On-board Live Storage of Atlantic Cod (*Gadus morhua*): Effects of Capture Stress, Recovery, Delayed Processing, and Frozen Storage on Fillet Color Characteristics

U. Erikson*, G. M. Tveit, M. Bondø, H. Digre

SINTEF Ocean, NO-7465 Trondheim, Norway

*Corresponding author: Tel: +47 98 24 50 31, Fax: +47 93 27 07 01,

E-mail: ulf.erikson@sintef.no

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18 **ABSTRACT**

19 On-board live storage of Atlantic cod caught by trawl was evaluated as a potential method to
20 improve color characteristics of fillets. Before slaughter and processing, the fish were: (i) stored
21 live for 3-6 h, (ii) kept in dry bin, or, (iii) stored on deck for 5 h post capture (without bleeding).
22 Blood chemistry and white muscle biochemistry were determined after capture and live storage.
23 Fillet color and presence of discolorations were assessed on board (fresh) and after frozen
24 storage (market quality). All fish were considerably stressed by capture and did not recover to
25 baseline levels after live storage. Processing just after capture, live or dry bin storage, resulted
26 in fillets with good color characteristics. Delayed processing (5 h) resulted in fillets with inferior
27 color characteristics. The color characteristics of fillets evaluated on board and after frozen
28 storage followed a similar pattern although fillets from all treatments exhibited a more
29 yellowish tint after frozen storage. Under the present catch and processing conditions, live
30 storage on board did not improve color characteristics of cod fillets. Color analysis indicated
31 that consumers would only be able to identify worst-case fillets cut from unbled fish as being
32 inferior to fillets from all other treatments.

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35 KEYWORDS Atlantic cod; Trawl capture; Handling stress; Fillet color*;* Frozen storage

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40 **Introduction**

41 Atlantic cod (*Gadus morhua*) represents one of the major whitefish species caught in the
42 Northeast Atlantic. In recent years, there has been a focus on improving product quality by
43 attempting to reduce the occurrence of unwanted effects of poor bleed-out and discolorations
44 commonly observed in the whitefish fisheries. It is well established that delayed bleeding after
45 capture should be avoided to achieve optimal fillet color characteristics and that the particular
46 bleeding method and other factors are of less importance in comparison (Kelly, 1969; Huss and
47 Asenjo, 1976; Valdimarsson et al., 1984; Botta et al., 1986; Olsen et al. 2014). Regarding
48 fishing method and quality, fillets cut from Atlantic cod caught by trawling have been shown
49 to have discolorations and bruises as determined by color and sensory analyses (Rotabakk et
50 al., 2011). Fillet color is one of the most important quality traits, especially for whitefish where
51 prime quality is associated with the highest possible degree of whiteness and lightness, without
52 reddish or yellowish tints. Furthermore, presence of discolored areas, blood spots and residual
53 blood in veins should be minimized.

54 As the sea-going fishing vessels in Norway have become fewer and larger over the last decades,
55 technological advances have made it possible to reduce the number of fishermen on each vessel
56 considerably. Particularly on larger vessels such as trawlers and demersal seiners, large
57 volumes of fish can be taken on board from a single haul. Once on board, the trawl-gear,
58 containing the catch, is emptied directly into a steel bin without water (dry bin) located below
59 deck. Depending on storage time in the dry bin, live, moribund or dead fish are subsequently
60 processed and frozen. Due to the comparatively low number of personnel on board, catch
61 processing may be delayed. Besides, it is usual practice on whitefish trawlers that fishermen
62 delay catch processing until the fish has become less active after capture to facilitate easy and
63 safe handling. Delay in catch processing can, however, result in poor bleed-out. For large catch

volumes, processing can take several hours. It is therefore questionable whether the whole catch can be processed before fish eventually die in the dry bin and the blood starts to coagulate.

Extending the period where the fish are kept alive by introducing short-term live storage tanks on board could represent a remedy to improve bleed-out and improve fillet color characteristics. By short-term live storage, we refer to a few hours after capture until the entire catch has been consecutively killed and processed immediately, not to be confused with live fish carriers and capture-based aquaculture. Another possible advantage of on-board live storage might be to minimize blood in white muscle (fillets) to let the fish recover from capture stress before processing. It has been hypothesized that during recovery, blood, initially distributed from internal organs to the white muscle during excessive swimming during capture (stress), may be gradually re-distributed back from the white muscle (Olsen et al., 2013). It is well known that cod captured by trawl are considerably stressed (Digre et al., 2010; Olsen et al., 2013). During the capture process, the fish can be stressed by factors such as capture depth, haul duration, fish density in the cod end, pressure (weight) from surrounding fish when the trawl is taken on board, and air exposure on the trawl deck. Since various stressors have a cumulative effect on fish (Wedemeyer et al., 1990), the stress level of fish ready for bleeding and processing represents the sum of all stressors the fish have experienced during capture.

Some fishing companies are currently considering introducing live fish tanks for possible better bleed-out on larger vessels. Preferably, such decisions should be backed up with solid evidence which is currently limited. From a live storage study (0, 3 and 6 h) of cod captured by trawl it was concluded that, for the best possible quality, the fish should be bled immediately, or alternatively, stored live for at least 6 h to allow the fish to recover from capture stress (Olsen et al., 2013). The authors also concluded that more research is necessary before the live-storage concept could be recommended for use on vessels. In the current research we aimed at providing more extensive and objective color analyses of cod fillets. For comparison of results, the cod

were stored live for a similar period of time (0-6 h). Moreover, previous on-board observations of cod kept alive did not show an evident time-dependent change in fillet color (unpublished results). Given the importance of fillet color characteristics for the fishing industry, the objectives were to assess whether:

(1) live storage on board would improve fillet color characteristics (due to possible recovery from capture stress) compared with fish processed from the dry bin, and fish stored for 5 h before processing started (delayed processing)

(2) frozen storage and thawing affected color characteristics of fillets (market quality)

(3) consumers would be able to identify potential differences in fillet color between various on-board processing methods.

Materials and methods

Experimental design

The experimental design and process flow is shown schematically in Figure 1. It was set up to cover the value chain from the moment the fish were hauled on deck (t = 0 h) to consumer after freezing and thawing. Effect of capture would then manifest itself as the cumulative stress load at t = 0 h. Fillet color was determined after the cod had been subjected to three onboard treatments, live storage (LS), commercial processing from a dry bin (DB), and delayed processing that can occur when large volumes of fish are caught (DE 5h). In case of live storage, we addressed whether possible recovery, for 3 and 6 h, from capture stress could be associated with improved fillet color characteristics. Finally, fillet color was assessed on board as well as

111 after frozen storage (market quality) to reveal whether point of color assessment on board could
112 be a confounding factor. Assessment of stress was carried out on fish stored live (LS) for 0, 3
113 and 6 h (n = 32) as well as on fish (n = 21 - 28) subjected to delayed processing (DE 5h). The
114 number of fish subjected to assessment of Fillet Quality Index and color analysis, before and
115 after frozen storage, were 24 - 49 (LS 0, 3 and 6 h), 12 (DB) and 33-44 (DE 5h).

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118 ***Capture and on-board fish processing***

119 Atlantic cod were captured using an Alfredo No. 3 two-panel Euronete trawl built entirely of
120 155 mm nominal mesh size polyethylene netting on board the vessel "M/S Helmer Hansen"
121 (63.8 m LOA and 4080 HP). The trawl had a 36.5 m headline and 19.2 m fishing line with 454
122 meshes of circumference. The fishing gear has been described and tested by Larsen et al. (2016).
123 Thirty-one hauls were conducted during the period 18-22 February 2015 off the coast of Troms,
124 in Northern Norway (70-71°N / 24-31°E). During the cruise, Atlantic cod from six of the hauls
125 were collected for the present research. The catch size, capture depth and haul duration of these
126 hauls were 0.5 - 2.5 metric tons, 50 – 316 m, and 39 - 90 min, respectively (Table 1). The table
127 also shows the total number of fish used in this research (Σn for all treatments = 353) and
128 number of experimental fish collected from each haul (Σn = 48 - 76) and how they were
129 assigned to the various treatments. This was solely done to increase the number of fish per
130 treatment since we were only able to process and measure a limited number of fish per haul. To
131 assess the concept of storing the catch live before bleeding and processing, a tank filled with
132 running seawater was placed on the trawl deck. The tank (Melbu Systems AS, Melbu, Norway)
133 volume was 2.4 m^3 where seawater was circulated at a rate of 97 - 156 L min^{-1} through a
134 perforated bottom plate and left the tank by simple overflow (flow-through principle). Table 2
135 shows the number of fish (n = 75 - 104), collected from four hauls, transferred to the live storage

136 tank. Of these fish, only LS 3h and LS 6h fish were sampled and analyzed. Therefore, the total
137 number of fish per haul in the live-storage tank (Table 2), was always higher than the total
138 number of fish shown in Table 1 (LS 0h, DE 5h and DB fish were never kept in the live-storage
139 tank). Furthermore, Table 2 shows the conditions in the live-storage tank where surface
140 seawater was pumped in continuously. Fish density varied between 105 and 235 kg m^{-3} and the
141 dissolved oxygen levels ranged from 74 to 108 % saturation (data from loggers not shown) and
142 85 to 102 % saturation (intermittent manual measurements). The water temperature in the tank
143 was between 3.5 and 4.4 °C (similar to surface seawater). Under these conditions, the immediate
144 survival rate varied between 51 and 100 %. As indicated in Table 2, survival rates seemed to
145 be lower for cod caught at greater depths (Table 1).

146 Since live fish had to be sampled directly from the trawl shortly after the gear was hauled on
147 board, we were only able to assess between 48 and 76 fish per haul (Table 1) before the trawl
148 gear was emptied into the dry bin. Fish were sampled from the trawl deck immediately after
149 capture (LS 0h) as well as after live storage for 3 h (LS 3h) and 6 h (LS 6h).

150 The normal, delayed, processing on board was carried out by the fishermen as follows: after
151 capture, the cod end was emptied into a bin without water (dry bin, DB). Starting from about
152 0.5 h after capture, fish were consecutively taken from the bin and subjected to direct gutting
153 and decapitation. Within about 2 h, all fish in the dry bin had been processed. The gutted fish
154 were subsequently subjected to bleed-out/washing for about 15 min in a tank containing
155 seawater before they were transferred to another tank also containing seawater for rinsing (30
156 min). The beheaded and gutted fish were subsequently frozen in a vertical plate freezer reaching
157 a block temperature of -18 to -20 °C after 3.5 h. The frozen fish blocks were then packed in
158 woven polypropylene/paper bags and stored on board at -23 °C until the fish were brought
159 ashore three days later.

160 As an *a priori* worst-case treatment to simulate delayed processing from the dry bin, fish were
161 collected from the codend just after the fish were taken on board and placed in a tub without
162 water at an air temperature of 0.5 ºC (on deck). We decided to wait for 5 h before processing
163 started which would be typical for delayed processing of a large catch. After 5 h, the dead fish
164 (DE 5h) were collected and gutted before they were either analyzed on board, or frozen for later
165 analysis (Figure 1). Note that the DE 5h fish were not subjected to bleed-out. On the other hand,
166 they were not subjected to pressure from the weight of surrounding fish as would be the case
167 for the fish in the dry bin.

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170 ***Assessment of stress and fillet color characteristics***

171 Individual vigorous fish, without injuries, from the LS 0h (control), LS 3h and LS 6h treatments
172 were rapidly sampled and killed by a blow to the head before blood samples were drawn by
173 inserting a heparinized syringe into the caudal vein and blood pH, glucose and lactate levels
174 were measured. White muscle excitability (twitches) and initial pH, along with body
175 temperature, were subsequently determined. Afterwards, the throats were cut and the fish were
176 bled in clean seawater for 30 min. Subsequently, total length and body weight were determined
177 before the fish were labeled, gutted and subjected to determination of gender. The liver and
178 gonads were weighed before the fish were rinsed in seawater. Right-hand side fillets were cut
179 and washed in seawater (4 ºC) for 30 s before excess surface water was wiped off with tissue
180 paper. The fillets were then visually examined for possible presence of discolorations, bruises
181 or residual blood in veins. Finally, each fillet was photographed for later objective assessment
182 of color.

183 Twenty-four fish processed from the dry bin (DB) were collected from the rinsing tank between
184 40-80 min post capture. Rigor status was evaluated before filleting and subsequent
185 determination of fillet color characteristics as mentioned for the live-stored fish.

186 All fish to be analyzed on board were subsequently filleted and subjected to the various
187 assessments. Afterwards, the fish were frozen and sent to our laboratory for human
188 consumption (no further analyses were carried out). The remaining experimental fish from each
189 treatment (see Figure 1) were bled, gutted and beheaded before they were labelled, packed and
190 frozen as described above. Temperature loggers (iButton DS1922F, Thermochron, Maxim
191 Integrated, San Jose, USA) were placed inside the body cavity of six fish. The fish were sent to
192 our laboratory where they were stored at -28 °C for 61 days before thawing. Later extraction of
193 data showed that the core temperature, from freezing to thawing, was -24.2 ± 3.8 °C. The frozen
194 blocks of fish were thawed in 1000-L tubs using air flow and a continuous water flow (10 °C)
195 for 2 h before the water temperature was lowered to -0.5 °C. After 18 h at -0.5 °C, the fish were
196 transferred to Styrofoam boxes with ice and stored for another 2-3 h before filleting. Fillets, cut
197 from the right-hand side of the fish, were washed in cold tap water for 10-15 s whereby surface
198 water was wiped off using tissue paper before evaluation of their color characteristics. Images
199 of fillets were obtained by using a machine vision system for subsequent evaluation of fillet
200 color in the Commision Internationale de l'Eclairage (CIE) L*a*b* color space using algorithms
201 developed previously (Erikson and Misimi, 2008). Since lighting conditions were different on
202 board the vessel and in our laboratory, color comparisons between images of fresh and
203 frozen/thawed fillets were not performed statistically.

204 The body weight, total length, heptosomatic index (HSI = (liver weight / body weight) x 100
205 %), and Fulton's condition factor of the experimental cod were (mean values ± SD): 3.1 ± 1.8
206 kg (n=353), 68 ± 14 cm (n=353), 3.9 ± 1.5 % (n=163), and 0.9 ± 0.1 (n=353), respectively. The
207 population consisted of 43 % females and 57 % males (n=149), with gonadosomatic indexes

208 (GSI = (gonad weight / body weight) x 100 %) of 3.5 ± 3.9 % (n=64) and 5.9 ± 5.2 % (n=85),
209 respectively.

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212 **Analytical methods**

213 ***Water and fish***

214 For logging of dissolved oxygen (DO) levels and temperatures in the live storage tank, two
215 oxygen sensors were used (Oxybox, Nortek AS, Norway). Temperature and DO were also
216 measured intermittently during the experiment using a YSI ProODO meter (YSI Inc., Yellow
217 Springs, OH, USA). Glucose and lactate test strips were briefly dipped in blood immediately
218 after the throat was cut before they were inserted into an Ascencia Contour Meter (Bayer
219 HealthCare LLC, Mishawaka, Indiana, USA) or a Lactate Scout+ meter (EKF Diagnostics
220 GmbH, Magdeburg, Germany), respectively. After a short delay, the metabolite concentrations
221 were read in mmol L^{-1} on the instrument display. Blood acidity was measured in same blood
222 sample as glucose and lactate. A shielded glass electrode (WTW SenTix 41, WTW, Weilheim,
223 Germany) connected to a portable pH meter (model WTW 315i) was used. After making a 2-3
224 cm long incision with a scalpel through the skin, the initial pH in white epaxial muscle was
225 measured between the lateral line and the 1st dorsal fin. Two similar pH electrodes and meters
226 were used. A Twitch Tester Quality Assessment Tool (AQUI-S Ltd., Lower Hutt, New
227 Zealand) was used to measure the excitability of muscle tissues when stimulated by an electrical
228 pulse (9 V DC for 0.6 s) supplied by the instrument. A few (1-3) measurements were performed
229 on one side of each fish. For each measurement, the electrodes were in contact with the fish for
230 about 1 - 2 s. Onset of rigor mortis was determined just before filleting on board according to
231 the Rigor Status Method (Erikson, 2001). Description of scores for both methods are shown in

232 Table 3. The fish body temperature was measured through the incision made for measuring
233 initial pH. A Testo 110 thermometer (Testo AG, Lenzkirch, Germany) was used.

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236 ***Fillet color characteristics***

237 Three experienced workers evaluated fillets cut from fresh (on board) and frozen/thawed fillets
238 according to a modified version of the Fillet Quality Index (FQI) method (Olsen et al., 2013).
239 Four attributes were assessed on the scale ranging from 0 to 2 and the sum of these attributes
240 represents the FQI score (Table 4).

241 On board, images of fillets were captured after the visual assessment of fillet discolorations. A
242 DSLR camera (Nikon D7000, Tokyo, Japan) with a 50 mm lens and manual settings (shutter
243 speed 1/200 s, ISO 400, aperture F 5.6, and external flash with manual settings) was used.
244 Images were acquired in the RAW format with maximum resolution (4928 x 3264) and stored
245 on the computer for later evaluation. Processing was carried out on the captured images. The
246 images were converted from the RAW format with Adobe Lightroom, corrected for color and
247 white balance, sharpened and filtered from noise. After thawing in our laboratory, images of
248 fillets were captured with a USB 3.0 Point Grey Grasshopper 3 color camera (Point Grey,
249 Richmond BC, Canada) connected to a computer. Illumination used was white and yellow LED
250 light and a red laser. Camera and LEDs were controlled/triggered using an external controller.
251 The camera system captured one white image, one yellow image and a 3D-image of the fillet.
252 For automated color analysis of the fillets in the CIE L*a*b* color space (Erikson and Misimi,
253 2008), we decided to study the potential effects of poor bleed-out on white muscle only. Since
254 the color parameters are pixel-averaged over the selected region of interest (ROI), we thereby
255 excluded color contributions from the peritoneum, backbone, cartilage, and residual blood in

256 the neck region due to beheading. The fillet images were color-calibrated using the
257 GretagMacbeth ColorChecker chart with 24 color patches (Colour-Science AG, Hinwil,
258 Switzerland). Since both a* and b* values turned out to be positive, they represented redness
259 and yellowness, respectively. Chroma (color saturation), hue angle (0° = red hue; 90° = yellow
260 hue) and whiteness (Park, 1994) were calculated as $C^*_{ab} = \sqrt{(a^{*2} + b^{*2})}$, $H^o_{ab} = \arctan\left(\frac{b^*}{a^*}\right)$
261 and $W = L^* - 3b^*$, respectively. Total color differences between treatments relative to control
262 (LS 0h) were calculated as $\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$. In terms of color
263 differences perceivable to the human eye, universally valid ΔE* values can be used as follows,
264 0-1: observer does not notice the difference; 1-2: only an experienced observer can notice the
265 difference; 2-3.5: unexperienced observer also notices the difference; 3.5-5: a clear difference
266 in color is noticed; > 5: observer notices two different colors (Mokrzycki and Tatol, 2011).

267 Images of fresh and frozen/thawed fillets were ranked manually through a computer-assisted
268 sorting algorithm implemented in a LabVIEW program (National Instruments Co., Austin,
269 Texas, USA). The program works by doing an insertion sort. All images were randomized and
270 then visually compared. The program displays each image candidate in between two previously
271 sorted images. By sliding a slide bar, the program image candidate was inserted to the set of
272 sorted images. When sliding the bar to the left, the candidate was compared to the most perfect
273 fillets (least discolorations), and by sliding to the right, the candidate was compared to the
274 imperfect fillets (most discolorations). Since three images were arranged side by side for
275 manual evaluation of the fillets, it was easier to perform color comparisons of several samples
276 as opposed to evaluating the color of a single sample at a time. When all images were evaluated
277 and inserted in their positions, the image array was indexed and thus scored "best" to "worst".
278 The overall "redness impression" of each fillet was the main criterion. Otherwise, general fillet
279 color, blood spots, and red tail areas were also taken into account. Surface blood, if present,
280 originating from the cutting of fillets, was ignored. The images of the fresh fillets on board (n

281 = 154) were scored from 1 to 154. The sorted image set was then grouped as follows: 1 – 50 =
282 white fillets, 51 – 90 = slight redness or one blood spot, and > 91 = reddish fillets and/or fillets
283 with more than one blood spot. The images of the frozen/thawed (n=178) fillets were scored
284 from 1 to 178 using same grouping.

285

286 ***Statistical analyses***

287 When the stress and color characteristics data from the various treatments passed both the
288 Shapiro-Wilk normality test and Levene Median test for homogeneity of variance, a one-factor
289 analysis of variance (ANOVA) was used to test significance ($P<0.05$) followed by a Holm-
290 Sidak post hoc test when significance was indicated. In most cases, however, normality and/or
291 homogeneity of variance tests failed. Such data were analyzed by using the Kruskal-Wallis One
292 Way Analysis of Variance on Ranks method followed by an All Pairwise Multiple Comparison
293 Procedure (Tukey or Dunn's methods). The data are reported as mean values ± 95% confidence
294 intervals (CIs).

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297 **Results**

298 ***Condition of fish after capture, live storage and delayed processing***

299 The cumulative stress effect of capture is given by the values for the LS 0h treatment (Table 5).
300 At the same time, these values also represented the starting point for our experiment to assess
301 whether the fish can subsequently recover in the live holding tank. As shown in Table 1, fish
302 were collected from different hauls. No significant differences between hauls that constituted
303 the LS 0h treatment were observed except from blood glucose where the values from Haul 222

304 were significantly higher (mean ± CI) at 4.2 ± 0.9 mmol L^{-1} than was the case for Hauls 207
305 and 214 at 2.4 ± 0.5 and 3.0 ± 0.8 mmol L^{-1}, respectively (data not shown). Blood chemistry
306 and muscle biochemistry as affected by on-board handling practices, including possible delayed
307 effects of capture stress, are shown as LS 3h and LS 6h values in Table 5. Although the mean
308 blood pH values varied between 7.25 and 7.56 during live storage, the values were not
309 significantly different. In the case of blood glucose, however, the concentrations continued to
310 increase during live storage from the capture-related value of 3.3 mmol L^{-1} to 7.0 mmol L^{-1} (3
311 h) and 8.7 mmol L^{-1} (6 h) ($P<0.05$). The mean blood lactate concentration was 4.6 mmol L^{-1}
312 just after capture and no significant changes took place during subsequent live storage for 3 h
313 (5.8 mmol L^{-1}) and 6 h (5.2 mmol L^{-1}).

314 Just after capture, the mean initial pH in the white muscle was 7.16 (Table 5). A significant
315 effect of live storage was identified after 6 h since the mean pH increased to 7.31. The unbled
316 fish (DE 5h) had a pH of 6.96 as evaluated 5 h post mortem. Just after capture and live storage,
317 the ability of the muscle to produce twitches was clearly present in all cases since strong
318 twitches were always observed except from in unbled fish which had almost lost their ability to
319 twitch altogether (Table 5).

320 Since only vigorous fish were sampled from the LS groups, no onset of rigor mortis was
321 accordingly observed when the fish were filleted and assessed about 30 min post mortem (Table
322 5). The same was true for DB fish evaluated no later than about 3 h post mortem. In contrast, a
323 mean rigor score of 1.3 (rigor onset) was observed in DE 5h fish when they were filleted from
324 5 to 7 h post capture.

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327 ***Visual assessment of discolorations and residual blood in fillets***

The fillets were visually evaluated, by using the FQI method after about 30 min post mortem, on board, and after 61 days of frozen storage (Table 6). For all FQI parameters of fresh fillets, there were no significant differences between treatments, except from the unbled fish (DE 5h) where the scores were higher, showing inferior color characteristics. Otherwise, discoloration scores and the number of bruises were low although some residual blood was found in some veins with mean scores of 0.7 to 0.9 on a scale from 0 to 2. This corresponds to the category "one or two small blood stains in the belly or tail" (Table 4). Generally, these fillets were considered to represent a high-quality product. The occurrence of bruises was minimal in all cases.

After frozen storage and thawing, basically the same overall trend was observed, except from a few minor differences between LS 3h and DB treatments (Table 6). The DE 5h fillets were also in this case, as expected, clearly inferior to those from all other treatments ($P<0.05$). FQI results show that frozen storage did not exacerbate discolorations already present in fresh fillets ($P<0.05$). On the contrary, fresh fillets were slightly more discolored than frozen/thawed fillets for several of the parameters and for all treatments.

Fillet color as assessed by machine vision

The CIE L*a*b* color space was used to assess fillet color integrated over the selected ROI. As shown in Table 6, there were significant differences among fresh fillets according to treatment. The most evident feature was that DE 5h fillets exhibited higher redness and chroma values whereas hue angles were lower ($P<0.05$), corresponding to a color tint towards red in the 3D color space. Some differences among treatments were found in case of lightness and yellowness ($P<0.05$). However, these differences were always small. Whiteness was generally

352 unaffected by treatment. Color differences (ΔE) between treatments were calculated relative to
353 fillet color just after capture (LS 0h). The values indicated that DB fresh fillet color was a
354 borderline case for what a trained observer can observe (ΔE* = 1.5). Only DE 5h fresh fillets
355 could be regarded different as the ΔE* was 2.5.

356 After freezing and thawing, lightness and redness values were similar for all treatments except
357 from slightly lower L* and higher a* values for DE 5h fillets ($P<0.05$). Regarding yellowness,
358 chroma and whiteness, several minor differences among treatments were observed. The same
359 was true for hue angle where the mean value of the DE 5h fillets was clearly lower compared
360 with fillets from all other treatments.

361 Overall, frozen storage per se resulted in changes in color since the fillets now exhibited a
362 yellowish tint (higher b* values), higher color saturation (higher C_{ab}^* values), hues changed
363 from reddish towards yellowish tints (higher H_{ab}^o values), and they became less white (lower
364 W values) compared with fresh fillets. On the average, after these changes occurred, the inferior
365 color characteristics of DE 5h seemed to be somewhat less evident in terms of visual inspection
366 since ΔE* was reduced to a value of 1.5.

367 For a better understanding of what the measured CIE L*a*b* values translated to in terms of
368 what the consumers can perceive, photos of a typical control fillet (LS 0h) versus best and
369 worst-case DE 5h fillets (chosen by visual inspection of fillets) are shown in Figure 2. The
370 related color variables are shown in Table 7. The fact that the fillets had been subjected to frozen
371 storage before color analysis is evident by the levels of yellowness, chroma, hue and whiteness.
372 Additionally, that the worst-case fillet was redder (higher a* value) than the two other fillets.
373 By visual inspection, it is difficult to spot any color difference by comparing photos A (LS 0h)
374 and B (best case DE 5h). In contrast, it is fairly easy to see the difference between Photo A (or
375 B) and Photo C. The related ΔE* values were 1.2 (Photos A vs B) and 5.3 (Photos A vs C).

Ranking of fillets based on image sorting

By the results from the computer-aided visual ranking of fillet images according to color characteristics, the following ranking of treatments came up (best to worst): DB > LS 0h or LS 6h > LS 3h >> DE 5h. By comparison, a ranking based on the Fillet Quality Index (total scores, Table 6) for fresh fillets lead to: DB > LS 6h> LS 0h and LS 3h >> DE 5h, that is, almost following a similar pattern. When color was measured objectively, the small differences in CIE L*a*b* values made ranking difficult and less systematic, apart from the fact that several of the DE 5h fillets were clearly inferior to all other fillets.

Discussion

Condition of fish after capture and during on-board live storage

A potential benefit of live storage could be to minimize the amount of blood in white muscle by possible recovery from capture stress before processing. Since recovery would require adequate storage conditions, assessments of water quality and stress levels after recovery were consequently of interest. The levels of DO in the live-storage tank varied between 74 and 108 % saturation (Table 2) showing that the fish had ample access to oxygen at all times. At water temperatures between 2 and 6 °C, cod mortality rates are high at DO levels < 16 - 22 % whereas no mortalities have been observed at DO levels > 34 % saturation (Plante et al., 1998). This suggests that the mortality rates observed for cod of this trial might be ascribed to factors related to capture, or perhaps to transfer from fishing gear to LS tank. The fish density varied between 105 – 235 kg m^{-3} (Table 2). By comparison, it could be mentioned that cod exhibit only

400 moderate stress responses when they are kept at 540 kg m^{-3} at 8 ºC (Staurnes et al., 1994).
401 Furthermore, the authors concluded that cod can be transported in good condition at very high
402 densities provided adequate levels of oxygen are supplied. Overall, we conclude that water
403 quality was good and that adequate conditions for possible post-capture recovery were present.

404 Just after capture, the mean blood pH was 7.30 which increased to pH 7.56 after live storage
405 for 6 h (Table 5). Blood pH in rested Atlantic cod, quickly netted and killed, has been
406 determined as pH 7.69 (Hultmann et al., 2012) indicating that blood acidity in the cod in this
407 trial was reduced to pH 7.30 by capture stress (LS 0h). After the fish had been stored live for 3
408 h (pH 7.25) and 6 h (pH 7.56), blood pH did not change significantly although acidity tended
409 to be less severe after 6 h suggesting the fish were recovering from capture stress. For haul
410 durations < 5 h, pH 7.20 has been reported after capture whereas after 3 h and 6 h, blood pH
411 increased significantly to 7.36 and 7.50, respectively (Olsen et al., 2013).

412 In case of LS 0h fish, the mean glucose concentration was 3.3 mmol L^{-1}. During live storage, it
413 increased to 7.0 (LS 3h) and 8.7 mmol L^{-1} (LS 6h) ($P<0.05$). Due to the slow response time of
414 glucose, the increasing trend could be interpreted as a delayed stress response to capture,
415 although it cannot be ruled out that live storage did in fact impose an additional stress response
416 to the fish. Reported blood glucose values after 0, 3 and 6 h storage showed a similar, although
417 a more prominent trend with respective values 5.34, 9.99 and 10.78 mmol L^{-1} (Olsen et al.,
418 2013).

419 Compared with blood lactate values of < 0.5 mmol L^{-1}, typical of unstressed cod (Brown et al.,
420 2008), the mean concentration of 4.6 mmol L^{-1} showed that the cod of this trial were affected
421 be capture stress. The subsequent live storage did not significantly affect the levels of lactate
422 (Table 5). By comparison, crowding of farmed cod in cages for 20 - 120 min, resulted in blood
423 lactate levels between 2 and 4 mmol L^{-1} as measured by a similar type of portable lactate meter

424 (Brown et al., 2008). In another study, the water level was lowered to 10 cm in a tank containing
425 cod before the fish were chased for 15 min at 8 °C before they were allowed to recover under
426 optimal conditions. Plasma lactate then peaked at 5.5 – 8.1 mmol L^{-1} after 15 min. This level
427 was maintained for 4 h before lactate reached basal levels 8 h after the stress incident. Plasma
428 glucose rose from the basal level of 3 – 4 mmol L^{-1} to 6.5 – 7.0 mmol L^{-1} 4 h after the stress
429 incident. The latter level was maintained for at least 24 h before basal levels were reached after
430 48 h (Olsen et al., 2008). Thus, it is possible that our cod had already reached their near-peak
431 lactate levels (Table 5) before live storage and that this level was subsequently maintained
432 during 6 h of live storage ($P>0.05$). Based on lactate levels alone, where lactate has a
433 considerably shorter stress response time than glucose (Stoot et al., 2014), it seemed that the
434 fish did not recover during the 6 h of live storage. A previous study of live storage reported that
435 the mean blood lactate value just after capture (0 h) was 3.49 mmol L^{-1} which increased to 7.87
436 and 6.36 mmol L^{-1} after live storage for 3 and 6 h, respectively, possibly indicating the cod were
437 slowly recovering after 6 h (Olsen et al., 2013). It should generally be pointed out though that
438 point-of-care glucose and lactate meters, when used on fish such as in the present study, should
439 be regarded as convenient field methods capable of producing relative values rather than being
440 able to produce accurate absolute values (Stoot et al., 2014).

441 Just after capture, the initial pH in white muscle was 7.16 (Table 5) showing that the cod were
442 considerably stressed due to excessive muscle activity during capture. By comparison, the
443 initial pH in rested farmed cod is about 7.6 whereas attempts (Atlantic cod is a rather sedate
444 fish species) to chase such fish to exhaustion resulted in an initial pH of about 7.1 (Erikson et
445 al., 2011). On a commercial trawler, the initial pH of cod after several hauls lasting for about 5
446 h, ranged from pH 7.2 to 7.3 (Digre et al., 2010). Regarding the lower pH of 6.96 in unbled fish
447 (DE 5h), it is likely that the drop from around pH 7.16 just after capture was caused by early
448 postmortem glycolysis. Considering live storage, a recovery trend was observed since the

449 muscle pH increased to 7.21 after 3 h (P>0.05) and then to pH 7.31 after 6 h (P<0.05). As judged
450 by pH, the cod studied previously (Olsen et al., 2013) were somewhat more stressed (pH 7.01)
451 than ours just after capture. Subsequently, a similar recovery trend was observed where pH
452 increased to 7.12 (P>0.05) and 7.26 (P<0.05) after 3 and 6 h, respectively (Olsen et al., 2013).

453 The twitch ability of LS fish was close to the maximum score of "3". Thus, the fish must have
454 had ample amounts of ATP for contraction in their white muscle (as well as functional nervous
455 system). In unbled fish, on the other hand, a storage period of 5 h resulted in hardly noticeable
456 twitches (score 0.2) due to postmortem ATP catabolism. In line with the depletion of energy
457 stores in the muscle, onset of rigor during processing was observed only in the case of unbled
458 fish (Table 5).

459 When all stress indicators are considered collectively, it was evident that 6 h of live storage was
460 by no means sufficient for recovery to baseline levels. Since the fish after 6 h were still in a
461 considerably stressed condition, it was questionable whether significant amounts of blood had
462 been re-distributed from the white muscle.

463

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465 ***Fillet color on board and after frozen storage***

466 The results from the visual (subjective) assessment of fillet discolorations and residual blood
467 showed only minor differences between treatments except from several (but not all) fillets cut
468 from fish subjected to delayed bleeding (DE 5h). However, the latter fillets exhibited, perhaps
469 somewhat surprisingly, only moderately higher scores. Similar trends were observed for fresh
470 and frozen/thawed fillets, although freezing generally resulted in an increase in yellowness
471 (including related chroma, hue and whiteness values, see Table 6). Considering on-board
472 bleeding and processing, the data confirm previous findings that acceptable cod fillets can be

473 obtained if the fish are bled within 0.5 h (Olsen et al., 2014) or 1-2 h (Kelly, 1969; Botta et al.,
474 1986) post mortem. Furthermore, considering unbled fish left in air for 5 h before processing
475 and washing, it should be mentioned that if cod are not bled before 3 h post mortem, the flesh
476 color has been reported to be similar to that of unbled fish (Olsen et al., 2014). When it comes
477 to the effect of live storage, however, it has been reported that compared with bleeding
478 immediately after capture, live storage for 3 h actually increases muscle discoloration
479 significantly (Olsen et al., 2013). After 6 h, however, most of the red discoloration was gone
480 and the fillets became considerably lighter as indicated by FQI scores. Compared with our
481 results just after capture, the total scores obtained by Olsen et al. (2013) were higher due to
482 more discoloration of loin and belly. Their "starting point" in terms of stress before live storage
483 for 6 h was somewhat more severe than in our case. One explanation for this could be the
484 considerably longer haul durations and higher total catch amounts compared with this study.
485 After live storage for 3 h, all FQI parameters were assigned higher scores than ours, indicating
486 that residual blood in their fillets was more prominent compared with fillets from fish bled just
487 after capture. We did not observe this effect in our study. After 6 h, their parameters related to
488 residual blood exhibited lower scores (Olsen et al., 2013). The authors explained this by
489 redistribution of blood away from the white muscle to other organs during the additional 3 h of
490 live storage. By this time, however, their fillets still had a higher mean FQI total score (1.5)
491 than ours (0.7).

492 A relevant question seems to be whether full recovery from stress could have improved fillet
493 color characteristics at all. In controlled laboratory studies, anesthetized farmed Atlantic cod,
494 displaying basically no white muscle activity at all before killing, were compared with cod
495 subjected to forced swimming (chased for 30 min) to produce severely stressed fish. In terms
496 of fillet color, the stress bout resulted in a few minor statistical differences as evaluated after
497 ice storage for 7 d (Erikson et al., 2011). In another study, anesthetized (rested) farmed cod

498 displayed slightly higher b*, hue and chroma values than stressed fish as evaluated shortly after
499 killing. Furthermore, whiteness, calculated as L* - 3b*, was in fact lower for the anesthetized
500 fish whereas no differences were observed in case of L* and a*. After 7 d of chilled storage,
501 whiteness of anesthetized fish was slightly higher as assessed visually by a sensory panel,
502 whereas no differences were observed from CIE L*a*b* color measurements except from
503 slightly higher whiteness values of the stressed fish (Digre et al., 2011a). Moreover, excessive
504 swimming activity did not significantly affect the amount of visually assessed residual blood in
505 fillets. Just after killing, stressed cod were darker (lower L* values) with slightly lower b* and
506 chroma values compared with anesthetized fish although this effect was offset by ice storage
507 for 7 d (Digre et al., 2011b). Collectively, these studies suggest that the blood distribution in
508 fish is not a crucial factor to produce fillets with good overall color characteristics. Furthermore,
509 muscular tissues only contain about 20 % of the total blood volume and it has been stated that
510 this distribution is not changed during exercise since white muscle is poorly vascularized (Huss
511 and Børresen, 1995). Rather, the blood flow-rate to the white muscle increases considerably
512 during muscular activity (Neumann et al., 1983). The use of live storage tanks to improve fillet
513 color by recovering fish from capture stress before slaughter can therefore be questioned from
514 that point of view. Consequently, the potential benefit of employing such tanks on board may
515 then be narrowed down to keep large catches alive until all fish have been processed since the
516 fish should be bled no later than 1 to 2 h post mortem (see above).

517

518 As shown in Table 6, objective machine vision analysis of fresh and frozen/thawed fillets in
519 CIE L*a*b* color space resulted in some minor differences between treatments apart from the
520 inferior unbled fillets. Furthermore, frozen storage resulted in a more yellowish tint of all fillets.
521 By comparison, CIE L*a*b* values of Atlantic cod loins were measured using a calibrated
522 digital photo imaging system. After capture of about 3 metric tons of cod by trawling (1.3 h

523 haul duration at a depth of 250-350 m), cod were gutted, beheaded (although it was not stated
524 for how long the fish were stored on board before gutting/processing took place), and frozen on
525 board. The fish were then stored for 3 months at - 23ºC. Just after thawing, the fish were filleted
526 and analyzed. In what the authors defined as poorly bled cod (60 % of the catch), the mean L*,
527 a* and b* values were 77.9, 5.07, and 14.26, respectively (Rotabakk et al., 2011). After frozen
528 storage, our corresponding values (range) were 87.1 to 88.5, 1.4 to 2.9, and 6.6 to 7.4 (Table
529 6). Thus, if we assume comparable calibration procedures of the imaging systems, their fillets
530 were darker, more red and yellow than our fillets (including DE 5h fish), suggesting that our
531 fish had been subjected to less stress during capture and/or a better bleed-out procedure.

532 Several fillets from all treatments exhibited an even, pinkish tint. Notably, such fillets were
533 measured along with less tinted fillets by the computer vision method (integrated over the
534 selected ROI) and presented as group averages as shown in Table 6. The source of the pinkish
535 background color was not clear. Perhaps the pinkish tint could be related to pre-capture factors
536 such as that individual fish had been feeding on different organisms like crustaceans containing
537 carotenoids.

538 The computer-aided method for visually based ranking of fillets worked reasonably well
539 compared with visually obtained FQI data. However, compared with objective data (CIE
540 L*a*b* variables), the results were non-conclusive considering ranking of all fillets. The latter
541 finding makes sense since it is known that ΔE* values of 2 to 3.5 represent borderline values of
542 what untrained human eyes (consumers) can detect (Mokrzycki and Tatol, 2011). Most fillets
543 subjected to ranking were of good quality with considerably lower ΔE* values. This effect is
544 remarkably well demonstrated by comparing the photos shown in Figure 2 where only the
545 worst-case DE 5h fillet can be quite easily distinguished from the control fillet.

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548 **Conclusions**

549 Cod, severely stressed by capture, did not recover to baseline levels after 6 h of live storage.
550 Fillets cut from fish immediately after capture, live storage and dry bin all exhibited good color
551 characteristics. In contrast, several fillets cut from fish subjected to delayed processing for 5 h
552 exhibited inferior color characteristics. Fillet color characteristics evaluated fresh on board and
553 after frozen storage (market quality) basically showed a similar pattern regarding effects of
554 capture and processing. However, frozen storage *per se* resulted in a more yellowish tint after
555 thawing regardless of on-board processing method. Color analysis indicated that consumers
556 would only be able to distinguish worst-case unbled fillets from all other fillets (treatments).
557 This study may have represented a relatively optimal case due to the comparatively short haul
558 durations and modest catch volumes. For larger catch volumes, a possible asset of short-term
559 live storage on board would be to avoid the detrimental effects of delayed processing on fillet
560 color characteristics.

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578 **References**

579 Botta, J.R., Squires, B.E., and Johnson, J. 1986. Effect of bleeding/gutting procedures on the sensory quality of
580 fresh raw Atlantic cod (*Gadus morhua*). Can. Inst. Food Sci. Technol. J. 19: 186-190.

581 Brown, J.A., Watson, J., Bourhill, A., and Wall, T. 2008. Evaluation and use of the Lactate Pro, a portable lactate
582 meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). Aquaculture 285: 135-
583 142.

584 Digre, H., Erikson, U., Misimi, E., Standal, I.B., Gallart-Jornet, L., Riebroy, S., and Rustad, T. 2011b. Bleeding
585 of farmed Atlantic cod: residual blood, color, and quality attributes of pre- and postrigor fillets as affected by
586 perimortem stress and different bleeding methods. J. Aquatic Food Prod. Technol. 20: 391-411.

587

588 Digre, H., Erikson, U., Skaret, J., Lea, P., Gallart-Jornet, L., and Misimi, E. 2011a. Biochemical, physical and
589 sensory quality of ice-stored Atlantic cod (*Gadus morhua*) as affected by pre-slaughter stress, percussion stunning
590 and AQUI-S™ anaesthesia. Eur. Food Res. Technol. 233: 447-456.

591

592 Digre, H., Jes Hansen, U., and Erikson, U. 2010. Effect of trawling with traditional and 'T90' trawl codends on
593 fish size and on different quality parameters of cod *Gadus morhua* and haddock *Melanogrammus aeglefinus*. Fish.
594 Sci. 76: 549-559.

595 Erikson, U. 2001. Rigor measurements. In: Kestin, S., Warriss, P. (Eds.), Farmed Fish Quality. Blackwell Sci,
596 Oxford, pp. 283-297.

597 Erikson, U., Digre, H., and Misimi, E. 2011. Effects of perimortem stress on farmed Atlantic cod product quality:
598 A baseline study. J. Food Sci. 76: S251-S261.

599 Erikson, U., and Misimi, E. 2008. Atlantic salmon skin and fillet color changes as effected by perimortem handling
600 stress, rigor mortis, and ice storage. J. Food Sci. 73: C50-C59.

601 Hultmann, L., Phu, T.M., Tobiassen, T., Aas-Hansen, Ø., and Rustad, R. 2012. Effects of pre-slaughter stress on
602 proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). Food Chem. 134: 1399-
603 1408.

604 Huss, H.H., and Asenjo, I. 1976. Some factors influencing the appearance of fillets from whitefish. Technological
605 Laboratory – a report, 8 p. Ministry of Fisheries, Lyngby, Denmark.
606

607 Huss, H.H., and Børresen, T. 1995. Anatomy and physiology. In: Huss, H.H. (Ed.), Quality and Quality Changes
608 in Fresh Fish. FAO Fisheries Technical Paper 348, Rome, pp. 11-17.
609

610 Kelly, T.R. 1969. Discolouration in sea-frozen fish fillets. In: Kreuzer, R. (Ed.), Freezing and Irradiation of Fish.
611 Fishing News (Books), London, pp. 64-67.
612

613 Larsen, R.B., Herrmann, B., Sistiaga, M., Grimaldo, E., Tatone, I., and Onandia, I. 2016. Size selection of redfish
614 (*Sebastes* spp.) in a double grid system: Estimating escapement through individual grids and comparison to former
615 grid trials. Fish. Res. 183: 385-395.

616 Mokrzycki, W., and Tatol, M. 2011. Color difference Delta E – A survey. Machine Graphics & Vision Int. J. 20:
617 383-411.

618 Neumann, P., Holeton, G.F., and Heisler, N. 1983. Cardiac output and regional blood flow in gills and muscles
619 after exhaustive exercise in rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 105: 1-14.
620

621 Olsen, R.E., Sundell, K., Ringø, E., Myklebust, R., Hemre, G.-I., Hansen, T., and Karlsen, Ø. 2008. The acute
622 stress response in fed and food deprived Atlantic cod, *Gadus morhua* L. Aquaculture 280: 232-241.

623 Olsen, S.H., Joensen, S., Tobiassen, T., Heia, K., Akse, L., and Nilsen, H. 2014. Quality consequences of bleeding
624 fish after capture. Fish. Res. 153: 103-107.

625 Olsen, S.H., Tobiassen, T., Akse, L., Evensen, T.H., and Midling, K.Ø. 2013. Capture induced stress and live
626 storage of Atlantic cod (*Gadus morhua*) caught by trawl: Consequences for the flesh quality. Fish. Res. 147: 446-
627 453.
628 Park, J.W. 1994. Functional protein additives in surimi gels. J. Food Sci. 59: 525-527.
629
630 Plante, S., Chabot, D., and Dutil, J.-D. 1998. Hypoxia tolerance in Atlantic cod. J. Fish Biol. 53: 1342-1356.

631 Rotabakk, T.B., Skipnes, D., Akse, L., and Birkeland, S. 2011. Quality assessment of Atlantic cod (*Gadus morhua*)
632 caught by longlining and trawling at the same time and location. Fish. Res. 112: 44-51.

633 Staurnes, M., Sigholt, T., Pedersen, H.P., and Rustad, T. 1994. Physiological effects of simulated high-density
634 transport of Atlantic cod (*Gadus morhua*). Aquaculture 119: 381-391.

635 Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., and Cooke,
636 S.J. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a
637 review. Conserv. Physiol. 2: 1-21.

638 Valdimarsson, G., Matthiasson, A., and Stefansson, G. 1984. The effect of on board bleeding and gutting on the
639 quality of fresh, quick frozen and salted products. In: Moller, A. (Ed.), Fifty Years of Fisheries Research in Iceland.
640 Icelandic Fisheries Laboratory, Reykjavik, pp. 61-72.

641 Wedemeyer, G.A., Barton, B., and McLeay, D.J. 1990. Stress and acclimation. In: Methods for Fish Biology,
642 Schreck, C.B., Moyle, P.B. (Eds.), Am. Fish. Soc., Bethesda, Maryland, pp.451-489.

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651 TABLES

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653 Table 1. Haul number, duration, capture depth and relationship between the number of fish (Σ n) collected from
654 different hauls and how they were assigned to different treatments. The catch size per haul varied between 0.5 to
655 2.5 metric tons. Before filleting on board or frozen storage, the fish were either stored live (LS) for 0, 3 or 6 h after
656 capture, stored on deck for 5 h without bleeding (DE 5h), or they were subjected to consecutive processing from
657 dry bin (DB). The sea temperature and wind velocity during fishing varied between 3.7 to 4.5°C and 1.9 to 9.2 m
658 s^{-1}, respectively.

Haul #	**Haul duration (min)**	**Capture depth (m)**	**LS 0h**	**LS 3h**	**LS 6h**	**DE 5h**	**DB**	**Σ n**
207	45	61 - 74	20	20	15	20	-	**75**
214	39	53	21	15	21	-	-	**57**
215	45	50 - 53	-	-	-	24	24	**48**
222	60	311 - 316	25	-	-	24	-	**49**
223	66	308 - 314	-	24	24	-	-	**48**
232	90	287 - 303	20	21	15	20	-	**76**
Σ n			**86**	**80**	**75**	**88**	**24**	

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662 **Table 2.** Total number of Atlantic cod transferred from each haul to the flow-through live storage tank, subsequent
663 live-storage conditions (fish density, dissolved oxygen and temperature), and immediate survival rates 3 or 6 h
664 after capture.

Haul #	**Fish**			**Water**	
	n[1]	**Survival rate[2] (%)**	**Fish density[3] ($kg\ m^{-3}$)**	**DO[4] (% saturation)**	**Temperature (°C)**
207	75	100	105	92 - 101	3.9 - 4.1
214	98	94	127	97 - 101	3.5 - 3.9
223	104	51	235	85 - 101	4.2 - 4.4
232	84	67	116	97 - 102	4.2 - 4.4

665 *[1]The number includes fish from LS 3h and LS 6h treatments (where only live fish were sampled) plus additional fish not*
666 *subjected to further analysis; [2]Calculation based on Σ LS 3h + LS 6h + remaining fish in tank; [3]Initial fish density in tank,*
667 *i.e. before sampling of LS 3h and LS 6h fish; [4]Dissolved oxygen (DO) measured intermittently with a hand-held meter*

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671 **Table 3.** Scores for assessment of early postmortem loss of muscle excitability and subsequent development of
672 rigor mortis.

Score	White muscle excitability[(a)]	Score	Rigor status
3	Strong tail twitch (electrodes placed along the entire lateral line, behind the head and near the caudal fin)	0	Pre- or postrigor
2	Weak tail twitch (electrodes placed as above)	1	Rigor onset (first sign of stiffness, for instance in neck or tail region)
1	Minor muscle contractions in (small) restricted areas on the fish surface (electrodes placed a few cm apart)	2	Rigor (a larger area is clearly in rigor)
0	No contractions whatsoever (rigor onset is imminent)	3	Whole fish in rigor
		4	Stronger rigor
		5	Very strong rigor (extremely stiff, rod-like fish)

673 (a) *Valid during the time interval from just after death to rigor onset*

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677 **Table 4.** Visual assessment of discoloration, residual blood and bruises in fillets (Fillet Quality Index).

Score	Discoloration (loins and belly flap)	Residual blood (belly veins)	Bruises
0	Homogeneous white	No visible blood in veins	No visible blood stains
1	Pink	Less than five veins partly filled with blood	One or two small blood stains in the belly or tail
2	Red	More than five veins, partly, or fully filled with blood	Several small blood stains on fillets or bruises in loins

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Table 5. Blood pH, glucose, lactate**,** white muscle initial pH, muscle twitch ability, and rigor status at the time of filleting of live-stored (LS) Atlantic cod for 0, 3 and 6 h after capture by trawling. Unbled fish were analysed and filleted after 5 h post capture (DE 5h). Commercial processing, where the fish were consecutively processed from dry bin (DB), was completed about 2 h post capture.

Treatment	**Blood**			**White muscle**				
	Blood pH	**Glucose (mmol L^{-1})**	**Lactate (mmol L^{-1})**	**Initial pH**	**Temperature (°C)**	**Twitch (0-3)**	**Time post mortem[1] (h)**	**Rigor[2] (0-5)**
LS 0h	7.30 ± 0.04^{A}	3.3 ± 0.5^{A}	4.6 ± 0.5	7.16 ± 0.07^{B}	4.7 ± 0.1	2.8 ± 0.1^{B}	0.5	0
LS 3h	7.25 ± 0.46^{B}	7.0 ± 1.0^{B}	5.8 ± 0.6	7.21 ± 0.07^{BC}	4.8 ± 0.1	2.9 ± 0.1^{B}	0.5	0
LS 6h	7.56 ± 0.04^{B}	8.7 ± 1.1^{B}	5.2 ± 1.0	7.31 ± 0.06^{C}	4.7 ± 0.1	2.9 ± 0.1^{B}	0.5	0
DE 5h	*n.a.*	*n.a.*	*n.a.*	6.96 ± 0.06^{A}	5.1 ± 0.5	0.2 ± 0.2^{A}	5-7	1.3
DB	*n.a*	*n.a*	*n.a*	*n.a*	*n.a*	*n.a*	< 0.7-1.3*	0

*Mean values ± 95% CIs, n = 32 (LS 0h, LS 3h, LS 6h), n = 21 or 28 (DE 5h); Different letters (A, B or C) represent significant differences between treatments (P< 0.05); n.a. = not analyzed; 1 – time post mortem when fish from each treatment were filleted; 2 - the fish were rinsed in seawater for 30 min before the assessments were carried out. *The DB fish were collected from the dry bin between 0.7 and 1.3 h post capture, exact time of death was unknown*

Table 6. Visual assessments of discolorations and residual blood (FQI), and objective analysis by machine vision of CIE L* a* b*, hue, chroma and whiteness of fresh and frozen/thawed Atlantic cod fillets. Color differences (ΔE*) between treatments were calculated relative to just after capture (LS 0h). Comparison between live stored (LS) fish kept in seawater for 0, 3 and 6 h, fish consecutively taken from dry bin where all fish had been processed after about 2 h post capture (DB), and unbled fish stored in air for 5 h (DE 5h). Fresh fillets were evaluated on board 0.5 h post mortem (after each treatment) whereas the frozen/thawed fillets were evaluated after frozen storage for 61 days.

Parameter	LS 0h	LS 3h	LS 6h	DB	DE 5h
			Fresh		
n	49	32	36	12	44
Discoloration loin (0-2)	$0.1 \pm 0.0^{A*}$	$0.1 \pm 0.1^{A*}$	$0.1 \pm 0.1^{A*}$	$0.0 \pm 0.0^{A*}$	0.6 ± 0.1^{B}
Discoloration belly (0-2)	$0.5 \pm 0.1^{A*}$	$0.5 \pm 0.1^{A*}$	$0.4 \pm 0.1^{A*}$	$0.5 \pm 0.2^{A*}$	$1.1 \pm 0.1^{B*}$
Residual blood in belly veins (0-2)	$0.9 \pm 0.1^{A*}$	$0.9 \pm 0.1^{A*}$	$0.8 \pm 0.1^{A*}$	$0.7 \pm 0.1^{A*}$	$1.1 \pm 0.1^{B*}$
Bruises (0-2)	0.2 ± 0.1	$0.2 \pm 0.1^{*}$	0.2 ± 0.1	0.1 ± 0.1	$0.2 \pm 0.1^{*}$
Total score (0-8)	$1.7 \pm 0.2^{A*}$	$1.7 \pm 0.2^{A*}$	$1.5 \pm 0.2^{A*}$	$1.3 \pm 0.3^{A*}$	$3.0 \pm 0.2^{B*}$
n	41	38	29	12	33
L* (lightness)	87.3 ± 0.4^{AB}	87.6 ± 0.4^{B}	87.9 ± 0.3^{B}	88.2 ± 0.4^{B}	86.6 ± 0.4^{A}
a* (redness)	3.7 ± 0.4^{A}	3.7 ± 0.5^{A}	3.1 ± 0.4^{A}	2.5 ± 0.5^{A}	6.0 ± 0.6^{B}
b* (yellowness)	2.2 ± 0.3^{B}	2.2 ± 0.3^{B}	2.1 ± 0.3^{AB}	2.4 ± 0.4^{B}	1.7 ± 0.2^{A}
C_{ab}* (chroma)	4.4 ± 0.4^{A}	4.3 ± 0.5^{A}	3.8 ± 0.4^{A}	3.5 ± 0.5^{A}	6.3 ± 0.6^{B}
H_{ab}^{o} (hue, ^{o})	31.3 ± 3.3^{B}	34.2 ± 5.8^{B}	35.1 ± 5.0^{BC}	44.8 ± 4.6^{C}	16.8 ± 2.8^{A}
W (whiteness)	80.7 ± 1.0	81.0 ± 0.9	81.7 ± 0.8	81.0 ± 1.3	81.6 ± 0.7
ΔE* (color difference)	-	0.3	0.6	1.5	2.5
			Frozen/Thawed		

n	24	41	39	12	44
Discoloration loin (0-2)	0.3 ± 1.0^{AB*}	0.4 ± 0.2^{B*}	0.3 ± 0.2^{AB*}	0.2 ± 0.2^{A*}	0.5 ± 0.2^{AB}
Discoloration belly (0-2)	0.1 ± 0.1^{A*}	0.1 ± 0.1^{A*}	0.0 ± 0.0^{A*}	0.0 ± 0.0^{A*}	0.3 ± 0.1^{B*}
Residual blood in belly veins (0-2)	0.2 ± 0.1^{AB*}	0.3 ± 0.1^{B*}	0.1 ± 0.1^{AB*}	0.1 ± 0.1^{A*}	0.4 ± 0.1^{C*}
Bruises (0-2)	0.2 ± 0.1^{A}	0.3 ± 0.1^{A*}	0.2 ± 0.1^{A}	0.2 ± 0.2^{A}	0.8 ± 0.1^{B*}
Total score (0-8)	0.7 ± 0.2^{AB*}	1.0 ± 0.2^{B*}	0.7 ± 0.2^{AB*}	0.5 ± 0.2^{A*}	2.0 ± 0.2^{C*}
n	24	40	39	12	44
L*(lightness)	88.0 ± 0.3^{B}	88.0 ± 0.3^{B}	88.3 ± 0.3^{B}	88.5 ± 0.5^{B}	87.1 ± 0.4^{A}
a* (redness)	2.2 ± 0.3^{A}	2.1 ± 0.2^{A}	1.6 ± 0.3^{A}	1.4 ± 0.3^{A}	2.9 ± 0.3^{B}
b* (yellowness)	7.4 ± 0.2^{C}	7.0 ± 0.2^{B}	6.6 ± 0.2^{A}	7.4 ± 0.3^{BC}	7.0 ± 0.2^{B}
C_{ab}* (chroma)	7.8 ± 0.2^{C}	7.3 ± 0.2^{B}	6.8 ± 0.2^{A}	7.5 ± 0.4^{BC}	7.6 ± 0.2^{BC}
H_{ab}^{o} (hue, º)	74.2 ± 1.9^{BC}	73.6 ± 1.6^{B}	76.9 ± 1.9^{CD}	79.1 ± 2.1^{D}	67.7 ± 1.8^{A}
W (whiteness)	65.7 ± 0.7^{A}	66.9 ± 0.8^{A}	68.4 ± 0.6^{B}	66.4 ± 1.3^{AB}	66.0 ± 0.7^{A}
ΔE* (color difference)		0.5	0.9	0.6	1.5

Mean values ± 95% CIs. Different letters, A, B or C, represent significant differences between treatments for either fresh or frozen/thawed fillets whereas an asterisk (*), highlighted in bold, denotes significant differences between fresh and frozen/thawed fillets for each parameter within each treatment (FQI only)

Table 7. Comparison between typical (B) best and (C) worst-case fillets from unbled cod stored in air before processing (DE 5h), and (A) fillets from fish sampled just after capture (LS 0h). Fillet color was determined after frozen storage by machine vision in the CIE L*a*b* color space. ΔE* values were calculated relative to the LS 0h group. The corresponding fillets (A-C) are shown in Fig. 2.

Fillet	L*	a*	b*	C^*_{ab}	H^o_{ab} (^{O})	W	ΔE*
A – LS 0h (control)	88.7	1.8	7.0	7.3	75.5	67.6	-
B – DE 5h (best case)	87.6	1.9	6.7	7.0	73.9	67.5	1.2
C – DE 5h (worst case)	84.3	4.7	7.5	8.8	57.6	61.9	5.3

FIGURES

Fig.1

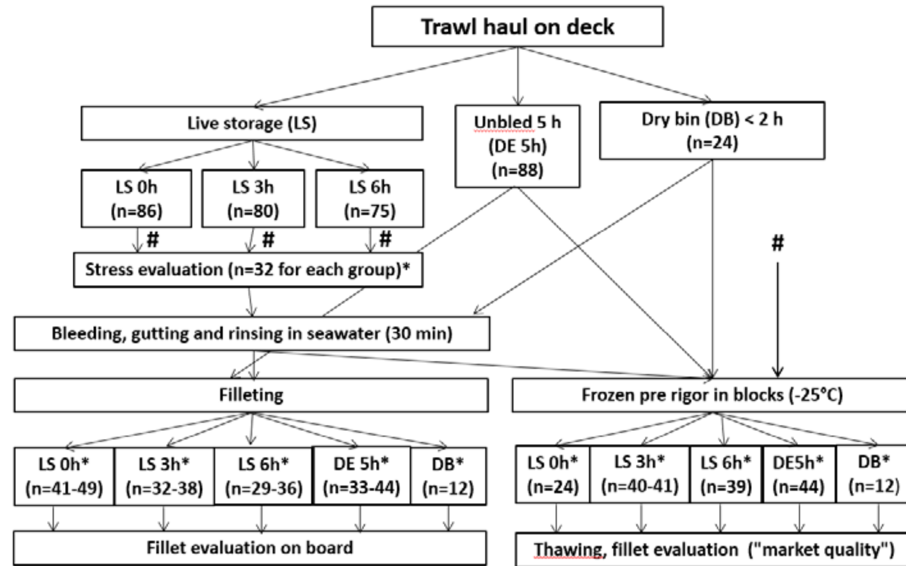

Fig. 2

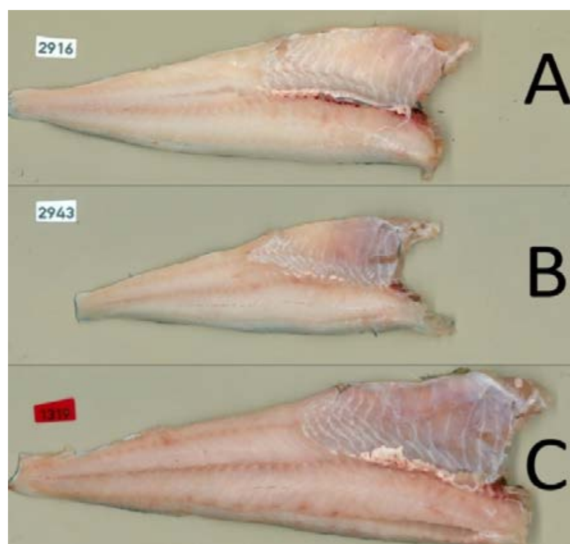

FIGURE LEGENDS

Figure 1. Experimental design and process flow-chart after trawl capture of Atlantic cod. The total number of experimental fish collected from different hauls was n = 353 and the number of fish subjected to each treatment is shown in the figure. Evaluation of stress was only carried out on fish filleted and evaluated for possible discolorations on board. The remaining fish were frozen pre-rigor on board along with the commercial catch (from dry bin) except from the DE 5h fish where rigor mortis had started when they were frozen. After frozen storage, the fish were filleted and evaluated accordingly. The asterisk (*) denotes number of fish subjected to the various analytical methods within each treatment and the hashtag (#) means that a portion of the live-stored fish (LS 0h, LS 3h and LS 6h groups) was frozen on board for later analysis.

Figure 2. Comparison of Atlantic cod fillets cut after frozen storage from: (Photo A) fish bled immediately after the catch was hauled on board, (Photo B) best, and (Photo C) worst-case fillets from the unbled fish stored for 5 h on deck before processing and freezing. The images were selected on a visual basis to represent consumers perception of the product. The corresponding CIE L*a*b* values of the three fillets are shown in Table 7.