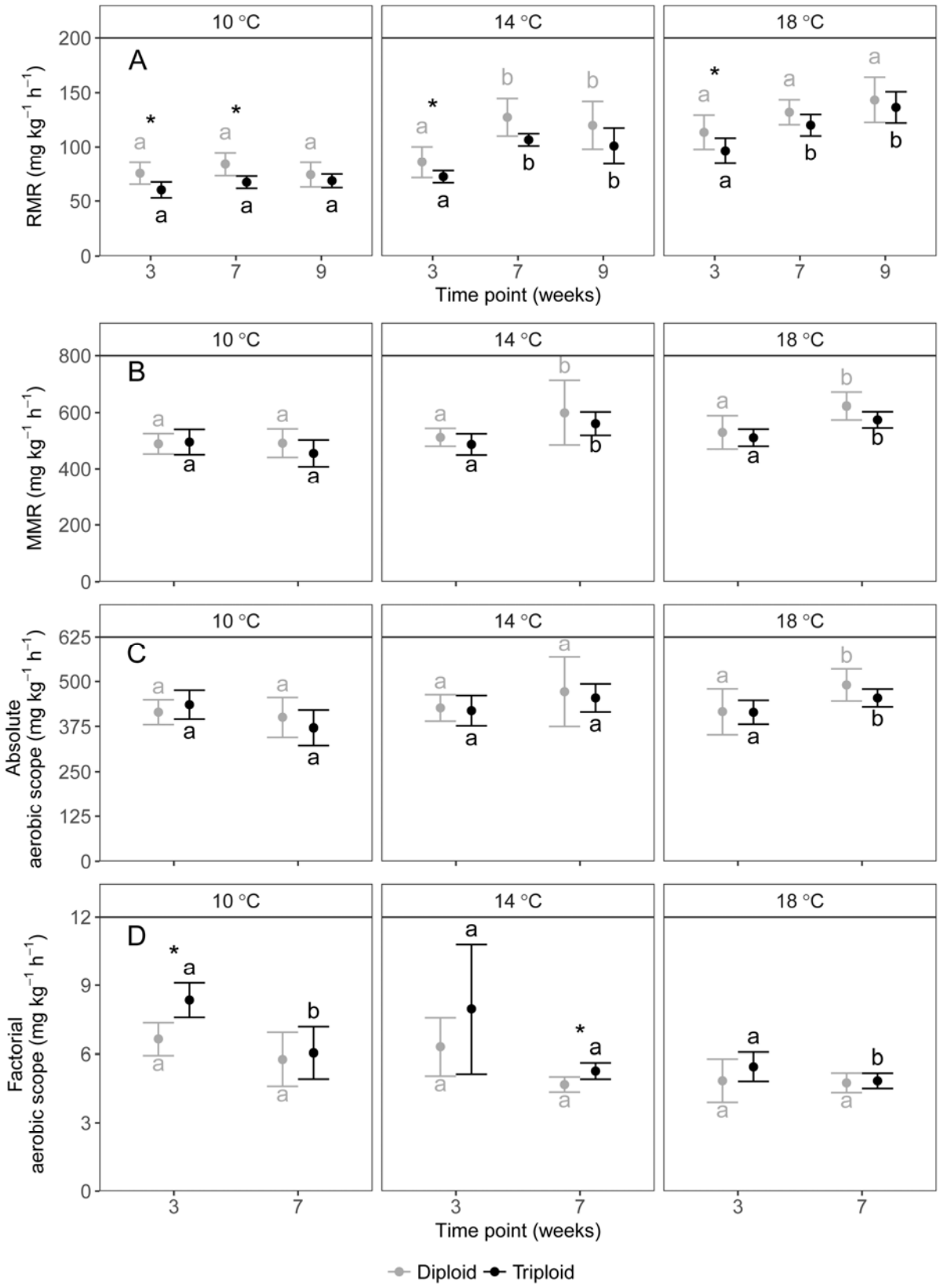

Figure 2: (A) Minimum oxygen consumption (RMR), (B) maximum oxygen consumption (MMR), (C) absolute aerobic scope, and (D) factorial aerobic scope for diploid (grey) and triploid (black) Atlantic salmon measured during acclimation to 10, 14, and 18°C. Values are mean ± 95%

confidence intervals. Significance between ploidies is denoted by (*) and differences between measuring time points (weeks) within a ploidy are signified by different lower case letters (Bonferroni p-value adjustments for pairwise comparisons). See Fig. 1A for sample sizes.

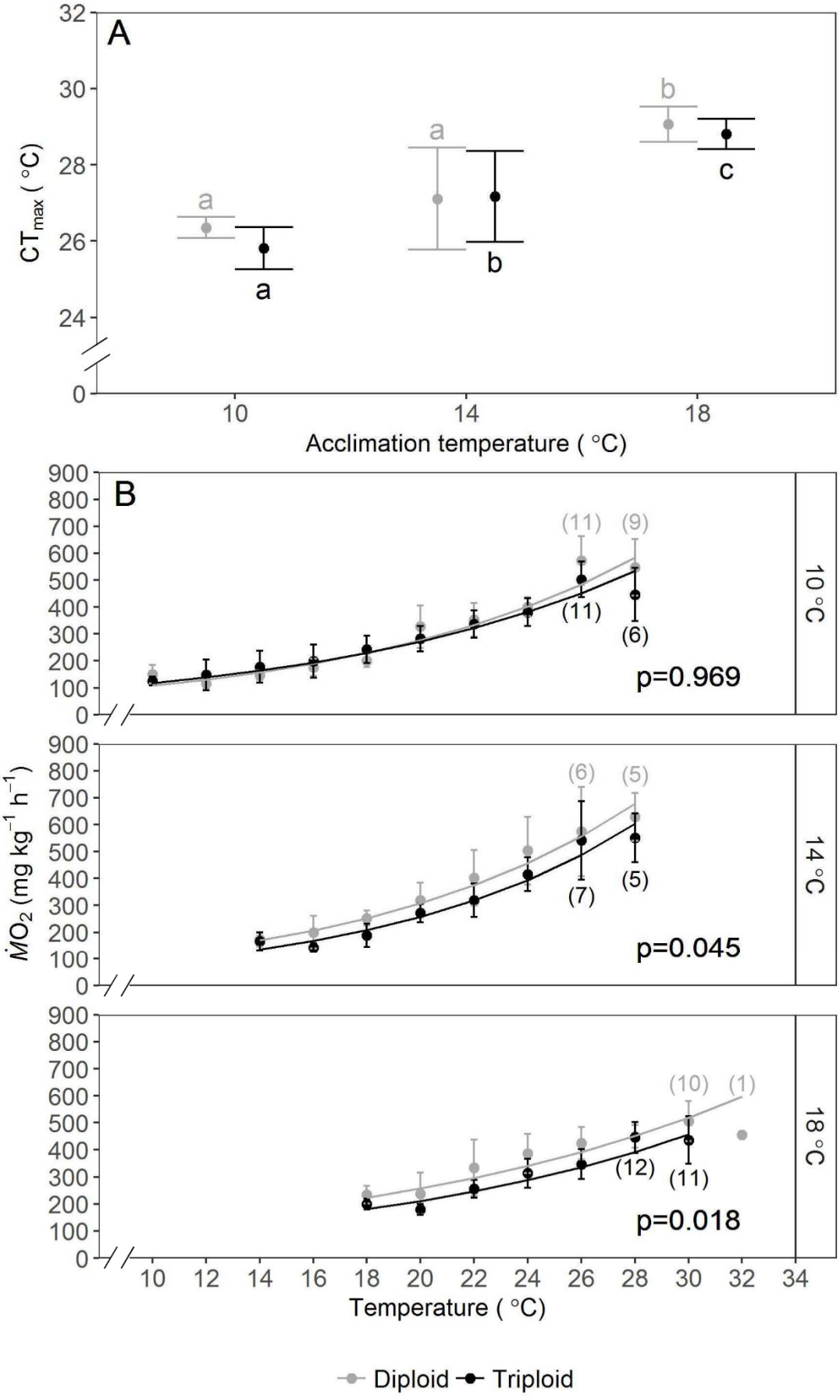

Figure 3: (A) CT_{max} temperatures for diploid (grey) and triploid (black) Atlantic salmon across acclimation temperatures and (B) oxygen consumption rate during the CT_{max} protocol. (A) Values are mean ± 95% confidence intervals. Lower case letters show differences within a ploidy across acclimation temperatures. (B) Values are mean ± 95% confidence intervals fitted with exponential regressions with the equations: diploids at 10°C: $y = 42.385(0.088)*e^{0.094(0.004)x}$ (R^2=0.84); triploids at 10°C: $y = 50.135(0.099)*e^{0.085(0.004)x}$ (R^2=0.81); diploids at 14°C: $y = 42.571(0.114)*e^{0.099(0.005)x}$ (R^2=0.92); triploids at 14°C: $y = 30.291(0.133)*e^{0.107(0.006)x}$ (R^2=0.86); diploids at 18°C: $y = 63.089(0.186)*e^{0.070(0.007)x}$ (R^2=0.60); triploids at 18°C: $y = 45.139(0.157)*e^{0.077(0.006)x}$ (R^2=0.68). P-values represent significance between the two regressions. Numbers in parentheses indicate when sample sizes decreased.