Heart rate bio-loggers as welfare indicators in Atlantic salmon (*Salmo salar*) aquaculture

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A B S T R A C T

In this study, 12 farmed Atlantic salmon (~1200 g) were tagged with commercially available heart rate (HR) bio-loggers and maintained in a controlled fish tank laboratory environment at 9 °C on a 12 h day/night cycle for 13 weeks. Apart from one fish that had obtained severe wounds on the tail region in the beginning, the remaining fish survived the entire test period and displayed consistent and similar HR in response to the day/night cycles with peak HR midday during feeding. At the end of the experiment, untagged conspecifics had significantly higher weights, fork lengths and conditions factors, showing the bio-logger may have a long term negative impact on growth. However, tagged fish still gained weight during the trial. Resting HR, as measured at night and early morning, decreased significantly over the first 2–3 weeks, and remained stable at ~25 beats min^{-1} between week 3 and 10, highlighting that substantial time is required for complete recovery following implantation of the bio-logger. At the start of week 11, 12 and 13, crowding stress trials of 30 min were performed which elevated HR to 55.7 beats min^{-1}, whereafter it took 24 h to recover normal HR. Emerging bio-logger technologies can provide otherwise unobtainable information on the physiology and behaviour in free swimming individual fish over long periods and has great potential as welfare assessment tools in aquaculture. However, the impact of the tag must be considered with regards to the general representativeness of untagged counterparts when interpreting data.

1. Introduction

Fish welfare in Atlantic salmon (*Salmo salar*) aquaculture is gaining increased attention from both consumers and producers (Branson, 2008; Noble et al., 2018). Simply put, good welfare in aquaculture means that the fish remains healthy, show normal behaviours and have high growth rates (Huntingford and Kadri, 2014). This is obviously advantageous for the producer and also allows the consumer to enjoy a product made in an ethical and responsible way.

To understand whether the fish are thriving, measurements of key parameters in the farm environment such as temperature, dissolved oxygen and current speeds in conjunction with observations of fish behaviour and health status are possible (Johansson et al., 2007; Oppedal et al., 2011; Hvas et al., 2017a). Commonly used methods for this purpose in scientific studies, and which are emerging in commercial production, includes detailed monitoring of water parameters per depth layer, underwater cameras that provide information of momentary fish behaviour and register health status, lice infestation level and size in free swimming fish, and sonar technology to assess fish densities and positioning within the sea cage environment (Føre et al., 2018a). In

addition, for thorough individual assessments of farmed Atlantic salmon, the salmon welfare index model has been developed where a range of physical traits are scored on netted fish (Stien et al., 2013; Folkedal et al., 2016; Noble et al., 2018). However, these methods either assess welfare indirectly through environmental monitoring, at the group level in rather crude manners, or only provide a single time point measurements of random individuals.

Recent advancements and commercialization in bio-logging technologies opens up for new possibilities in how fish behaviour and welfare potentially can be monitored in aquaculture settings (e.g. Føre et al., 2018a). A bio-logger is either inserted into the abdominal cavity via a simple surgical procedure, or fixated externally to measure behavioral, environmental or physiological parameters in free swimming fish over long periods of time, for instance heart rate (HR), acceleration, orientation, temperature and depth (e.g. Johansson et al., 2009; Clark et al., 2010; Brijs et al., 2018; Wright et al., 2019).

Of the available types of measurements, HR is particularly interesting from a welfare perspective, as it reflects the level of activity and stress of the fish (Heath and Hughes, 1973; Laitinen and Valtonen, 1994; Lucas, 1994), which then could be directly related to the

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prevailing environmental conditions in the sea cage, disease outbreaks, or to major operational procedures such as crowding, transport and delousing. Hence, continuous measurements of HR in free swimming farmed Atlantic salmon could become a powerful welfare indicator as it allows us to obtain new fundamental knowledge of the physiology and behaviour in individual fish that was not possibly with previously established methods.

Recently, commercially available HR bio-loggers have successfully been used to study the behaviour and stress response of farmed rainbow trout and farmed Atlantic cod (Brijs et al., 2018, 2019; Bjarnason et al., 2019). In these studies it was confirmed that such bio-loggers can provide accurate HR measurements, and that stressful events were associated with notable increases in HR. moreover, rainbow trout, another salmonid species, displayed a strong diurnal pattern in HR, where disturbance to this pattern could serve as an indicator of poor welfare, while recovery of the diurnal rhythm ideally should be allowed before a subsequent stressor is experienced (Brijs et al., 2018).

A methodological concern that emerges with all bio-logging technologies is how much the logger itself impact the physiology and behaviour of the tagged fish, and whether the measurements obtained represents a normal situation and is representative for untagged fish generally (Cooke et al., 2011; Jepsen et al., 2015). For instance, it was recently found that bio-loggers affects the buoyancy of Atlantic salmon meaning that tagged fish were restricted to more shallow depths to maintain neutral buoyancy, and therefore showed different swimming behaviours, depth preferences and reduced coping ability in new surface restricting technology compared to untagged fish (Wright et al., 2019). Hence, some caution is warranted when interpreting data obtained from bio-loggers. To minimize potential biased measurements, the size of the bio-logger relative to the fish must be considered, and ample time allowed for recovery after the tagging procedure before data are considered representative. Moreover, if longer tagging periods are desired, potential effects on growth, appetite and survival should also be assessed.

The purpose of this study was to investigate the long-term feasibility of a commercially available HR bio-logger implanted in the abdominal cavity in farmed Atlantic salmon with regards to survival, growth and welfare. We held tagged fish in groups within a highly controlled tank laboratory environment for up to 13 weeks, to allow for a proper evaluation of the prospects of such HR bio-loggers for long-term studies in sea cage environments. In addition, measurements of routine and resting HR in stable conditions over many weeks should provide a valuable baseline reference when interpreting measurements obtained in unpredictable and fluctuating environments, as well as reveal the time required for Atlantic salmon to recover from the tagging procedure. Finally, in the beginning of each of the three last weeks of the experiment, we performed a 30 min crowding stress trial that simulated a major farming operation to assess the acute stress response, subsequent time to recover normal HR, and potential stress related mortality.

2. Materials and methods

2.1. Animal husbandry

Atlantic salmon post-smolts were kept in the indoor fish tank laboratories at Matre Research Station, Matredal, Institute of Marine Research, Norway. Two holding tanks of 2 m in diameter and a water volume of 2.4 m^3 were used, each containing ~25 fish which provided an adequate stocking density of approximately 12–15 kg m^{-3} over the course of the experiment. An open flow of filtered, UVC treated and aerated seawater (34‰) into the fish tanks was supplied from the local fjord at 90 m depth. The water flow was set to ensure high oxygen saturation levels at all times, prevent waste products from accumulating, and to keep the ambient temperature stable at 9 °C. The fish were fed excessively (Spring Supremeplus, Skretting, Norway) for 1 h each day between 13:30 and 14:30 via automated feeding devices, and

were kept on a 12 h light/dark cycle from 08:00 to 20:00.

This study was conducted between June and September 2019 in accordance with the Norwegian laws and regulations on animal experimentation in scientific research under permit identification number 19452.

2.2. Implantation of the bio-logger

Twelve fish (6 from each tank) were tagged with commercially available DST milli-HRT bio-loggers (Star-Oddi, Iceland). The size of the bio-logger was 3.95 cm in length and 1.3 cm in diameter, weighing 11.8 g in air, and is designed for fish of 800–1600 g.

Confirmation of accurate heart rate measurements, recommendations of procedures, as well as a thorough method description of the surgical implantation of the DST milli-HRT bio-logger was recently provided by Brijs et al. (2018). We largely used a similar tagging protocol in the present study. First, randomly chosen individual fish were gently netted form a holding tank and anaesthetized in a 100 l water filled tank containing 150 mg l^{-1} Finquel (Tricaine methanesulfonate). Time to enter surgical anesthesia was ~4 min, inferred from the cessation of active gill ventilation. The fish was then placed on a foam bed with an appropriately sized elongated cavity covered in a plastic sheet to minimize friction and drying out the skin of the fish. To maintain anesthesia, a milder dose of 75 mg l^{-1} Finquel in a 25 l water volume was re-circulated through both gills with a "Y" connection of plastic tubes connected to a water pump. Using a scalpel, a 3 cm long incision was made on the ventral midline starting approximately 4 cm behind the perceived position of the pericardium and cutting in the posterior direction of the fish. The bio-logger was then inserted into the abdominal cavity and pushed up anteriorly so that the flat end was positioned in close proximity to the pericardium, while the rounded end was positioned at the start of the incision. The side of the tag with two electrodes was ensured to be oriented in the ventral direction towards the abdominal muscles as recommended by Brijs et al. (2018). Using the channel on the rounded end, the bio-logger was sutured to the body wall so that it would remain in this position. The incision was subsequently closed with 4 interrupted stiches using a Resolon 3–0 sterile monofilament non-absorbable suture (RESORBA Medical GmbH, Germany), after which antibacterial surgical skin glue was applied on the wound (Histoacryl; B. Braun Surgical, Germany). Then fork length and weight were recorded and the fish were returned to the holding tanks where they quickly started to ventilate, and within minutes were swimming apparently normal again. The entire procedure took 12–15 min to complete per fish.

2.3. Measurements and protocols

Prior to the experiment, a pilot study with three tagged fish maintained in a separate tank for 1 week was made. Here the ECG signal was recorded along with a calculated heart rate in each measurement point which allowed us to confirm that heart rates were in fact being measured from the QRS waveform interval (e.g. Brijs et al., 2018). Heart rates were measured using a 7.5 s sampling duration at 80 Hz, which was the longest sampling configuration available for the DST milli-HRT bio-logger. Since we were working with larger sized fish (~1 kg) at a fairly low temperature (9 °C), resting heart rates will be low, and longer sampling periods therefore reduces the risk of poor quality measurements, as it should allow for two QRS cycles from which heart rate can be derived.

For the actual experiment, each bio-logger was programmed using the Mercury application software (Star-Oddi, Iceland) to measure and save heart rate at 10:00, 14:00, 18:00, 22:00, 02:00 and 06:00 every day. Hence, three measurements were made during the lit day and during the dark night in 4 h intervals, where the 14:00 measurement midday coincided with the feeding time of the fish. Tagged fish were maintained in their respective holding tanks with untagged conspecifics

for 10 weeks where they experienced the exact same conditions each day.

On the first day of week 11, a crowding stress test was performed from 09:00 to 09:30. This was done by opening the bottom outlet of each tank until the water level had been reduced to just a few cm, which exposed part of the fish to air. These low water levels were then carefully maintained for 30 min by manually opening and closing the bottom outlet. Meanwhile, flow of new water into the tanks remained the same during this procedure to avoid hypoxic conditions. The same crowding stress test was repeated on the first day of week 12 and week 13.

After week 13, all the fish were euthanized with an overdose of Finquel, whereafter weight and fork length were recorded for both tagged and untagged fish. In addition, prior to retrieval of the bio-logger, all the tagged fish were X-rayed using a HiRay PLUS X-ray machine (Eickemeyer, UK) to assess whether the bio-logger still was positioned adequately in the abdominal cavity 13 weeks post-surgery.

2.4. Data analyses

The bio-logger grades each HR measurement with a quality index (QI) between 0 and 3, where 0 is great, 1 is good, 2 is fair and 3 is poor. By comparing measurements from a non-invasive wireless system, it has previously been confirmed that QI 0 data points are highly accurate while the potential error margin when also including QI 1 data points rapidly declines to < 3 beats min^{-1} when several measurements are averaged (Brijs et al., 2019). In our study 54.3% of the data points were graded QI 0, while the sum of QI 0 and 1 data points covered 91.2% of the dataset (Table 1). To avoid working with fragmented data, we therefore decided to include both QI 0 and 1 measurements in our analyses, as it should still allow for sufficiently accurate results. The incorrectness of QI 2 and 3 measurements were most often quite obvious due to either unrealistically low or high measurements.

To assess changes in resting and routine heart rates over the course of the experiment, a repeated measure ANOVA followed by a Tukey's post hoc test was used on mean heart rates per week at each time of day measurement for the first 10 weeks, after normality and equal variance had been confirmed with the Shapiro-Wilk and Levene's mean test, respectively. The response and recovery trajectory following crowding stress was assessed by merging the 3 stress weeks together and plotting them against the preceding 3 weeks merged together. A *t*-test was then performed at each time point to identify differences in HR, after normality and equal variance had been confirmed. Similarly, a t-test was used to compare size parameters between tagged and untagged fish at the end of the experiment. A *P*-value below 0.05 was considered significant and all data are presented as mean ± s.e.m.

3. Results

At the end of the experiment, untagged fish had significantly higher body weight, fork length and condition factor than tagged fish, while all tagged fish still had increased in size since the start of the experiment (Table 2). One tagged fish died in the beginning of the second week and was left out of the analyses. It had obtained severe wounds on the tail region, and we suspect that physical handling of the fish during the surgical protocol unfortunately had damaged the delicate mucus layer on the skin and made it susceptible to infections. As such, we do not

Table 1
Quality index (QI) fractions for the entire dataset: The QI is automatically given to each data point by the bio-logger with 0 being best and 3 being worst. Combining QI 1 and 0 allows for the majority of the measurements to be used.

QI 3	QI 2	QI 1	QI 0	QI 1 + 0
5.4 ± 1.1%	3.4 ± 1.3%	36.9 ± 1.9%	54.3 ± 2.9%	91.2 ± 2.2%

Table 2
Size parameters: Weight, fork length, condition factor (K) and number of Atlantic salmon at the start of the experiment, 13 weeks later, and in untagged conspecifics. Data are mean ± s.e.m.

	Weight (g)	Fork length (cm)	K	n
Tagged, week 0	1138 ± 49	46.7 ± 0.6	1.11 ± 0.02	11
Tagged, week 13	1248 ± 65	48.8 ± 0.7	1.06 ± 0.02	11
Untagged, week 13	1509 ± 52	50.8 ± 0.5	1.13 ± 0.01	47

believe the tagging procedure in itself was the cause of death.

From X-ray photographs we were able confirm that the bio-loggers still were positioned adequately in the abdominal cavity in close proximity to the pericardium and that the side with two electrodes were oriented ventrally after 13 weeks (Fig. 1).

An overview of the HR measurements during the entire experimental period is shown in Fig. 2. Overall, HR remained stable and consistent during the first 10 weeks with distinct peaks at the start of week 11, 12 and 13 owing to the crowding stress trials.

The resting HR measured at night at 02:00 decreased significantly from 33.2 ± 1.5 beats min^{-1} in the first week to 27.8 ± 1.5 beats min^{-1} in the second week, and decreased slightly further in the third week where it remained stable at ~25 beats min^{-1} for the following 7 weeks (Fig. 3A). A similar pattern in resting HR was measured early in the morning (06,00) prior to the light was turned on, where a statistically identical plateau was reached by the third week (Fig. 3B). The HR measured at 14:00 which corresponded to feeding time midday remained statistically unaffected in the entire period at ~38 beats min^{-1} (Fig. 3D). At 10:00, 18:00 and 22:00 the HR was elevated in the first week, but had decreased to a stable plateau in the second week (Fig. 3C, E and F).

As water levels were lowered during the crowding stress trials, swimming activity increased and the fish eventually reached a state of hyperactivity. In the latter part of the trial, the fish became motionless and showed hyperventilation, suggesting physiological exhaustion. At 10:00, half an hour after experiencing prolonged crowding stress, HR was drastically elevated to 55.7 ± 1.1 beats min^{-1}, and then gradually recovered fully over the following 24 h to similar levels as in the preceding weeks (Fig. 4). Here, a strong diurnal cycle in HR can also be seen, with peak routine HR at 14:00 during feeding at midday. When a crowding stress trial had been performed in the morning, fish behaviour was observed 4 h later at 13:30 when feeding started. The fish responded strongly to obtain feed pellets and were eating vigorously despite of having experienced a severe stressor only a few hours earlier.

4. Discussion

4.1. Long term impact of the tagging procedure

We were able to successfully maintain farmed Atlantic salmon tagged with HR bio-loggers for up to 13 weeks in a controlled fish tank laboratory environment. Apart from one individual that died in the second week of the experiment owing to wounds and infections in the tail region, the remaining 11 tagged fish showed consistent and similar HR patterns in response to the day/night cycles, feeding time midday, and to the crowding stress trials in the final weeks of the study. In addition, X-ray photography at the end of the experimental period confirmed that the bio-logger in all fish remained adequately situated in the abdominal cavity close to the pericardium.

However, implantation of the bio-logger may have had some negative consequences for the fish with regards to growth since untagged counterparts were significantly larger at the end of the experiment. This is perhaps not surprising as the bio-logger is relatively large and situated in the anterior part of the fish where it could interfere with the ability for food intake by pressing on the digestive tract and stomach
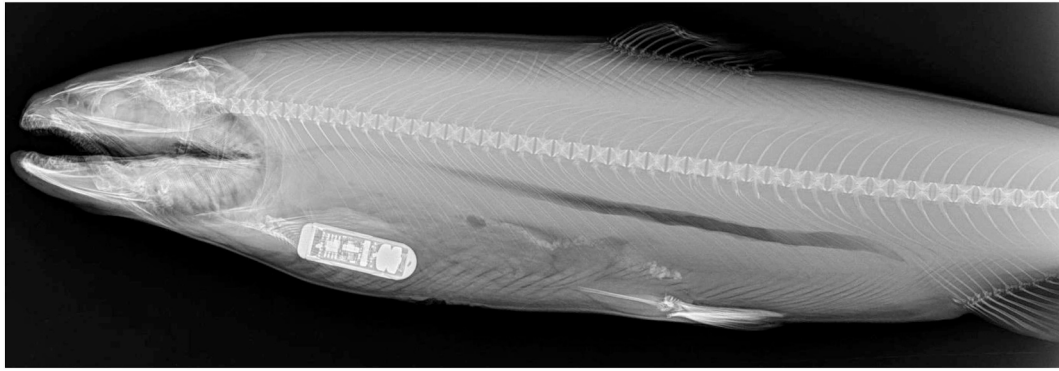
Fig. 1. X-ray photograph of a tagged fish: The placement of the bio-logger in close proximity of the pericardium can be seen. The X-ray was taken at the end of the experiment to confirm that the bio-logger still was positioned adequately inside the fish. Moreover, it can be seen that the side with two electrodes is oriented ventrally and the side with one electrode is oriented dorsally.
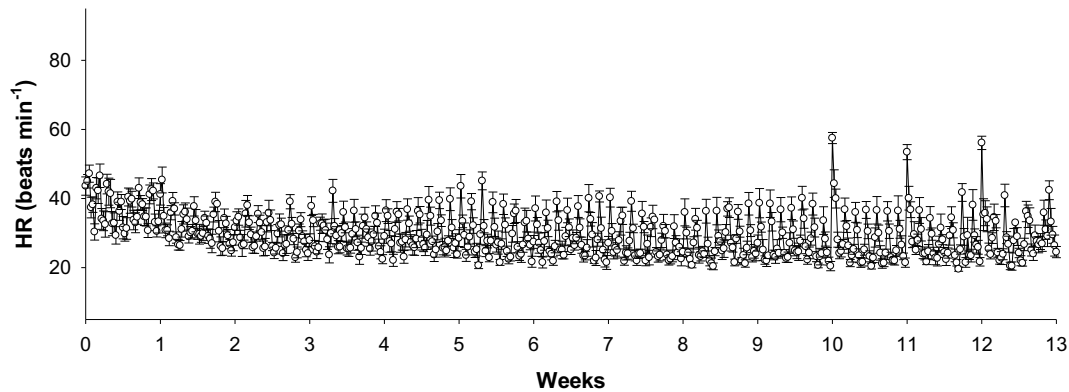

Fig. 2. Overview of the entire experimental period: The routine heart rate of Atlantic salmon in holding tanks over a 10 week undisturbed period and followed by crowding stress tests at the start of week 11, 12 and 13. Data are mean ± s.e.m. of 11 individuals.
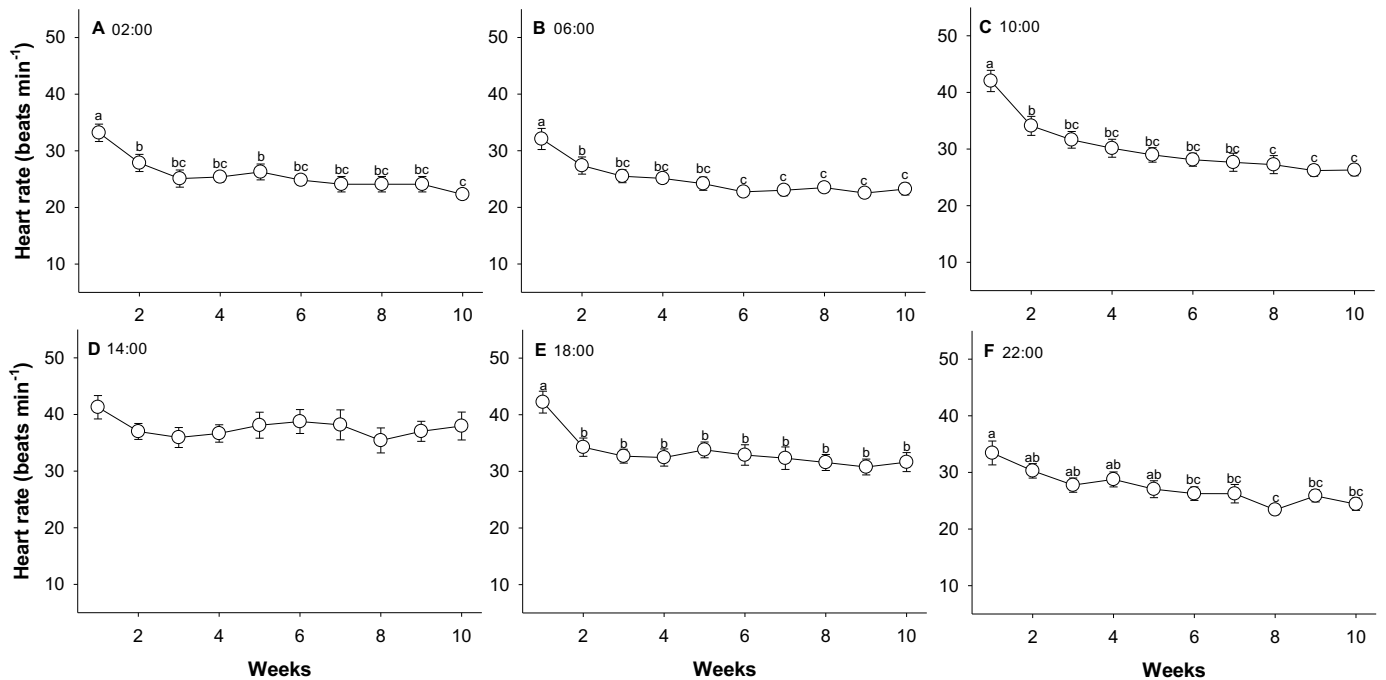

Fig. 3. Heart rates at different times of day: The heart rates at night at 02:00 (A), early morning before the light came on at 06:00 (B), at 10:00 in the morning (C), during feeding midday at 14:00 (C), at 18:00 in the afternoon (E), and 22:00 in the evening (F) over 10 weeks of otherwise undisturbed conditions following the tagging procedure. Statistical differences between weeks are indicated with different letters. Data are mean ± s.e.m.
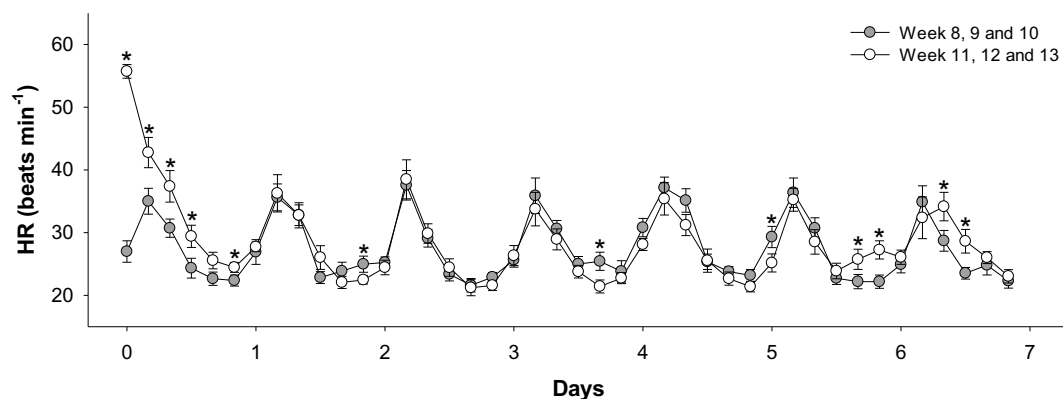
Fig. 4. Stress response and diurnal cycle: Heart rate measurements combined from week 8, 9 and 10 are used as a baseline for normal diurnal rhythm (grey circles), from which the combined measurements from week 11, 12 and 13 (white circles) are compared. These latter weeks all started with a 30 min crowding stress test prior to the first measurement on Monday morning at 10.00. Statistical differences between normal and stress weeks at specific time points are indicated with asterisks. Data are mean ± s.e.m.

region. The tagged fish must still have been eating some, as they did increase in weight during the experiment. Whether feed intake was consistently reduced throughout the experimental period or gradually recovered towards the latter part was not directly assessed here, but we optimistically expect that appetite would improve over time following the tagging procedure. Hopefully this will be confirmed in future field studies where even longer tagging periods ideally should be used. Moreover, it is well documented in farmed Atlantic salmon that they possess great capabilities for compensatory growth following long periods of restricted feeding or feed withdrawal (Reimers et al., 1993; Johansen et al., 2002). Hence, if appetite gradually will recover following implantation of a bio-logger, tagged fish may catch up in size with untagged individuals, provided the study period is sufficiently long. However, in studies of short to medium duration, one must consider that measurements likely are being made on fish with impaired growth performance derived from reduced appetite. Whether this is a concern when interpreting data will depend on the specific purpose of a particular study.

4.2. Resting heart rates

Although the tagging procedure is brief and fairly simple, it is associated with several stressors such as netting, air exposure, anesthesia, and the subsequent surgical implantation where a deep incision on the ventral midline is necessary. Knowledge of the time required to recover adequately, including sufficient wound healing, is therefore crucial when establishing a new tagging method. Generally, salmonids are highly sensitive to acute stressors and it typically takes many hours to recover from the associated acid-base, osmotic and endocrine disturbances (Pagnotta et al., 1994; Wendelaar Bonga, 1997; Hvas et al., 2018). Similarly, when estimating resting metabolic rates of Atlantic salmon in respirometers, a minimum measurement period of 20 h is needed to ensure that fish have ample time to calm down completely (Hvas and Oppedal, 2019). Since HR is directly related to metabolic rate and stress levels, at best, a similar prolonged period is required to reestablish normal HR following a stressful event (Anderson et al., 1998; Donaldson et al., 2010). Owing to the multiple stressors associated with the implantation of the bio-logger, it was previously concluded that rainbow trout needed 3–4 days to recover normal HR, inferred from the reestablishment of a strong diurnal HR pattern (Brijs et al., 2018, 2019). These authors therefore argued that one must allow tagged fish at least 3–4 days of recovery from the surgical procedure in studies where true resting HR is needed. Interestingly, in the present study, we found that resting HR of Atlantic salmon, as measured at night and early morning, continued to decrease until the third week of the experiment whereafter it remained stable for the remaining weeks.

This suggests that the impact of the tagging procedure perhaps takes substantially longer to completely recover from than thought previously, or that Atlantic salmon need more time to recover, possibly due to its shorter time of domestication to aquaculture conditions compared to rainbow trout. Nevertheless, this observation was likely not made earlier since previous HR bio-logger studies did not maintain fish under stable conditions for sufficiently long periods as in the present study.

When comparing measurements of resting HR between studies, apart from species differences, one must consider that both water temperature and fish size will affect the results, where smaller fish at higher temperatures can be expected to have a higher resting HR owing to metabolic scaling and metabolic acceleration, respectively (Labat, 1966; Hvas et al., 2017b; Oldham et al., 2019). In the present study, the resting HR of Atlantic salmon measured at 02:00 and 06:00 between the third and tenth week was ~25 beats min^{-1}, and was obtained at a constant temperature of 9 °C on fish weighing ~1.2 kg. In confirmation with this, resting HR of Atlantic salmon weighing 1.5–3 kg at 10 °C was also reported to be 25 beats min^{-1} (Lucas, 1994). In rainbow trout of 900–1500 g at 9–10.5 °C resting HR was reported to be 32 beats min^{-1} (Kiceniuk and Jones, 1977), while sockeye salmon of 2.5–4.2 kg at 12 °C had a resting HR of 37 beats min^{-1} (Clark et al., 2010). In rainbow trout at 10–11 °C of 600 g tagged with the same HR bio-logger as the present study, resting HR was reported to be 42 beats min^{-1} (Brijs et al., 2019). Hence, the resting HR of Atlantic salmon reported here is generally similar to previous findings in salmonids, and in those cases where it was higher, this may be explained either by having used smaller fish, higher test temperatures, or using a briefer recovery period which may overestimate the true resting HR.

4.3. Crowding stress and subsequent recovery

In Atlantic salmon aquaculture, major farming operations such as thermal and mechanical delousing, other health treatments, and transportations necessitate crowding of the fish where they are confined at high densities. This unfamiliar situation imposes substantial acute stress by causing a state of panic and hyperactivity that may result in physiological exhaustion as well as substantial collision damage. Increased activity has previously been documented via implanted acoustical tags (Føre et al., 2018b). Furthermore, anecdotes from Atlantic salmon farmers suggest that some mortality is the norm during any crowding operation where the fish otherwise appeared healthy beforehand, while certain delousing methods specifically are associated with increased mortality risks (Overton et al., 2018). Salmonids have a powerful acute stress response, and it has been known for a while that hyperactivity and exhaustion alone may kill the fish during subsequent recovery, presumably because they are unable to correct for the massive

acid-base and osmotic disturbances experienced in a timely manner (Black, 1958; Wood et al., 1983). Hence, this may explain mortalities related to crowding operations in Atlantic salmon aquaculture.

In the present study, we therefore anticipated that some fish would not survive the crowding stress trials and that full recovery could take several days. To our surprise, no fish died following the stress trials, and furthermore, the fish were observed eating already during feeding 4 h after having experienced prolonged and intense crowding stress.

While HR was significantly elevated compared to normal, recovery was rapid and complete after 24 h. The peak HR measured at 55.7 ± 1.1 beats min^{-1} half an hour after the crowding stress trial finished, is within the range of previous reports, for instance rainbow trout swimming close to its critical limit had a HR of 52 beats min^{-1} (Kiceniuk and Jones, 1977), while the maximum HR observed in Atlantic salmon was 65 beats min^{-1} (Lucas, 1994). Both these studies were made at a similar water temperature as in the present study on fish of comparable sizes.

A likely explanation to why the fish in the present study demonstrated impressive recovery capabilities following a severe stressor is that they were in excellent health owing to having been maintained indoor, with filtered and UV treated water supply minimizing parasite, bacteria and virus infections, in stable and calm conditions for several months. This contrasts the situation of Atlantic salmon exposed to an ambient sea cage environment where they encounter fluctuating environmental conditions and multiple pathogens, where the cumulative effect of these could decrease the available scope in HR, as seen for rainbow trout subjected to a series of stressful events (Brijs et al., 2018). During crowding stress, this may force the fish to become anaerobic earlier, exemplified by high amoebic gill disease loads (Hvas et al., 2017c), and thereby increase mortality risks, while the fish in the present study still had a complete HR scope available to cope adequately with the crowding stress trial. Hence, observations made in environmentally controlled indoor fish tank facilities may not necessarily be a realistic reflection of the life of a farmed Atlantic salmon in a commercial sea cage environment.

5. Conclusion

The purpose of this study was to obtain basic data sets from HR bio-loggers to evaluate them as a suitable method to document fish welfare in Atlantic salmon aquaculture in ways that otherwise was not possible with other available tools. While it is a concern that implantation of the bio-logger impair growth for quite some time, the benefits of obtaining high quality physiological measurements of free swimming individuals over long periods of time in their ambient environment makes up for this concern, in our opinion. However, a thorough evaluation of the representativeness of untagged conspecifics in terms of behaviour, growth and survival will be even more important in future field studies where fish will be subject to multiple stressors in a much less controlled environment (e.g. Wright et al., 2019).

Several questions remains unanswered with regards to experienced stress levels and welfare status of growing Atlantic salmon in sea cages when subjected to the interactive effects of major farming operations, various pathogens and specific environmental conditions (e.g. water temperature, dissolved oxygen, current velocity, waves, algae blooms, jellyfish), as well as coping capabilities in new farm concepts (Bui et al., 2019). Of particular importance for management purposes is the time required for physiological recovery in fish subjected to certain challenges, especially when considering the added burden of prevailing stressors. This will allow to better identify mortality risks and to make nuanced guidelines for how often it is feasibly to subject fish to major farming operations. For instance, at elevated temperatures and moderate hypoxia it may be particularly risky to perform a delousing operation owing to a reduced scope for activity (Hvas et al., 2017b; Oldham et al., 2019). Emerging HR-bio-loggers such as those employed in the present studies could help us answer these questions so that more

robust recommendations for fish management can be developed.

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