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Is it advantageous for Atlantic salmon to be triploid at lower temperatures?

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ABSTRACT

Marine organisms living at low temperatures tend to have larger genomes and larger cells which suggest that these traits can be beneficial in colder environments. In fish, triploidy (three complete sets of chromosomes) can be induced experimentally following fertilization, which provides a model system to investigate the hypothesis that larger cells and genomes offers a physiological advantage at low temperatures. We tested this hypothesis by measuring metabolic rates and swimming performance of diploid and triploid Atlantic salmon (*Salmo salar*) post smolts acclimated to 3 or 10.5 °C. At 10.5 °C, triploids had significantly lower maximum metabolic rates which resulted in a lower aerobic scope compared to diploids. In addition, triploids initiated ram ventilation at lower swimming speeds, providing further evidence of a reduced capacity to meet oxygen demands during strenuous activity at 10.5 °C. However, at 3 °C, metabolic rates and critical swimming speeds were similar between both ploidies, and as expected substantially lower than at 10.5 °C. Therefore, triploidy in colder environments did not provide any advantage over diploidy in terms of metabolic rate traits or swimming performance in Atlantic salmon. We therefore conclude that traits, other than aerobic scope and swimming performance, contribute to the trend for increased cell and genome size in marine ectotherms living in cold environments.

1. Introduction

A curious and widespread phenomenon in marine ecosystems is that ectotherms living at lower temperatures tend to have larger genomes and cell sizes (Arendt, 2007; Rees et al., 2007; Hessen et al., 2013). This suggests that larger genomes and/or larger cell sizes provide some general advantages in colder environments, or that they are selected against in warmer environments. A key factor directly affected by these observations, which may also play a role in explaining them, is metabolism. For example, larger genomes and cell sizes generally correlate negatively with mass-specific metabolic rate at a given temperature (Maciak et al., 2011; Kozlowski et al., 2003), while higher temperatures elevate metabolic rates owing to the acceleration of all biochemical processes (Gillooly et al., 2001). Furthermore, food availability may be scarcer in colder environments. Hence, the pace of life is slower at lower temperatures, and larger cells with lower metabolic demands may therefore be advantageous in these conditions. In contrast, growth is expected to be positively associated with temperature and the lower surface area to volume rate of larger cells may limit nutrient and gas exchange, which could become a disadvantage at higher temperatures.

One way to obtain larger genomes and cells is by polyploidization, where the genome is duplicated. In fish, diploidy (two complete chromosome sets) is the norm. However, polyploidy is not uncommon, where examples of species with 3, 4, 6, 8 and 16 complete chromosome sets are known (Leggatt and Iwama, 2003). Of interest, some instances of polyploidy can be easily induced in fish, such as triploidy (three complete chromosome sets), by preventing the expulsion of the second polar body during meiosis (Benfey and Sutterlin, 1984).

Induced triploidy provides a model system for studying the physiological consequences of larger genomes and cell sizes within species (Benfey, 1999; Maxime, 2008). As larger cells have a lower surface area to volume ratio that may impair gas exchange as well as the rate of other cell membrane functions, it can be hypothesized that larger genomes and cell sizes in triploids should become a disadvantage relative to

Abbreviations: AS, aerobic scope; MMR, maximum metabolic rate; MO_2, oxygen uptake rate; Q_{10}, temperature coefficient; SMR, standard metabolic rate; U_{crit}, critical swimming speed; U_{ram}, onset of ram ventilation.

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diploids at elevated temperatures. For example, triploid Atlantic salmon (*Salmo salar*) have a lower thermal maximum, as well as reduced growth and appetite at elevated temperatures compared to diploid counterparts (Atkins and Benfey, 2008; Hansen et al., 2015; Sambraus et al., 2017). In contrast, triploid Atlantic salmon have higher rates of food consumption compared with diploids at 3 and 9 °C (Sambraus et al., 2018).

When evaluating the physiological adaptations of fish to different environmental conditions, aerobic scope (AS) models are often used (Fry, 1971; Claireaux and Lefrancois, 2007; Clark et al., 2013). The AS is the difference between standard metabolic rate (SMR) and maximum metabolic rate (MMR), where SMR represents the minimum energetic requirement to maintain basal homeostasis in inactive and non-digestive fish at their acclimation temperature, while MMR is the highest rate of oxygen consumption achieved during strenuous activity. The AS thereby represents a measurement of the capacity to perform fitness related aerobic activities such as foraging, locomotion, digestion and reproduction, meaning that environmental or biological conditions that compromise the AS can be considered suboptimal (Fry, 1971; Lefevre, 2016).

Earlier studies on triploid salmonids have suggested a reduced aerobic capacity relative to diploids owing to an increased SMR (O'Donnell et al., 2017) and a reduced oxygen carrying capacity of the blood (Bernier et al., 2004), or found no differences as evidenced by similar swimming capabilities (Small and Randall, 1989; Stillwell and Benfey, 1997) and similar metabolic rates (Hyndman et al., 2003; Bowden et al., 2018). However, these studies were all performed at the mid to high end of the thermal niche in salmonids.

The natural thermal range of Atlantic salmon is 3 to 18 °C (Hvas et al., 2017a). Our objective was to compare the AS between diploid and triploid Atlantic salmon at the low end of their thermal niche. This was done by measuring the SMR and MMR of fish acclimated to either 3 or 10.5 °C. Moreover, we also assessed effects on swimming performance by measuring the onset of ram ventilation (U_{ram}) and the critical swimming speed (U_{crit}) (Brett, 1964). We hypothesized that triploid Atlantic salmon would perform better at 3 °C compared to diploid Atlantic salmon because we presumed that larger genomes and cells are advantageous in colder environments.

2. Materials and methods

2.1. Animal husbandry

Diploid and triploid Atlantic salmon (provided by Aquagen, Norway) were reared at Matre Research Station, Institute of Marine Research, Norway. Triploidy was induced following fertilization by subjecting fish eggs to a hydrostatic pressure of 655 bar (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) to interfere with the release of the second polar body, as described previously (Piferrer et al., 2009; Sambraus et al., 2017, 2018). Ploidy status of all fish was assessed by measuring mean red blood cell diameter under a microscope after the experiments had concluded (e.g. Sambraus et al., 2017). Diploid red blood cell diameter was 13.2 ± 0.2 μm (range: 12.7–13.7 μm), and mean triploid red blood cell diameter was 15.3 ± 0.3 μm (range 14.5–15.9 μm). No size overlaps between ploidy groups suggested a 100% triploidization rate.

After smoltification the fish were kept in holding tanks with a water volume of 405 l. Here, an open flow of UVC treated and filtered seawater was supplied from the local fjord, which ensured oxygen levels above 85% at all times and prevented waste products accumulating. Constant temperatures of either 3 °C or 10.5 °C in the holding tanks were achieved using customized computer software (SD Matre, Normatic AS, Nordfjordeid, Norway) by adequate mixing of water from cooled (2 °C), ambient (9 °C) and heated (25 °C) water reservoirs. The fish were fed to satiation every day with feed pellets (3 mm pellet size, Nutra, Skretting, Norway) via automated feeding devices, and kept under a continuous light regime. Prior to the experimental trials, diploid and triploid Atlantic salmon had been acclimating in these conditions for a minimum of one month.

This work was performed between October and December 2017 in accordance with the Norwegian laws and regulations regarding use of animals in scientific research under permit number 12827.

2.2. Swim tunnel respirometry setup

Swimming performance and metabolic rates were measured with a submerged 90 l intermittent-flow swim tunnel respirometer (Loligo systems, Denmark) that was described previously (Hvas et al., 2018). Mass-specific oxygen uptake rates (MO_2) provided an indirect measurement of aerobic metabolism, while swimming capabilities of fish could be assessed systematically by controlling the flow speed within the tunnel via a motor driven propeller, after having established the relationship between motor output and flow speed using a handheld flow meter (Höntzsch Flow Measuring Technology, Germany).

The rectangular dimensions of the swim section were 66 × 20 × 19.5 cm, where a flow straightener with honeycomb shaped cells upstream provided approximated laminar flow properties. Further upstream a temperature sensor and a fibre optic oxygen sensor were installed, while an inlet downstream of the swim section was connected to a flush pump (57 l min^{-1}). These devices were connected to computer software (AutoResp Respirometry Software; Loligo Systems, Denmark), where temperature and oxygen concentration were logged every second during experimentation, while the flush pump could be turned on and off automatically in desired repeated intervals so that oxygen levels could be re-established after a measurement period. Constant water temperatures were obtained by having an open flow running through the outer tank containing the respirometer, using the same method of water supply as for the holding tanks. The majority of the respirometer was covered in black plastic sheets to reduce visual disturbances to the fish. To further prevent unwanted disturbances during measurements, the entire setup was placed in a small secluded room.

2.3. Experimental protocol

Prior to all respirometry trials, food had been withheld for 24 h to minimize metabolic effects from digestion.

In the afternoon, one fish was gently netted from one of the holding tanks and quickly transferred in a lidded water filled bucket to the respirometer that was located in a neighbouring room. The fish was then allowed to acclimate in the experimental setup overnight at a flow speed of 15 cm s^{-1} (~0.4 body lengths s^{-1}), which was too low to initiate swimming. The following morning swimming was enforced by increasing the water flow in increments of 15 cm s^{-1} every 30 min. At higher flow speeds the fish was eventually unable to continue swimming and consequently either got stuck on the rear grid or was resting against it. When this happened, the flow speed was momentarily reduced to allow the fish to resume its swimming activity whereafter the speed was increased again to the intended test speed. The point of fatigue was then defined as when the fish had stopped its swimming activity 3 times within 1 min, and the time was recorded. After the swimming trial, the fish was sedated in 100 mg l^{-1} Finquel (Scanvacc, Norway) and weight (W), fork length (L_f), maximum height and maximum width were measured. The fish was then returned to its holding tank. A measurement period was subsequently made in the empty respirometer to account for possible bacterial respiration. However, in all cases background respiration rates were low, probably due to the combination of a continuous open flow through the buffer tank, thorough daily cleaning of the setup and the low test temperatures used. Consequently, bacterial respiration had a negligible influence on the results.

At moderate to high swimming intensities salmonids start to ram ventilate as it becomes energetically more efficient than actively ventilating the gills (Steffensen, 1985; Hvas et al., 2017b). When ram ventilation was first observed during the swim trials, the time and flow speed

was noted.

The MO_2 was measured intermittently by automatically alternating between closed measurement periods and open flush periods throughout the experiment. At 10.5 °C, measurement cycles of 15 min were used, consisting of a 570 s closed period followed by 240 s of flushing and a 90 s wait period. However, at 3 °C the oxygen solubility in water is higher while the metabolic rate of the fish is lower, meaning a longer time period was required to obtain a reliable trace of the decrease in oxygen concentration. Therefore, the measurement cycle was increased to 30 min, consisting of a 1210 s closed measurement period, 500 s of flushing and a 90 s wait period.

2.4. Calculations

The MO_2 was calculated in each measurement period by fitting a linear regression to the decrease in dissolved oxygen over time as:

$$MO_2 = \frac{\frac{\Delta O_2}{\Delta t}(V_{sys} - V_b)}{M_b}$$

here ΔO2/Δt is the slope of the linear regression, representing the change in dissolved oxygen over time (mg O_2 h^{-1}), V_{sys} is the volume of the respirometer (90 l), and V_b and M_b are the volume (l) and mass of the fish (kg), respectively. A fish density of 1 kg L^{-1} was assumed.

The automated measurement loops allowed for several MO_2 measurements while the fish was acclimating in the respirometer overnight prior to the swim trial. To estimate the SMR, the average of the 10% lowest values was calculated, and any outliers (±2 standard deviations from the mean) were then removed whereafter a new average was calculated from the remaining data (e.g. Clark et al., 2013).

The MMR was defined as the highest MO_2 measured, which coincided with the final swimming speeds achieved prior to reaching fatigue. The AS was then calculated as MMR minus SMR.

The rate of change in SMR and MMR from 3 °C to 10.5 °C was quantified by calculating the temperature quotient (Q10):

$$Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)}$$

where R is MO_2 and T is temperature.

The U_{crit} was calculated according to Brett (1964):

$$U_{crit} = U_f + \frac{t_f U_i}{t_i}$$

where U_f is the highest completed flow speed (cm s^{-1}), U_i is the flow increment (cm s^{-1}), t_f is the time before fatigue at the final speed (min) and t_i is the increment interval (30 min). In a similar fashion, the swimming speed threshold for ram ventilation, U_{ram}, was calculated by using the onset of ram ventilation rather than the point of fatigue.

An object within a fixed volume obstructs the flow and consequently increases it. Since flow speeds were calibrated when the swim tunnel was empty, the fish would theoretically therefore experience slightly higher flow speeds than prescribed. This is termed solid blocking, and the actual current velocity experienced by the fish in the swim tunnel can then be approximated according to Bell and Terhune (1970):

$$V_f = V_t(1 + \varepsilon_s)$$

here V_f is the water velocity when a fish is present, V_t is the water velocity in the empty swim tunnel, and ε_s is the fractional error owing to solid blocking effects in V_t. ε_s is calculated as:

$$\varepsilon_s = \tau\lambda(A_0/A_t)^{3/2}$$

here τ is a dimensionless factor of 0.8, λ is a shape factor calculated as 0.5(length/thickness) for streamlined shapes such as Atlantic salmon, A_0 is the cross sectional area of the fish, and A_t is the cross sectional area of the swim tunnel. Some of the fish tested had cross sectional areas relative to the swim tunnel exceeding 10%, which imposes an appreciable theoretical current increase. For a consistent comparison, all U_{crit} values reported have therefore been compensated for solid blocking effects.

2.5. Statistics

The data were transferred to R version 3.65.03 (R Development Core Team 2019; http://www.r-project.org). Significance was assigned at $p < 0.05$. Initially we compared body size parameters between diploids and triploids using linear models. Body size was log transformed as the model residuals had a right skew following examination of a qqplot. We used the Akaike information criteria with a correction for small sample sizes (AICc) to compare models with and without an interaction between ploidy and temperature (i.e. ploidy × temperature versus ploidy + temperature). The model with the lowest AICc score was considered the best data fit when weighted against the number of model parameters, and this model was then subsequently compared to a null model, again using AICc to determine the best data fit.

Our hypothesis was that triploids would perform better at 3 °C compared to 10.5 °C with regard to their AS. Previously, it has been shown that aerobic scope increases between 3 and 10 °C in Atlantic salmon due to a mismatch in the increase of SMR and MMR with increasing temperature (Hvas et al., 2017a). Therefore, to test our hypothesis, we compared two models. Firstly, we generated a linear mixed effect (LME) model with a 3-way interaction between the categorical variables ploidy (diploid versus triploid), temperature (3 versus 10.5 °C), and metabolic parameter (SMR versus MMR) that would provide support for our hypothesis (i.e. metabolic rate ~ ploidy × temperature × parameter + body mass). Body mass was included as a continuous variable as it is known to scale with SMR and MMR (Oldham et al., 2019), and fish was included as a random effect as the SMR and MMR was calculated from the same individuals. We then generated a second model that would provide no evidence to refute our hypothesis, whereby we removed the 3-way interaction, but allowed all 2-way interactions between ploidy, temperature, and metabolic parameter. Initial inspection of the fitted versus standardised residuals suggested heteroskedasticity, therefore the dependent variable (metabolic rate) was log transformed. These two models were then compared using AICc. Initially we also included body mass as a continuous variable (i.e. metabolic rate ~ ploidy × temperature × parameter + body mass), as triploids were slightly heavier than diploids (Table 1) and SMR and MMR scale with body mass (Oldham et al., 2019). However, the models with body mass included had a higher AICc value than those without, therefore body mass was removed. The model with the lowest AICc score was considered the best data fit when weighted against the number of model parameters, and further compared against a null model. The marginal and conditional R^2 of the final model was determined using the command "r.squaredGLMM", which returns the variance explained when excluding or including the random effect, respectively (Nakagawa and Schielzeth, 2013).

Following this we also looked for ploidy interactions with temperature on AS, U_{crit} and U_{ram}. For AS and U_{crit}, two linear models were compared, one that allowed ploidy to interact with temperature (i.e. ploidy × temp) versus a model without the interaction (i.e. ploidy × temperature versus ploidy + temperature). For U_{ram}, only those fish at 10.5 °C were compared, as ram ventilation was not observed at 3 °C, therefore only ploidy was included in the model. Finally, we assessed the relationship between U_{crit} and AS using a 3-way interaction model (i.e. aerobic scope × temperature × ploidy). All of the above described models were compared with and without body mass. The model including mass had a higher AICc score compared to the model without mass for AS, but the opposite was true for the models looking at U_{crit} and U_{ram} and so mass was included in the latter two models.

For all models, the fit was assessed using standardised versus fitted residuals to check for systematic errors within the residuals and qqplots

Table 1
Size parameters. Number of replicates (n), fork length (L_f), weight (W) and condition factor (K) in each experimental group. Statistics are from linear models and report main effects. For condition factor, no statistics are provided as the null model had a lower AICc score than the model including ploidy and temperature. Data are presented as mean ± s.e.m.

Parameter		3.0 °C		10.5°Cv	Ploidy				Temperature				R^2
	Diploid	Triploid	Diploid	Triploid	SS	df	*F*	*p*	SS	df	*F*	*p*	
n	10	9	8	8									
W (g)	432 ± 16	485 ± 27	584 ± 53	643 ± 43	0.1	1	3.2	0.080	0.7	1	20	<0.001	0.42
L_f (cm)	33.6 ± 0.3	34.6 ± 0.6	36.5 ± 1.0	38.2 ± 0.9	18.4	1	4.3	0.047	92.0	1	22	<0.001	0.44
K	1.14 ± 0.02	1.16 ± 0.02	1.18 ± 0.02	1.14 ± 0.03	–	–	–	–	–	–	–	–	–

to check for normality. Post-hoc tests were done using least square means with a Tukey adjustment from the "emmeans" library, whereby means for groups are adjusted for means of other factors within the model (Lenth, 2016). For all models, the "ANOVA" command within the "car" library was used to extract the results for the main effects of the model with the lowest AICc. Type II sum of squares were used for models without interactions, whereas main effects were calculated using type III sum of squares when interactions were present within the final model.

3. Results

A minimum of eight replicate respirometry trials with novel fish were completed at 3 °C and 10.5 °C on both diploid and triploid Atlantic salmon. Model comparison suggested no interaction between ploidy and temperature on body mass, length, or condition factor (Table 1). However, triploids were on average significantly longer than diploids, with a tendency to be heavier, while fish maintained at 10.5 °C generally were longer and heavier than those reared at 3 °C. There was no effect of temperature or ploidy on condition factor.

SMR and MMR values and their corresponding statistical results are summarized in Table 2, and in addition, the modelled effects of temperature and ploidy on these parameters are illustrated in Fig. 1. The model with the 3-way interaction had a lower AICc score than the model with only 2-way interactions (−38 versus −35, respectively). A post hoc analysis showed that at 10.5 °C triploids had a significantly lower MMR compared to diploids while SMR was higher in triploids, although not significantly different from diploids. Consequently, this resulted in a significantly lower AS in triploids at 10.5 °C (Table 3). However, at 3 °C diploid and triploid Atlantic salmon had similar MMR, SMR and AS. Regardless of ploidy, fish reared at 3 °C had significantly lower SMR, MMR and AS compared to fish reared at 10.5 °C (Tables 2 and 3).

Q_{10} values for SMR were 2.74 and 2.91 in diploids and triploid individuals, respectively; and those for MMR were 1.94 in diploids and 1.76 in triploids, respectively.

Fish reared at 10.5 °C had a higher U_{crit} than those reared at 3 °C, but there was no difference between ploidy at each temperature (Table 4). However, although not significantly different, at 10.5 °C triploids tended to have a lower U_{crit} than diploids (Δ0.26 body lengths s^{-1}). Furthermore, at 10.5 °C triploids had a significantly lower U_{ram} meaning that they would initiate ram ventilation at lower swimming speeds than diploids. Neither of the ploidy groups was observed to ram ventilate at 3 °C. There was a significant positive association between U_{crit} and AS (LM, $SS = 0.7$, df = 1, $F = 12.5$, $p = 0$ .0.002), but no effect of temperature, ploidy, or any interaction (data not shown).

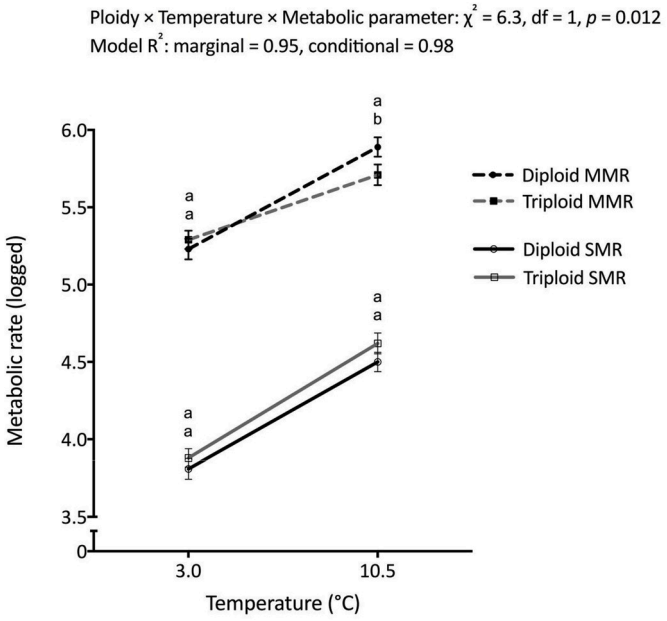

Fig. 1. Standard (SMR) and maximum (MMR) metabolic rates in diploid and triploid Atlantic salmon at 3.0°C and 10.5°C. The statistics are from a linear mixed effect model showing logged data. Different lowercase letters indicate significant ploidy effects within ploidy, temperature, metabolic parameter comparisons (lsmeans, $p < 005$, see Table 2). Results are lsmeans ± s.e.m.

4. Discussion

4.1. Is it advantageous to be triploid at lower temperatures?

We hypothesized that triploid Atlantic salmon should gain a physiological advantage relative to diploid Atlantic salmon at the lower end of their thermal niche owing to having larger cells and genomes. Here, we chose to assess metabolic rates and swimming performance due to their importance in the environmental adaptation of fish. We found that triploids and diploids performed equally at 3 °C, while 10.5 °C imposed a physiological disadvantage in triploids. Hence, although triploids performed relatively better at the lower temperature, they did not gain an advantage over diploid counterparts. Therefore, we reject our initial hypothesis and conclude that the trend for increased genome and cell

Table 2
Standard metabolic rate (SMR) and maximum metabolic rate (MMR) of diploid and triploid Atlantic salmon at 3.0°C and 10.5°C. Statistics are from lsmeans used as a post hoc analysis of a significant 3-way interaction model (see Fig. 1). Data are presented as mean ± s.e.m.

Parameter	Temperature	Diploid	Triploid	Estimate	SE	df	t-ratio	P
SMR (mg O_2 kg^{-1} h^{-1})	3.0 °C	46.5 ± 2.7	49.5 ± 3.1	−0.0583	0.0835	29	−0.698	0.491
MMR (mg O_2 kg^{-1} h^{-1})	3.0 °C	194 ± 19	205 ± 16	−0.054	0.0835	29	−0.646	0.523
SMR (mg O_2 kg^{-1} h^{-1})	10.5 °C	89.6 ± 3.0	100.0 ± 3.0	−0.1134	0.0859	29	−1.32	0.197
MMR (mg O_2 kg^{-1} h^{-1})	10.5 °C	358 ± 10	300 ± 15	0.1814	0.0859	29	2.111	0.044

Table 3
The aerobic scope (AS) of diploid and triploid Atlantic salmon at 3.0°C and 10.5°C. Statistics are from linear models and report main effects. Different superscript letters indicate a significant ploidy effect within temperature. Data are presented as mean ± s.e.m.

Parameter	3.0 °C		10.5 °C		Ploidy × Temperature			
	Diploid	Triploid	Diploid	Triploid	SS	df	p	R^2
AS (mg O_2 kg^{-1} h^{-1})	148 ± 16	155 ± 14	269 ± 11[a]	200 ± 15[b]	11753	1	0.012	0.59

Table 4
Swimming performance parameters. The critical swimming speed (U_{crit}) and onset of ram ventilation (U_{ram}) of diploid and triploid Atlantic salmon at 3.0 °C and 10.5 °C. Unit is body lengths per second (bl s^{-1}). Statistics are from linear models and report main effects. Data are presented as mean ± s.e.m.

Parameter	3.0 °C		10.5 °C		Ploidy				Temperature				R^2
	Diploid	Triploid	Diploid	Triploid	SS	df	F	p	SS	df	F	p	
U_{crit} (bl s^{-1})	1.51 ± 0.12	1.49 ± 0.11	2.14 ± 0.11	1.88 ± 0.11	<0.1	1	0.04	0.847	2.5	1	22.8	<0.001	0.45
U_{ram} (bl s^{-1})	NA	NA	1.48 ± 0.11	1.09 ± 0.08	0.4	1	6.9	0.021	na	na	na	na	0.55

size at lower temperatures may not be related to improvements in key whole-organismal metabolic traits such as the AS, as shown here for Atlantic salmon. Instead, triploids perform similarly at low temperatures, and this may allow for selection on other traits offered by polyploidy and/or larger cell size that would otherwise be unavailable at higher temperatures owing to an increasing metabolic burden.

4.2. Ploidy effects on metabolic rate and swimming performance

As cell size is generally inversely related to metabolic rate (Maciak et al., 2011; Kozlowski et al., 2003), one would expect triploids to have a lower SMR than diploids. However, larger cells with lower surface are to volume ratios may become more costly to maintain at higher temperatures when metabolism accelerates. Therefore, any cell size effect of polyploidy is likely to be temperature dependent. Unexpectedly, we observed no ploidy effect on SMR at either 3 or 10.5 °C..

Only two other studies have investigated ploidy effects on SMR in Atlantic salmon. Similar to our study, neither found evidence of a ploidy effect on SMR at 15 °C (Lijalad and Powell, 2009) or at 10, 14 and 18 °C (Bowden et al., 2018). Neither was a ploidy effect on SMR observed in two other species of salmonids, the brook trout at 16 °C (Hyndman et al., 2003) or the rainbow trout at 12 °C (Scott et al., 2015). However, the SMR of brook trout was higher in triploids at 16 °C (O'Donnell et al., 2017), a temperature that is in the upper range for this species. Hence, the present study is the only study that could be considered to test a triploid salmonid at the lower end of the thermal niche. Nevertheless, our data provide little evidence that larger cell size offers an advantage with regards to a reduced basal maintenance costs on whole organism level even at low temperatures.

In contrast to the SMR, ploidy interacted with temperature on the MMR where triploid had a reduced MMR at 10.5 °C, but not at 3 °C. A similar reduction in MMR in triploid brook trout was reported at 16 °C (Hyndman et al., 2003). In contrast, two other studies did not find a ploidy effect on MMR in Atlantic salmon between 10 and 18 °C (Lijalad and Powell, 2009; Bowden et al., 2018). However, these studies employed a chasing protocol to estimate MMR, which is known to underestimate the MMR by 50% in Atlantic salmon when compared to prolonged swimming until exhaustion (Hvas and Oppedal, 2019), which was the method we used in the present work. Therefore, the conflicting reports on ploidy effects with regards to the MMR could be due to methodological differences, where a chase protocol specifically may be unable to detect real differences since the full aerobic potential of the fish is not achieved with this method (Hvas and Oppedal, 2019).

Key physiological processes take place on cell membranes where the available surface area can be a rate limiting factor (Choleva and Janko, 2013; Schoenfelder and Fox, 2015), while smaller cells typically have higher uptake affinities owing to more surface area per volume (Tambi et al., 2009). Hence, the reduced surface to volume ratio in triploids may impose additional limitations on physiological capacities such as oxygen delivery and nutrient supply on the cellular level that here manifested into a compromised whole-animal MMR at 10.5 °C in Atlantic salmon. In addition, the MMR is first and foremost defined by the functionality of the cardiovascular and respiratory systems in uptake, transport and delivery of oxygen to the respiring tissues (Wang and Malte, 2011; Norin and Clark, 2016). In this regard, reduced gill surface area have been reported in triploids (Sadler et al., 2001), although others did not find a difference (Leclercq et al., 2011). Nevertheless, a reduced functional gill surface area should compromise the maximum capacity for oxygen uptake in salmonids (Duthie and Hughes, 1987; Hvas et al., 2017c). Moreover, triploid Atlantic salmon of a similar life stage to those used in the present study had a higher relative heart size which could indicate an increased burden on the cardiovascular system (Fraser et al., 2015).

While triploidy reduced the AS at 10.5 °C, primarily owing to a reduced MMR, there was no ploidy effect on these traits at 3 °C. A possible explanation for this is that factors compromising MMR in triploids at mid to high temperatures are negated at low temperatures. For example, being in a colder environment reduces the maximum velocity of red muscle fibre shortening and their power production in fish (Rome, 1990; Rome et al., 1992). This reduces the maximum tail beat frequency in Atlantic salmon at 3 °C while recruitment of white anaerobic muscles become necessary at lower swimming speeds compared to higher temperatures (Hvas et al., 2017a). Hence, low temperatures restrict the biomechanics of swimming in ways that likely are unrelated to the capacity for oxygen uptake and delivery. Moreover, since the rates of virtually all biochemical processes are reduced when temperature decreases, perhaps Atlantic salmon simply were unable to utilize more oxygen regardless of ploidy when acclimated to 3 °C. For similar reasons, the U_{crit} was also unaffected by ploidy at 3 °C. Moreover, since ram ventilation was not observed at this temperature in either ploidy, the limitation in attainable swimming speeds in these conditions was likely unrelated to the capacity for oxygen uptake.

At 10.5 °C, the U_{crit} was highly associated with the increased AS in diploids, although the difference in U_{crit} between ploidies was not statistically significant. Interestingly, triploids started to ram ventilate at lower swimming speeds, suggesting that they had a reduced efficiency to meet oxygen uptake demands earlier on. This supports our finding that MMR indeed was lower in triploids at 10.5 °C and that this may be related to the previously observed reduced gill surface area in triploid Atlantic salmon (Sadler et al., 2001). Onset of ram ventilation in triploid Atlantic salmon has also been observed during exposure to moderate hypoxia at 19 °C (Hansen et al., 2015), which similarly to a swim trial imposes increased oxygen uptake requirements that evidently are more challenging for triploids compared to diploid counterparts.

Previous studies also did not find significant ploidy effects on U_{crit} in Atlantic salmon at 12–17 °C (Lijalad and Powell, 2009), chinook salmon at 9 °C (Bernier et al., 2004), rainbow trout at 12 °C (Scott et al., 2015),

coho salmon at 6 °C (Small and Randall, 1989), or brook trout (Stillwell and Benfey, 1997, temperature not reported). Here, it is noteworthy that the study on chinook salmon also reported a reduced AS in triploids without a significant effect on U_{crit} (Bernier et al., 2004). Hence, due to the incremental nature of the U_{crit} test protocol, it is possible that more subtle differences go undetected. Perhaps a more thoroughly designed test protocol with lower increment steps in the range of the expected U_{crit} would reveal a slightly lower U_{crit} in triploids to support the observed lower AS. Alternatively, triploids may be able to compensate for reduced oxygen uptake rates by having an improved anaerobic capacity, which could be explored in a future study.

4.3. Ecological advantages of polyploidy at low temperatures

The AS is widely used to infer the optimal environmental conditions of fish, especially in relation to water temperature (Fry, 1971; Farrell, 2016; Lefevre, 2016). The reduced AS of triploids at higher temperatures reported here would therefore suggest that the environments in which polyploidy becomes beneficial are limited to lower temperature ranges.

We found no advantage or disadvantage of triploidy at 3 °C in metabolic and locomotory performance in contrast to 10.5 °C where triploidy reduced MMR and AS. Therefore, an alternative hypothesis can be generated, being that the increased metabolic maintenance cost and surface area limitations of larger cells becomes negligible at lower temperatures, and this then allows for selection of other beneficial traits associated with large cell sizes such as polyploidy.

The main advantages of polyploidy are considered to be heterosis, gene redundancy, and asexual reproduction (Comai, 2005). These traits could explain the many whole genome duplications that have occurred during the evolution of plants and vertebrates and may allow for greater short term adaptation whilst leading to an increase in biological complexity over time. For example, polyploidy in plants is known to increase disease resistance and polyploidy is often found in the most extreme limits of a species natural range, suggesting it confers an advantage under certain environmental stress (Van de Peer et al., 2017). Similarly, invasive plant species worldwide are more likely to be polyploid when a diploid counterpart exist (Pandit et al., 2011). Polyploidy may also allow for increased genetic diversity. For instance, it has been observed in plants that although many genes rapidly returned to a single copy after one whole genome duplication, those that maintained duplicate copies were related to conditional responses to abiotic and biotic stress and these would be available for novel use (Li et al., 2016).

4.4. Implications for aquaculture

As a final word, triploidy is used in aquaculture and fisheries management in order to produce sterile fish that have a reduced environmental impact due to their inability to interbreed with genetically distinct wild stocks that are under threat (Benfey, 2016). After smoltification and transfer to sea water production cages, farmed Atlantic salmon may experience temperatures varying from 3 to 20 °C depending on season, water depth and location (Oppedal et al., 2011). As such, 10.5 °C represents the midrange of temperatures typically encountered while higher temperatures are common during summer and autumn.

Since the SMR and MMR of Atlantic salmon continues to increase until lethal temperatures (Hvas et al., 2017a), and considering that the Q_{10} of the SMR was higher while the Q_{10} of the MMR was lower in triploids (present study), the projected AS at temperatures above 10.5 °C should therefore become even more reduced compared to diploids.

While the AS most often is used to infer environmental adaptations in the wild, it can also be utilized to predict performance and welfare in aquaculture (Hvas and Oppedal, 2019). For instance, a reduced AS may compromise appetite, digestion and growth (Farrell, 2016; Remen et al., 2016). Moderate hypoxia is a recurring issue in salmon sea cages and reduces the AS by lowering the MMR (Oldham et al., 2019). Furthermore, diseases and parasites are inevitable in salmon aquaculture and will also have some effects on physiological capacities, as is the case of gill parasites that reduces the AS and U_{crit} in Atlantic salmon (Hvas et al., 2017c). In addition, periods of strong current conditions at off-shore farm sites will force the fish to divert a substantial amount of their aerobic capacity towards swimming which means that less energy will be left for digestion and growth (Solstorm et al., 2015; Hvas and Oppedal, 2017). Hence, having a high AS in aquaculture is advantageous as it renders the fish more robust to cope with the various biological and environmental challenges encountered. Therefore, a reduction in the AS of triploid Atlantic salmon at mid to high temperatures will make them more vulnerable to all kinds of stressors, especially when several are present simoultanously owing to the potential accumulating negative effect on the AS. However, if unfavourable conditions in the sea cage environment largely can be avoided such as very high temperatures, severe hypoxia or strong water currents, while diseases and parasites are managed appropriately, triploid Atlantic salmon should still have sufficient aerobic capacities to thrive in most aquaculture settings.

Author contributions

This work was conceived and designed by all authors. E.N.R. performed experiments. E.N.R., T.W.K.F and M.H. analysed the data. M.H. wrote the first draft of the manuscript, and all co-authors provided valuable feedback before approving the final version.

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Animal ethics

This work was performed between October and December 2017 in accordance with the Norwegian laws and regulations regarding use of animals in scientific research under permit number 12827.

Declaration of competing interest

The authors declare no competing or financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102548.

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