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RESEARCH ARTICLE

Energetic costs of ectoparasite infection in Atlantic salmon

Malthe Hvas* and Samantha Bui

ABSTRACT

Parasites are widespread in nature, where they affect the energy budget of hosts, and depending on the imposed pathogenic severity, this may reduce host fitness. However, the energetic costs of parasite infections are rarely quantified. In this study, we measured metabolic rates in recently seawater adapted Atlantic salmon (*Salmo salar*) infected with the ectoparasitic copepod *Lepeophtheirus salmonis* and used an aerobic scope framework to assess the potential ecological impact of this parasite–host interaction. The early chalimus stages of *L. salmonis* did not affect either standard or maximum metabolic rates. However, the later mobile pre-adult stages caused an increase in both standard and maximum metabolic rate yielding a preserved aerobic scope. Notably, standard metabolic rates were elevated by 26%, presumably caused by increased osmoregulatory burdens and costs of mobilizing immune responses. The positive impact on maximum metabolic rates was unexpected and suggests that fish are able to transiently overcompensate energy production to endure the burden of parasites and thus allow for continuation of normal activities. However, infected fish are known to suffer reduced growth, and this suggests that a trade-off exists in acquisition and assimilation of resources despite an uncompromised aerobic scope. As such, when assessing impacts of environmental or biotic factors, we suggest that elevated routine costs may be a stronger predictor of reduced fitness than the available aerobic scope. Furthermore, studying the effects on parasitized fish in an ecophysiological context deserves more attention, especially considering interacting effects of other stressors in the Anthropocene.

KEY WORDS: Aerobic scope, Pathophysiology, Respirometry, Salmon lice *Lepeophtheirus salmonis*, Conservation biology

INTRODUCTION

Parasites are ubiquitous in nature, where they play a pivotal role in shaping food webs and regulating host abundances (Dobson et al., 2008). The pathogenic effects on parasitized hosts are sometimes obvious, but often effects are subtle, indirect and nonlethal, which make them difficult to quantify. Nevertheless, hosts will to some extent suffer an increased energetic burden from coping with parasites, and depending on the severity, this can ultimately affect host fitness (Booth et al., 1993; Gooderham and Schulte-Hostedde, 2011; Hicks et al., 2018). Since animals have a limited sustained energy budget (Hammond and Diamond, 1997), additional energetic costs from coping with parasites may impose trade-offs with other life-history traits such as foraging, growth and reproduction (Norris and Evans, 2000; Klemme and Karvonen, 2017; Terui et al., 2017). Increased costs caused by parasites include mobilization of immune and stress responses, although it is not well understood exactly how costly these responses are among different animal taxa and their respective host–parasite interactions (Sheldon and Verhulst, 1996; Martin et al., 2003; Sandland and Minchella, 2003). Parasites also exert a direct cost by draining resources from the host and thereby causing a negative impact on energy budgets which may affect overall performance (McElroy and de Buron, 2014; Smyth and Drea, 2016; Binning et al., 2017).

In fish, one of the most widely studied host–parasite interactions is between Atlantic salmon (*Salmo salar*) and the ectoparasitic salmon lice (*Lepeophtheirus salmonis*) owing to the persistent issues with pest management in aquaculture that has become the most prominent sustainability obstacle for the industry. In parallel to the rapid expansion of salmon aquaculture and associated proliferation of the parasite population (Dempster et al., 2021), there are conservation concerns for wild salmon populations as aquaculture sea cages act as reservoirs for the parasite, which freely disperses into the adjacent environment (Brooker et al., 2018; Johnsen et al., 2020).

Newly smoltified wild Atlantic salmon en route to the sea pose a significant population bottleneck in survival. At this critical stage, Atlantic salmon are under substantial predation pressure from birds and larger fish as they migrate through rivers, estuaries and fjords, while simultaneously undergoing challenging physiological transformations in preparation for a marine life (Thorstad et al., 2012). Since salmon farms are located along migration routes, wild Atlantic salmon also have to endure an inflated infection pressure of *L. salmonis*, which is believed to further reduce marine survival chances (Johnsen et al., 2020; Vollset et al., 2021).

The ecology of *L. salmonis* consists of three pelagic, lecithotrophic larval stages that disperse with water currents until the infective copepodid stages, whereby lice will begin host-searching behaviours (Costello, 2006). Once a salmonid host has been found and successfully infected, lice will progress through two sessile chalimus stages, followed by mobile pre-adult and adult stages where they are able to move around the surface of the host (Johnson and Albright, 1991). Whilst attached, the lice feed on mucus, skin and blood of the salmonid host (Costello, 2006).

Atlantic salmon mobilize immunological defence responses at the early infection stages of *L. salmonis* (Tadiso et al., 2011; Braden et al., 2020). However, known pathogenic effects are primarily associated with the later mobile pre-adult life-stages, where general visible impacts on infected fish are skin damage and lesions (Grimnes and Jakobsen, 1996; Dawson et al., 1999). Physiological disturbances assessed from haematological analyses include elevated levels of plasma ions and osmolality, higher cortisol and lactate levels, and a decrease in haematocrit, which suggest that infected fish can suffer osmoregulatory impairments, increased stress, and anaemia (Grimnes and Jakobsen, 1996; Wagner and McKinley, 2004; Wells et al., 2006; Fjelldal et al., 2020). *L. salmonis* may also reduce swimming performance (Wagner et al., 2003; Bui et al., 2016) and transiently reduce feed intake and growth (Dawson et al., 1999; Fjelldal et al., 2020). At sufficiently high

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infection levels, fish eventually become moribund (Grimnes and Jakobsen, 1996; Fjelldal et al., 2020), whereas fish with fewer parasites are able to recover (Dawson et al., 1999).

Measurements of energy usage in response to increasing parasite levels can provide a quantification of otherwise elusive nonlethal effects, which allow for predictions of the ecological impact of a given parasite–host interaction. In fish, energy budgets and how they are influenced by environmental factors are commonly assessed with an aerobic scope (AS) framework (Fry, 1971; Claireaux and Lefrancois, 2007; Pörtner and Farrell, 2008). In this framework, the AS is defined as the difference between standard and maximum metabolic rates (SMR and MMR), where SMR is the minimum energetic requirement to maintain basal homeostasis and MMR corresponds to the highest aerobic performance that can be achieved during exhaustive activities. As such, all fitness-related behaviour and functions, including coping with parasites, will have a certain metabolic cost restricted by a finite scope for activity.

Although the use of AS frameworks are widely used in many areas of fish biology (Wood et al., 2012; Clark et al., 2013; Brauner and Richards, 2020), assessments of energy budgets in parasitized fish are rarely reported (Hvas et al., 2017a). We are therefore unaware of previous attempts to quantify the metabolic costs of *L. salmonis* infections in Atlantic salmon.

In Fig. 1 we hypothesize how increasing parasite levels theoretically may affect SMR, MMR and AS based on known pathogenic effects of *L. salmonis* on Atlantic salmon. Specifically, the SMR may increase owing to costs associated with immune and stress responses as well as increased basal osmoregulatory requirements. The MMR may decrease owing to anaemia, and a reduced oxygen uptake capacity imposed by the need to prioritize active branchial ion regulation since the gills are subjected to an osmorespiratory compromise in physiologically demanding situations (Sardella and Brauner, 2007). An increasing severity of parasite load should therefore reduce the AS, meaning that less respiratory capacity can be diverted to other activities.

To test the hypothesis outlined in Fig. 1, we used respirometry to measure oxygen uptake rates ($\dot{M}_{O_2}$) as a proxy for aerobic metabolic rates at rest and following exhaustive stress in recently smoltified and seawater adapted Atlantic salmon infected with low and high amounts of *L. salmonis* and at different times following infection coinciding with the sessile and mobile stages.

MATERIALS AND METHODS

Fish husbandry

Atlantic salmon (Aquagen) were raised at the Institute of Marine Research, Matre, Norway following standard protocols. Fish had completed the smoltification process immediately prior to being transferred to the experimental holding tanks (26 cm deep, 55 cm wide, 55 cm long, water volume of 79 l), with 10 fish per tank and 4 tanks per subsequent parasite treatment (see next section). Temperature was maintained at 9°C in full strength seawater (33 ppt), and each tank had a continuous inflow of 10 l min^{-1} from an aerated, filtered and sterilized water supply which ensured normoxic conditions and removal of waste products. A test temperature of 9°C was chosen to represent ecologically relevant conditions of newly smoltified seaward migrating Atlantic salmon in spring (Thorstad et al., 2012). The fish were kept under a natural simulated photoperiod and fed to satiation daily between 14:00 h and 18:00 h via automatic feeding devices (Nutra Olympic, 2 mm pellet size, Skretting). Prior to infection, the fish had been acclimating in these conditions and in seawater for 2 weeks.

This study was performed between April and June of 2021 and was approved by The Norwegian Food Safety Authorities under permit number 25907, adhering to ethical and legal obligations to vertebrate animals in scientific research.

Infection with *Lepeophtheirus salmonis*

Experimental fish were divided among three treatment groups: control (uninfected), low or high infection intensities. Categorical infection intensities were estimated from Fjelldal et al. (2020), where low infection was considered ∼0.3 louse g^{-1} and high was ∼1 louse g^{-1}. Treatment groups were further divided into those tested when lice were at the sessile (chalimus) stage or the mobile (pre-adult) stage.

Salmon lice were produced at the same research facility using a wild population of adult female lice sourced from nearby fish farms (Norwegian Food Safety Authorities permit number 12935). Egg strings were collected from females and incubated at the

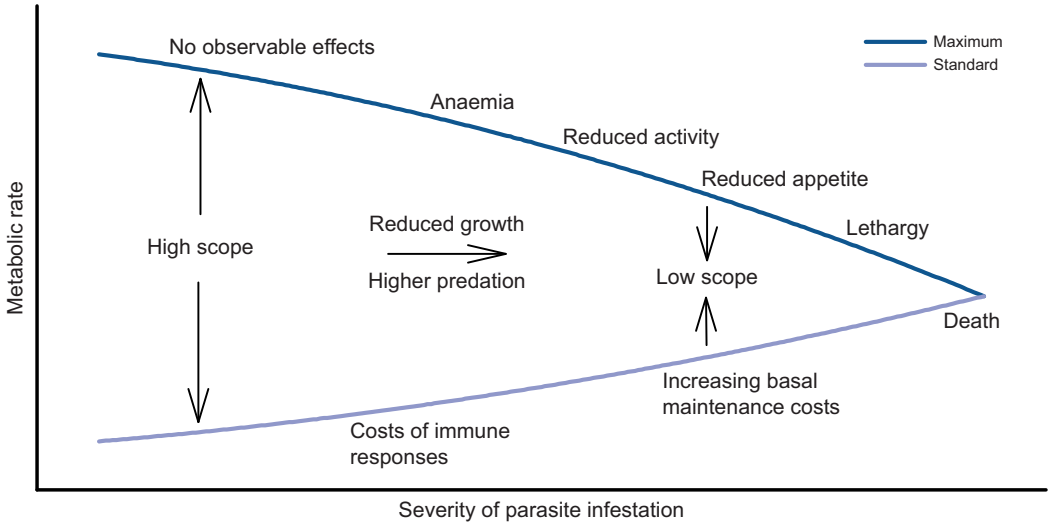

Fig. 1. Conceptual diagram of parasite impact on metabolic rates and aerobic scope in fish. Curves shows maximum and standard metabolic rates in response to an increasing severity of parasite load, while the aerobic scope corresponds to the difference between the two curves. The onset of some important nonlethal effects is suggested.

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experimental temperature (Hamre et al., 2009). Two days after larvae had developed to the infective copepodid stage (when infection success is optimal; Skern-Mauritzen et al., 2020), they were enumerated to generate a low or high infection pressure. The number of larvae introduced to each tank was calculated for the intended infection intensity, with an estimated infection success of 30% while considering the mean fish mass per holding tank.

The infection protocol was applied to all tanks, including uninfected controls who experienced the procedure without the addition of infective lice. Water level in tanks was lowered to 50% and inflow reduced to 2 l min^{-1}, whereafter infective copepodids were added to the water (number depending on treatment group). Fish were left in these conditions for 30 min with constant monitoring of behaviour, and water quality using a ProSolo Digital Water Quality Meter (YSI, Xylem Analytics, Ohia, USA) with oxygen, salinity and temperature sensor. After this period, water flow was restored and eventually returned to the original state.

Three days prior to the first respirometry tests per treatment group, all fish were sedated within their tanks (metomidate hydrochloride, 0.01 g l^{-1}, Aquacalm vet, Scan Aqua AS, Norway) and louse abundance was recorded for all fish, to confirm successful infection.

Respirometry setup

To measure oxygen uptake rates ($\dot{M}_{O_2}$) of Atlantic salmon post-smolts infected with *L. salmonis*, a 4-chamber automated intermittent-flow respirometry system was used, allowing for parallel testing of 4 individual fish (Loligo Systems, Denmark). The cylindrical acrylic chambers were 30 cm in length and with an 8 cm internal diameter. Each chamber was connected with gas-tight PVC tubes running through a water pump and a flow-through oxygen sensor cell, forming an internal circulation loop. The volume of this closed system, including tubes, was 1.584 l. For the purpose of intermittent flushing, the respirometry chambers also had an open loop connected to a flush pump (5 l min^{-1}), where the upstream PVC tube reached above the water surface. Each chamber together with flush pump, circulation pump, and oxygen sensor was submerged in its own rectangular tank with a water volume of 140 l. A constant in- and outflow through all 4 tanks with water from the same source as used for the holding tanks ensured that the temperature was maintained at 9°C and minimized waste accumulation and bacterial proliferation during experimental trials. Oxygen sensors were connected via optic fiber cables to computer software (AutoResp, Loligo Systems) along with the flush pumps, allowing for automatic periodic flushing and logging of oxygen concentration at 1 Hz. The oxygen sensors were carefully calibrated beforehand following the manufacturer's instructions.

The complete setup, including water supply and electronics, was located in a secluded room. This ensured that potential disturbances from other activities were avoided.

Experimental protocols

Respirometry trials were performed on fish from the low and high parasite treatments at 11–15 days post-infection (sessile chalimus stages) and 24–27 days post-infestation (mobile pre-adult stages). Uninfected controls were tested randomly between these times on 12, 16, 21 and 24 days post-infection. Sixteen fish were measured individually in each of these 5 treatment groups, with 4 fish being tested simultaneously per run. In addition, fish were removed sequentially from replicate tanks (i.e. 4 fish from one tank, then 4 fish from the next tank on the following day), so that all tanks were exposed to the same disturbance over time.

Before being introduced into the respirometer, the fish were netted from their holding tanks and subjected to an exhaustive chase test. This was done to obtain an estimate of the MMR at the onset of the measurement protocol (Norin and Clark, 2016). Here, each fish was moved to a new tank with rounded corners containing approximately 100 l water and forced to burst swim in circles via tactile stimulation. Chasing of the fish was done for 4 min, and at the end of this period the fish would have limited responsiveness allowing it to be handled almost as if sedated, suggesting physiological exhaustion. A 4 min chase period was found to be optimal based on earlier pilot trials on Atlantic salmon of similar sizes and at similar temperatures. Shorter periods were found to not consistently exhaust the fish (e.g. 3 min), and longer periods would contain extended durations where the fish barely were able to elicit an escape response (e.g. 5 min). To avoid parasite detachment during the chase test, efforts were made to not touch or grip the fish too roughly; inspection of the chase tank water afterwards showed that parasites did not fall off during this procedure.

Following the chase test, the fish was transferred into the respirometry chamber that then was sealed off as quickly as possible before starting $\dot{M}_{O_2}$ measurements. There was a delay of approximately 1 min between the end of the chase protocol and the start of the first $\dot{M}_{O_2}$ measurement period.

An automated intermittent-closed protocol was then repeated in 6 min cycles that consisted of a 4 min closed measurement period, followed by a 1.5 min open flush period to reestablish oxygen levels, and a 0.5 min wait period to stabilize flow conditions in preparation for the next closed measurement period. All trials were initiated prior to the beginning of the daily feeding schedule at 14:00 h to ensure that fish had been fasting overnight for a minimum of 18 h. This was done to reduce confounding effects associated with the metabolic costs of digestion when attempting to estimate the SMR in fish (Chabot et al., 2016). The fish where then kept in the respirometers overnight for a minimum of 21 h.

The next day, the fish were removed from their chambers and euthanized in an overdose of anaesthetic (metomidate hydrochloride, 0.05 g l^{-1}). Mass and fork length were then measured, whereafter number of lice and their life-stage were recorded for each fish.

After removal of the fish, the chambers were resealed to measure background respiration rates. A minimum of four measurement cycles were made in the empty chambers, and the mean value of these measurements was subtracted from all prior measurement periods to correct for background respiration. The setup with acrylic chambers, tubes, pumps, and sensors were then disassembled and thoroughly cleaned to prepare for the next trial.

Calculations and statistics

The $\dot{M}_{O_2}$ was calculated in all closed measurement periods from the change in dissolved oxygen over time as:

$$\dot{M}_{O_2} = \frac{\frac{\Delta O_2}{\Delta t}(V_{sys} - V_b)}{M_b}, \quad (1)$$

where $\Delta O_2/\Delta t$ is the slope of the linear decrease in oxygen (mg O_2 h^{-1}), V_{sys} is the volume of the respirometer (1.584 l), and V_b and M_b are the volume (litres) and mass of the fish (kg), respectively, assuming a fish density of 1 kg l^{-1}. The R^2 of the linear regressions used to calculate $\dot{M}_{O_2}$ were most often >0.98.

The SMR was estimated from the mean of the 10% lowest MO_2 values measured during the respirometry protocol. However, if any

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Table 1. Size parameters and parasite infestation levels

	Infection level	Fish body mass (g)	Fork length (cm)	Condition factor	Infection intensity (no. lice g^{-1})	Louse life-stage
Control	–	62.8±2.9	18.0±0.2	1.07±0.02	0	–
Sessile stage	Low	57.1±1.9	17.5±0.2	1.06±0.02	0.15±0.02	Chalimus
	High	60.1±3.3	17.8±0.3	1.06±0.03	0.79±0.09	Chalimus
Mobile stage	Low	57.3±2.7	17.6±0.3	1.05±0.02	0.12±0.01	Pre-adults
	High	65.0±1.8	18.0±0.2	1.11±0.01	0.56±0.06	Pre-adults

Mass, fork length and condition factor were statistically similar between groups (one-way ANOVA, $P>0.05$). $N=16$ per group and data are means±s.e.m.

outliers (±2 s.d. from the mean) were found, these were removed, and a new average was calculated based on the remaining data points as the reported SMR (Clark et al., 2013).

The MMR was defined as the highest $\dot{M}_{O_2}$ measured, which in most cases coincided with the beginning of the trial where the fish had been subjected to the chase test. However, in some cases, fish were able to approach and exceed this initial peak $\dot{M}_{O_2}$ at seemingly random times in latter part of the trials, presumably as an expression of spontaneous escape behaviour. The absolute and factorial AS were calculated as MMR minus SMR and MMR divided by SMR, respectively. The influence of lice respiration rates on total MO_2 were considered negligible owing to their small sizes relative to the fish. In the present study, the total biomass of lice would likely range between approximately 0.03% and 0.3% of the fish (based on estimated mass of lice from L. Hamre, pers. comm.).

The condition factor of each fish was calculated as 100 M_b (fork length^3)$^{-1}$, and infection levels were expressed as lice g^{-1}.

A one-way ANOVA along with Tukey's *post hoc* test was used to test for differences in size parameters between groups, after having confirmed equal variance and normal distribution of the data with Levene's test and Shapiro-Wilks tests, respectively. Linear mixed effect (LME) models were used to evaluate the effect of louse stage (mobile, sessile or control) and infection intensity (no. of lice g^{-1}) on the four metabolic rate parameters: SMR, MMR, AS and Factorial AS. The full model included the factors Stage, Intensity and Fish mass, with Tank as a random factor and using the maximum likelihood method. Different iterations of the inclusion or exclusion of factors generated 8 models, which were compared using the second-order Akaike information criterion (AICc). The models were fit using the 'lme' function from the package nlme (https://cran.r-project.org/package=nlme), and model selection achieved using the 'aic.tab' function from AICcmodavg package (https://cran.r-project.org/package=AICcmodavg), in the statistical software R v. 3.6.1 (http://www.r-project.org). *Post hoc* multiple comparisons of factors were conducted using the 'lsmeans' function in the lsmeans package (https://cran.r-project.org/package=lsmeans). Model residuals were checked for linearity and normality with visual examination of diagnostic plots. A *P*-value below 0.05 was considered significant and data are reported as means±s.e.m. unless specified otherwise.

RESULTS

Body mass, fork length and condition factors of the fish were similar between stage and infection intensity groups (one-way ANOVA, d.f.=79, $P=0.08$, 0.37 and 0.30, respectively) (Table 1). The mean size parameters across all treatments were 60.5±1.2 g mass, 17.8 ±0.1 cm fork length and 1.07±0.01 condition factor ($N=80$). No fish died or became moribund during this study.

The two infection trials with low and high levels of *L. salmonis* resulted in distinct parasite levels of 0.15±0.02 and 0.79±0.09 lice g^{-1} at 11–14 days post-infection, respectively. At this time, the louse stages were chalimus 1 and chalimus 2, and they were mainly attached to pectoral and dorsal fins (Fig. 2A,B). At 25–28 days post-infection, parasite levels had decreased to 0.12±0.01 and 0.56±0.06 lice g^{-1} in the low and high groups, respectively. The stages at this time were mobile pre-adult 1 and 2

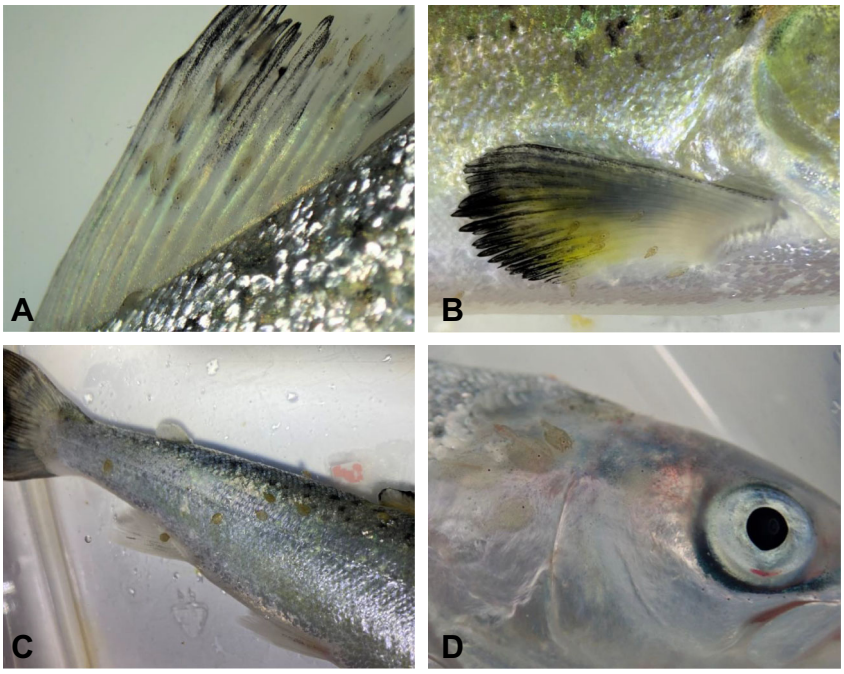

Fig. 2. Site of attachment of *Lepeophtheirus salmonis* at different life-stages. The early sessile chalimus stages (1–2 mm in length) were found primarily on the fins (A,B), and the later and larger mobile preadult stages (≈4 mm in length) were found all over the body (C,D).

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Table 2. Estimated parameters from selected models for each respirometry measure

	Selected model AICc	Selected model factor	χ^2	d.f.	*P*
SMR	634.1	Stage	90.8	2	<0.0001
MMR	823.9	Stage	37.7	2	<0.0001
		Mass	15.6	1	<0.0001
AS	824.3	Stage	14.4	2	<0.0001
		Mass	18.1	1	<0.0001
Factorial AS	122.5	Stage	11.7	2	0.003
		Mass	15.6	1	<0.0001

For each measure, the AICc value and factors included in the selected model is shown, along with results of the summarized model.

males and pre-adult 1 females and they were mainly attached along the body of the fish (Fig. 2C,D).

Final selected LME models for SMR, MMR, AS and factorial AS are represented in Table 2. All metabolic measures were affected by louse stage; however, none was influenced by infection intensity (Table 2). The SMR was higher in the two mobile lice infection groups compared with the control (estimate=−25.2, t_{68}=−6.8, P<0.0001) and sessile infection groups (estimate=−26.1, t_{68}=−8.6, P<0.0001), whereas sessile groups remained similar to controls (estimate=0.9, t_{68}=0.25, P=1.0). Hence, the sessile chalimus stages did not affect the SMR, while the mean SMR of the low and high intensity mobile groups (123.5±2.5 mg O_2 kg^{-1} h^{-1}) on average were 25.5% higher than the control group (98.4±2.2 mg O_2 kg^{-1} h^{-1}) (Fig. 3A).

The MMR was significantly higher for the mobile louse infection groups (mean 391.8±7.3 mg O_2 kg^{-1} h^{-1}) compared with both the control group (estimate=−73.1, t_{67}=−5.3, P<0.0001; mean 315.9±7.7 mg O_2 kg^{-1} h^{-1}) and the sessile groups (estimate=−43.3, t_{67}=−4.5, P=0.0001; mean 352.6±8.7 mg O_2 kg^{-1} h^{-1}). This difference corresponded to a 24.0% higher MMR in mobile lice groups compared with the controls. MMR was similar among control and sessile-infected fish (estimate=−29.8, t_{67}=−2.1, P=0.09) (Fig. 3B).

The AS was elevated by the presence of mobile louse stages (mean 268.2±7.6 mg O_2 kg^{-1} h^{-1}), compared with uninfected fish (estimate=−47.7, t_{67}=−3.7, P=0.0014; mean 217.6±8.5 mg O_2 kg^{-1} h^{-1}), while fish with sessile lice (mean 255.2±8.5 mg O_2 kg^{-1} h^{-1}) had a similar AS to both the control fish (estimate=−30.1, t_{67}=−2.3, P=0.06) and fish with mobile lice (estimate=−17.5, t_{67}=−1.8, P=0.1817) (Fig. 3C). The factorial AS was slightly higher in sessile compared with mobile louse stages (estimate=0.4, t_{67}=3.2, P=0.0058), while control fish had a similar factorial AS as both sessile and mobile groups (Fig. 3D).

Although none of the metabolic rate parameters were influenced by infection intensity (Table 2), there was an observed, but non-significant, positive correlation between mobile lice infection levels and the SMR, along with the elevated metabolic rates in fish with mobile louse stages as revealed by the LME (Fig. 4A). However, a similar positive correlation was not observed for the MMR (Fig. 4B).

Fish mass was found to be a significant factor that influenced MMR, AS and factorial AS together with louse stage (Fig. S1; Table 2). These negative correlations were relatively weak (estimate=−1.6 and −1.8 for MMR and AS, respectively, and −0.02 for FAS) and appeared to be largely influenced by individuals with elevated body mass compared with the majority of the cohort (i.e. >80 g; see Fig. 1).

For a descriptive overview of the respirometry trials, the $\dot{M}_{O_2}$ over time in the 5 treatment groups are shown in Fig. 5. Within the first 5 h of the trial, the control fish appeared to have ceased stress responses more rapidly and to a more stable, lower level following the exhaustive chase test than infected fish, whereas $\dot{M}_{O_2}$ approaches a baseline level after approximately 12 h in all treatments which

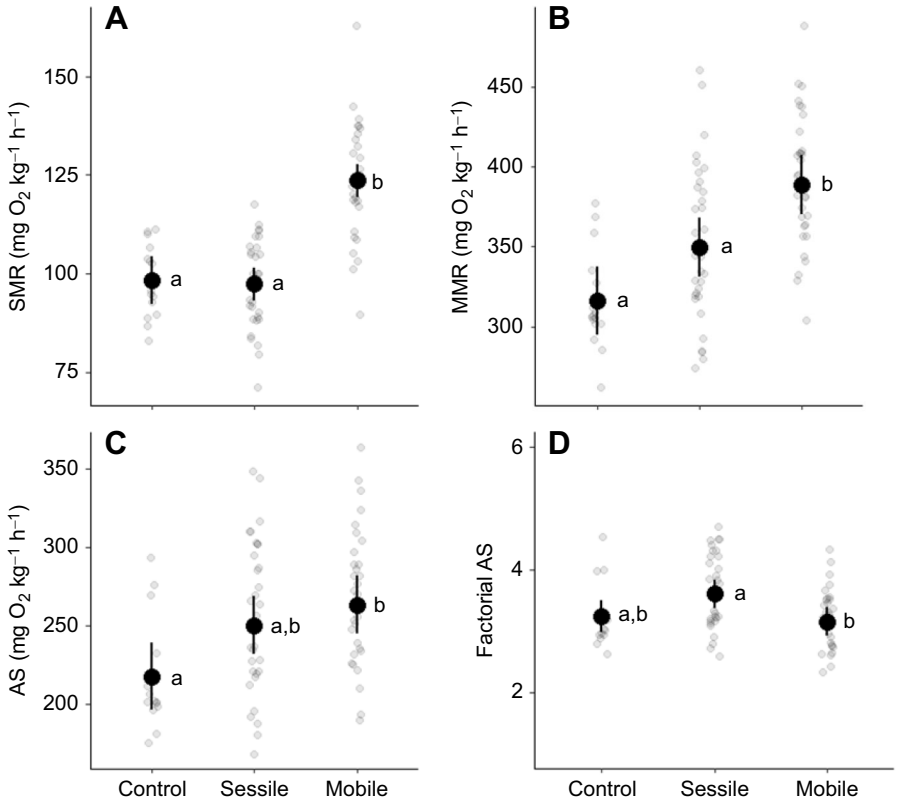

Fig. 3. Metabolic rate parameters for *L. salmonis* at different stages of louse infection. Mean standard (SMR; A) and maximum (MMR; B) metabolic rates, aerobic scope (AS; C), and factorial AS (D), as shown by the black marker with bars representing±95% confidence intervals. Spread of values from individual fish is also shown (smaller grey markers). Different letters indicate statistical differences from the *post hoc* contrasts (P<0.05). N=16 for controls and N=32 for sessile and mobile groups.

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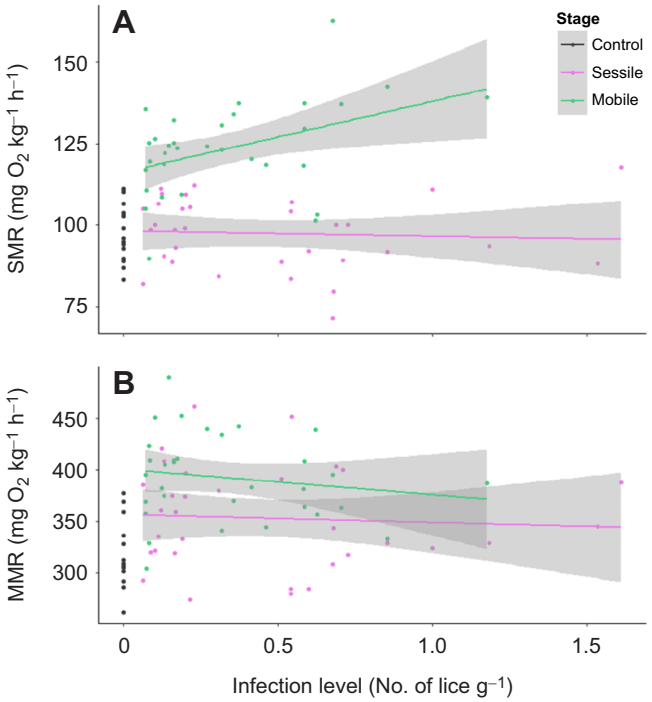

Fig. 4. Metabolic rate correlations with *L. salmonis* infection level and stage. (A) Standard (SMR) and (B) maximum (MMR) metabolic rates of individual fish and their corresponding parasite levels are shown, together with linear regressions (and shaded 95% confidence interval) for control fish (*N*=16) and the two main louse stages assessed (sessile and mobile, *N*=32 for both).

suggests recovery from the chase test and that the protocol length was adequate for SMR estimations. In the latter part of the trials from 10 to 21 h, the routine $\dot{M}_{O_2}$ of the two mobile louse groups are notably higher than the control and two sessile infection groups, in line with an elevated SMR in the mobile infection groups (i.e. Fig. 3A).

DISCUSSION

Effects on metabolic rates

We found that recently smoltified Atlantic salmon infected with the ectoparasitic copepod *L. salmonis* experienced an increased metabolic burden once *L. salmonis* had progressed to the pre-adult stages. Approximately 12,000 species of copepods have been described and about one-third of these are parasitic, using either fish or other invertebrates as hosts (Humes, 1994). However, to our knowledge, the present study is the first quantification of energetic costs in a host infected with a parasitic copepod.

Detecting subtle nonlethal pathogenic effects in animals is generally difficult, and similarly to previous studies of infected Atlantic salmon (Grimnes and Jakobsen, 1996; Dawson et al., 1999; Bui et al., 2016), we did not observe physiological changes during the early chalimus stages of *L. salmonis*, regardless of parasite numbers. The chalimus stages in this study were mainly attached on the fins, which are poorly vascularized, and it is therefore perhaps not surprising that we were unable to detect a metabolic impact at the whole-animal level at this point.

The later pre-adult stages on the other hand caused an increase in the SMR. However, while this effect tended to be greater in fish with a more severe parasite load, as hypothesized, the mere presence of mobile lice rather than infection intensity was the main cause for elevating the SMR. An elevated basal maintenance cost in parasitized Atlantic salmon was presumably caused by a combination of immune responses and increased osmoregulatory requirements (Wagner et al., 2008; Fast, 2014; Fjelldal et al., 2020). Although, it is unclear which one of these physiological functions that is energetically more expensive.

The energetic cost associated with mobilizing immune responses is poorly understood in fish and reported results are somewhat conflicting. For instance, rainbow trout (*Oncorhynchus mykiss*) injected with a DNA vaccine transiently increased their routine metabolic rates (Skinner et al., 2010) and mosquitofish (*Gambusia affinis*) exposed to a bacterial lipopolysaccharide had increased resting metabolic rates (Bonneaud et al., 2016), while zebrafish (*Danio rerio*) immune challenged with a heat-killed bacteria showed an increase in routine metabolic rates at 27.5°C but not at 22°C (Bennoit and Craig, 2020). However, formalin-killed *Aeromonas salmonicida* injected into rainbow trout had no effect on resting metabolic rates (Zanuzzo et al., 2015) and neither did high loads of piscine orthoreovirus (Zhang et al., 2019), or infections with the gill parasite *Paramoeba perurans* in Atlantic salmon (Hvas et al., 2017a). This suggests that immune responses in fish both can be energetically expensive and inexpensive relative to whole-animal metabolic rates, depending on species, environmental contexts and pathogens involved.

While *L. salmonis* is known to induce immune responses on its own (Braden et al., 2020), secondary infections facilitated by damage to the skin and mucus layer during the pre-adult stages (e.g. Bjørn and Finstad, 1998) likely also played a role in the present study. Specifically, since the SMR did not increase at the early chalimus stages, despite Atlantic salmon already mobilizing an immune response at this point (Tadiso et al., 2011), secondary infections rather than a direct reaction to the parasite could also be a driver for the observed increase in the SMR during later infection phases.

Similarly to immune responses, the energetic costs of maintaining osmotic balance in fish is also context dependent and may vary from a few percent to above 30% of resting metabolic rates (Ern et al., 2014). However, in salmonids, osmoregulatory costs are generally considered to be on the lower side (Morgan and Iwama, 1999). Moreover, Atlantic salmon had similar metabolic rates when swimming at high intensities in hypoosmotic, isosmotic and hyperosmotic salinities (Hvas et al., 2018), further suggesting that osmoregulation is relatively inexpensive, even during osmotically challenging situations when assessed at the whole-animal level.

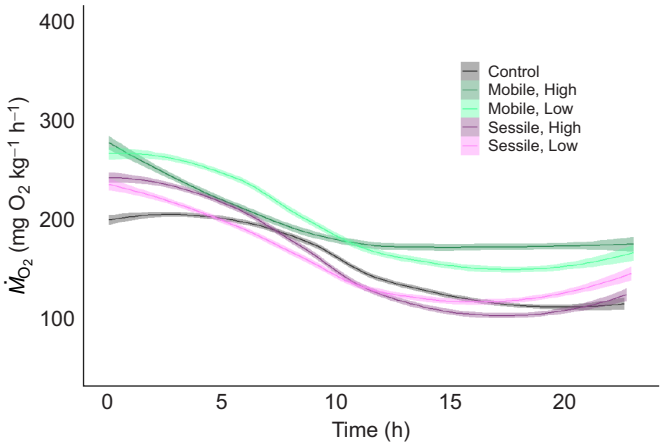

Fig. 5. Oxygen uptake rates over time. The $\dot{M}_{O_2}$ of each consecutive measurement period during the 21 h respirometry trials of the 5 treatment groups [separated into louse stage (sessile/mobile) and infection intensity (high/low); *N*=16 per group]. Lines are the smoothed conditional means (fitted using a LOESS regression) for each group over time, with the shaded area representing the 95% confidence interval for the respective group.

Nevertheless, osmoregulatory impairment is one of the major pathogenic effects of *L. salmonis* and is specifically associated with the pre-adult infection stages (Bjørn and Finstad, 1998; Fjelldal et al., 2020), and since these stages coincided with an increased SMR in the present study, it was likely a contributing factor for this observation.

Additionally, a higher SMR could perhaps also be partly explained by skin irritation, as the mobile stages of lice crawl around on the fish, causing a general state of discomfort and stress not directly related to specific physiological functions such as immune defence and osmoregulation. Hence, constantly sensing the presence of lice may induce a state of chronic alertness that is energetically costly.

The pre-adult stages also caused an increase in the MMR, but in contrast to the SMR, this was not in accordance with our hypothesis. We expected that the MMR would decrease owing to anaemia and an osmorespiratory trade-off, where the need to prioritize osmotic integrity would limit maximum branchial oxygen uptake rates, as inferred from the main pathogenic effects of *L. salmonis* (Grimnes and Jakobsen, 1996; Wells et al., 2006; Fjelldal et al., 2020).

It may seem paradoxical that a severe parasite infection would increase the aerobic capacity of a fish. However, a similar observation was made on mosquitofish challenged with a bacterial lipopolysaccharide (Bonneaud et al., 2016). These authors suggested that the fish overcompensate ATP production to offset the costs of immune responses as an adaptive strategy to preserve the AS and thereby allow continuation of normal activities. As in the present study, they unfortunately did not test whether a seemingly improved aerobic capacity translated into an improved athletic performance such as higher maximum swimming speeds. However, in the case of *L. salmonis*, severely infected Atlantic salmon and pink salmon (*Oncorhynchus gorbuscha*) (Wagner et al., 2003; Nendick et al., 2011) have previously been shown to have reduced critical swimming speeds, suggesting that immune-challenged fish with allegedly higher MMRs were unlikely to achieve an improved functional performance. Nevertheless, it is an interesting idea that fish may be able to transiently improve their MMR to preserve their AS when challenged with pathogens.

Another possibility for the observed effects on the MMR could be related to methodology. We used an exhaustive chase test, and whether this method underestimates the MMR in salmonids compared with a swim challenge until fatigue has been debated (Hvas and Oppedal, 2019; Raby et al., 2020; Zhang et al., 2020). If the chase method used in the present study failed to capture the true MMR, our data may be interpreted differently. Specifically, all fish tested received the same 4 min chase treatment and the subsequent measurement in peak $\dot{M}_{O_2}$ therefore provides an indicator of the energetic requirement to endure a defined stressor. More severely infected Atlantic salmon had a higher $\dot{M}_{O_2}$, which suggests that they then became more stressed and were required to use a higher proportion of their AS to cope with an identical challenge. Conversely, a lower $\dot{M}_{O_2}$ response in uninfected controls becomes an indicator of higher robustness to acute stress as they presumably utilized a lower fraction of their AS. Nevertheless, this highlights a shortcoming of the chase method in that the achieved peak $\dot{M}_{O_2}$ is not directly related to a quantifiable performance trait as is the case in swim challenge tests. Regardless of interpretation, neither provided evidence for that the capacity for oxygen uptake was compromised by *L. salmonis* infections, as initially hypothesized.

Ecological impacts

In the present study, neither the absolute nor the factorial AS were reduced owing to *L. salmonis* infections in Atlantic salmon when compared with uninfected controls. Moreover, the increase in the SMR during the pre-adult infection phase was accompanied by an increase in the MMR, which increased the absolute AS and preserved the factorial AS.

While these results contrasted with our initial hypothesis of a decreasing AS with increasing parasite load, infection still caused a substantial shift in energy budgets where maintenance of homeostasis became energetically more expensive once lice had developed into mobile stages. In an ecological context, this is disadvantageous owing to resource limitations of wild fish, as increased costs of living need to be offset by increasing efforts in foraging to maintain similar growth rates as uninfected individuals (Barber and Wright, 2005). Pre-adult *L. salmonis* were previously reported to reduce growth in Atlantic salmon (Fjelldal et al., 2020), and similarly, immune-challenged mosquito fish with preserved or elevated AS also had reduced size gains (Bonneaud et al., 2016), showing that pathogens can impose a trade-off in acquisition and assimilation of resources despite an uncompromised AS. Moreover, parasitized birds spend less time actively foraging because of presumed restrictions on energy budgets (Hicks et al., 2018). As such, if infected Atlantic salmon also spend less time foraging, this could reduce their growth potential, which would be further exacerbated by an increased maintenance burden. Indeed, in wild Atlantic salmon returning from sea, increasing parasite levels were associated with reduced body condition (Susdorf et al., 2018), providing indirect evidence for an energy trade-off.

A similar shift in energy budgets is seen at high water temperatures above optimal ranges in Atlantic salmon and other fish species. Here, increasing temperature accelerates the SMR, but the MMR also increase, meaning that the AS either is preserved or elevated, yet appetite and growth still declines (Hvas et al., 2017b; Jutfelt et al., 2021). This suggests that environmental or biotic factors that impose excess energetic requirements on routine costs generally may be stronger predictors of ecological impacts than the available AS.

Numerically, we found that pre-adult *L. salmonis* infection caused, on average, a 26% increase in basal maintenance costs. Provided that fish are able to survive their infection (e.g. Dawson et al., 1999), over time this will accumulate into a substantial deficit on resource allocation, and this will be particularly troublesome for small Atlantic salmon post smolts starting the marine phase of their lifecycle, where rapid growth is paramount to reduce predation risks and to increase overall survival chances.

Wild Atlantic salmon populations have historically suffered from overfishing, habitat destruction and pollution (Parrish et al., 1998; Thorstad et al., 2012), with more recent threats being increased parasite infection pressures from aquaculture sites (Krkošek et al., 2007; Johnsen et al., 2020). Although high mortality risks are typically associated with the seaward migration phase of newly smoltified fish (Thorstad et al., 2012), emerging evidence also points to increasing population decline during the marine phase, where the cause is speculated to be unreported and unregulated fishery (Dadswell et al., 2021; Pardo et al., 2021). However, considering the present study, increased struggle at sea could also be driven by the long-term energetic disadvantage imposed by parasites.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.H., S.B.; Methodology: M.H., S.B.; Validation: M.H., S.B.; Formal analysis: M.H., S.B.; Investigation: M.H., S.B.; Resources: M.H., S.B.; Data curation: M.H., S.B.; Writing - original draft: M.H.; Writing - review & editing: S.B.; Funding acquisition: M.H., S.B.

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