Simulated trawling: Exhaustive swimming followed by extreme crowding may be a significant contributor to variable fillet quality in trawl-caught Atlantic cod (*Gadus morhua*)

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Abstract

Fillet quality can vary tremendously in trawl-caught Atlantic cod (*Gadus morhua*). Poor quality may be caused by capture stress, crowding or exhaustion. To investigate mechanisms involved in causing variable quality, commercial-sized (size 3.5±0.9 kg) Atlantic cod were swum to exhaustion in a large swim tunnel and exposed to extreme crowding (736±50 kg m^3) for 0, 1 or 3 hours in an experimental cod-end. Further, fish were recuperated for 0, 3 or 6 hours in a net pen prior to slaughter to assess the possibility to quickly reverse the reduced quality. We found that exhaustive swimming and crowding were associated with increased metabolic stress, as indicated by increased plasma cortisol, blood lactate and blood haematocrit levels, and a reduced quality of the fillets in terms of increased visual redness and a drop in muscle pH. The observed negative effects of exhaustive swimming and crowding were only to a small degree reversed within 6 hours of recuperation. The results from this study suggest that exhaustive swimming followed by extreme crowding is a likely significant contributor to the variable fillet quality seen in trawl-caught Atlantic cod, and that recuperation for more than six hours may be required to reverse these effects.

Introduction

Fish captured in a trawl encounter a number of strenuous and stressful events such as forced swimming, crowding, confinement, crushing and barotrauma [1]. Because a trawl is an active fishing gear that involves herding the fish into the mouth of the trawl, fish will swim until exhaustion in an attempt to avoid capture. Fatiguing/fatigued fish drift back into the cod-end, where they are retained. With the increasing number of fish in the cod-end, animals will be compressed resulting in an extreme crowding situation.

Physiological measurements of trawl-captured cod, show fish in near homeostatic crisis that are highly variable in quality [2]. This indicates that the stressors to which the fish are exposed, plays a role in the degradation of quality. An increasing number of studies suggest that pre-mortem stress can strongly influence the quality of the final fish product [2-6]. Stress causes an elevation of circulating catecholamines and corticosteroids (e.g. cortisol), which in turn will alter metabolism, hydro-mineral balance and increase heart- and ventilation rate [7]. An ultimate function of the short-term stress response is mobilization of stored fuels for the physiological reactions known as "fight or flight" [8]. This pre-slaughter stress is known to cause textural changes of fish meat by altering the rate and extent of pH decline, and inducing a more rapid onset of rigor mortis [9, 10]. Furthermore, pre-mortem stress is associated with a change in muscle colour, which is considered an aesthetic quality defect in white fish [11]. Both discolouration of the fillet and textural changes play a role in downgrading of the fish and economic loss for the producer. Therefore, finding ways to reduce or reverse detrimental effects of capture stress will be of economic interest for both fishermen and producers.

During commercial trawling, it is challenging to separate the various parameters that could have an effect on quality. This also includes a variable size and length of the hauls, which is of great importance to both quality and survival of the catch [2]. Investigating trawl related stress in an

experimental setting may give a better understanding on how fillet quality parameters are influenced by different pre-mortem stressors. Previously, we have shown that neither the poor physiological state or negative fillet quality features of trawled cod could be reproduced by exhaustive swimming alone, and argue that variable fillet quality more likely is the result of several factors operating during the trawling process [12, 13]. In addition, studies performed on board commercial trawlers, have shown that it is possible to improve the quality of cod by keeping them alive in holding tanks for a few hours prior to slaughter [2].

In the current study, our aim was to experimentally simulate some aspects of a trawl capture, namely exhaustive swimming followed by extreme crowding, and investigate how this affects some key metabolic stress parameters and subsequent fillet quality in Atlantic cod. A second aim of the study was to investigate if post-stress recuperation for 0, 3 or 6 hours could reverse potential negative effects on fillet quality. We have addressed these issues by measurements of blood glucose, blood lactate, plasma cortisol, haematocrit, muscle pH, and fillet redness in cod swum to exhaustion in a swim tunnel and subsequently crowded (retained) in an experimental cod-end attached to the tunnel.

Materials and Methods

Animals and husbandry

A total of 197 wild Atlantic cod (body mass 3.5 ± 0.9 kg, body length 75 ± 7 cm, mean ± SD) (group means in Table 1, trial means in S1 Table) were captured by Danish seine in mid May 2014 outside the coast of Finnmark, Norway. The fish were kept live on board in tanks

supplied with running seawater and delivered to a live fish storage facility in Nordvågen, Norway, for recuperation for three weeks. From here, the fish were transported in a wellboat approximately 300 km to the Tromsø Aquaculture Research Station in, Norway. At the research station, the fish were held in two outdoor tanks (4 m diameter, 10 m^3) supplied with filtered seawater at natural water temperature and day-length (69ºN), until the start of the experiment in February 2015. The fish were fed three times a week, using a mixture of capelin (*Mallotus villosus*) and commercial feed (Skretting Amber 5 mm, Skretting ASA, Norway), until 48 hours before transfer of fish into an outdoor swimming tunnel (1400 L swim chamber, maximum speed 1.2 m^{-1}, we have previously described tunnel in detail [12]). There were no differences in gender distribution (N= 107 females and N = 90 males).

Table 1. Overview of biological parameters per treatment group

Group	N	Weight (g)	Length (cm)	CF	GSI	HSI
Rested ctrl	21	3477 ± 1035	74 ± 6.61	0.83 ± 0.1	4.33 ± 6.04	4.41 ± 1.21
Swum ctrl	42	3336 ± 895	73 ± 6.44	0.84 ± 0.15	4.95 ± 4.92	4.29 ± 1.39
C1.0	21	3487 ± 1015	74 ± 7.51	0.86 ± 0.13	6.57 ± 6.05	4.32 ± 1.45
C1.3	21	3761 ± 874	77 ± 4.85	0.81 ± 0.11	5.02 ± 4.96	4.2 ± 1.43
C1.6	21	3498 ± 821	74 ± 7.41	0.87 ± 0.22	3.68 ± 4.07	4.85 ± 1.41
C3.0	21	3729 ± 774	76 ± 7.21	0.84 ± 0.14	6.72 ± 6.12	4.58 ± 1.4
C3.3	21	3358 ± 922	75 ± 7.96	0.77 ± 0.12	5.03 ± 6.21	4.2 ± 1.8
C3.6	22	3497 ± 744	74 ± 5.76	0.87 ± 0.13	6.13 ± 6.52	4.75 ± 1.3

Overview of group distribution of number of fish (N), weight, length, condition factor (CF), gonadosomatic index (GSI) and hepatosomatic index (HSI). Each row show data from separate recovery groups; rested control (sampled from the holding tanks), swum control (sampled immediately after exercise), crowded for 1 hour and recuperated for 0 (C1.0), 3 (C1.3) and 6 hours (C1.6) respectively, and crowded for 3 hours and recuperated for 0 (C3.0), 3 (C3.3) and 6 hours (C3.6), respectively.

Experimental set-up

The experiment was conducted in three replicates over 26 days. There were 7 fish in each crowding group in each replica, adding up to a total of 21 individuals in each group by the end of the experiment. Three crowding durations of 1, 3 and 5 hours were selected in the original set-up to represent short, medium and long trawl hauls based reports from commercial trawl hauls [2]. However, mortality of the 5 hour crowding group reached over 80 % in the first trial and this group was therefore omitted in subsequent trials.

Control fish

Two days before each swimming trial, 7 fish were randomly dip-netted from the two holding tanks. In each trial, 3 fish were taken from one tank and 4 from the other. These fish were used to establish baseline levels for measured parameters for rested, unstressed fish (rested control). The fish were taken out and sampled within 1 min.

Swimming trial

Immediately after sampling of the control, 28 fish were transferred to a large swim tunnel housed in an 11 m tank and designed for swimming groups of large fish (sette inn referense). The fish were acclimated to the swim tunnel for 36 hours at a water speed of 0.15 m s^{-1} prior to the swimming trial. The fish density in the tunnel was on average 54 kg m^{-3}. The swimming trial started with a water velocity of 0.15 m s^{-1} and increased to 1.2 m s^{-1} in 1200 steps in 20 minutes (1 step s^{-1}). As fish ceased swimming and rested on the grid in the back of the tunnel (Fig 1), they were pinched in the tail with use of fingers to see if they would continue swimming. Non-responsive fish were considered exhausted [13] and subsequently released into the retention chamber, where water flow kept them on the grid (Fig 1). When all 28 fish in each

trial were in the retention chamber, 7 were randomly selected and sampled as swum control fish.

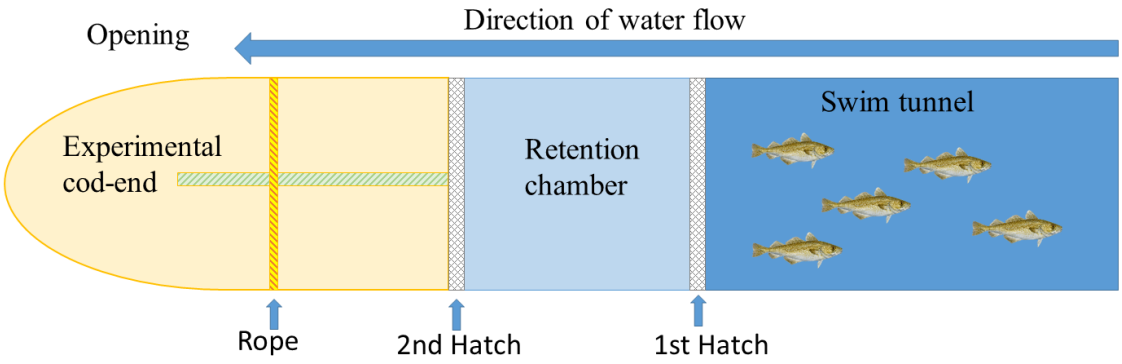


Fig 1. Schematic overview of the swim tunnel/trawl simulator. Graphic illustration of the swim tunnel and fish chamber, retention chamber and the experimental cod-end.

Crowding in the experimental cod-end.

Following removal of the 7 swum control fish, the remaining 21 fish were released from the retention chamber and into an experimental cod-end (Fig 1). The experimental cod-end was constructed as a four-panel cylindrical bag (length 200 cm height 58 cm with tension) using the same material as in a commercial cod-end (8 cm diamond cod-end mesh, 0.3 cm twine). The cod-end could be opened via a joint at the top (Fig 1). A rope was placed at a fixed position to close the cod-end, and tightened to ensure the fish were crowded. (Fig 1). When the cod-end was closed it was sphere shaped with a diameter of about 58 cm (S2 Fig) yielding a volume of about 100 L. For each trial, fish density was estimated based on the average weight of total individuals in the cod-end (S1 Table). Oxygen inside the cod-end was continuously monitored using an YSI ProODO handheld dissolved oxygen metre with a ProODO Optical probe (Yellow Spring Instruments, Ohio, USA). The fish were crowded for 1 or 3 hours. Afterwards, the fish

were taken out of the bag and randomly assigned to recuperation cages, where they were allowed to rest for 0, 3 or 6 hours.

Recuperation

The recuperation groups (0, 3 or 6 hours) were kept in 1×1×1 m lid-covered, floating steel mesh (4×4 cm) cages placed in the same tank as the swim tunnel. They fish were supplied with seawater at natural water temperature to ensure oxygen-saturated water.

Sampling procedure

All fish were euthanized by a blow to the head and blood was collected from the caudal vessels within 1 min, using 4 ml heparinized vacutainers with 4×0.9 mm needles (BD Diagnostics, Franklin Lakes, NJ, USA). Measurements of pH were then obtained by inserting a Hamilton double pore glass electrode (WTW330/set-1 pH-metre,Wissenscaftliche-Technische Werkstätten, Weilheim, Germany. Electrode: Hamilton Bonaduz AG, Bonaduz, Switzerland) via an incision (1 cm×2 cm) in the epaxial part of the white muscle tissue, rostrally to the dorsal fin on the left side of the fish. During the post-mortem pH measurements, a new incision was made 1 cm caudal to the previous incision for each measurement. pH was measured immediately after euthanasia, then there was a 20 hour period without measurements followed by measurements approximately every 8-15 hour. The instrument was calibrated frequently using pH 4.01 and 7.00 buffers at 2°C, and the electrode was cleaned with demineralized water between each measurement.

Concentrations of blood lactate and glucose were obtained from samples of whole blood, using the hand-held meters Lactate Scout+ (SensLab GmbH, Germany) and FreeStyle Lite (Abbott Diabetes Care, Inc., Alameda, CA), respectively. To calculate haematocrit, whole blood was spun using a microhaematocrit capillary tube centrifuge (Critocaps; Oxford Lab, Baxter,

Deerfield, IL) and the resulting red blood cell and total fraction measured using a millimeter ruler. The remaining blood was then centrifuged at 2700 × *g* for 5 minutes at 4°C, and plasma was transferred to cryo tubes, frozen in liquid nitrogen and stored at −80° C for later analysis of plasma cortisol. Immediately after blood collection and peri-mortem pH-measurements, all fish were exsanguinated by cutting the *Bulbus arteriosus* and *Vena cardinalis communis* on both sides. The fish were then bled for 30 min in a tank supplied with running seawater. Afterwards, weight (g), length (cm) and gender of each fish was registered. The liver and gonads were then taken out and weighed (g) to determine hepatosomatic (HSI) and gonadosomatic indices (GSI) by tissue weight x 100/total weight. The fish were then gutted, covered with plastic film and placed on ice in standard plastic fish boxes and stored at 4ºC.

Fillet redness

After approximately 72 hours storage all fish were filleted by trained personnel. The fillets were not de-skinned, but the black lining of the peritoneum was removed. Each fillet was evaluated by a sensory panel of three trained and experienced persons. To avoid expectation bias, the sensory panel was unaware of which group of fish they were evaluating. The fillets were given a score from 0 to 2, where 0 was a white fillet, 1 was a pinkish fillet and 2 was a clearly red fillet.

Imaging VIS/NIR Spectroscopy

After filleting, the muscle haemoglobin was evaluated by hyperspectral imaging of the fillets in diffuse reflectance mode. Imaging was performed with a push-broom hyperspectral camera with a spectral range of 430-1000 nm and spatial resolution of 0.5 mm across-track by 1.0 mm along track (Norsk Elektro Optikk, model VNIR-640). The camera was fitted with a

lens focused at 1000 mm, and mounted 1020 mm above a conveyor belt. By characterizing the incoming light, those spectra were transformed into absorbance spectra. Following the procedure outlined in Skjelvareid, Heia (14) the haemoglobin concentration was then estimated, on pixel level, for each fillet.

Cortisol analysis

Plasma concentrations of cortisol were analysed by use of radioimmunoassay (RIA), in accordance with previously described methods [15, 16]. In short, cortisol was extracted from 300 μL plasma with 4 mL diethyl ether under shaking for four min. The aqueous phase was frozen in liquid nitrogen and the organic phase was decanted to tubes and evaporated in a water bath at 45°C for ca 20 min and reconstituted by addition of 900 μL assay buffer before assaying by RIA. The antibody used was obtained from New Zealand white (NZW) rabbits and the detection limit for the assay was 0.6 ng mL^{-1} [15].

Statistical analysis and data management

The data was analysed with the statistical software R, version 3.4.0 [17]. The relationships between response variables (plasma cortisol (ng L^{-1}), lactate (mM L^{-1}), glucose (mM L^{-1}), pH, fillet redness, muscle pH) and corresponding potential explanatory variables (as factor; groups: crowding 1 or 3 hours, recuperated 0, 3 o 6 hours, rested control and swum control), sex (as factor), plasma cortisol, blood glucose, blood lactate, muscle haemoglobin (mg g^{-1}), hepatosomatic index (HSI), gonadosomatic index (GSI) and Fulton's condition factor (100 g cm^{-3})), were investigated using Generalised Linear Modelling (GLM) [18, 19]. Muscle pH was modelled with time post-mortem and groups: crowding 1 or 3 hours, recuperated 0, 3 o 6 hours, rested control and swum control) and curvature were checked by testing with different polynomials and interactions to determine significant differences between slopes. Note that

some variables are both response and explanatory, depending on which response is under investigation. Before proceeding with the GLM analysis, the data were checked and prepared for modelling following procedures previously described [20].

Briefly, most of the response variables had only positive values and were therefore best modelled using Gamma distribution, which accounts for skewed distribution of model errors and prevents negative predictions. In those cases where distribution was normal and there was no risk of predicting negative values, data was modelled using Gaussian (Normal) error distribution. In the case for sensory evaluation of redness, data were strictly bound between 1 and 4 and therefore fitted to a quasi-binomial distribution to make sure that predicted values also falls within this range. Link function (identity, log, inverse or logit) was chosen based on which link gave the best fit to data in terms of lowest Akaike information criterion (AIC) and by visual evaluation of the graphics. All model details are available in S3 Model details.

Results

Fish density in the cod-end varied between trials from 672 to 803 kg (S1 Table) and the oxygen saturation of the water in the cod-end always remained above 95% at any position. There were no mortalities during the swim-trial (i.e. swim tunnel and retention chamber) or following crowding for one hour, but for the group crowded for 3 hours 18 % of the fish where considered dead or moribund. The first run with 3 hours crowding had 48 % mortality, whereas the last two runs had 5 and 0 % mortality, respectively (S1 Table).

The plasma level of cortisol was clearly affected by swimming, crowding and recuperation ($p < 0.001$), but was also correlated with GSI ($p < 0.001$) (S4 Fig 1). The fish that were only swum (and not crowded) experienced a slight increase in plasma cortisol compared to the resting control. The highest levels of cortisol were found after 0 hours recuperation in the 3 hours

crowding group and after 3 hours recuperation for the 1 hour crowding group. After 6 hours of recuperation, the cortisol levels were still elevated (Fig 2A).

Blood glucose was affected by crowding and recuperation ($p<0.001$) and was positively correlated with HSI ($p < 0.001$) (S4 Fig 2). Blood glucose was higher after crowding for 1 and 3 hours compared to both resting and swum controls and remained elevated throughout the recuperation period (Fig 2B).

Blood lactate was clearly affected by swimming ($p<0.001$) and duration of crowding ($p<0.001$) (Fig 2C). Fish crowded for 1 hour had significantly higher lactate levels compared to resting and swum control ($p<0.001$), the levels remained elevated throughout the recuperation period. The animals crowded for 3 hours showed an almost 2-fold increase in lactate levels compared to 1 hour ($p<0.001$). The lactate stayed elevated throughout the recuperation period. Blood lactate levels were also negatively correlated to muscle pH ($p<0.001$) (S4 Fig3), this correlation was strongest for the 3 hours crowding group.

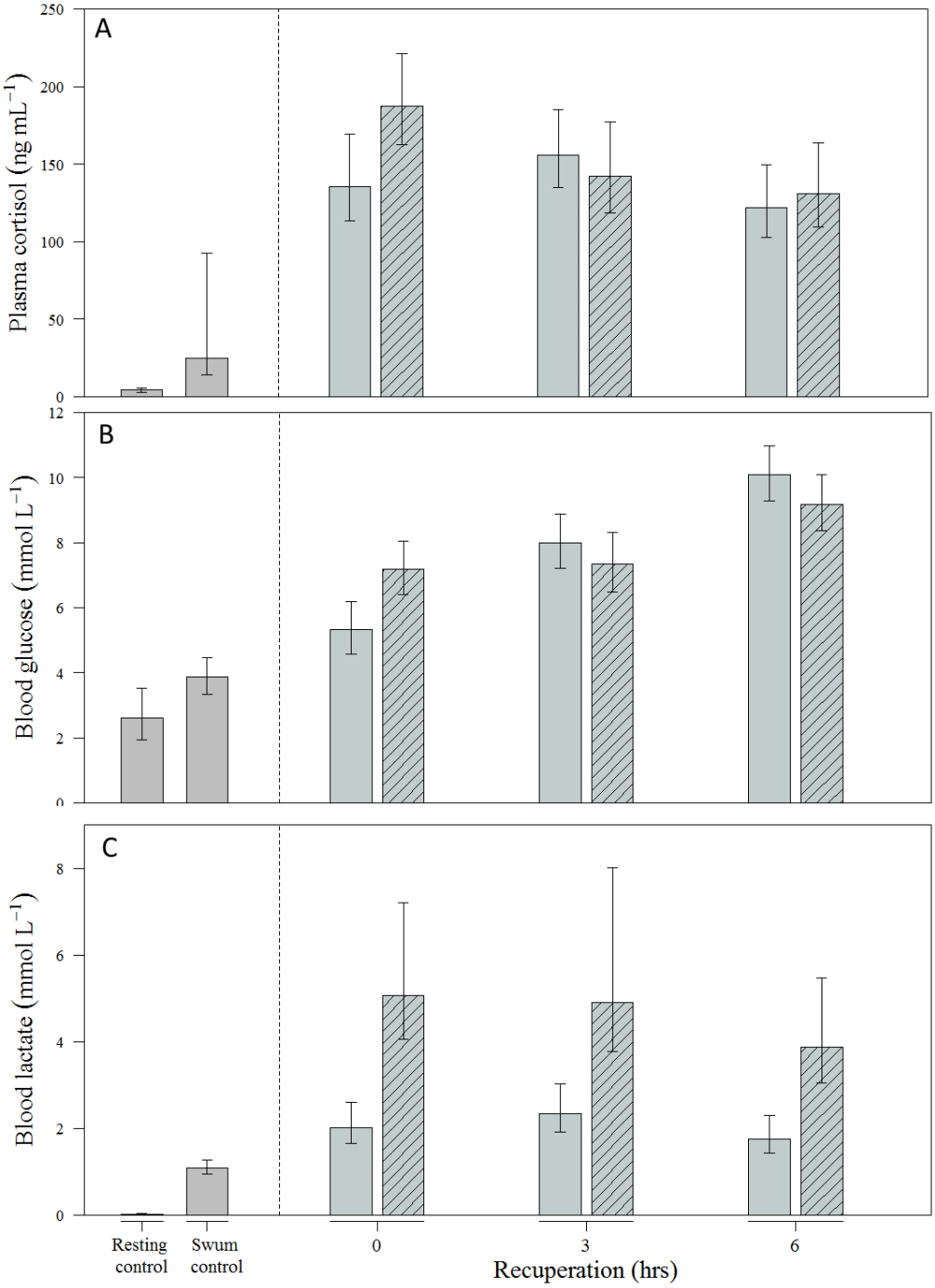
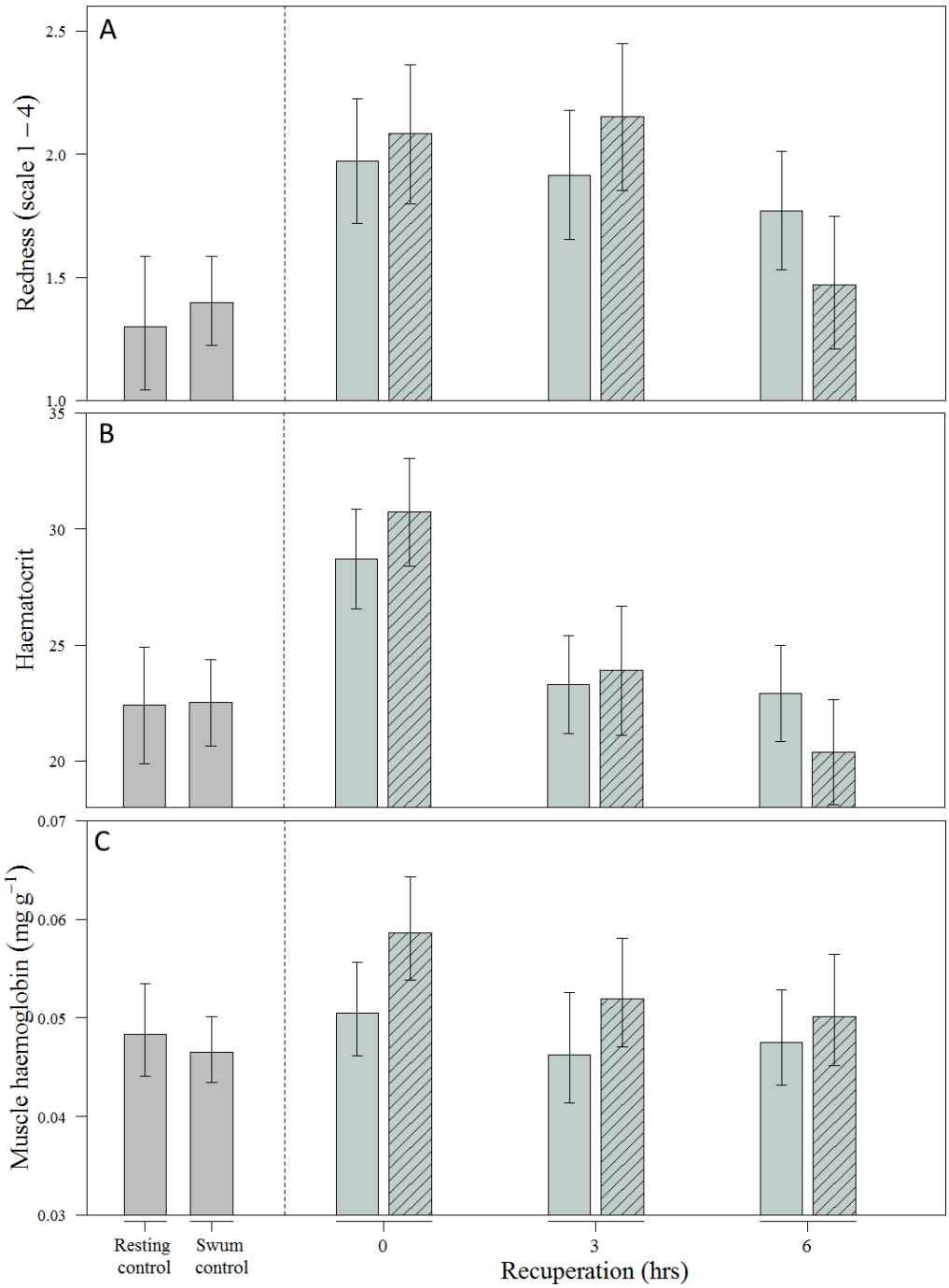


Fig 2. Physiological stress response to crowding and recuperation. Plasma cortisol (A), blood glucose (B) and blood lactate (C) in Atlantic cod during recuperation following exhaustive exercise and severe crowding for 1 hour (open bars) or 3 hours (dashed bars). Resting control are sampled from tank and swum controls are sampled immediately following exhaustive swimming exercise. Data are presented as estimated mean and errors indicate 95% confidence intervals fitted from GLM. See S3 for model details

Fillet redness was affected by swimming, crowding and recuperation and was positively correlated with muscle haemoglobin levels (S4 Fig 3). There were no major differences between fillets of fish crowded for 1 hour versus those crowded for 3 hours. After 6 hours of recuperation, the level of redness was still higher than for resting and swum control, but lower than after 0 and 3 hours of recuperation (Fig 3A). In the GLM without haemoglobin as explanatory variable, swimming, crowding and recuperation remained significant explanatory variables ($p<0.001$). In addition, a positive correlation between cortisol level and redness was found ($p=0.043$) (S4 Fig 5).

Crowding and recuperation affected muscle haemoglobin ($p=0.007$), but only the fish crowded for 3 hours without recuperation had increased muscle haemoglobin compared to the swum and rested control (Fig 3B). When modelled together with haematocrit, this effect disappeared and only haematocrit remained a significant explanatory variable ($p=0.02$) (S4 Fig 6). Because it can be argued that haemoglobin and haematocrit are dependant, a second GLM without haematocrit was run. In the second run, a positive correlation between cortisol level and muscle haemoglobin was found ($p=0.012$), also the swimming, crowding and recuperation was significant when modelled together with cortisol ($p=0.008$) (S4 Fig 7).

Swimming, crowding and recuperation affected haematocrit ($p < 0.001$) and was positively correlated to plasma cortisol levels ($p = 0.038$) (S4 Fig 8). The haematocrit increased during crowding, was highest immediately after crowding and had decreased to control levels after 3 hours of recuperation (Fig 3B).



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297 **Fig 3. Redness, haematocrit and muscle haemoglobin**. Sensory evaluation of redness (A),
298 haemotocrit (B) and muscle haemoglobin in the surface area of fillets measured by spectroscopy (C) in
299 Atlantic cod during recuperation following exhaustive exercise and severe crowding for 1 hour (open
300 bars) or 3 hours (dashed bars). Resting control are sampled from tank and swum controls are sampled
301 immediately following exhaustive swimming excercise. Data are presented as estimated mean and errors
302 indicate 95% confidence intervals fitted from GLM. See S3 for model details

Muscle pH was affected by swimming, crowding and recuperation (Fig 4). The peri-mortem pH was lowest in un-recuperated, crowded fish, but there were no differences between groups crowded for 1 and 3 hours. However, the fish crowded for 1 hour recovered faster than fish crowded for 3 hours. The rate and shape of the slope of the post-mortem muscle pH drop was significantly affected by crowding and recuperation ($p<0.001$, Fig 4). The muscle pH drop rate was highest in control fish and fish recuperating from 1 hours crowding. Furthermore, there were significant differences in the shape of pH drop slopes that were dependant on crowding time. Fish crowded for 3 hour appeared to level at minimum pH ca 48 hours post-mortem, whereas the other groups seemed to continue the drop beyond measured time.

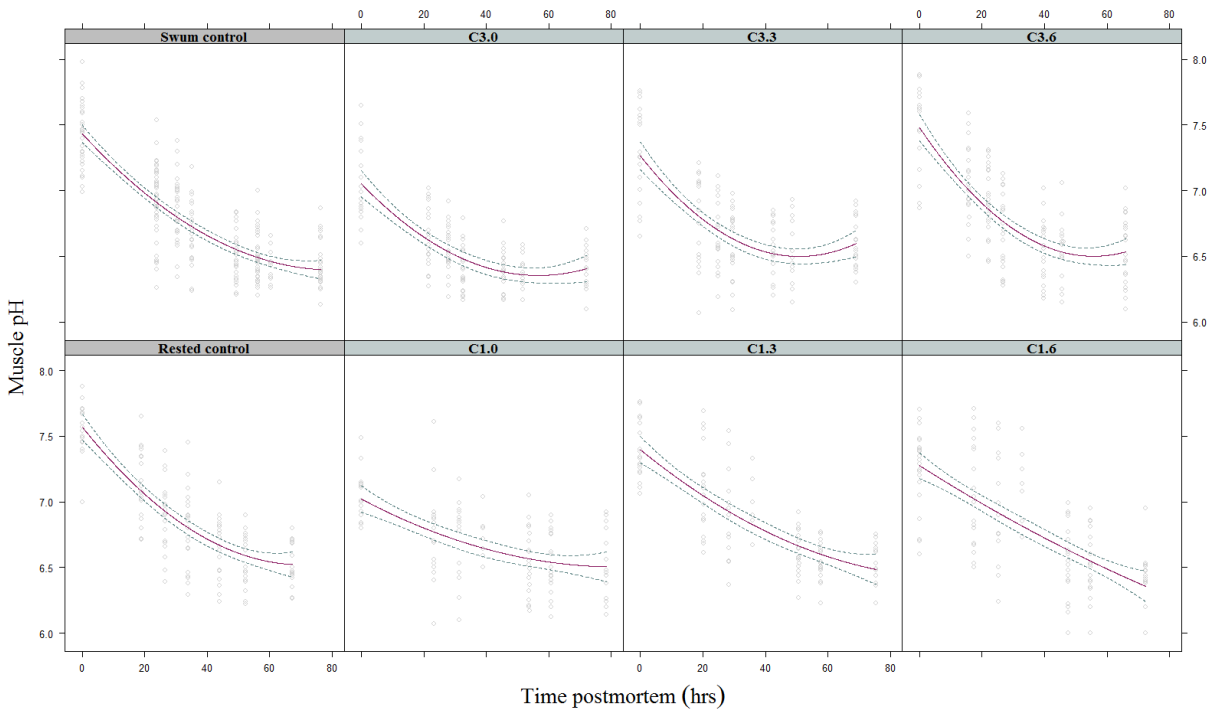

Fig 4. Postmortem change in muscle pH. Relationship between muscle pH and time postmortem. Each panel represents data from separate recovery groups: rested controls (sampled from tank), swum control (sampled immediately after swimming exercise), crowded for 1 hour and recuperated for 0 h (C1.0), 3 h (C1.3) and 6 h (C1.6), crowded for 3 hours and recuperated for 0 h (C3.0), 3 h (C3.3) and 6 h (C3.6). Data are presented as open circles; fitted values from the GLM are shown as a solid red line and the corresponding 95% confidence interval as dashed grey lines. See S3 for model details.

Discussion

There is growing interest in the fishing industry to improve the quality of fish caught by commercial trawlers. Large catches and lengthy hauls often result in lower muscle pH, muscle segment gaping and a reddish coloration of the fillet, all of which are considered quality defects that may lead to downgrading of the fish and financial loss for the producer [21, 22]. One way to circumvent this problem is to temporarily store the fish live in tanks supplied with running seawater to let the fish recover from the capture process. This procedure has successfully improved fillet quality in Atlantic cod caught by trawl [2].

We have previously demonstrated that exhaustive swimming alone does not cause the variable or reduced fillet quality frequently seen in Atlantic cod caught by trawl and suggested that crowding in the cod-end may be an important factor causing reduced fillet quality in trawl-caught fish [13]. Hence, the purpose of this study was to experimentally study the effects of exhaustive swimming and crowding in the cod-end on physiological stress parameters and fillet quality traits in Atlantic cod. We found that exhaustive swimming followed by crowding caused a severe metabolic stress response, as demonstrated by high plasma cortisol levels and elevated blood lactate and glucose levels. The metabolic stress was accompanied by a reduction in muscle pH and increased fillet redness, similar to that reported for cod caught by trawl [2, 6]. The direct cause of the stress induced by crowding is not clear, but a gradual build-up of blood lactate, which correlated with the duration of the crowding, is an indication of insufficient oxygen uptake and prolonged anaerobic metabolism during the period of confinement. Our initial expectation was that there would be less oxygen available inside the cod-end during crowding which could affect the oxygen uptake of the fish, but oxygen saturation of the water always remained above 95% at any position inside the experimental cod-end. It seems more likely, therefore, that our cod may have experienced hypoxia as a consequence of impaired

opercular movement and thus insufficient ventilation due to the very high fish density inside the cod-end.

In the present experiment, post-exercise crowding for 1 and 3 hours, were associated with 0 and 18% mortality after 6 hours of recovery, respectively. This suggests that the majority of Atlantic cod can handle extreme crowding (about 700 kg m^{-3}) for 3 hours. However, the mortality in the 3 hour crowding group varied greatly between the three trials (48, 5 and 0 %, S1 Table). The first trial of fish crowded for 3 hours had higher fish density (*i.e.* about 800 kg m^{-3}) than the last two trials. The density was similar to that in the first trial with 1 hour crowding. This indicates that crowding time is particularly important when the fish density is high and that there may be a threshold for tolerable crowding between 700 and 800 kg m^{-3}. A study from commercial trawlers found that hauls longer than 5 hours led to up to 27 % mortality [2]. This is in contrast to the initial trial in our experiment where confinement in the cod-end for 5 hours resulted in over 80% mortality. We speculate that the discrepancy between our experiment and the observations from commercial trawls, may be due to the gradual filling of the trawl under natural conditions, in which case the fish would not experience extreme crowding until the cod-end is filled up to some degree. For example, another large scale trawl study found a less severe cortisol response (~ 60 ng mL^{-1}) in cod after hauls lasting 15-55 min [6], compared to the fish in our study that were confined in the experimental cod-end for 1 hour (~ 200 ng mL-1).

During hypoxia, the metabolic fuel preference is thought to shift from mainly lipids and proteins to carbohydrates [23]. We found a marked elevation in blood glucose after crowding, which continued to increase throughout the recuperation period. This is most likely due to catecholamine and cortisol-mediated stimulation of glycogenolysis and gluconeogenesis, respectively, which is not met by a comparable increase in glucose utilisation [24, 25]. We also found that fillet redness increased as a response to crowding, and that it correlated with elevated plasma cortisol levels and muscle haemoglobin. This suggests that the sensory evaluation of

redness is a valid method for assessing amount of blood in cod fillets. In addition, the haemoglobin measurement was positively correlated with haematocrit, indicating that the amount of red blood cells also have a contributing effect to observed increase in fillet redness. In Atlantic cod, hypoxic conditions are reported to increase resistance of vessels supplying the stomach, intestines and other digestive organs, while somatic circulation is dilated [26], thereby redistributing blood flow to the muscle. Furthermore, in rainbow trout 80 % of cardiac output is found to be routed to the white muscle of during recovery from strenuous exercise [27]. It seems likely, therefore, that the increase in haematocrit, together with a presumed increased blood perfusion of the white muscle during recovery may be the most important factors causing increased redness of the fillet during recovery.

In the present study, the strong lactate response in crowded fish was negatively correlated to muscle pH. High peri-mortem lactate levels may have consequences for shelf-life of the fillets because lactate, as a carbohydrate, can be a substrate for microbial growth and production of volatiles [28]. It is frequently claimed that the formation of lactic acid causes the post-mortem decrease in muscle pH. However, the concept of lactic acidosis has been questioned [29-33]. It is now more accepted that the major source of protons is hydrolysis of ATP and formation of reduced nicotinamide adenine dinucleotide during glycolysis, with lactate production being a proton-consuming process that retards, not causes, acidosis [34].

In accordance with other studies [2, 35-38] we found that the stress associated with crowding lead to a low peri-mortem muscle pH that continued to decline post-mortem. A rapid decline in post-mortem muscle pH has been associated with softening of the muscle in cod [39]. We found that fish crowded for 3 hours reached minimum pH faster than the other groups and appeared to level out or even increase muscle pH after approximately 48 hours storage on ice. A previous study on meagre (*Argyrosomus regius*) found that a late post-mortem increase in pH was associated with decomposition of nitrogenated compounds, caused primarily by

393 microbial activity [40]. This means that an early increase in post-mortem muscle pH as
394 observed in the current study, may influence shelf-life of the final product. Interestingly, the
395 tendency of pH to increase 60-80 hours post-mortem occurred for all fish crowded for 3 hours,
396 even after 6 hours of recuperation when there were no differences in the peri-mortem muscle
397 pH. This suggests that the severity of stress fish are exposed to pre-mortem affects how muscle
398 pH changes post-mortem, and thereby may influence final quality

399 Conclusion

400 In this study, we found that exhaustive swimming together with crowding for 3 hrs cause
401 physiological responses comparable to what is seen in trawl-captured cod. The same responses
402 were not seen in fish subjected only to exhaustive swimming. This indicates that the additional
403 physiological stress caused by crowding in the cod-end is an important contributor to the often-
404 observed reduction in fillet quality of cod caught by trawl. A complete recovery from
405 exhaustive exercise and extreme crowding, most likely requires more than 6 hours.

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410 research station is also gratefully acknowledged.

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Supporting information

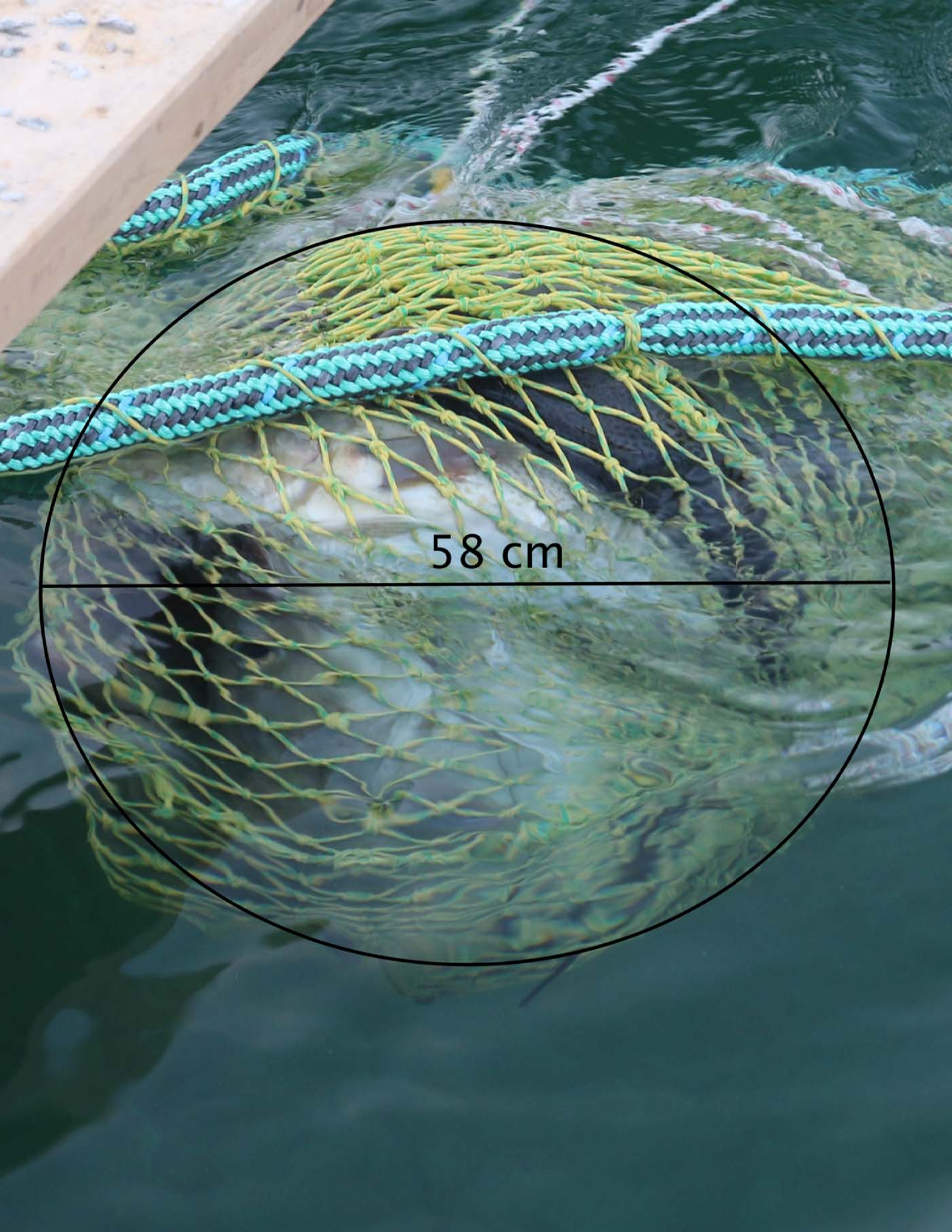
S1 Table. Overview and summary information of each trial. Trial number, dates, air temperature, biological information, fish density and mortality for each trial.

S2 Fig. Extreme crowding of Atlantic cod. Image showing the extreme crowding of cod in the experimental cod-end. The shape of the closed cod-end resembled a sphere with diameter 58 cm.

S3 Model detail. Model parameters and ANOVA output from the generalized linear models.

S4 Figures. GLM correlation plots.

Trial no.	Treatment	Recuperation time (hrs)	Date	♂	♀	N	Weight	Length	CF	GSI	HSI	Fish density in cod-end (kg m-3)	Mortality (%)	Air temp. °C
1	Rested control	Na	01.02.2015	2	5	7	3771 ± 1330	75 ± 7	0.85 ± 0.07	6.23 ± 9.17	4.80 ± 1.09	Na	0	-5±0.4
	Swum control	0	03.02.2015	3	4	7	3939 ± 616	77 ± 5	0.88 ± 0.15	7.64 ± 6.33	4.38 ± 1.39	Na		
	1 hr crowding	0	03.02.2015	3	4	7	4211 ± 1186	78 ± 10	0.89 ± 0.19	9.57 ± 6.15	4.76 ± 1.62		0	-7.8±0.4
	1 hr crowding	3	03.02.2015	2	5	7	4171 ± 1124	81 ± 6	0.78 ± 0.12	4.87 ± 4.21	4.41 ± 1.77	803		
	1 hr crowding	6	03.02.2015	6	1	7	3343 ± 839	73 ± 10	0.92 ± 0.38	5.69 ± 5.31	4.48 ± 1.76			
1	Swum control	0	10.02.2015	2	5	7	3397 ± 1220	75 ± 8	0.78 ± 0.08	3.12 ± 2.99	3.98 ± 0.90	Na	0	-0.9±1.1
	3 hrs crowding	0	10.02.2015	2	5	7	3603 ± 804	77 ± 6	0.78 ± 0.14	7.03 ± 6.81	3.93 ± 1.09			
	3 hrs crowding	3	10.02.2015	5	2	7	3934 ± 248	80 ± 5	0.78 ± 0.13	6.19 ± 4.46	3.83 ± 1.02	802	48	
	3 hrs crowding	6	10.02.2015	3	5	8	3645 ± 597	77 ± 6	0.79 ± 0.13	6.08 ± 5.74	4.34 ± 0.95			
2	Rested control	Na	08.02.2015	1	6	7	3626 ± 911	76 ± 6	0.81 ± 0.11	3.48 ± 4.34	4.34 ± 1.30	Na	0	-6±0.4
	Swum control	0	12.02.2015	2	5	7	3293 ± 889	75 ± 6	0.77 ± 0.15	4.79 ± 5.62	4.69 ± 1.30	Na	0	
	1 hr crowding	0	12.02.2015	2	5	7	2683 ± 601	68 ± 5	0.83 ± 0.12	5.79 ± 6.98	3.56 ± 1.34			-4.9±0.8
	1 hr crowding	3	12.02.2015	4	3	7	3423 ± 852	75 ± 4	0.79 ± 0.11	4.73 ± 4.86	3.40 ± 1.44	672	0	
	1 hr crowding	6	12.02.2015	4	3	7	3706 ± 889	76 ± 7	0.84 ± 0.06	1.74 ± 2.19	5.28 ± 1.15			
2	Swum control	0	17.02.2015	2	5	7	3418 ± 706	73 ± 5	0.87 ± 0.18	5.75 ± 5.96	4.72 ± 2.14	Na	0	
	3 hrs crowding	0	17.02.2015	4	3	7	3776 ± 975	76 ± 10	0.88 ± 0.17	7.37 ± 7.11	5.30 ± 1.47			1.3±0.54
	3 hrs crowding	3	17.02.2015	2	5	7	3304 ± 1104	74 ± 10	0.79 ± 0.12	8.49 ± 8.28	4.22 ± 1.74	706	5	
	3 hrs crowding	6	17.02.2015	4	3	7	3222 ± 454	71 ± 4	0.91 ± 0.11	9.29 ± 8.78	4.25 ± 1.22			
3	Rested control	Na	22.02.2015	4	3	7	3034 ± 784	72 ± 7	0.82 ± 0.12	3.27 ± 3.52	4.07 ± 1.28	Na	0	-1.3±2.27
	Swum control	0	24.02.2015	3	4	7	3364 ± 898	72 ± 5	0.90 ± 0.11	5.11 ± 4.71	4.51 ± 0.85	Na	0	
	1 hr crowding	0	24.02.2015	4	3	7	3567 ± 539	74 ± 4	0.87 ± 0.07	4.34 ± 4.31	4.65 ± 1.23			0.9±1.0
	1 hr crowding	3	24.02.2015	3	4	7	3690 ± 472	75 ± 3	0.86 ± 0.10	5.48 ± 6.35	4.78 ± 0.66	733	0	
	1 hr crowding	6	24.02.2015	2	5	7	3446 ± 818	73 ± 5	0.86 ± 0.10	3.62 ± 3.63	4.78 ± 1.36			
3	Swum control	0	26.02.2015	5	2	7	2608 ± 676	69 ± 8	0.81 ± 0.17	3.26 ± 3.29	3.44 ± 1.40	Na	0	
	3 hrs crowding	0	26.02.2015	5	2	7	3808 ± 609	76 ± 6	0.86 ± 0.09	5.78 ± 5.11	4.52 ± 1.46			0.1±1.1
	3 hrs crowding	3	26.02.2015	4	3	7	2836 ± 921	72 ± 8	0.74 ± 0.11	0.43 ± 0.22	4.56 ± 2.55	702	0	
	3 hrs crowding	6	26.02.2015	4	3	7	3604 ± 1089	73 ± 5	0.92 ± 0.11	3.04 ± 3.23	5.71 ± 1.35			


58 cm

S3 Model details

Output from Generalized linear models

Cortisol

```
Call:
glm(formula = cort ~ treatment + gsi, family = gaussian(inverse),
    data = df)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-165.60   -23.93    -3.34    30.73   163.00

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         2.237e-01  7.357e-01   0.304    0.762
treatmentpack.1.0  -2.184e-01  7.357e-01  -0.297    0.767
treatmentpack.1.3  -2.189e-01  7.357e-01  -0.298    0.766
treatmentpack.1.6  -2.168e-01  7.357e-01  -0.295    0.769
treatmentpack.3.0  -2.203e-01  7.357e-01  -0.299    0.765
treatmentpack.3.3  -2.182e-01  7.357e-01  -0.297    0.767
treatmentpack.3.6  -2.180e-01  7.357e-01  -0.296    0.767
treatments.control -1.855e-01  7.359e-01  -0.252    0.801
gsi                 4.471e-04  7.317e-05   6.111 9.17e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 2737.255)

    Null deviance: 1112242  on 149  degrees of freedom
Residual deviance:  385946  on 141  degrees of freedom
  (40 observations deleted due to missingness)
AIC: 1623.6

Number of Fisher Scoring iterations: 9
```

Analysis of Deviance Table

```
Model: gaussian, link: inverse

Response: cort

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                        149    1112242
treatment  7   554006       142     558235 28.913 < 2.2e-16 ***
gsi        1   172290       141     385946 62.943 5.956e-13 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


>
```

Glucose

```
Call:
glm(formula = glu ~ treatment + hsi, family = gaussian(log),
    data = df)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.9957  -1.1436  -0.0911   0.9309   6.4114
```

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        0.62007    0.16346   3.793 0.000205 ***
treatmentpack.1.0  0.71497    0.16867   4.239 3.63e-05 ***
treatmentpack.1.3  1.13090    0.15933   7.098 3.04e-11 ***
treatmentpack.1.6  1.31248    0.15660   8.381 1.66e-14 ***
treatmentpack.3.0  0.99200    0.16123   6.153 5.04e-09 ***
treatmentpack.3.3  1.04070    0.16217   6.417 1.26e-09 ***
treatmentpack.3.6  1.22771    0.15805   7.768 6.47e-13 ***
treatments.control 0.39689    0.16786   2.364 0.019157 *
hsi                0.07691    0.01334   5.764 3.64e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 3.330925)

    Null deviance: 1809.80  on 183  degrees of freedom
Residual deviance:  582.91  on 175  degrees of freedom
  (6 observations deleted due to missingness)
AIC: 754.34

Number of Fisher Scoring iterations: 5
```

Analysis of Deviance Table

```
Model: gaussian, link: log

Response: glu

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                        183    1809.80
treatment  7   1111.8       176     698.01 47.682 < 2.2e-16 ***
hsi        1    115.1       175     582.91 34.555  2.05e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Lactate

```
Call:
glm(formula = lac ~ treatment + mpH, family = Gamma(inverse),
    data = df)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-2.58366  -0.33619  -0.00047   0.27781   0.99015

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        36.53743    4.37497   8.351 1.99e-14 ***
treatmentpack.1.0 -39.24542    4.35518  -9.011 3.46e-16 ***
treatmentpack.1.3 -39.46750    4.35499  -9.063 2.51e-16 ***
treatmentpack.1.6 -39.24458    4.35523  -9.011 3.47e-16 ***
treatmentpack.3.0 -39.48641    4.35498  -9.067 2.44e-16 ***
treatmentpack.3.3 -39.51815    4.35497  -9.074 2.34e-16 ***
treatmentpack.3.6 -39.55227    4.35491  -9.082 2.22e-16 ***
treatments.control -38.97400    4.35532  -8.949 5.11e-16 ***
mpH                 0.45794    0.05559   8.238 3.96e-14 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.2488991)

    Null deviance: 318.337  on 183  degrees of freedom
Residual deviance:  95.924  on 175  degrees of freedom
```

```
  (6 observations deleted due to missingness)
AIC: 442.75

Number of Fisher Scoring iterations: 5
```

Sensory evaluation of redness with muscle haemoglobin

```
Call:
glm(formula = (sens + 1)/4 ~ treatment + mbr, family = quasibinomial(),
    data = df)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.39348  -0.15090  -0.03632   0.10519   0.84174

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         -2.1613     0.1912 -11.302  < 2e-16 ***
treatmentpack.1.0    0.6153     0.1465   4.200 4.25e-05 ***
treatmentpack.1.3    0.7535     0.1601   4.708 5.10e-06 ***
treatmentpack.1.6    0.4951     0.1472   3.363 0.000948 ***
treatmentpack.3.0    0.4948     0.1497   3.306 0.001151 **
treatmentpack.3.3    0.6906     0.1469   4.701 5.25e-06 ***
treatmentpack.3.6    0.3723     0.1459   2.552 0.011566 *
treatments.control   0.1552     0.1307   1.188 0.236567
mbr                 29.2960     3.2643   8.975 4.49e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 0.0516939)

    Null deviance: 17.5746  on 182  degrees of freedom
Residual deviance:  9.3824  on 174  degrees of freedom
  (7 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 4
```

Analysis of Deviance Table

```
Model: quasibinomial, link: logit

Response: (sens + 1)/4

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                        182    17.5746
treatment  7   3.8829       175    13.6917 10.731 3.288e-11 ***
mbr        1   4.3093       174     9.3824 83.362 < 2.2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Sensory evaluation of redness with plasma cortisol

```
Call:
glm(formula = (sens + 1)/4 ~ treatment + cort, family = quasibinomial,
    data = df)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.47022  -0.16657  -0.03464   0.09097   1.17908
```

```
Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)        -0.7397988  0.1585117  -4.667 7.07e-06 ***
treatmentpack.1.0   0.5154926  0.2228334   2.313  0.02216 *
treatmentpack.1.3   0.4308118  0.2269462   1.898  0.05971 .
treatmentpack.1.6   0.3279003  0.2163676   1.515  0.13191
treatmentpack.3.0   0.5452524  0.2421873   2.251  0.02592 *
treatmentpack.3.3   0.6802684  0.2398435   2.836  0.00524 **
treatmentpack.3.6   0.0074835  0.2289634   0.033  0.97397
treatments.control  0.0812914  0.1875940   0.433  0.66544
cort                0.0014818  0.0007247   2.045  0.04275 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 0.07156997)

    Null deviance: 14.116  on 148  degrees of freedom
Residual deviance: 10.392  on 140  degrees of freedom
  (41 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 3
```

Analysis of Deviance Table

```
Model: quasibinomial, link: logit

Response: (sens + 1)/4

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                        148     14.116
treatment  7   3.4236       141     10.692 6.8338 5.525e-07 ***
cort       1   0.2999       140     10.392 4.1901   0.04253 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Muscle haemoglobin modelled with haematocrit

```
Call:  glm(formula = (mbr) ~ hct, family = gaussian(), data = df)


Deviance Residuals:
      Min         1Q     Median         3Q        Max
-0.022056  -0.007248  -0.000999   0.006001   0.038645

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.0372313  0.0050855   7.321 7.22e-11 ***
hct         0.0005040  0.0002062   2.444   0.0163 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 0.0001254236)

    Null deviance: 0.012915  on 98  degrees of freedom
Residual deviance: 0.012166  on 97  degrees of freedom
  (91 observations deleted due to missingness)
AIC: -604.47

Number of Fisher Scoring iterations: 2
```

Analysis of Deviance Table

```
Model: gaussian, link: identity
```

```
Response: (mbr)

Terms added sequentially (first to last)


     Df   Deviance Resid. Df Resid. Dev      F  Pr(>F)
NULL                     98   0.012915
hct   1 0.00074912        97   0.012166 5.9727 0.01634 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Muscle haemoglobin without haematocrit

```
Call:
glm(formula = mbr ~ treatment + cort, family = gaussian(), data = df)

Deviance Residuals:
      Min         1Q     Median         3Q        Max
-0.021899  -0.007157  -0.001759   0.005926   0.036879

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)         4.977e-02  2.970e-03  16.761   <2e-16 ***
treatmentpack.1.0  -2.771e-03  4.329e-03  -0.640   0.5231
treatmentpack.1.3  -7.339e-03  4.591e-03  -1.598   0.1123
treatmentpack.1.6  -6.657e-03  4.182e-03  -1.592   0.1138
treatmentpack.3.0   2.019e-03  4.741e-03   0.426   0.6709
treatmentpack.3.3  -3.577e-04  4.677e-03  -0.076   0.9391
treatmentpack.3.6  -4.212e-03  4.378e-03  -0.962   0.3377
treatments.control -4.062e-03  3.518e-03  -1.155   0.2503
cort                3.767e-05  1.473e-05   2.557   0.0117 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 0.0001145962)

    Null deviance: 0.018644  on 144  degrees of freedom
Residual deviance: 0.015585  on 136  degrees of freedom
  (45 observations deleted due to missingness)
AIC: -893.54

Number of Fisher Scoring iterations: 2
```

Analysis of Deviance Table

```
Model: gaussian, link: identity

Response: mbr

Terms added sequentially (first to last)


          Df   Deviance Resid. Df Resid. Dev      F   Pr(>F)
NULL                         144   0.018644
treatment  7 0.00230938       137   0.016334 2.8789 0.007785 **
cort       1 0.00074931       136   0.015585 6.5387 0.011652 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Haemotocrit

```
Call:
glm(formula = (hct) ~ treatment + cort, family = gaussian(),
    data = df)

Deviance Residuals:
```

```
     Min        1Q    Median        3Q       Max
-17.0103   -1.6971    0.3383    2.3208    7.3038

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       22.326168   1.511959  14.766   <2e-16 ***
treatmentpack.1.0  4.194346   2.165753   1.937   0.0568 .
treatmentpack.1.3 -1.464930   2.119666  -0.691   0.4917
treatmentpack.1.6 -1.446498   2.112939  -0.685   0.4958
treatmentpack.3.0  5.400214   2.233996   2.417   0.0182 *
treatmentpack.3.3 -0.838822   2.452710  -0.342   0.7334
treatmentpack.3.6 -5.592258   2.114374  -2.645   0.0101 *
treatments.control -0.234210   1.882553  -0.124   0.9013
cort               0.016383   0.006802   2.409   0.0186 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 15.99685)

    Null deviance: 2265.3  on 79  degrees of freedom
Residual deviance: 1135.8  on 71  degrees of freedom
  (110 observations deleted due to missingness)
AIC: 459.27

Number of Fisher Scoring iterations: 2
```

Analysis of Deviance Table

```
Model: gaussian, link: identity

Response: (hct)

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev     F    Pr(>F)
NULL                        79     2265.3
treatment  7  1036.69        72     1228.6 9.258 4.331e-08 ***
cort       1    92.81        71     1135.8 5.802   0.01861 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Muscle pH

```
Call:
glm(formula = pH ~ adj.hrs * treatment + I(adj.hrs^2) * treatment,
    family = gaussian(), data = rigor)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.73550  -0.15952  -0.01063   0.16668   0.84081

Coefficients:
                                Estimate Std. Error t value Pr(>|t|)
(Intercept)                    7.571e+00  5.091e-02 148.715  < 2e-16 ***
adj.hrs                       -2.988e-02  3.150e-03  -9.486  < 2e-16 ***
treatmentpack.1.0             -5.497e-01  7.239e-02  -7.594 6.32e-14 ***
treatmentpack.1.3             -1.744e-01  7.192e-02  -2.425  0.01548 *
treatmentpack.1.6             -2.957e-01  7.130e-02  -4.147 3.61e-05 ***
treatmentpack.3.0             -5.178e-01  7.236e-02  -7.155 1.47e-12 ***
treatmentpack.3.3             -3.065e-01  7.441e-02  -4.120 4.06e-05 ***
treatmentpack.3.6             -9.082e-02  7.252e-02  -1.252  0.21073
treatments.control            -1.370e-01  6.119e-02  -2.239  0.02534 *
I(adj.hrs^2)                   2.126e-04  4.425e-05   4.804 1.75e-06 ***
adj.hrs:treatmentpack.1.0      1.731e-02  4.334e-03   3.993 6.93e-05 ***
adj.hrs:treatmentpack.1.3      1.052e-02  4.435e-03   2.371  0.01788 *
adj.hrs:treatmentpack.1.6      1.479e-02  4.547e-03   3.252  0.00118 **
```

```
adj.hrs:treatmentpack.3.0        5.229e-03  4.310e-03   1.213  0.22530
adj.hrs:treatmentpack.3.3       -2.206e-04  4.533e-03  -0.049  0.96120
adj.hrs:treatmentpack.3.6       -5.302e-03  4.606e-03  -1.151  0.24994
adj.hrs:treatments.control       4.093e-03  3.700e-03   1.106  0.26884
treatmentpack.1.0:I(adj.hrs^2)  -1.362e-04  5.827e-05  -2.337  0.01960 *
treatmentpack.1.3:I(adj.hrs^2)  -1.161e-04  6.060e-05  -1.915  0.05568 .
treatmentpack.1.6:I(adj.hrs^2)  -1.795e-04  6.343e-05  -2.830  0.00473 **
treatmentpack.3.0:I(adj.hrs^2)   4.963e-06  5.844e-05   0.085  0.93234
treatmentpack.3.3:I(adj.hrs^2)   8.367e-05  6.195e-05   1.350  0.17714
treatmentpack.3.6:I(adj.hrs^2)   1.035e-04  6.448e-05   1.606  0.10865
treatments.control:I(adj.hrs^2) -5.230e-05  5.072e-05  -1.031  0.30274
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 0.06071145)

    Null deviance: 185.904  on 1198  degrees of freedom
Residual deviance:  71.336  on 1175  degrees of freedom
  (265 observations deleted due to missingness)
AIC: 69.225

Number of Fisher Scoring iterations: 2
```

Analysis of Deviance Table

```
Model: gaussian, link: identity

Response: pH

Terms added sequentially (first to last)


                        Df Deviance Resid. Df Resid. Dev         F    Pr(>F)
NULL                                    1198    185.904
adj.hrs                  1   86.570      1197     99.334 1425.9288 < 2.2e-16
***
treatment                7   11.190      1190     88.144   26.3295 < 2.2e-16
***
I(adj.hrs^2)             1   10.265      1189     77.880  169.0742 < 2.2e-16
***
adj.hrs:treatment        7    4.168      1182     73.712    9.8070 6.355e-12
***
treatment:I(adj.hrs^2)   7    2.376      1175     71.336    5.5904 2.353e-06
***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fig 1. Correlation between plasma cortisol and GSI

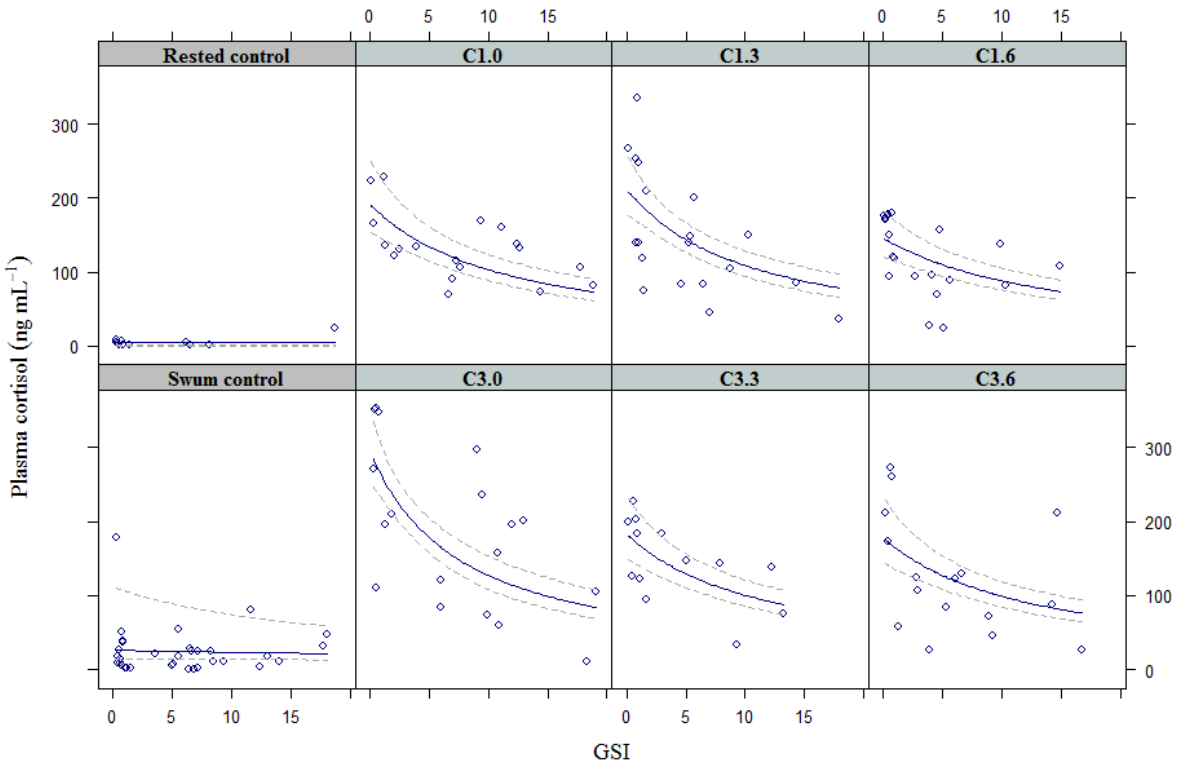


Fig 2. Correlation between blood glucose and HSI

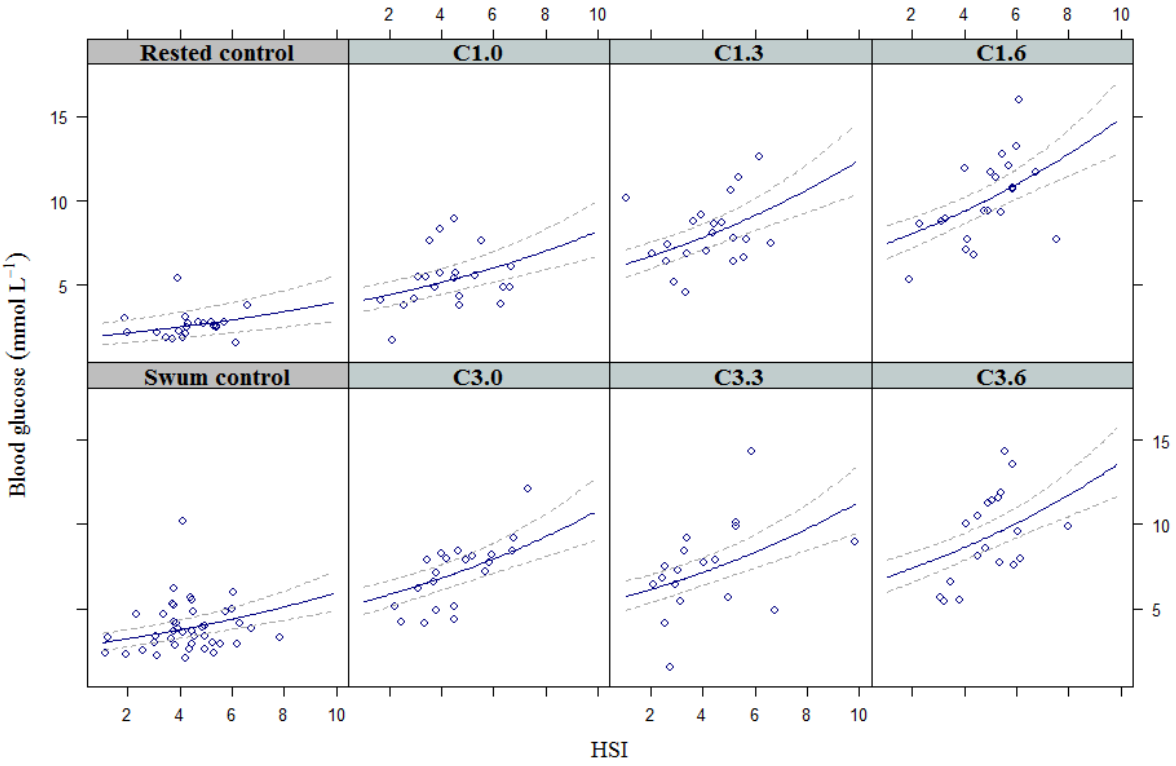


Fig 3. Correlation between blood lactate and muscle pH.

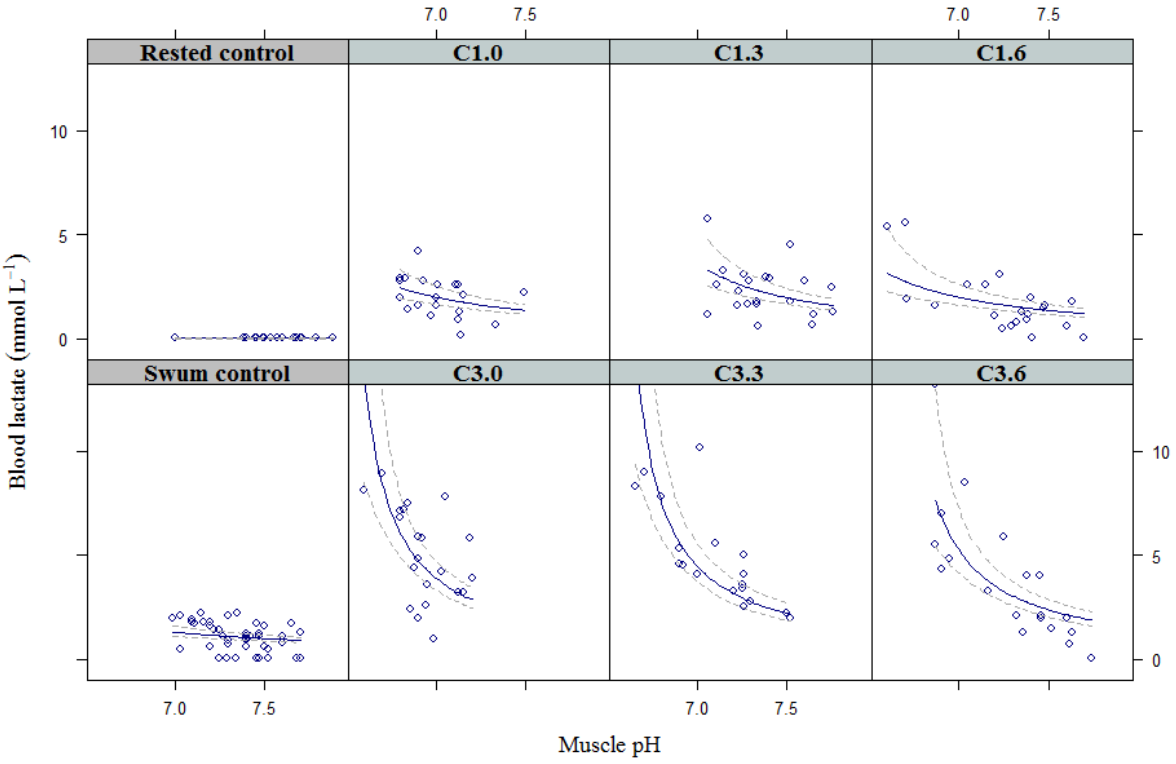


Fig 4. Correlation between fillet redness and muscle haemoglobin

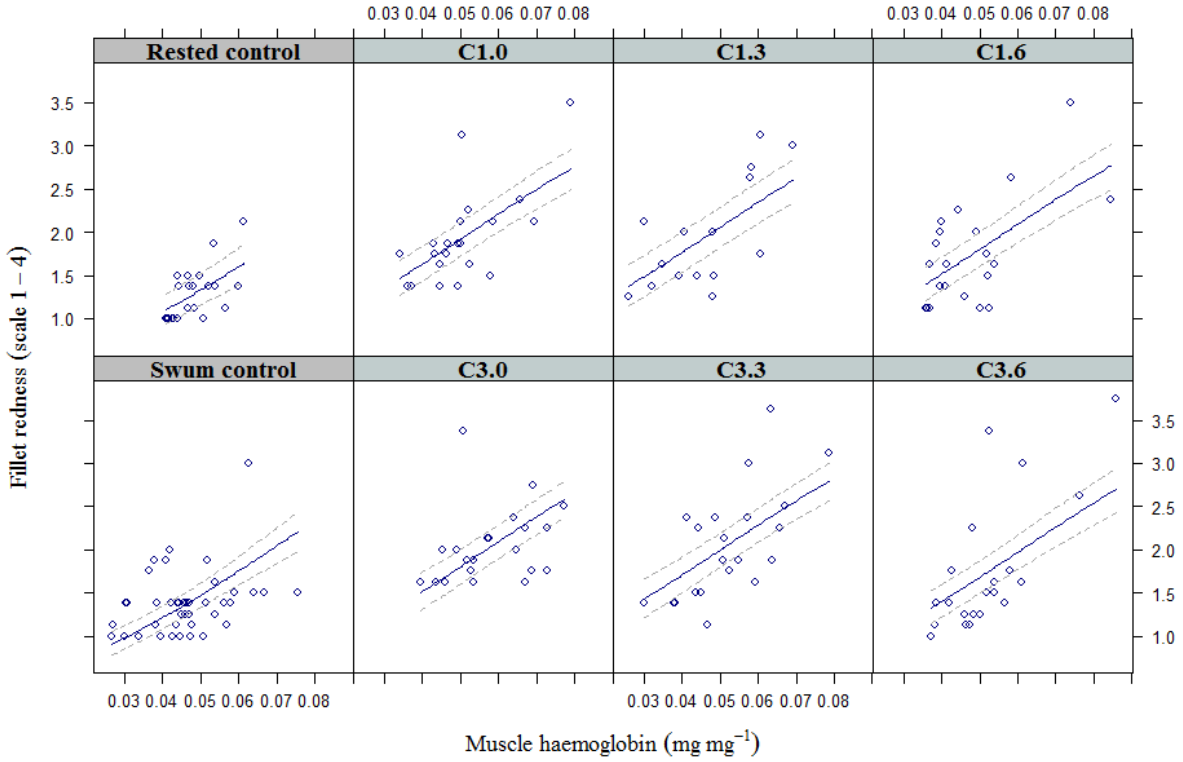


Fig 5. Correlation between fillet redness and plasma cortisol

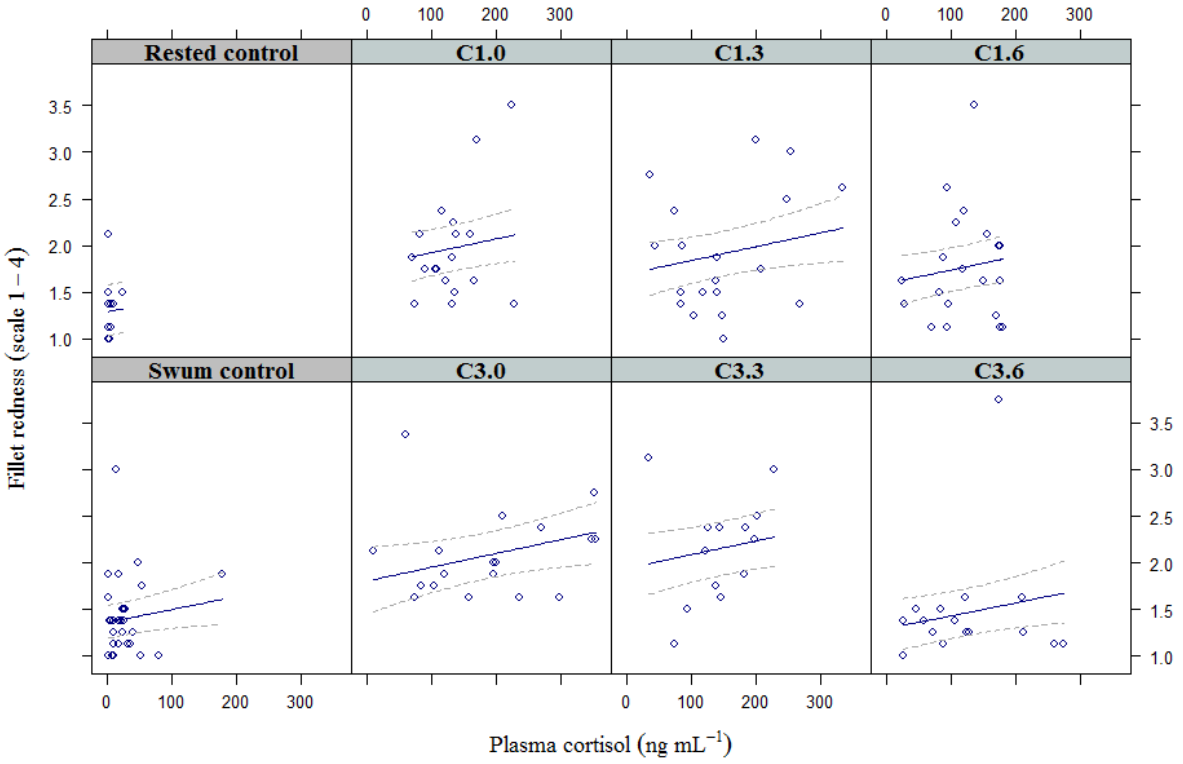


Fig 6. Correlation between muscle haemoglobin and haematocrit

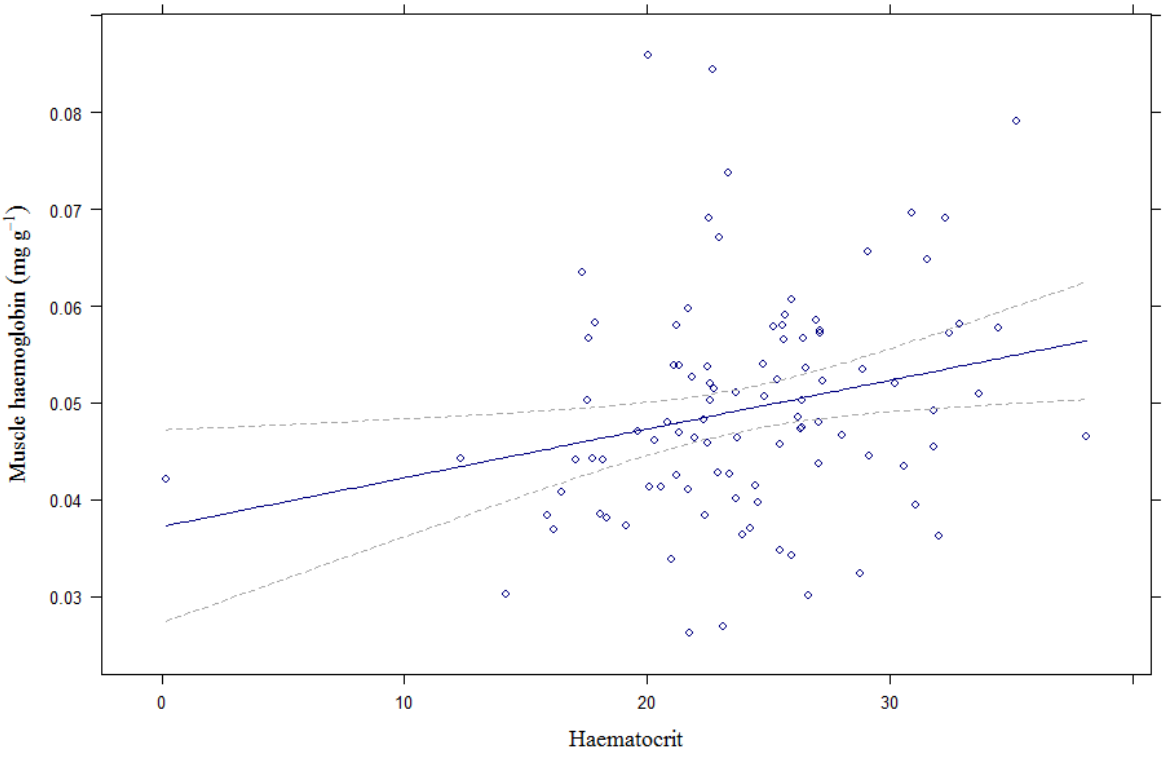


Fig 7. Correlation between muscle haemoglobin and cortisol

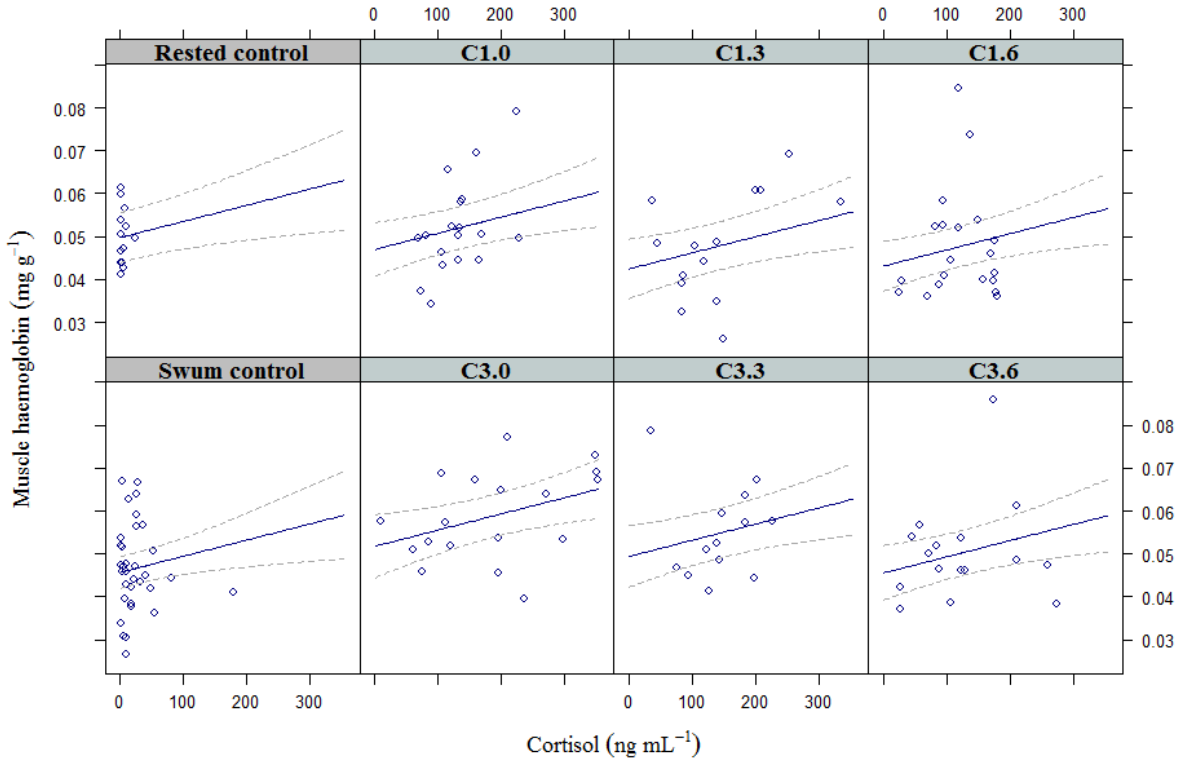


Fig 8. Correlation between haematocrit and plasma cortisol