



Under the sea: How can we use heart rate and accelerometers to remotely assess fish welfare in salmon aquaculture?

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ABSTRACT

Recent advances in bio-sensing technologies open for new possibilities to monitor and safeguard the welfare of fishes in aquaculture. Yet before taken into practice, the applicability of all novel biosensors must be validated, and the breadth of their potential uses must be investigated. Here, we investigated how ECG and accelerometry-derived parameters measured using bio-loggers, such as heart rate, acceleration and variance of acceleration, relate to O₂ consumption rate (MO₂) and blood borne indicators of stress and tissue damage to determine how biologgers may be used to estimate stress and welfare. To do this, we instrumented 13 fish with a biologger and an intravascular catheter and subjected them to a swimming protocol followed by a stress protocol throughout which the physiological parameters were measured and analyzed a posteriori. Additionally, based on the empirical data obtained, we calculated the mathematical relationships between the bio-logger data and the other parameters and tested the relationship between the calculated parameters using the linear regression algorithms and the measured parameters. Our results show that acceleration is a good proxy for swimming activity as it is closely related to tail beat frequency. In addition, we show that heart rate, acceleration and variance of acceleration all can be used as predictors for metabolic rate. Accelerometry based data, especially variance of acceleration, significantly explain some of the variation in venous partial pressure of O₂, blood lactate and plasma cortisol concentration. Variance of acceleration also significantly explains some of the variation in pH and mean corpuscular hemoglobin concentration. These relationships are explained by variance of acceleration being a good indicator of the onset of burst-swimming activity, which is often followed by acid-base imbalances and release of catecholamines. The results herein indicate that bio-logger data can be used to extrapolate a range of stress-related physiological events when these are accompanied by increases in activity and highlight the great potential of biosensors for monitoring fish welfare.

1. Introduction

As one of the fastest growing food sectors worldwide, the aquaculture industry is undergoing a rapid increase in production intensification, a characteristic often associated with sustainability issues (Edwards, 2015; Edwards et al., 2019; Henriksson et al., 2018; Naylor et al., 2021). For example, the elevated stocking densities used in

intensive aquaculture may result in poorer fish health, welfare and yield (Ellis et al., 2002; North et al., 2006), which reduce profitability and increase resource wastefulness and reliance in environment-polluting chemicals for disease control (Santos and Ramos, 2018). Activities routinely performed in fish farms are also critical from an animal welfare perspective (e.g., handling, grading, crowding, transportation, delousing) and are often followed by periods of increased mortality

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Table 1Summary of swimming activity and cardiac, respiratory and blood status of Atlantic salmon (*Salmo salar*) swimming at increasing speeds and following stress.

| | Swimming speed (BL s ⁻¹) | | | Time post-stress (hours) | | |
|---|--------------------------------------|--------------------|--------------------|--------------------------|--------------------|--------------------|
| | 0.5 | 1.0 | 1.5 | 0 | 1 | 24 |
| Acceleration (1·10 ⁻³ G) | 10.1 ± 1.1ab (13) | 14.2 ± 1.3cd (12) | 16.9 ± 0.8d (13) | 13.4 ± 1.6bcd (13) | 12.1 ± 1.4abc (13) | 8.9 ± 0.9a (12) |
| VAR (1·10 ⁻³ G ²) | 36.5 ± 8.2a (13) | 160.0 ± 35.3b (12) | 383.2 ± 69.9c (13) | 152.0 ± 32.2b (13) | 91.0 ± 26.0ab (13) | 18.8 ± 2.5a (12) |
| TBF (Hz) | 1.5 ± 0.1a (7) | 2.2 ± 0.1b (7) | 2.5 ± 0.1c (7) | - | - | - |
| TBA (mm s ⁻¹) | 24.2 ± 3.3a (7) | 53.9 ± 6.6b (7) | 69.1 ± 15.6b (6) | - | - | - |
| Heart rate (beats min ⁻¹) | 59.3 ± 1.2abc (13) | 62.7 ± 1.2bc (13) | 63.2 ± 1.2c (13) | 58.3 ± 1.2ab (11) | 59.3 ± 1.2abc (12) | 56.1 ± 1.3a (12) |
| MO ₂ (mg O ₂ h ⁻¹ kg ⁻¹) | 83.2 ± 7.4a (13) | 176.7 ± 11.4b (12) | 257.4 ± 10.8c (12) | - | - | - |
| MOF(Hz) | 0.9 ± 0.1 (5) | 1.1 ± 0.1 (7) | 0.9 ± 0.1 (7) | - | - | - |
| MOA (mm s ⁻¹) | 2.9 ± 0.4 (7) | 3.2 ± 0.4 (7) | 3.7 ± 0.4 (7) | - | - | - |
| PvO ₂ (kPa) | 4.4 ± 0.2a (11) | 3.2 ± 0.2bcd (12) | 2.8 ± 0.2d (12) | 3.1 ± 0.2cd (12) | 3.6 ± 0.2bc (10) | 3.9 ± 0.2ab (10) |
| pH | 7.54 ± 0.07a (12) | 7.48 ± 0.7ab (12) | 7.30 ± 0.07b (12) | 7.01 ± 0.07c (12) | 7.59 ± 0.08a (10) | 7.41 ± 0.08ab (10) |
| [Lactate] (mmol L ⁻¹) | 1.2 ± 0.1a (12) | 1.8 ± 0.3a (12) | 3.2 ± 0.2b (12) | 4.8 ± 0.5c (11) | 5.0 ± 0.8bc (9) | 1.2 ± 0.1a (10) |
| [Glucose] (mmol L ⁻¹) | 8.6 ± 0.7ab (12) | 7.7 ± 0.7a (12) | 8.2 ± 0.7ab (12) | 9.5 ± 0.7b (12) | 8.9 ± 0.8ab (10) | 7.6 ± 0.8ab (10) |
| Hematocrit (%) | 26.2 ± 1.9ab (12) | 25.9 ± 1.9ab (12) | 26.9 ± 1.9ab (12) | 30.2 ± 1.9a (12) | 22.4 ± 2.1b (10) | 25.3 ± 2.1ab (10) |
| [Hemoglobin] (g L ⁻¹) | 75.2 ± 5.6 (12) | 66.8 ± 5.6 (12) | 70.7 ± 5.6 (12) | 75.5 ± 5.6 (12) | 66.2 ± 5.9 (10) | 69.9 ± 6.1 (10) |
| MCHC (g L ⁻¹) | 286.3 ± 10.1a (12) | 254.5 ± 6.0ab (12) | 257.4 ± 7.6ab (12) | 245.0 ± 8.7b (12) | 293.5 ± 17.1a (9) | 274.0 ± 13.6ab (9) |

Statistical analyses to determine significant differences at different sampling points were performed using linear mixed model with significant differences ($P < 0.05$) represented by different letters. Abbreviations are: acceleration, variance of acceleration (VAR), tail beat frequency (TBF), tail beat amplitude (TBA), O₂ consumption rate (MO₂), mouth opening frequency (MOF), mouth opening amplitude (MOA), venous partial pressure of O₂ (PvO₂) and mean corpuscular hemoglobin content (MCHC). Sample size at each sampling point is indicated between brackets. Further statistical details are provided in Supplementary tables 1 and 2. Values are estimated marginal means ± SEM.

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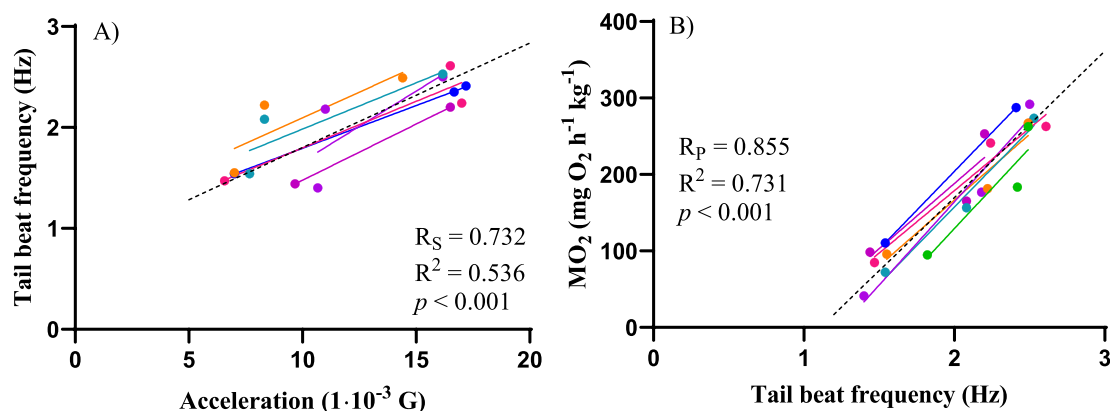


Fig. 1. Relationship between A) acceleration and tail beat frequency and B) tail beat frequency and O₂ consumption rate (MO₂) in Atlantic salmon. Simple linear regression was performed between tail beat frequency measured using video imaging ($n = 6$) and acceleration measured using bio-loggers or MO₂ using respirometry ($n = 7$). The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) Tail beat frequency = $0.104 \cdot \text{Acceleration} + 0.767$ and B) $\text{MO}_2 = 191.24 \cdot \text{Tail beat frequency} - 212.70$. Spearman's rho was used between calculated tail beat frequency and actual tail beat frequency. Pearson's correlation was used between calculated MO₂ and actual MO₂. The R , R^2 and p for the models are displayed in the figure.

(Huntingford et al., 2006; Overton et al., 2019; Sviland Walde et al., 2021). Safeguarding the welfare of the animals is therefore a vital aspect of sustainable aquaculture. This practice benefits not just the fish, but also the farmers and consumers, as it improves the quality and cost-efficiency of the product (Toni et al., 2019). Accordingly, new technologies able to reliably quantify the welfare status of farmed fishes are needed to further develop and industrialize fish farming both sustainably and efficiently.

Animal welfare is a multifaceted concept that englobes many aspects of the animals' lives, from their physical and mental health to their ability to express natural behaviours and adequately regulate biological functions in response to changes in the environment (Ashley, 2007; Segner et al., 2012). Consequently, multiple physiological and

behavioural parameters can be used to assess welfare. In aquaculture, physiological welfare indicators have traditionally been assessed via e. g., sampling individuals for growth, injury or diseases, which is generally terminal for the sampled fish and may disturb the rest of the cohort. Visual methods are the most common method for obtaining behavioural indicators, but may often prove challenging to use particularly due to poor water visibility or the increasingly larger numbers of individuals resulting from production intensification (Barreto et al., 2021). An alternative approach for assessing animal welfare is direct stress estimation (Barton, 2002; Chrousos and Gold, 1992). Stress responses can be categorized as primary, secondary and tertiary, although secondary and tertiary stress responses may be overlappingly categorized depending on the author (Sopinka et al., 2016; Wendelaar Bonga, 1997).

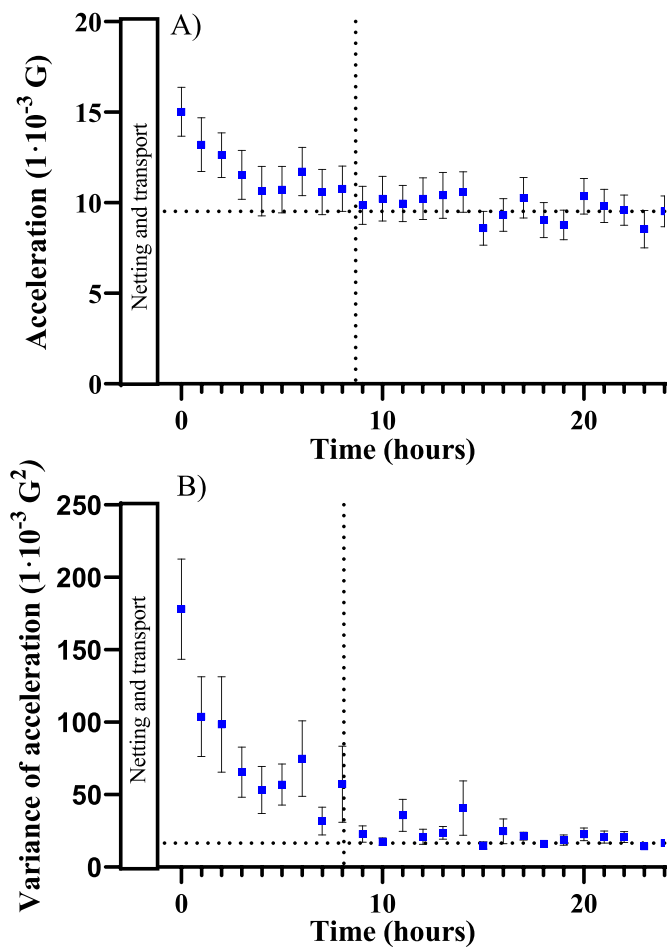


Fig. 2. Hourly dynamics of A) acceleration and B) variance of acceleration following netting and transport stress in Atlantic salmon over a 24-h period. Dashed lines indicate the intersection point between the low reference accelerometry value (i.e., acceleration or variance of acceleration) at 24 h post-stress with the accelerometry traces. Data are means \pm SEM. Sample sizes are 13.

The primary hormonal stress response is well conserved among vertebrates and entails the release of catecholamines (i.e., adrenaline and noradrenaline) and corticosteroids (Wendelaar Bonga, 1997). This neuroendocrine release leads to secondary stress responses, which include energy mobilization, changes in ion and metabolite composition of the blood and tissues, as well as cardiorespiratory and hematological changes (Barton, 2002). While stress does not necessarily lead to poor welfare, severe or repeated stress often lead to tertiary stress responses. These can be maladaptive and affect overall animal performance, as the stress-driven neuroendocrine release also suppresses functions that are not immediately essential to address the stressor, such as immune and reproductive activity or growth (Ashley, 2007; Wendelaar Bonga, 1997). Thus, tools that improve our ability to identify events of aversive stress during rearing can be used to optimize husbandry practices and reduce the likelihood of long-term negative effects on fish welfare.

The recent development, miniaturization and greater accessibility to biosensing systems have allowed for their increasingly widespread adoption in animal research (Fahlman et al., 2021; Gräns et al., 2009; Whitford and Klimley, 2019). The expanding body of scientific studies utilizing biosensors (e.g., bio-loggers, bio-telemetry systems) to measure physiological parameters linked to stress on free swimming fish, may pave the way for their routine implementation in aquaculture settings (Brijs et al., 2019b; Føre et al., 2018; Hjelmstedt et al., 2021; Hvas et al., 2020; Warren-Myers et al., 2021). Still, before their use in aquaculture becomes widespread, these techniques must be validated and our understanding of the relationship between these parameters and other stress-related physiological variables must improve (Brijs et al., 2021; Brijs et al., 2019a). Previous studies have shown that heart rate and acceleration, which often increase during and following stressful events, positively correlate with stress indicators. For example, fish may respond to an acute stressor with behavioural changes (e.g., swimming activity), which alter their energetic costs. This has allowed accelerometers to be used as tools to predict MO_2 in a number of species (Clark et al., 2010; Gleiss et al., 2010; Wright et al., 2014). Similarly, changes in O_2 demand are generally accompanied by cardiovascular adjustments to support tissue metabolism. As one of the factors influencing cardiac function, heart rate often correlates with O_2 consumption rate (Clark et al., 2010; Lucas, 1994). Concentration of cortisol, the main corticosteroid in teleosts, during recovery from surgery in rainbow trout (*Oncorhynchus mykiss*) is strongly correlated with resting heart rate (Brijs et al., 2019a). Similarly, in Atlantic salmon (*Salmo salar*) subjected to consecutive bouts of stress, blood salt-water imbalances and elevated

Table 2
Plasma chemistry of Atlantic salmon (*Salmo salar*) swimming at increasing speeds and following stress.

| | Swimming speed (BL s ⁻¹) | | | Time post-stress (hours) | | |
|---|--------------------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-----------------------|
| | 0.5 | 1.0 | 1.5 | 0 | 1 | 24 |
| [Cortisol] (ng mL ⁻¹) | 129.0 \pm 24.7ab (12) | 181.5 \pm 24.7bc (12) | 184.5 \pm 24.7bc (12) | 221.8 \pm 24.7c (12) | 172.1 \pm 25.2bc (11) | 88.4 \pm 24.7a (12) |
| Osmolality (mOsm Kg ⁻¹) | 338.7 \pm 6.1ab (12) | 331.2 \pm 6.1a (12) | 335.5 \pm 6.1ab (12) | 344.2 \pm 6.1ab (12) | 350.1 \pm 6.2bc (11) | 359.8 \pm 6.1c (12) |
| [Cl ⁻] (mmol L ⁻¹) | 147.9 \pm 2.4ab (12) | 147.6 \pm 2.4ab (12) | 146.7 \pm 2.4ab (12) | 144.3 \pm 2.4a (12) | 153.8 \pm 2.5b (11) | 169.3 \pm 2.4c (12) |
| [Na ⁺] (mmol L ⁻¹) | 152.9 \pm 1.6a (11) | - | - | 155.5 \pm 1.9a (10) | - | 164.7 \pm 3.9b (7) |
| [K ⁺] (mmol L ⁻¹) | 2.7 \pm 0.1a (11) | - | - | 3.0 \pm 0.1b (10) | - | 2.4 \pm 0.1a (7) |
| [Ca ²⁺] (mmol L ⁻¹) | 2.9 \pm 0.2 (11) | - | - | 2.8 \pm 0.2 (10) | - | 2.7 \pm 0.2 (8) |
| [ALP] (U L ⁻¹) | 184.8 \pm 13.7a (12) | - | - | 205.8 \pm 19.4a (10) | - | 127.2 \pm 15.8b (8) |
| [ALT] (U L ⁻¹) | 1.9 \pm 0.6 (12) | - | - | 1.7 \pm 0.7 (10) | - | 2.1 \pm 0.8 (8) |
| [AST] (U L ⁻¹) | 81.2 \pm 14.7a (12) | - | - | 107.9 \pm 18.0a (10) | - | 33.3 \pm 18.1b (8) |
| [CK] (U L ⁻¹) | 35.4 \pm 8.1a (11) | - | - | 47.0 \pm 8.2b (9) | - | 25.4 \pm 8.5a (6) |
| [LDH] (U L ⁻¹) | 531.7 \pm 74.3 (9) | - | - | 434.6 \pm 14.9 (7) | - | 338.7 \pm 30.7 (7) |
| [Cholesterol] (mmol L ⁻¹) | 6.0 \pm 0.4a (12) | - | - | 6.0 \pm 0.4a (10) | - | 4.4 \pm 0.5b (8) |

Statistical analyses to determine significant differences at different sampling points were performed using linear mixed model with significant differences ($P < 0.05$) represented by different letters. Abbreviations are: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LDH). Further statistical details are provided in Supplementary tables 1 and 3. Sample size at each sampling point is indicated between brackets. Values are estimated marginal means \pm SEM.

Statistical analyses to determine significant differences at different sampling points were performed using linear mixed model with significant differences ($P < 0.05$) represented by different letters. Abbreviations are: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LDH). Further statistical details are provided in Supplementary tables 1 and 3. Sample size at each sampling point is indicated between brackets. Values are estimated marginal means \pm SEM.

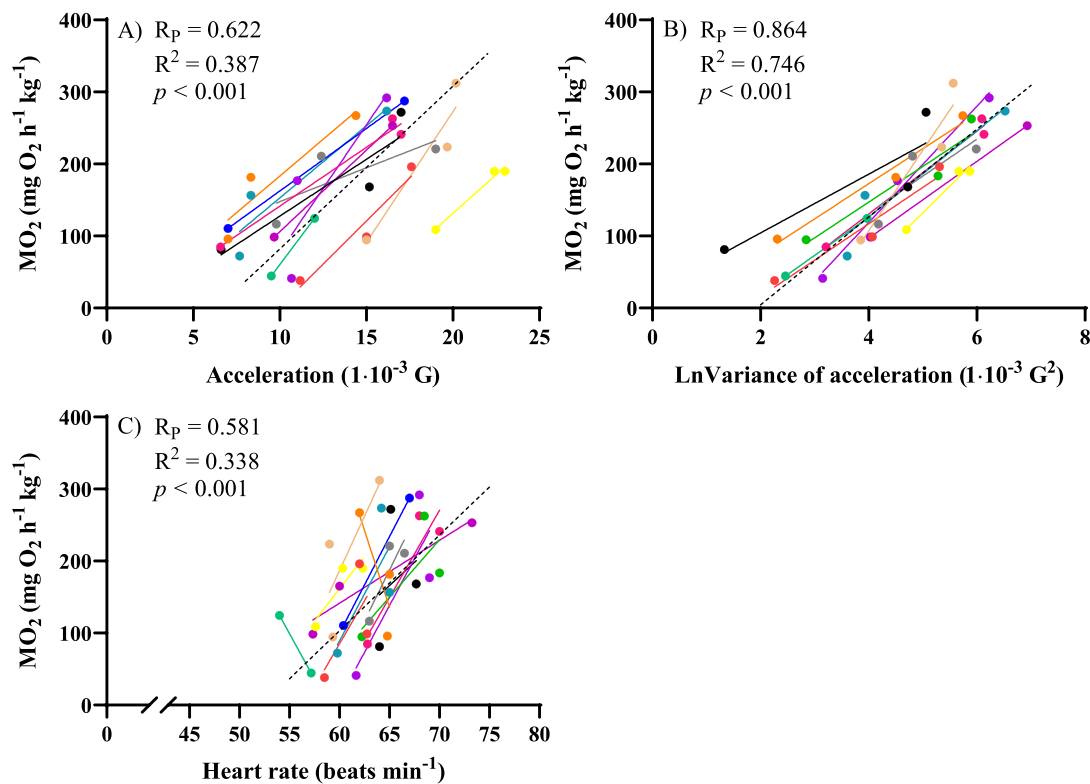


Fig. 3. Relationship of MO_2 with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 $BL\ s^{-1}$. Sample sizes were 12 for acceleration and variance of acceleration and 13 for heart rate. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) $MO_2 = 22.581 \cdot \text{Acceleration} - 143.612$, B) $MO_2 = 60.945 \cdot \text{LnVariance of acceleration} - 117.292$ and C) $MO_2 = 13.33 \cdot \text{Heart rate} - 696.585$. Pearson's correlation was used between calculated MO_2 and actual MO_2 and the R , R^2 and p are displayed in the figures.

plasma concentrations of cortisol, glucose and lactate co-occur with elevated heart rate and acceleration (Svendsen et al., 2021). Still, previous studies comparing blood parameters influenced by stress with bio-logger data were performed on terminally sampled fish, which impedes taking consecutive blood samples while simultaneously obtaining bio-logger data at different conditions. To further validate the use of bio-loggers to estimate stress in fish it is necessary to include methods that allow for repeated sampling with minimal disturbance to the fish, e.g., intravascular cannulations.

The objective of our study was to assess how well parameters (i.e., activity and heart rate) measured in adult Atlantic salmon, using a commercially available bio-logger relate to blood and respiratory parameters often used as indicators of acute stress and poor animal welfare. Towards this end, we performed respirometry in fish simultaneously implanted with a bio-logger and an intravascular catheter for sequential blood sampling. Additionally, we used video recording combined with machine learning-based analyses to estimate ventilatory and swimming activity during the swim trials.

2. Materials and methods

2.1. Experimental fish and ethical permit

Atlantic salmon of mixed sex were obtained as smolts in 2020 from a hatchery (Marine Harvest AS, Slørdal, Norway) and kept at the facilities of SINTEF Ocean in Trondheim until the time of the experiments in 2022. The fish were maintained with 24 h lighting and held in 5 m^3 tanks supplied with aerated seawater obtained at 70 m depth in the Trondheim fjord. During housing the fish were fed every two hours with dry pellets (EWOS Rapid HP 500, Norway) totalling to $\sim 1.5\%$ of the total estimated tank biomass per day. The temperature of the incoming

water during the duration of the experiments ranged 6.2–8.3 $^{\circ}C$. At the time of the experiment, the fish had a body mass of 1207.2 ± 65.1 g and a length of 46.4 ± 0.7 cm. The study was approved by the Norwegian Food Safety Authority (FOTS ID: 22913).

2.2. Surgery and instrumentation

Feed was withheld for one day prior to the implantation of the loggers. To measure heart rate and swimming activity (via acceleration), 13 fish were surgically equipped with pre-programmed data storage tags (milli-HRT ACT, Star-Oddi LTD, Gardabaer, Iceland). Before the surgery, fish were anesthetized in seawater containing a knock-out dose of MS-222 (150 mg L^{-1} , Tricaine methanesulphonate, Pharmaq AS, Overhalla, Norway). Following the cessation of opercular movements, the fish were placed dorsally, and the gills were perfused with a continuous flow of seawater containing a maintenance dose of 75 mg L^{-1} MS-222. Implantation of the bio-loggers was performed using a similar procedure as that described in Føre et al. (2021). Briefly, to insert the bio-logger, an incision of ~ 15 mm was performed along the midline of the abdomen. The two ends of a nylon suture were inserted ~ 2 mm below the transverse septum with the assistance of two 20G stainless steel cannulas and used to anchor the bio-logger to the peritoneal wall. The incision was closed using 3–4 sutures. Initially, immediately following the implantation of the bio-logger we performed the cannulation of the sinus venosus and allowed the fish to recover for >18 h before the start of the experiments. Nevertheless, upon observing a gradual drop in hematocrit throughout the protocol in some of the fish, we decided to perform the logger implantation and cannulation on different days, which solved the issue. Therefore, following logger implantation, the fish were then allowed to recover for ~ 48 h in individual holding tanks (0.4 m^3) to allow for a longer clotting before heparin was injected

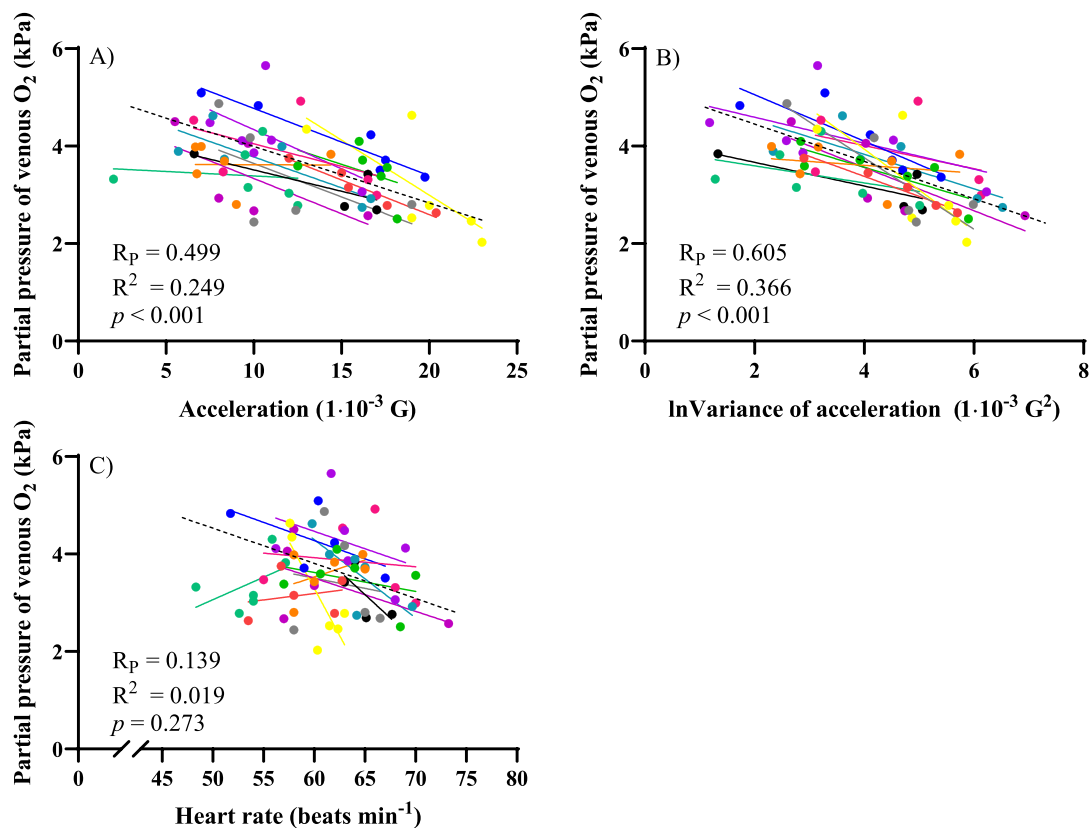


Fig. 4. Relationship of partial pressure of venous O₂ (P_VO₂) with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 BL s⁻¹ and 0, 1 and 24 h following netting and transport stress. Sample sizes were 12. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) $P_{V}O_2 = -0.116 \cdot \text{Acceleration} + 5.155$, B) $P_{V}O_2 = -0.383 \cdot \ln\text{Variance of acceleration} + 5.217$ and C) $P_{V}O_2 = -0.072 \cdot \text{Heart rate} + 8.135$. Pearson's correlation was used between calculated MO₂ and actual MO₂ and the R, R² and p are displayed in the figure.

intravenously during the cannulation procedure. The fish were then re-anesthetized in a knockout solution of MS-222 and placed laterally on wet foam with a continuous flow of maintenance MS-222 solution perfused over the gills. To allow for sequential blood sampling, the sinus venosus was then non-occlusively cannulated following the protocol described by Sandblom et al. (2006). The catheter was secured with two 3–0 silk sutures to the skin of the fish in front of the dorsal fin. From the 13 fish included in the dataset, 11 received 48 h of recovery between procedures, while 2 fish that had the cannulation and implantation done on the same day and did not display a gradual decrease in hematocrit were also included in the dataset.

2.3. Experimental protocol

After cannulation, the fish were transferred to either a Blazka swim-tube respirometer (Thorstad et al., 1997) or a Loligo (Brett type) swim tunnel respirometer (<https://www.loligosystems.com>) and allowed to recover for >18 h. During the recovery period, the water flow inside the respirometer was maintained at 0.5 body lengths per second (BL s⁻¹) until the start of the swim challenge. To encourage the fish to swim, the upstream half section of the respirometer was covered with black plastic while a light was placed above the downstream half of the respirometer, creating a darker section within the swimming chamber where the fish generally preferred to position themselves. Prior to the start of the swim challenge, one respirometry cycle (i.e., a closed period when the flush pump is off followed by a flush period) was performed, during the final minute of which a 0.5 mL blood sample was taken to determine blood variables at 0.5 BL s⁻¹ (i.e., routine). Water flow was then increased stepwise in 0.5 BL s⁻¹ increments, up to 1.5 BL s⁻¹, keeping water velocity constant for 30 min at each speed increment. If fish were unwilling

to swim and instead rested the tail against the back-grid, the speed was quickly reduced followed by a burst-increase in water velocity, after which the settings were quickly returned to the desired velocity. This generally resulted in the fish resuming swimming. Nevertheless, some fish struggled to maintain a steady swimming speed at 1.5 BL s⁻¹ and therefore, the time at sustained 1.5 BL s⁻¹ for these fish was reduced to 20 min. Before each increase in swimming speed, one respirometry cycle and a sampling of blood (0.25 mL) was performed. After the respirometry cycle at 1.5 BL s⁻¹ ended, water velocity was reduced to 0.5 BL s⁻¹. Fish were then netted and air exposed for 10 s during transfer to a black opaque 15 L bucket for transport, where they remained for 5 min. The fish were then netted out of the bucket and air-exposed for an additional 10 s during transfer into 0.4 m³ holding tanks, where 0.5 mL of blood were immediately sampled (0 h post-stress). Next, the fish were sampled for blood 1 h (0.25 mL) and 24-h post-stress (0.5 mL). After the final sampling, the fish were euthanized via blunt trauma to the head. The bio-loggers were retrieved and the integrity of the pericardium of each fish was confirmed post-mortem.

2.4. Data acquisition and calculations

MO₂ was measured using intermittent stop-flow respirometry and calculated from the decline in water O₂ saturation during a closed respirometry cycle using the following formula:

$$MO_2 = [(V_r - V_f) \times (\Delta\% \text{Sat}/t) \times \alpha] / (M_b)$$

Where V_r is the volume of the respirometer, V_f is the volume of the fish, assuming that 1 g of fish is equivalent to 1 mL of water, Δ% Sat/t is the decline in % air saturation over time during a closed respirometry

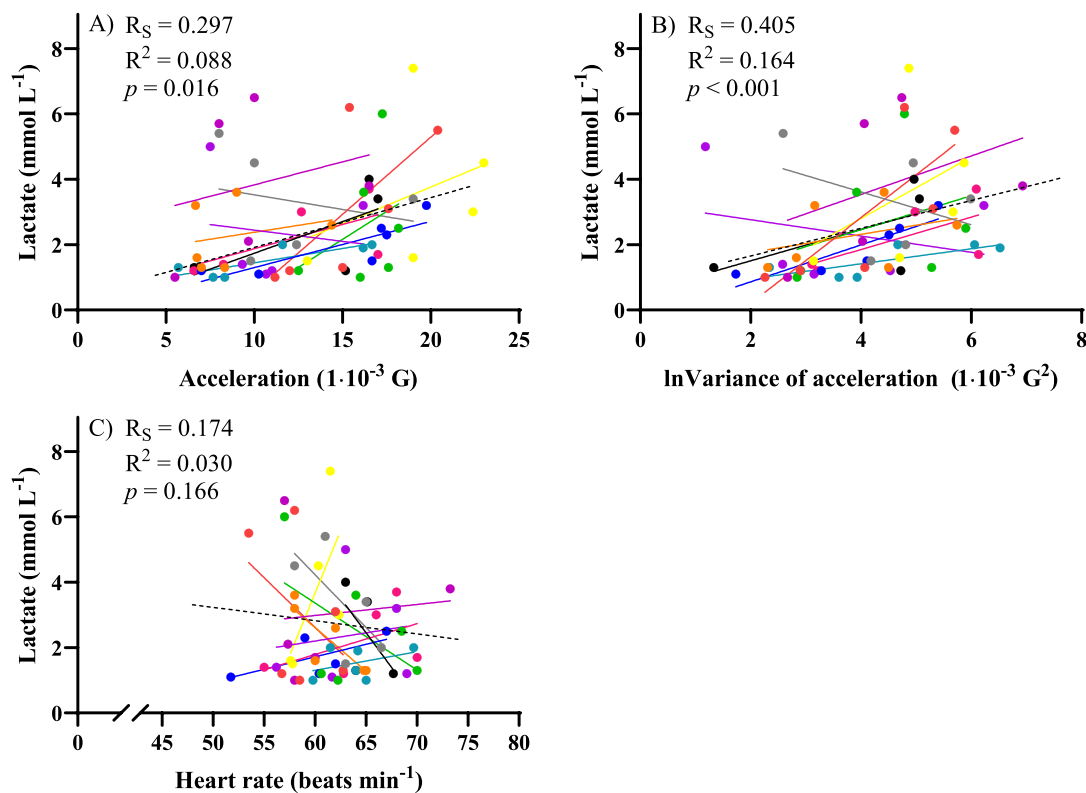


Fig. 5. Relationship of blood lactate concentration with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 BL s⁻¹ and 0, 1 and 24 h following netting and transport stress. Sample sizes were 11. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) Lactate = 0.125*Acceleration + 0.854, B) lactate = 0.373*lnVariance of acceleration + 1.147 and C) lactate = -0.083*Heart rate + 7.664. Spearman's rho was used between calculated lactate concentration and actual lactate concentration and the R , R^2 and p are displayed in the figure.

cycle, α is the temperature-adjusted solubility of O₂ in water and M_b is the body mass of the fish (Clark et al., 2013). The duration of each respirometry cycle was adjusted manually, to ensure that the O₂ saturation within the respirometer remained >80%. Thus, the time between flush cycles ranged from 8 to 12 min. Background O₂ consumption was assessed in the empty respirometer at the end of each protocol and the slope obtained was subtracted or added (depending on whether the slope was negative or positive, respectively) from the slopes used to calculate the MO₂ of the fish. The swim challenge was performed in two different types of swim-flume respirometers. One group was swum in a 90 L Brett-type respirometer ($n = 9$, test section 66 cm length × 20 cm width × 20 cm height, www.loligosystems.com), while another was swum in a 55 L Blazka-type respirometer ($n = 4$, test section 16 cm diameter × 116 cm length). The optode in the Brett-type system was connected to a Witrox O₂ meter and data was recorded using AutoResp software (Loligo Systems, Tjele, Denmark), while the optode within the Blazka system was connected to a FireSting O₂ system (PyroScience, Aachen, Germany) and collected using PowerLab system (ADInstruments, Castle Hill, Australia). Both optodes were 2-point calibrated at 100% air saturation and 0% air saturation using water supersaturated with sodium sulfite.

The fish in the Brett-type swim respirometer were continuously recorded using a custom build stereo vision camera at 15 frames per second at 1920 × 1080 resolution. The camera system consisted of two IR-sensitive cameras (Allied Vision, Alvim C-501 NIR, 5MP) with a fisheye lens (TECHSPEC, #39-937, 3.5 mm) connected to a Nvidia Jetson Xavier NX for real-time video encoding. The fish were illuminated using 1 Meter of IR LED strips (ledlightworld.com, 850 nm, 28.8 W/Meter) during the experiment to avoid disturbance to the fish. The video footage was post-processed using Python 3.6. During post processing, five landmarks were automatically detected on the fish: three on

the tail (i.e., the upper- and lower-lobe and along the middle axis of the caudal fin) and two on the jaw (i.e., upper- and lower jaw). The landmarks were detected using DDRNet (Deep Dual-resolution Networks for Real-time and Accurate Semantic Segmentation of Road Scenes; Pan et al., 2023) trained on a random selection of 555 images of the fish in the respirometer (Supplementary Fig. 1). By locating the landmarks in both the left- and right camera of the stereo camera setup, the position (in x, y, z [mm]) for each landmark could be calculated using the stereo module in OpenCV (<https://github.com/opencv/opencv>, Bradski, 2000), achieving a continuous time-series of the position of each landmark relative to the camera. Tail beat frequency, tail beat amplitude, mouth opening frequency and mouth opening amplitude were estimated for a total of seven fish. Tail beat frequency and tail beat amplitude were derived from the derivative of the position of the middle axis of the tail (Supplementary Fig. 1). Mouth opening frequency and mouth opening amplitude were derived from the derivative of Euclidean distance between the upper- and lower jaw position (Supplementary Fig. 1). Using Fast Fourier Transformation on a sliding window of 300 samples (i.e., 20 s of video data), the frequency- and amplitude components were extracted from the time series data. The frequency component with the highest amplitude was then selected as the estimated frequency for both mouth opening and tail beat. Datapoints (in x, y, z [mm]) outside the boundaries of the raceway were ignored in the calculations, although fewer than 0.2% of datapoints had to be removed for each fish. Tail beat frequency was limited between 0.1 Hz and 4 Hz, if the tail beat amplitude was <0.5 the fish was assumed to be still (not moving its tail). Mouth opening frequency was limited between 0.5 Hz and 2 Hz, if the mouth opening amplitude was <0.4, the mouth opening frequency was set to zero. From the 9 fish swum in the Brett respirometer, only 7 were successfully recorded due to technical issues in 2 of them.

The acceleration parameter registered by the biologists was

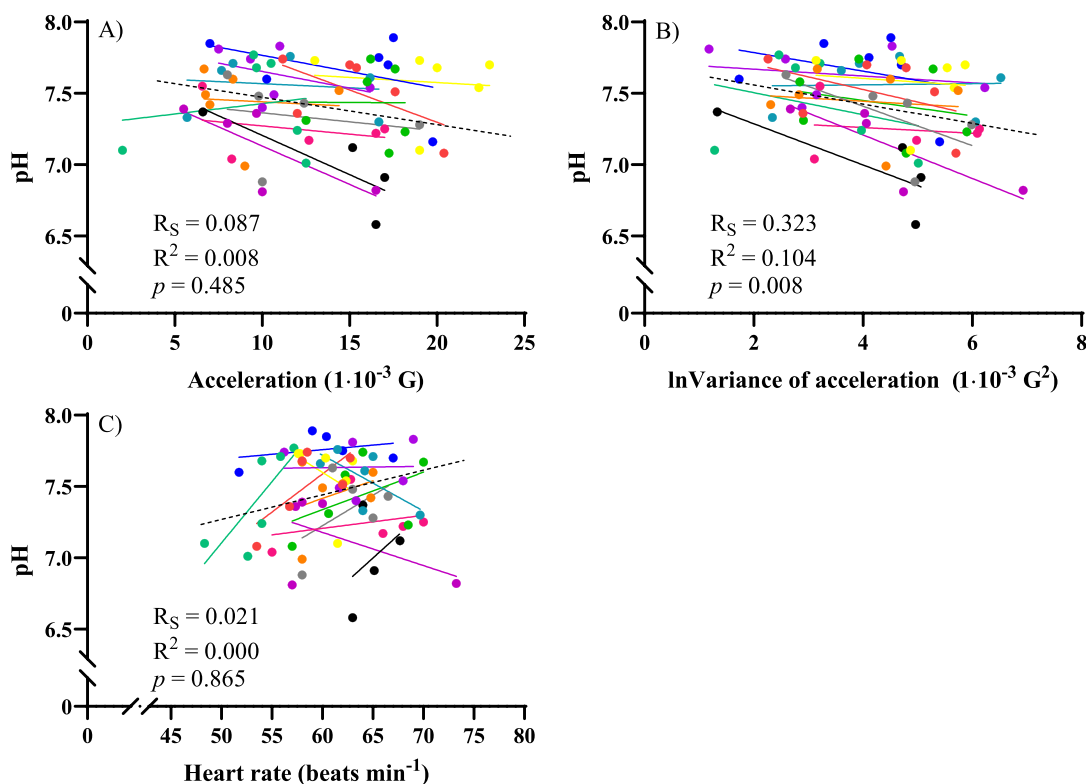


Fig. 6. Relationship of blood pH with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 BL s^{-1} and 0, 1 and 24 h following netting and transport stress. Sample sizes were 12. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) $pH = -0.019 \cdot \text{Acceleration} + 7.663$, B) $pH = -0.067 \cdot \ln\text{Variance of acceleration} + 7.693$ and C) $pH = 0.017 \cdot \text{Heart rate} + 6.405$. Spearman's rho was used between calculated pH and actual pH and the R_s , R^2 and p are displayed in the figure.

calculated as the square root of the vector sum measured on three axes (x, y and z) and reported in $1 \cdot 10^{-3}$ G (1 G being the acceleration due to gravity, i.e., 9.81 m s^{-2}) and can be used as a proxy for swimming activity, while variance of acceleration was calculated as the square of the standard deviation of this computed acceleration value over a given time (i.e. 7.5 s) and can be used as a proxy for discontinuous swimming activity. The bio-loggers were pre-programmed, using Star-Oddi's Mercury software, to record temperature, heart rate and acceleration at a rate of 200 Hz and 10 Hz, respectively, for 7.5 s twice an hour for 64 h starting immediately after implantation. During the following 6 h, the sampling rate was increased to 7.5-s measurements (200 Hz and 10 Hz for heart rate and acceleration, respectively) every 2 min. These higher definition measurements thus encompassed approximately 2 h before the start of the swim protocol, the swim and stress protocols and 2 h post-stress. After this 6-h period, the recordings were performed again twice an hour until the end of the protocol when the bio-loggers were retrieved. Average temperature, heart rate, acceleration and variance of acceleration were obtained from the same time periods as the MO_2 measurements at 0.5, 1.0 and 1.5 BL s^{-1} , and at the time of blood sampling at 0, 1 and 24 h post-stress. Only heart rate measurements graded with a quality index (QI) of 0 were included in the analyses (Brijs et al., 2019a). Heart rate measurements with a QI of 0 ranged from 53 to 91% for the different fish, with an average of 74%. Events with unusually large variance of acceleration, i.e., outside of the range previously recorded for this species at the measured swimming speeds (for reference see Zrini and Gamperl, 2021), were inspected in the video recordings. In extreme cases, these time periods generally matched in the footage with instances where the fish was not swimming in a coordinated fashion or in an upright position but was instead struggling or had even turned around. Thus, upon confirming the source of such unusual measurements for variance of acceleration, the datapoints for both acceleration and variance of acceleration were removed. In case such unusual

measurements occurred at timepoints when no video recordings were available (e.g., swim protocols performed in the Blazka respirometer), the data points were equally removed. Datapoints were removed for 7 out of 13 fish, all occurring at either 1 or 1.5 BL s^{-1} . Although for one fish, these comprised 67% of the measurements performed during the last 12 min at 1.5 BL s^{-1} (when MO_2 measurements were performed), the rest ranged 17–40% of the measurements during this time interval. Other instances of elevated variance of acceleration matched in the video recordings with instances where the fish switched between steady swimming and burst-and-glide swimming and therefore such datapoints were not removed from the dataset. Graphpad Prism 9.1.2. was used to calculate recovery time from stress using acceleration and variance of acceleration. Acceleration and variance of acceleration during recovery from stress were plotted over 24 h, including 0-h post-stress (i.e., the mean values first 5–7 min immediately following stress), hour 1 following stress (i.e., mean values between hour 0 to 1 following stress excluding the first 5–7 min), hour 2 following stress (i.e., mean values between hour 1 to 2 h following stress) and so on until hour 23 to 24 (i.e., 25 datapoints). The resulting pattern was an exponential decay which was then used to estimate the recovery time of each individual fish as the point in which the trace became equal to the datapoint at hour 24 (i.e., mean value between hour 23 to 24). Datapoints $>40\%$ larger than the nearest value were removed from the analyses to minimize the effects of spontaneous surges of activity.

2.5. Blood and plasma measurements

Blood samples taken at 0.5, 1.0 and 1.5 BL s^{-1} as well as 0, 1 and 24 h post-stress were immediately analyzed for venous partial pressure of O_2 (P_{vO_2}), pH, hematocrit, concentration of hemoglobin, glucose and lactate. Mean corpuscular hemoglobin concentration (MCHC) was calculated as $[\text{Hemoglobin}] / \text{hematocrit} \times 100$. The remaining blood was

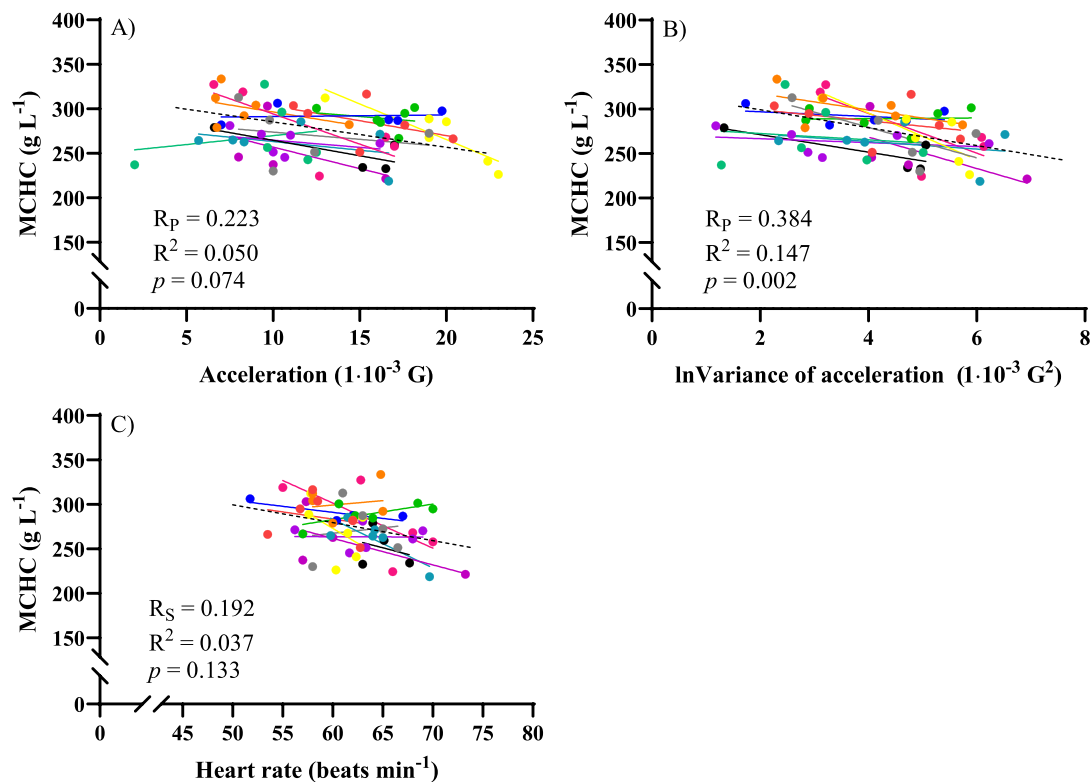


Fig. 7. Relationship of mean corpuscular hemoglobin concentration (MCHC) with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 BL s⁻¹ and 0, 1 and 24 h following netting and transport stress. Sample sizes were 12 for acceleration and variance of acceleration and 11 for heart rate. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) $MCHC = -2.852 \cdot \text{Acceleration} + 313.908$, B) $MCHC = -10.062 \cdot \ln\text{Variance of acceleration} + 319.317$ and C) $MCHC = -2.021 \cdot \text{Heart rate} + 400.755$. Pearson's correlation or Spearman's rho was used between calculated MCHC and actual MCHC and the R, R² and *p* are displayed in the figure.

centrifuged at 10,000 rpm and the plasma was frozen and stored at -20 °C for further analyses, which were performed within 3 months of being obtained. P_vO₂ was measured using a fibre optic optode 2-point calibrated at 7.8 °C connected to a FireSting O₂ system (PyroScience, Aachen, Germany). Once a blood sample was drawn through the catheter using a tuberculin syringe, the injector was removed from the syringe and replaced with a modified cap with an optode inserted and sealed to the cap with silicone. The syringe was then submerged in water cooled to 7.8 °C. A descending PO₂ value was immediately observed, which then plateaued following ~3 min. Thus, all PO₂ values were taken as the average value 3 min after the optode was inserted into the blood sample, as in [McArley et al. \(2021\)](#). The blood was then transferred to a 1 mL Eppendorf tube placed in the water bath at 7.8 °C and pH was measured using a two-point calibrated handheld pH meter (Sentron SI400, Sentron Europe, Leek, Netherlands). Hematocrit was determined by spinning the blood in microcapillary tubes at 10000 rpm for 5 min and measuring the fraction of red blood cells. [Hemoglobin] was measured using a handheld Hb 201+ analyser (Hemocue, Ängelholm, Sweden) and all values were adjusted for fish blood according to [Clark et al. \(2008\)](#). [Glucose] was measured using a FreeStyle Blood Glucose Monitoring Systems (Abbott Diabetes Care Ltd., Oxon, UK) and [Lactate] was measured using a Lactate Pro 2 test meter (Arkray factory INC, Shiga, Japan), following the manufacturers' instructions. Plasma [Cortisol] was measured using an ELISA kit (RE52061, IBL International, Hamburg, Germany). Plasma osmolality was determined using a Fiske Model 210 osmometer (Advanced Instruments, Norwood, MA, USA). Plasma [Cl⁻] was estimated by titration (M926S Chloride Analyser, Sherwood Scientific, Cambridge, UK). The abovementioned plasma analyses were performed for all sampling points. In the instances when 0.5 mL of blood samples were taken (i.e. at 0.5 BL s⁻¹, 0 h post-stress and 24 h post-stress), further plasma analyses were performed using a Pentra

C400 Clinical Chemistry Analyser (HORIBA Medical). These included [K⁺], [Na⁺] and [Ca²⁺], the concentrations of intracellular enzymes creatine kinase [CK], alanine transaminase [ALT], lactate dehydrogenase [LDH], aspartate transaminase [AST] and alkaline phosphatase [ALP] as well as [cholesterol]. Briefly, on the day of the analysis, frozen samples were thawed and immediately pipetted into pediatric cups and run using standard kits for the instrument and analyzed as described by the manufacturer.

2.6. Statistics

Statistical analyses were performed using SPSS statistics 28 (IBM Corp., Armonk, NY, USA), with statistical significance accepted at *P* < 0.05. While 13 individuals were instrumented with bilogger and a catheter, the catheter of one individual remained blocked for the duration of the experiment and therefore, the maximum sample size for blood parameters at any given timepoint is 12. Differences in cardiorespiratory, activity and blood/plasma variables at the different sampling points (i.e., time: 0.5, 1.0 and 1.5 BL s⁻¹, and 0, 1 and 24 h post-stress) were analyzed using linear mixed models. Fixed effects were time as repeated within subjects factor and surgical protocol (surgeries performed on the same day vs 48 h of recovery between implantation and cannulation) and respirometer (Brett-type or Blazka-type) as between subjects factors. Interactions between surgery/respirometer type and time were included as fixed effects, but subsequently removed if no significant effects were found. Body mass was included as a covariate and subsequently removed if no significant effects were found. Covariance structure was first-order autoregressive or heterogeneous first-order autoregressive depending on which provided the best fit to the data (as indicated by the Akaike information criterion, AIC; Supplementary table 1). Homoscedasticity and normal distribution of data was

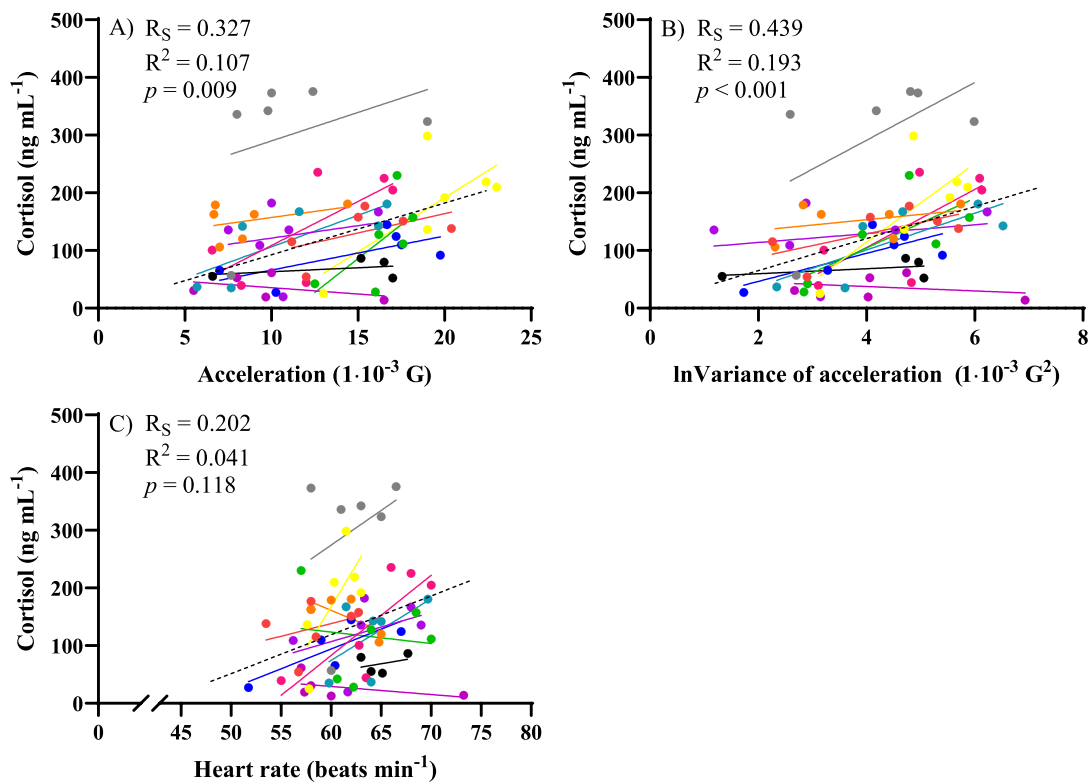


Fig. 8. Relationship of plasma cortisol concentration with A) acceleration, B) the natural logarithm of variance of acceleration and C) heart rate in Atlantic salmon swimming at 0.5, 1.0 and 1.5 BL s⁻¹ and 0, 1 and 24 h following netting and transport stress. Sample sizes were 11. The slopes and intercept of these regression were averaged resulting in one linear regression for each model (black dashed line): A) Cortisol = 6.750*Acceleration + 36.468, B) cortisol = 21.995*lnVariance of acceleration + 39.775 and C) cortisol = 4.673*Heart rate - 160.908. Spearman's rho was used between calculated cortisol concentration and actual cortisol concentration and the R, R² and p are displayed in the figure.

assessed by visual inspection of the residual plots. To assess how much of the variance of other physiological indicators of stress is explained by acceleration, variance of acceleration and heart rate simple linear regression analyses were carried out between the bio-logger data as explanatory variables and the physiological parameters as dependent variables. Regressions were performed for each individual fish and, subsequently, all individual slopes (independently of goodness of fit) and intercepts were averaged, resulting in a single regression model. To test the goodness of fit of our model, a Pearson's correlation or Spearman's rho (if non-normally distributed) were performed using the measured physiological parameters and the estimated physiological parameters, calculated by integrating the measured bio-logger data into the regression equation. Normality of distribution was assessed by Shapiro-Wilk's test. Individuals with a slope ± 2 standard deviations from the average slope for each model were removed as outliers.

3. Results

MO₂, acceleration, variance of acceleration, tail beat frequency and amplitude all increased significantly with swimming speed (Table 1). There was a significant relationship between acceleration and tail beat frequency, where acceleration explained 54% of the variation in tail beat frequency (Fig. 1 A). Similarly, when tail beat frequency was used to predict MO₂ it explained 73% of the variation in MO₂ from 0.5 to 1.5 BL s⁻¹. (Fig. 1 B). Acceleration and variance of acceleration dropped immediately after netting and transport stress (0 h), following which both gradually decreased, with acceleration plateauing at 8.7 h and variance of acceleration 8.1 h into the recovery period (Fig. 2). Heart rate increased by 3.9 beats min⁻¹ between 0.5 and 1.5 BL s⁻¹, and slightly dropped upon release in the holding tanks following stress, where it remained relatively stable for the remainder of the 24-h post

stress recovery phase (Table 1).

Upon increasing water velocity from 0.5 to 1.5 BL s⁻¹, P_vO₂ and pH significantly decreased (Table 1). P_vO₂ was lowest when swimming at 1.5 BL s⁻¹, while the lowest pH was measured immediately post stress (Table 1). During recovery, P_vO₂ and pH rapidly increased from 0 to 1 h and remained relatively stable between 1 and 24 h post stress (Table 1). Anaerobic metabolism surged during the swimming challenge, as indicated by the increase in [Lactate] from 0.5 to 1.5 BL s⁻¹, and further increased following stress, reaching its peak at 0 and 1 h-post stress and being back to baseline after 24 h (Table 1). [Glucose] was stable throughout the swimming protocol and increased slightly following stress (Table 1). Even though hematocrit and [Hemoglobin] did not change significantly throughout the swimming challenge, there was significant red blood cell swelling as indicated by a reduction in MCHC (Table 1). Hematocrit was highest at 0-h post-stress mainly due to red blood cell swelling, as indicated by the elevation in MCHC and lack of change in [Hemoglobin], but both hematocrit and MCHC had returned to baseline 1 h post-stress (Table 1). Plasma [Cortisol] sharply increased upon increasing the swimming speed to 1.0 BL s⁻¹ and remained elevated throughout the rest of the swimming protocol (Table 2). [Cortisol] then further increased and peaked at 0 h post stress and had gone back to baseline 24 h post stress (Table 2).

The close relationship among many of the blood and plasma variables measured here is well established, thus only relationships between parameters measured using bio-loggers and respiratory and blood/plasma variables were explored. When acceleration, variance of acceleration, or heart rate were used to predict MO₂, they explained 39, 75 and 34% of the variation in MO₂, respectively (Fig. 3 A-C). The slopes of the different models indicated that for a 1·10⁻³ G increase in acceleration, MO₂ would have concomitantly increased by 22.6 mg O₂ h⁻¹ kg⁻¹, an increase of 1·10⁻³ G² in the natural logarithm of the variance of

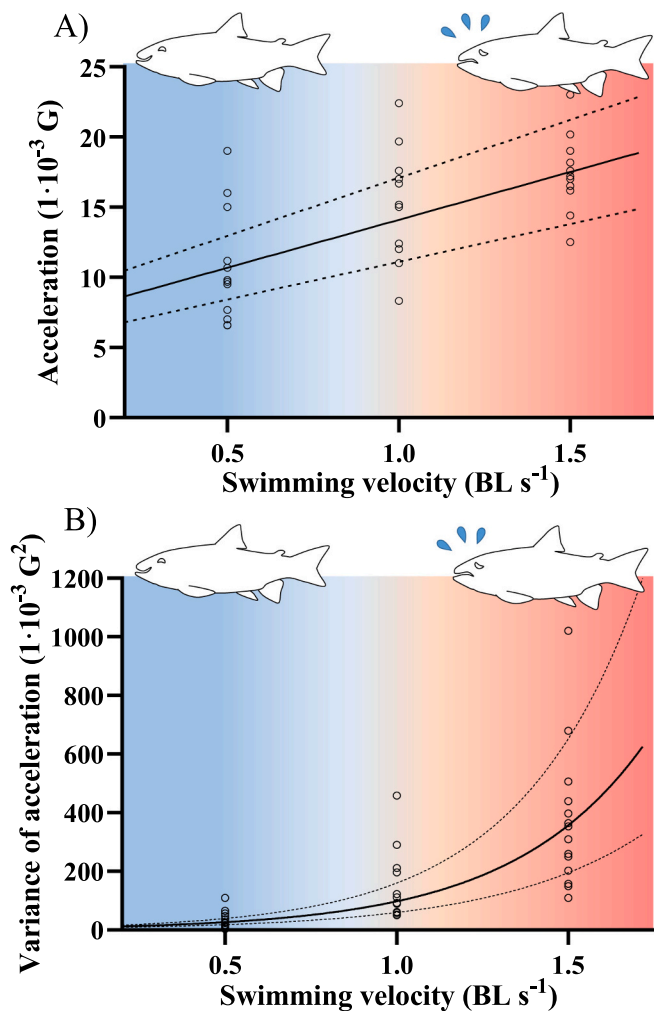


Fig. 9. A) Acceleration and B) variance of acceleration of Atlantic salmon ($n = 13$) during a swimming protocol at swimming speeds set to 0.5, 1.0 and 1.5 $BL s^{-1}$ to 1.5 $BL s^{-1}$. Background colours represent a gait transition from steady swimming (blue) to unsteady swimming (i.e., burst-and-glide, red) as fish approach exhaustion. Regressions for acceleration = $6.83 \cdot BL s^{-1} + 7.27$ and variance of acceleration = $7.33 \cdot e^{(2.59 \cdot BL s^{-1})}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acceleration, will entail an increase of $60.9 \text{ mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$ and MO_2 would increase by $13.3 \text{ mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$ for every 1 beat min^{-1} increase in heart rate (Fig. 3. A-C). Models including acceleration and variance of acceleration explained 25 and 37% of the variation in PvO_2 , respectively (Fig. 4 A-C). Similarly, the models including acceleration and variance of acceleration significantly explained 9 and 16% of the variation in [Lactate] (Fig. 5 A and B). Only the model including VAR significantly explained some of the variation in pH and MCHC, being 5 and 15%, respectively (Figs. 6 and 7). The algorithms including acceleration and variance of acceleration significantly explained 11 and 19% of the variation in plasma [Cortisol] (Fig. 8).

3.1. Effects of surgery protocol and type of respirometer on cardiorespiratory, blood and plasma variables

From the 13 fish used in this study, 11 were instrumented allowing for 48 h of recovery between the implantation of the bio-logger and the cannulation, while 2 fish were implanted with the bio-logger during the same procedure as the cannulation. Still, no major differences in cardiac, swimming or blood parameters were found between the fish intervened

with the two different surgical protocols, with the exception that fish that had 48 h of recovery between surgeries responded to exercise with significantly larger elevations in variance of acceleration (mean difference: $94.9 \pm 44.4 \cdot 10^{-3} G^2$; Supplementary table 1). Additionally, the fish were also swum in either a Brett-type ($n = 9$) or a Blazka respirometer ($n = 4$). Linear mixed model analyses showed that these two groups displayed differences in acceleration, heart rate, MO_2 , [Lactate], [Glucose], [Cortisol], $[\text{Cl}^-]$, $[\text{Na}^+]$, $[\text{Ca}^{2+}]$, [CK] and [LDH] (Supplementary table 1). Swim respirometer-specific averages of cardiac, swimming, blood and plasma parameters are summarized in Supplementary table 2.

4. Discussion

The main goal of this study was to establish whether bio-logger data can be used to extrapolate other physiological parameters related to stress that are often harder to measure as they generally require handling, blood sampling or for the fish to be confined in an airtight container (i.e., for respirometry). The results herein verify that such data, which can be obtained from off-the-shelf bio-loggers, can indeed be used as activity and stress indicators during various commonplace events in the life of a fish.

The usefulness of accelerometry data for predicting other physiological responses is largely dependent on its accuracy in approximating swimming activity. Recent studies on salmonids have shown that parameters of acceleration measured using bio-loggers can be used as robust proxies of swimming activity, as they correlate well with swimming speed and tail beat frequency (Kawabe et al., 2003; Warren-Myers et al., 2023; Wilson et al., 2013; Zrini and Gamperl, 2021). Here, we also show that acceleration and variance of acceleration increased with swimming speed, largely agreeing with the results from a recent study using similar Star-Oddi accelerometry and heart rate bio-loggers (Zrini and Gamperl, 2021). Additionally, acceleration significantly predicted tail beat frequency, accounting for 54% of the variability, further validating the use of these accelerometry data as proxy for swimming activity. Still, it must be considered that 46% of the variability remains unaccounted for, which leaves room for error for the approximation of swimming activity. A possible source for this large margin of error could be that tail beat frequency is not the only factor affecting the swimming activity and the position of the accelerometer. Other factors such as tail beat amplitude may have a large effect on swimming activity, specially at high swimming speeds (Warren-Myers et al., 2023). In this study, initial attempts to factor in tail beat amplitude in our predictive models proved unsuccessful, which may relate to the difficulty of accurately estimating tail beat amplitude visually from a lateral point view, as opposed to, for example, from above.

The changes in the properties of the blood and plasma followed the classic pattern generally observed with exercise. O_2 demand of the skeletal muscle increased with swimming speed, as reflected by elevations in MO_2 , which in turn reduced PvO_2 as tissue O_2 extraction increased (Kiceniuk and Jones, 1977). Since increased skeletal muscle work is the event originating these cascading physiological events, MO_2 increased and PvO_2 decreased as the proxies for swimming activity, acceleration, and variance of acceleration increased. As muscle work intensified, O_2 delivery to these increasingly metabolically active tissues became insufficient, which results in increasing proportions of energy being obtained from the anaerobic breakdown of glycogen resulting in an accumulation of lactate in blood and tissues (Wang et al., 1994). Additionally, the release of H^+ from the hydrolysis of ATP surpasses the usage of these protons for oxidative phosphorylation, resulting in metabolic acidosis, as reflected by the reduced blood pH (Robergs et al., 2004). Consequently, fish at their peak aerobic performance that are approaching exhaustion also have an elevated anaerobic metabolism, a higher blood [Lactate] and a lower blood pH (see Table 1, Jones, 1982). Despite acceleration being a good predictor for MO_2 , acceleration was a weaker predictor for blood lactate and did not significantly explain

variation in pH. Fish closer to exhaustion engage in gait transitions, such as burst-and-glide swimming, which mobilizes fast-twitching white muscle fuelled via anaerobic glycolysis, greatly contributing to the metabolic acidosis and lactate accumulation (Parkhouse et al., 1988; Peake and Farrell, 2004; Tudorache et al., 2010). Given that acceleration is simply an estimate of the average swimming activity at each water velocity setting, it does not directly reflect the locomotory behaviour of the fish. Variance of acceleration, on the other hand, could be used to significantly explain some of the variation in lactate and pH, since it exponentially increased as fish commenced to swim at an unsteady pace, incurred in bursts of swimming more frequently and became more reliant in unsustainable anaerobic metabolism (see Fig. 9). The blood acidosis attained during swimming and particularly following the acute stress caused by netting and transport, also lowers intraerythrocytic blood pH, which reduces hemoglobin-O₂ binding and may impair O₂ transport to the tissues. This leads to catecholamine-mediated activation of erythrocytic sodium-proton exchangers, which increase intracellular blood pH and cell volume, the latter being evidenced by a reduced MCHC (Nikinmaa et al., 1984; Wood, 1991). Therefore, catecholamines play an important role in maintaining O₂ transport capacity during burst swimming (Primmett et al., 1986), which explains why variance of acceleration describes some of the variation in MCHC, as acidosis during strenuous exercise is associated with catecholamine-driven erythrocyte swelling. Additionally, fish closer to exhaustion often have a larger cortisol release (Hvas et al., 2018). Accordingly, acceleration and variance of acceleration significantly explained 10 to 19%, respectively, of the variation in cortisol. Thus, while acceleration is clearly a good predictor for swimming activity and both acceleration and variance of acceleration can be used as proxies for elevated aerobic metabolism, the novel relationships with blood chemistry data presented here suggest that variance of acceleration is overall a better indicator of stress. Although, the accelerometry data cannot be used to accurately estimate the blood-borne stress-related parameters given the low percentage of variation explained, their significant relationships highlight that increases in acceleration and variance of acceleration are likely accompanied by changes in the stress-related parameters and thus may be used to infer periods of exacerbated stress. An interesting question remains whether and how the relationships between accelerometry data and blood variables related to stress are affected in more complex environments, e.g., net pens, as opposed to the controlled laboratory settings. Similarly, the length of recovery following surgery is likely to affect these relationships.

As MO₂ increases, elevated tissue O₂ delivery is sustained via a combination of two main mechanisms, summarized by the Fick equation: $MO_2 = \text{cardiac output} \times \text{arteriovenous } O_2 \text{ difference}$, where cardiac output is the product of heart rate and stroke volume, and the arteriovenous O₂ difference is the amount of O₂ extracted by the tissues per volume of blood. Blood O₂ content is influenced by partial pressure of O₂, thus, P_vO₂ can be used as a proxy for tissue O₂ extraction. Here, P_vO₂ at 0.5 BL s⁻¹ was within range of oxygen tensions previously reported (Steffensen and Farrell, 1998) and decreased with swimming speed. In contrast, heart rate increased, suggesting that the increased O₂ consumption is achieved via simultaneous elevations in tissue O₂ extraction and cardiac output. In accordance with previous studies showing good correlations between heart rate and metabolic rate (Butler et al., 2004; Clark et al., 2010; Lucas, 1994), heart rate was associated with MO₂. Still, compared to previous studies in adult Atlantic salmon, the elevations in heart rate with swimming speed are rather small [increase of 3.9 beats min⁻¹ between 0.5 and 1.5 BL s⁻¹ at 7.3 °C vs. 11.0 beats min⁻¹ at 10 °C (Lucas, 1994)]. While it is possible that our fish preferentially regulated cardiac output via changes in stroke volume (Thorarensen et al., 1996), given the negative correlation between resting heart rate and heart rate scope (Brijs et al., 2019a; Brijs et al., 2019b), it is more likely that the scope available for regulating heart rate was reduced due to an elevated routine heart rate [e.g., 59.3 at 0.5 BL s⁻¹ (current study) vs 34.7 at 10 °C (Lucas, 1994)]. Thus, a limitation of

this study is the short recovery period following surgery (≥ 18 h), since it is estimated that Atlantic salmon require at least 3 days of recovery before heart rate stabilizes, although it can often take up to 3 weeks (Føre et al., 2021; Hvas et al., 2020; Yousaf et al., 2022; Zrini and Gamperl, 2021). While intravascular cannulations allow for repeated sampling with minimal disturbance to the fish, it poses important trade-offs as the likelihood of catheter blockage due to thrombus formation increases with time (Formanek et al., 1970). Thus, for such experiments, the recovery time following surgery may need to be minimized, influencing some of the physiological parameters, such as heart rate. The need for a longer recovery period following surgery is equally reflected by the elevated [Cortisol] at the start of the swimming protocol, and the fact that some of the markers of tissue damage such as [ALP] and [AST], despite being within the range of normal values for healthy adult Atlantic salmon at any given sampling point (Rozas-Serri et al., 2022), were lowest at the sampling point furthest from surgery. Likewise, [Cholesterol] was elevated at 0.5 BL s⁻¹ and 0 post-stress, likely in part due to cortisol-induced energy mobilization (Mommensen et al., 1999), with the lowest cholesterol coinciding with the lowest [Cortisol] following 24 h of recovery from stress. Still, despite the short recovery time, the associations between heart rate and MO₂, and the strong relationship between variance of acceleration and multiple stress-related physiological variables, further reinforces the notion that ECG and accelerometry-derived data can be used as robust indicators of metabolic consequences of stress.

Intracellular acidosis has been suggested to be one of many potential drivers of delayed mortality in fish exercised to exhaustion (Wood et al., 1983). Even though it is estimated that only a relatively small proportion of the protons accumulated within the muscle cells are released into the bloodstream (Turner and Wood, 1983; Turner et al., 1983), severe blood acidosis is a reflection of a large intracellular acidosis (Wood et al., 1983). Thus, via its relationships with indirect measures of intracellular acidosis (i.e., low blood pH and MCHC, onset of burst swimming), variance of acceleration has the potential of being a useful tool for predicting post-stress mortality in fishes. In addition, we found associations between variance of acceleration and [Lactate] and, while not necessarily a causal factor of mortality, [Lactate] has been shown to be a good predictor of mortality in red snapper (*Lutjanus campechanus*, Diamond and Campbell, 2009) and high blood [Lactate] is generally found in moribund sharks (Moyes et al., 2006). Indeed, accelerometers have been proposed as useful tools for predicting post-release survivability in fish (Holder et al., 2022); here we highlight that especially variables derived from accelerometry data such as variance of acceleration, may prove particularly useful towards achieving this goal.

Following netting and transport stress, fish maintained elevated acceleration and variance of acceleration for 8.1–8.7 h. O₂ consumption rate remained elevated for some time following events of high energy demand, a phenomenon known as excess-post exercise O₂ consumption. During this period, fish consume additional O₂ to recover from the imbalances and repay the metabolic debt acquired during the exhaustive exercise (Scarabello et al., 1991; Wang et al., 1994). While pH had already been restored to routine levels at hour 1 post-stress, [Lactate] was still elevated and P_vO₂ reduced. In fact, [Lactate] may continue increasing for several hours following stress as it gradually leaks from the tissues (Tang et al., 1989). The reduced P_vO₂ is indicative of an elevated MO₂ which, in Atlantic salmon, may remain above standard metabolic rate (the minimum O₂ consumption rate to maintain basic functions) anywhere between 4 and 27 h (depending on the estimation method), although it is estimated that 64–73% of the metabolic debt is already paid within 4–6 h (Zhang et al., 2018). Recovery from exhaustive exercise has been shown to be facilitated by swimming at a low speed (Farrell et al., 2001; Milligan et al., 2000), therefore, it is possible that this elevated swimming activity during the first hours of recovery entails a behaviour-mediated mechanism to facilitate the repayment of the O₂ debt by increasing branchial ventilation and gas exchange, but this requires further investigation. Still, even though we show that most

of the metabolic imbalances measured had been corrected following 24 h, at least compared to the routine values (i.e., at 0.5 BL s^{-1}), salmon may take much longer to recover from a stressful event, if estimated via activity (> 3 days; Føre et al., 2021). Indeed, here we show that [Cortisol] was still elevated 24 h post-stress compared to estimated unstressed values for salmonids (Jepsen et al., 2001; Svendsen et al., 2021). Thus, while the plateaus in acceleration and variance of acceleration observed do not necessarily indicate that a new steady state has been reached, they might be indicative of the bulk of the homeostatic imbalances following stress having been rectified.

Interestingly, there were pronounced differences depending on the type of respirometer used, such as differences in acceleration, MO_2 , osmolality, lactate, glucose and salt concentrations. There are multiple potential explanatory factors for these differences, most relating to differences in cross-sectional area of the respirometer with respect to that of the fish, which alters the velocity of the water flowing around the fish (i.e., solid-blocking effect; Beamish, 1979; Kline et al., 2015) and to water flow dynamics within the respirometer (i.e., turbulent vs laminar flow) which may alter the swimming mode of the fish (e.g., tail locomotion). Additionally, depending on the position of the fish within the test section, water velocity may also change (Beamish, 1979). Nevertheless, these conclusions must be drawn with caution given the low sample size in the Blazka respirometer ($N = 4$). Independent of the underlying cause of the differences between respirometers, to build our algorithms, individual regressions were calculated for each individual fish and therefore the influence of the type of respirometer on the relationships of our parameters is accounted for. Furthermore, fish in the field are not generally swimming against a homogeneous flow and, therefore, the goal of our model is to approximate what a stress-related parameter might be at a given bio-logger value and not at a specific environmental condition.

5. Conclusions and future perspectives

To the best of our knowledge, this is the first study to simultaneously measure blood variables influenced by stress combined with acceleration, swimming activity, O_2 consumption and heart rate using a combination of bio-loggers and indwelling central venous cannulas in fish to evaluate their relationship. We show that variables that can be easily measured with readily available bio-logger technologies can be used to infer a posteriori a whole array of stress-related physiological parameters. These linkages between bio-logger data and physiological parameters, combined with the growing literature on the numerous applications of these systems, incentivises the continuation of the development of such biosensing tools for monitoring fish and set the foundation for their future widespread use in commercial aquaculture (Brijs et al., 2021). For example, even though bio-loggers require retrieval of the tag to access the data and therefore the information obtained using these systems can only be used retrospectively (Cooke et al., 2016), similar biosensing technologies coupled to a telemetry system allow for real-time visualization of the physiological status of the fish. Utilizing such monitoring systems in aquaculture would allow for immediate remedial measures upon events of elevated levels of stress, thus reducing negative impacts on fish welfare and productivity. This ability to gather information on the physiological status of the fish is fundamental to integrate aquaculture management within the framework of Precision Fish Farming (Føre et al., 2018). Although the relationships between parameters obtained via the bio-logger and blood sampling indicate that heart rate, acceleration and specially variance of acceleration can be used to infer periods of exacerbated acute stress, the usefulness of these parameters in assessing chronic stress remains to be studied. Furthermore, whether these parameters would be good stress indicators in situations in which exposure to a stressor induce hypoactivity or lethargy remains to be answered yet provides an interesting venue for future research.

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Author contributions

Study design: DM; Conceptualization: DM, BK, FØ, BF, REO, ES, CR, MA, NB, MF, AG; Investigation: DM, BK, FØ, REO; Data analysis: DM, BK, REO; Technical assistance: BF, ES, CR, MA, NB, MF, AG; Resources: BF, REO, MA, MF, AG; Funding acquisition: BF, REO, MA, MF, AG; Writing original manuscript: DM; Revision of original manuscript: DM, BK, FØ, BF, REO, ES, CR, MA, NB, MF, AG; Project coordination: NB; Supervision: AG.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2023.740144>.

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