Aquaculture 534 (2021) 736258

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Continuous physiological welfare evaluation of European whitefish (*Coregonus lavaretus*) during common aquaculture practices leading up to slaughter

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ARTICLE INFO

Keywords:
Heart rate
Cortisol
Stress
Crowding
Brailing
Transport
CO_2 exposure

ABSTRACT

European whitefish (*Coregonus lavaretus*) is an aquaculture species with the potential for expanded cultivation in the fresh and brackish waters of Northern Europe. Yet, relatively little species-specific information is available regarding the stress responses and associated welfare implications for this species in captivity. We addressed this knowledge gap by using a combination of implantable heart rate bio-loggers and a range of traditional stress indicators (*e.g.* haematological parameters and plasma concentrations of cortisol, glucose and ions) to comprehensively evaluate the physiological responses of freely swimming whitefish in captivity, as well as when subjected to aquaculture practices and stressors that commonly occur prior to and during slaughter. Whitefish appeared to recover rapidly from surgery, as resting heart rate decreased within 36 h to stabilize at ~25 beats min^{-1} for the next 18 days when fish were left relatively undisturbed (*i.e.* personnel were only present when feeding fish). In contrast with previous studies on farmed rainbow trout and Atlantic salmon, whitefish did not exhibit a clear circadian heart rate rhythm, which may be related to species-specific differences in diurnal locomotor activity. Whitefish also appear to have a well-developed capacity for thermal acclimation of heart rate, as daily resting heart rate did not change during the undisturbed period despite an increase in body temperature from ~6.8 to 11.2 °C. Following acute stressors such as crowding and transportation, the physiological response of whitefish typically involved transient elevations in heart rate, plasma cortisol and glucose, and red blood cell swelling, while plasma $[K^+]$ decreased. In contrast, the heart rate of whitefish plummeted following the combination of brailing (*i.e.* to haul in fish with a brail/net) and CO_2 exposure prior to slaughter, while plasma cortisol, glucose and $[Ca^{2+}]$ significantly increased. An unforeseen finding concerns the substantial and long-lasting physiological stress response observed in whitefish when held in close proximity (*i.e.* within ~10 m) to a rainbow trout net pen, as the mean heart rate of whitefish increased from ~32 to 43 beats min^{-1} (*i.e.* an increase of ~34%). This may represent an innate physiological response to the threat of predation, which consequently increases the allostatic load and energetic expenditure of whitefish when farmed alongside salmonids. To conclude, this study highlights the importance of performing long-term, species-specific evaluations of freely swimming fish in real aquaculture settings, and provides a platform for further research aiming to determine the welfare implications of simultaneously farming predatory and prey species in close proximity.

1. Introduction

Due to the ever-growing demand for safe and nutritious food, aquaculture continues to grow faster than other major food producing sectors (FAO, 2020). Alongside, and perhaps partly due to the rapid expansion of the aquaculture industry, the welfare of farmed fish has

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https://doi.org/10.1016/j.aquaculture.2020.736258
Received 18 August 2020; Received in revised form 26 October 2020; Accepted 7 December 2020
Available online 10 December 2020

become increasingly important for consumers, producers, retailers, interest groups and the authorities (Ashley, 2007; Cooke, 2001; EFSA, 2009; Frewer et al., 2005; OIE, 2019). The concept of animal welfare is complex and takes into account an animal's subjective mental state, the capacity to express their natural behaviour, and their biological ability to adapt to their environment and remain in good health (Ashley, 2007; Segner et al., 2012). While the debate regarding whether or not fish possess the mental capacity to experience pain and suffering is still ongoing, the balance of evidence indicates that fish may indeed have such capabilities and should therefore be given the benefit of the doubt with regards to their welfare (Ashley, 2007; EFSA, 2009; FAO, 2020; Huntingford et al., 2006; OIE, 2019; van de Vis et al., 2012). Despite the growing awareness, understanding and concern for the welfare of farmed fish, an overall shortage of scientific data still exists, especially when one considers the high diversity of fishes currently being exploited for aquaculture purposes (*e.g.* ~369 species of finfish were estimated to be farmed world-wide in 2016; FAO, 2020; OIE, 2019).

Scientific approaches to assess the welfare of fish in aquaculture are continually developing and largely revolve around evaluating the responses of fish to environmental and/or anthropogenic stressors (Ashley, 2007; Segner et al., 2012). Stress can be defined as any condition or state that threatens an animal's homeostasis such that it needs to induce a range of responses to re-establish homeostasis (*i.e.* allostasis; Korte et al., 2007; Segner et al., 2012). The initial response to stress typically includes the release of catecholamines and the stimulation of the hypothalamic-pituitary-interrenal axis culminating in the release of corticosteroids such as cortisol (*i.e.* primary stress responses; Barton, 2002; Wendelaar-Bonga, 1997). This neuroendocrine stress response elicits subsequent changes such as an increase in cardiorespiratory activity, redistribution of blood flow according to altered tissue oxygen demands, splenic release of red blood cells and mobilization of energy stores. Collectively, this serves to enhance the probability of survival for an individual facing a threatening situation (*i.e.* secondary stress responses; Ashley, 2007; Barton, 2002). However, overly severe or long-lasting stress disrupts the negative feedback mechanism of the stress response, which ultimately increases the allostatic load on the animal and may result in detrimental tertiary stress responses such as impairments of appetite, growth rate, swimming ability, immune responses, behavioural repertoire, reproductive ability and cardiac function (Ashley, 2007; Eliason et al., 2013; Korte et al., 2007; Segner et al., 2012). Thus, from both an animal welfare and economic perspective, it is essential that the origin and/or causes of stress responses are identified early enough so that an intervention can take place before physiological mechanisms are compromised and become detrimental to the fish's health and well-being (*i.e.* state of distress or allostatic overload; Korte et al., 2007; Moberg, 2000; Segner et al., 2012; Huntingford et al., 2006; van de Vis et al., 2012).

Since neurohumoral factors released during the primary stress response (*i.e.* adrenalin, noradrenalin and cortisol) are known to increase heart rate and energy mobilization, the measurement of heart rate can be used as a proxy to assess the stress responses of fish (Cooke et al., 2016). Moreover, since heart rate is often correlated with metabolic rate in various fish species (Armstrong, 1986; Campbell et al., 2004; Clark et al., 2005; Eliason et al., 2008; Eliason et al., 2011), it can also provide insights into the relative energetic consequences of different environmental and/or anthropogenic stressors (Cooke et al., 2016). Recent advances in the development and miniaturization of heart rate bio-loggers and bio-telemetry systems have thus far made it possible to determine the magnitude and duration of stress responses induced by various aquaculture practices in species such as rainbow trout (*Oncorhynchus mykiss*; Brijs et al., 2018, 2019a, 2019b), Atlantic salmon (*Salmo salar*; Hvas et al., 2020), and Atlantic cod (*Gadus morhua*; Bjarnason et al., 2019). However, due to the wide variety of ecological adaptations and evolutionary histories of fishes, different species can react very differently to environmental and/or anthropogenic stressors. Thus, there is an urgent need for consideration of species-specific differences when developing guidelines, regulations and legislations intended to safeguard fish welfare in aquaculture (Barton, 2002; EFSA, 2009; FAO, 2020; OIE, 2019). To achieve this, further research is required for a vast number of species to identify situations in the rearing environment that may compromise fish health and welfare, as well as to quantify the potential negative impacts of common practices employed in aquaculture.

One of the species that warrants further investigation is the European whitefish (*Coregonus lavaretus*). This highly valued cold-water species has a relatively high growth rate and favorable feed conversion ratio, and thus has the potential to become an important commercial species in both fresh and brackish water in northern Europe (Jobling et al., 2010; Siikavuopio et al., 2012). Despite anecdotal reports stating that this species is highly susceptible to stress (*e.g.* fish have been observed to immediately reduce feed intake and/or lose equilibrium following common aquaculture practices), their ability to tolerate the varying environmental and/or anthropogenic stressors that they are regularly exposed to in aquaculture settings has not been systematically investigated. Therefore, the aim of the present study was to comprehensively evaluate the stress responses of European whitefish subjected to aquaculture practices that commonly occur before and during slaughter. Specifically, by using surgically implanted heart rate bio-loggers in focal fish, we aimed to continuously record heart rate and body temperature of whitefish for an extended period of time when left undisturbed in the sea cage to identify 'normal' heart rate patterns of this species, as well as to identify deviations from this pattern in response to a range of common aquaculture practices (*e.g.* crowding, well-boat transport, brailing and CO_2 exposure). In addition, we aimed to use 'early' physiological stress indicators such as plasma cortisol levels and a range of haematological and blood chemistry parameters to further assess the severity of the stress responses, as well as to validate the implications of the observed heart rate responses. Detailed species-specific information regarding the welfare of European whitefish in aquaculture is urgently required for the development of guidelines regarding the ethical production of this food source.

2. Materials and methods

2.1. Experimental animals

The experimental part of the study took place at Brändö Lax AB facilities, Brändö, Åland Islands, Finland between the 9th and 30th of May 2017 (hereafter referred to as day 0 to 21. European whitefish (n: 116, *Coregonus lavaretus*, hereafter referred to as 'whitefish') with body masses of 752 ± 255 g were used in the present study (all data in the materials and methods section are presented as means±s.d.). They were kept in a circular sea cage (diameter: 40 m, depth: 4 m) together with ~3000 conspecifics, which was located near the slaughterhouse facilities in the Djurholms Sound. All experimental protocols were approved by the Åland provincial government project approval committee (decision 2/2016).

2.2. Bio-logger details and surgical implantation

From the 116 fish used in the present study, a sub-sample of 20 individuals with body masses of 901 ± 310 g were individually implanted with a 12 mm Passive Integrated Transponder tag (PIT-tag, Oregon RFID, Portland, Oregon, USA) for future identification, as well as a DST milli-HRT bio-logger (Logger version 8 DM/CRC16/4800, STAR-ODDI, Gardabaer, Iceland). The bio-loggers (diameter: 13.0 mm, length: 39.5 mm, volume: 5 cm^3, mass in air: 11.8 g) monitor heart rate *via* a single channel electrocardiogram (ECG) amplifier that uses three measuring electrodes incorporated into the ceramic casing. Each logged heart rate value is derived from the mean RR-interval (*i.e.* time between two consecutive R waves in the ECG) from a burst of 600 measurements, which corresponds to a 6-s period when sampling at 100 Hz. For

validation purposes, all logged heart rate measurements are graded with a data verification quality index (QI) which range from 0 to 3 where 0 = Great, 1 = Good, 2 = Fair and 3 = Poor. A temperature sensor with a resolution of 0.032 °C and an accuracy of ±0.2 °C is also located within the casing of the bio-logger. Additionally, the sensor contains a real-time clock with an accuracy of ±1 min month^{-1}. The bio-loggers were programmed with the application software Mercury v 4.28 and the associated Communication Box (STAR-ODDI, Gardabaer, Iceland) to measure heart rate and body temperature every 10 min from 6:00 on day 0 till 6:00on day 20, after which measurements were taken every 2 min until 24:00 on day 21. All times are reported in Eastern European Summer Time (EEST or UTC + 2).

PIT-tags and bio-loggers were surgically implanted between 8:00and 15:00on day 0. To achieve this, fish were individually dip netted from the sea cage and placed in a bin containing seawater from the Djurholms Sound with 150 mg L^{-1} ethyl-3-aminobenzoate methanesulphonic acid (MS222, Sigma-Aldrich Inc., St. Louis, Missouri, USA) buffered with 300 mg L^{-1} $NaHCO_3$ at 5 °C to induce surgical anaesthesia. When anaesthetised (as indicated by loss of opercular movements), the fish was placed on an operating table and anaesthesia was maintained by continuously flushing aerated water containing MS-222 (100 mg L^{-1}) and $NaHCO_3$ (200 mg L^{-1}) over the gills. A 25–30 mm long mid-ventral incision was then made with a scalpel ~40 mm posterior to the pectoral fins. The DST milli-HRT bio-logger was subsequently inserted into the abdominal cavity and placed in close proximity to the pericardium, which is a position previously determined to be optimal for salmonids with regards to signal strength and quality (Brijs et al., 2018; Svendsen et al., 2021). The bio-logger was anchored to the abdominal muscle with a 3–0 sterile monofilament non-absorbable Prolene™ suture (Ethicon, LLC, Puerto Rico, USA). The PIT-tag was then inserted into the abdominal cavity *via* the same incision prior to closing the wound with interrupted non-absorbable Prolene™ sutures. To promote wound healing, a mixture consisting of a protective paste (Orabase®, ConvaTec, Bromma, Sweden), an antifungal agent (Pevaryl®, McNeil Sweden AB, Solna, Sweden) and an antibacterial agent (Bacibact®, Orion Corporation, Espoo, Finland) was applied on the surface of the wound while a broad-spectrum antibiotic (10 mg kg^{-1} Baytril®, Bayer Healthcare, Berlin, Germany) was injected intraperitoneally. To facilitate the retrieval of the bio-logger during slaughter, fish were tagged with blue dots in the area between the pectoral fins using Alcian Blue dye injected with a pressure injector (AKRA Dermojet Polymedical, Barthou, France). After the surgical procedure, which took ~10 min to complete, fish were released back into the sea cage with their conspecifics.

2.3. Monitoring of whitefish in sea cage and timing of events during aquaculture procedures

Whitefish remained in the sea cages with conspecifics during days 0–20. Fish were fed *ad libitum* once per day with commercial feed pellets (BioMar, Aarhus, Denmark) on days 0–13. Fish were fasted on days 14–20 in accordance with the pre-slaughter procedures employed at Brändö Lax AB. With the exception of the abovementioned daily feeding events, the fish used in the present study remained relatively undisturbed until 21:00 on day 19 when a sea cage containing ~5000 rainbow trout (individual body masses ranging between ~2 to 4 kg) was towed by a well-boat and secured in close proximity (*i.e.* within 10 m) to the sea cage containing the whitefish.

On days 20–21, the procedures associated with the slaughter of whitefish took place. Before and during a number of the aquaculture practices described below, sub-samples of randomly selected whitefish were carefully captured with a hand net, euthanised by a sharp blow to the head, sampled for blood (~1 mL) from the caudal vessels using a heparinised syringe, and then weighed for body mass and measured for fork length (Table 1). These blood samples were immediately placed on ice for further analyses (see *2.5. Blood analyses*).

Between 11:45 and 12:15 on day 20, whitefish were crowded by lifting the bottom of the sea cage during which the first sub-sample of fish (n: 10) were captured and sampled as quickly as possible in order to collect 'pre-stressor' blood samples (*i.e.* blood samples representative of event 1, see *2.4. Data retrieval from bio-loggers and analysis of heart rate*). The bottom of the sea cage was subsequently released to allow the whitefish to disperse until the well-boat arrived. Whitefish were then crowded between 13:45 and 14:20, and subsequently brailed (*i.e.* the act of hauling in fish with a brail/net) into the well-boat between 14:20 and 14:35. The fish were then transported around the Djurholms Sound in the well-boat between 14:35 and 15:45 during which the second sub-sample of fish (n: 30) were captured and sampled for blood (*i.e.* blood samples representative of event 4–6, see *2.4. Data retrieval from bio-loggers and analysis of heart rate*). Dissolved oxygen levels of the water within the well-boat were also monitored throughout the transportation event using a hand-held oxygen meter (HQ40D Portable Multi Meter, Hach Lange GmbH, Düsseldorf, Germany). Following transportation, whitefish were released from the well-boat into a holding cage near the slaughterhouse *via* a water chute. It was noted that this holding cage was also in close proximity to other holding cages containing rainbow trout. To investigate the recovery of whitefish from the procedures described above, a third sub-sample of fish (n: 19) were randomly captured from the holding cage and sampled for blood between 16:26 and 20:12 (*i.e.* a different individual was sampled every ~12 min). Fish were then left undisturbed overnight.

The slaughter of whitefish on day 21 occurred between 08:15 and 11:15. Between 06:20 and 06:40, the fourth sub-sample of fish (n: 10) were captured and sampled as quickly as possible in order to collect 'pre-slaughter' blood samples (*i.e.* blood samples representative of event 8, see *2.4. Data retrieval from bio-loggers and analysis of heart rate*). The slaughter procedure included the crowding and subsequent brailing of whitefish from the holding cage to an air chute, which led to a tank in the slaughterhouse containing CO_2-saturated seawater. Fish were then

Table 1
Description of the various events and when those events took place, as well as details regarding the sampling duration, times and sizes for the heart rate analyses and blood sampling associated with the various events.

Event	Event description	Day	Time	Mean heart rate sampling		Blood sub-sampling	
				Sampling duration (min)	*n*	Sampling time	*n*
1	Undisturbed	20	11:45–12:15	20	20	12:03–12:11	10
2	Peak response to first crowding	20	13:45–14:20	20	20		
3	Prior to second crowding	20	14:20–14:35	20	20		
4	Peak response to second crowding	20	14:35–15:45	20	20	14:23–15:35	30
5	Beginning of transportation			20	20		
6	End of transportation			20	20		
7	Peak heart rate during day 20	20	16:26–20:20	20	20	every 12 min	19
8	Prior to initiation of slaughter procedures	21	06:20–06:40	20	20	06:25–06:37	10
9	Peak response to final crowding	21	08:15–11:15	10	20	08:22–08:57	17
10	Response following brailing and CO_2 exposure	21		10	20	08:32–09:48	30[a]

[a] Consisted of sampling blood from 20 instrumented and 10 uninstrumented whitefish.

held in this tank until they could no longer maintain equilibrium and were deemed unconscious by the slaughterhouse personnel, whereupon they were mechanically lifted onto a grid designated as the 'gill cutting station'. Personnel then manually cut the gill arches and ventral aorta of each individual, and transferred the fish to an adjacent tank for exsanguination. The fifth and sixth sub-sample of fish were sampled for blood during the crowding phase (n: 17) and directly following brailing and CO_2 exposure (n: 30, which consisted of 10 uninstrumented fish and the 20 fish instrumented with bio-loggers), respectively. These blood samples were representative of event 9 and 10, respectively (see *2.4. Data retrieval from bio-loggers and analysis of heart rate*).

2.4. Data retrieval from bio-loggers and analysis of heart rate

The data was retrieved from the bio-loggers by placing the loggers in the associated Communication Box and the application software Mercury v 4.28 was used to extract the recordings. The data was then organised using Excel (Microsoft office 2016). To ensure that subsequent analyses were based on highly accurate heart rate measurements, only measurements with the highest grade (*i.e.* grade 0 which represented 57 ± 18% of the recorded data) were included in the analyses. The measurement error associated with grade 0 recordings has previously been demonstrated to be <1 beat min^{-1} (Brijs et al., 2019a).

Prior to procedures associated with slaughter (*i.e.* during days 0–20), mean heart rate and resting heart rate of whitefish were calculated from 12 hourly periods between days 0–3 (to investigate recovery from handling, anaesthesia, surgery and reintroduction with conspecifics in the sea cage), from 24 hourly periods between days 3–19 (to investigate the temporal dynamics of heart rate when fish were left undisturbed), and from 6 hourly periods between days 19–20 (to investigate the effects of the arrival of the sea cage containing rainbow trout). Mean heart rate includes periods of spontaneous activity and/or postprandial responses, whereas resting heart rate accounts for the theoretical temporal variations in the heart rate of individuals during periods of inactivity. The analysis of resting heart rate was based on a method used for determining standard metabolic rate in fish (Chabot et al., 2016), and consists of calculating the 20th percentile of recorded heart rate values for each individual. This method has previously been shown to provide a good approximation of heart rate of fish during periods of rest (Hjelmstedt et al., 2020).

To evaluate the severity of, and recovery from, the different acute stressors associated with slaughter, the mean heart rates of whitefish were calculated from ten specific time periods or 'events' during days 20–21 (Table 1). Mean heart rates were calculated from 20 min periods within events 1–8 (*i.e.* 1–10 measurements) and from 10 min periods within events 9–10 (*i.e.* 1–5 measurements). Finally, to assess the cumulative effects of the different stressors that whitefish were subjected to during the procedures associated with slaughter, mean heart rates during each of the abovementioned 'events' on days 20–21 were compared to the mean heart rates calculated for the corresponding time of day during days 16–18. These days were used for comparison as the fish were considered undisturbed and maximally recovered from anaesthesia and surgery, and best represented the conditions the fish were exposed to leading up to slaughter with respect to feeding status and water temperature (Brijs et al., 2018).

2.5. Blood analyses

Blood samples were analysed for haematocrit (Hct, %) and haemoglobin concentration ([Hb], g dL^{-1}). The Hct was determined as the fractional red cell volume after centrifugation of a subsample of blood in 80 μl heparinised microcapillary tubes at 10,000 rcf for 5 min in a Hct centrifuge (Haematokrit 210, Hettich, Tuttlingen, Germany). A handheld Hb 201+ meter (Hemocue® AB, Ängelholm, Sweden) was used to determine [Hb] and values were corrected for fish blood (Clark et al., 2008). Mean corpuscular haemoglobin concentration (MCHC, g dL^{-1}) was subsequently calculated as [Hb]/Hct × 100.

Following the haematological analyses, the remaining blood samples were centrifuged at 10000 rcf for 5 min in a microcentrifuge (Eppendorf 5415D, Eppendorf, Hamburg, Germany). The plasma was subsequently collected and frozen at −80 °C for analyses to determine the concentration of plasma glucose (mmol L^{-1}) and plasma cortisol (ng mL^{-1}). Concentration of plasma glucose was determined using a glucose assay kit (GAHK20, Sigma-Aldrich, St. Louis, Missouri, USA). Plasma cortisol concentration was determined by a radioimmunoassay (RIA) previously described by Young (1986) that uses a cortisol antibody (Code: S020; Lot:1014–180,182, Guildhay Ltd., Guildford, Surrey, UK) validated by Sundh et al. (2011). As a tracer, tritiated hydrocortisone-[1,2,6,7–3H (N)] (NET 396; NEN Life Sciences Products, Boston, Massachusetts, USA) was used and cortisol standards were prepared from hydrocortisone (Sigma, St. Louis, Missouri, USA). Radioactivity was determined with a Wallac 1409 liquid scintillation counter (LKB Instruments, Turku, Finland). Intra- and interassay coefficients of variation for this cortisol RIA has been shown to be 3.9% and 5.4%, respectively, with a detection limit of 0.7 ng mL^{-1} (Sundh et al., 2011). Plasma ion concentrations (*i.e.* $[K^+]$, $[Na^+]$, $[Cl^-]$, and $[Ca^{2+}]$ in mmol L^{-1}) were determined using a Convergys®ISE comfort Electrolyte Analyzer (Convergent technologies, Coelbe, Germany).

2.6. Statistical analyses

Statistical analyses were performed using SPSS Statistics 26 (IBM Corp., Armonk, New York, USA). All data were assessed to ensure that they did not violate the assumptions of the specific models outlined below. F-, t- and *p*-values obtained from the statistical analyses are reported throughout the text and all *p*-values of <0.05 were considered statistically significant. All data in the results section are presented as means±s.e.m. All data supporting the paper is readily available in the supplementary information (S1-S11).

To statistically analyse body temperature (*i.e.* days 0–20) and heart rate (*i.e.* days 0–3, days 3–19, and days 19–20) of instrumented whitefish prior to the initiation of slaughter procedures, we used one-way repeated measures ANOVAs with Bonferroni adjusted post-hoc tests. Linear regressions were used to assess the relationship between heart rate and body temperature for each individual whitefish during the entire undisturbed period (*i.e.* days 3–19), as well as separately during the fed (*i.e.* days 3–11) and fasted period (*i.e.* days 12–19).

To statistically analyse heart rate of instrumented whitefish during the ten specific 'events' that occurred during the slaughter days (*i.e.* days 20–21), as well as in comparison to the mean heart rates calculated for the corresponding time of day during the days leading up to slaughter (*i.e.* days 16–18), we used a two-way mixed ANOVA with a Bonferroni adjusted post-hoc test. This model used 'events' as the within-subjects factor (fixed factor, ten levels: event 1–10), the specific days as the between-subjects factor (fixed factor, two levels: slaughter day and days leading up to slaughter), and the interaction between these factors. Since a significant interaction was detected between the fixed factors of this model ($F_{6.33,240.42} = 48.67$, $p < 0.001$), the simple main effects of the model were determined by using one-way ANOVAs for each category of the within-subjects factor or by using one-way repeated measures ANOVAs for each category of the between-subjects factor. Since the assumption of sphericity was violated (assessed by Mauchly's test of sphericity), a Greenhouse-Geisser correction was applied to each of the abovementioned repeated measures analyses involving heart rate,

To statistically analyse the differences in body and blood/plasma parameters of whitefish that were representative of different events (*i.e.* event 1, 4–6, 8, 9 and 10), we used one-way ANOVAs with Tukey's post-hoc tests when no assumptions were violated (*i.e.* for body mass, $[Ca^{2+}]$ and plasma glucose).When the assumption of homogeneity of variance was violated, we instead used Welch ANOVAs with Games-Howell post-hoc tests (*i.e.* for body length, plasma cortisol, [Hb], Hct, MCHC, $[K^+]$, $[Na^+]$ and $[Cl^-]$). Despite significant differences in the body mass and

length of the sampled whitefish (*i.e.* body mass and length of whitefish sampled during event 10 > event 1, 4–6, 8 and 9; Table 2), neither of these body parameters were found to be significantly related to any of the measured blood/plasma parameters and were therefore not included as covariates in the abovementioned models. Following the combination of all the stressors that occurred on day 20 (*i.e.* after event 7), a logarithmic regression was used to predict the decrease in plasma cortisol over time since this model proved to be the best fit (as assessed by the R^2 value).

3. Results

3.1. Heart rate responses of whitefish before slaughter (days 0–20)

Visual inspection of the instrumented whitefish at the end of the experiment indicated that the incision made to insert the bio-logger had healed satisfactorily in all fish and no obvious signs of infection were detected. During the 21 days following the surgical implantation of bio-loggers, the instrumented fish had significantly increased in body length by 5 ± 1 mm (~1% increase in body length, $t_{19} = 4.22$, $p < 0.001$) and decreased in body mass by 22 ± 2 g (~2% decrease in body mass, $t_{19} = -10.58$, $p < 0.001$). The mean body temperature of instrumented fish significantly increased from ~6.0 to 11.2 °C throughout the experimental period ($F_{20,380} = 3660.55$, $p < 0.001$, Fig. 1A), as a result of similar increases in ambient water temperature.

Between days 0–3, the mean heart rate of whitefish was initially elevated following handling, anaesthesia, surgery and reintroduction with conspecifics in the sea cage, yet decreased rapidly during the recovery period (Fig. 1A). Temporal changes in mean heart rate (*i.e.* from ~44 to 30 beats min^{-1}) and resting heart rate (*i.e.* from ~38 to 24 beats min^{-1}) indicated that fish recovered from the stress associated with the surgical implantation of heart rate bio-loggers in less than two days (Fig. 1B). Between days 3–19, mean heart rate typically fluctuated between 25 and 45 beats min^{-1}, yet no clear circadian heart rate rhythm was observed (Fig. 1A). Despite a significant increase in body temperature from ~6.8 to 11.2 °C during this period, as well as changes in feeding state (*i.e.* fish were fed daily to satiation on days 0–13 and then fasted on days 14–20 in preparation for slaughter), resting heart rates did not significantly change (Fig. 1C). Visual inspection of scatterplots revealed no clear or significant relationships between heart rate and body temperature of individual whitefish during the entire undisturbed period or within the fed and fasted periods. At ~21:00 on day 19, mean heart rate rapidly increased and remained elevated thereafter (Fig. 1A). It was noted that this increase in heart rate coincided with the introduction of a sea cage containing ~5000 rainbow trout in close proximity (*i.e.* within 10 m) to the sea cage containing the whitefish. Statistical analyses of the temporal changes in heart rate revealed significant increases in both mean heart rate (*i.e.* from ~32 to 43 beats min^{-1}) and resting heart rate (*i.e.* from ~27 to 36 beats min^{-1}) shortly after the arrival of the rainbow trout (Fig. 1D).

3.2. Heart rate responses of whitefish during slaughter (day 20 to 21)

Prior to the commencement of slaughter procedures, the mean heart rate of whitefish was significantly elevated by ~11 beats min^{-1} ($F_{1,38} = 18.89$, $p < 0.001$) when compared to the mean heart rates observed at the same time of day during the days leading up to slaughter (*c.f.* black square and grey circle during event 1, Fig. 2A). The heart rate response to crowding was relatively consistent, as mean heart rate significantly increased by ~15 beats min^{-1} (*c.f.* black squares in event 1–2, 3–4 and 8–9, Fig. 2A). When the whitefish were allowed to recover within the sea cage following a crowding event, the mean heart rate significantly decreased to values observed prior to crowding within 1.5 h (*c.f.* black squares in events 1–3, Fig. 2A).

Following the second crowding event, the brailing of whitefish from the sea cage into the well-boat appeared to induce a slight decrease in the mean heart rate (between 14:20 and 14:35, Fig. 2A). However, closer examination of the data revealed that out of the whitefish with reliable heart rate recordings during brailing (n: 16) only 31% of fish experienced a clear reduction in heart rate (*e.g.* from ~51 to 23 beats min^{-1}), whereas heart rate remained unchanged in the other 69% of fish. When whitefish were transported around the Djurholms Sound in the well-boat, the mean heart rate significantly increased by ~13 beats min^{-1} during the initial 30 min, yet decreased during the last 40 min of transportation to reach values that were similar to those observed prior to transport (*c.f.* black squares in events 4–6, Fig. 2A). This decrease in heart rate was not related to the levels of dissolved oxygen in the water within the well-boat, as values always remained between 10.80 and 11.84 mg L^{-1} (*i.e.* ~100% air saturation). When whitefish were released from the well-boat into the holding cage, which was in close proximity to other holding cages containing rainbow trout, mean heart rate significantly increased to peak at 73 ± 2 beats min^{-1} (*c.f.* black squares in events 6–7, Fig. 2A).

Mean heart rate subsequently decreased overnight to reach values that did not significantly differ to that observed prior to the commencement of slaughter procedures (*c.f.* black squares in events 1 and 8, Fig. 2A). However, it remained significantly elevated ($F_{1,38} = 53.84$, $p < 0.001$) when compared to the values observed at the same time of day during the days leading up to slaughter (*c.f.* black squares and grey circles in event 8, Fig. 2A). Following the final crowding event,

Table 2
Body measurements, haematological parameters and plasma ion/glucose concentrations of European whitefish sampled at specific time points or events during the final 2 days leading up to slaughter. Statistical analyses were generated using a one-way ANOVA for each variable and significant differences between groups are represented by different letters ($p < 0.05$).

Measured variables	Events					Statistical summary
	Pre-stress (event 1)	Crowding/transportation (event 4–6)	Pre-slaughter (event 8)	Crowding (event 9)	Brailing/CO_2 narcosis (event 10)	
Body measurements						
Mass (g)	549 ± 45^{a}	741 ± 44^{a}	681 ± 75^{a}	714 ± 40^{a}	947 ± 36^{b}	$F_{(4,92)} = 9.253$, $p < 0.001$
Length (mm)	367 ± 10^{a}	392 ± 6^{a}	381 ± 12^{a}	389 ± 6^{a}	414 ± 3^{b}	Welch's $F_{(4,29.16)} = 8.38$, $p < 0.001$
Haematological parameters						
[Hb] (g dL^{-1})	11.3 ± 0.4^{a}	12.6 ± 0.5^{a}	11.4 ± 0.8^{a}	11.3 ± 0.4^{a}	12.3 ± 0.2^{a}	Welch's $F_{(4,27.72)} = 2.88$, $p = 0.041$
Hct (%)	33.3 ± 1.5^{a}	42.5 ± 2.0^{b}	34.6 ± 2.3^{a,b}	39.1 ± 1.5^{a,b}	54.6 ± 1.1^{c}	Welch's $F_{(4,30.57)} = 39.08$, $p < 0.001$
MCHC (g Hb dL^{-1})	34.0 ± 0.5^{a}	30.8 ± 1.4^{a,b}	33.0 ± 0.6^{a}	29.0 ± 0.5^{b}	22.7 ± 0.3^{c}	Welch's $F_{(4,30.31)} = 106.31$, $p < 0.001$
Blood plasma chemistry						
[K+] (mmol L^{-1})	2.6 ± 0.1^{a}	1.4 ± 0.2^{b}	2.4 ± 0.1^{a}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	Welch's $F_{(4,28.84)} = 43.25$, $p < 0.001$
[Na+] (mmol L^{-1})	131.2 ± 0.8^{a,c}	134.3 ± 0.5^{b}	136.0 ± 1.0^{b}	128.9 ± 0.3^{a}	131.8 ± 0.4^{c}	Welch's $F_{(4,24.44)} = 28.50$, $p < 0.001$
[Cl-] (mmol L^{-1})	120.9 ± 0.9^{a}	122.4 ± 0.9^{a}	114.5 ± 0.5^{b}	121.0 ± 0.8^{a}	120.1 ± 0.5^{a}	Welch's $F(4,27.36) = 26.75$, $p < 0.001$
[Ca2+] (mmolL^{-1})	1.00 ± 0.02^{a}	0.98 ± 0.02^{a}	0.65 ± 0.03^{b}	1.05 ± 0.03^{a}	1.2 ± 0.02^{c}	$F_{(4,71)} = 54.98$, $p < 0.001$
Glucose (mmol L^{-1})	4.3 ± 0.2^{a}	5.0 ± 0.1^{a,b}	4.8 ± 0.3^{a,b}	5.7 ± 0.2^{b}	6.7 ± 0.2^{c}	$F_{(4,83)} = 19.12$, $p < 0.001$

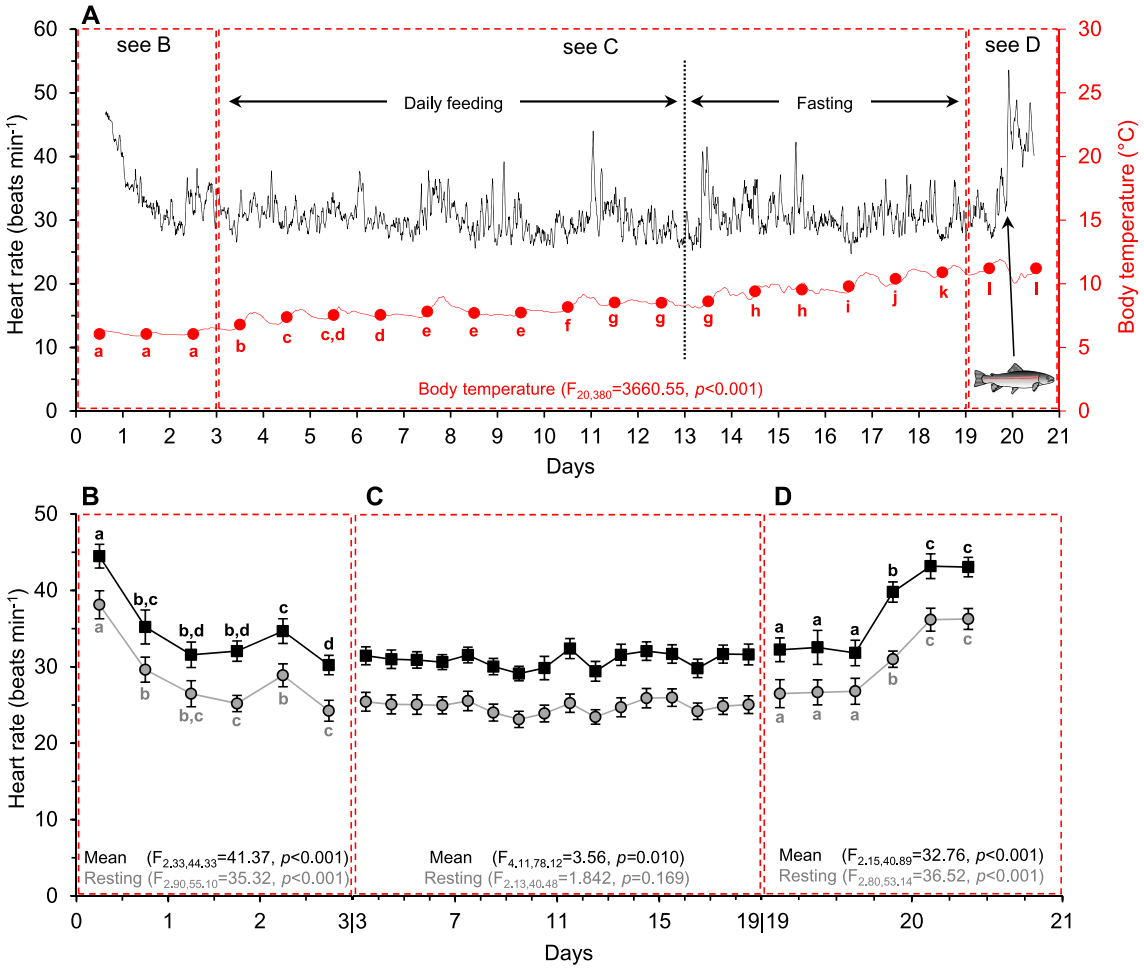

Fig. 1. (A) Hourly moving mean heart rate (black line) and body temperature (red line) of European whitefish (n: 20) from when all fish were reintroduced into the sea cage with conspecifics (~15:00 on day 0) until the slaughter procedures commenced (~24:00 on day 20). The fish symbol with the arrow illustrate the time when the cage with rainbow trout was introduced. (B-D) Detailed temporal changes in mean heart rate (black squares) and resting heart rate (grey circles, defined as the 20th percentile of recorded heart rate values) of European whitefish during (B) recovery from handling, anaesthesia, surgery and reintroduction with conspecifics, (C) the period when fish were left relatively undisturbed, and (D) the period during which a sea cage of rainbow trout were secured in close proximity (*i.e.* within ~10 m). Note that the time scale on the x-axis differ between A, B, C and D. Repeated measures ANOVAs were used to statistically analyse the temporal changes in (A) mean daily body temperature (red circles) and (B-D) mean and resting heart rates of European whitefish. The fixed effects of each model are reported and the different letters represent significant differences ($p < 0.05$) within each period. For the undisturbed period, minor significant differences in mean heart rate occurred sporadically (*e.g.* mean heart rate significantly differed on day 9 *vs.* day 7 and 14, and on day 12 *vs.* day 11,13 and 14), however, these differences are not highlighted in the figure for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the mean heart rate was significantly elevated before dramatically decreasing by ~37 beats min^{-1} (from ~60 to ~23 beats min^{-1}) following brailing and CO_2 exposure (*c.f.* black squares in events 8–10, Fig. 2A), which was significantly lower ($F_{1,38} = 4.20, p = 0.047$) than the mean heart rate observed at the same time of day during the days leading up to slaughter (*c.f.* black squares and grey circles during event 10, Fig. 2A).

3.3. Blood and plasma chemistry of whitefish during slaughter (day 20 to 21)

The circulating levels of plasma cortisol in the 'pre-stressor' blood sample of whitefish was 13 ± 4 ng mL^{-1} (event 1, Fig. 2B). Following the combined stressors of crowding, brailing and transportation, plasma cortisol significantly increased to 50 ± 3 ng mL^{-1} (*c.f.* event 1 and 4–6, Fig. 2B). When examining the immediate recovery of whitefish following the combination of all the stressors that occurred on day 20, it was found that a logarithmic regression could significantly predict the decrease in plasma cortisol over time using $y = -8.184\ln(x) + 60.429$, where y represents plasma cortisol and x represents duration of recovery in minutes (Fig. 2C). Despite a significant decrease in plasma cortisol overnight (*c.f.* event 4–6 and 8, Fig. 2B), values tended ($p = 0.052$) to be elevated the next morning when compared to pre-stressor values (*c.f.* event 1 and 8, Fig. 2B). Following the combined stressors of crowding, brailing and CO_2 exposure, plasma cortisol significantly increased and peaked at 60 ± 5 ng mL^{-1} (event 8–10, Fig. 2B).

Crowding, as well as the combined stressors of brailing and CO_2 exposure, were observed to induce significant increases in Hct and significant decreases in MCHC, whereas [Hb] remained relatively similar throughout the slaughter period (Table 2). With regards to plasma ion concentrations, the greatest changes were observed for $[K^+]$, which consistently decreased by ~44% in response to the different stressors (*c.f.* event 1 to 4–6, event 8 to 9–10 in Table 2). Both $[Ca^{2+}]$ and plasma glucose peaked following brailing and CO_2 exposure, whereas only minor and relatively inconsistent changes were observed in $[Na^+]$ and $[Cl^-]$ throughout the slaughter period (Table 2).

4. Discussion

This is the first study to comprehensively evaluate the physiological

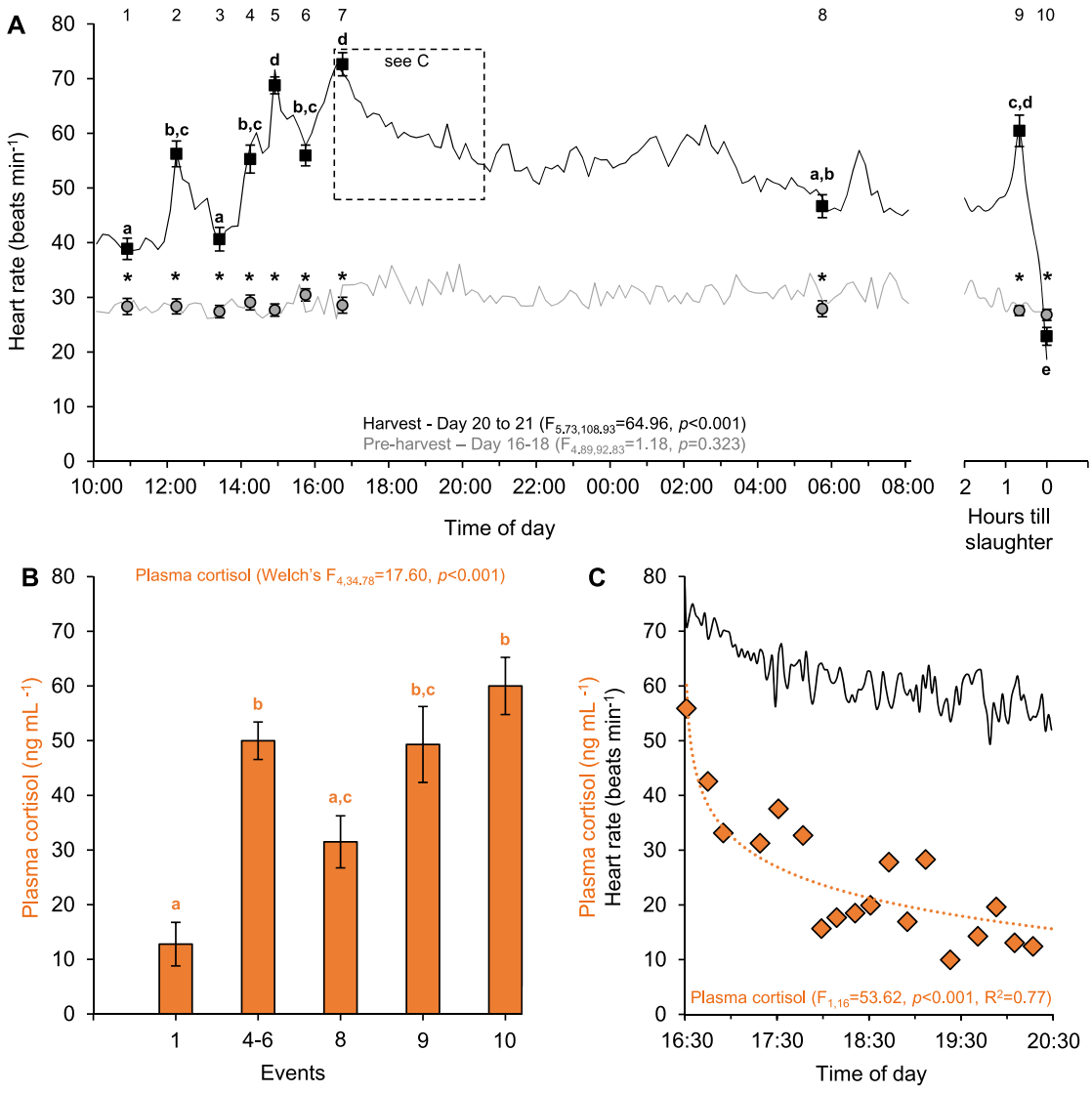

Fig. 2. (A) Mean heart rate of European whitefish (n: 20) during the final two days leading up to slaughter (black line, calculated from days 20–21) compared to the heart rate prior to slaughter in the same individuals (grey line, calculated from days 16–18, see materials and methods for details). Mean heart rate during this period was statistically analysed at specific time points or events (see black squares or grey circles within shaded bars) to evaluate the severity of, and recovery from, a range of different acute stressors. These time points or events represent (1) the period prior to the initiation of harvest procedures, (2) the peak response to the first crowding event, (3) the period prior to the second crowding event, (4) the peak response to the second crowding event, (5) the beginning of the transportation event, (6) the end of the transportation event, (7) peak heart rate during day 20, (8) the period prior to the initiation of slaughter procedures, (9) the peak response to the final crowding event, and (10) following brailing and CO_2 narcosis. (B) Circulating levels of plasma cortisol in whitefish representative of the abovementioned time periods or events (n: 10, 30, 10, 17 and 30 fish for event 1, 4–6, 8, 9, and 10, respectively). (C) The decrease in mean heart rate (black line) and plasma cortisol (orange diamonds) following the combination of all the stressors that occurred on day 20. The decrease in plasma cortisol over time could be significantly predicted using a logarithmic regression (orange dotted line). Statistical analyses were generated using (A) a two-way mixed ANOVA (within-subject and between-subject differences represented by different letters and *, respectively), (B) a one-way ANOVA (between-subject differences represented by different letters), and (C) a logarithmic regression analysis. All p-values of <0.05 were considered statistically significant.

responses of freely swimming European whitefish when left undisturbed in a commercial sea cage and in response to common aquaculture practices. The continuous recordings of heart rate combined with the 'snapshots' provided by the blood/plasma stress indicators revealed unique insights into the impact of, and recovery from, a range of acute and chronic stressors experienced by whitefish in aquaculture.

4.1. Heart rate responses of undisturbed and freely swimming whitefish (days 0 to 20)

Resting heart rate of whitefish decreased and stabilised within 36 h following the permanent implantation of the bio-logger. Thus, post-surgical recovery in whitefish appeared to be relatively rapid when compared to the time required for resting heart rates of rainbow trout to decrease and stabilize in response to an identical procedure at the same location (*i.e.* >72 h, Brijs et al., 2018). When fully recovered, whitefish did not display a clear circadian heart rate rhythm during the ~20-day period prior to slaughter, which is in contrast to the strong circadian heart rate rhythms previously demonstrated in other salmonid species such as rainbow trout, brown trout (*Salmo trutta*) and Atlantic salmon (Brijs et al., 2018; Føre et al., 2018; Hjelmstedt et al., 2020; Holliday et al., 1974; Hvas et al., 2020; Priede, 1978; Priede and Young, 1977; Young et al., 1972). Since the circadian heart rate rhythms observed in salmonids have previously been suggested to reflect daily changes in locomotor activity, the lack of a circadian heart rate rhythm in whitefish may be related to the less pronounced or complete lack of clear daily

changes in their locomotor activity. Indeed, when observing whitefish and rainbow trout swimming in the sea cages at the study site there appeared to be clear species-specific differences in circadian activity patterns. For example, rainbow trout were visibly more active during the day than at night (Brijs et al., 2018), whereas this did not appear to be the case for whitefish, which tended to continuously swim in a circular pattern within the sea cage regardless of the time of day. However, an alternative explanation for the lack of a circadian heart rate rhythm in whitefish could be related to the relatively long periods of daylight that the fish experienced in the present study (*i.e.* 17:7 light:dark cycle). For example, closely related Lake whitefish (*Coregonus clupeaformis*) displayed clear circadian swimming activity patterns in the wild and under controlled laboratory conditions when subjected to a 12:12 light:dark cycle, whereas these patterns were suppressed under constant light conditions (Anras et al., 1999; Bégout et al., 1998; Scherer and Harrison, 1988).

Acute warming in fish typically results in an increase in resting heart rate, whereas during more chronic seasonal temperature changes, compensatory physiological adjustments can be initiated to counteract the thermal effects (*i.e.* thermal acclimation) (Ekström et al., 2016; Sandblom et al., 2016; Seebacher et al., 2015). Here, we demonstrate the thermal acclimation capacity of whitefish for the first time, as whitefish maintained a resting heart rate of ~25 beats min^{-1} despite an increase in body temperature from ~6.8 to 11.2 °C during the undisturbed period (*i.e.* a ~ 1.6-fold increase). This indicates that, at least within the observed temperature range, whitefish were able to adjust physiologically to counter the effects of the temperature increase on resting heart rate. Similar to other fish species, this was most likely achieved *via* either an increased neural (vagal) inhibition of heart rate and/or a reduction in the intrinsic pacemaker rate (Sandblom and Axelsson, 2011; Ekström et al., 2016; Sandblom et al., 2016).

The processing and digestion of a meal coincides with an increase in energetic expenditure (*i.e.* specific dynamic action) and gastrointestinal blood flow in a diverse range of vertebrates (Secor, 2009; Seth et al., 2011). To meet the elevated metabolic and circulatory demands associated with feeding, fish typically increase cardiac output *via* an increase in heart rate and/or stroke volume (Seth et al., 2011). In the present study, the heart rate of whitefish did not differ with regards to feeding state, which contrasts with the pronounced post-prandial increase in heart rate previously reported in species such as rainbow trout (Eliason et al., 2008; Gräns et al., 2009), sea bass (*Dicentrarchus labrax*; Altimiras et al., 2008; Axelsson et al., 2002; Dupont-Prinet et al., 2009), northern pike (*Esox Lucius*; Lucas et al., 1991), shorthorn sculpin (*Myoxocephalus scorpius*, Seth and Axelsson, 2009), white sturgeon (*Acipenser transmontanus*; Gräns et al., 2010) and bald notothens (*Pagothenia borchgrevinki*; Sandblom et al., 2012). This finding could be due to a number of reasons such as i) surgical implantation of the bio-logger may have disrupted the feeding behaviour/activity of whitefish, ii) the amount of food ingested was not enough to induce a clear post-prandial increase in heart rate, iii) voluntary feeding and freely swimming fish may not exhibit similar post-prandial responses as those documented for gavage fed fish in the laboratory (Brijs et al., 2018, 2019b; Seth et al., 2011), and/or iv) whitefish may increase cardiac output *via* stroke volume instead of heart rate, which would be similar to the situation in Atlantic cod (Axelsson and Fritsche, 1991). Since body mass decreased by ~2% over the course of the study, it is likely that reasons i and/or ii underlie this finding and thus research on the effects of implanted devices on behavioural and physiological aspects of feeding is warranted. A potential recommendation for future research investigating the temporal dynamics of feeding in freely swimming fish is to allow longer recovery times prior to recording (*e.g.* several weeks before recording is initiated).

4.2. Physiological responses of whitefish during slaughter (days 20 to 21)

The physiological response of whitefish to acute stressors such as crowding and transportation typically consisted of increases in heart rate, cell swelling (as indicated by the reductions in MCHC; Nikinmaa, 1983) and circulating levels of plasma cortisol and glucose, while plasma $[K^+]$ decreased. The heart rate response of whitefish to crowding and transportation (*i.e.* an increase of ~15 and 13 beats min^{-1}, respectively) were relatively similar to the responses observed in rainbow trout subjected to similar procedures (*i.e.* increase of ~13 and 9 beats min^{-1}, respectively; Brijs et al., 2018). However, whitefish appeared to recover relatively rapidly from these acute stressors, as heart rate recovered within 1.5 h following crowding or even began to decrease during transportation to reach pre-stressor levels by the end of the transportation event. The present study also highlights some species-specific differences in the usefulness of varying indicators for gauging the severity of acute stressors such as crowding and transportation. For example, changes in haematological (*e.g.* Hct and MCHC) and plasma stress indicators (*e.g.* $[K^+]$) were more pronounced in whitefish than in rainbow trout, as the latter exhibited no changes in these parameters following similar crowding and transportation events in the same location (Brijs et al., 2018). On the contrary, the plasma cortisol response to the combination of crowding and transportation was low in whitefish, as plasma cortisol only increased by ~37 ng mL^{-1} compared to ~148 ng mL^{-1} in rainbow trout (Brijs et al., 2018).

Another acute stressor that farmed fish are exposed to is brailing, which induced an immediate and transient decrease in the heart rate of rainbow trout that is likely due to inadequate oxygen availability during air exposure (Brijs et al., 2018). However, only 31% of the whitefish with reliable heart rate recordings experienced a clear and substantial reduction in heart rate during brailing (*i.e.* reduction of ~38 beats min^{-1}), while the heart rate remained unchanged in the other 69% of whitefish. The underlying reason for this finding remains unknown, but it could be related to variations in brailing duration or position of the fish within the brail during brailing. Consistent with previous studies on other fish species (Brijs et al., 2018; Gräns et al., 2016; Sandblom et al., 2013; Seth et al., 2013), CO_2 exposure induces a severe stress response in whitefish, as circulating levels of plasma cortisol, glucose and $[Ca^{2+}]$ significantly increased along with substantial cell swelling. In addition, heart rate plummeted during CO_2 exposure, which is likely the result of a cardiac collapse caused by severe acidosis (Seth et al., 2013).

An unforeseen, yet extremely relevant, finding of the present study concerns the substantial and long-lasting physiological response of whitefish when held in close proximity (*i.e.* within ~10 m) to rainbow trout. Directly after a sea cage containing ~5000 large rainbow trout (~2 to 4 kg) was towed and secured in close proximity to the sea cage containing the whitefish, the mean heart rate of the latter increased from ~32 to 43 beats min^{-1} (*i.e.* an increase of ~34%) and remained elevated by at least this amount thereafter. Since whitefish are often among the dominant prey species in the wild for piscivores across their range (Jensen et al., 2008; Kahilainen and Lehtonen, 2003), this finding may represent an innate physiological response to the threat of predation. Indeed, previous studies have demonstrated an immediate and often maintained increase in heart rate following a predatory attack or threat in a range of fish species (Holopainen et al., 1997; Höjesjö et al., 1999; Johnsson et al., 2001; Sundström et al., 2005). This physiological response has been suggested to enhance the probability of escape and/or to maintain preparation for flight in the near future (Höjesjö et al., 1999; Ydenberg and Dill, 1986). While this response is crucial for survival in the wild, this sustained physiological response would most likely serve to increase the allostatic load on farmed whitefish, and could result in detrimental tertiary stress responses (Korte et al., 2007; Moberg, 2000; Segner et al., 2012). This may consequently have substantial economic implications, as less energy would be available for growth due to the potentially elevated metabolic demands of whitefish when held captive in close proximity to a potential predator (Fraser and Gilliam, 1992). Furthermore, there is a risk that the allostatic load imposed by the presence of rainbow trout may approach or turn into an allostatic overload when whitefish are simultaneously subjected to additional stressors such as those mentioned above. For example, the mean heart

rate of whitefish was ~44 beats min^{-1} higher than 'normal' due to the stress imposed by the presence of rainbow trout and the range of common aquaculture practices employed on day 20 (*c.f.* black squares and grey circles during event 7, Fig. 2A). The allostatic load imposed by the combination of these stressors most likely impacts the ability of whitefish to perform normal physiological processes (Brijs et al., 2019a) and may ultimately result in cardiac collapse, and even death, if conditions become more unfavourable (Eliason et al., 2013; Priede, 1977). Furthermore, the potentially high allostatic load imposed by the presence of rainbow trout may also explain why this species has previously been perceived by commercial fish farmers to be highly susceptible to the stress induced by common aquaculture practices, as it is quite common to have both species at the same farm with varying physical distances.

Finally, the present study demonstrates the advantages of using continuous heart rate recordings from bio-loggers to identify and quantify the impacts of both acute and chronic stressors when compared to the 'snapshot' provided by more traditional measures such as circulating plasma cortisol levels. Despite the introduction of rainbow trout on day 19 inducing a substantial and maintained elevation in heart rate, plasma cortisol of whitefish was within the previously reported unstressed range (<30 ng mL^{-1}; Lappivaara and Oikari, 1999; Lappivaara, 2001) on the morning of day 20. This may reflect a relatively rapid recovery of plasma cortisol in whitefish following a perceived stressor. For example, plasma cortisol returned to pre-stressor levels within 6 h after whitefish had been subjected to a combination of acute stressors on day 20 (see logarithmic regression equation in *3.3. Blood and plasma chemistry of whitefish during slaughter*). This result is not unexpected, as circulating levels of plasma cortisol has been reported to be a poor indicator of chronic stress in several fish species including Atlantic salmon (Sundh et al., 2010, 2019), brown trout (Pickering and Stewart, 1984), rainbow trout (Person-Le Ruyet et al., 2008) sea bream (*Sparus aurata*; Tort et al., 1996) and common carp (*Cyprinus carpio*; Aerts et al., 2015).

5. Conclusion

The present study highlights the importance of performing long-term, species-specific evaluations of freely swimming fish in real aquaculture settings when developing guidelines or regulations governing fish welfare in aquaculture. Although the acute stress responses of whitefish to a range of common aquaculture practices such as crowding, transportation, brailing and CO_2 exposure were comparable to those previously described in farmed rainbow trout (Brijs et al., 2018), there were several important species-specific differences. This information will be useful for safeguarding, as well as assessing, the health and welfare of farmed European whitefish in the future. The most pronounced differences in whitefish relative to other salmonid species included an increased sensitivity of some blood/plasma stress indicators to perceived stressors, the presence of a substantial physiological stress response when held captive in the proximity of a potential predator and the lack of a circadian heart rate rhythm in undisturbed whitefish. Thus, given the welfare and economic implications, further investigations are urgently needed to systematically evaluate the effects of farming both predatory and prey species simultaneously so that methods or techniques can be developed to reduce or prevent the physiological stress response of the prey species.

Declaration of Competing Interest

None.

Acknowledgements

The authors greatly appreciate the on-site facilities and experimental animals provided by Brändö Lax AB (https://brandolax.fi) and the organizational/practical assistance of Rosita Broström from the Ålands farm association (https://fiskodlarna.ax). The work was funded by grants from the Swedish Board of Agriculture, the Agricultural Sciences and Spatial Planning (Formas), the Swedish Research Council (VR), and the Helge Ax:son Johnsson foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.736258.

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