

HEART RATE RESPONSIVENESS AS A PREDICTOR OF WEIGHT CHANGE IN FARMED ATLANTIC SALMON

Advancements in welfare monitoring



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Abstract

The aquaculture industry is rapidly expanding with an increasing demand of fish for human consumption, and this comes with challenges. Early and accurate predictions of stress in farmed fish is critical for improving welfare and economic return. Further advancements are needed in the sector to provide sufficient welfare monitoring, especially in large scale systems. This study was conducted on Atlantic salmon (*Salmo salar*), a promising species for recirculating aquaculture systems in Sweden, and examined the individual and combined effects of operational welfare indicators (both pre-existent and a manually inflicted fin clip) and simulated stress events common in aquaculture practices (i.e., crowding, fasting and anthropogenic disturbance) on physiological and behavioural stress indicators. The data was collected over three trials, each 14 days long, on a total of 30 individuals with surgically implanted biologgers measuring heart rate and swimming activity via tri-axial accelerometry. Heart rate was found to be an effective measurement to predict weight change; individuals who lost the most weight responded with a significantly lower heart rate compared to fish with a larger appetite. This indicates a need for stimulation in order to differentiate them. While accelerometry measurements showed less obvious effects, similar trends were observed, suggesting potential for future welfare monitoring purposes. These indicators were also correlated to behavioural observations of feeding motivation and activity from video recordings. Lastly, heart rate and accelerometry did not manage to show differences in fish based on fin clipping. Reliably connecting physiological cues to camera footage could form the basis for automatic, AI-based systems for future welfare assessment in fish, ultimately removing the need for manual evaluation.

Sammanfattning

Vattenbruksindustrin expanderar snabbt med en ökande efterfrågan på fisk för mänsklig konsumtion, och detta medför utmaningar. Tidig och korrekt detektion av stress hos odlad fisk är avgörande för att förbättra välfärd och ekonomisk avkastning. Ytterligare framsteg krävs inom sektorn för att säkerställa tillfredsställande välfärdsövervakning, särskilt i storskaliga system. Denna studie genomfördes på atlantlax (*Salmo salar*), en lovande art för recirkulerande akvakultursystem i Sverige, och undersökte de individuella och kombinerade effekterna av operativa välfärdsindikatorer (befintliga samt manuella fenklipp) och simulerade stressevent som är vanliga inom vattenbruk (dvs. trängsel, fasta och antropogen störning) på fysiologiska och beteendemässiga stressindikatorer. Data samlades in under tre försök, vardera 14 dagar långa, på totalt 30 individer med kirurgiskt implanterade *biologgers* som mätte hjärtfrekvens och simaktivitet via triaxiell accelerometri. Hjärtfrekvens visade sig vara ett effektivt mått för att förutsäga viktförändring; individer som gick ner mest i vikt reagerade med en signifikant lägre hjärtfrekvens jämfört med fisk med större aptit. Detta indikerar ett behov av stimulering för att differentiera dem. Medan accelerometriska mätningar visade mindre uppenbara effekter kunde liknande trender observeras, vilket tyder på potential för användning inom framtida välfärdsövervakning. Dessa indikatorer korrelerade även med beteendeobservationer av födomotivation och aktivitet från videoinspelningar. Slutligen lyckades inte hjärtfrekvens eller accelerometri visa skillnader hos fisk baserat på fenklippning. Att tillförlitligt koppla fysiologiska signaler till kameramaterial skulle kunna utgöra grunden för automatiska, AI-baserade system för framtida välfärdsbedömning av fisk, vilket i slutändan eliminerar behovet av manuell bedömning.

1. Introduction

1.1 Aquaculture

In order to meet the increasing demand of fish for human consumption, more fish farms are being established around the world. The global fisheries and aquaculture production of finfish experienced a 50% increase between the years 2000 and 2023, setting a record in 2023 with 189 million tonnes produced (Food and Agriculture Organization of the United Nations, 2025). The growth of the aquaculture industry is responsible for the majority of this increase, having an average growth of 5% per year and surpassing the capture production in terms of weight in 2022. According to the Swedish Board of Agriculture, the number of recirculating aquaculture systems (RAS) for food production in Sweden were 152 in 2024, compared to 53 in the previous year, which corresponds to a total aquaculture production of 9100 tonnes live weight in 2024 where >85% consist of salmonids (*Salmonidae*) (Jordbruksverket, 2025).

More companies are investing in research and establishing new facilities and techniques to ensure future, sustainable production (Sarosh et al., 2024). Smögenlax Aquaculture AB is one of them, aiming at producing 6000 tonnes of Atlantic salmon (*Salmo salar*) per year in RAS by 2030 (Andersson, 2025). From a sustainability perspective, one advantage of RAS is the reduced risk of disease transmission compared with off-coast farms. As recently as February 2026, an outbreak of the contagious IPN virus occurred at an off-coast fish farm in Malgomaj, Sweden (Bergström, 2026). The event resulted in the slaughter of 600 tonnes of fish, that were otherwise healthy, to prevent further spread and caused large economic losses for the farmers.

1.2 Welfare monitoring

With rapidly growing, industrial fish farming comes challenges related to animal welfare. During the last decade, these issues have raised increasing concern in both the public and scientific scene, and the importance of promoting good animal welfare for fish is becoming a critical subject (Brown, 2015; Iversen et al., 2018; Martins et al., 2012; Stien et al., 2020). There is extensive evidence for the capacity for fish to experience pain and suffering (Nilsson et al., 2019; Sneddon, 2015; Sneddon, 2019) and their cognitive function is receiving more attention. Fish are included in the Swedish Animal Welfare Legislation (SFS 2018:1192, 2 §), but the delayed recognition of their sentience and multitude of farmed species in comparison to other vertebrates has put them behind in species-specific welfare research. Further advancements are needed in the aquaculture sector to provide sufficient welfare monitoring, especially in large scale systems (Maia et al., 2024; Martins et al., 2012; Saraiva et al., 2019).

There is currently no universal definition of animal welfare, including fish, however there are several interpretations of it that complement each other. Stien et al. (2020) describes animal welfare as the quality of life as perceived by the animal itself, but this approach can be seen as subjective and inaccessible to scientific enquiry. Other approaches include functional, nature- and feeling-based ones. The functional definitions of animal welfare revolve around biological functioning, whereas the nature-based definitions focus on the animal's ability to express natural behaviours, and feeling-based definitions emphasize the animals emotional state (Iversen et al., 2018; Martins et al., 2012).

Welfare monitoring on farms today mostly consists of individual removal from the water and sedation for visual evaluation based on predetermined protocols. This analysis is not only resource intensive, but also exposes the fish to stress and risks providing a misleading picture of the health

status at the farm with insufficient sample sizes (Cook et al., 2015; Martins et al., 2012). The process is also limited in time as it only allows for snap shots of the situation, i.e., during the examination window, which may not be representative of overall health. Furthermore, several individuals of the same species may also exhibit divergent coping mechanisms: a stressor being tolerated by one individual may be detrimental to another (Martins et al., 2012). This highlights the importance of advancing the field to provide continuous monitoring on an individual level, which could result in a more accurate and comprehensive picture of the welfare status of each fish and allow the farmer to respond to problematic indicators before they become detrimental.

1.3 Operational welfare indicators

Operational Welfare Indicators (OWIs) are widely used to evaluate fish health and welfare because they provide practical, measurable signs of an animal's condition that can be assessed directly on farms or during experiments (Iversen et al., 2018). Most often, these are used as a standardized way to assess the welfare of fish through different physical indicators, such as damage to scales, fins, and eyes, by providing information about potential welfare problems and their causes, as well as serving as a basis to implement interventions (Iversen et al., 2018; Martins et al., 2012). However, these physical indicators may have limited sensitivity, are often subjectively assessed and may only detect problems once welfare has already deteriorated. Therefore, they should ideally be used in combination with behavioural and environmental OWIs, as well as physiological laboratory welfare indicators (LABWIs). In line with this, there is a need to develop more extensive OWIs for behaviour (e.g., movement patterns and feeding activity), environment (e.g., water quality, stocking density, parasite levels) and physiology (e.g., stress hormones, heart rate).

Behavioural analysis through video surveillance can turn qualitative assessment into quantitative measures to detect early signs of stress in a non-invasive way (Føre et al., 2018; Martins et al., 2012; Stien et al., 2020; Wiese et al., 2023). Although showing promising potential, video alone is currently insufficient to characterize the status of the fish. Before video-based welfare assessment can be relied upon, there is a need to establish which behavioural and physiological indicators are associated with different welfare-states and how these indicators are reflected in video recordings. In other words, the physiological and behavioural basis of fish welfare first needs to be understood and linked to observable video cues before video surveillance can be used as a reliable standalone assessment tool. It could then form the basis for automatic, AI-based systems for future welfare assessment in fish, ultimately removing the need for manual evaluation (Føre et al., 2018).

1.4 Stress response in fish

Common stressors for farmed fish include poor water quality (Marino, 2008; Wiese et al., 2023), physical injuries (Wiese et al., 2023), handling, transportation, incorrect stocking densities and disease (Jones & Sloman, 2024). The stress response in fish consists of physiological, biochemical and behavioural adaptations to overcome the stressor that threatens homeostasis (Damsgård, 2008). This response is initiated through the release of the catecholamines adrenalin and noradrenalin, as well as the glucocorticoid cortisol (Wendelaar Bonga, 1997). These are stress hormones which elicit the secondary stress response where energy is redirected from essential functions like digestion to depletion of glycogen stores. The subsequent increase of glucose and lactate in the blood, as well as increased circulation, help deal with the stressor.

Jones and Sloman (2024) argue that a certain amount of environmental challenge might be beneficial to the fish, resulting in eustress: an amount and duration of stress that allows the fish to maintain homeostasis, so that welfare is not threatened. This can promote neurogenesis and encourage learning on how to handle certain stressors in the future. Stress is often referred to as a negative response, but from an allostasis-based approach to welfare, too low levels of environmental variation may cause hypo-stimulation and boredom, which in turn decreases welfare (Korte et al., 2007; Jones & Sloman, 2024). On the other hand, prolonged, frequent or high intensity stress can lead to tertiary stress responses, which inhibits growth and immune function, as well as makes the fish more susceptible to diseases (Damsgård, 2008; Opinion et al., 2023; Wendelaar Bonga, 1997). Chronic stress can also cause a depression-like state in fish, where reduced feeding intake (or anorexia) is a characterizing behaviour (Vindas et al., 2025), further stunting growth (Leal et al., 2011; Wendelaar Bonga, 1997). This can be detrimental to fish health and potentially fatal, but also has a negative economical impact for the farmer due to reduced slaughter yields. Thus, promoting good animal welfare and preventing high allostatic loads are important from several aspects.

2. Aim

The aim of this project is to aid in the development of non-invasive methods for comprehensive monitoring of health and welfare in Atlantic salmon, a promising species for RAS in Sweden. This was done using a combination of video and advanced data analysis, and linked these to assessments of behavioural and physiological cues. The main objective was to identify and evaluate behavioural and visual indicators that can be detected through video observations and linked to physiological and welfare-related states in Atlantic salmon. In relation to this, heart rate and tri-axial accelerometry logging was evaluated as a method to identify fish who suffer from commonly observed fin damage in aquaculture. As an extension, the long-term goal is to utilize AI and machine learning for welfare assessment, with data from this project forming the foundation. A system that can evaluate fish health based on OWIs in an automatic and non-invasive way could reduce stress and subjectiveness in the visual evaluation, as well as be more standardized and consistent, while reducing dependencies on manual labour and thus improving staff safety in RAS facilities. By developing new methods that can provide more comprehensive information about the fish well-being, the farmers could detect problems in an early stage and implement measures.

3. Materials and methods

3.1 Experimental animals and housing conditions

Female Atlantic salmon with body masses ranging between 495 and 1450 g (mean $\pm$ SEM 839 ± 39.2 g, N=30) were obtained from Smögenlax Aquaculture AB. The salmon were housed for at least two weeks in 525 L tanks receiving aerated, recirculating seawater at 11.9-12.4°C on a 12:12 h light:dark photoperiod. They were initially fed twice a day with dry commercial salmon pellets (Skretting ® RCX 300-50A, Stavanger, Norway) and as appetite grew stronger, the feeding rate was reduced to once per day. Cleaning was performed on a daily basis by flushing the tanks for 10 s.

3.2 Experimental arena

The experimental part of the study was conducted at the University of Gothenburg in Natrium, Medicinaregatan, Gothenburg, Sweden between 6th of March and 24th of April 2026. Upon surgery (see 3.3.1 Surgery and identification of operational welfare indicators) the fish were transferred to an experimental tank (4.2 m; 2800 L) for a period of three weeks. The salmon received recirculating, aerated seawater at 11.9-12.4°C and were kept on a 12:12 h light:dark photoperiod (07:00-19:00;19:00-07:00). The experimental tank was divided into two sections. One section constituted the experimental arena (1.9.2 m; 1330 L; Figure 1) where data collection occurred, while the other section (1.7.2 m; 1190 L) constituted a recovery site where surgically instrumented salmon of upcoming trials were placed during one week of recovery. Wire fencing was put up around the tank to prevent the fish from potentially jumping out and a panel was installed in the middle of the experimental arena to provide an appropriate background for underwater footage. This panel contained large cutouts, allowing the fish to move between the two sides of the experimental arena. The salmon was fed dry commercial salmon pellets (Skretting ® RCX 300-50A, Stavanger, Norway) once per day at 11:30, starting with a burst feeding of 10 g (feeding three to five pellets at a time in a fast pace) to catch their attention and then continuing with one-two pellets at a time until visual satiation was reached (ranging between 10-85 g). Removal of faeces, uneaten food and other debris was performed daily after the measurement window for heart rate and accelerometry logging (see 3.3.4. Recordings) to maintain good water quality. Animal care and experimental procedures were in accordance with national regulations and covered by an ethical permit (5.8.18-04990/2023) approved by the ethical committee on animal research in Gothenburg, Sweden.

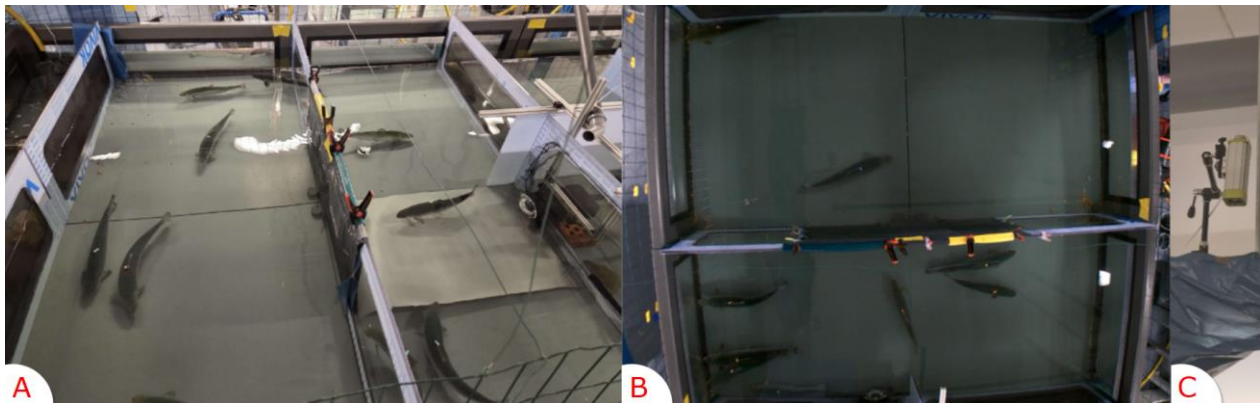


Figure 1. (A) Atlantic salmon in experimental arena with the underwater camera visible on the right side. (B) View from camera above experimental arena. (C) Camera above experimental arena.

3.3 Data collection

The data was collected in three separate trials, each including ten individuals of Atlantic salmon and running for 14 days. Fin clipping, as described in Roques et al. (2010), was selected as a target OWI of impairment, because fin damage is one of the most prevalent injuries observed in land-based fish farms (Noble et al., 2012), and was performed on half of the individuals. The rest had a mock fin clip performed as a placebo treatment (i.e., handling only) and acted as an experimental control group. No individuals in trial one were fin clipped, whereas all individuals in trial two and half in trial three were fin clipped. All individuals were marked with, for the trial, unique combinations of colored beads for identification purposes.

3.3.1. Surgery and identification of operational welfare indicators

Individual fish were netted and anesthetized in seawater with 150 mg·L⁻¹ MS-222 (Pharmaq AS ® Tricaine methanesulfonate, Overhalla, Norway) until regular ventilation ceased and the fish was deemed as insensible, while being controlled for OWIs. Detected OWIs were graded in line with *FISHWELL Morphological Operational Welfare Indicators (OWI's) for farmed Atlantic salmon v1.1* found in Iversen et al. (2018) and included scale loss, opercular damage/deformities, cataracts, eye pop, eye bleed, snout damage, fin erosion, fin deformities, jaw deformities, red/blood spots and wounds. Length and weight were obtained before transferring the fish to a surgical station (Figure 2A/B).

During the surgery, the fish was positioned in a bath of 10°C seawater and was continuously irrigated through the mouth and over the gills with 10°C seawater containing 75 mg·L⁻¹ MS-222 to maintain surgical anaesthesia. A ~4 cm incision was made in the middle part of the abdomen with a scalpel and a biollogger (Star-Oddi ® DST milli-HRT ACT, Garðabær, Iceland) was inserted into the peritoneal cavity, close to the pericardium, securing it with an anchor stitch anterior to the incision (Figure 2A). The incision was then closed with three stitches using 3.0 prolene sutures (Ethicon Inc ® Prolene, Somerville, New Jersey, USA) and the wound was treated with povidone-iodine powder (Vetofish ® Châteauneuf-les-Martigues, France) for wound healing. The fish was turned on its belly and a unique combination of coloured plastic beads for individual identification were attached with sutures ~1 cm in front of the dorsal fin (Figure 2B). When applicable, a fin clip was performed with scissors (Figure 2C). With the surgery complete, the fish was moved to a bucket with aerated seawater to promote adequate recovery and oxygen intake. When needed, extra water flow from a hose was used to irrigate the gills. Once the fish recovered from the anaesthesia (identified by visual inspection of regained ventilation and movement), it was transferred into the recovery section of the experimental tank for one week before data collection started.

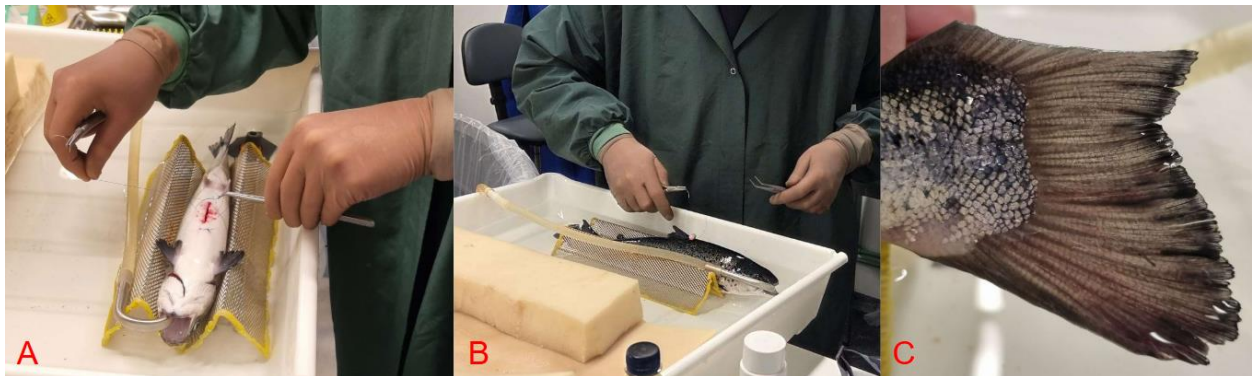


Figure 2. Pictures showing wound closing after surgical biollogger implantation (A), bead attachment (B) and caudal fin clip (C) on an individual Atlantic salmon (*Salmo salar*).

3.3.2. Simulated events

Three separate, potentially stressful, routine farming procedures were simulated during each trial. The first one (crowding) occurred on day 6. A person entered the tank at 10:50 and herded all the fish into one side, where they were then restricted by a barrier from returning to the other side until 11:15. During this time, the person remained in the tank for maintenance simulation. The barrier was then removed and the person exited the tank, leaving the fish undisturbed, before entering the

tank again between 11:30-11:35. The second event, feed-withdrawal, was performed on days 9 and 10, by fasting the fish for a total of 72 h. In aquaculture, this is typically performed prior to stressful procedures or can occur as a result of clogged feeding tubes. The third event (anthropogenic disturbance) occurred on day 13. A group of 13-15 people entered the room at 10:50, remaining there while moving in close proximity and maintaining a high volume around the tank until 11:05. This was performed to simulate an investor or student visit, a common practice in aquaculture facilities.

3.3.3. *End sampling and blood analyses*

The experimental end point for all respective trials occurred on day 15 from the start of data collection, 21 days after surgery. The fish were euthanized by blunt force through sharp cranial blows to avoid potential effects on blood parameters from overdosing with anesthesia. Blood (2 mL) was then sampled with heparinated syringes from the caudal vein. Hemoglobin (HemoCue® Hb 801, Ängelholm, Sweden), lactate (Arkray® Lactate Pro 2, Kyoto, Japan) and glucose (Abbott Laboratories® Freestyle Lite, Chicago, USA) was measured directly after sampling to prevent enzyme activity to alter the parameters. Microcapillary tubes were filled with blood and centrifuged in a microcapillary centrifuge (Hettich® Haematokrit 210, Tuttlingen, Germany) for 5 min at 10000 rcf before measuring hematocrit (Hawksley® Micro Hematocrit Reader, London, England). The remaining blood was centrifuged for 5 min at 10000 rcf (Eppendorf® 5415D, Sigma-Aldrich Sweden AB, Stockholm, Sweden) before the plasma was transferred to 1 mL tubes and kept frozen (-80°C) until plasma cortisol analysis. A picture of each individual was taken on a solid, white background with an ID note beside. Weight and length were obtained for each fish, OWIs were noted (see 3.3.1 Surgery and identification of operational welfare indicators) and the biologger implant was removed from the abdomen for data extraction.

Plasma cortisol concentrations were measured using a radioimmunoassay (RIA) described by Young (1986), with the cortisol antibody validated by Sundh et al. (2011). In short, sheep cortisol antibody (Guildhay Ltd® Guildford, Surrey, UK, no longer in activity) was used. Hydrocortisone [1,2,6,7-3H (N)] (NEN Life Science Products® NET 396, Boston, USA) was used as a tracer and the standard was prepared using hydrocortisone (Sigma-Aldrich® St. Louis, USA). Radioactivity was measured using a β -counter (Wallac® 1409 Liquid Scintillation Counter, Turku, Finland). Samples below the detection limit (N=3) were assigned the value of the detection limit (0.5 ng·mL⁻¹).

3.3.4. *Recordings*

Heart rate at 200 Hz and tri-axial accelerometry at 10 Hz was measured by the surgically implanted biologgers for 15 s every two minutes and measurements occurred daily between 11:00-14:06 in seven minute sessions every half hour. A camera (Basler® Ace 2 Basic, Ahrensburg, Germany) was installed above the tank (Figure 2B/C) to perform scheduled video recordings for manual behaviour assessment. The different scheduled recordings were programmed by Svenska Miljöinstitutet (IVL) to override each other with priority in the order they are mentioned: Stress event sessions on day 6 and 13 (10:50-11:29); daily feeding sessions (11:30-12:28); daily biologger synchronization in seven minute intervals every half hour (10:59-14:06); daily intermittent 2.5 minute sessions every half hour during the light period. A camera (TiVA AB® TiVA UVC,

Kalmar, Sweden) positioned under the water surface (Figure 1A) was also installed to take pictures of passing fish for OWI detection, but was mainly used by IVL outside the scope for this project.

3.4 Manual behaviour assessment

Manual behaviour assessment was performed on the video material recorded during the first ten minutes of the daily feeding sessions (11:30-11:40) during day 8-12. The fish were graded individually, logging the highest level observed in terms of feeding motivation, aggression and activity (Table 1). The grading system was created specifically for this project and the behaviours chosen for analysis were based on easily distinguishable motifs on camera that could be indicative of poor health and welfare (Martins et al., 2012).

Table 1. Grading template for manual behaviour assessment in Atlantic salmon.

PARAMETER	LEVELS	GRADING
Feeding motivation	NA, 0-2	NA, no feeding performed; 0, no feeding attempted; 1, some feeding attempted; 2, actively feeding
Aggression	0-2	0, no aggression displayed; 1, some displays of aggression (e.g., chasing other individuals once or twice); 2, repeated displays of aggression (e.g., chasing other individuals several times)
Activity	0-2	0, still; 1, swimming; 2, high speed swimming/darting

3.5 Statistical analyses

Statistical analyses were performed in RStudio. The raw biologger data was filtered to remove faulty measurements, only keeping data where quality index > 2 and 100 > heart rate > 20. Implausible weight calculations where individuals were reported having lost > 20% of their body weight, most likely due to scale calibration errors, were set to not applicable (N=2). Repeated heart rate and accelerometry measurements from the same individual are dependent on each other and can cause type 1 errors of false significance due to inflated p-values if not considered. Thus, linear mixed models were used to test how response variables changed with different predictors (fixed effects) while accounting for repeated measurements within individuals and grouping structure (random effects). That way, each individual received their own baseline. The significance of fixed effects was assessed using ANOVA. This was performed to test the effects of trial, treatment (i.e., fin clip or placebo) and health index (sum of OWI scores) on mean heart rate and accelerometry, as well as difference in mean heart rate and accelerometry between 11:00- and 11:30-measurements. The effects of the same parameters were also tested on relative weight change with a linear model and ANOVA, not having to account for repeated measurements.

Linear regressions were made to evaluate the relationship between relative weight change and mean heart rate and accelerometry, as well as difference in mean heart rate and accelerometry

between 11:00- and 11:30-measurements. The variables were tested separately against relative weight change and performed for data from day 10, 11 and on averages across all 14 days. Lastly, Shapiro-Wilk tests were used to test for normality in the distribution of relative weight change from surgery to end sampling for both treatment groups. Due to non-normality in both distributions (fin clip: $W=0.732$, $p<0.001$; control: $W=0.820$, $p<0.01$), a Wilcoxon rank-sum test was performed to test for differences in relative weight change between them.

4. Results

4.1 Blood parameters, manual behaviour assessment and weight change

Results (raw data) from blood analyses (Table A4), manual behaviour assessment (Table A5), as well as weight and fork length changes (Table A2) are presented individually for each fish in Appendix 1.

4.2 Heart rate and accelerometry

4.2.1 Effects of treatment, trial and health index

Results (raw data) of operational welfare indicators and health index are presented individually for each fish in Appendix 1 (Table A3). Small sample sizes per OWI and the fact that all fish were affected by several (except for one who had no observed OWIs; Table A3) made it difficult to get conclusive results about their individual effects on physiological parameters. There were no differences in activity score between treatments, although a significant effect of trial and treatment was found on blood variables. No significant difference in relative weight change between the treatment groups was found ($W=158$, $p>0.05$).

Table 2. Effects of treatment, trial and health index on mean heart rate (HR) and accelerometry (AvgEA), difference in heart rate and accelerometry between 11:00- and 11:30-measurements, and relative weight change.

Response variable	Fixed effect	F-value	Df1	Df2	P-value
Mean HR	Treatment	1.099	1	22	0.306
Mean HR	Trial	0.599	2	22	0.558
Mean HR	Health index	1.656	4	22	0.196
Mean AvgEA	Treatment	0.007	1	22	0.935
Mean AvgEA	Trial	0.728	2	22	0.494
Mean AvgEA	Health index	0.941	4	22	0.459
Δ HR	Treatment	0.406	1	21.68	0.531
Δ HR	Trial	2.434	2	21.74	0.111
Δ HR	Health index	0.944	4	21.59	0.458
Δ AvgEA	Treatment	0.147	1	21.74	0.706
Δ AvgEA	Trial	0.345	2	21.84	0.712
Δ AvgEA	Health index	0.919	4	21.58	0.471
Relative weight change	Treatment	1.015	1	-	0.326
Relative weight change	Trial	1.421	2	-	0.265
Relative weight change	Health index	0.732	4	-	0.581

No evidence for effects of treatment, trial or health index was found on daily mean heart rate or accelerometry (Table 2). Similarly, there were no differences found between treatment, trial or health index group on mean heart rate and accelerometry between the 11:00- and 11:30-measurements (reflecting prior to and during feeding on most days; Table 2).

4.2.2 Heart rate, accelerometry and weight change group

Weight change groups are presented individually for each fish in Table A2, with categorization described in Table A1, based on weight change from surgery to end sampling. Although no significance was found for differences within weight change groups, both the crowding and visit event showed tendencies for elevated heart rate in comparison to a baseline (Figure 3). The great loss group had the largest standard error during all three measurements, indicating larger individual differences in heart rate response within the group.

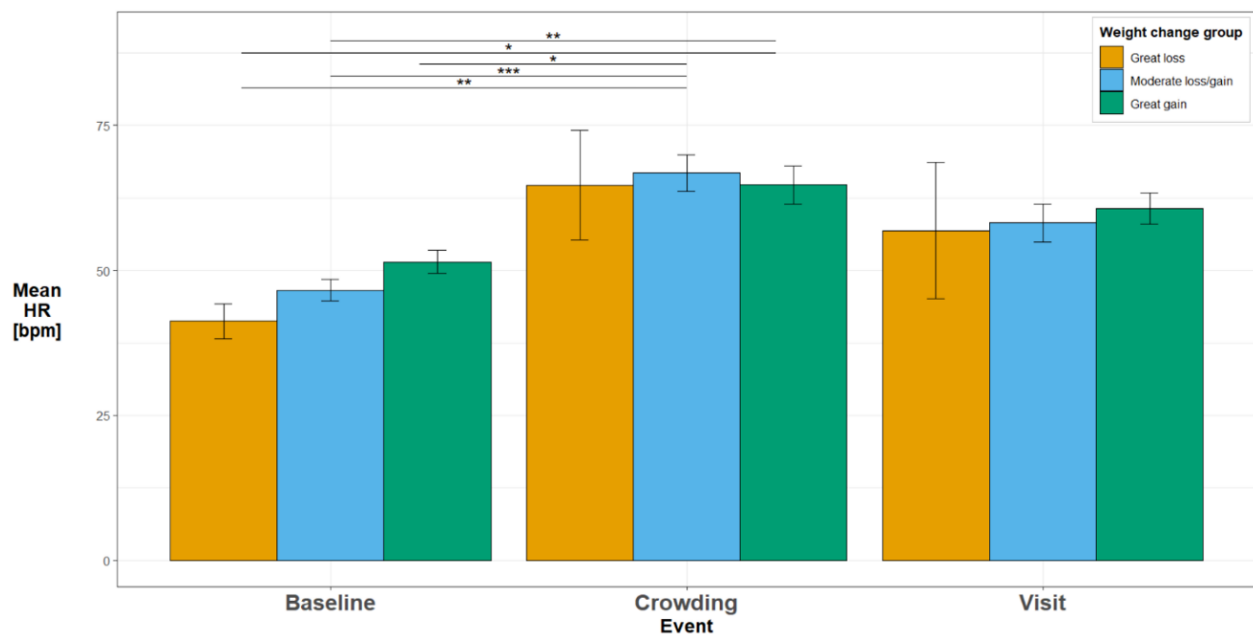


Figure 3. Average heart rate during 11:00-measurements on day 5 (baseline), 6 (crowding/maintenance simulation) and 13 (visit/anthropogenic disturbance) in Atlantic salmon (N=28), categorized in groups “great loss” (N=4), “moderate loss/gain” (N=13) and “great gain” (N=11) based on weight change over 21 days. Error bars represent standard error. Significance: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Average heart rate across the 14-day period varied between weight change groups, where the great loss group distinguished itself most prominently with a lower value (Figure 4A). Average accelerometry across the same time period exhibited substantial overlap in the group’s distributions (Figure 4C). Average heart rate remained elevated on days where feeding occurred in both the great gain and moderate loss/gain groups, while decreasing gradually during fasting (Figure 4B). The great loss group did not display as drastic changes in average heart rate. Average accelerometry displayed similar patterns for the great gain and moderate loss/gain groups, being generally lower on fasted days, while the great loss group fluctuated more (Figure 4D).

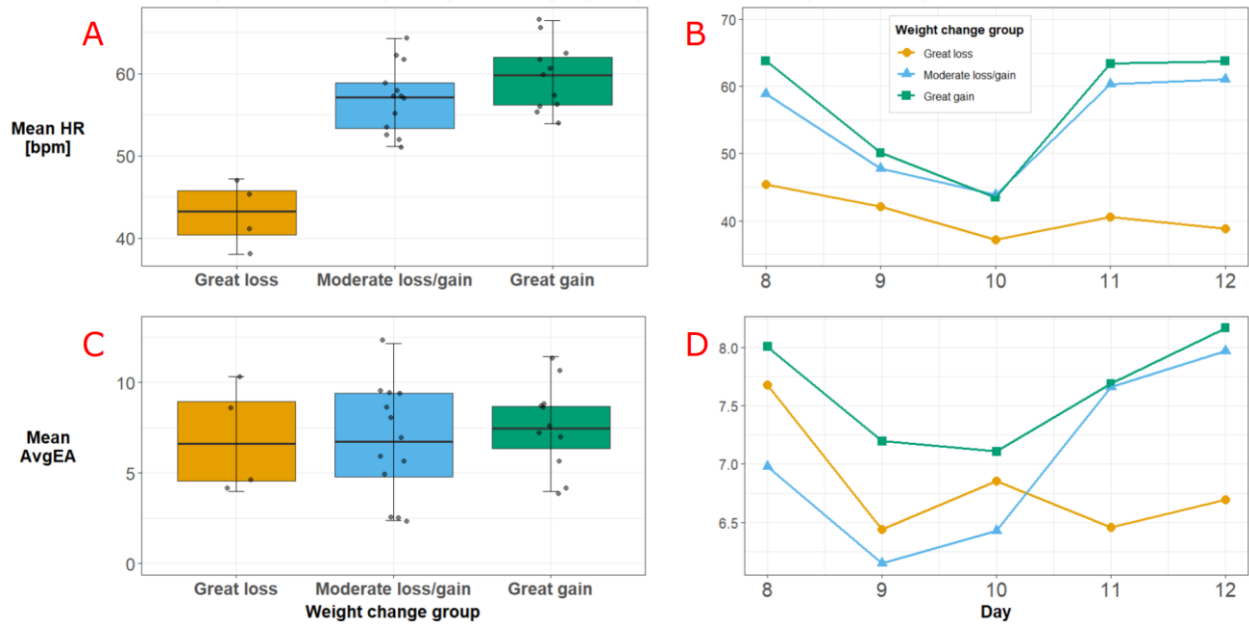


Figure 4. Average heart rate and accelerometry over 14 days (A, C) and days 8-12 (B, D) in Atlantic salmon (N=28), categorized in groups “great loss” (N=4), “moderate loss/gain” (N=13) and “great gain” (N=11) based on weight change over 21 days. Feed was withheld on days 9-10. Please note that all axes do not begin at 0.

4.2.3 Heart rate and relative weight change

Strong evidence that mean heart rate difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) across 14 days increased with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon by 0.956 bpm per % body weight increase ($\pm SE=0.2833$, $t=3.376$, 95% CI 0.374 to 1.539, $p=0.002$, $R^2=0.30$) was found (Figure 5).

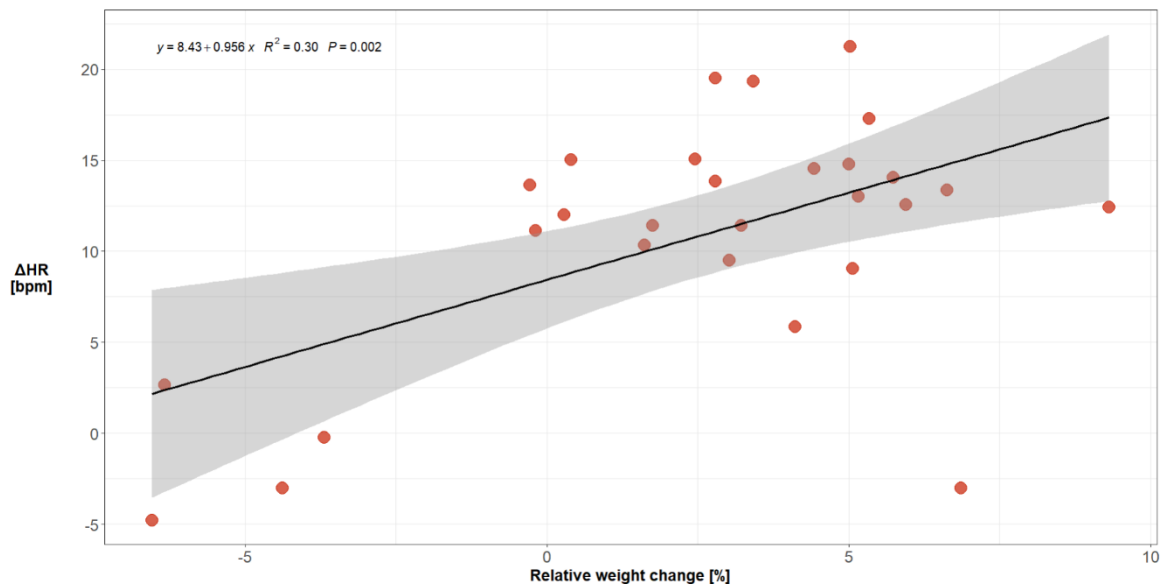


Figure 5. Relationship between average heart rate difference between 11:00- and 11:30-measurements across 14 days (reflecting prior to and during feeding on most days) and relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon (N=28). The ribbon represents 95% confidence interval. Please note that the axes do not begin at 0.

No evidence that mean heart rate difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) on day 10 changed with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon ($\pm\text{SE}=0.2974$, $t=0.133$, 95% CI -0.573 to 0.652, $p>0.1$, $R^2<0.01$) was found (Figure 6A).

Strong evidence that mean heart rate difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) on day 11 increased with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon by 2.32 bpm per % body weight increase ($\pm\text{SE}=0.8514$, $t=2.727$, 95% CI 0.568 to 4.075, $p=0.012$, $R^2=0.23$) was found (Figure 6B).

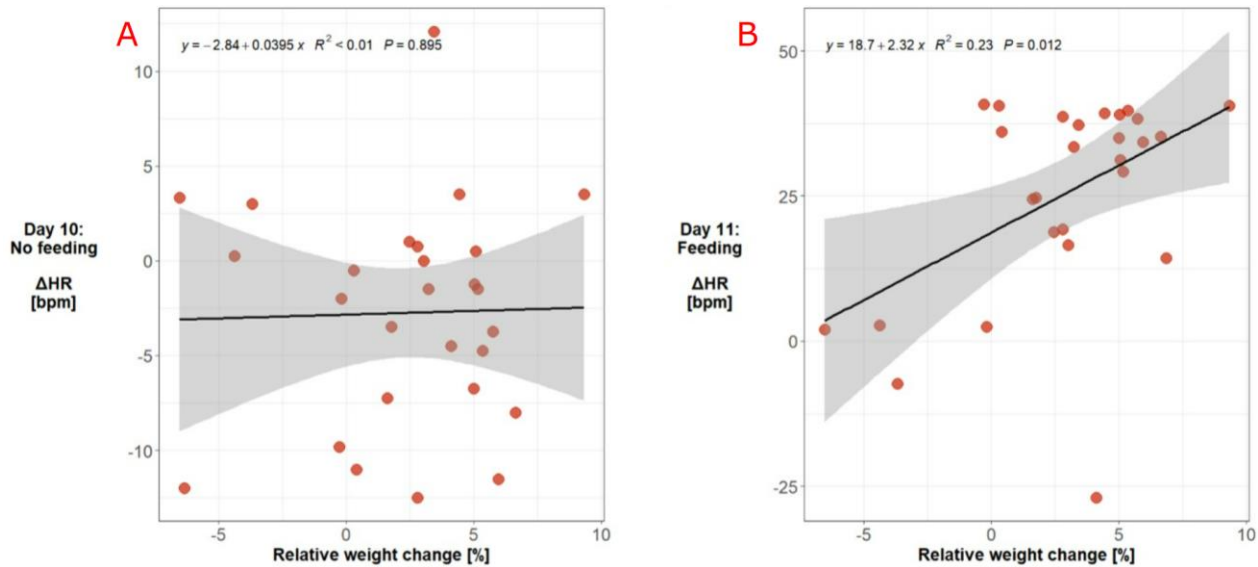


Figure 6. Relationship between average heart rate difference between 11:00- and 11:30-measurements and relative weight change (measured over 21 days, starting 7 days before data collection) on a fasted day (A) and a day where feeding occurred at 11:30 (B) in Atlantic salmon ($N=28$). The ribbons represent 95% confidence interval. Please note that the axes do not begin at 0.

4.2.4 Accelerometry and relative weight change

Very strong evidence that mean accelerometry difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) across 14 days increased with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon by 0.230 per % body weight increase ($\pm\text{SE}=0.0613$, $t=3.756$, 95% CI 0.104 to 0.356, $p<0.001$, $R^2=0.35$) was found (Figure 7).

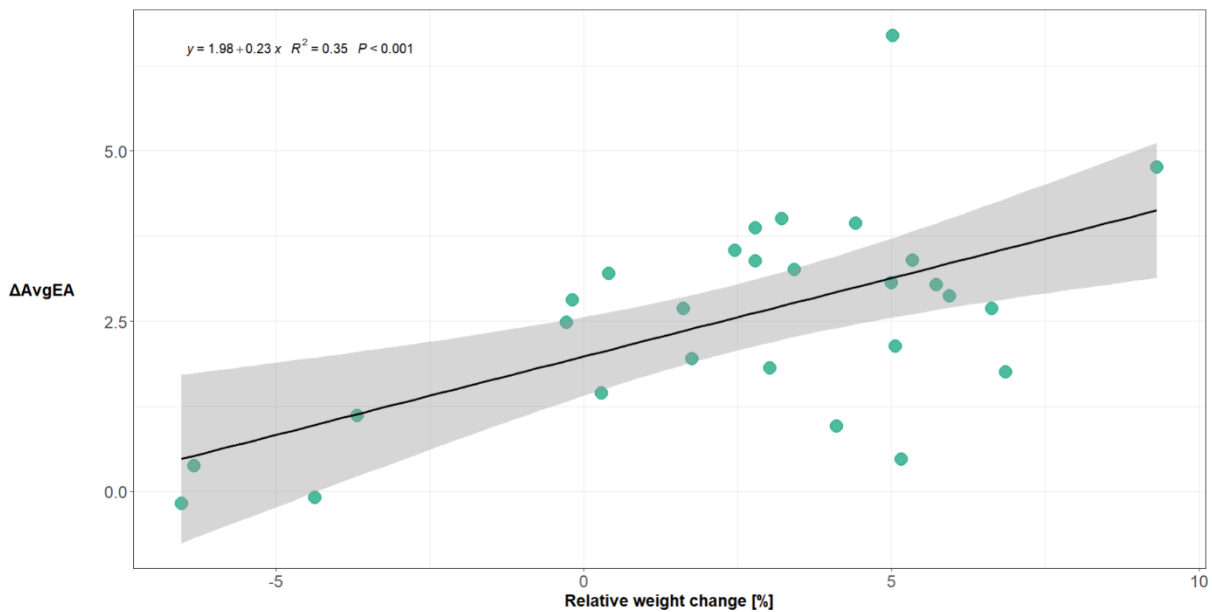


Figure 7. Relationship between average accelerometry difference between 11:00- and 11:30-measurements across 14 days (reflecting prior to and during feeding on most days) and relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon ($N=28$). The ribbon represents 95% confidence interval. Please note that the axes do not begin at 0.

No evidence that mean accelerometry difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) on day 10 changed with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon ($\pm SE=0.2974$, $t=0.133$, 95% CI - 0.573 to 0.652, $p>0.1$, $R^2<0.01$) was found (Figure 8A).

Weak evidence that mean accelerometry difference between 11:00-window (11:00-11:06) and 11:30-window (11:30-11:36) on day 11 increased with relative weight change (measured over 21 days, starting 7 days before data collection) in Atlantic salmon by 0.464 per % body weight increase ($\pm SE=0.2330$, $t=1.992$, 95% CI -0.016 0.944, $p>0.05$, $R^2=0.14$) was found (Figure 8B). While no statistical significance was found, there was a tendency for elevated accelerometry difference with relative weight change.

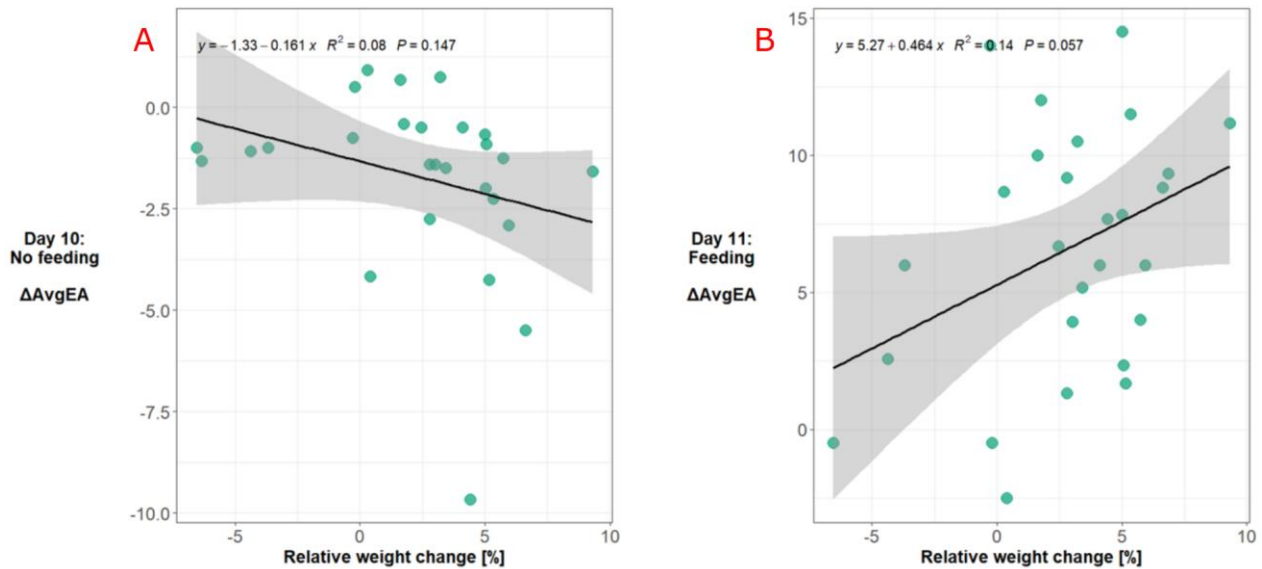


Figure 8. Relationship between average accelerometry difference between 11:00- and 11:30-measurements and relative weight change (measured over 21 days, starting 7 days before data collection) on a fasted day (A) and a day where feeding occurred at 11:30 (B) in Atlantic salmon ($N=27$). The ribbons represent 95% confidence interval. Please note that the axes do not begin at 0.

5. Discussion

5.1 Operational welfare indicators

Although we found no evidence for an effect of health index on heart rate, accelerometry or relative weight change (i.e., no significant differences between individuals based on the amount of morphological OWIs), future studies may benefit from weighting OWIs based on their severity or biological relevance, as the present index treated all indicators equally. For example, a level two cataract might have affected fish more than a level two scale loss, but they would end up contributing to the health index the same amount. This compromises any possibility to understand and conclude anything about the extent to which the different OWIs may have contributed to their overall well-being. Considering this, no definite conclusions can be drawn about the effect of OWIs on the measured physiological parameters in this study. However, these results do not imply that the fish had not been affected at all, but rather that the investigated parameters failed to reflect it. Further research on that subject would therefore be recommended to include other parameters (e.g., the effect of scale loss on immune function or osmoregulatory capacity). For the goal of automatic, camera-based welfare assessment, the chosen parameters failed to reflect the injuries on the fish, including the fin clip, perhaps since most of them were of low scores and possibly even tolerable at these levels.

5.2 Relative weight change

Substantial weight loss has been previously identified as an indicator of poor health or mental state in fish, which subsequently can impact both welfare and economy negatively (Leal et al., 2011; Martins et al., 2012; Wendelaar Bonga, 1997). Days where feeding occurred were more successful at highlighting individual differences in heart rate and accelerometry response, thus indicating a need for stimulation for differentiation purposes. Fish with less appetite displayed a lower response in heart rate and accelerometry during feeding and were also the ones who lost the most weight

during the experiment. Differentiating individuals based on physiological parameters such as these, and potentially being able to predict which ones might be in poor shape or losing weight, is a significant step on the way to comprehensive and automatic welfare monitoring. Average heart rate and accelerometry means and differences across all 14 days of data collection also showed potential in weight differentiation. This is likely due to large daily differences in response during feeding. All fish experienced an elevated heart rate compared to baseline during the crowding event; however, it reached a roughly equal level over all weight change groups. Similarly, the elevated heart rate during the anthropogenic disturbance was not significantly different between weight change groups, indicating the responsiveness during feeding as the primary predictor of weight change.

The anthropogenic disturbance event did not elicit as strong of a response in heart rate as the crowding event, which could indicate it as a less stressful experience for the fish. Crowding may be interpreted as a more immediate danger, but there is also a question of whether the fish had acclimatized to humans. The laboratory space where the fish were held was shared with other researchers, who from time to time moved in close proximity to the tank and generated noise. Thus, the reaction from the visit simulation could possibly be more in line with eustress, and in that case, according to Jones and Sloman (2024), may even have beneficial effects for fish by combatting hypo-stimulation from a lack of variation in the environment. Commercial fish in RAS are generally not acclimatized to human interaction or presence, but regular visits from investors or students may lead to it, leaving it as a positive experience rather than a negative one. Considering that, the findings from this study encourage RAS facilities to host more frequent visits for educational purposes to enhance general knowledge about the farming practice.

5.3 Manual behaviour assessment

The manual behaviour assessment was mainly used as a supporting measure to investigate different behaviours and how they correlated with the physiological parameters. Fish with lower feeding motivation and activity scores tended to lose more weight and experienced a similar trend for heart rate and heart rate responsiveness. Aggression was relatively rare and typically displayed by the same few individuals, hence not much can be concluded about it from this study. A realized limitation of the grading template was that many individuals received a similar score, especially for the feeding motivation and activity parameters. This was not due to them behaving the exact same way during the whole session, but rather based on that the highest observed level was graded. For example, two fish receiving a level two for feeding motivation could spend the majority of the session behaving differently. More detailed logging of observed behaviours (e.g., grading them in several intervals per session or incorporating more levels in the grading template) would have been preferable to further distinguish behavioural differences. More intricate behaviours (e.g., gill/opercular rate) would also be beneficial to include in the assessment, but limited camera resolution prevented us from doing this. Overall, the manual behaviour assessment was not used to conclude findings due to its subjectiveness, but the insights gained showed potential for camera footage as a component in future welfare assessment.

5.4 Blood parameters

Blood was sampled on day 15 when data collection finished. A single sampling at the end of the experiment can be useful for detecting chronic stress, but at the same time limiting as it mainly provides information about the fish current health status. It could not provide information about

how they reacted to the stress events since the last one occurred on day 13 and showed a relatively mild response, leaving the fish time to recover physiologically and reinstate homeostasis (Wendelaar Bonga, 1997). Continuous measurements of blood parameters would have been preferable when testing for individual effects of the stress events, but was not possible since the sampling itself is a stressful and invasive procedure; it would require the fish to be netted out of the water and anesthetized, which they need to recover from afterwards (Cook et al., 2015). Insufficient recovery time before being subjected to another stressor can be harmful for the fish and act as a confounding effect for the next sampling (Brijs et al., 2019; Hjelmstedt et al., 2020). Repetitive sampling of blood from the same fish is also not ideal as it can cause injuries from handling or infection from puncturing. Furthermore, potential confounding time effects from blood sampling being halted for a period of time after the first fish in trial one compromised data, leaving only 20 viable samples from the remaining trials. Since that group contained 10 out of 15 individuals without a fin clip, we were left without an appropriate sample size for a treatment control group; following tests on the effect of treatment on blood parameters would not be statistically reliable.

In presumably healthy, adult Atlantic salmon living in seawater, some reference intervals include 53-104 g·L⁻¹ hemoglobin, 28.0-48.0% hematocrit, 2.8-17.9 mmol·L⁻¹ lactate and 2.8-10.1 mmol·L⁻¹ glucose (Keitel-Gröner et al., 2025). Since most measurements from trial two and three fall within these reference intervals and the timing of sampling makes it difficult to get conclusive results, we were not able to corroborate the assumption that some of the fish might have been experiencing chronic stress. Lactate was the parameter that contained the most measurements outside the reference interval (N=12). These values were below the interval limit, which is rather an indicator that the muscle tissue is receiving adequate oxygen supply, not having to perform anaerobic respiration, i.e., not a sign of stress or poor health.

A baseline for cortisol is more complex to define and is typically compared between groups within the same experiment. Since we had no control group (i.e., individuals not submitted to stress events), it is a subjective task to define which values are considered high and may indicate stress. Considering this, the plasma cortisol concentrations measured in this study were relatively low, but that does not inherently mean that all were experiencing the same emotional state or degree of stress; once again, a single blood sampling at the end cannot be used to corroborate a claim that the fish had not been stressed during the experiment. Previous studies investigating the effects of chronic stress in Atlantic salmon have found that it causes a down-regulation of the cortisol response and slower or stunted growth (Madaro et al., 2015; Opinion et al., 2023). As seen in Madaro et al. (2015) and Opinion et al. (2023), fish exposed to stress protocols even had cortisol concentrations below control levels, due to negative feedback regulation. In summary, the blood measurements from this study could not corroborate potential stress in the fish or be adequately compared between treatment groups as we had hoped.

5.5 Conclusions & implications

In conclusion, the results from this study indicate that heart rate and heart rate responsiveness can be used as a potential predictor of weight change in Atlantic salmon. In order to distinguish individual differences, stimulation such as feeding was shown to be a key component. While heart rate is currently a difficult parameter to apply for practical use in commercial RAS facilities, the manual behaviour assessment showed potential of being able to detect feeding motivation, which was linked to weight change similarly to heart rate, through behavioural video analysis instead. Swimming activity measured through tri-axial accelerometry and accelerometry responsiveness

also showed potential for individual differentiation. Compared to heart rate, accelerometry can be measured through camera footage, making it directly applicable for video-based welfare monitoring. This methodology also enables the possibility of detecting different behavioural motifs, which can further strengthen evidence of poor welfare indicated by accelerometry measurements. However, heart rate and accelerometry did not reflect differences in treatment. To implement a non-invasive way to accurately determine OWIs in RAS, a possible solution is to instead use a different methodology altogether. Pictures taken by the underwater camera mounted in the tank are currently being used to train and validate an AI-based program to detect them visually, instead of using physiological proxies. Data from this project will also be used for machine learning to create a video-based system for automatic and comprehensive welfare monitoring in RAS.

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Most of all, I would like to honor the 32 fish whose lives were taken to enable this project. During the short time I was allowed to get to know you, you managed to show me your resilience and trust. Although you will never know this, your presence in this world will contribute to a brighter future for your peers subjected to human farming practices. I wish you a peaceful rest.

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Appendix 1. Raw data

Unique ID=[Trial]_[Biologger number]_[Bead colour combination]

Table A1. Weight change group categorization ($\Delta g = [\text{Start weight}] - [\text{End weight}]$).

Weight change group [Δg]
Great loss < -30
-30 < Moderate loss/gain < +30
+30 < Great gain

Table A2. Weight and fork length over 21 days per fish. End weight was measured with the biologger implanted and start weight without. The relative weight change is based on end weight with biologger weight subtracted (12g).

Unique ID	Start weight [g]	End weight [g]	Relative weight change [%]	Weight change group	Start fork length [cm]	End fork length [cm]
1_1139_PB	825	860	2.79	Moderate loss/gain	40.0	40.5
1_1148_PY	520	555	4.42	Moderate loss/gain	35.5	36.3
1_1150_BY	555	600	5.95	Great gain	36.8	37.5
1_1147_PG	700	760	6.86	Great gain	37.5	38.8
1_1143_BG	595	625	3.03	Moderate loss/gain	37.5	37.6
1_1149_PW	750	805	5.73	Great gain	40.0	40.4
1_1144_BW	645	675	2.97	Moderate loss/gain	38.0	38.1
1_1152_RW	900	855	-6.33	Great loss	41.0	40.8
1_1146_YW	1025	970	-6.54	Great loss	44.5	44.9
1_1142_YG	660	705	5.00	Great gain	38.3	39.0
2_1159_OW	980	790	-20.6 (NA)	Great loss (NA)	42.4	43.2
2_1155_BW	935	970	2.46	Moderate loss/gain	42.2	42.5
2_1157_YW	1055	1065	-0.19	Moderate loss/gain	43.3	43.9
2_1158_OY	960	930	-4.38	Great loss	44.2	44.2
2_1163_BG	750	800	5.07	Great gain	39.6	40.2
2_1160_YG	1030	1045	0.29	Moderate loss/gain	42.3	43.3
2_1161_BY	495	515	1.62	Moderate loss/gain	35.5	35.4
2_1162_OB	1450	1070	-27.0 (NA)	Great loss (NA)	43.6	43.8
2_1154_BB	715	750	3.22	Moderate loss/gain	38.4	38.4
2_1164_OO	1005	980	-3.68	Great loss	44.6	44.4
3_1146_OO	730	810	9.32	Great gain	40.0	40.6
3_1144_OW	805	860	5.34	Great gain	41.0	40.8
3_1139_BW	560	595	4.11	Moderate loss/gain	36.5	36.8
3_1148_RW	745	760	0.40	Moderate loss/gain	40.5	40.5
3_1142_YW	1025	1055	1.76	Moderate loss/gain	43.2	44.6
3_1149_BB	950	1025	6.63	Great gain	41.5	42.2
3_1143_YY	1110	1160	3.42	Great gain	45.0	45.1
3_1150_PO	715	725	-0.28	Moderate loss/gain	39.0	39.1
3_1152_OB	930	990	5.16	Great gain	42.0	42.4
3_1147_PY	1055	1120	5.02	Great gain	43.0	43.0

Table A3. OWIs and health index per fish.

Unique ID	Treatment	Cataract	Scale loss	Operculum damage	Eye pop	Snout damage	Wound	Fin deformity	Health index
1_1139_PB	0	0	1	0	0	1	0	0	2
1_1148_PY	0	1	1	0	0	1	0	1	4
1_1150_BY	0	2	0	0	0	1	0	0	3
1_1147_PG	0	0	2	0	0	1	0	0	3
1_1143_BG	0	0	1	0	0	1	0	0	2
1_1149_PW	0	1	1	1	0	1	0	1	5
1_1144_BW	0	0	1	0	0	1	0	0	2
1_1152_RW	0	0	1	0	0	1	0	0	2
1_1146_YW	0	1	1	0	0	1	0	1	4
1_1142_YG	0	1	1	0	0	1	0	1	4
2_1159_OW	1	0	1	0	0	1	0	0	2
2_1155_BW	1	1	1	0	0	0	0	0	2
2_1157_YW	1	2	1	0	0	1	1	0	5
2_1158_OY	1	3	1	0	0	0	0	1	5
2_1163_BG	1	1	1	0	0	1	0	0	3
2_1160_YG	1	0	1	1	0	1	0	0	3
2_1161_BY	1	0	0	0	0	0	0	0	0
2_1162_OB	1	0	1	0	0	1	0	0	2
2_1154_BB	1	1	0	0	0	0	0	1	2
2_1164_OO	1	1	2	0	1	0	0	0	4
3_1146_OO	0	2	1	0	0	0	0	0	3
3_1144_OW	1	0	1	0	0	1	0	0	2
3_1139_BW	0	1	1	0	0	0	0	1	3
3_1148_RW	1	1	1	0	0	0	0	0	2
3_1142_YW	0	0	1	1	0	0	0	1	3
3_1149_BB	1	0	1	0	0	1	1	0	3
3_1143_YY	0	0	0	0	0	1	0	1	2
3_1150_PO	1	1	0	0	1	0	1	0	3
3_1152_OB	0	2	0	1	0	0	0	1	4
3_1147_PY	1	1	1	0	0	1	0	0	3

Table A4. Blood values per fish.

Unique ID	Hemoglobin [g·L ⁻¹]	Hematocrit [% red blood cells]	Lactate [mmol·L ⁻¹]	Glucose [mmol·L ⁻¹]	Cortisol [ng·mL ⁻¹]
1_1139_PB	77	30.5	1.9	4.2	32.5
1_1148_PY	58	22.0	1.1	5.1	6.5
1_1150_BY	54	21.5	1.3	5.0	33.5
1_1147_PG	78	33.0	1.4	3.8	48.4
1_1143_BG	74	30.0	3.0	4.2	93.1
1_1149_PW	85	35.5	4.9	4.4	50.4
1_1144_BW	76	29.0	1.3	4.2	73.6
1_1152_RW	97	40.5	4.1	3.8	81.0
1_1146_YW	80	32.5	1.5	3.6	59.2
1_1142_YG	88	35.5	1.3	4.1	43.4
2_1159_OW	94	34.5	1.1	2.9	30.8
2_1155_BW	79	30.0	1.3	4.4	17.1
2_1157_YW	87	33.0	4.7	3.4	15.6
2_1158_OY	79	28.5	1.3	2.9	20.6
2_1163_BG	93	37.0	4.1	3.1	4.2
2_1160_YG	84	21.5	3.2	4.3	25.3
2_1161_BY	45	16.0	1.0	5.0	0.5
2_1162_OB	76	30.0	3.4	4.4	0.5
2_1154_BB	46	18.5	1.4	5.6	12.0
2_1164_OO	85	31.0	0.9	3.1	13.0
3_1146_OO	50	17.5	1.6	4.4	4.0
3_1144_OW	52	21.0	2.0	5.1	18.2
3_1139_BW	67	25.5	1.9	4.8	1.1
3_1148_RW	69	26.5	1.6	4.4	3.6
3_1142_YW	97	38.0	2.5	4.4	13.2
3_1149_BB	97	37.5	4.0	3.6	13.1
3_1143_YY	95	36.0	5.5	3.4	14.1
3_1150_PO	107	39.0	2.9	3.9	10.8
3_1152_OB	105	42.5	4.3	3.5	5.3
3_1147_PY	66	20.5	1.6	5.1	0.5

Table A5. Average manual behaviour assessment scores per fish based on grading template in Table 1.

Unique ID	Feeding motivation	Aggression	Activity
1_1139_PB	2.00	0.00	1.60
1_1148_PY	2.00	0.40	1.40
1_1150_BY	2.00	0.00	1.40
1_1147_PG	1.67	0.60	1.80
1_1143_BG	2.00	0.00	1.60
1_1149_PW	2.00	0.00	1.40
1_1144_BW	2.00	0.00	1.60
1_1152_RW	0.33	0.00	0.60
1_1146_YW	0.00	0.00	0.40
1_1142_YG	2.00	0.00	1.40
2_1159_OW	1.33	0.00	0.80
2_1155_BW	2.00	0.00	1.20
2_1157_YW	1.33	0.00	0.80
2_1158_OY	0.33	0.40	1.20
2_1163_BG	2.00	0.00	1.60
2_1160_YG	2.00	0.00	1.20
2_1161_BY	2.00	0.00	1.20
2_1162_OB	1.67	0.00	1.20
2_1154_BB	2.00	1.00	2.00
2_1164_OO	0.00	0.00	0.4
3_1146_OO	2.00	0.00	1.40
3_1144_OW	2.00	0.00	1.60
3_1139_BW	2.00	0.00	1.40
3_1148_RW	2.00	0.00	1.80
3_1142_YW	2.00	0.00	1.60
3_1149_BB	2.00	0.00	1.60
3_1143_YY	2.00	0.00	1.20
3_1150_PO	2.00	0.00	1.40
3_1152_OB	2.00	0.00	1.60
3_1147_PY	2.00	0.00	1.40

Appendix 2. R-script

```
#Examensarbete----

#Hanna Piikki (16/5-2026)

#Libraries----

library(tidyverse)
library(performance)
library(see)
library(GGally)
library(ggplot2)
library(dplyr)
library(ez)
library(lme4)
library(lmerTest)
library(emmeans)
library(plotly)
library(car)
library(broom)
library(DiagrammeR)
library(broom.mixed)
library(ggh4x)
library(grid)
library(ggbreak)
library(ggpubr)

#Adding unique ID's----
#Creating a new column with unique ID's for all individual based
on trial, biollogger-ID and color
df$Unique_ID <- paste(df$Trial, df$ID, df$Color, sep = "_")

#Checking that it worked properly (should print 30 for 30
individuals)
length(unique(df$Unique_ID))

#Adding weight difference----
#Creating a new column with weight change [g] from surgery to
sampling
df <- df %>%
  mutate(Weight_diff = (Weight_end - 12) - Weight_start)

#Alternative weight change ([%], e.g. -0.40 for 40% weight loss)
df <- df %>%
  mutate(Weight_diff_prop =
    ((Weight_end - 12) - Weight_start) / Weight_start)

#Adding a column that has the clean percentage for weight
difference
df <- df %>%
```

```

mutate(Weight_diff_percent = Weight_diff_prop * 100)

#Replacing implausible weight_diff values with NA
df <- df %>%
  mutate(Weight_diff = ifelse(Weight_diff > 120 | Weight_diff < -
120,
                                NA,
                                Weight_diff))

#Same thing for proportional weight change
df <- df %>%
  mutate(Weight_diff_prop = ifelse(Weight_diff_prop < -0.1,
                                NA,
                                Weight_diff_prop))

#Making sure that parameters are treated as factors
df$General_activity <- factor(df$General_activity)
df$Trial <- factor(df$Trial)
df$Treatment <- factor(df$Treatment)
df$Cataract <- factor(df$Cataract)

#Replacing commas with dots for AvgEA and HR
df$AvgEA <- gsub(",", ".", df$AvgEA)
df$HR <- gsub(",", ".", df$HR)

#Making AvgEA and HR numeric instead of character
df$AvgEA <- as.numeric(df$AvgEA)
df$HR <- as.numeric(df$HR)

#Creating new data frames----

#Creating a new data frame with filtered data, only keeping rows
where QI < 2, HR < 100 and HR > 20)
df_filtered <- df %>% filter(QI < 2,
                                HR < 100,
                                HR > 20)

#Creating new columns from the date_time column in a more
appropriate time format (new column = "time")
df_filtered <- df_filtered %>%
  mutate(
    date_time_clean = gsub(",", ".", date_time),
    datetime = dmy_hms(date_time_clean),
    time = format(datetime, "%H:%M:%S")
  )

#Creating a new column "window" to mark 11:00-11:06 and 11:30-
11:36
df_filtered <- df_filtered %>%
  mutate(
    window = case_when(

```

```

      time %in% c("11:00:00", "11:02:00", "11:04:00", "11:06:00")
~ "11:00_window",
      time %in% c("11:30:00", "11:32:00", "11:34:00", "11:36:00")
~ "11:30_window",
      TRUE ~ NA_character_
    )
  )

```

```

#Grouping the OWI-parameters

```

```

owi_cols <- c("Cataract", "Scale_loss", "Operculum",
              "Eye_pop", "Snout", "Wound", "Fin_deformity")

```

```

#Making sure that the OWI-parameters are treated as numeric
df_filtered[owi_cols] <- lapply(df_filtered[owi_cols],
as.numeric)

```

```

#Creating a new data frame with HR means for each individual
during 11:00-11:06 and 11:30-11:36 each day

```

```

df_HR_diff <- df_filtered %>%
  filter(day!=15) %>%
  filter(!is.na(window)) %>%
  rowwise() %>%
  mutate(
    Health_index = sum(c_across(all_of(owi_cols)), na.rm = TRUE)
  ) %>%
  ungroup() %>%
  group_by(Unique_ID, Trial, day, window, Weight_diff,
Weight_diff_prop, Weight_diff_percent, Treatment,
General_activity, Hb, Hct, Glucose, Lactate, Cortisol, Cataract,
Scale_loss, Operculum, Eye_pop, Snout, Wound, Fin_deformity,
Health_index) %>%
  summarise(
    mean_HR = mean(HR, na.rm = TRUE),
    mean_AvgEA = mean(AvgEA, na.rm = TRUE),
    .groups = "drop"
  )

```

```

#Adding HR_diff (difference in mean HR between windows 11:00-
11:06 and 11:30-11:36) for each individual and day

```

```

df_HR_diff_wide <- df_HR_diff %>%
  select(Unique_ID, Trial, day, Weight_diff, Weight_diff_prop,
Weight_diff_percent, Treatment, General_activity, Hb, Hct,
Glucose, Lactate, Cortisol, Cataract, Scale_loss, Operculum,
Eye_pop, Snout, Wound, Fin_deformity, Health_index, window,
mean_HR, mean_AvgEA) %>%
  pivot_wider(
    names_from = window,
    values_from = c(mean_HR, mean_AvgEA)
  ) %>%
  mutate(
    HR_diff = `mean_HR_11:30_window` - `mean_HR_11:00_window`,

```

```

    AvgEA_diff = `mean_AvgEA_11:30_window` -
`mean_AvgEA_11:00_window`
  )

#Creating new data frames for day 10 and 11
df_HR_diff_10 <- df_HR_diff_wide %>%
  filter(day == 10)

df_HR_diff_11 <- df_HR_diff_wide %>%
  filter(day == 11)

#Creating a new data frame with means for AvgEA_diff and HR_diff
df_behaviour <- df_HR_diff_wide %>%
  group_by(Unique_ID) %>%
  summarise(
    Weight_diff = first(Weight_diff),
    Weight_diff_prop = first(Weight_diff_prop),
    mean_AvgEA_diff = mean(AvgEA_diff, na.rm = TRUE),
    mean_HR_diff = mean(HR_diff, na.rm = TRUE),
    Weight_diff_percent = first(Weight_diff_percent)
  )

#Linear models----

#Mean HR diff ~ Weight diff----
lm_weight_HR_diff <- lm(mean_HR_diff ~ Weight_diff_percent, data
= df_behaviour)
summary(lm_weight_HR_diff)
confint(lm_weight_HR_diff)

#HR diff ~ Weight diff (day 10)----
lm_weight_HR_diff_10 <- lm(HR_diff ~ Weight_diff_percent, data =
df_HR_diff_10)
summary(lm_weight_HR_diff_10)
confint(lm_weight_HR_diff_10)

#HR diff ~ Weight diff (day 11)----
lm_weight_HR_diff_11 <- lm(HR_diff ~ Weight_diff_percent, data =
df_HR_diff_11)
summary(lm_weight_HR_diff_11)
confint(lm_weight_HR_diff_11)

#Mean AvgEA diff ~ Weight diff----
lm_weight_AvgEA_diff <- lm(mean_AvgEA_diff ~ Weight_diff_percent,
data = df_behaviour)
summary(lm_weight_AvgEA_diff)
confint(lm_weight_AvgEA_diff)

#AvgEA diff ~ Weight diff (day 10)----
lm_weight_AvgEA_diff_10 <- lm(AvgEA_diff ~ Weight_diff_percent,
data = df_HR_diff_10)

```

```

summary(lm_weight_AvgEA_diff_10)
confint(lm_weight_AvgEA_diff_10)

#AvgEA diff ~ Weight diff (day 11)----
lm_weight_AvgEA_diff_11 <- lm(AvgEA_diff ~ Weight_diff_percent,
data = df_HR_diff_11)
summary(lm_weight_AvgEA_diff_11)
confint(lm_weight_AvgEA_diff_11)

#HR_diff ~ Treatment/Trial/Health_index/day----

#Linear mixed model
#Testing for effects of treatment, trial, health index and day on
HR difference
model_Treatment_diff_H <- lmer(
  HR_diff ~ Treatment + Trial + Health_index + day + (1 |
Unique_ID),
  data = df_HR_diff_wide
)

#ANOVA on LMM
anova(model_Treatment_diff_H)

#AvgEA_diff ~ Treatment/Trial/Health_index/day----

#Linear mixed model
#Testing for effects of treatment, trial, health index and day on
AvgEA difference
model_Treatment_diff_A <- lmer(
  AvgEA_diff ~ Treatment + Trial + Health_index + day + (1 |
Unique_ID),
  data = df_HR_diff_wide
)

#ANOVA on LMM
anova(model_Treatment_diff_A)

#mean_HR ~ Treatment/Trial/Health_index/day----

#Linear mixed model
#Testing for effects of treatment, trial, health index and day on
mean HR
model_Treatment_H <- lmer(
  mean_HR ~ Treatment + Trial + Health_index + day + (1 |
Unique_ID),
  data = df_OWI
)

#ANOVA on LMM
anova(model_Treatment_H)

```

```

#mean_AvgEA ~ Treatment/Trial/Health_index/day----

#Linear mixed model
#Testing for effects of treatment, trial, health index and day on
mean AvgEA
model_Treatment_A <- lmer(
  mean_AvgEA ~ Treatment + Trial + Health_index + day + (1 |
Unique_ID),
  data = df_OWI
)

#ANOVA on LMM
anova(model_Treatment_A)

#Weight_diff_prop ~ Treatment/Trial/Health_index/day----

#Creating a new data frame with only one row per Unique_ID
df_weight_unique <- df_OWI %>%
  group_by(Unique_ID) %>%
  summarise(
    Weight_diff_prop = first(Weight_diff_prop),
    Treatment = first(Treatment),
    Trial = first(Trial),
    Health_index = first(Health_index)
  )

#Linear model
#Testing for effects of treatment, trial, health index and day on
relative weight change
model_weight <- lm(
  Weight_diff_prop ~ Treatment + Trial + Health_index,
  data = df_weight_unique
)

#ANOVA on LM
anova(model_weight)

#Relative weight change ~ Treatment----

#Testing for normality of relative weight change distribution in
the two treatment groups with Sharpiro-Wilk tests
shapiro.test(df_HR_diff_10$Weight_diff_percent[df_HR_diff_10$Trea
tment == 0])
shapiro.test(df_HR_diff_10$Weight_diff_percent[df_HR_diff_10$Trea
tment == 1])

#Wilcoxon rank-sum test to test for differences in the
distribution of weight change between the two treatment groups
(used due to non-normality in the distributions)
wilcox.test(
  Weight_diff_percent ~ Treatment,

```

```
data = df_HR_diff_10,  
exact = FALSE  
)
```