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Physiological responses of farmed Atlantic salmon and two cohabitant species of cleaner fish to progressive hypoxia

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ABSTRACT

To mitigate salmon lice infestations in Atlantic salmon (*Salmo salar*) sea cages, deployment of cleaner fish have become a widespread strategy. However, species of cleaner fish may experience poor welfare in the highly fluctuating farm environment owing to differences in physiological adaptations and niche requirements. In particular, occurrences of reduced oxygen levels are common in salmon cages. The purpose of this study was therefore to compare hypoxia responses of Atlantic salmon and two commonly used cleaner fish species, the lumpfish (*Cyclopterus lumpus*) and the ballan wrasse (*Labrus bergylta*). We used respirometry to measure metabolic rates (MO_2) during progressive hypoxia down to 20% oxygen saturation. In addition, we also measured key haematological parameters before, during and after hypoxia exposure. While all fish survived exposure down to 20% oxygen saturation, distinct differences in metabolic and haematological responses were found, reflecting species specific adaptations and lifestyles. In Atlantic salmon, MO_2 was independent of ambient oxygen levels until 27% saturation, after which it decreased linearly. In lumpfish, MO_2 steadily decreased throughout the hypoxia trial. In ballan wrasse, MO_2 was notably lower than in the other species and unaffected by the levels of hypoxia encountered. Hypoxia induced changes in plasma cortisol, plasma lactate and plasma osmolality were substantially greater in Atlantic salmon compared to both cleaner fish species. This suggests that similar magnitudes of hypoxia exposure were more stressful to Atlantic salmon. Hence, neither cleaner fish species should be in immediate danger as long as hypoxia levels that are known to be detrimental to Atlantic salmon are avoided. However, lumpfish had markedly reduced activity levels at the early onset of progressive hypoxia, and is therefore likely to require near normoxic conditions to efficiently function as cleaner fish.

1. Introduction

The global production of farmed Atlantic salmon (*Salmo salar*) has increased from 0.2 million tonnes in 1990 to above 2 million tonnes since 2012, and in terms of total economic value it is presently the largest commodity in finfish aquaculture (FAO, 2018, 2019). However, further growth is currently haltered primarily owing to the salmon lice (*Lepeophtheirus salmonis*), an ectoparasite that attach on salmon and feed on their skin, mucus and blood (Costello, 2006). Substantial costs are associated with controlling salmon lice infestations in sea cages (Abolofia et al., 2017), and lice outbreaks may also harm wild salmonids (Krkošek et al., 2011, 2013).Various control measures against salmon lice are currently in use and include thermal, mechanical and chemical treatments. However, these methods can be harmful to the fish and have been associated with increased mortalities (Overton et al., 2018a, 2018b).

A less harmful and more cost effective preventive method is the deployment of cleaner fish in sea cages, as it has been found that they can be effective in removing salmon lice from Atlantic salmon (Leclercq et al., 2014; Imsland et al., 2014, 2018). In Norway, the largest producer of Atlantic salmon, cleaner fish usage has therefore increased tremendously in recent years and 55 million fish were deployed in salmon sea cages in 2017 (Norwegian Directorate of Fisheries, 2018). The most popular cleaner fish species is the lumpfish (*Cyclopterus lumpus*) since it is easily cultured, but several species of wrasses (*labridae*) are also used (Powell et al., 2018). Most wrasse species deployed in sea cages are wild-caught and currently it is only the ballan wrasse (*Labrus bergylta*) that is cultured successfully for cleaner fish deployment (Skiftesvik et al., 2013; Powell et al., 2018).

Cleaner fish are vertebrate species with high cognitive capabilities and they are therefore protected by animal welfare legislations in Norway and other countries (e.g. Branson, 2008; Dyrevelferdsloven, 2009). From ethical and legal points of view, their wellbeing in the sea cage environment is therefore equally important as for Atlantic salmon

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despite that cleaner fish have no commercial value as food. It is therefore worrisome that anecdotal reports of very high unaccounted mortalities of cleaner fish in salmon sea cages are quite common.

Since the widespread use of cleaner fish in salmon aquaculture is a recent trend, much of the fundamental biology of these species has not yet been properly studied (Hvas et al., 2018a; Yuen et al., 2019). Moreover, the sea cage environment can be highly variable on both temporal and spatial scales in temperature, salinity, current speeds, light conditions and oxygen levels (Johansson et al., 2006, 2007; Oppedal et al., 2011a). Lumpfish and wrasses have different ecologies than Atlantic salmon, and their environmental preferences and thresholds are likely also different. Hence, knowledge of the physiological adaptations and responses to the fluctuating environmental conditions encountered in salmon sea cages is therefore important if cleaner fish are to be used efficiently and responsibly.

In particular, periods of environmental hypoxia (low oxygen levels) is a well-documented issue in Atlantic salmon aquaculture and is caused by a range of factors such as high stocking densities, insufficient water exchange and heat waves (Oppedal et al., 2011b; Burt et al., 2012; Stien et al., 2012; Dempster et al., 2016). The severity of hypoxia in sea cages varies, but moderate hypoxia that is known to reduce feed intake of Atlantic salmon is common (Solstorm et al., 2018), and in extreme cases oxygen levels may be as low as 21% saturation (Dempster et al., 2016; Stehfest et al., 2017).

Hypoxia causes numerous potential detrimental physiological disturbances in fish, depending on the severity. First the capacity for aerobic metabolism gradually becomes more compromised as oxygen levels decreases, which causes a reduction in appetite, growth and activity (Claireaux et al., 2000; Remen et al., 2016; Oldham et al., 2019). In severe hypoxic conditions fish will struggle to meet the oxygen demands required to maintain basal homeostasis, making them acidotic from anaerobic metabolism, and if exposed for prolonged periods they will eventually suffocate (Fry, 1971; Farrell and Richards, 2009).

It is well established that different species of fish show tremendous variation in hypoxia tolerance and coping strategies owing to differences in habitat distribution and diverse evolutionary adaptations (Nilsson and Renshaw, 2004; Richards et al., 2009; Mandic et al., 2009; Iftikar et al., 2010). Considering such differences between Atlantic salmon and cleaner fish species, variation in hypoxia tolerance is therefore expected. For instance, wild Atlantic salmon sometimes endures hypoxia during river migration (Sergeant et al., 2017). Ballan wrasses are coastal and sedentary species with short home-ranges (Villegas-Ríos et al., 2013), and may also occasionally experience hypoxia in their natural habitat. Moreover, high hypoxia tolerances have been reported in other closely related temperate wrasse species (Corkum and Gamperl, 2009). However, it is likely rare for lumpfish to encounter hypoxic conditions naturally since they are semi-pelagic species (Blacker, 1983; Davenport, 1985). Hence, it can be hypothesized that lumpfish should be more vulnerable to hypoxia.

The purpose of this study was to compare the physiological responses to environmental hypoxia between Atlantic salmon, lumpfish and ballan wrasse subjected to similar experimental conditions. This was done by performing respirometry trials where aerobic metabolic rates were measured continuously during progressive hypoxia down to 20% oxygen saturation, and by measuring relevant haematological parameters before, during and after hypoxia exposure.

2. Material and methods

2.1. Experimental animals and husbandry

Atlantic salmon (Aquagen, Norway) were produced on site at the Matre Research Station, Institute of Marine Research in Norway. After smoltification the fish were kept inside in holding tanks with a water volume of ~0.5 m^3. Fish were subjected to a simulated natural photoperiod and fed to satiation through automatic feeding devices daily (Spirit, 3 mm pellet size, Skretting, Norway).

Ballan wrasse were obtained from a commercial supplier (MOWI site Øygarden, Rong, Norway) and transported to the Matre Research Station where they were kept at the same fish tank facilities as used for Atlantic salmon. Artificial kelp hides were put in the holding tanks to provide shelters. Ballan wrasses were fed in excess with commercial food pellets every day (Otohime S3, Pacific Trading Aqua, Ireland).

Lumpfish were obtained from a commercial supplier (MOWI site Vanylven, Åheim, Norway), and transported to the Matre Research station. Lumpfish were kept in ~1.0 m^3 cylindrical shaped holding tanks provided with vertical oriented half-circular plastic pipes that the fish could attach to with their ventral suction disc. Food pellets were given daily in excess through automatic feeding devices (Atlantic Gold, 2 mm pellet size, Pacific Trading Aqua, Ireland).

All species were maintained at a water temperature of 9 °C via an open water flow of UVC treated and filtered sea water (34 ppt) obtained from the nearby fjord. A continuous open water flow through the holding tanks ensured a constant high water quality by maintaining oxygen saturation above 90% at all times and preventing waste products such as carbon dioxide and ammonia to accumulate. All species had been acclimating in their holding tanks for minimum one month prior to experimentation.

The experiments were approved under permit number 16290 by The Norwegian Food Safety Authority in accordance with Norwegian legislations on the ethical use of animals in scientific research.

2.2. Respirometry setup

The oxygen uptake rate (MO_2) during progressive environmental hypoxia was measured with a 90 l intermittent-flow respirometer (Loligo Systems, Denmark). This setup was described previously (Hvas et al., 2018a). A constant water temperature of 9 °C was maintained by having an open water flow running through the buffer tank containing the respirometer. Proper mixing of water within the respirometer was ensured by having a propeller running at a minimum speed opposite of the measurement section of the setup. A fibre optic oxygen sensor, a temperature sensor and a powerful flush pump (57 l min^{-1}) was part of the setup and was connected to computer software (AutoResp Respirometry Software, Loligo Systems). This allowed for systematic measurements of MO_2 and automatic flushing of the respirometer chamber to re-establish oxygen levels in predefined 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 experimental trials.

2.3. Experimental protocols

Prior to movement into the respirometer, fish had been starved for one day to reduce confounding metabolic effects from digestion. For Atlantic salmon 4 fish were used per trial, for Lumpfish 5 fish were used per trial and for ballan wrasse 10 fish were used per trial. An uneven number of fish between species was used to provide similar biomass to volume ratios in the respirometer and to ensure that the rate of gradual hypoxia experienced between species was similar. In addition, group respirometry on evenly sized fish can provide similar MO_2 estimates as for fish tested individually while also allowing for more fish to be tested for subsequent blood sampling in substantial less time (Hvas and Oppedal, 2019). 6 replicate respirometry trials were performed for each species.

Fish were allowed to acclimate in the respirometer overnight with intermittent flushing to maintain normoxic conditions (above 90% O_2 saturation) and to monitor routine metabolic rates. The following morning the flush pump was turned off to cause natural gradual hypoxia as the fish used up the remaining oxygen in the respirometer. In this way oxygen levels were allowed to decrease to 20% saturation.

At this point in half of the trials, fish were quickly removed from the respirometer and knocked unconscious whereafter a blood sample was drawn from the caudal vein with a heparinized syringe and then momentarily stored on ice. Since fish already were calm and easy to net while in hypoxia, anaesthetics were not used at this sampling time. In the other half of the trials, the respirometer was thoroughly flushed to re-establish normoxic conditions, and the fish were then allowed to recover for 1 h. After recovery fish were anaesthetized within the respirometer in 5 mg l^{-1} metomidate hydrochloride (AquaCalm vet, Scanvacc, Norway) to minimize handling stress prior to being sampled for blood. All Atlantic salmon and lumpfish were successfully sampled for blood. However, it was more difficult to obtain a sufficient blood sample (~1 ml) from ballan wrasse owing to their smaller sizes and only 4 fish were therefore sampled per trial.

After all fish had been removed from the respirometer, oxygen uptake rates were measured in the empty respirometer in normoxia for 20 min to account for microbial respiration. Since the entire setup was thoroughly cleaned after each trial and owing to the fairly low test temperature of 9 °C, background respiration rates were low and had negligible effects on the results.

To obtain control values, blood sampling was performed on all species after they had been anaesthetized within the holding tanks in 5 mg l^{-1} metomidate hydrochloride (AquaCalm vet, Scanvacc, Norway).

2.4. Haematological analyses

Immediately following blood sampling, small subsamples were transferred to capillary tubes and centrifuged (StatSpin MP Centrifuge) to measure the haematocrit (Hct) as the visible fraction of red blood cells. The haemoglobin concentration (Hb) was then measured in 10 µl blood with a haemoglobin assay kit (MAK115, Sigma-Aldrich). In addition, having measured both Hct and Hb allowed for calculation of the mean corpuscular haemoglobin concentration (MCHC). To separate plasma from the red blood cells, the remaining blood was centrifuged at 5000 *g* for 5 min whereafter plasma samples were stored at −80 °C for later analyses.

The concentration of Na^+ and Cl^- in the plasma samples were measured in an electrolyte analyser (Cobas 9180, Roche Diagnostics). Plasma osmolality was measured via freeze point determination with a Fiske 210 Micro-Sample Osmometer (Advanced Instruments). Concentrations of plasma lactate and glucose were measured in a spectrophotometer using MaxMat PL (MaxMat), and plasma cortisol was measured with ELISA (IBL International GmbH).

2.5. Calculations of metabolic rates

The MO_2 in a given time interval could be calculated by fitting a linear regression of the decrease in water oxygen concentration as a function of time:

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

$\Delta O_2/\Delta t$ is the slope of the regression line (mg O_2 h^{-1}), V_{sys} is the volume of the respirometer (l), and V_b and M_b are the volume (l) and mass of the fish (kg), respectively, where a fish density of 1 kg l^{-1} was assumed. MO_2 is therefore reported as mg O_2 kg^{-1} h^{-1}.

For all respirometry trials, the MO_2 was calculated for each time period that caused a 2% further decrease in oxygen saturation in the water. By starting calculations at 90% and stopping at 20% oxygen saturation, this allowed MO_2 to be represented at 35 different ambient oxygen levels during the cause of progressive hypoxia.

Table 1
Morphometric parameters. Weight, fork length and condition factor of Atlantic salmon (N = 24), lumpfish (N = 30) and ballan wrasse (N = 60) used in the respirometry trials. Data are mean ± s.e.m.

	Weight (g)	Length (cm)	Condition factor
Atlantic salmon	164 ± 6	26.1 ± 0.3	0.92 ± 0.01
Lumpfish	185 ± 5	16.6 ± 0.2	4.06 ± 0.09
Ballan wrasse	92 ± 2	17.6 ± 0.1	1.68 ± 0.02

2.6. Statistical analyses

The same statistical analyses were performed on all species. A repeated measures ANOVA was used to determine changes in oxygen uptake rates during progressive hypoxia. Differences in haematological parameters between control samples, during hypoxia and following recovery was tested with a one-way ANOVA. A Tukey's post hoc test was applied afterwards to identify which ambient oxygen levels or sampling times differed from each other. Compliance to test assumptions regarding equal variance and normal distribution of the data were ensured beforehand with Levene's test and the Shapiro–Wilk test, respectively, where in some cases a log transformation was required. A P-value below 0.05 was considered significant. All data are reported as mean ± standard error of the mean.

3. Results

The weight, length and condition factor of the fish used in the respirometry trials are summarized in Table 1. Owing to different morphologies, each species had a distinct condition factor with Atlantic salmon being lean, lumpfish being rounded and ballan wrasse falling somewhere in between. Atlantic salmon and lumpfish were of comparable size classes with weights or 164 ± 6 g and 185 ± 5 g, respectively. Ballan wrasses were smaller, weighing 92 ± 2 g. None of the fish kept in the allocated holding tanks prior to the experiments or during the experiments died.

3.1. Metabolic rates

The average rate of progressive hypoxia from 90% to 20% oxygen saturation in the respirometry trials were −8.3 ± 0.5% O_2 h^{-1} for Atlantic salmon, −9.0 ± 0.6% O_2 h^{-1} for lumpfish, and −6.5 ± 0.4% O_2 h^{-1} for ballan wrasse.

The MO_2 in response to progressive hypoxia for each species is shown in Fig. 1. Initially in normoxia, Atlantic salmon and lumpfish had similar metabolic rates of ~100 mg O_2 kg^{-1} h^{-1}, while ballan wrasse had a notable lower MO_2 of ~60 mg O_2 kg^{-1} h^{-1}. However, each species showed a distinct response in MO_2 following progressive hypoxia.

For Atlantic salmon, MO_2 remained independent of ambient oxygen levels until 27% oxygen saturation, whereafter MO_2 decreased linearly (Fig. 1A). During the majority of the time, Atlantic salmon remained calm and inactive in the respirometer, but some individuals started to show occasional bursts of escape behaviours once oxygen levels declined below 30% saturation. Notable increases in gill ventilation rates as hypoxia became more severe were also observed.

For lumpfish, MO_2 gradually declined throughout the different oxygen levels assessed. In the beginning of the trials, lumpfish were more active and many of the individuals swam around within the respirometer chamber. This is reflected by larger error bars in the MO_2 measurements when in normoxia and early onset of hypoxia. Between 60% and 70% oxygen saturation, the error bars started to become smaller which coincided with the fish ceasing to move around spontaneously. Instead all lumpfish now remained attached to the bottom floor by using their ventral suction disc. This was followed with more

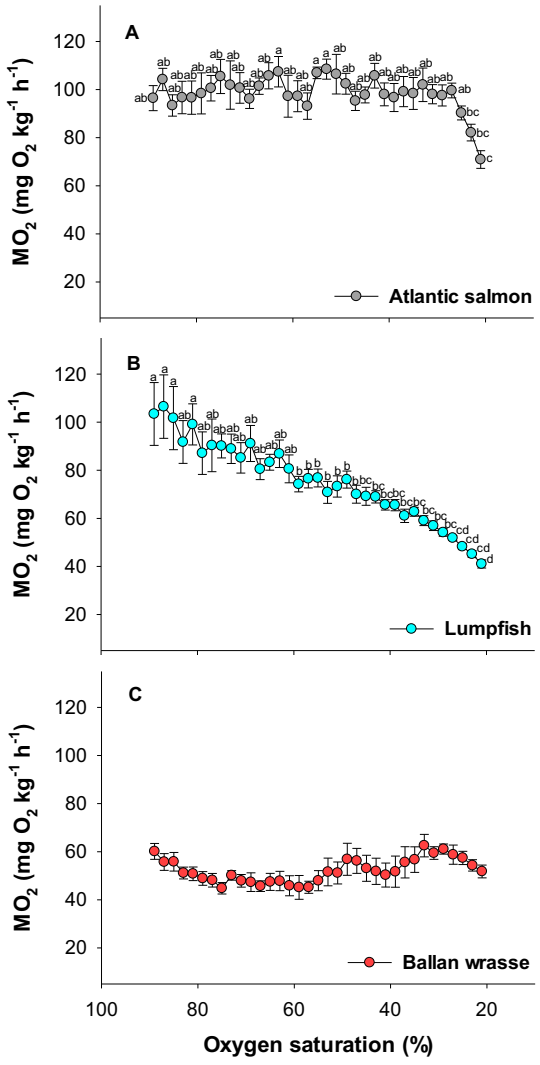

Fig. 1. Oxygen uptake rates (MO_2) during progressive hypoxia of Atlantic salmon (A), lumpfish (B) and ballan wrasse (C). MO_2 values are shown for each 2% decrease in ambient oxygen saturation from 90% to 20%. For Atlantic salmon, statistical differences between all assessed oxygen levels are indicated with superscript letters. For lumpfish, representative statistical differences are indicated with superscript letters, where a, b, c and d are measurements that are statistically similar to 89%, 47%, 29% and 21% oxygen saturation, respectively. For ballan wrasse, all MO_2 values were statistically similar. $N = 6$. Data are mean ± s.e.m.

pronounced gill ventilations, as also seen in Atlantic salmon. Hereafter the MO_2 continued to decrease steadily until the end of the trial at 20% oxygen saturation (Fig. 1B).

In ballan wrasse the MO_2 remained unaffected by the ambient oxygen levels experienced throughout the trials (Fig. 1C). The ballan wrasses were generally calm and inactive within the respirometer chamber, although increased ventilation rates were observed towards the final phase of the experimental trials.

3.2. Blood parameters

The Hct, Hb and MCHC of Atlantic salmon, lumpfish and ballan wrasse during control conditions, acute hypoxia exposure and following 1 h of recovery are summarized in Table 2. Distinct species specific differences were found where Atlantic salmon had higher haematocrit and haemoglobin concentration in control conditions compared to both species of cleaner fish. Furthermore, in Atlantic salmon acute hypoxia caused a significant increase in haematocrit and haemoglobin concentration that had returned to control levels after 1 h of recovery (df = 34, $P < .001$ and $P = .005$, respectively). However, in lumpfish these parameters were statistically unaffected by hypoxia and a subsequent recovery period (df = 44, $p = .343$ and $P = .149$, respectively). In ballan wrasse, both haematocrit and haemoglobin concentration increased significantly in acute hypoxia and was still elevated at a statistically similar level following 1 h of recovery (df = 35, $P < .001$ and $P = .016$, respectively). The MCHC was unaffected in Atlantic salmon and ballan wrasse, but a significant decrease following hypoxia exposure and brief recovery was found in lumpfish (df = 44, $P < 0001$).

Table 2
Haematology. Haematocrit (Hct), haemoglobin concentration (Hb) and mean corpuscular haemoglobin concentration (MCHC) of Atlantic salmon, lumpfish and ballan wrasse in control conditions, during acute hypoxia exposure and after 1 h of subsequent recovery in normoxia. $N = 12$ for Atlantic salmon and ballan wrasse, and $N = 15$ for lumpfish. Statistical differences between sampling time for each species are indicated with superscript letters. Data are mean ± s.e.m.

		Control	Hypoxia	Recovery
Atlantic salmon	Hct (%)	25.9 ± 0.8^a	30.7 ± 1.0^b	23.4 ± 0.7^a
	Hb (mM)	1.16 ± 0.04^a	1.30 ± 0.04^b	1.09 ± 0.03^a
	MCHC (mM)	4.48 ± 0.40	4.26 ± 0.57	4.65 ± 0.07
Lumpfish	Hct (%)	20.0 ± 0.6	21.6 ± 1.0	21.1 ± 0.8
	Hb (mM)	0.88 ± 0.04	0.77 ± 0.05	0.81 ± 0.04
	MCHC (mM)	4.44 ± 0.14^a	3.53 ± 0.13^b	3.83 ± 0.15^b
Ballan wrasse	Hct (%)	16.6 ± 0.6^a	24.3 ± 1.2^b	21.3 ± 1.3^b
	Hb (mM)	0.93 ± 0.05^a	1.23 ± 0.07^b	1.22 ± 0.10^b
	MCHC (mM)	5.56 ± 0.25	5.03 ± 0.16	5.68 ± 0.24

Baseline cortisol levels were notable lower in lumpfish (5.6 ± 1.3 ng ml^{-1}) compared to Atlantic salmon (37 ± 4 ng ml^{-1}) and ballan wrasse (44 ± 9 ng ml^{-1}). Plasma cortisol increased significantly in hypoxia in all species, and subsequent recovery caused a decrease to an intermediate level for Atlantic salmon and ballan wrasse while plasma cortisol remained at a statistically similar level in lumpfish (df = 35 for Atlantic salmon and ballan wrasse, df = 43 for lumpfish, $P < .001$ for all species) (Fig. 2A). Atlantic salmon demonstrated a substantially greater cortisol response between control conditions and hypoxia exposure compared to both cleaner fish species, where the change in cortisol was Δ343 ng ml^{-1} in Atlantic salmon, Δ154 ng ml^{-1} in lumpfish and Δ165 ng ml^{-1} in ballan wrasse. However, expressed as a factorial increase, the cortisol response was more dramatic in lumpfish where cortisol increased 28.5 fold in hypoxia relative to in control conditions, while the factorial cortisol increase was 10.3 in Atlantic salmon and 4.75 in ballan wrasse.

Plasma lactate was barely detectable in control conditions for both cleaner fish species, and for lumpfish the hypoxia trials had no statistical effect on plasma lactate (df = 44, $P = .256$), while a small significant increase was found in ballan wrasse during hypoxia and after 1 h recovery relative to controls (Δ0.46 mM) (df = 33, $P < .001$) (Fig. 2B). However, in Atlantic salmon plasma lactate was notably higher already in control conditions and increased substantially following hypoxia exposure (Δ3.35 mM) (df = 35, $P < .001$) (Fig. 2B). Similar patterns were found for plasma glucose, where Atlantic salmon showed elevated levels compared to both cleaner fish species (Fig. 2C).

Plasma osmolality, [Na^+] and [Cl^-] in control conditions, during acute hypoxia exposure and following 1 h of recovery in Atlantic salmon, lumpfish and ballan wrasse are shown in Fig. 2D, E and F, respectively. Compared to the other species, only minor effects on ion balance where observed in lumpfish, although [Cl^-] did increase significantly in hypoxia (df = 44, $P = .002$), while osmolality and [Na^+] increased significantly between hypoxia and recovery measurements (df = 44, $P = .017$ and $P = .031$, respectively). Furthermore, lumpfish generally showed an elevated osmolality and higher plasma [Na^+] and

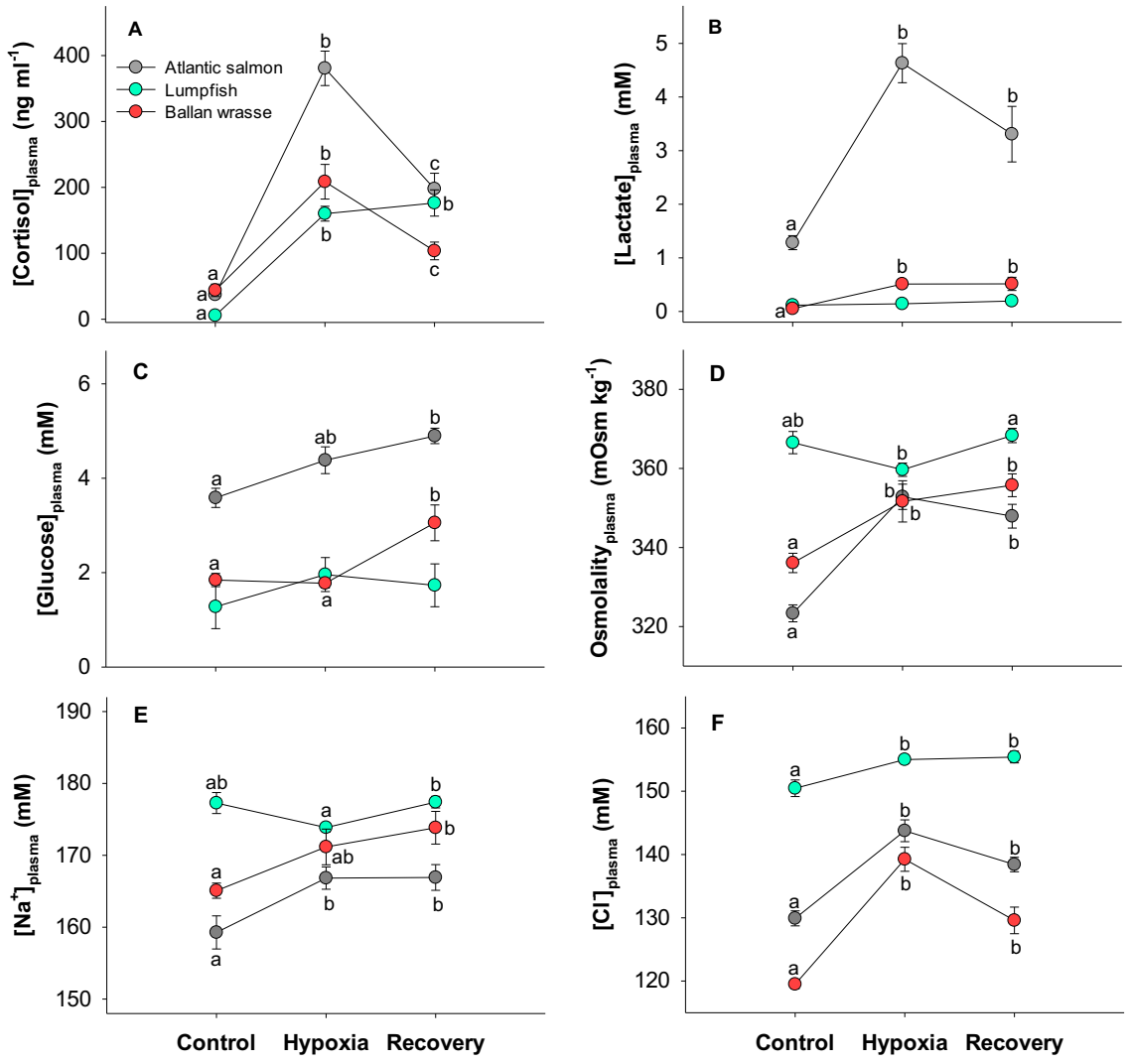

Fig. 2. Blood plasma parameters. Cortisol (A), lactate (B), glucose (C), Osmolality (D), Na^+ (E) and Cl^- (F) of Atlantic salmon (grey circles), lumpfish (cyan circles) and ballan wrasse (red circles) in control conditions, during acute hypoxia exposure and after 1 h of subsequent recovery in normoxia. N = 12 for Atlantic salmon and ballan wrasse, and N = 15 for lumpfish. Statistical differences between sampling time for each species are indicated with superscript letters. Data are mean ± s.e.m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$[Cl^-]$ compared to Atlantic salmon and ballan wrasse at all sampling times. In Atlantic salmon, acute hypoxia exposure and subsequent brief recovery caused significant increases in both plasma osmolality and plasma $[Na^+]$ and $[Cl^-]$ relative to control conditions (df = 35, $P < .001$, $P = .027$ and $P < .001$, respectively). Ballan wrasse responded similarly to Atlantic salmon with elevated plasma osmolality and plasma $[Na^+]$ and $[Cl^-]$ following hypoxia exposure (df = 35, $P = .002$, $P = .015$, and $P < .001$, respectively).

4. Discussion

4.1. Is hypoxia posing a risk to cleaner fish in salmon sea cages?

The purpose of this study was to compare the respiratory and haematological responses to progressive hypoxia in Atlantic salmon and two commonly used cleaner fish species, lumpfish and ballan wrasse. To provide a serious challenge, the fish were exposed acutely down to 20% oxygen saturation. This represents an extreme hypoxia event when considering typical oxygen fluctuations documented in salmon sea cages (Johansson et al., 2006; Oppedal et al., 2011a; Stien et al., 2012; Solstorm et al., 2018), although, oxygen saturations in the low twenties have been observed in extraordinary circumstances (Dempster et al., 2016).

Since both cleaner fish species survived the hypoxia trials in the present study, it can be concluded that they will not be at immediate risk from the less severe hypoxia levels typically encountered in salmon sea cages. Furthermore, the largest haematological disturbances were found in Atlantic salmon, where especially plasma cortisol, lactate and osmolality increased substantially more compared to both cleaner fish species (Fig. 2). This suggests that the similar hypoxia exposures were more stressful to Atlantic salmon.

This is further supported by the MO_2 patterns in response to progressive hypoxia. Atlantic salmon that generally remained calm throughout the trial showed a linear decrease in MO_2 from 27% oxygen saturation and onwards, which suggest that basal metabolic requirements no longer could be met aerobically at this point. Meanwhile MO_2 remained unaffected in ballan wrasse, corroborating earlier observations of high hypoxia tolerance in temperate wrasse species (Corkum and Gamperl, 2009). In lumpfish MO_2 started to decrease at earlier hypoxia levels than in Atlantic salmon. However, the initial reduction in MO_2 could be ascribed to behavioural changes in activity, and while MO_2 subsequently continued to decrease, plasma lactate remained unaffected in lumpfish. Hence, a defined threshold in oxygen saturations for the onset of anaerobic metabolism to maintain basal

homeostasis were not obtained in either cleaner fish species in these trials. Although, lumpfish have previously been reported to have critical oxygen tensions of ~34% saturation (54.4 mmHg) at 10 °C (Ern et al., 2016), suggesting that they are less hypoxia tolerant than Atlantic salmon. However, lactate was not measured in that study to assess whether lumpfish became anaerobically stressed already at 34% oxygen saturation.

Hypoxia effects and environmental thresholds are well-documented for farmed Atlantic salmon (Barnes et al., 2011; Remen et al., 2012, 2013, 2016; Hansen et al., 2015), and in aquaculture management strategies and technological developments this knowledge is actively applied to promote better farm environments and to avoid negative effects from hypoxia such as poor appetite and reduced growth (Stien et al., 2012; Solstorm et al., 2018). When considering cleaner fish welfare, it is therefore good news that neither lumpfish nor ballan wrasse demonstrated notably poorer hypoxia tolerance compared to Atlantic salmon. High unaccounted mortalities of cleaner fish in salmon sea cages are therefore unlikely to be driven by insufficient oxygen supply.

These trials were performed at 9 °C, a representative temperature for coastal Norwegian waters for large part of the year. However, the risks of hypoxia in sea cages become greater at higher temperatures owing to increased metabolic rates of the fish and lower oxygen solubility in the water (Johansson et al., 2006; Dempster et al., 2016). Furthermore, hypoxia tolerance of fish is lower at higher temperatures owing to accelerated metabolic rates (Fry and Hart, 1948; Claireaux et al., 2000; Remen et al., 2016). Fish species have different thermal niches and thermal tolerances, reflecting the conditions in their natural environments. As such, relative to the eurythermal Atlantic salmon, the lumpfish is a cold water species that struggle at temperatures above 15 °C (Hvas et al., 2017, 2018a). However, the ballan wrasse is a warm water species that thrives at 25 °C, which is a lethal temperature to Atlantic salmon (Hvas et al., 2017; Yuen et al., 2019).

In the sea cage all species must endure the same environmental conditions regardless of differences in thermal preference. Hence, while Atlantic salmon and lumpfish in the present study were tested in the midrange of their respective thermal niches, this corresponded to the lower end of the thermal niche in ballan wrasse. This explains an overall lower MO_2 in ballan wrasse compared to Atlantic salmon and lumpfish at 9 °C, and why 20% oxygen saturation was not enough to cause a significant reduction in MO_2 for this species (Fig. 1C.). Furthermore, these differences in thermal niches should also make ballan wrasse more hypoxia tolerant relative to Atlantic salmon and lumpfish at elevated temperatures.

This study investigated responses to severe hypoxia exposure on an acute time scale, and it therefore still remains to be seen how longer term moderate hypoxia affect cleaner fish species and their ability to eat salmon lice. The lumpfish showed reduced spontaneous activity at the early onset of progressive hypoxia, and hereafter remained attached to the bottom floor by using its ventral suction disc while displaying pronounced hyperventilation. Hence, lumpfish in particular may require close to normoxic conditions in the sea cage to stay active and function efficiently as cleaner fish.

4.2. Species specific hypoxia responses

Some notable differences in hypoxia responses were observed between the three species investigated in the present study that likely reflect different biological adaptations and lifestyles.

For instance, Atlantic salmon and other salmonids are active and athletic fish with impressive aerobic and anaerobic capacities (Black et al., 1962; Farrell, 2007; Hvas and Oppedal, 2017). Moreover, they elicit a strong adrenergic driven response to acute stressors such as chasing, crowding and hypoxia to fully mobilize the cardio-respiratory system (Barton and Iwama, 1991; Milligan, 1996; Reid et al., 1996, 1998). The dramatic increases in plasma cortisol and lactate following acute hypoxia were therefore expected in Atlantic salmon. The increase in both haematocrit and haemoglobin concentration were likely caused by additional recruitment of erythrocytes via splenic contraction to further boost the oxygen carrying capacity of the blood (Yamamoto, 1988; Kita and Itazawa, 1989; Hvas et al., 2018b). Elevated plasma osmolality, mainly from increased plasma concentration of Na^+ and Cl^-, are also typically observed in salmonids subjected to an acute stressor, which is largely caused by fluid shifts between body compartments, and may be further augmented in hyperosmotic sea water from increased Na^+ and Cl^- influxes as ventilation increases (Wood, 1991; Kieffer, 2000; Hvas et al., 2018b).

The lumpfish is a sluggish fish and contrasts Atlantic salmon in its response to exhaustive exercise stress, as it do not recruit additional erythrocytes, show minimal or no change in ion balance, plasma lactate and plasma glucose, while the cortisol response is slow and lower (Hvas et al., 2018a). In addition, contrary to salmonids, lumpfish show no excess post-exercise oxygen consumption following a critical swim speed test (Milligan, 1996; Lee et al., 2003; Hvas et al., 2018a). Similar contrasting physiological patterns were seen during acute hypoxia exposure in the present study, where no changes in haematocrit, haemoglobin concentration, plasma lactate and glucose were observed. However, a decrease in MCHC was observed, which suggest some swelling of erythrocytes during hypoxia exposure.

The increase in plasma cortisol was higher in hypoxia (Δ154 ng ml^{-1}, present study) compared to exhaustive exercise stress (Δ49 ng ml^{-1}, Hvas et al., 2018a), which indicate that acute severe hypoxia exposure was more stressful to the lumpfish. Moreover, expressed as a factorial increase form resting conditions, the cortisol response during progressive hypoxia was substantially greater in lumpfish (28.5) than in both Atlantic salmon (10.3) and ballan wrasse (4.75). Considering the large interspecific variation in cortisol between fish species, it can therefore be argued that the factorial increase is a better indicator of the stress levels experienced. However, increased stress levels in lumpfish were not reflected in the other haematological parameters measured. For instance, plasma lactate remained unaffected at barely detectable levels. This was surprising when considering the continuing decrease in MO_2 and the large factorial cortisol increase. However, it is possible that lactate was metabolised in the white muscles to be utilized for glycogen re-synthesis in situ, as has been shown in other sedentary species (Turner et al., 1983). Still, these results suggest that lumpfish have a poor capacity for anaerobic metabolism and overall are less flexible in responding to sudden challenges in their environment compared to Atlantic salmon.

Ballan wrasse responded similarly to Atlantic salmon in the hypoxia trials. However, the haematological changes were less pronounced, where especially the increase in plasma lactate was modest (Δ0.46 mM in ballan wrasse and Δ3.35 mM Atlantic salmon). Since MO_2 remained independent of ambient oxygen levels down to 20% saturation at 9 °C in ballan wrasse, substantial reliance on anaerobic metabolism to maintain basal homeostasis was not yet necessary, which explains lower plasma lactate concentrations. If the experimental trials had been continued to lower oxygen levels or were performed at a higher temperature, the magnitude of the physiological responses may have approached those seen in Atlantic salmon.

5. Conclusion

Hypoxia is a recurring environmental issue in salmon sea cages, and although lumpfish and ballan wrasse responded differently in some physiological parameters in the present study, Atlantic salmon appeared to be more sensitive when acutely exposed to low oxygen levels. While moderate hypoxia may reduce activity levels and consequently compromise parasite control efficiency, unexplained mortalities of cleaner fish in salmon cages likely originates from other factors such as diseases, injuries and suboptimal temperatures.

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Author contribution

This work was conceived and designed by both authors. M.H. performed experiments, analysed data and wrote the first draft, and F.O. provided valuable additions before approving the final version.

Declaration of Competing interest

We declare no competing interest.

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