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The effect of fasting period on swimming performance, blood parameters and stress recovery in Atlantic salmon post smolts

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ABSTRACT

In this study, Atlantic salmon post smolts (~250 g, ~29 cm) were fasted for four weeks at 12 °C in full strength seawater. During this period, the critical swimming speed (U_{crit}) was measured after 1, 2 and 4 weeks of fasting, as well as in a fed control group. Furthermore, blood samples were taken in each treatment group prior to the swim test, at fatigue, and following 3 h and 24 h of subsequent recovery. Four weeks of fasting gradually reduced the condition factor from 1.03 to 0.89. However, the U_{crit} remained statistically unaffected at 3.5 body lengths s^{-1}. Exhaustive exercise stress caused large increases in plasma osmolality, [Cl^-], [Na^+], [Ca^{2+}], [lactate] and [cortisol], while haematocrit and [haemoglobin] also increased. Plasma ions and lactate had increased further after 3 h recovery, and osmolality, [Cl^-] and [Na^+] were still elevated above control levels after 24 h while other blood parameters were fully recovered. Osmotic disturbances may therefore be considered the most challenging stressor during strenuous exercise in seawater. Only minor effects of fasting period on blood parameters in response to exhaustive exercise were detected, which included slightly higher osmotic disturbances and a repressed response in red blood cell recruitment at fatigue in fasted fish. Furthermore, the 4-week fasting group had a reduced cortisol response following fatigue compared to the other treatment groups. In conclusion, these results show that Atlantic salmon maintain their full swimming capacity as well as their ability to respond and recover from acute stress during an extended period of food deprivation.

1. Introduction

All animals must eat to obtain energy for growth, locomotion, reproduction, and the overall sustenance of life (Porter and Gates, 1969). The required frequency of meals varies tremendously among species where at one extreme, small birds and mammals may starve to death within a day of not eating owing to their high metabolism, while some larger ectotherms such as fish and reptiles can fast for months without suffering detrimental effects (Wang et al., 2006; McCue, 2010).

In species of fish, prolonged fasting periods naturally occur owing to seasonal fluctuations in food supplies, migration or in relation to reproduction (Green and Farwell, 1971; Van Ginneken et al., 2005; Miller et al., 2009). As such, studying the feeding patterns and adaptations to food deprivation in fish can reveal fundamental aspects of their ecology and evolution. Fish show a range of adaptive responses during prolonged periods of food deprivation; for instance, whole animal metabolic rates are down regulated to preserve energy (Mehner and Wieser, 1994; Fu et al., 2005; Hvas et al., 2020a), while on the biochemical level beneficial changes in enzyme activities, gene expression and mitochondrial functions occur in targeted organs (Méndez and Wieser, 1993; Bermejo-Nogales et al., 2015; Cassidy et al., 2016; Salin et al., 2018). Moreover, upon regaining access to food these physiological changes can be rapidly reversed, and loss of growth can be compensated for over time (Méndez and Wieser, 1993; Reimers et al., 1993; Morgan and Metcalfe, 2001; Hvas et al., 2020a). Hence, fish are highly flexible in coping successfully with temporal variability in food supplies. However, long term starvation will cause detrimental consequences eventually, where resilience will depend on species and environmental conditions.

Whether certain physiological functions are prioritized for preservation during periods of limited resources reflect their overall importance for imminent survival. In fish, swimming capability should therefore be particularly important to preserve owing to its use in foraging, predator avoidance and migration. Swimming muscles can be divided into slow red fibers and fast white fibers, where the former is used for sustained aerobic swimming and the latter for anaerobic high speed burst swimming (Hudson, 1973; Bone et al., 1978; Wilson and Egginton, 1994). A general trend that has been reported in Atlantic cod

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(*Gadus morhua*), Pollock (*Pollachius virens*) rainbow trout (*Oncorhynchus mykiss*) and plaice (*Pleuronectes platessa*) is that white muscle fibers are more affected by starvation than red muscle fibers with regards to fiber size, protein synthesis, glycogen levels and capillary supply, and moreover, the impact is larger on the glycolytic than on the oxidative capacity regardless of fiber types (Johnston and Goldspink, 1973; Beardall and Johnston, 1983; Loughna and Goldspink, 1984; Scarabello et al., 1991; Martínez et al., 2003). Hence, prolonged fasting in fish should therefore first reduce the capacity for high-speed burst swimming while the ability to perform routine aerobic swimming activities is preserved.

Swimming performance is most often measured as the critical swimming speed (U_{crit}) in swim tunnels by forcing fish to swim at increasingly higher speeds in increments until they become exhausted (Brett, 1964). The U_{crit} thereby signifies the prolonged swimming capacity of fish, and at the final increment speeds swimming is powered by both aerobic and anaerobic muscle fibers (Wilson and Egginton, 1994). As such, long fasting periods should reduce the U_{crit} mainly owing to a reduced capacity for anaerobic work, which previously was shown to be the case for Atlantic cod (Martínez et al., 2004). Furthermore, known limits of sustained aerobic swimming could in theory predict how much the U_{crit} should be affected by food deprivation. For instance, in salmonids 70–85% of the U_{crit} is sustained aerobically (Burgetz et al., 1998; Beddow and McKinley, 1999; Hvas and Oppedal, 2017), meaning that those percentages of the normal U_{crit} perhaps will remain attainable following periods of food deprivation after degradation of fast white muscle fibers.

High speed swimming until exhaustion causes substantial physiological disturbances to acid-base, osmotic, ionic and endocrine balances, and in salmonids and other athletic fish species, these disturbances may take several hours to recover from (Wood, 1991; Milligan, 1996; Kieffer, 2000). However, following a prolonged period of food deprivation, provided that the capacity for anaerobic work is reduced, the magnitude of exercise stress should therefore also be reduced at the point of exhaustion, particularly through a lower acidosis from lactate production. Moreover, if less time is endured at strenuous activity levels, the magnitude of other physiological disturbances associated with exercise stress would presumably also be lower.

The purpose of this study was to investigate the effect of up to 4 weeks of food deprivation on the U_{crit} in Atlantic salmon (*Salmo salar*) acclimatized to seawater, and moreover, to assess the magnitude of exercise stress following exhaustion and the subsequent recovery trajectories by measuring relevant blood parameters at different time points. Atlantic salmon is a powerful sustained swimmer and has a high capacity for anaerobic work that results in massive physiological disturbances upon exhaustion (Hvas et al., 2018). Exercise physiology in response to food deprivation should therefore be interesting to study in this species. We hypothesized that food deprived Atlantic salmon eventually would elicit a reduced U_{crit} coinciding with lower osmotic, ionic and endocrine disturbances owing to adaptive metabolic adjustments and reduced functionality of anaerobic muscle fibers.

2. Materials and methods

2.1. Fish husbandry

Atlantic salmon post smolts (Aquagen, Norway) were maintained in three large holding tanks (diameter: 3 m, volume: 5.3 m^3, ~150 fish per tank) in the environmental laboratory facilities at the Matre Research Station, Institute of Marine Research, Norway. The holding tanks were supplied with unused, filtered, aerated, and UVC treated full strength sea water at a constant flow of 120 l min^{-1} which always ensured high water quality. Water temperature was maintained at 12 °C via controlled mixing of ambient and heated water supplies before aeration. The fish were subjected to a simulated natural photoperiod and were fed 4.5 mm commercial pellets (Skretting, Norway) in excess each day via automated feeding devices. Prior to the fasting regime, the fish had been acclimating in these conditions for two months.

The present study was performed in April and May of 2020, and the use of animals was approved by the Norwegian Food Safety Authorities under permit identification number 20474.

2.2. Swim tunnel setup and sampling protocols

A 1905 l custom build Brett-type swim tunnel, as described in Remen et al. (2016), was used for all the swim trials. The swim section part of the tunnel was 248 cm long and 36 cm in diameter, and the top lid at the downstream end could be removed for access when fish had to either be put in or removed. A camera was placed behind the rear grid downstream of the swim section to observe the fish during the swim trials. Controlled current speeds were generated with a motor driven propeller (Flygt 4630, 11° propeller blade, Xylem Water Solutions Norge AS, Norway), after first having carefully calibrated motor output with its corresponding current speed with a handheld flow meter (Höntzsch Flow Measuring Technology, Germany). A constant flow of water into the tunnel via a large pipe connected to the same water supply that was used for the holding tanks ensured a stable temperature of 12 °C and normoxic conditions during the swim trials.

In the afternoon, 12 fish were netted and transferred to the swim tunnel that was located in the same room next to the holding tanks. The fish were then allowed to acclimate overnight in the swim tunnel at a low current speed of 20 cm s^{-1} (~0.7 body lengths s^{-1}). The swim test started on the following morning by increasing the current speed incrementally in steps of 10 cm s^{-1} every 20 min. Eventually the fish would struggle to maintain swimming and fall back on the rear grid or attempt to rest against it. At this time, by reaching through the opening in the rear end, the fish were gently touched by the experimenter's hand to assert whether they were truly fatigued. If not, the fish would become startled and resume swimming efforts. However, if they were deemed fatigued, the fish could easily be removed as if sedated. Once removed, time was recorded, and individual fish were allocated in an alternating order to one of two recovery tanks next to the swim tunnel (3 h or 24 h) or euthanized with a blow to the head. Euthanized fish were immediately sampled for blood (~1 ml) via caudal puncture with a heparinized syringe, and weight (W) and fork length (L_f) were recorded. Blood samples were momentarily stored on ice until further processing, while the swim trial continued until all fish had reached fatigued.

Fish that had been moved to a designated recovery tank were sampled for blood 3 h or 24 h after the time of fatigue. To minimize handling stress when sampling recovering fish, they were lightly sedated within the holding tanks in 25 mg l^{-1} Finquel (Tricaine methanesulfonate). This procedure allowed for gentle netting of the fish without initiating a panic response, whereafter they were stunned with a blow to the head prior to blood sampling and measurement of size parameters. To provide control blood samples of fish that had not been subjected to a swim test, 12 fish were netted and transferred to recovery tanks were they were kept for 24 h before being sampled, as described for recovering fish.

Feed was withheld for 4 weeks, and during this time swim trials were performed after 1, 2 and 4 weeks of fasting as well as in the week prior to the onset of fasting on fed fish. Three replicate swim trials were performed for each treatment group on consecutive days using fish from one of the three holding tanks to provide identical fasting times between replicate trials. Moreover, control blood samples were taken from each treatment group. Hence, this study design provided 36 U_{crit} measurements as well as 12 blood samples from unswum control fish, at fatigue and following 3 h and 24 h of recovery per treatment group. Group testing of fish in a larger swim tunnel was chosen here as it provides some advantages compared to smaller setups for single fish. For instance, the fish will have more space to express natural swimming behaviours, a less confined setup is considered less stressful, and more importantly, it allows for more individuals to be tested within a limited time to provide sufficient replication of blood samples (Hvas and

Oppedal, 2019).

2.3. Analyses of blood parameters

Momentarily after blood sampling, the haematocrit (Hct) was measured in duplication as the fraction of red blood cells from small subsamples in capillary tubes after having used a haematocrit centrifuge (StatSpin MP Centrifuge). At the same time, the haemoglobin (Hb) concentration was measured in 10 μl of blood with an assay kit (MAK115, Sigma-Aldrich). The mean corpuscular haemoglobin concentration (MCHB) was then calculated as Hb divided by the Hct percentage. Remaining blood was centrifuged at 5000*g* for 5 min in Eppendorf tubes whereafter the plasma supernatant was transferred to new Eppendorf tubes and stored at −80 °C for later analyses.

Plasma osmolality was measured by freeze point determination in 20 μl subsamples with a Fiske 210 Micro-Sample Osmometer (Advanced Instruments). The concentration of plasma cortisol was measured with an ELISA assay kit in 20 μl subsamples (standard range: 20 to 800 ng ml^{-1}, IBL International GmbH). The concentration of other reported plasma parameters (Na^+, Cl^-, Ca^{2+} and lactate) were measured in 65 μl subsamples with an ABL90 FLEX blood gas analyzer (Radiometer).

2.4. Calculations and statistical analyses

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

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

Were U_f is the final completed current speed (cm s^{-1}), U_i is the increment magnitude (10 cm s^{-1}), t_f is time endured at the uncompleted speed before fatigue (min) and t_i is the increment interval (20 min). The large cross sectional area of the swim tunnel relative to the fish sizes tested meant that solid blocking effects would be minimal, and they were therefore not corrected for in the reported U_{crit} values (Bell and Terhune, 1970).The condition factor (K) of each fish was calculated as 100 W $(L_f^3)^{-1}$ (Fulton, 1904; Nash et al., 2006).Changes in U_{crit}, K, W, L_f with fasting week were analysed by generalized linear models (function glm, family = Gaussian, R Core Team 2019). Changes in blood parameters were analysed similarly, but with interaction to sampling (unswum control fish, at fatigue and following 3 h and 24 h) included as categorical factor. Models were also created with week as a binary factor (week 0 = TRUE otherwise FALSE or week 4 = TRUE otherwise FALSE), and the model with best fit was chosen. Chosen models were then simplified and compared to confirm if the simplifications were justified (function ANOVA, R Core Team 2019), otherwise the simplification was rejected. The constancy of variance and normality of errors assumptions of each model where checked by model checking plots (function plot, R Core Team 2019). In case of heavily skewed data, the model distribution was changed to 'inverse.gaussian(link = $1/mu^2$)'. In the reported boxplots the central markers indicate the median, the top and bottom edges the 75- and 25-percentiles, and the whiskers the maximum and minimum observed values. Data are reported in the text as mean ± s.e.m. and *p*-values of <0.05 were considered significant.

3. Results

The changes in W and L_f over a 4-week fasting period were not significant ($P = 0.140$ and $P = 0.357$, respectively, Table 1). However, when combined as K, there was a gradual significant decrease over time from 1.03 ± 0.02 in fed control fish to 0.89 ± 0.01 after 4 weeks of fasting ($P < 0.001$, Table 1). Fasting period did not cause mortalities, and no apparent injuries (snout damage, skin wounds, eye damage etc.) or deviations in behaviour (increased aggression, irregular swimming patterns etc.) were observed during the daily inspection of the fish in the holding tanks.The U_{crit} remained unaffected by fasting period with a mean of 3.47 ± 0.05 body lengths s^{-1} across all treatments ($P = 0.332$, Fig. 1A). Similarly, the U_{crit} expressed in absolute units was also unaffected with a mean of 102 ± 0.7 cm s^{-1} across all treatments ($P = 0.264$, Fig. 1B).Plasma osmolality, Cl^-, Na^+, and Ca^{2+} all increased substantially in response to exhaustive exercise and had increased further following 3 h of recovery, whereafter they were approaching control values following 24 h of recovery (Fig. 2). However, complete recovery had not been attained for plasma osmolality, Cl^- and Na^+ after 24 h ($P < 0.001$, Fig. 2A, B and C), while plasma Ca^{2+} did fully recover ($P > 0.5$, Fig. 2D). The only observed effect of fasting period for plasma osmolality was a greater increase at the point of fatigue in the 4-week group ($P = 0.014$, Fig. 2A). This was similar to plasma Na^+, where the only effect of fasting period detected was a significantly increasing trend for higher levels with increasing length of fasting period at the point of fatigue ($P = 0.015$, Fig. 2C). However, plasma Cl^- was unaffected by fasting period at all sampling points ($P > 0.077$, Fig. 2B), while plasma Ca^{2+} generally was lower in the 4 week group regardless of sampling time compared to the shorter duration fasting groups ($P < 0.001$, Fig. 2D).Plasma lactate increased in response to exhaustive exercise ($P = 0.004$), increased further after a 3 h recovery period ($P < 0.001$), and had returned to control levels after 24 h ($P = 0.552$) (Fig. 3A). The only effect of fasting period observed was that in the fed treatment group, plasma lactate was less elevated after 3 h recovery compared to the fasted fish groups ($P = 0.025$, Fig. 3A). Plasma cortisol also increased in response to exhaustive exercise and remained elevated at a similar level following 3 h recovery ($P < 0.001$), while it returned towards control levels after 24 h (Fig. 3B). However, at the 24 h recovery time point, all the fasted fish groups had lower cortisol levels than at the control sampling point ($P = 0.007$), while the fed fish group had higher cortisol levels after 24 h compared to the control sampling point ($P = 0.002$) (Fig. 3B). In addition, the 4 week fasting group had lower cortisol levels at fatigue ($P = 0.034$) and near significant lower cortisol levels at 3 h recovery ($P = 0.068$) compared to the other treatment groups (Fig. 3B).Both Hct and Hb increased at fatigue, and had returned to or decreased slightly below control levels after 3 h, while the 24 h sampling point was similar to controls (Fig. 4A and B). Furthermore, a notable observed effect on both Hct and Hb was that the response to exhaustive exercise with elevated values at fatigue gradually decreased with increasing fasting period ($P < 0.001$) (Fig. 4A and B). The MCHC generally remained stable between fasting groups and sampling points, except for slightly elevated levels at the 3 h recovery point ($P = 0.003$) (Fig. 3C). In addition, the 4-week fasting group had a lower MCHC at the first 3 sampling points, and a higher MCHC at the final 24 h sampling point compared to the other treatment groups ($P < 0.001$) (Fig. 3C).

Table 1
Size parameters of Atlantic salmon subjected to different periods of feed withdrawal.

	Fed	1 Week	2 Weeks	4 weeks	P
W (g)	253 ± 10	264 ± 11	234 ± 8	237 ± 10	0.140
L_f (cm)	28.8 ± 0.3	29.6 ± 0.3	29.1 ± 0.3	29.7 ± 0.4	0.357
K	1.03 ± 0.02^a	0.99 ± 0.01^a	0.93 ± 0.01^b	0.89 ± 0.01^c	<0.001

The weight (W), fork length (L_f) and condition factor (K) at each sampled fasting period ($N = 48$). Superscript letters indicate statistical differences. The *p*-values (P) indicate if there is significant change between treatments. Data are mean ± s.e.m.

4. Discussion

4.1. Swimming performance and fish condition

Contrary to our hypothesis, we did not observe a reduction in the U_{crit} after up to 4 weeks of food deprivation in Atlantic salmon. This was a surprising result when considering that the latter part of the U_{crit} test exploits the anaerobic capacity of the fish to the point of complete

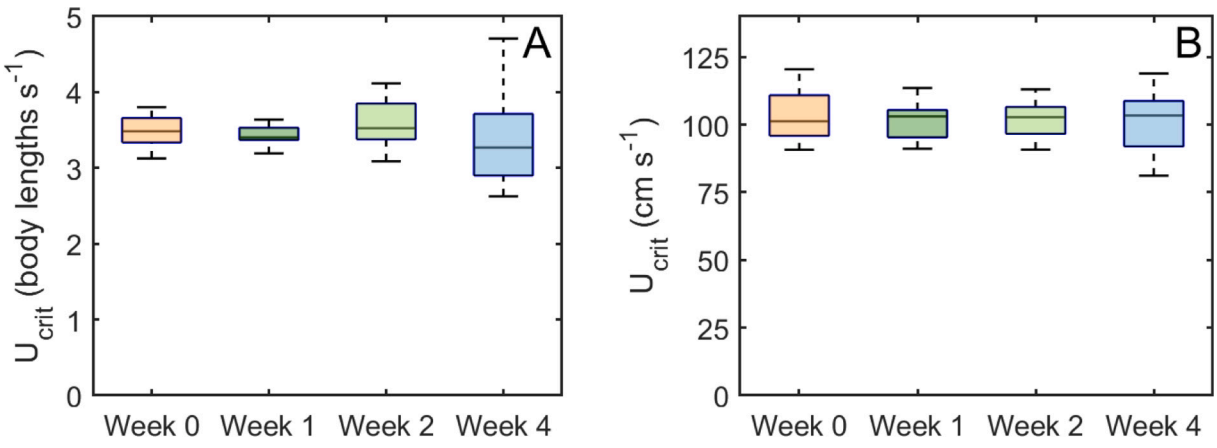

Fig. 1. Boxplot of critical swimming speed (U_{crit}) of Atlantic salmon subjected to different periods of feed withdrawal. The data in A are expressed in relative units (body lengths s^{-1}) while B shows data points for individual fish in absolute units (cm s^{-1}). There were no significant changes in U_{crit} with fasting week.

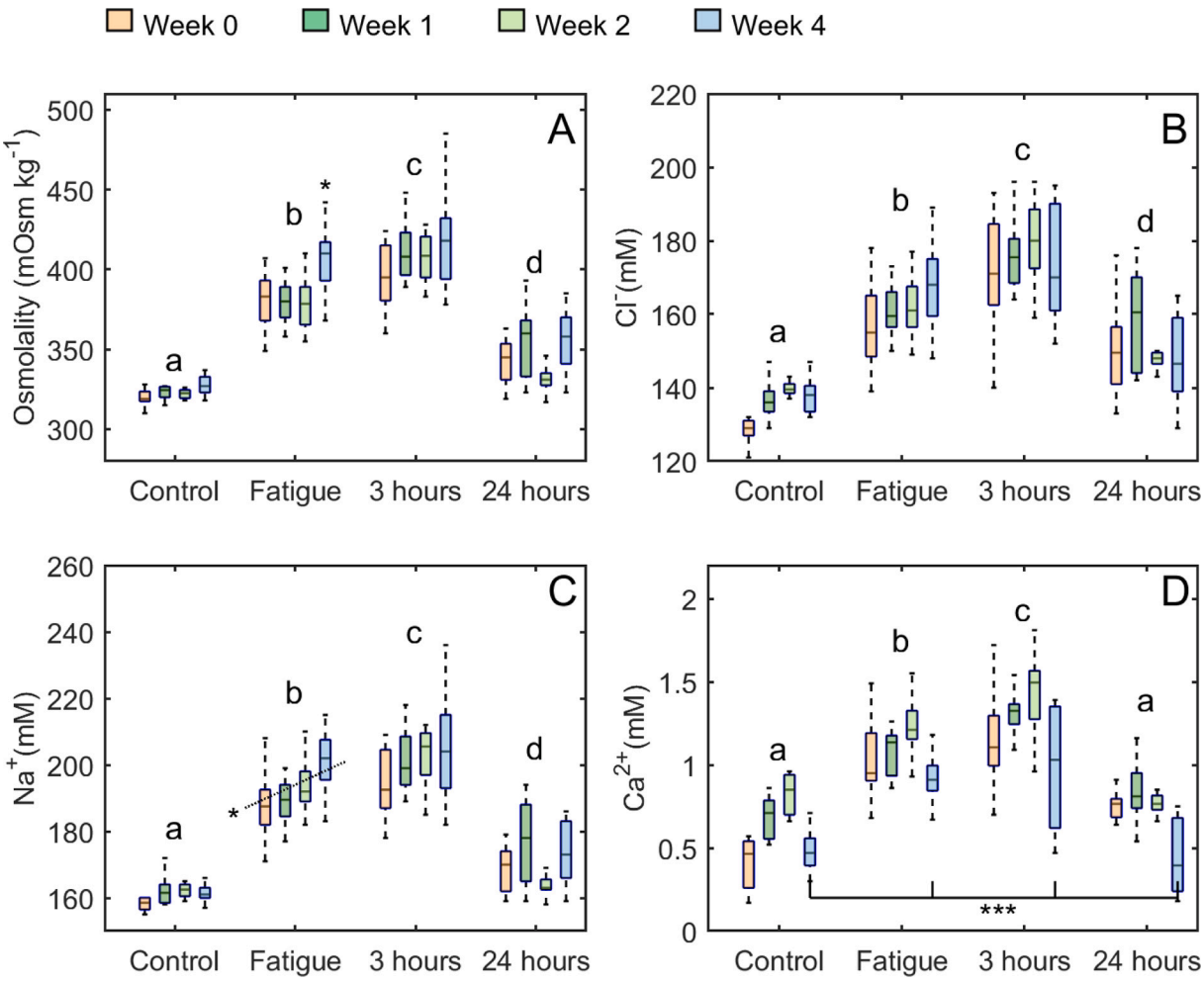

Fig. 2. Boxplot of plasma osmolality (A), plasma [Cl^-] (B), plasma [Na^+] (C) and plasma [Ca^{2+}] (D) in control conditions, at fatigue and after 3 h and 24 h of recovery in Atlantic salmon following different periods of feed withdrawal. Different letters indicate significant effect from blood sampling time, stars indicate significant effect from fasting period (first or last week), stars and dotted line indicate significant change with fasting length: * ≤0.05, ** ≤0.001 and *** ≤0.001.

exhaustion, and several studies have specifically reported reduced functionality of white muscle fibers in response to fasting (e.g. Loughna and Goldspink, 1984; Martínez et al., 2003). For instance, in Atlantic cod and rainbow trout fasting caused significant reductions in glycogen stores of white muscles that consequently limited anaerobic capacities as inferred from weaker burst swimming and a lower production of lactate (Scarabello et al., 1991; Martínez et al., 2003, 2004). However, in the present study plasma lactate at the point of exhaustion was similar between fed controls and fasting groups, and moreover, the fasting groups had accumulated higher lactate levels in the blood plasma 3 h after

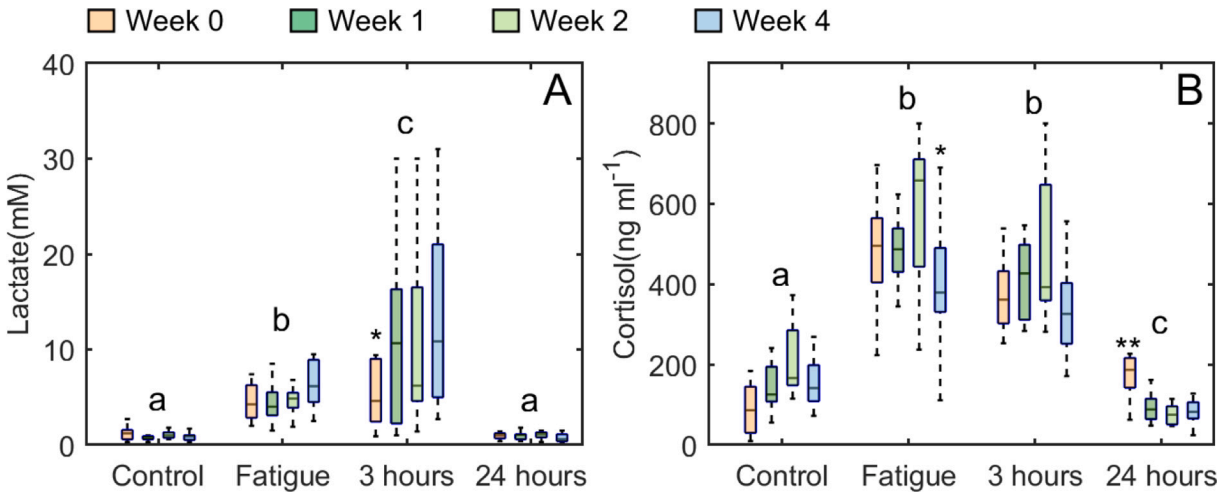

Fig. 3. Boxplot of plasma lactate (A) and cortisol (B) in control conditions, at fatigue and after 3 h and 24 h of recovery in Atlantic salmon following different periods of feed withdrawal. Different letters indicate significant effect from blood sampling time, stars indicate significant effect from fasting period (first or last week): * ≤0.05, ** ≤0.001 and *** ≤0.001.

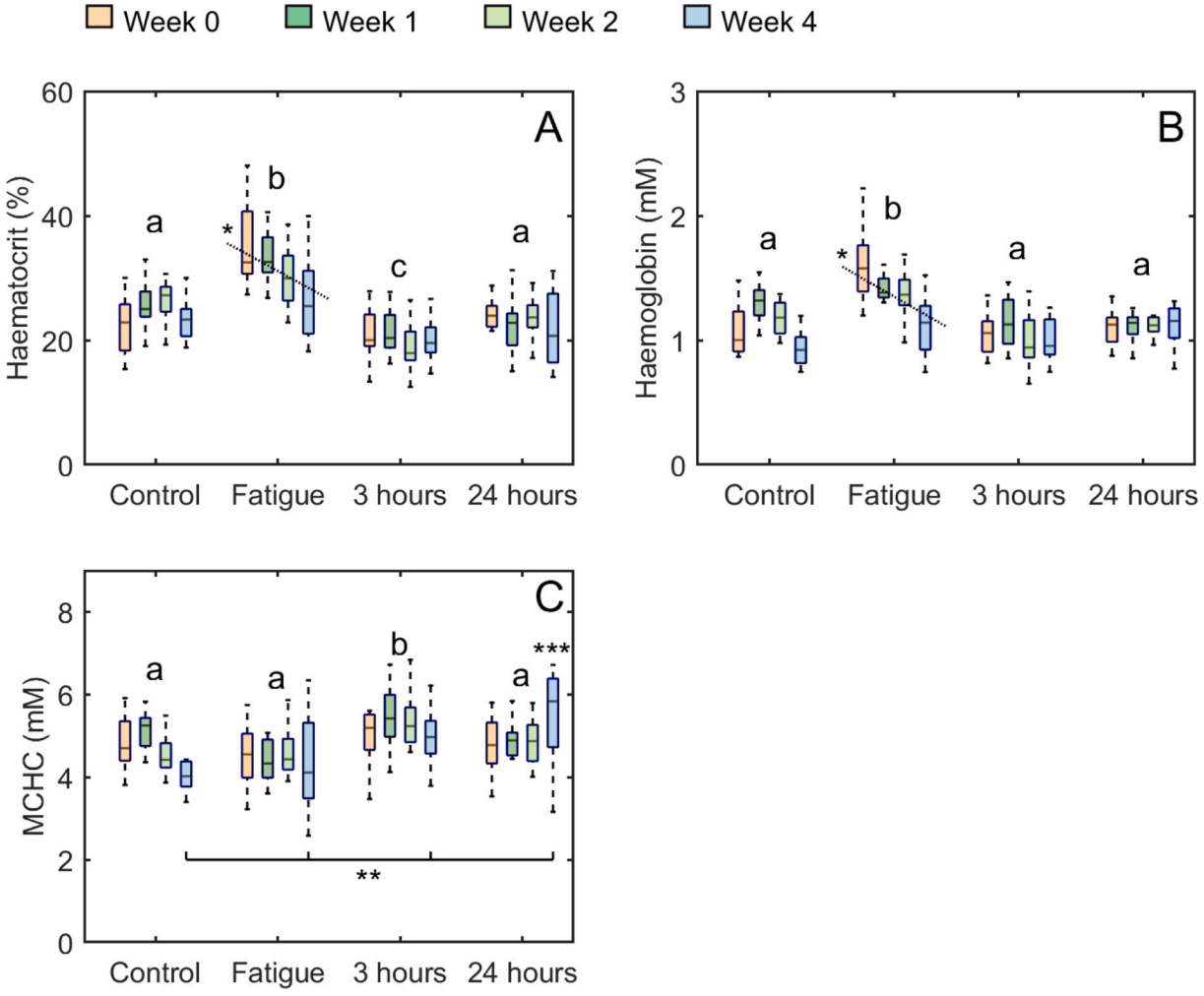

Fig. 4. Boxplot of haematocrit (A), [haemoglobin] (B), mean corpuscular haemoglobin concentration (MCHC) (C) in control, at fatigue and after 3 h and 24 h of recovery in Atlantic salmon following different periods of feed withdrawal. Different letters indicate significant effect from blood sampling time, stars indicate significant effect from fasting period (first or last week), stars and dotted line indicate significant change with fasting length: * ≤0.05, ** ≤0.001 and *** ≤0.001.

exhaustion. Together with an unaffected U_{crit}, this shows that the capacity for anaerobic work was not reduced in Atlantic salmon following the fasting periods used in the present study. Furthermore, a tendency for higher lactate levels during recovery in fasted fish could mean that the dynamics of lactate metabolism was altered and perhaps less lactate was metabolized in situ which allowed for a larger proportion to enter the blood. This may have caused a higher acute metabolic blood acidosis but considering that lactate had completely recovered to control levels after 24 h regardless of fasting period, this was unlikely to cause long term detrimental consequences.Atlantic salmon post-smolts reduce their resting metabolic rates progressively over a 4 week fasting period at 12 °C (Hvas et al., 2020a), the same test temperature and fasting regime as used in the present study. A general down regulation of whole-animal metabolism could therefore be expected to also affect swimming performance. However, since the U_{crit} here was found to be unaffected, maintaining good swimming capabilities in the absence of food for as long as possible is presumably an adaptive survival strategy owing to the crucial importance of swimming for Atlantic salmon in all aspects of its life. Lower whole-animal metabolic rates during prolonged fasting instead likely originate from reduced costs associated with digestive and growth-related processes.Previously Atlantic cod was found to have a reduced U_{crit} following a 12-week fasting period that concomitantly decreased the condition factor from 1.0 to 0.5 (Martínez et al., 2004). Apart from a substantially longer fasting regime and a different species studied, this was a rather dramatic reduction in condition factor compared to a more modest reduction from 1.1 to 0.89 in the present study. In hindsight, to determine the tolerance limit to food deprivation before a negative effect on swimming performance can be observed in Atlantic salmon, we should therefore have used a longer fasting period. However, it should be noted that it is difficult to compare and predict the consequences of fasting periods between studies as they typically are made on difference species, at a range of temperatures, and at various sizes and life-stages. For instance, Atlantic cod is known to endure long periods with low food supply in winter (Lambert and Dutil, 1997), and may therefore be expected to be better adapted to food deprivation than Atlantic salmon. Moreover, we tested smaller post-smolts that compared to adult Atlantic salmon have lower condition factors, less fat reserves available and higher mass specific metabolic rates (e.g. Oldham et al., 2019). They should presumably therefore be more vulnerable to long periods of food deprivation compared to older and larger fish; however, 4 weeks evidently did not exceed their limit with regards to preservation of swimming performance.

4.2. Exhaustive exercise stress and recovery

We hypothesized that the magnitude of exhaustive exercise stress would be lower in food deprived Atlantic salmon owing to a reduced capacity for strenuous anaerobic swimming. However, stress parameters measured in blood samples were mostly similar between fasting periods at the point of exhaustion, showing that the amount of exercise stress endured in the swim tests was of a comparable magnitude in agreement with an unaffected U_{crit}. Although, some subtle differences in blood parameters in response to fasting period were observed, namely a slightly increased osmotic disturbance and a decline in red blood cell recruitment following fatigue in the latter feed withdrawal groups. The 4-week fasting group also showed a lower increase in plasma cortisol following exhaustion, which along with a lack of increase in Hct and Hb may indicate the onset of a repressed stress response or alternatively, a disturbance in the HPI axis. However, this effect was not yet sufficient to significantly affect swimming performance. Long periods of food deprivation can also reduce the maximum aerobic metabolic rate as inferred from lower oxygen uptake rates following acute stress, presumably owing to an overall down regulation of metabolism (Fu et al., 2005; Hvas et al., 2020a). A lower oxygen uptake capacity would affect aerobic swimming limits which should translate into a reduced U_{crit} (Hvas et al., 2017). Moreover, as a multifunctional organ simultaneously responsible for gas exchange and ion regulation, the gill is subjected to an osmorespiratory compromise, meaning that facilitation of increased branchial oxygen uptake may impose an increased osmoregulatory burden (Sardella and Brauner, 2007). Specifically, owing to the large osmotic gradient between the fish (~325 mOsm kg^{-1}) and the external environment in sea water (~1025 mOsm kg^{-1}), exhaustive exercise results in substantial increases in plasma osmolality, chloride and sodium owing to either passive water loss or passive ion uptake (Hvas et al., 2018). As such, if the capacity for oxygen uptake indeed was reduced, the resultant osmotic and ionic disturbances following exhaustion should also be reduced. Since this was not the case, the present results suggest that the maximum aerobic metabolic rate was preserved together with the capacity for anaerobic work in Atlantic salmon over a 4 week fasting period. Recovery from exhaustive exercise stress in salmonids is a slow process and blood chemistry parameters such as arterial pH, plasma lactate and the major plasma ions are typically reported to take 8-12 h to fully return to control levels (Wood, 1991; Pagnotta et al., 1994), roughly coinciding with the duration of excess post-exercise oxygen consumption (Zhang et al., 2018), while plasma cortisol levels may take up to 24 h to fully recover (Pagnotta et al., 1994; Kieffer, 2000). Interestingly, most studies on exercise physiology in salmonids have been made in freshwater where the osmotic gradient is reversed and substantially smaller compared to full strength seawater. In the present study, plasma osmolality, Cl^- and Na^+ had not yet fully recovered after 24 h, corroborating that exercising in seawater results in a larger osmoregulatory challenge and a prolonged recovery period compared to lower salinities (Hvas et al., 2018). Hence, an increased osmoregulatory burden could be an important limiting factor for repeated exercise challenges in seawater acclimated Atlantic salmon rather than lactate clearance and resynthesis of glycogen and phosphocreatine in swimming muscles.To boost the oxygen carrying capacity of the blood during strenuous swimming, salmonids are able to recruit additional red blood cells via splenic contractions (Yamamoto, 1988; Kita and Itazawa, 1989), while exercise stress also causes swelling of red blood cells owing to acidosis, increase catecholamine levels and fluid changes between intra and extracellular compartments (Nakano and Tomlinson, 1967; Wood, 1991). These factors explain the increases in Hct and Hb following exhaustion observed in the present study. However, this response became less pronounced with increasing fasting period. A reduced ability to recruit additional red blood cells in response to exercise stress could suggest a state of functional anaemia. Albeit this effect did not become sufficiently influential to negatively affect the U_{crit} during the fasting periods explored here. Food deprivation may also be expected to cause progressive anaemia, as was the case in common carp (*Cyprinus carpio*) following a 10-week fasting period (Sultan, 2013), although no effect on Hct, Hb or MCHC was found in the Olive Flounder (*Paralichthys olivaceus*) following 12 weeks of food deprivation (Park et al., 2012). In the present study, control values of haematological parameters were not affected by a 4-week fasting period in Atlantic salmon.

4.3. Some considerations for fish welfare in aquaculture management

An increased attention to animal welfare has emerged in recent years in commercial Atlantic salmon aquaculture production (Branson, 2008; Noble et al., 2018). Here, farmed Atlantic salmon are occasionally subjected to both voluntary and involuntary prolonged fasting periods owing to unfavorable conditions in the net cage environment such as pathogens, hypoxia and extreme temperatures (McLoughlin and Graham, 2007; Stehfest et al., 2017; Wade et al., 2019), or for management purposes prior to transport or slaughter (Kristiansen and Samuelsen, 2006; Waagbø et al., 2017). Since extended periods of fasting may violate ethical and legal obligations to farm animals (Webster, 2001; Branson, 2008; Norwegian Ministry of Agriculture and Food, 2009), the above-mentioned conditions have raised concerns with regards to fish welfare. However, no clear science-based recommendations currently

exist that explicitly defines when fish welfare is compromised owing to food deprivation (Hvas et al., 2020a). Hence, the present study may contribute to this account by having documented unaltered swimming capacities and preserved stress responses in smaller Atlantic salmon post smolts over a 4-week fasting period. Moreover, maintaining swimming capacities are particularly relevant owing to the current industry wide trend of expansion to exposed offshore farm sites subjected to rougher current conditions (Hvas et al., 2020b). While we did observe a significant reduction in condition factor, as expected, this may not warrant fish welfare concerns as Atlantic salmon have a profound capacity for growth compensation following periods of fasting or restricted feeding (Reimers et al., 1993; Morgan and Metcalfe, 2001; Johansen et al., 2002). Hence, evidently Atlantic salmon are well able to cope with prolonged periods of limited food supply and concerns with regards to fish welfare on this account are likely exaggerated.

Author contributions

This work was conceived by M.H. that also performed the experiments, analysed the data, prepared figures and wrote the first draft of the manuscript. L.H.S. contributed with data analyses and figure preparations, and L.H.S. and F.O. both provided feedback before approving the final version of the manuscript.

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Declaration of Competing Interest

The authors declare no competing or financial interests.

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