Simulated trawling: Exhaustive swimming followed by extreme crowding as contributing reasons to variable fillet quality in trawl-caught Atlantic cod (*Gadus morhua*)

Ragnhild Aven Svalheim[1]*, Øyvind Aas-Hansen[1,#a], Karsten Heia[1], Anders Drangsholt-Karlsson [2,#b], Stein Harris Olsen[1], Helge Kreutzer Johnsen [2]

[1] Nofima - the food research institute, Muninbakken 9-13, 9291 Tromsø, Norway

[2] University of Tromsø, Faculty of Biosciences, Fisheries and Economics, Norwegian College of Fishery Science, Muninbakken 21, N-9037 Tromsø, Norway

[#a] Current address: The Norwegian Radiation Protection Authority, Section High North, The Fram Centre, Tromsø, Norway

[#b] Current Address, Bellona, Vulkan 11, 0178, Oslo, Norway

*Corresponding author

Email address: ragnhild.svalheim@nofima.no

Abstract

Trawl-caught Atlantic cod (*Gadus morhua*) often yield high variable fillet quality potentially related to capture stress. 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 showed 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 the swim tunnel and acclimated 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 commended with increasing water velocity from 0.15 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 steel mesh (4×4 cm) cages placed in an 11 m diameter fiberglass tank supplied with running seawater at natural water temperature to ensure flow-through of 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 were subsequently 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 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. Haematocrit measurements were performed with a microhaematocrit capillary tube reader (Critocaps; Oxford Lab, Baxter, Deerfield, IL). 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 were 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

211 300 µL plasma with 4 mL diethyl ether under shaking for four min. The aqueous phase was
212 frozen in liquid nitrogen and the organic phase was decanted to tubes and evaporated in a water
213 bath at 45°C for ca 20 min and reconstituted by addition of 900 µL assay buffer before assaying
214 by RIA. The antibody used was obtained from New Zealand white (NZW) rabbits and the
215 detection limit for the assay was 0.6 ng mL^{-1} [15].

216

217 Statistical analysis and data management

218 The data was analysed with the statistical software R, version 3.4.0 [17]. The
219 relationships between response variables (plasma cortisol (ng L^{-1}), lactate (mM L^{-1}), glucose
220 (mM L^{-1}), pH, fillet redness, muscle pH) and corresponding potential explanatory variables (as
221 factor; groups: crowding 1 or 3 hours, recuperated 0, 3 o 6 hours, rested control and swum
222 control), sex (as factor), plasma cortisol, blood glucose, blood lactate, muscle haemoglobin (mg
223 g^{-1}), hepatosomatic index (HSI), gonadosomatic index (GSI) and Fulton's condition factor (100
224 g cm^{-3})), were investigated using Generalised Linear Modelling (GLM) [18, 19]. Muscle pH
225 was modelled with time post-mortem and groups: crowding 1 or 3 hours, recuperated 0, 3 o 6
226 hours, rested control and swum control) and curvature were checked by testing with different
227 polynomials and interactions to determine significant differences between slopes. Note that
228 some variables are both response and explanatory, depending on which response is under
229 investigation. Before proceeding with the GLM analysis, the data were checked and prepared
230 for modelling following procedures previously described [20].

231

232 Briefly, most of the response variables had only positive values and were therefore best
233 modelled using Gamma distribution, which accounts for skewed distribution of model errors
234 and prevents negative predictions. In those cases where distribution was normal and there was
235 no risk of predicting negative values, data was modelled using Gaussian (Normal) error

236 distribution. In the case for sensory evaluation of redness, data were strictly bound between 1
237 and 4 and therefore fitted to a quasi-binomial distribution to make sure that predicted values
238 also falls within this range. Link function (identity, log, inverse or logit) was chosen based on
239 which link gave the best fit to data in terms of lowest Akaike information criterion (AIC) and
240 by visual evaluation of the graphics. All model details are available in S3 Model details.

241 Results

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

248 The plasma level of cortisol was clearly affected by swimming, crowding and recuperation (p
249 < 0.001), but was also correlated with GSI ($p < 0.001$) (S4 Fig 1). The fish that were only swum
250 (and not crowded) experienced a slight increase in plasma cortisol compared to the resting
251 control. The highest levels of cortisol were found after 0 hours recuperation in the 3 hours
252 crowding group and after 3 hours recuperation for the 1 hour crowding group. After 6 hours of
253 recuperation, the cortisol levels were still elevated (Fig 2A).

254 Blood glucose was affected by crowding and recuperation ($p<0.001$) and was positively
255 correlated with HSI ($p < 0.001$) (S4 Fig 2). Blood glucose was higher after crowding for 1 and
256 3 hours compared to both resting and swum controls and remained elevated throughout the
257 recuperation period (Fig 2B).

258 Blood lactate was clearly affected by swimming ($p<0.001$) and duration of crowding ($p<0.001$)
259 (Fig 2C). Fish crowded for 1 hour had significantly higher lactate levels compared to resting

260 and swum control (p<0.001), the levels remained elevated throughout the recuperation period.
261 The animals crowded for 3 hours showed a 2-fold increase in lactate levels compared to 1 hour
262 (p<0.001). The lactate stayed elevated throughout the recuperation period. Blood lactate levels
263 were also negatively correlated to muscle pH (p<0.001) (S4 Fig3), this correlation was strongest
264 for the 3 hours crowding group.

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

271

272 Fillet redness was affected by swimming, crowding and recuperation and was positively
273 correlated with muscle haemoglobin levels (S4 Fig 4). There were no major differences between
274 fillets of fish crowded for 1 hour versus those crowded for 3 hours. After 6 hours of
275 recuperation, the level of redness was still higher than for resting and swum control, but lower
276 than after 0 and 3 hours of recuperation (Fig 3A). In the GLM ran without haemoglobin as
277 explanatory variable, swimming crowding and recuperation remained a significant explanatory
278 variable (p<0.001). In addition a positive correlation between cortisol level and redness was
279 found (p=0.043) (S4 Fig 5).

280 Crowding and recuperation did have an effect on muscle haemoglobin (p=0.007), but only for
281 the fish crowded for 3 hours without recuperation (Fig 3B). When modelled together with
282 haematocrit, this effect disappeared and only haematocrit remained a significant explanatory
283 variable (p=0.02) (S4 Fig 6). Because it can be argued that haemoglobin and haematocrit are
284 dependant, a second GLM without haematocrit was run. In the second run, a positive correlation

285 between cortisol level and muscle haemoglobin was found (p=0.012), also the swimming,
286 crowding and recuperation was significant when modelled together with cortisol (p=0.008) (S4
287 Fig 7).

288 Swimming, crowding and recuperation had a transient effect on haematocrit ($p < 0.001$), which
289 was influenced by both crowding and recuperation time and was positively correlated to plasma
290 cortisol levels ($p = 0.038$) (S4 Fig 8). The haematocrit response was highest immediately after
291 1 and 3 hours of crowding, but decreased to control levels after 3 hours (Fig 3B).

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

298

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

308

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. The problem is that 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 been used successfully to improve fillet quality in Atlantic cod caught by trawl [2].

We have previously demonstrated that exhaustive swimming alone is not a major cause of the variable or reduced fillet quality frequently seen in Atlantic cod caught by trawl, and we therefore 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 simulate trawling by means of an experimental cod-end attached to a swim tunnel to study effects of exhaustive swimming and crowding in an experimental 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 experiemental 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 Atlantic cod can handle extreme crowding (about 700 kg m^{-3}) for 3 hours. However, we did find a mortality of 48 % in the first run of fish crowded for 3 hours (S1 Table). This trial had higher fish density (*i.e.* about 800 kg m^{-3}) than the last two trials. The density was however not higher than the first trial with 1 hour crowding. This indicates that crowding time is particularly important when the fish density is high. 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).

359 During hypoxia, the fuel preference is thought to shift from mainly lipids and proteins to
360 carbohydrates [23]. We found a marked elevation in blood glucose after crowding, which
361 continued to increase throughout the recuperation period. This is most likely due to
362 catecholamine and cortisol-mediated stimulation of glycogenolysis and gluconeogenesis,
363 respectively, which is not met by a comparable increase in glucose utilisation [24, 25]. We also
364 found that fillet redness increased as a response to crowding, and that this correlated with
365 elevated plasma cortisol levels and muscle haemoglobin. This suggests that the sensory
366 evaluation of redness is a valid method for assessing amount of blood in cod fillets. In addition,
367 the haemoglobin measurement was positively correlated with haematocrit, indicating that the
368 method is indeed measuring amount of blood in the fillets. In Atlantic cod, hypoxic conditions
369 are reported to increase resistance of vessels supplying the stomach, intestines and other
370 digestive organs, while somatic circulation is dilated [26], thereby redistributing blood flow to
371 the muscle. Furthermore, in rainbow trout 80 % of cardiac output is found to be routed to the
372 white muscle of during recovery from strenuous exercise [27]. It seems likely, therefore, that
373 the increase in haematocrit, together with a presumed increased blood perfusion of the white
374 muscle during recovery may be the most important factors causing increased redness of the
375 fillet during recovery.

376 In the present study, the strong lactate response in crowded fish was negatively correlated to
377 muscle pH. High peri-mortem lactate levels may have consequences for shelf-life of the fillets
378 because lactate, as a carbohydrate, may serve as substrate for the productions of microbial
379 growth and volatiles [28]. It is frequently claimed that the formation of lactic acid causes the
380 post-mortem decrease in muscle pH. However, the concept of lactic acidosis has been
381 questioned [29-33] and it is now more accepted that the major source of protons is hydrolysis
382 of ATP and formation of reduced nicotinamide adenine dinucleotide during glycolysis, with

383 lactate production being a proton-consuming process that retards acidosis rather the causing it
384 [34].

385 In accordance with other studies [2, 35-38] we found that the stress associated with crowding
386 lead to a low peri-mortem muscle pH that continued to decline post-mortem. A rapid decline
387 in post-mortem muscle pH has been associated with softening of the muscle in cod [39]. We
388 found that fish crowded for 3 hours reached minimum pH faster than the other groups and
389 appeared to level out or even increase muscle pH after approximately 48 hours storage on ice.
390 A previous study on meagre (*Argyrosomus regius*) found that a late post-mortem increase in
391 pH was associated with decomposition of nitrogenated compounds, caused primarily by
392 microbial activity [40]. This means that an early increase in post-mortem muscle pH as
393 observed in the current study, may influence shelf-life of the final product. Interestingly, the
394 tendency of pH to increase 60-80 hours post-mortem occurred for all fish crowded for 3 hours,
395 even after 6 hours of recuperation when there were no differences in the peri-mortem muscle
396 pH. This suggests that the severity of stress fish are exposed to pre-mortem affects how muscle
397 pH changes post-mortem, and thereby may influence final quality

398 Conclusion

399 In the present experiment, exhaustive swimming together with crowding for 3 hrs cause
400 physiological responses comparable to what is seen in trawl-captured cod. This indicates that
401 the additional physiological stress caused by crowding in the cod-end is an important
402 contributor to the often-observed reduction in fillet quality of cod caught by trawl. A complete
403 recovery from exhaustive exercise and extreme crowding, most likely requires more than 6
404 hours.

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References

1. Suuronen P. Mortality of fish escaping trawl gears In: Food AOotU, Nations, editor. FAO Fish Tech Pap. Rome, Italy: Food and Agriculture Organization of the United Nations; 2005.

2. Olsen SH, Tobiassen T, Akse L, Evensen TH, Midling KØ. Capture induced stress and live storage of Atlantic cod (Gadus morhua) caught by trawl: Consequences for the flesh quality Fish Res. 2013;147:446-53.

3. Botta JR, Bonnell G, Squires BE. Effect of method of catching and time of season on sensory quality of fresh raw Atlantic cod (*Gadus morhua*). J Food Sci. 1987;52(4):928-31. doi: 10.1111/j.1365-2621.1987.tb14245.x.

4. Esaiassen M, Nilsen H, Joensen S, Skjerdal T, Carlehög M, Eilertsen G, et al. Effects of catching methods on quality changes during storage of cod (*Gadus morhua*). LWT - Food Science and Technology. 2004;37(6):643-8.

5. Rotabakk BT, Skipnes D, Akse L, Birkeland S. Quality assessment of Atlantic cod (*Gadus morhua*) caught by longlining and trawling at the same time and location. Fish Res. 2011;112(1):44-51. doi: https://doi.org/10.1016/j.fishres.2011.08.009.

6. Digre H, Rosten C, Erikson U, Mathiassen JR, Aursand IG. The on-board live storage of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) caught by trawl: Fish behaviour, stress and fillet quality. Fish Res. 2017;189:42-54.

429 7. Barton BA. Stress in Fishes: A Diversity of Responses with Particular Reference to
430 Changes in Circulating Corticosteroids1. Integr Comp Biol. 2002;42(3):517-25. doi:
431 10.1093/icb/42.3.517.
432 8. Wendelaar Bonga SE. The stress response in fish. Physiol Rev. 1997;77(3):591-625.
433 doi: 10.1152/physrev.1997.77.3.591. PubMed PMID: 9234959.
434 9. Dunajski E. Textre of Fish Muscle J Texture Stud. 1980;10(4):301-18. doi:
435 doi:10.1111/j.1745-4603.1980.tb00862.x.
436 10. Sigholt T, Erikson U, Rustad T, Johansen S, Nortvedt TS, Seland A. Handling Stress
437 and Storage Temperature Affect Meat Quality of Farmed-raised Atlantic Salmon (Salmo
438 Salar). J Food Sci. 1997;62(4):898-905. doi: doi:10.1111/j.1365-2621.1997.tb15482.x.
439 11. Margeirsson S, Jonsson GR, Arason S, Thorkelsson G. Influencing factors on yield,
440 gaping, bruises and nematodes in cod (Gadus morhua) fillets. J Food Eng. 2007;80(2):503-8.
441 doi: http://dx.doi.org/10.1016/j.jfoodeng.2006.05.032.
442 12. Karlsson-Drangsholt A, Svalheim RA, Aas-Hansen Ø, Olsen S-H, Midling K, Breen
443 M, et al. Recovery from exhaustive swimming and its effect on fillet quality in haddock
444 (Melanogrammus aeglefinus). Fish Res. 2018;197(Supplement C):96-104. doi:
445 https://doi.org/10.1016/j.fishres.2017.09.006.
446 13. Svalheim RA, Karlsson-Drangsholt A, Olsen SH, Johnsen HK, Aas-Hansen Ø. Effects
447 of exhaustive swimming and subsequent recuperation on flesh quality in unstressed Atlantic
448 cod (*Gadus morhua*). Fish Res. 2017;193(Supplement C):158-63. doi:
449 https://doi.org/10.1016/j.fishres.2017.04.008.
450 14. Skjelvareid MH, Heia K, Olsen SH, Stormo SK. Detection of blood in fish muscle by
451 constrained spectral unmixing of hyperspectral images. J Food Eng. 2017;212:252-61. doi:
452 https://doi.org/10.1016/j.jfoodeng.2017.05.029.

15. Tveiten H, Bjørn PA, Johnsen HK, Finstad B, McKinley RS. Effects of the sea louse Lepeophtheirus salmonis on temporal changes in cortisol, sex steroids, growth and reproductive investment in Arctic charr Salvelinus alpinus. J Fish Biol. 2010;76(10):2318-41. doi: doi:10.1111/j.1095-8649.2010.02636.x.

16. Schulz R. Measurement of five androgens in the blood of immature and maturing male rainbow trout, Salmo gairdneri (Richardson). Steroids. 1985;46(2):717-26. doi: https://doi.org/10.1016/0039-128X(85)90051-0.

17. R Core Team. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. 2017.

18. McCullagh P, Nelder J. Generalized Linear Models. second ed. London: Chapman and Hall/CRC; 1999.

19. Buckley YM. Generalized linear models In: Fox GA, Negrete-Yankelevich S, Sosa VJ, editors. Ecological Statistics: Contemporary Theory and Application. Oxford: Oxford University Press.; 2015. p. 131-43.

20. Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol. 2010;1(1):3-14. doi: doi:10.1111/j.2041-210X.2009.00001.x.

21. Michie I. Causes of downgrading in the salmon farming industry. In: S.C. Kestin PDW, editor. Farmed Fish Quality. Oxford, UK: Blackwell; 2001. p. 129-36.

22. Borderías AJ, Sánchez-Alonso I. First processing steps and the quality of wild and farmed fish. J Food Sci. 2011;76(1):R1-R5. doi: 10.1111/j.1750-3841.2010.01900.x.

23. Haman F, Zwingelstein G, Weber J-M. Effects of hypoxia and low temperature on substrate fluxes in fish: plasma metabolite concentrations are misleading. Am J Physiol Regul Integr Comp Physiol. 1997;273(6):R2046-R54. doi: 10.1152/ajpregu.1997.273.6.R2046.

24. Wright PA, Perry SF, Moon TW. Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. J Exp Biol. 1989;147(1):169-88.

25. Mommsen TP, Vijayan MM, Moon TW. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish. 1999;9(3):211-68. doi: 10.1023/a:1008924418720.

26. Axelsson M, Fritsche R. Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. J Exp Biol. 1991;158(1):181-98.

27. Neumann P, Holeton GF, Heisler N. Cardiac Output and Regional Blood Flow in Gills and Muscles after Exhaustive Exercise in Rainbow Trout (Salmo Gairdneri). J Exp Biol. 1983;105(JUL):1-14. PubMed PMID: WOS:A1983RB62400001.

28. Gram L, Huss HH. Microbiological spoilage of fish and fish products. Int J Food Microbiol. 1996;33(1):121-37. doi: https://doi.org/10.1016/0168-1605(96)01134-8.

29. Brooks GA. Lactate doesn't necessarily cause fatigue: why are we surprised? J Physiol. 2001;536(Pt 1):1-. doi: 10.1111/j.1469-7793.2001.t01-1-00001.x. PubMed PMID: PMC2278833.

30. A. BG. Cell–cell and intracellular lactate shuttles. J Physiol. 2009;587(23):5591-600. doi: doi:10.1113/jphysiol.2009.178350.

31. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. J Physiol. 2004;558(Pt 1):5-30. doi: 10.1113/jphysiol.2003.058701. PubMed PMID: PMC1664920.

32. Weber J-M, Choi K, Gonzalez A, Omlin T. Metabolic fuel kinetics in fish: swimming, hypoxia and muscle membranes. J Exp Biol. 2016;219(2):250-8. doi: 10.1242/jeb.125294.

33. Teye Omlin, Karolanne Langevin, Jean-Michel Weber. Exogenous lactate supply affects lactate kinetics of rainbow trout, not swimming performance. Am J Physiol Regul

502 Integr Comp Physiol. 2014;307(8):R1018-R24. doi: 10.1152/ajpregu.00200.2014. PubMed
503 PMID: 25121611.
504 34. Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic
505 acidosis. Am J Physiol Regul Integr Comp Physiol. 2004;287(3):R502-R16. doi:
506 10.1152/ajpregu.00114.2004. PubMed PMID: 15308499.
507 35. Helge SL, Elisabeth H, Marit B, Ørjan K, Ragnar N, Bencze RAM, et al. The effects
508 of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod
509 (Gadus morhua L.). Aquacult Res. 2005;36(12):1197-206. doi: doi:10.1111/j.1365-
510 2109.2005.01339.x.
511 36. Richards MP, Hultin HO. Contributions of blood and blood components to lipid
512 oxidation in fish muscle. J Agric Food Chem. 2002;50(3):555-64. Epub 2002/01/24. PubMed
513 PMID: 11804529.
514 37. Jørpeland G, Imsland A, Stien LH, Bleie H, Roth B. Effects of filleting method, stress,
515 storage and season on the quality of farmed Atlantic cod (*Gadus morhua* L.). Aquacult Res.
516 2015;46(7):1597-607. doi: doi:10.1111/are.12312.
517 38. Kristoffersen S, Tobiassen T, Steinsund V, Olsen RL. Slaughter stress, postmortem
518 muscle pH and rigor development in farmed Atlantic cod (Gadus morhua L.). Int J Food Sci
519 Tech. 2006;41(7):861-4. doi: doi:10.1111/j.1365-2621.2005.01149.x.
520 39. F. AJ, F. HN. Chemical composition and postmortem changes in soft textured muscle
521 from intensely feeding Atlantic cod (*Gadus morhua* L.). J Food Biochem. 1985;9(1):49-64.
522 doi: doi:10.1111/j.1745-4514.1985.tb00338.x.
523 40. Hernández MD, López MB, Álvarez A, Ferrandini E, García García B, Garrido MD.
524 Sensory, physical, chemical and microbiological changes in aquacultured meagre
525 (Argyrosomus regius) fillets during ice storage. Food Chem. 2009;114(1):237-45. doi:
526 https://doi.org/10.1016/j.foodchem.2008.09.045.

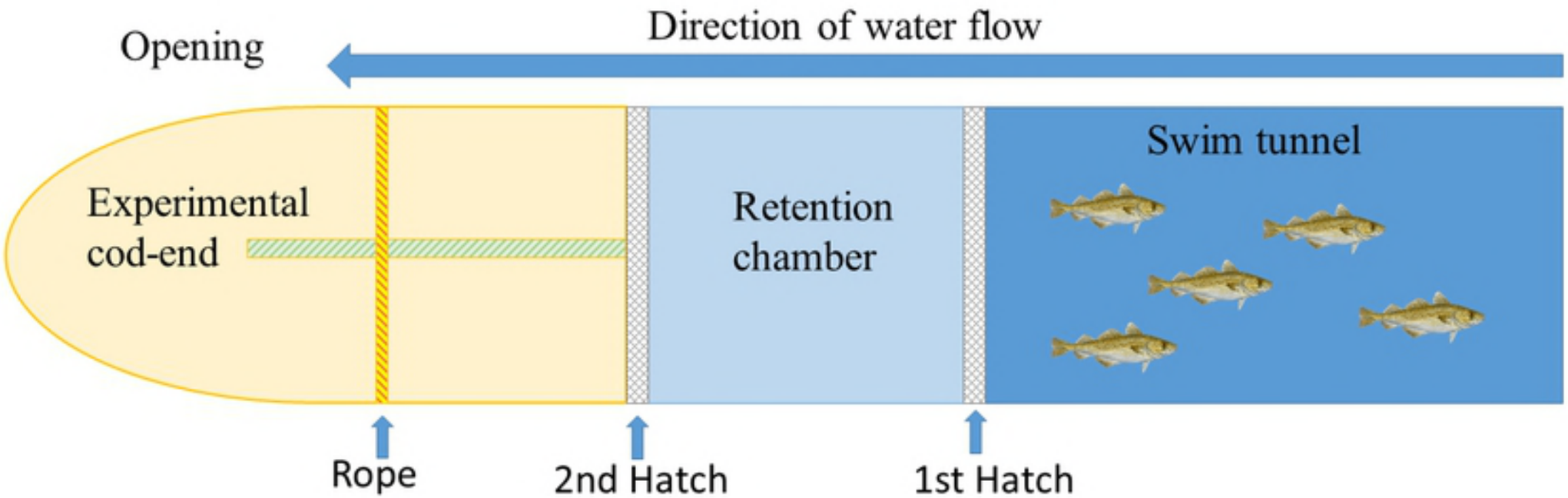
Supporting information

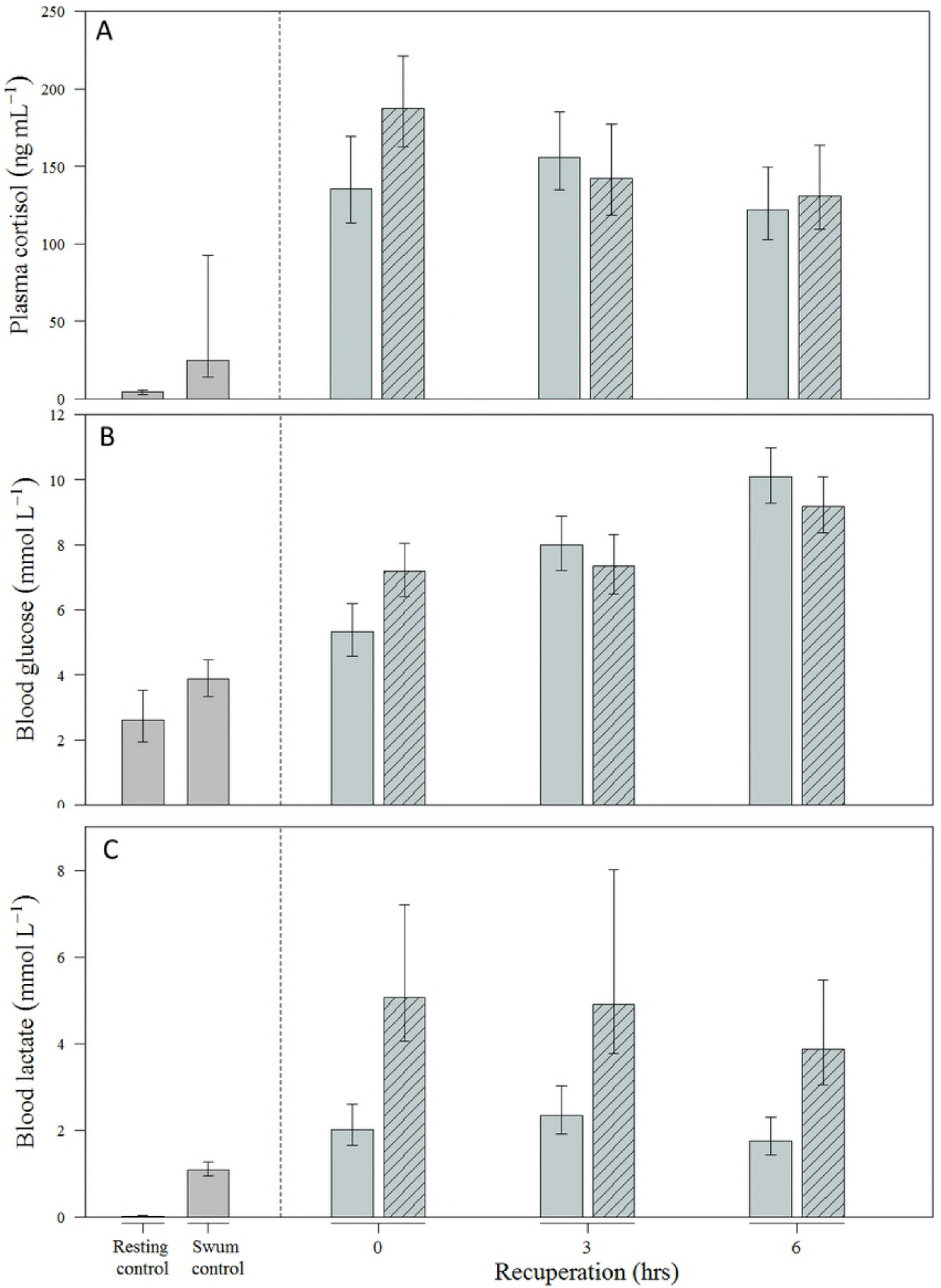
S1 Table. Overview and summary information of each trial. Trial number, dates, air temperature, biological information, fish density and mortality for each trial.

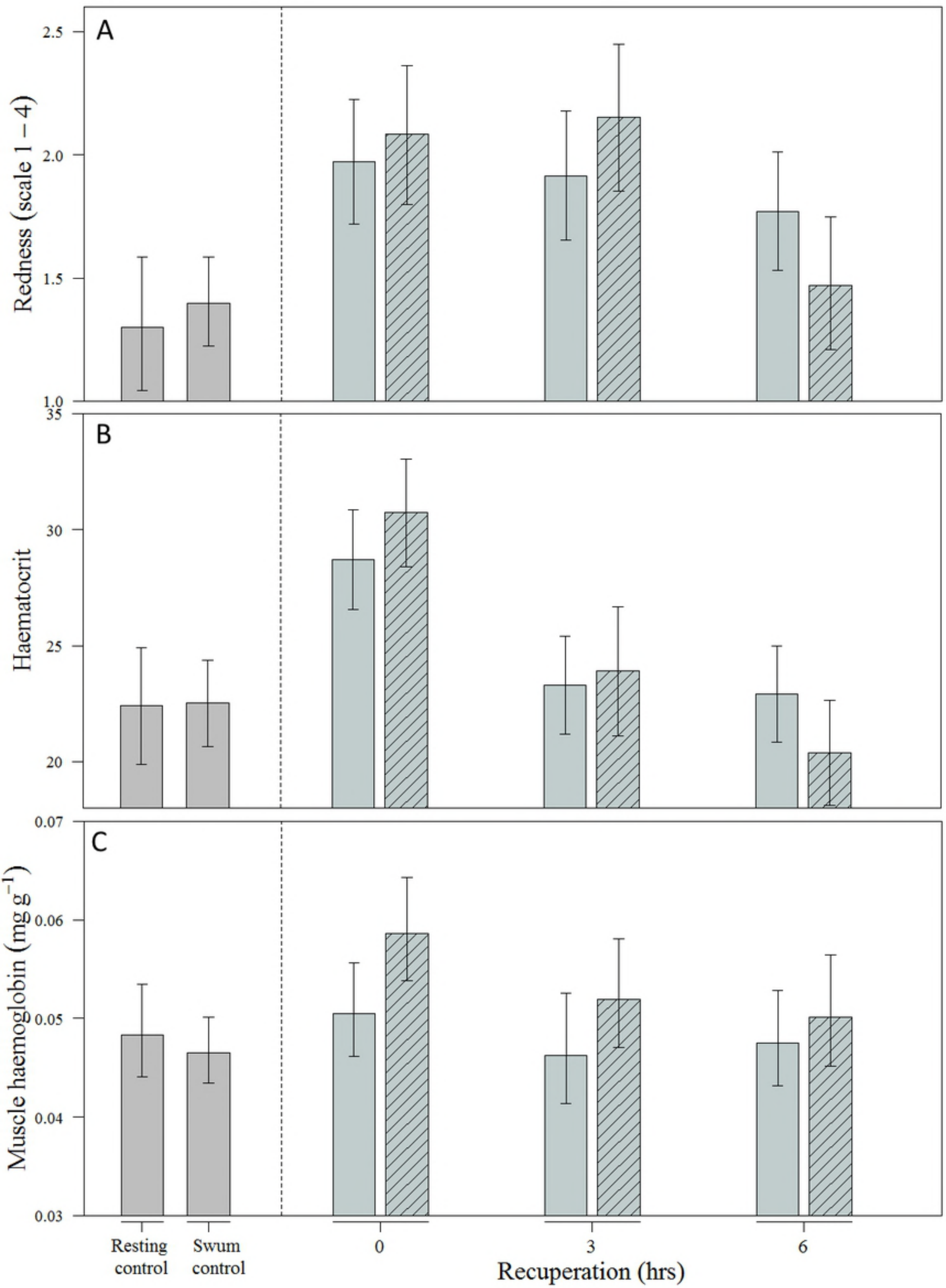
S2 Fig. Extreme crowding of Atlantic cod. Image showing the extreme crowding of cod in the experimental cod-end. The shape of the closed cod-end resembled a sphere with diameter 58 cm.

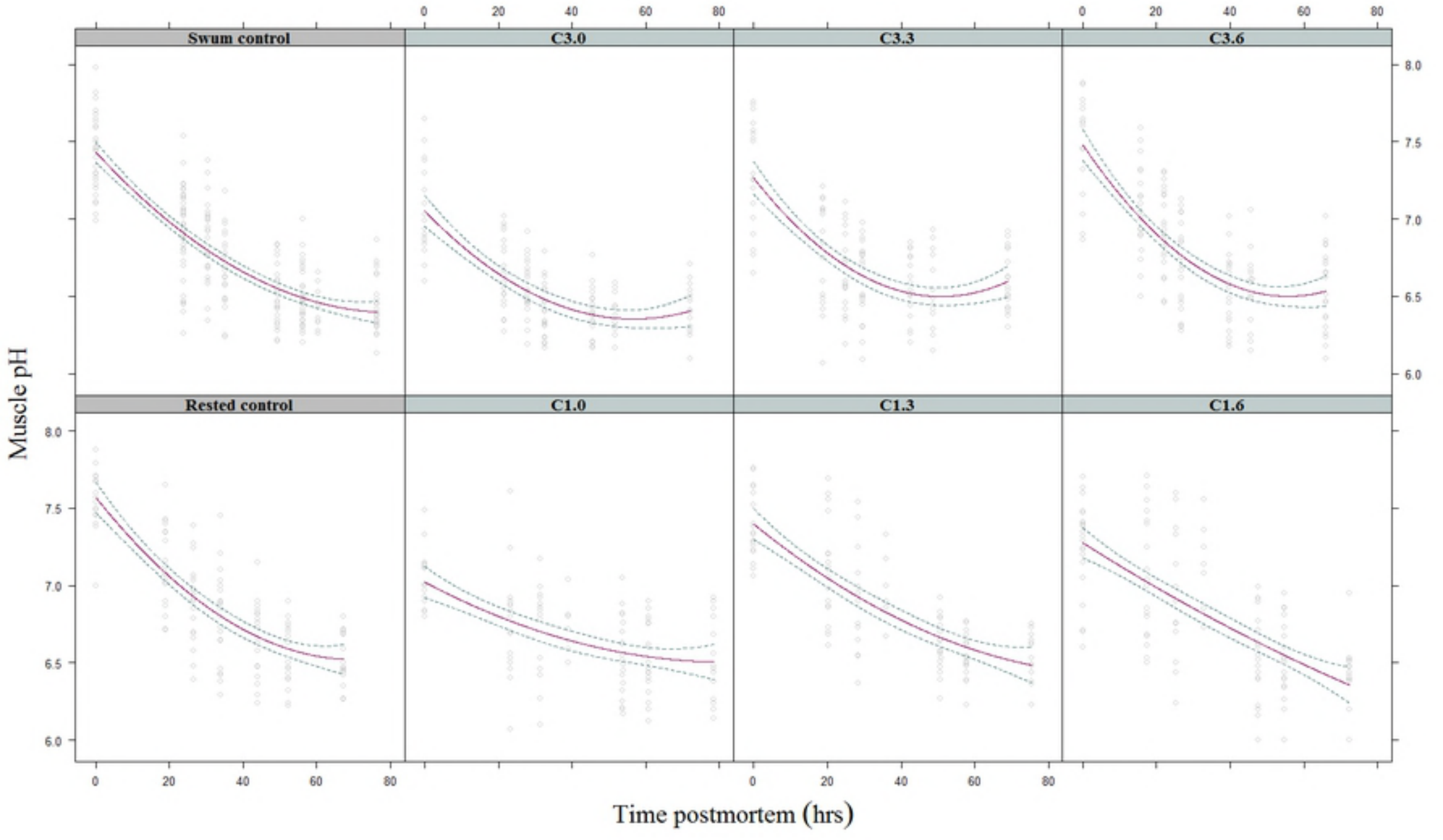
S3 Model detail. Model parameters and ANOVA output from the generalized linear models.

S4 Figures. GLM correlation plots.


Opening
Direction of water flow
Experimental
cod-end
Retention
chamber
Swim tunnel
Rope
2nd Hatch
1st Hatch


A
Plasma cortisol (ng mL^{-1})
250
200
150
100
50
0
B
Blood glucose (mmol L^{-1})
12
10
8
6
4
2
0
C
Blood lactate (mmol L^{-1})
8
6
4
2
0
Resting control
Swum control
0
3
6
Recuperation (hrs)


A
B
C
Redness (scale 1 – 4)
2.5
2.0
1.5
1.0
Haematocrit
35
30
25
20
Muscle haemoglobin (mg g^{-1})
0.07
0.06
0.05
0.04
0.03
Resting control
Swum control
0
3
6
Recuperation (hrs)


Swum control
C3.0
C3.3
C3.6
Rested control
C1.0
C1.3
C1.6
Muscle pH
Time postmortem (hrs)
0
20
40
60
80
6.0
6.5
7.0
7.5
8.0