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Aquaculture

Heart rate and swimming activity as stress indicators for Atlantic salmon (*Salmo salar*)

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ABSTRACT

We investigated the relationship between telemetry measurements of heart rate and swimming activity and the physiological status in farmed Atlantic salmon (*Salmo salar*) to assess the potential to use telemetry measurements as proxies for stress. Sensor tags measuring heart rate and swimming activity were surgically implanted into the peritoneal cavity of Atlantic salmon individuals kept in tanks. Four tanks were stocked with three tagged fish and four untagged cohabitants, while two additional tanks containing 16 untagged fish were used as reference groups. Following surgery, tagged fish were kept undisturbed for 14 days as acclimation period. All fish were then subjected to physical stress by reducing the tank water level in 4 consecutive rounds, after which they were left undisturbed for another ten days before the experiment ended. Plasma cortisol, glucose, lactate and osmolality were measured to assess stress levels from fish in the reference groups before and after being subjected to stressing and from all fish at the end of the experiment. Both heart rate and swimming activity rose after the stress treatment, remaining elevated for 24.5 and 16.2 Hrs respectively. Glucose, plasma cortisol, lactate and osmolality levels were significantly greater when measured immediately after stress. Results from the experiment indicate that heart rate and swimming activity can be used as proxies for fish stress, thus opening the possibility for on-line stress monitoring in full scale production.

1. Introduction

Atlantic salmon (*Salmo salar*) farming is a growing global industry (Directorate of Fisheries, 2019) responding to increased global need for ocean-based protein production (Olafsen et al., 2012). The industry faces challenges regarding fish welfare and is under pressure to improve production methods and farm operations. Operations involving fish handling (e.g. crowding using sweep-nets followed by pumping and treatment against sea lice (*Lepeophtheirus salmonis*) or disease) are common in Norwegian aquaculture and detrimental to fish welfare (Hjeltnes et al., 2018). To increase production volumes efforts are made to develop larger, higher capacity production systems at more exposed locations (Bjelland et al., 2015) which will entail that such operations must handle fish in larger groups and under more challenging conditions. Handling related welfare hazards are believed to be major contributors to the annual mortality in salmon production (Bleie and

Skrudland, 2014). With a mortality of 14.7% for the sea stage in 2018 (Norwegian Food Safety Authority, 2018), this highlights the need for better control of how operations are conducted and their impact on fish welfare.

Monitoring fish welfare during aquaculture operations is a challenge and extensive resources and research efforts have been allocated over the last decades to developing new methods to monitor and reduce negative welfare effects of common aquaculture practices on fish. Existing tools to evaluate fish welfare are typically referred to as Operational Welfare Indicators (OWIs) derived from an extensive literature review (Noble et al., 2018). Most state-of-the-art methods fish farmers employ to evaluate fish welfare and quantify OWIs in an industrial production setting are based on visual observations from e.g. sampling of fish or various camera feeds. However, the outcomes of these may rely on the personal experience of the observer and can thus be influenced by subjective observation bias. In addition, with each sea

cage being up to 50 m deep and containing up to 200,000 fish, only a fraction of the individuals can be observed from the surface at any given time making the total population difficult to monitor through surface observations. Underwater cameras are therefore often used to supplement such methods as they enable data collection from a larger part of the cage volume, essentially making more of the total population observable. However, placing cameras within sea-cages may conflict with cage operations and be of limited use because low visibility and high biomass densities (e.g. during crowding) may prevent a sufficiently wide field of view to extract useful information (Shieh and Petrell, 1998). Other instruments such as active hydroacoustic instruments (e.g. sonars and echo sounders) may provide additional insight into the distribution and behaviour of the fish but are of limited use when biomass density exceeds a certain threshold as signal saturation makes fish density estimates uncertain. Because of these technological limitations, visual inspection and subjective evaluation of relevant welfare indicators remain the most common fish welfare monitoring methods (Føre et al., 2018a).

The observability of the fish in aquaculture can be improved by equipping individual fish with electronic devices ("tags") containing sensors that can measure various parameters in or near the fish (Thorstad et al., 2013). Using tags will result in individual data histories for the tagged fish, which is complementary information to that obtained through e.g. cameras, echo sounders and direct observations. Acoustic telemetry is a type of biotelemetry where tags contain a transmitter and data are transferred wirelessly to the user in real time using acoustic signals. This method has recently been shown to be viable for individual based data collection in commercial salmon cages (e.g. Føre et al., 2017; Stehfest et al., 2017). Data Storage Tags (DSTs) represent a different branch in biosensing where the tags store data internally and thus must be retrieved for the user to access the data. This method has been employed to log e.g. swimming depth and temperature for individual salmon in aquaculture production cages (Johansson et al., 2009). Since DSTs are not limited by acoustic bandwidth, this method may result in higher data density than the acoustic counterpart but faces challenges related to tag retrieval in commercial size fish populations in addition to data being available only for retrospective analysis.

Recent studies have used acoustic telemetry to study the swimming activity of salmon during crowding and delousing (Føre et al., 2018) and DSTs to study the effects of crowding and transport on heart rate in rainbow trout (*Oncorhynchus mykiss*) (Brijs et al., 2018). These studies imply that swimming activity and heart rate may be useful indicators of welfare during such operations and demonstrate that these parameters can be measured in industrially relevant situations. If the characteristics in these parameters during welfare critical operations in fish farms could be detected and linked to stress, they can potentially serve as OWI's.

Stress as defined within the context of biology is the non-specific bodily response to demands for change (Selye, 1973). The first step in identifying the link between stress and measurable physiological parameters is to conduct laboratory studies through which it is possible to collect detailed data under controlled conditions. In this study we investigate the links between variables that can be measured using existing sensor tags (heart rate, swimming activity) and the stress level of the fish quantified by blood analysis. This included a laboratory study where off-the-shelf sensors measuring heart rate and swimming activity were implanted into fish which were then subject to stress. Blood was sampled and analysed at different times during the experiment to determine the actual stress level of the fish and to evaluate if the data from the implants provided valid proxies for stress based on the hypothesis that heart rate and swimming activity are affected by and linked to stress.

2. Materials and methods

2.1. Ethical statement

All fish handling and surgery was done in compliance with the Norwegian Animal Welfare Act (2009). The experiment was approved by the local responsible laboratory animal science specialist under the surveillance and approval of the Norwegian Animal Research Authority (NARA) (ID 18/18431). The fish were allowed to habituate to the experimental tanks for a month before surgery. During this period, inspection and feeding operations were limited to once per day to minimize the potential stress induced upon the fish. Several experimental refinement strategies were applied in the trials, including fish sampling using a knotless dip net, immediately transferring sampled fish to an anaesthetic bath to minimize stress, and continuously irrigating the gills with aerated water containing maintenance anaesthetic and covering the heads of all fish with a moist cloth during surgery. Mortality after surgery was 0%.

2.2. Experimental animals, housing and husbandry

The experiment was conducted between January 1st and March 3rd, 2019, at the Norwegian Institute for Nature Research's (NINA) Aquatic Research Station, Ims (Sandnes municipality, Norway). Sixty Atlantic salmon (55.5 ± 5.7 cm fork length) of the Aqua Gen genetic strain were randomly selected from a holding tank and distributed between the six experimental tanks (hereafter denoted Tank 1 - Tank 6). The tanks were square with rounded corners, had a volume of approximately 5.6 m^3, and were equipped with adjustable (flow and direction) seawater inlets from a shared manifold, overflow outlets, sensors for temperature and dissolved O_2 (DO) and oxygenation equipment to ensure a stable and controlled environment. Water temperatures and DO levels registered during the experimental period were 4.2 ± 0.2 °C and 97.7 ± 0.2% respectively. Husbandry was carried out by on-site personnel trained in handling experimental fish and entailed daily feeding and general supervision.

2.3. Study design and timeline

The experiment animals were distributed such that Tanks 1–4 each contained three tagged fish and four untagged fish. Tanks 5 and 6 each contained a reference group of 16 untagged fish. All tagged fish were equipped with DSTs (Centi HRT, Centi HRT ACT and Milli HRT, Star Oddi LTD, Gardabaer, Iceland) all of which registered heart rate, and some also registered tri-axial acceleration at 1 Hz (Centi HRT ACT). These are the same tags employed by Brijs et al. (2018), ensuring comparability with that study. The Centi HRT and Centi HRT ACT tags logged data with a sampling interval of 2 min for 25 days. Due to a lower internal storage capacity, the Milli HRT tags were set with a longer sampling interval of 10 min, which was reduced to 2 min between March 3rd 08:00 and March 6th 08:00 to achieve higher data resolution during and after stressing. Two fish in both Tanks 1 and 2 were also equipped with acoustic telemetry tags of the type A-MP 9 (Thelma Biotel AS, Trondheim, Norway) containing a three-axis accelerometer with a range of 0–2.1 m/s^2 sampling at 5 Hz. The tags were set to sample accelerations in consecutive 30 s windows from which the average Euclidean norm was calculated as in Føre et al. (2018), but with a lower maximum value for swimming activity (2.1 m/s^2) to obtain higher output resolution. The resulting swimming activity indicator was transmitted every 40s, providing a higher data density for this parameter than in Føre et al. (2018). Two acoustic receivers (Thelma Biotel TBR700 and TBR700-RT) were installed in each of the tanks containing acoustic telemetry tags (Tanks 1 and 2) for redundant data capture and storage. The distribution of fish and tags between tanks is illustrated in Fig. 1.

Tags were surgically implanted into the peritoneal cavity while the
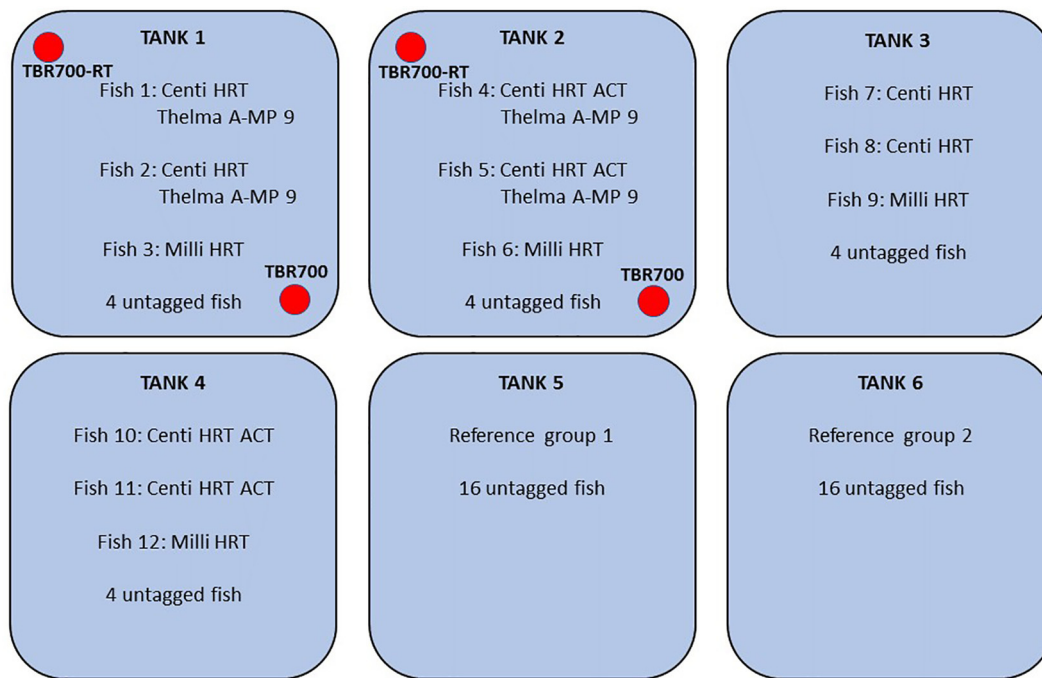
Fig. 1. Study design: Fish and tag distribution between experiment tanks. The red circles for tanks 1 and 2 denote the location of the hydroacoustic receivers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fish were anaesthetised using Benzoak Vet (70 mg/L knock-out solution and 35 mg/L maintenance dosage during surgery). The DSTs were implanted using a procedure similar to that of Brijs et al. (2018), where the tag was inserted through an incision in the abdomen. An extra step was taken in this study to prevent tag movement relative to the heart by anchoring the heart rate tags to the peritoneal wall using an anterior suture close to the peritoneal septum. For fish equipped with two tags, both were inserted through the same incision. After tag implantation, the incision was closed with 3–4 interrupted sutures. The tagged fish was then transferred to a recovery tank with circulating sea water where it was kept until it had regained full consciousness, upon which it was transferred back to the tank it had been collected from. These procedures and their potential impact on the fish are described and discussed in greater detail in Føre et al. (2019).

Surgery was conducted on February 18th and was followed by a fourteen-day recovery period until March 3rd, when the fish were subjected to stress by repeated draining of the water levels in all tanks simultaneously. Following this, the fish recovered for ten days until March 14th, when the experiment was concluded by euthanising all fish. Tag data were collected throughout the entire experimental period and blood samples were taken after surgery (February 18th), before and after stressing (March 4th), and prior to euthanasia (March 14th). In the periods between the main experimental events the fish were kept undisturbed in their tanks except for daily hand feeding and visual welfare evaluations. After conclusion of the study, data from the DSTs was downloaded using the proprietary base stations and software (Mercury v5.20) from Star Oddi, while data from the Thelma tags were downloaded from the TBR700-units using a native Bluetooth interface and software (ComPort v3.0.0).

2.4. Experimental procedures

Stressing of the fish was done by reducing tank water levels until the dorsal region of the fish was exposed to air (Fig. 2) in four iterations with different durations. For all iterations, lowering the water level took approximately 1 min. For the first iteration, the water level was restored immediately. For the second iteration the water level was kept low for 1 min. For the third and fourth iterations, the water level was kept low for 5 min. During the last iteration, the stressing also included chasing the fish around the tank with a dip net until fish exhaustion. For all iterations, re-filling the tanks took approximately 10 min. Each iteration was started on the hour, so the time between iteration was 49 min, 47 min, 42 min and 35 min respectively. The stress procedure was conducted simultaneously for all tanks so that all fish were subject to the same treatment. Four people participated when stressing the fish to ensure similar operations on all tanks.

Blood was sampled from fish in the reference groups and involved opening the tank lid, netting a random fish from the tank as quickly as possible using a knotless dip net, and placing it in an anaesthetic knock-out solution (Aqui-S, > 80 mg isoeugenol/L water). Blood was sampled from the caudal artery using heparinized syringes immediately after loss of consciousness. The fish was thereafter euthanized by a powerful blow to the head. Blood samples were centrifuged, and plasma was collected for analysis.

2.5. Data processing

2.5.1. Heart rate

Heart rates were derived from on-board, proprietary signal processing methods and stored in the DSTs. Outliers due to measurement errors were removed using the Median Absolute Deviation (MAD) approach (Huber, 1981; Leys et al., 2013). The MAD decision criterion was set to 3 (very conservative according to Miller (1991)) to exclude heart rate values outside published heart rate ranges ($15 < HR < 80$) for Atlantic salmon (Lucas, 1994) and comparable species (Brijs et al., 2019). Heart rate periodicity was evaluated by calculating the real, one-sided Fast Fourier Transform of the average of all heart rate time series collected in the 7 days before stressing.

2.5.2. Swimming activity

When using accelerometers to measure fish swimming activity, the gravity vector will introduce a slow varying gravity component convolved with both the low frequency fish attitude component and the high frequency motion component induced by fish motion. The impact

Fig. 2. Photograph of low-water stress imposed on the fish. Water level was lowered until the dorsal fins remained in air for up to 5 min (photo: Bengt Finstad).
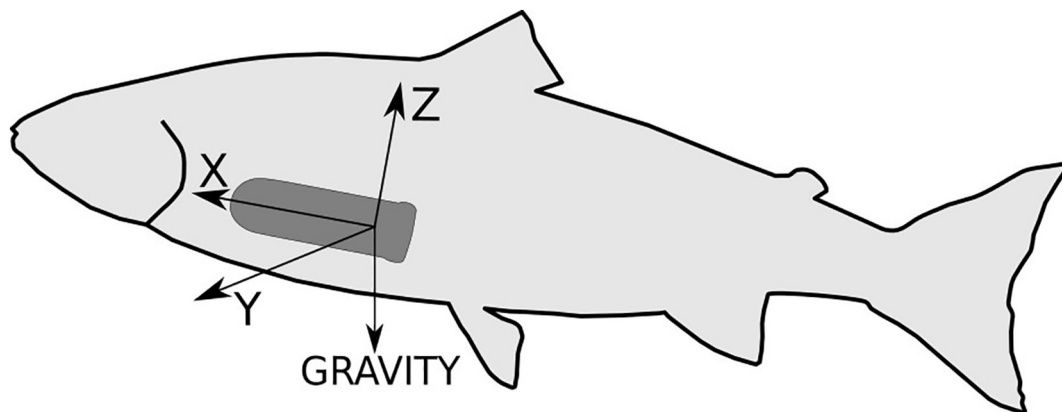

Fig. 3. DST and fish orientation relative to gravity vector. The gravity vector always points towards the centre of the earth. Hence the contribution from the gravity vector will depend on the orientation of the tag and the fish. The X, Y and Z axes denote the alignment of the accelerometer's sensing axes.

of this effect on the three sensing axes depends on the relative orientation between the tag and the fish's orientation relative to the earth's gravity vector (Fig. 3). In the Thelma Biotel tags, this effect is countermanded by low pass filtering the raw data and subtracting the resulting low frequency (LF) component from the raw signal, resulting in a high frequency (HF) component representing the swimming activity of the fish. A swimming activity proxy is achieved by calculating the magnitude (Zwillinger, 2007) of the HF signal and applying a moving average with a 30 s time window. To ensure that swimming activity values measured using the Centi HRT ACT could be compared to data collected by Thelma tags, a similar signal processing approach was applied to the raw acceleration data measured by the Centi HRT ACT tags. Low pass filtering was achieved using a Butterworth low pass filter with a cutoff frequency of 0.2 Hz. The resulting LF component was subtracted from the raw values for each axis, and the magnitude of the HF signal (swimming activity) was calculated as given in Eq. (1).

$$activity = \sqrt{|X|^2 + |Y|^2 + |Z|^2} \qquad (1)$$

The result was then smoothed by calculating the moving average over a 30 s time window. Although this results in swimming activity proxies with the same unit (m/s^2), measured accelerations differed between the Thelma and the Star-Oddi tags due to differences in accelerometer properties and intraperitoneal placement. To reduce the impact of these effects, swimming activity data were normalized to a mean of zero and a standard deviation of one (the effect of this procedure is demonstrated for Fish 2 in tank 2 during the first six days of the experiment in Fig. 4.

2.5.3. Blood sample analysis

Sampled blood was centrifuged at 3250 *g* for 5 min and plasma was removed and stored in cryo tubes at −36 °C until analyses were performed. A Freestyle Freedom Lite™ (Abbott Diabetes Care Inc.) handheld blood glucose measuring device for people with diabetes which registers concentrations from 1,1–27,8 mmol/L (mM) and uses a dosage of 0.3 μL per reading was used. A study by Wells and Pankhurst (1999), confirms that the use of such a device in animal glucose evaluation is adequate. A Lactate Scout + ™ (EKF Diagnostics for life) handheld blood lactate measuring device was used which registers concentrations of 0.5–25,0 mmol/L (mM) and uses a dosage of 0.2 μL per reading. A study by Wells and Pankhurst (1999) as well as EKF Diagnostics for life
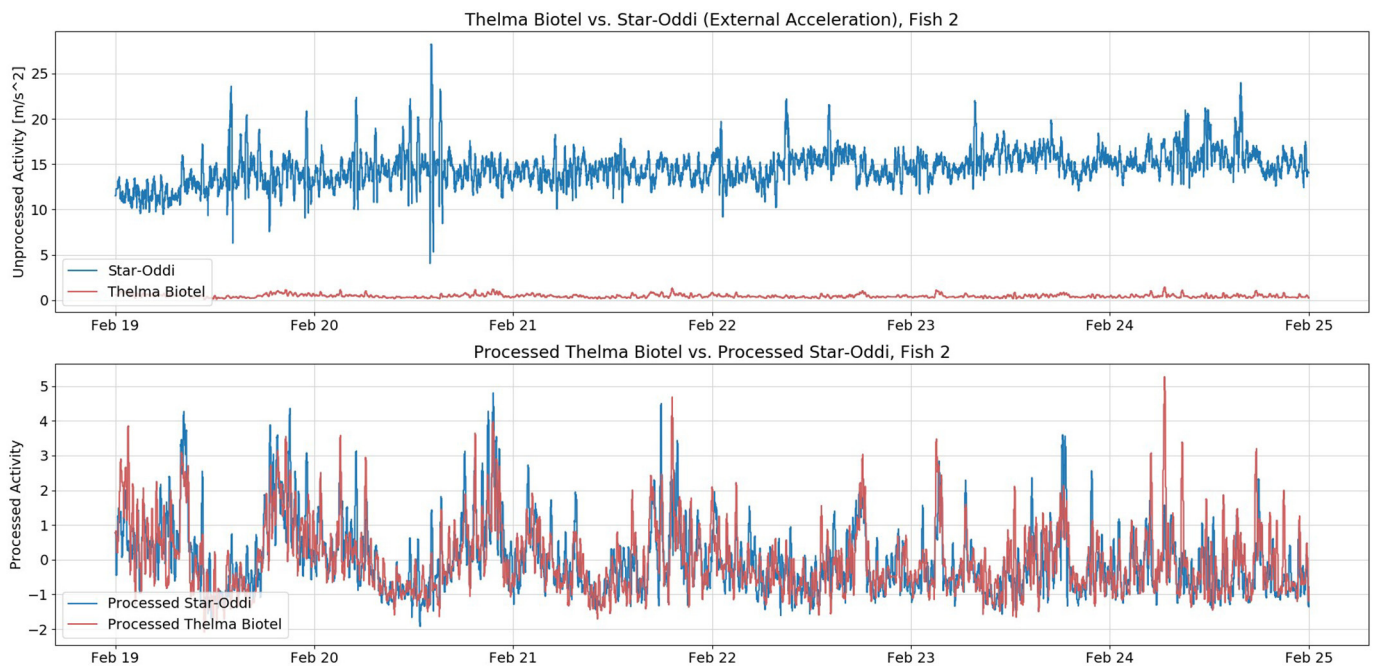
Fig. 4. Before (top) and after (bottom) Star-Oddi data processing.

confirm that the use of such a device in animal lactate evaluation is adequate. A radioimmunoassay technique was used to measure plasma cortisol concentrations as described by Iversen et al. (1998). Plasma osmolality was analysed using a Wescor 5500 osmometer (Wescor™, Elitech Goup, Salon-de-Provence, France).

2.6. Statistical methods

A binary recursive partitioning approach (Vignon, 2015) was employed to identify systemic changes in heart rate and swimming activity that could result from stressing. This approach recursively splits the explanatory variable (time in this case) into distinct segments such that the response variable values (heart rate or swimming activity) in each segment are maximally different from one another. The recursive approach means that the most significant split (in terms of deviance between the two segments) is identified first, and splits of lesser significance are subsequently identified in a recursive procedure. Binary recursive partitioning may overfit data, so some smoothing of the input data and/or simplification of the fitted model may be required. The procedure used was therefore as follows:

1) Time-series were processed to remove features that were not evident of systematic trends in physiometry related to stress. For heart rate, circadian trends in time-series were removed. For swimming activity, the scaled (mean = 0; SD = 1) time-series were smoothed using a kernel smoother (using the *ksmooth* function in R; bandwidth = 1 h, kernel type =" box") to remove short-peaks in swimming activity.
2) Time-series were then partitioned using the *rpart* function of the *rpart* library in R. After an initial partitioning, fitted models were simplified using cost-complexity pruning (using the *prune.rpart* function). The optimal model was that with the largest number of partition breaks where the cross-validated error was at least one SD greater than the minimum cross validated error of the full model. Differences in mean rank blood parameters (plasma cortisol, glucose, lactate and osmolality) between the start, pre-stressing, post-stressing and recovery samples were determined using Kruskal-Wallis tests. Post-hoc analysis was done using pairwise Wilcoxon tests.

3. Results

The FFT analysis revealed a dominating variation in heart rate with a period of 24.0 h, showing a circadian variation with a peak to peak difference in heart rate of 4.53 beats per min (BPM) between day and night in the 7 days preceding stressing (Fig. 5). A similar circadian pattern was identified for the 7-day period before the end of the experiment, with a period of 24.0 h and a peak to peak day/night heart rate difference of 5.19 BPM.

The average baseline heart rate for all fish equipped with heart rate DSTs ($N = 12$) in the 48 h prior to stressing was 24.2 BPM (SD = 2.3 BPM), while the average baseline swimming activity for all fish equipped with swimming activity DSTs ($N = 4$) before normalization was 0.48 m/s² (SD = 0.12 m/s²). Binary recursive partitioning showed that there were systematic changes in the average heart rate and swimming activity of the tagged fish throughout the study (Fig. 6). An immediate increase in heart rate occurred on initiation of the stressing period. From there, heart rate remained at an elevated level for 24.5 h, before returning to a level similar to that pre-stressing. There was not, however, a systematic increase in mean swimming activity immediately on commencement of the stressing period. Rather, mean swimming activity remained low for 2.4 h before showing a systematic increase. Mean swimming activity remained high for 16.2 h, before returning to a level similar to that pre-stressing. Heart rate remained at a systematically elevated level for 1.9 h after swimming activity has returned to a level similar to that pre-stressing. During the stress period four peaks in swimming activity corresponding to the points in time when stressing was initiated could be seen in the raw data for all fish equipped with activity tags as shown in Fig. 7.

Significant differences in blood parameter levels (Kruskal-Wallis test, $p < 0.05$) existed according to when they were measured (Fig. 8). All measured blood parameters (plasma cortisol, lactate, glucose and osmolality) rose from initial low levels at the start of the experiment (Feb 18th) and pre-stressing (10:54 Hrs March 4th) to reach maximum levels immediately post-stressing (15:38 Hrs March 4th) (Table 1). Significant differences between pre- and post-stressing samples were observed for all parameters (Wilcoxon-test $p < 0.05$), indicating a physiological response to treatment. Blood parameter levels sampled at the end of the experiment in the recovery period (March 14th) had
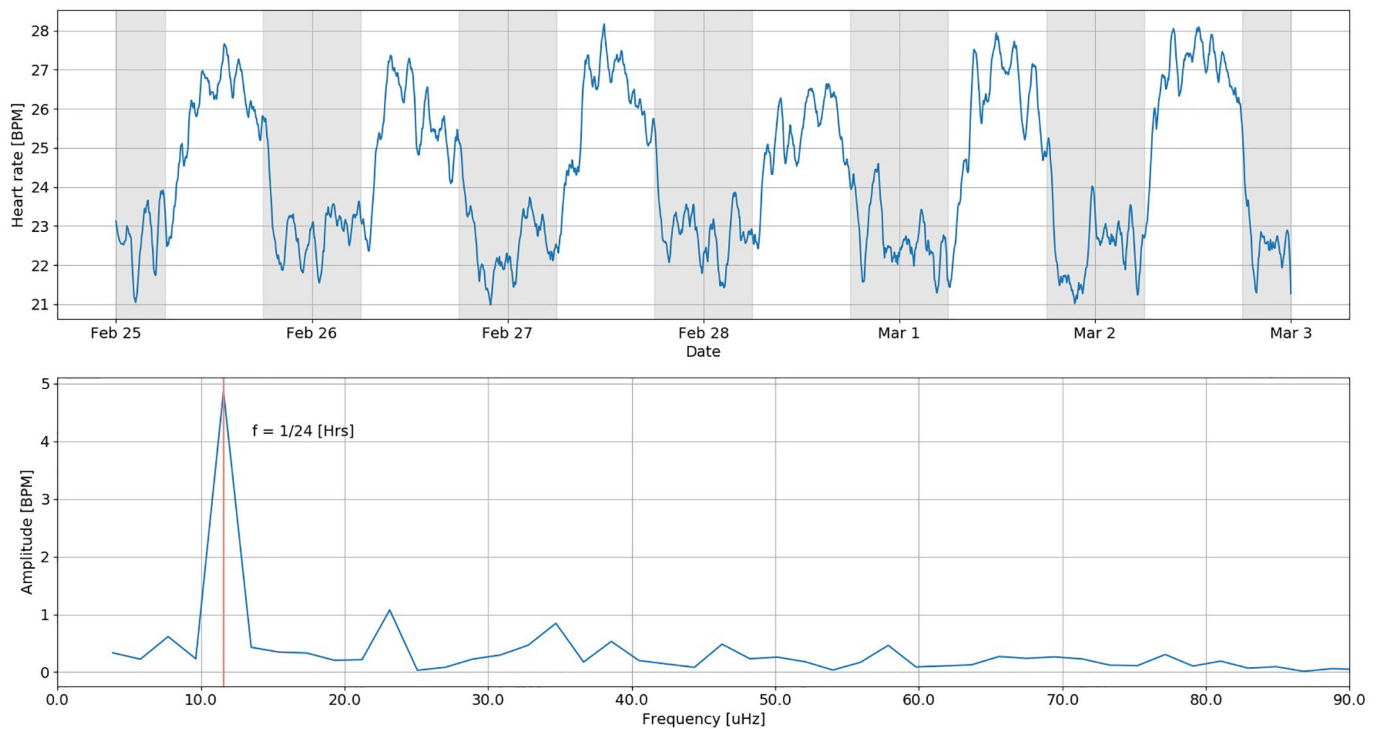
Fig. 5. Average heart rate (top) and the corresponding real, one-sided Fourier transform (bottom). For the average heart rate, night (18:00–06:00) is shaded grey. For the Fourier transform, frequency along the x-axis given in micro-Hertz (μHz). The highest peak (indicated by the vertical red line) resulting from the Fourier transform places the dominating variation in heart rate at 24.0 h (i.e. circadian). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fallen to levels similar to those at the start and pre-stressing samples (Table 1), indicating a physiological recovery.

4. Discussion

The results from this experiment demonstrate that there are changes in heart rate and swimming activity in response to stress events that are consistent with blood proxies for stress levels in Atlantic salmon. The physiological responses of plasma cortisol, lactate, glucose and osmolality followed the treatment timeline by increasing post-stress and levelling off during recovery. The data types measured in this study are therefore candidates for indirect stress monitoring in free-swimming salmon. Since both tag types applied in the experiment have been used in industrial settings (Føre et al., 2018; Brijs et al., 2018), their application in commercial facilities might be considered a more objective and operational tool for monitoring fish stress than conventional approaches involving manual observation and blood sampling.

Several blood parameters were included in the analysis both to evaluate stress levels and how they can be related to heart rate and swimming activity.

Plasma cortisol measurements can provide useful information on the primary stress response in fish. Studies carried out by Iversen and Eliassen (2012) reported a link between high resting levels of plasma cortisol during commercial smolt production and mortality at plasma cortisol levels above 50 nM after transfer to seawater. Similar studies have shown that the normal resting levels of plasma cortisol in fish can be as low as 13.8 nM, while fish with a chronically activated stress response can have a resting level > 27.5 nM (Maule et al., 1987; Pickering and Pottinger, 1989; Van Zwol et al., 2012; Noble et al., 2018). Similar to glucocorticoids (mainly cortisol), catecholamines (i.e. adrenalin and noradrenalin) also play a role in the primary stress response. But due to the difficulties associated with interpretation of catecholamines caused by their short half-life in circulation, they are of limited use as indicators of the primary stress response (Bonga, 1997,

2011) and have therefore been omitted from this study.

Lactate is produced by anaerobic ATP production (glycolysis) when oxygen is not available in sufficient amounts for the cells to utilise aerobic metabolism. The drivers behind this could be decreased oxygen levels in the water (Remen et al., 2012) or heavy physical exercise (Milligan and Girard, 1993). As lactate is primarily produced in muscle cells, it takes some time before it appears in blood and the response is delayed by a few hours after the event. In most cases the animal will recover after 6–12 h (Hatløy, 2015). The peak of plasma lactate during stressors such as transport and handling ranges from 6.4 to 13.3 mM (Hatløy, 2015; Iversen et al., 2003; Noble et al., 2018). Pre-stress levels of plasma lactate are normally between 3 and 5 mM (Carey and McCormick, 1998; Iversen et al., 2005). Elevations in plasma cortisol stimulate glycogenolysis which is the conversion of glycogen stored in the tissue to glucose released into the blood (Barton and Iwama, 1991).

Increased plasma glucose in the blood is a relatively slow response to a stressor and peaks after around 3–6 h in salmon (Olsen et al., 2003). In salmon, plasma glucose levels can increase to twice that of baseline levels 4 h after acute stress but can return to baseline levels much faster (2 h) in fasted fish than in fed fish. Pre-stress levels of plasma glucose are normally between 3.7 and 4.6 mM (Carey and McCormick, 1998; Skjervold et al., 2001).

In general, teleosts attempt to keep an osmolality of between 290 and 340 mOsm regardless of the surrounding salinity. Deviations from these levels for prolonged periods will result in mortality (McCormick et al., 2013). Arnesen et al. (1998) reported that typical osmolality in freshwater was approximately 320 mOsm, while osmolality ranged from 325 to 345 mOsm in seawater adapted Atlantic salmon. The selected blood parameters are therefore relevant when evaluating stress levels in fish.

Heart rate data showed an immediate response to induced stress through a significant increase that coincided with the stressing period implying that heart rate could serve as a sensitive and immediate stress indicator for salmon. In contrast, the swimming activity derived from
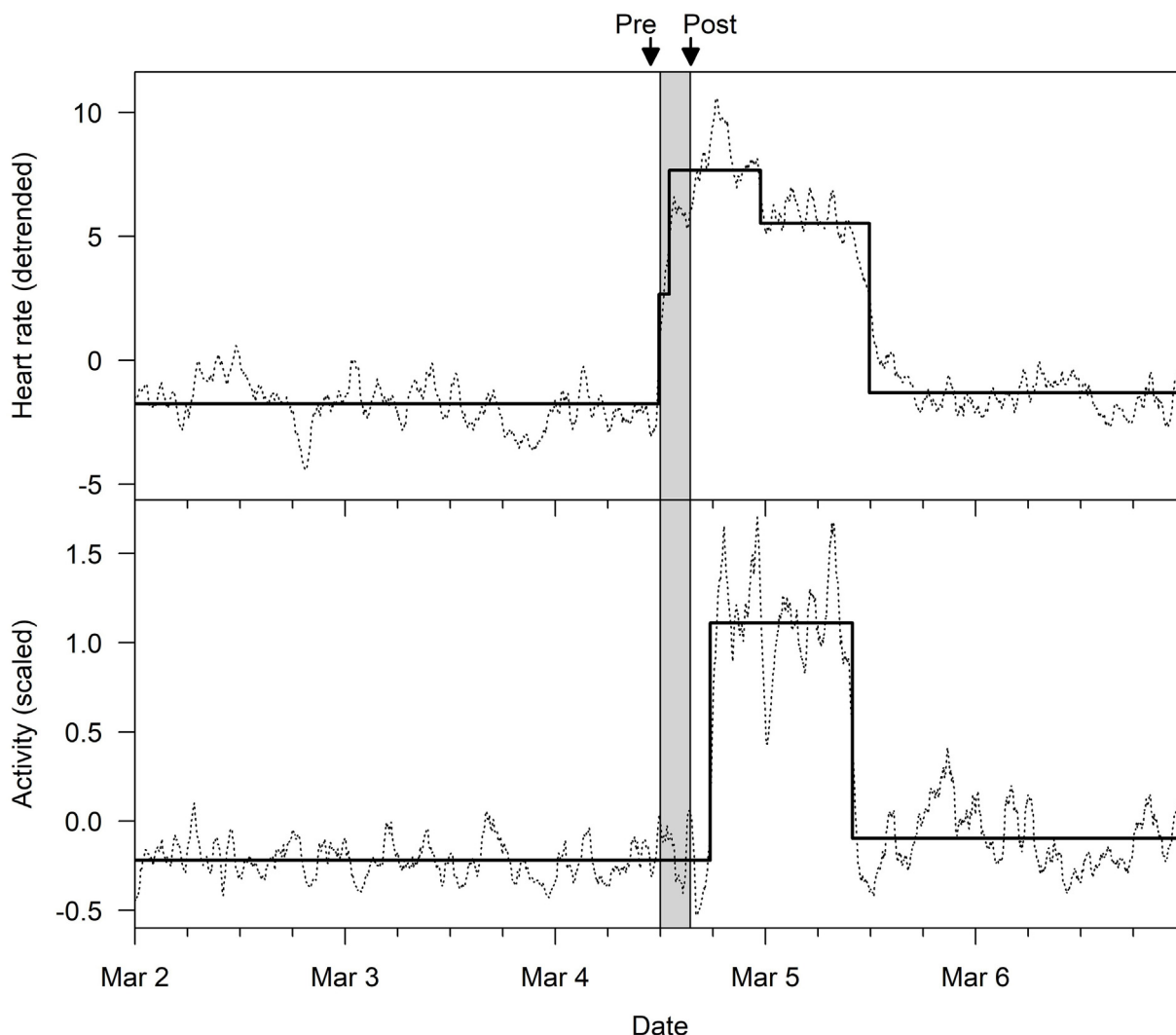
Fig. 6. Mean heart rate and mean activity partitions. Time-series are shown by dotted lines; partitions are shown by thick lines; the stress period is shown by the grey bar; the times of pre- and post-stressing blood samples in the reference group tanks are shown by dashed vertical lines. Time-series of heart rate have had circadian trends removed; time-series of activity have been smoothed and scaled.; time-series of heart rate have had circadian trends removed.

the accelerometers was low immediately after water levels were restored after stressing, a situation that lasted 2.4 h before swimming activity increased significantly to levels higher than before the stress period (Fig. 6). This was an unexpected result, as a previous study where salmon were subjected to crowding and delousing in a commercial cage found a gradual increase in swimming activity while the

fish were subjected to the stressor, and an immediate drop to lower levels as soon as they were released (Føre et al., 2018). A possible explanation for these dissimilar results could be that the four consecutive stress events in the present study drove the fish to a more complete state of exhaustion than the earlier cage-trials. The low swimming activity period could therefore represent a restitution period where the fish had
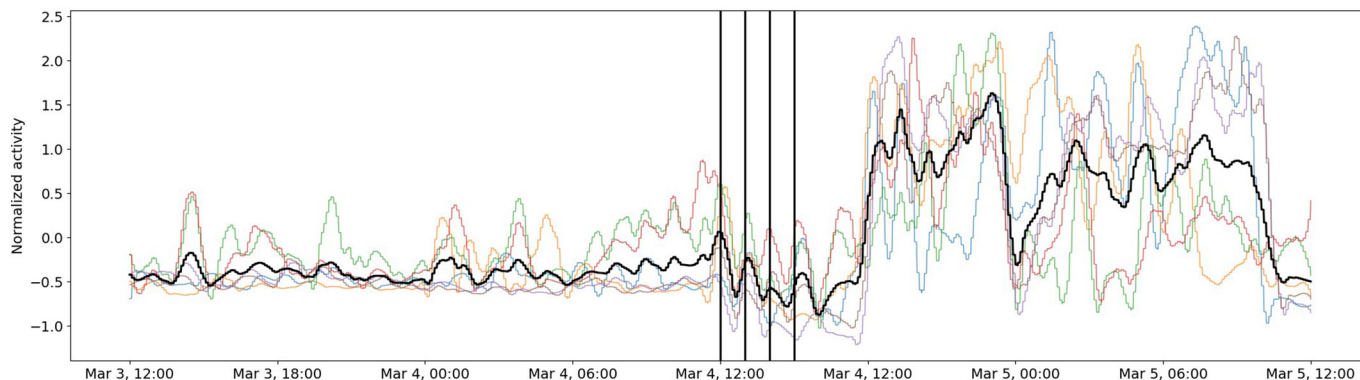
Fig. 7. Activity data for all fish during and post stressing with average activity shown by the solid black line. The vertical lines indicate the start time for each stressing.
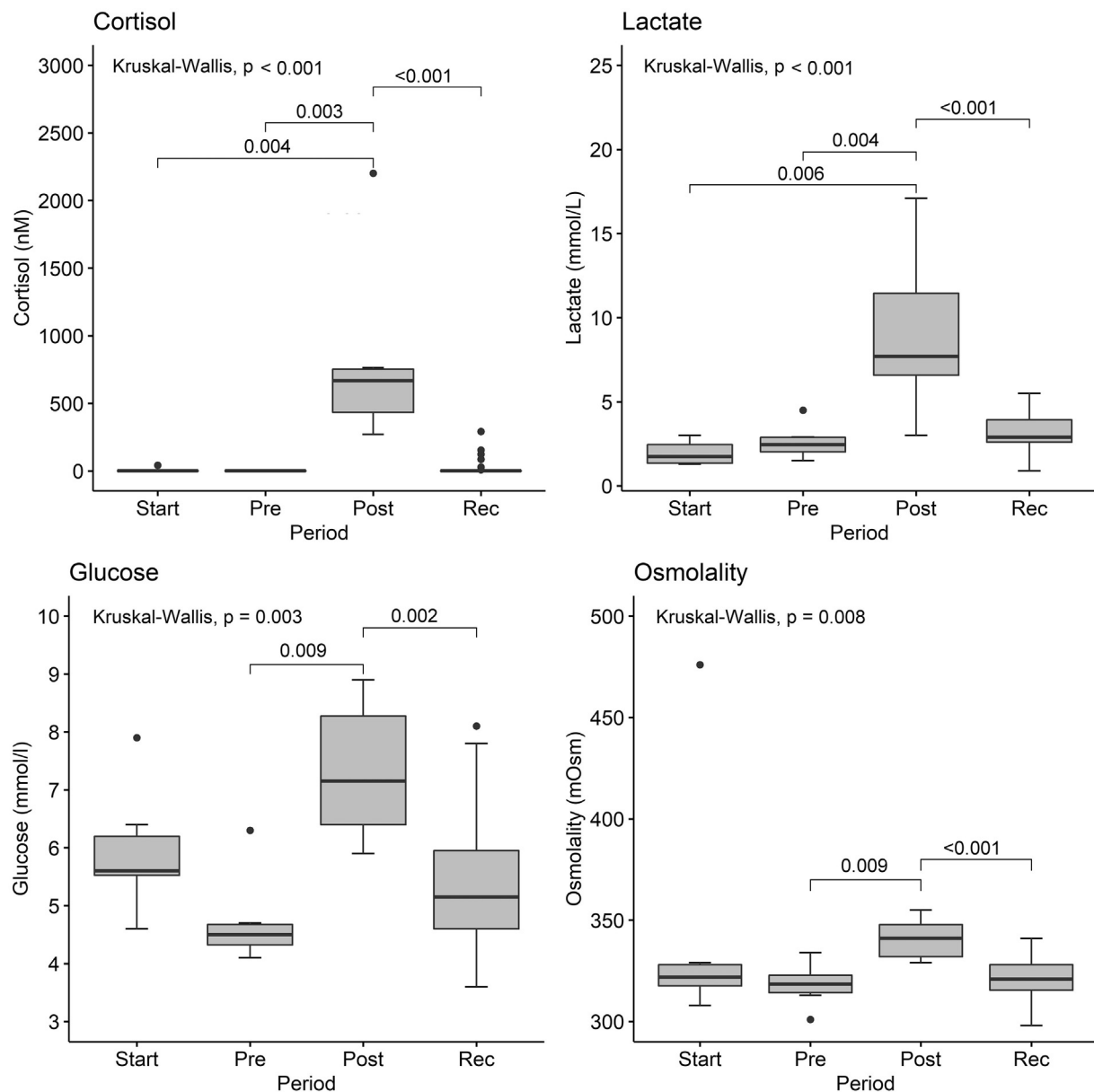
Fig. 8. Blood properties (cortisol, lactate, glucose and osmolality) measured from fish at the start of the experiment (Start, $N = 6$), pre-stressing (Pre, N = 6), post-stressing (Post, N = 6) and in recovery (Rec, $N = 40$). Note that the total number of blood samples are 58 and not 60 as in the total number of fish due to the two males with agonistic behaviour dying the day before the conclusion of the experiment.

to recover physiologically before being able to express their behavioural/flight response to the stressors.

Results from this experiment were robust because all attempts were made to remove extraneous factors that could influence the results. The experimental tanks were located outdoors, meaning that external disturbances such as noise from local traffic, fauna and weather could be suspected to influence the data in inducing responses in the fish. However, it is unlikely that this had a strong effect on stress levels since

all fish used in these experiments had been reared in outdoor tanks at the same site from fry to adult fish and thus were likely accustomed to local ambient sounds. Furthermore, the crew on site ensured that regular tasks such as feeding and daily husbandry were conducted at approximately the same times each day and with the same duration thereby reducing the chance that they could affect the stress levels. Furthermore, the homogeneity in the diurnal cycle throughout the experiment (outside the stress period), indicates that the fish were well

Table 1
Blood property values.

Date	Handling	Fish length (cm)	Plasma cortisol (nM)	Lactate (mM)	Glucose (mM)	Osmolality (mOsm)
February 2nd	Start	56.1 ± 5.9 (6)	8.5 ± 16.7	2.0 ± 1.0	5.9 ± 1.1	345.7 ± 64.3
March 4th	Pre-stressing	58.3 ± 4.3 (6)	1.7 ± 0.0	2.6 ± 1.1	4.7 ± 0.8	318.2 ± 11.0
March 4th	Post-stressing	58.9 ± 4.4 (6)	824.4 ± 702.1	9.1 ± 5.0	7.3 ± 1.2	340.8 ± 10.6
March 15th	Recovery	56.4 ± 3.1 (6)	27.0 ± 62.0	1.9 ± 0.7	5.4 ± 1.4	318.5 ± 11.2

habituated to their environment.

About half of the experimental fish reached sexual maturity during the trials. Two male fish kept in tank 3 (one tagged and one untagged individual) exhibited agonistic behaviour throughout the experimental period until they died the day before the conclusion of the experiment. Apart from this, the response patterns in the sensor measurements were similar across tanks, suggesting that the chosen data types are robust indicators of stress in salmon.

An approved anaesthetic was used for surgery and used in the recommended dosage by experienced personnel. As shown by Føre et al. (2019), the heart rate and swimming activity levels for fish having undergone surgery stabilises after 6 days. It is therefore likely that no adverse effects were introduced by either anaesthesia or surgery.

Due to the low temperature during the study (4.2 $\pm$ 0.2 °C), it was expected that all biological processes were slower than they would have been compared to higher temperatures. This may have had an impact on e.g. the fish's timely response to the stressors. Blood properties (Table 1), however, show the expected changes to stress. Posthumous examination and dissection revealed no signs of poor wound healing or infection. Hence, the impact of water temperature on the overall results could be negligible.

The carefully controlled conditions caused similar responses among tagged individuals, such that it was possible to identify the effect of stress despite the small sample size. Heart rate and activity responses were consistent across most tags, involving an immediate increase in heart rate and a delayed increased in activity, followed by a period of high heart rate and activity, after which levels declined to those pre-stress. This pattern was absent in only one heart rate tag (Fish 7, 1CHL0310; Fig. 11 in supplementary material) and one activity tag (Fish 11, 1CAL0165, Fig. 15 in supplementary material). Likewise, the controlled conditions allowed for identification of significant differences in blood parameter levels according to sample period. It is possible that an increased sample size will have provided a better indication of changes in blood parameters. For example, no significant difference in either glucose or osmolality was identified between the blood samples taken at the start of the experiment and those taken post-stressing. This lack of a significant difference was caused by a single outlier value for each of glucose and osmolality, and a greater sample size would have helped identify whether there was a systematic difference in parameter values between these sample periods.

The main reason for the slight variations in the swimming activity results between co-located Thelma Biotel and Star-Oddi tags (Fig. 7) was probably that the tags were located in different positions within the peritoneal cavity and would thus have experienced different accelerations due to fish movements. For the Thelma tags, which were not anchored using a suture (Føre et al., 2019), shifts in the tag's position within the peritoneal cavity could also result in transients not seen in Star-Oddi data. An additional source for these differences between tag types may also lie in that key components in the tags (e.g. the accelerometer) were not identical between types.

To further investigate the links between heart rate, swimming activity and stress response, at least two options for further work are considered. One is to gain a more detailed understanding of the responses by doing swim respirometer trials with cannulated fish in controlled laboratory conditions. The other is to increase scale and repeat the experiment in meso- or full scale to approach an industrial setting and application.

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Declaration of Competing Interest

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