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The metabolic rate response to feed withdrawal in Atlantic salmon post-smolts

Malthe Hvas*, Lars Helge Stien, Frode Oppedal

Animal Welfare Research Group, Institute of Marine Research, Matre, Norway

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ABSTRACT

Feed withdrawal is a widespread practice in Atlantic salmon (*Salmo salar*) aquaculture to empty the gut prior to major farming operations, while certain pathogens and suboptimal environmental conditions in production cages are known to induce prolonged fasting. However, these fasting periods may be in conflict with ethical and legal obligations to farm animals. Presently, science-based recommendations on responsible fasting times that consider fish welfare are lacking. In this study, we measured the standard metabolic rate (SMR) and metabolic rate following acute handling and confinement stress in Atlantic salmon post smolts (~575 g, ~38 cm) following 1, 2, 3 and 4 weeks of feed withdrawal and 1 week of subsequent refeeding at 12 °C. The purpose was to identify when changes in metabolic mode occurred and assess whether the capacity to respond to stress eventually was compromised, since such observations could serve as potential welfare indicators. The SMR decreased significantly from 84.4 ± 4.7 mg O_2 kg h^{-1} in control fish to 71.0 ± 1.8 mg O_2 kg h^{-1} following 1 week of fasting. A further significant decrease to 65.0 ± 3.7 mg O_2 kg h^{-1} was measured after 3 weeks, while refeeding returned SMR to control levels. The increase in MO_2 following acute stress was unaffected for the first three weeks of fasting. However, the 4 week group showed a reduced peak response compared to the preceding weeks (278 ± 13 vs. 310 ± 7 mg O_2 kg h^{-1}). Weight, fork length and condition factor did not change significantly during the fasting period, and the fish immediately resumed eating upon refeeding. We conclude that up to 4 weeks of feed withdrawal had negligible effects on fish welfare. Moreover, an improved aerobic scope owing to a reduced SMR may be advantageous prior to certain farm operations.

1. Introduction

In the wild, many species of fish occasionally experience prolonged periods without eating owing to a range of factors such as seasonal scarcity in food supplies, migration or in relation to reproduction (Green and Farwell, 1971; Van Ginneken et al., 2005; Miller et al., 2009). Since fish are ectotherms and therefore have much lower metabolic demands compared to birds or mammals, they are less reliant on frequent meals to stay healthy and internal energy deposits takes substantially longer to deplete during periods of fasting (Wang et al., 2006).

The metabolic response to food deprivation in animals can be divided into three phases (Cahill Jr., 1976; Cherel and Groscolas, 1999; Wang et al., 2006): First is the initial postabsorptive phase that relies on glycogenolysis in the liver to supply energy. In the second phase, following depletion of liver glycogen, the required energy is provided primarily from oxidation of fatty acids stored in adipose tissues. The third phase is severe starvation where all fat reserves are used, and the energy must then be supplied with metabolization of muscle protein, which eventually results in death. While these metabolic responses extensively have been studied in mammals and birds, existing studies in fish also show that starvation elicits a similar three phase response to food deprivation with the notable difference being that the duration of each phase is much longer owing to lower metabolic demands (Jobling, 1980; Blasco et al., 1992; Bar, 2014).

Studying the feeding patterns and adaptations to food deprivation in fish species contribute to understanding their ecology and evolution. Moreover, from an applied point of view, such knowledge is becoming increasingly more relevant in Atlantic salmon (*Salmo salar*) aquaculture management. Although Atlantic salmon aquaculture is characterized by continuous satiation feeding to optimise growth performance, periods of varying length of voluntary or involuntary fasting periods can occur. For instance, feed withdrawal is routinely used to empty the gut of the fish to reduce risks of poor water quality from waste products prior to major farming operations involving crowding, pumping, delousing, transportation and slaughter (Ashley, 2007; Waagbø et al., 2017; Noble

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* Corresponding author.
E-mail address: malthe.hvas@imr.no (M. Hvas).

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et al., 2018). The reduced metabolic rate of fasting fish may also increase stress tolerance prior to these handling procedures owing to an increased aerobic scope. Various pathologies, for example pancreas disease caused by the salmon alphavirus, are well known to cause prolonged voluntary fasting that results in low growth rates (McVicar, 1987; McLoughlin and Graham, 2007; Føre et al., 2016). Furthermore, suboptimal environmental conditions such as low winter temperatures or summer heat waves combined with hypoxia can also cause prolonged voluntary fasting (Bogevik et al., 2010; Dempster et al., 2016; Stehfest et al., 2017; Wade et al., 2019), while new off shore farm locations may encounter periods of stormy conditions where feeding simply becomes impossible (Hvas et al., 2017a).

Fasting regimes in Atlantic salmon aquaculture has recently led to increased concern regarding fish welfare as excessive periods of food deprivation violate ethical and legal obligations to farm animals (Webster, 2001; Branson, 2008; Norwegian Ministry of Agriculture and Food, 2009). However, no clear science-based recommendations currently exist that defines when fish welfare becomes a concern owing to prolonged fasting. This is further complicated because the effects of food deprivation will depend on water temperature, body size and initial condition of the fish. For instance, harvest sized salmon maintained at low temperatures show greater resilience to prolonged fasting (Lie and Huse, 1992; Einen et al., 1998) than smolts at higher temperatures (Hevrøy et al., 2011).

Previous studies on feed withdrawal in Atlantic salmon have assessed gut evacuation rates (Storebakken et al., 1999; Handeland et al., 2008), weight loss, fatty acid composition and fillet quality (Einen et al., 1998), routine oxygen consumption at the group level and body composition (Cook et al., 2000), and endocrine responses (Hevrøy et al., 2011).

A potential welfare marker for unacceptable periods of food deprivation could be the onset of the third phase that signifies serious starvation (e.g. Wang et al., 2006). However, Atlantic salmon can survive weeks and even months without food with only fairly minor impacts on body condition (e.g. Lie and Huse, 1992; Cook et al., 2000). It may therefore be difficult to define when Atlantic salmon are truly subjected to detrimental starvation. Moreover, since farmed Atlantic salmon clearly elicits a strong desire to eat in frequent intervals, welfare indicators ideally should be defined in a more nuanced manner.

For this purpose, metabolic rate traits can serve as powerful welfare indicators since they are modulated by environmental and biological factors, and therefore can be used to predict in which conditions fish are able or unable to thrive (Hvas and Oppedal, 2019). Of particular interest regarding food deprivation is the resting metabolic rate termed standard metabolic rate (SMR) in fish that reflect the minimum energetic requirement to maintain basal homeostasis (Fry, 1971; Chabot et al., 2016), since any change over time in SMR would imply changes in metabolic mode. Also of interest is the metabolic rate increase in response to acute stress, which indicates the capacity of the fish to respond and cope with sudden challenges in its environment (Hvas et al., 2018). Hence, if prolonged fasting compromises stress responses or causes a substantial decrease in SMR owing to the fish entering a state of energy saving, the required time points to trigger these effects could potentially be used as welfare indicators for recommended feed withdrawal periods in Atlantic salmon aquaculture.

Our objective in this study was to measure the metabolic rate of Atlantic salmon post-smolts following up to 4 weeks of feed withdrawal and after 1 week of consecutive refeeding. While the SMR in fish generally tends to decrease with fasting time (Mehner and Wieser, 1994; Fu et al., 2005), the primary purpose was to quantify the magnitude of this decrease and to identify potential time dependent phases in changes to the SMR that could reflect adaptive strategies to prolonged fasting. Another purpose of this study was to assess the effect of feed withdrawal on the acute stress response by measuring metabolic rates following brief handling and confinement stress. Here we hypothesized that fasted Atlantic salmon eventually would become more docile and therefore elicit a reduced acute stress response.

2. Materials and methods

2.1. Animal husbandry

Atlantic salmon (Aquagen, Norway) were maintained at Matre Research Station, Institute of Marine Research, Norway. The fish were kept in groups of 25 in 6 identical holding tanks with a water volume of 405 l and with an open flow of UVC treated, filtered and aerated seawater supplied from 90 m depth in the local fjord. The open flow ensured that oxygen saturation levels remained above 85% at all times and prevented waste products from accumulating. Temperatures in the holding tanks were maintained at 12 °C by mixing of water from ambient (9 °C) and heated (20 °C) water reservoirs, and the fish were fed to satiation every day with feed pellets (4.5 mm pellet size, Skretting, Norway) via automated feeding devices. Prior to the experiments, the fish had been acclimating in these conditions for two months.

This work was performed between July and October 2019 in accordance with the Norwegian laws and regulations regarding the use of animals in scientific research under permit number 20474.

2.2. Protocols and measurements

Metabolic rates of fish were measured with a submerged 90 l intermittent-flow respirometer (Loligo systems, Denmark) previously described in Hvas et al. (2018). Here, the mass-specific oxygen uptake rates (MO_2) provides an indirect measurement of aerobic metabolism. A stable water temperature of 12 °C was maintained in the setup by having an open water flow running through a buffer tank containing the respirometer. Adequate mixing within the respirometer was ensured with a propeller running at a minimum speed opposite of the measurement section. As part of the respirometer, a fibre optic oxygen sensor, a temperature sensor and a flush pump (57 $l\ min^{-1}$) were connected to computer software (AutoResp Respirometry Software, Loligo Systems). This allowed for automatic measurements of MO_2 and subsequent flushing of the respirometer chamber to re-establish oxygen levels at predefined repeated intervals. The entire setup was partially covered in black plastic sheets to prevent visual disturbances to the fish, and the measurements were performed in a secluded room to further avoid unwanted disturbances during the respirometry trials.

The experimental protocol started in the afternoon, where one fish was netted from one of the holding tanks and transferred to the respirometer. Immediately following the introduction to the respirometer, MO_2 measurement commenced. The MO_2 was measured intermittently by automatically cycling between a closed measurement period of 10 min, an open flush period of 3.5 min and a waiting period of 1.5 min. The entire measurement period lasted for a minimum of 22 h which allowed for at least 88 MO_2 measurements. Afterwards, the fish was removed from the setup and euthanized with a blow to the head. The weight and fork length were then recorded. In the following hour, 4 measurement cycles were completed in the empty respirometer to account for bacterial respiration. The average of these MO_2 measurements were subsequently subtracted from all measurement intervals where the fish was present. However, background respiration rates remained very low owing to a fairly low test temperature, a continuous flow through the surrounding buffer tank and daily cleaning of the system.

The treatment groups consisted of a control fed in excess, 4 feed withdrawal groups representing 1, 2, 3 and 4 weeks of fasting, and a refeeding group that had been fed for 1 week after having endured 4 weeks of fasting. Six novel fish from each treatment group were measured in the respirometer. Prior to the experimental trials of the control and the fasted refed groups, feed had been withheld for one day to minimize metabolic effects of digestion, which is a standard procedure when measuring SMR of fish in other contexts (Clark et al., 2013;

Hvas and Oppedal, 2019).

2.3. Calculations and statistics

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

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

where $\Delta O_2/\Delta t$ 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, where the density of the fish was assumed to be 1 kg L^{-1}.

The SMR was estimated from the average of the 10% lowest values. If any outliers (± 2 standard deviations from the mean) were found, they were removed and a new average was calculated based on the remaining data points (e.g. Clark et al., 2013).

Being introduced into the respirometer imposes significant stress to the fish owing to first being caught in a net, experiencing brief air exposure, and subsequently being confined to a small unfamiliar environment. The measured MO_2 is therefore always drastically elevated at the beginning of each trial, reflecting the amount of stress experienced by the fish (Hvas and Oppedal, 2019). The MO_2 measured at the onset of each trial was therefore used here as an indicator of the stress response. In addition, to assess whether the time required to calm down within the respirometer was affected by the fasting period, we calculated how long it took each fish to reach a MO_2 corresponding to 1.5 × SMR.

The data analysis was performed using R software version 3.6.1 (R Core Team, 2019). Change in SMR and stress levels from controls to increasing durations of fasting (1–4 weeks) and subsequent refeeding had a distinct step function (staircase) shape. We therefore used regression trees to objectively determine between which weeks these steps occurred (function tree, Ripley, 2019) in accordance with Crawley (2007). The expected decline in response values at each step were tested for significance by Welsh's *t*-test (function t.test, R Core Team, 2019). Welsh's t-test was preferred to Student's t-test due to unequal sample sizes between step-levels (e.g. Crawley, 2007). Expected increase in SMR and stress response after refeeding, and differences between fed and refed groups were tested with the same method. Potential differences in size parameters and recovery time following acute stress were tested with a one-way ANOVA after test assumptions had been confirmed. Significance levels were set at $p < .05$.

3. Results

The fish were on average 575 g in weight and 38 cm in fork length, with condition factors of 1.02 (summarized in Table 1). Significant differences were not detected between any of the groups for any of the size parameters. No fish died during the fasting period and upon refeeding fish were immediately eating vigorously.

The SMR decreased in two distinct steps (Fig. 1A). First between control fish and 1 week of fasting (84.4 ± 4.7 vs. 72.4 ± 1.4 mg O_2 kg h^{-1}, $t = 2.4$, $p = .026$), and then between week 2 and 3 of fasting (72.4 ± 1.4 vs. 65.9 ± 2.2 mg O_2 kg h^{-1}, $t = 2.5$, $p = .011$). Upon refeeding, the SMR returned to control levels (90.3 ± 2.10 vs. 84.4 ± 4.74 mg O_2 kg h^{-1}, $t = 1.2$, $p = .287$), and was significantly higher than all fasted fish groups.

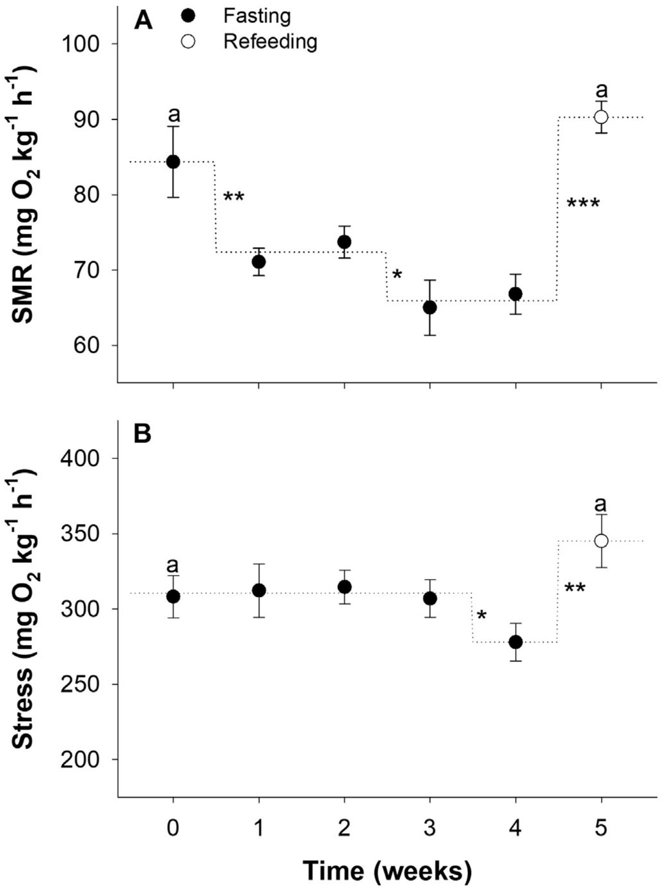

Fig. 1. The standard metabolic rate (SMR) (A) and the highest oxygen uptake rate measured in the beginning of each respirometry trial (Stress) (B) during up to 4 weeks of fasting and after 1 week of subsequent refeeding. * is $p < .05$, ** is $p < .01$, and *** is $p < .001$ for significant differences between the steps found by the tree-algorithm while statistical differences between controls and the re-fed groups are indicated with different letters. Data are mean ± s.e.m.

The acute stress response inferred from the initial peak MO_2 measurement remained preserved during the first three weeks of feed withdrawal. However, the tree-algorithm detected a step between week 3 and 4, where week 4 was significantly lower compared to the previous fasting weeks (278.0 ± 12.7 vs. 310.5 ± 6.6 mg O_2 kg h^{-1}, $t = 2.3$, $p = .026$) (Fig. 1B). There was also a statistical difference between the 4 week group and the refed group ($t = 3.1$, $p = .006$), but not between the refed group and the control ($t = 1.6$, $p = .13$) (Fig. 1B).

The time course of the MO_2 in the experimental trials for all treatment groups are shown in Fig. 2A. Overall, the response is similar where it takes several hours for the fish to completely calm down within the respirometer. However, the elevated metabolic rate in the refeed group and lowered metabolic rate in the two latter fasting groups are

Table 1
Morphometric parameters of Atlantic salmon used in the respirometry trials.

	Control	1 week	2 week	3 week	4 week	Refeeding
Weight (g)	578 ± 19	562 ± 47	587 ± 48	553 ± 47	608 ± 56	571 ± 44
Length (cm)	38.3 ± 0.7	38.0 ± 1.1	38.7 ± 1.0	37.8 ± 1.2	39.1 ± 1.3	38.1 ± 1.0
K	1.03 ± 0.02	1.02 ± 0.02	1.01 ± 0.03	1.01 ± 0.02	1.01 ± 0.02	1.02 ± 0.03

$N = 6$ for all groups. K is condition factor. Week number refers to the duration of feed withdrawal. Data are mean ± s.e.m.

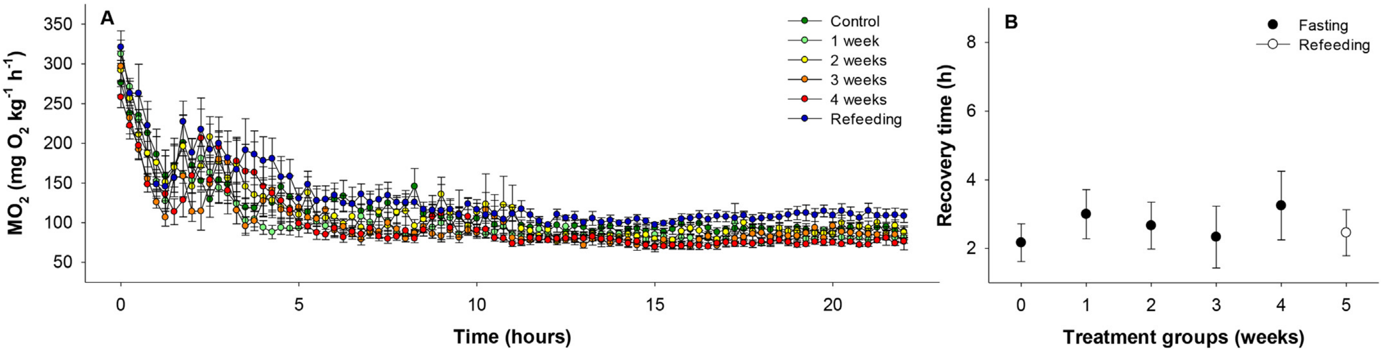

Fig. 2. The oxygen uptake rate (MO$_2$) over time for each treatment group in the respirometry trials (A), and the time required to recover 1.5 × SMR following acute confinement stress in each treatment group (B). Data are mean ± s.e.m.

notable at the latter part of the trial. Recover time from handling and confinement stress, inferred from the time required to reach 1.5 × SMR was statistically similar between all treatment groups (Fig. 2B).

4. Discussion

4.1. Standard metabolic rate

A reduction in SMR following prolonged fasting was expected as this has been shown previously in other species of fish (e.g. Mehner and Wieser, 1994; Fu et al., 2005), while routine oxygen uptake rates also decreased in a tank study of juvenile Atlantic salmon following periods of food deprivation (Cook et al., 2000). Interestingly, after the initial reduction in the first week, the SMR remained at a statistically similar plateau for the following week, and then it decreased again to a new plateau after 3 and 4 weeks that corresponded to a 22% reduction in SMR compared to the control fish. Hence, the basal maintenance cost of Atlantic salmon appeared to undergo two transitional adjustments during the periods of food deprivation assessed here. Considering that weight and condition factor did not change significantly in these experiments, it can be inferred that 4 weeks without eating did not induce a state of starvation in Atlantic salmon. It is therefore possible that the SMR could undergo a third adaptive transition to further preserve energy expenditure once a larger fraction of fat deposits had been metabolized, if the fish had been subjected to longer periods of food deprivation. Nevertheless, a reduction in SMR of 15% after one week and more than 20% after three to four weeks is substantial and shows that Atlantic salmon can adjust their metabolic mode beneficially during prolonged periods without access to food.

The metabolic increase associated with feeding and digestion, termed specific dynamic action (SDA), was not directly assessed in this study, since control and refed fish had been fasted for one day prior to the respirometry experiments. Based on studies on gut evacuation rates in Atlantic salmon while also considering fish size and water temperature (Storebakken et al., 1999; Handeland et al., 2008), this should have eliminated the majority of any SDA effects in the control and refed fish in the present study. Hence, the measured reduction in SMR after one week of fasting was likely caused by adaptive changes to preserve energy.

The physiological responses to food deprivation in fish may involve decreased activity of certain enzymes, reduced protein synthesis, down regulation of mitochondrial activity, and changed gene expressions. For instance, in common roach (*Rutilus rutilus*) the enzyme activities of major glycolytic and glycogenolytic enzymes in swimming muscles dropped to 60% of control levels initially and then remained constant following 7 weeks of food deprivation, while other enzymes' activities showed a continuous decrease during this fasting period (Méndez and Wieser, 1993). In Arctic char (*Salvelinus alpinus*) protein synthesis in the liver and muscle tissues decreased markedly following 36 days of fasting (Cassidy et al., 2016). In juvenile brown trout (*Salmo trutta*) the total number of mitochondria in the liver was reduced after 2 weeks of fasting, and moreover, state 3 mitochondrial respiration increased while state 4 decreased, suggesting that ATP production had become more efficient in terms of reducing excess energy waste via proton leakage (Salin et al., 2018). Also, in gilthead sea bream (*Sparus aurata*) subjected to 10 days of fasting, it was found that 72 genes in the liver were down regulated while 39 genes in muscle tissues were up regulated (Bermejo-Nogales et al., 2015). These adaptive responses likely also happened to the Atlantic salmon in our study that would have contributed to a lowered SMR in fasted fish.

After one week of refeeding the SMR had returned to control levels. Combined with an unchanged condition factor, this further corroborates that 4 weeks of feed withdrawal did not cause serious detrimental effects, since appetite was preserved and the fish quickly readjusted their metabolic mode as food became available again. This is in line with previous studies where fish easily were able to recover tissue reserves and blood metabolites once normal feeding was resumed, provided that the fasting regime was not too severe (Weatherley and Gill, 1981; Méndez and Wieser, 1993).

4.2. Stress response

Most species of fish are dependent on high aerobic activities and burst swimming doing foraging and predator avoidance, and a strong acute stress response that mobilizes the full capacity of the cardiorespiratory system is therefore a key adaptation to survival (Wendelaar Bonga, 1997). Since prolonged periods of food deprivation results in down regulation of metabolism in fish to preserve energy (Méndez and Wieser, 1993; Mehner and Wieser, 1994), this could consequently lead to a trade-off where the ability to efficiently respond to acute stressors becomes compromised owing to a reduced maximum aerobic capacity. Indeed, in the Yangtze catfish (*Silurus meridionalis*) the maximum metabolic rate, measured with a chase protocol, was significantly reduced after 15 days compared to 2–5 days of fasting (Fu et al., 2005). However, in the present study, while the SMR of Atlantic salmon already was down regulated, the metabolic rate following acute stress was statistically unaffected during the first 3 weeks of feed withdrawal. Hence, this fasting regime was likely not severe enough to substantially impact the ability of the fish to adequately respond to challenges in their environment. Moreover, this suggests that the initial metabolic down regulation during food deprivation in Atlantic salmon differentially select tissues and organs that do not directly interfere with the cardiorespiratory system. However, the fish from the final feed withdrawal group had a reduced peak MO$_2$ following acute stress, suggesting that 4 weeks starts to affect the acute responsiveness in Atlantic salmon, although the magnitude of change at this point was modest.

In this study, the acute stress response was quantified by measuring MO$_2$ immediately following netting, brief air exposure, and subsequent confinement to a small unfamiliar environment doing the initial movement of the fish into the respirometry chamber. Confinement stress trials are commonly used when studying the acute stress response in salmonids (Pottinger and Carrick, 1999; Vindas et al., 2016). It may also serve as a proxy for the maximum metabolic rate (MMR) in Atlantic salmon since it provides a higher MO_2 compared to an exhaustive chase protocol, which is a widespread method to measure MMR in fish (Hvas and Oppedal, 2019). However, confinement stress alone does not fully mobilize the cardiorespiratory system, as Atlantic salmon are able to attain a higher MO_2 during prolonged swimming until exhaustion (Hvas and Oppedal, 2019). Still, provided that the stress response and MMR were preserved for up to 3 weeks of feed withdrawal, as suggested here, the aerobic scope (the differences between MMR and SMR) would have increased owing to a reduction in the SMR. Increased aerobic scopes in fish are generally considered advantageous, as the magnitude of the capacity to increase aerobic metabolism above resting levels signifies the available potential to perform fitness related activities (Fry, 1971; Claireaux and Lefrancois, 2007). Hence, moderate fasting regimes may allow farmed Atlantic salmon to better cope with certain challenges, for instance those encountered during crowding, de-lousing and transport operations.

4.3. Fish welfare

The purpose of this study was to establish time points for the onset of changes in metabolic rate traits that potentially could be used as welfare indicators for fasting regimes in Atlantic salmon aquaculture. The increase in metabolic rate following stress remained similar between control fish and the feed withdrawal groups from the first 3 weeks while the 4 week group showed a minor reduction in acute responsiveness, suggesting that physiological capabilities only were marginally impaired after 4 weeks of fasting. Moreover, the initial reduction in SMR is an adaptive response that allows Atlantic salmon to preserve energy and rapidly reversed following refeeding. Also, considering that both appetite and condition factor were preserved after 4 weeks of feed withdrawal, fish welfare did not appear to be poor, apart from having denied Atlantic salmon to indulge in their farm-conditioned pattern to eat in frequent intervals.

The average time of feed withdrawal before transporting fish to slaughter is 9 days in Norwegian Atlantic salmon aquaculture (Kristiansen and Samuelsen, 2006). Considering that 4 weeks of feed withdrawal in the present study did not induce substantial detrimental and irreversible physiological changes, the current fasting regimes prior to slaughter currently in use should not compromise fish welfare. Moreover, our study was performed on ~575 g fish, and harvest sized Atlantic salmon weighing several kilos will inherently be even more robust to fasting owing to lower mass specific metabolic rates (e.g. Oldham et al., 2019), as well as having higher fat reserves.

Our study was performed at 12 °C, which is the midrange of the thermal niche of Atlantic salmon. The SMR of Atlantic salmon post-smolts gets drastically accelerated with increasing temperature (Hvas et al., 2017b), and resilience to food deprivation will therefore improve at low temperatures and worsen at high temperatures. Hence, it should be noted that the results reported here may not be representative of Atlantic salmon maintained at more extreme temperatures or of different size classes. For instance, in two other studies, large Atlantic salmon weighing 2 and 5 kg, respectively, were fasted during winter for 11 and 12 weeks (Lie and Huse, 1992; Einen et al., 1998). Here it was found that weight loss mainly was caused by depletion of fat reserves, primarily in the viscera while fillet protein levels remained constant, suggesting that these fish not yet had entered a stage of starvation. Moreover, while food deprivation eventually will cause weight loss, compensatory growth following longer periods of restricted feeding or feed withdrawal is well documented in farmed Atlantic salmon (Reimers et al., 1993; Morgan and Metcalfe, 2001; Johansen et al., 2002). Hence, the impressive flexibility to cope well in various highly fluctuating farm environments, including temporal variability in food supply is part of what makes Atlantic salmon such a successful species in aquaculture.

Author contributions

This work was conceived by all authors. M.H. performed the experiments, analysed the data and wrote the first draft of the manuscript while all co-authors provided valuable feedback before approving the final version.

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Declaration of Competing Interest

The authors declare no competing or financial interests.

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