Harvesting procedures, welfare and shelf life of ungutted and gutted short-fin pompano (*Trachinotus falcatus)* stored in ice

Ulf Erikson

SINTEF Ocean, 7010 Trondheim, Norway

Hanh T. M. Truong[a], Dung V. Le[ab], Phuong D. Pham[a], Niels Svennevig[a] and Van T. Phan[a]

[a]*Research Institute for Aquaculture No.1, Hanoi, Vietnam*

[b]*Vietnam National University of Agriculture, Hanoi, Vietnam*

ABSTRACT

Shortfin pompano (*Trachinotus falcatus*) were harvested from a floating cage culture. Handling stress (blood pH, white muscle pH, and rigor mortis) and welfare (''eye roll'') were determined for two harvesting methods: (i) batch netting and transfer to ice-slurry where the fish were killed and chilled (commercial method) and, (ii) transfer of fish to a tub immersed in the cage before it was lifted onto a barge where the fish were euthanised by an AQUI-S^{TM} overdose. Half of the ice-slurry fish were gutted. All fish were subsequently stored for 18 d in ice for assessment of freshness (total bacterial counts, TBC and Quality Index Method, QIM) as well as skin and eye color. A modified QIM scheme for European sea bass was used with demerit points ranging from 0 to 18. Due to excessive swimming during crowding, both ice-slurry and AQUI-S^{TM} fish were harvested in an exhausted condition. Upon sampling, none of the fish exhibited ''eye roll'', indicating they were either unconscious or dead. Since the ice-slurry fish were not stunned immediately, the welfare of the fish might have been compromised. During rapid chilling, the fish developed cloudy eyes. The asset of using AQUI-S^{TM} was possibly better fish welfare during the stunning and euthanising phase since exposure to ice-slurry was associated with escape swimming behaviour and slow death. Shortly after harvesting there were some significant differences between harvesting methods in terms of skin color. They were, however, largely offset by storage for one day. Only a few minor changes in skin color took place during further storage. Changes in eye color were more prominent than for skin. After storage for more than a week, the TBCs of gutted fish were significantly higher compared with ungutted fish ($P<0.05$). AQUI-S^{TM} fish (ungutted) exhibited the lowest TBCs for about the first two weeks indicating exposure to the anesthetic reduced bacterial growth. After 18 d, none of fish from all treatments had yet reached the generally accepted spoilage level of 7 log cfu g^{-1}. At this point, the fish had not reached the maximum attainable QI score of 18. High linear correlations ($R \geq 0.972$, $P < 0.001$) were achieved for development of QI during ice storage where the ungutted and AQUI-S^{TM} treatments were practically similar. The modified QIM scheme was considered suitable for pompano. The shelf life was tentatively considered to be

approximately 18 d, although this should be verified by other, supplementary methods. To prolong shelf life, it is recommended that pompano is not gutted before they are subjected to chilled storage.

Key words: Shortfin pompano, Harvesting, Stress, Welfare, Ice storage, QIM, TBC, AQUI-S™

1. Introduction

Since the 1960s, it has been recognized that farming of the different species of the genus *Trachinotus spp* of the Carangidae family seems to be promising due to its excellent flesh quality (pompano is one of the most highly desired marine fishes) and high market prices (Berry and Iverson 1967). Lazo et al. (1998) concluded, based on studies of growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*), that the species is suitable for aquaculture due to its ready adaption to culture systems, acceptance of formulated feeds, and rapid growth rates. Shortfin pompano (*Trachinotus falcatus*), endemic to the subtropical and tropical western parts of the Atlantic Ocean, was introduced to Asia in the 1990s and eventually to Vietnam. The annual production of pompano in Vietnam is about 700 tons where the market-sized fish (400-600 g) are traded locally or exported to USA, Korea and Japan (McMaster and Gopakumar 2016).

Before harvest, the quality of farmed fish is dependent on several factors such as genetics, feed composition, water quality, farming practices and health condition. Once harvested, freshness is the most important quality trait for raw fish. Freshness can be assured by effective and consistent chilling from farm or processing plant to consumers as well as by minimizing storage time before consumption. Harvesting procedures such as pre-slaughter crowding, pumping and killing methods can be stressful and might, depending on fish species, unfavorably affect flesh quality (Lowe et al. 1993; Sigholt et al. 1997; Bagni et al. 2007; Knowles et al. 2007; Roth et al. 2009; Bahaud et al. 2010; Erikson et al. 2011; Matos et al., 2011). Choice of stunning method is important to safeguard fish welfare (Erikson 2011). For example, to minimize handling stress during harvesting (rested harvest) and to provide good

welfare, AQUI-S^{TM} can be a good option (Jerrett et al. 1996; Bosworth et al. 2007; Erikson 2011). Alternatively, when used correctly, automated electrical or percussion stunning methods can also provide good fish welfare by rendering the fish unconscious instantly (Lambooij et al. 2008, 2010; Sattari et al. 2010). However, the shelf life of king salmon (*Oncorhynchus tshawytscha*) is not affected by harvesting method (Fletcher et al. 2003). Different aspects of fish welfare and stress related to slaughter methods and fish quality are reviewed by Poli et al. (2005). Following harvesting, quality changes during storage of fish are mostly attributed autolysis followed by bacterial activity (Liston 1980). Depending on storage temperature and storage time, a wide range of chemical reactions gradually take place in the flesh leading to reduced product quality (Huss 1995).

Regarding the effect of gutting on fish quality, it is in many cases found that shelf life is prolonged when the viscera is removed after slaughter including proper washing of the belly cavity. On the other hand, gutting implies that the belly cavities are exposed to either chilled water (such as in ice-slurry tubs after slaughter) or air (on ice during storage) rendering them susceptible to microbial invasion, oxidation or discoloration (Borderias and Sánchez-Alonso 2011). Shelf life is inherently linked to food safety by the activity of microorganisms. However, the eating quality of fish is a rather complex issue and shelf life can therefore be defined by various quality parameters. With rainbow trout (*Oncorhynchuss mykiss*) as example, the shelf life of ungutted chilled fish is considered 14 d (Dawood et al. 1986). Regarding the effect of lipids on the sensory quality of rainbow trout, they were resistant to oxidation during ice storage for up to 14 d. Changes were related to oxidation product decomposition being more intensive in whole than in gutted fish. It was concluded that this species of fish has a shelf life of at least 14 d, and since gutting resulted in delayed autolytic changes, the shelf life of gutted fish was extended by at least 2 to 3 d (Kolakowska et al. 2006). In contrast, based on postmortem changes of biogenic amines and nucleotide degradation of inosine monophosphate to hypoxanthine, combined with sensory evaluation, ungutted and gutted rainbow trout stored in ice were of acceptable quality for only 5 and 6 d, respectively (Rodriguez et al. 1999). In gilthead seabream (*Sparus aurata*), among several measured quality parameters, the only effects of gutting were lower intensity of rigor mortis and a

111 drop in the bacterial load. After around 12 to 14 d, all fish had passed sensory and bacterial rejection
112 thresholds (Tejada and Huidobro 2002). In case of sea bass (*Dicentrarchus labrax*) stored in ice, the
113 shelf life of ungutted and gutted fish, as assessed by overall acceptability sensory scores and
114 microbiological activity, was considered 13 and 8 d, respectively (Papadopoulos et al. 2003).

115 The Quality Index Method (QIM) is a method for evaluating fish freshness by sensory attributes
116 (Hylding and Green-Petersen 2004). The method is robust and is relatively simple to use in practice. QI
117 schemes have therefore been adapted for several fish species (see Seafish 2010). A review of the
118 usefulness of employing QIM in different contexts for assessing freshness and shelf life of several fish
119 species is given by Bernardi et al. (2013).

120 Regarding pompano, data on postharvesting issues, flesh quality and storage are scarce. Gao et al.
121 (2014) studied the use of rosemary extract in combination with nisin to extend shelf life of pompano
122 (*Trachinotus ovatus*) fillets over a period of 15 d at 4^{o}C. The following indices of quality were employed:
123 peroxide value, thiobarbituric acid, total volatile basic nitrogen, trimethylamine, pH, K-value, texture,
124 color as well as sensory and microbial characteristics. Since addition of rosemary and nisin improved
125 quality by affecting several of the mentioned parameters including microbial growth, it was concluded
126 that the shelf life of fillets may be extended by adding these constituents. The proximate composition
127 of the cultured 400-500 g pompano used in the study was: 68.4 % moisture, 17.3 % crude protein, 10.3
128 % lipid and 1.8 % ash.

129 To our knowledge, no peer-reviewed studies are available concerning effects harvesting method and
130 gutting of shortfin pompano. Therefore, the objective of the study was to provide such data. Another
131 objective was to evaluate whether the Quality Index Method scheme, originally intended for sea bass
132 *(Dicentrarchus labrax)*, would also be appropriate for assessing loss of freshness during ice storage.
133 Since pompano are most commonly traded as whole fish in the market, the external appearance of
134 the fish is also important. In such a context, skin and eye color are relevant factors for the consumers
135 perception of the product. Hence, emphasis was also put on color assessment throughout storage.

2. Materials and Methods

2.1 Fish harvesting and ice storage

Shortfin pompano were farmed in floating cages (19 m in diameter) located in Van Phong Bay north of the city of Nha Trang in Vietnam. During sampling of fish, the seawater temperature and pH in the cage were 28.7 ^{o}C and 8.54, respectively. The total biomass in the cage was about 8 metric tons corresponding to a fish density of 3.3 kg m^{-3}. Before fasting for one day, the fish had been offered Barramundi Stella B5 feed from Skretting (Stavanger, Norway) with the following composition: 44 % crude protein, 12-14 % crude fat, 5 % fiber, 11 % moisture, 2.3 % lysine, 1.4 % methionine + cysteine, 1.5 – 3.0 % calcium and 0.5 – 2.0 % phosphorus. The weight and fork length (mean ± SD, n = 108) of the experimental fish were 467 ± 80 g and 25 ± 1 cm (6 months post hatching).

To examine the effect of harvesting and gutting on fish quality, the following procedures were carried out. For harvesting, the fish were crowded in the cage by using a sweep net. Crowded fish were then rapidly netted batchwise to 1000-L tubs placed on the deck of a barge next to the cage. One of the tubs was used for the experimental fish whereas the other ones were used for commercial harvesting. Prior to harvesting, seawater and crushed ice had been mixed in the tubs to make an ice-slurry where the fish were killed and chilled at about 2^{o}C (normal harvesting procedure at the farm). Seventy-two experimental fish were transferred from the cage to one of the ice-slurry tubs over a period of 6 min. Sampling of fish started after all swimming activity had ceased and the fish were apparently unconscious or dead.

As an alternative stunning method, AQUI-S^{TM} (AQUI-S Ltd., Lower Hutt, New Zealand) was tested to assess whether the level of peri-mortem stress could be reduced compared with the current slurry-based method. A 1000-L tub was immersed into the crowded volume of the cage. Fish (n = 36) in water

were hoisted onto the deck of the barge by using a crane. AQUI-S^{TM} was immediately added to the tub where fish were euthanised by an anesthetic overdose (93 mg AQUI-S^{TM} L^{-1}). Subsequently, the fish were transferred to expanded polystyrene (EPS) boxes filled with crushed ice. Notably, the fish were not washed after exposure to AQUI-S^{TM}.

In case of both harvesting methods, vestibulo-ocular reflex, handling stress (blood pH and initial pH in white muscle), and body temperature were measured before the fish (n = 10) were subjected to analysis of skin and eye color. After sampling was completed, the fish were transported by the barge for 2 h to quay where half of the slurry fish were gutted 30 min after arrival. The fish were gutted in the traditional local way, that is, by removing the gill arches along with intestines through the throat (without opening the belly). Subsequently, the body cavities of gutted fish were thoroughly rinsed using bottled drinking water. The AQUI-S^{TM} fish were not gutted. All fish were repacked in EPS flight boxes filled with crushed ice before transport to the Research Institute for Aquaculture No. 1 (RIA-1) in Hanoi (15 h transport) where the fish were subjected to chilled storage. Six fish from each treatment were sampled and analysed (length, weight, rigor mortis, ultimate pH, core temperature, skin and eye color, bacterial counts and Quality Index) on Days 1, 3, 7, 10, 14 and 18 post mortem. Melted ice was replenished with crushed ice and excess water was drained off daily.

2.2 Analytical Methods

2.2.1 Vestibulo-ocular reflex

Vestibulo-ocular reflex (VOR or ''eye roll') was assessed according to Kestin et al. (2002) as an indicator of whether the fish were unconscious (or dead) after instant chilling in ice-slurry or after treatment with AQUI-S^{TM}.

2.2.2 pH in blood and muscle

184 A shielded glass electrode (SenTix 41, WTW, Wilhelm, Germany) connected to a portable pH meter
185 (model WTW 330i WTW) was used for the determination of pH in blood and white muscle. Just after
186 sampling of fish from the AQUI-S^{TM} bath or ice-slurry tub, gill arches were cut by using a scalpel before
187 the pH electrode was brought in contact with the flowing blood to measure its acidity. A similar pH-
188 electrode was inserted directly in epaxial white muscle in front of the dorsal fin after an incision had
189 been made by a scalpel. The initial pH was recorded after some seconds when the fluctuating pH values
190 had stabilized. In addition to frequent electrode cleaning and re-calibration using buffers 4.01 and 7.00,
191 it was important to ensure good contact between the electrode surface and the flesh. The initial pH
192 describes peri-mortem anaerobic white muscle activity (e.g. escape behaviour). During ice storage, the
193 ultimate pH was determined in approximately in the same location where the initial pH was measured.

194 *2.2.3 Rigor mortis*

195 Rigor mortis onset was determined by using the rigor status method (Erikson 2001): 0 = pre rigor or
196 post rigor; 1 = rigor onset (first signs of rigor, neck or tail region); 2 = rigor (a large area of fish body is
197 clearly in rigor); 3 = whole fish in rigor; 4 = stronger rigor; 5 very strong rigor (fish extremely stiff like a
198 rod).

199 *2.2.4 Body and core temperatures*

200 The body temperature of the fish was measured by using a Testo 110 thermometer (Testo AG,
201 Lenzkirch, Germany) just after killing, between the dorsal fin and lateral line, next to the backbone in
202 the thickest part of the fish. Core temperatures during ice storage were measured similarly.

203 *2.2.5 Quality Index Method*

204 We chose to apply the QIM scheme intended for farmed sea bass (*Dicentrarchus labrax*) as shown in
205 Seafish (2010) for our QI assessment of pompano. The sea bass scheme ranges from 0 – 22 demerit
206 points and includes assessments of fillet color (0-2) and viscera (0-2). Since pompano is normally traded
207 as round fish, assessments of fillets and viscera were omitted. Thus, three assessors evaluated the

following QI attributes of pompano: skin color/appearance (0: bright, iridescent pigmentation, 1: rather dull, becoming discoloured (head), 2: green, yellowish, mainly near abdomen); skin odor (0: fresh seaweedy, neutral, 1: cucumber, metal, hay, 2: sour, dish cloth, 3: rotten); skin texture (0: in rigor, 1: finger mark disappear rapidly, 2: finger mark returns slowly (> 3 s); eye pupils (0: clear and black, metal shiny, 1: grey, 2: matt, grey; 2); eye form (0: convex, 1: flat, 2: sunken); gill color (0: blood red/orange, 1: pale red, pink/light brown, 2: grey-brown, brown, grey); gill odor (0: fresh, seaweed, neutral, 1: metal, grass, 2: sour, moldy, dish cloth, 3: rotten); gill mucus (0: transparent, 1: milky, clotted, 2: brown, clotted). Accordingly, the modified total QI score ranged from 0 (very fresh fish) to 18 (spoiled fish). For gutted pompano, where gills and viscera were removed, the total attainable QI score became 11.

2.2.6 Mesophilic bacterial counts

On each sampling day, approximately 10 g of fish white muscle of three fish from each treatment was excised with sterile scalpels and forceps. Before sampling, the skin around the sampling area was rinsed with 70 % ethanol. The skin of the sampled cube was removed aseptically before the samples were mixed with 90 mL of 0.1% peptone water. From the 10^{-1} dilution, other decimal dilutions were prepared. Total viable mesophilic bacterial counts were determined using plate count agar (PCA, Merck) after incubation for 24 h at 29 ^{o}C.

2.2.7 Skin color

Color was determined objectively with a Minolta Chroma Meter CR-200 (Minolta, Osaka, Japan) with probe diameter 8 mm. Skin color during ice storage was measured on four different locations (two dorsal, belly and eye), see Fig. 1. Changes in eye color were assessed to evaluate whether eye color could be used as a freshness indicator during storage. Color analysis was performed within the CIE L*a*b* color space where L* is lightness (ranging from 0 to 100), a*>0 describes redness and a*<0 describes greenness, whereas b*>0 corresponds to yellowness and b*<0 to blueness. Hue angle (0^{o} = red hue; 90^{o} = yellow hue) was calculated as: $H_{ab}{}^{o}$= arctan (b^*/a^*) for $a^*$and b^*> 0, $H_{ab}{}^{o}$= 180° + arctan (b^*/a^*)

for a^*< 0, or $H_{ab}{}^{o}$= 360° - arctan (b^*/a^*) for a^*> 0 and b^*< 0. Chroma, where higher values correspond to a more intense color saturation, was calculated as: $C_{ab}{}^{*}$=sqrt ($a^{*2} + b^{*2}$).

2.3 Statistical analysis

Initial pH and core temperature of ungutted and AQUI-S^{TM} fish on Day 0 were compared by using Mann-Whitney U statistics since Shapiro-Wilk normality tests failed. The effect of storage time (Day 1 to 18) on flesh pH, rigor status, core temperature, skin color, TBC and QI was tested using a one-way ANOVA followed by a Holm-Sidak post hoc test when significance was indicated. Where normality tests or Levene Median tests of homogenity of variance failed, the Kruskal-Wallis One Way Analysis of Variance on Ranks method was applied followed by a Tukey post hoc test. The relationship between the quality index (QI) and storage time was analysed by linear regression. Unless otherwise stated, all data are presented as mean values ± standard error of mean (SEM).

3. Results

3.1 Harvesting procedures and handling stress

Frenzied burst of activity (escape behaviour) occurred immediately once the crowding process started as well as during transfer to the ice-slurry tub. In the ice-slurry, the fish still exhibited vigorous swimming activity for 20 to 30 s as they tried to force themselves downwards into the ice-slurry. After less than 5 min, practically all signs of movement had ceased. At this stage sampling of individual fish started. In the AQUI-S^{TM} tub, all fish lost equilibrium 2 to 3 min after transfer from cage. No escape swimming behaviour took place during this period. The fish were subsequently lying sideways on the bottom of the tub exhibiting weak and sporadic ventilation movements. Three min later, no signs of respiration were observed and none of the fish responded to handling. No VORs altogether were observed for fish sampled from the ice-slurry tub as well as from the AQUI-S^{TM} tub. Simultaneous

measurements of blood pH in AQUI-S™ and ice-slurry fish showed significantly different pH values of 6.77 ± 0.07 and 7.11 ± 0.04, respectively. The effect of excessive peri-mortem swimming activity was measured as low initial pH values in white muscle, pH 6.55, similar for both treatments (Table 1). Mean body temperatures shortly after killing of ice-slurry and AQUI-S™ fish were 9.7 and 29.2 °C, respectively. During the assessments, it was noticed that the fish were already very stiff.

3.2 Ice storage and market quality

From Day 1 onwards, the measured acidity represented ultimate pH. Except from a slightly lower pH in the AQUI-S™ fish on Day 1, there were no differences between treatments during the entire storage period (P>0.05). For all treatments, however, from Day 7 onwards an increase in flesh acidity of about 0.2 to 0.4 pH units was observed. The body temperature of the AQUI-S™ fish was about 29 °C immediately after sampling whereas the ice-slurry fish were almost 20 °C colder after chilling for up to 30 min. The mean temperature of the AQUI-S™ fish were still a little higher one day later, although not significantly so. Throughout storage, the core temperatures of all fish basically were within the range of 2.0 ± 0.8 °C. All fish from both treatments were already extremely stiff during the assessments carried out at the farm. Two very stiff fish from the ice-slurry were left in ambient air (about 30 °C) for 20 min to check whether stiffness still persisted. No change in stiffness occurred during this period. Rigor strength gradually decreased to Rigor Status values of 3 – 4 (*'strong rigor in whole fish'*) on Day 1 to about 2 – 3 (*'whole fish still in rigor'*) on Day 3. Traces of rigor were still present on Day 7 (Rigor Status about 1 corresponding to *'weak rigor in some areas of the fish'*). Subsequently, the fish were clearly in the post-rigor state. The development of rigor was similar for all treatments (P>0.05).

Table 2 shows skin and eye color shortly after harvesting (Day 0). The most striking difference between anterior and posterior dorsal and belly locations were in lightness where the belly section, as expected, was considerably lighter. Several color differences between AQUI-S™ and ungutted fish were observed. The AQUI-S™ fish consistently displayed higher yellowness and color saturation values,

whereas the hue values were lower ($P<0.05$). Regarding eye color, except from chroma, all color variables were clearly affected by that the ungutted fish had been immersed in ice-slurry ($P<0.05$). Shortly after transfer to ice-slurry, it was observed that all fish developed cloudy eyes (grayish, less clear appearance). Thus, the difference in the CIE L*a*b* color space between the two harvesting methods actually reflected difference between clear and cloudy eyes.

After ice storage for one day, the skin and eye color would be approximately representative of fish traded at the local market (< 1 day post mortem, Table 3). As perhaps could be expected, basically no differences in skin color were observed between gutted and ungutted fish. At this storage time, the initial differences in color between AQUI-S^{TM} and ungutted fish had been offset in most cases, and in general, no dramatic differences in color between the three groups of fish were observed. Moreover, the initial effect of immersion in ice-slurry on eye color was no longer evident ($P>0.05$). During further storage, no dramatic changes in skin and eye color occurred although some minor, significant differences did occur on each sampling day (Days 3, 7, 10 and 14). However, these minor differences occurred in a random and unsystematic fashion (data not shown). The final evaluation of color was carried out when the experiment was terminated after 18 days (Table 4). Clearly, neither harvesting method nor gutting had an effect on skin and eye color at this stage. Compared with fish stored in ice for one day, the most prominent changes in color during storage were as follows: lightness (L*) – somewhat darker skin on dorsal posterior skin and lighter eyes; higher a* and b* values for eyes; higher hue values for dorsal skin and lower hue values for eyes. Changes in chroma were less consistent. Overall, changes in eye color were more pronounced than changes in skin color. On all sampling days, the eyes had a cloudy appearance while the fish were stored in ice. Once the fish were removed from the ice for sampling, the eye color changed gradually due to the increase in temperature. Frequent color measurements during the first few minutes confirmed that significant changes in color occurred. The reported values were recorded when fluctuations in L*, a* and b* values more or less levelled out after a few minutes at room temperature.

307 The development of TBCs in the flesh of ungutted, gutted and AQUI-S^{TM} fish during ice storage is shown
308 in Fig. 2. After 3 d post mortem there were no significant differences among treatments where the TBC
309 values varied around 4.7 – 4.9 log cfu g^{-1}. After 7 and 10 d, the AQUI-S^{TM} fish had lower bacterial counts
310 ($P<0.05$) than ungutted and gutted fish. On Day 18, both AQUI-S^{TM} and ungutted fish had significantly
311 lower TBC values than gutted fish, 5.6 - 5.8 log cfu g^{-1} versus 6.2 log cfu g^{-1}, respectively. Throughout
312 storage, AQUI-S^{TM} fish had consistently the lowest TBC mean values. After ice storage for a week, the
313 bacterial counts of gutted fish were somewhat higher than for ungutted fish ($P<0.05$).

314 Development of individual QI demerit scores during storage for parameters related to skin, eyes and
315 gills are shown in Fig. 3. Since all QI parameters of AQUI-S^{TM} and ungutted fish developed similarly,
316 their demerit scores were pooled. The same was true for gutted fish (skin and eyes) although data
317 from these fish are not shown since the gills had been removed. Texture did not change for the first
318 seven days meaning signs of rigor (stiffness) were visible until then. Thereafter, the fish gradually grew
319 softer. The color and appearance of the skin started to change after three days reaching its maximum
320 score of 2 after 18 days post mortem. Odor showed a related development with respect to time
321 although the fish did not yet attain a "rotten" odor (score 3) on Day 18. After three days, the form of
322 the eyes started to gradually change from "convex" to "flat" (score 1) after more than a week. The
323 clear, black and metal shiny appearance of the pupils was lost after three days when they turned grey
324 (score 1). The maximum score of 2 ("matt grey") was reached after 18 days. The blood red appearance
325 of the gills started to change after two to three days, and after a week, the color had changed to "pale
326 red, pink/light brown" (score 1) which persisted throughout storage. For three days after harvest, the
327 gill mucus remained "transparent". From Day 7 to Day 18, the mucus changed from a "milky, clotted"
328 (score 1) to a "brown, clotted" appearance (score 2). Until Day 3, the gill odor was typical of very fresh
329 fish ("fresh, seaweed, neutral"). Subsequently, the odor score increased gradually to score 3 ("rotten")
330 on Day 18.

Linear regression of data show that the QI of ungutted and AQUI-S^{TM} fish increased steadily throughout storage from QI = 0 on Days 0 and 1 to values between approximately 14 to 15 after 18 d post mortem (Fig. 4). Significant differences between the two treatments occurred on Days 10, 14 and 18. The gutted fish, with their gills removed, therefore exhibited lower values increasing to QI values of 9 to 10 after 18 d. If demerit points related to gills were omitted from the ungutted and AQUI-S^{TM} Quality Indexes, they would basically resemble the QI development of gutted fish. The regression equations [QI = f (storage time)] for the AQUI-S^{TM}, ungutted and gutted fish were $y = -0.514 + 0.864x$ ($R = 0.985$, $P<0.001$), $y = -0.548 + 0.893x$ ($R = 0.996$, $P<0.001$), and $y = -0.134 + 0.620x$ ($R = 0.972$, $P<0.001$), respectively.

4. Discussion

4.1 Harvesting procedures and handling stress

The fish instantly responded with intensive muscle activity (escape behaviour) once the sweep-net was being prepared for crowding. Crowding *per se* further exacerbated the degree of handling stress. The extreme level of activity is a consequence of the fact that the pompano is a very active fish species with high metabolic rates, constantly swimming at high speeds (Tutman et al. 2004). When transferred to ice-slurry, the fish became quiescent. Consequently, the low initial pH in white muscle (pH 6.55, Table 1) and the low pH values in blood (pH 6.8 - 7.1), indicated the fish were either severely stressed or more probably, exhausted. Based on our observations of behavior, crowding was likely to be the main stressor. We are not aware of relevant stress physiology data for adult pompano. If we compare with well-established data for another active species, Atlantic salmon (*Salmo salar*), the initial muscle pH of rested and exhausted salmon are pH 7.5 ± 0.1 and pH 6.7 ± 0.1, respectively (Erikson and Misimi 2008). In case of blood acidity of salmon, corresponding values are pH 7.848 and pH 7.316 (Tufts et al. 1991). If we assume that the pH ranges in pompano are approximately similar, our present data

357 suggests that the harvested pompano were in fact exhausted. After a few minutes in the ice-slurry,
358 none of the fish exhibited VORs indicating the fish were either unconscious or dead. It is likely that this
359 was caused by a cold shock reaction, known to occur when chilling tropical fish (Curran et al. 1986).
360 Cold-shock can occur when fish are exposed to a rapid decrease in temperature resulting in a cascade
361 of physiological and behavioural responses which, under severe circumstances, can lead to death
362 (Donaldson et al. 2008). It was also observed that the pompano developed cloudy eyes when immersed
363 in ice-slurry. This phenomenon has also been reported when gilthead seabream are killed in ice-slurry.
364 Since seabream are often traded as whole fish, it was considered that cloudy eyes significantly reduce
365 the commercial value of the fish (Huidobro et al. 2001). Similar findings have also been reported for
366 sea bass in ice-slurry where a loss of quality was reported related to the appearance of eyes and gills
367 (Cakli et al. 2006). See below for further results related to changes in pompano eye color during ice
368 storage.

369 During sampling of ice-slurry fish, they were very stiff. The core temperature in these fish was by then
370 9.7 ^{o}C (Table 1). The stiffness was irreversible as observed after a ''heating'' period of 20 min in air
371 which suggests that the stiffness was related to early rigor mortis, due to exercise to exhaustion during
372 harvesting. Alternatively, the observed fish stiffness, different from rigor mortis, might have been due
373 to a cold shock reaction (Curran et al. 1986). In our case it seems plausible that exercise to exhaustion
374 was the predominant factor causing early onset of rigor mortis. By comparison, when Atlantic salmon
375 are stressed to exhaustion, time to onset of rigor is dramatically reduced from about 24 h in rested fish
376 to only 1-2 h post mortem in exhausted fish (Erikson and Misimi 2008).

377 Fish from the volume confined by the sweep-net were collected by immersing a tub into the cage. Due
378 to excitability of pompano, it turned out that it was not possible to carry out such an operation without
379 inflicting severe stress reactions. Even though the fish became calm after AQUI-S^{TM} was added to the
380 tub it would not matter anymore since they were already severely stressed by crowding. The
381 behavioural pattern was reflected in a low initial pH in the muscle, similar to the ice-slurry harvesting

382 method (Table 1), and to a low blood pH of 6.77. As with the ice-slurry fish, the AQUI-S^{TM} fish, with
383 core temperature 29.2 ^{o}C, were also very stiff during sampling, strongly indicating vigorous muscle
384 activity during crowding caused rapid depletion of ATP leading to early rigor onset. Thus, achieving
385 rested harvest of pompano in cage cultures did not seem to be a feasible option. When reared in tanks
386 (e.g. in RAS) however, rested harvest could be possible to achieve by adding the anesthetic to influent
387 water without intervention of personnel until the fish have been sedated or anesthetized. Another
388 aspect of using AQUI-S^{TM} is to improve fish welfare (Erikson 2011) since it has been shown by
389 measurements of brain activity that AQUI-S^{TM} can render fish unconscious without recovery (Erikson
390 et al. 2012). Absence of VORs of the AQUI-S^{TM} fish in the present study indicated that good welfare was
391 achieved during the stunning process per se. Conversely, fish harvested in ice-slurry were subjected to
392 a cold shock and a comparatively slow death (few minutes) possibly exposing the fish to a certain
393 period of distress.

394

395 *4.2 Ice storage and market quality*

396 During the 18 d of ice storage, the mean core temperatures were always within the range of 1.2 and
397 4.0 ^{o}C (Table 1) showing that fish were stored under adequate chilling conditions. From Day 1 onwards,
398 when the Rigor Status method was used to assess stiffness, the results showed that the fish were in a
399 state of strong rigor on Day 1 gradually diminishing to weak rigor on Day 7. It was our impression that
400 the fish were at least as stiff just after harvesting on Day 0 as on Day 1. Probably, peak rigor (Rigor
401 Status score 5) had occurred during the 15 h transport to the laboratory. Rapid onset of rigor also takes
402 place in exhausted Atlantic salmon after 1-2 h, whereas peak rigor and post-rigor states occur after
403 about 10 h and 30 h post mortem, respectively (Erikson and Misimi 2008). Apparently, time to
404 completion of rigor in exhausted pompano (warm-water adapted) is considerable longer than in
405 salmon (cold-water adapted). This could be ascribed differences in habitat temperatures and lower
406 enzyme activity in pompano when stored in ice (Tsuchimoto et al. 1986).

Except from some small differences in muscle pH between treatments on Day 1 ($P<0.05$), no differences were observed thereafter (Table 1). The modest drop in acidity, from pH 6.55 (Day 0) to levels around pH 6.2 – 6.3 the following days (ultimate pH) provided further evidence that due to excessive struggling during harvesting, most of glycogen stores had been depleted to form lactate and H^+. In other words, the harvested fish were killed in an exhausted state. To our knowledge, it is unknown whether exercise to exhaustion has detrimental effects on pompano flesh quality. From Day 7 to Day 18, the slight increase in pH can probably be ascribed the observed increased bacterial activity (Fig. 2). A similar pattern in pH development during ice storage was reported by Gao et al. (2014) where flesh acidity ranged from pH 6.56 to pH 6.77.

Fish skin color can be affected *in vivo* by stress (Iger et al. 2001; Pavlidis et al. 2006; Erikson and Misimi 2008) by the action of chromatophores (Fujii 2000). Just after harvesting, there were some differences in color between AQUI-S^{TM} and ice-slurry fish. However, since both groups of fish were exhausted, the observed results must have other explanations such the initial differences in temperature or exposure to the AQUI-S^{TM} solution (yellowish color). After the fish had been stored in ice for one day, the initial differences in color were basically no longer present (Table 3). Subsequent storage for up to 18 d post mortem had no dramatic effects on skin color. This finding is in line with results of salmon stored in ice, where initial differences in skin color were offset by storage time (Erikson and Misimi 2008).

When the ice-slurry harvesting method was used, the fish rapidly developed cloudy eyes (see above). The corresponding changes in color are shown in Table 2. It was evident that eye color of ice-slurry fish was very different from the normal eye color of the AQUI-S^{TM} fish just after harvesting. When the fish were stored in ice for one day, however, the eye color of fish from the two harvesting methods were similar (Table 3). Thus, the issue of cloudy eyes in ice-slurry would hardly matter in cases where the fish are destined for ice storage during transport to market. On each sampling day during ice-storage, there were largely no significant differences in eye color between treatments although there were differences on each sampling day (color data for Days 3, 7, 10 and 14 not shown). However, since the

color changes did not follow a consistent pattern, no further attempts were made to correlate eye color changes with increasing storage time. In case of gilthead seabream, however, except from the a* values, the CIE L*a*b* variables showed a consistent increasing trend during ice storage. This property of eyes and gills was exploited to assess freshness rapidly by using machine vision (Dowlati et al. 2013).

The total bacterial counts of flesh samples from the three treatments increased from around 4.8 log cfu g^{-1} on Day 3 to around 5.7 log cfu g^{-1} on Day 18 (Figure 2). It has been estimated that it takes 3 to 5 d for bacteria, present in surface mucus, gills and intestinal tract, to penetrate the skin into the flesh (Martin et al. 1978). After 18 d, the pompano TBCs were still below the maximum level of 7 log cfu g^{-1} generally recommended for acceptance of fish for human consumption (ICMSF 1986). Notably, elevated levels of TBC do not necessarily mean a high spoilage potential, since a further characterisation of the bacterial flora would then be necessary (Hansen et al. 2009). It was not until Day 10 that a significant difference in TBC between ungutted and gutted fish was observed, that is, at a time when increasing bacterial activity typically can be expected to occur during chilled storage. From this point onwards, the TBCs of gutted fish were significantly higher than those of ungutted fish ($P<0.05$). Even if the belly was not cut open, this indicated that bacteria still had access to the body cavity of gutted fish, and hence, further into the flesh. Concerning microbial counts of flesh from several fish species, such as for Atlantic croaker (*Micropogon undulatus*) and grey trout (*Cynoscion regalis*) (Townley and Lanier 1981), gutting has an advantageous effect during ice storage. The positive effect of gutting of fishes is, however, not universal. In the flesh of orange roughy (*Hoplosethus atlanticus*), no differences in bacterial counts has been reported (Scott et al. 1986) whereas in seabass, the bacterial counts of ungutted fish were in fact lower than in gutted fish (Cakli et al. 2006; Erkan and Özden 2006).

Interestingly, for up to about the first 14 d of storage, the AQUI-S^{TM} fish (ungutted) exhibited lower TBCs than found in ungutted fish ($P<0.05$). Since the AQUI-S^{TM} fish were transferred to ice directly after exposure to the anesthetic without washing, it could be that residual amounts of AQUI-S^{TM} on the skin

surface suppressed growth of bacteria. Eventually, it could be that residual AQUI-S^{TM} was gradually washed away be melting ice minimizing the observed positive effect. Essential oils contain a wide variety of metabolites capable of inhibiting or slowing down the growth of bacteria, yeasts and moulds (Nazzaro et al. 2013). The chief constituents of clove oil and AQUI-S^{TM} are eugenol and isoeugenol, the constituents that produce the anesthetic effect. These compounds are also known to have an antiseptic effect (see references in Nazzaro et al., 2013). In countries where AQUI-S^{TM} is approved for use in connection with rested harvest (fish for human consumption), the antiseptic effect of the essential oils might be exploited further for storage or transport of fish to market. However, this effect should be weighed against possible uptake and accumulation of isoeugenol or eugenol in the edible tissues of the fish (Kildea et al. 2004). By comparison, when pompano fillets were immersed in distilled water before they were individually packed in air-proofed polyethylene packs (control) prior to storage at 4 ^{o}C, the microbial counts increased from 1.5 log cfu g^{-1} on Day 0 to 8.5 log cfu g^{-1} on Day 15. Immersion in a rosemary/nisin solution before packing increased shelf life by at least 6 d compared with control fillets (Gao et al. 2014).

The development of each quality attribute of skin, eyes and gills during ice storage is shown in Fig. 3. As can be seen from the figure, all of them contributed to the total QI scores to various extents. After a lag phase lasting for a few days, the most pronounced changes took place after storage for about a week, except from the changes in eye pupils and form that occurred after three days. Thus, the well-known relationship between increases in QI scores with increased microbial activity (Fig. 2) was, as expected, also corroborated in case of shortfin pompano. Gradually increasing microbial activity did in turn affect the appearance and odor of the skin and gills (Fig. 3). The increase in skin texture after Day 7 correlated with the completion of rigor mortis as measured by the Rigor Status method (Table 1). The subsequent increasing QI "texture" values (softening of muscle) has been shown to correlate well with post-mortem degradation of connective tissue as shown by histology of cobia (*Rachycentron canadium*) flesh. After 14 and 21 days on ice, between QI texture scores of 1 and 2, the collagen fibrils in the pericellular connective tissue were disorganized and degraded leading to spaces between

muscle fibers (Fogaca et al. 2017). The skin color/appearance increased distinctively after storage for three days (Fig. 3). Correlation with CIE L*a*b* color was poor since the latter data did not show consistent patterns during storage (Tables 2, 3 and 4). Concerning the eyes, however, there was a relationship between the two methods. From Day 1 to Day 18, the color of pupils changed from black to matt grey (Fig. 3). During the same period, the lightness (L*) values increased together with clear changes in redness, yellowness and hue (Tables 2 and 4).

The present QI scheme, with maximum total score of 18, was considered useful for ungutted shortfin pompano since all quality attributes contributed to the total QIM score. Moreover, they basically developed in a similar fashion to cobia (Fogaca et al. 2017), another tropical fish species. It should be pointed out though that the present scheme seems to be suitable for up to 18 days only (under the current storage conditions). This was because five out of eight quality attributes reached their maximum values after 18 days (Fig. 3) meaning that further deterioration of quality cannot be monitored effectively.

Good linear correlations between QI and storage time were observed for all treatments, where the spoilage rate of AQUI-S^{TM} and ungutted fish were basically similar. Due to the removal of gills, the QI values of gutted fish were generally lower (Fig. 4). On Day 18, none of the treatments exceeded the set maximum demerit scores of 18 (AQUI-S^{TM} and ungutted fish) and 11 (gutted fish). Since the bacterial counts of the fish were always lower than 7 log cfu g^{-1}, it could tentatively be concluded that the shelf life of exhausted pompano is at least 18 d post mortem when chilled properly throughout storage. However, since pompano is a rather fatty fish (Gao et al. 2014), it is recommended that measurements of rancidity, or decomposition of oxidation products (Kolakowska et al. 2006), in addition to an overall verification by a sensory panel, should be performed before categorical conclusions are made. For example, based on sensory evaluation (flavor), it has been reported that gilthead seabream stored in ice were judged unacceptable after 14 d, whereas maximum bacterial counts and QI points were reached after 16 d (Huidobro et al. 2000). The modified QIM scheme, here

intended for whole shortfin pompano, was considered appropriate for assessment of freshness during storage. When gutted traditionally, which included removal of gill arches, the QIM was, however, considered a less powerful method to assess loss of freshness.

Since the bacterial counts of gutted fish were significantly higher than for ungutted fish after 7 d in ice, together with the fact that the QI demerit points of ungutted fish minus their gill scores were equal to the QI points of gutted fish, we conclude that gutting of this species of fish is not necessary to slow down the rate of loss of freshness. If the fish are to be stored for more than a week, the better way is to leave the fish ungutted. The shelf life was tentatively considered be approximately 18 d, although this needs to be verified by other, supplementary methods.

5. Conclusions

Due to the extreme excitability of shortfin pompano, the outlook for achieving rested harvest in floating cage systems seemed to be rather far-fetched. Since both ice-slurry and AQUI-S^{TM} harvesting methods were preceded by very stressful fish crowding and transfer operations, all fish were harvested in an exhausted condition showing method of harvesting did not matter when the principles of rested harvest is considered. From a welfare point of view though, stunning and euthanising fish by using an anesthetic overdose may be preferable to exposing the fish to ice-slurry where a cold shock reaction can occur. On the other hand, batch netting of fish from cage to ice-slurry was considered a convenient harvesting method where the fish were instantly chilled in transport containers. Exposure of fish to ice-slurry rapidly resulted in development of cloudy eyes. Since AQUI-S^{TM} fish developed cloudy eyes during storage, fish from both treatments had cloudy eyes by the time they would have been presented to consumers. The skin color of fish harvested by the two methods were initially somewhat different. However, this effect was offset after ice storage for one day. Overall, skin color and appearance changed little during ice storage. In contrast, changes in eye color were more evident. Due to transient changes during assessment, however, eye color was not considered a simple and

straightforward method to assess the post-mortem age of pompano. Exposure to AQUI-S^{TM} during harvesting may be exploited to reduce growth of microorganisms during ice storage. The modified QIM scheme was considered suitable for assessment of shortfin pompano quality during storage. The present study indicated that the shelf life of exhausted shortfin pompano stored in ice at constant low temperatures was about 18 d. Gutting of shortfin pompano was not considered necessary, and for longer storage times it cannot be recommended.

Acknowledgements

The present research was carried out as a part of the project ''Building advanced research, education and extension capacity of Research Institute for Aquaculture No.1, Phase III 2012-2016'' co-funded by the Norwegian Agency for Development Cooperation (NORAD).

555 **References**

556

557 Bagni, M., Civitareale, C., Priori, A., Ballerini, A., Finoia, Brambilla, M.G., Marino, G., 2007. Pre-
558 slaughter crowding and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus*
559 *labrax*) and sea bream (*Sparus aurata*). Aquaculture 263, 52-60.

560 Bahuaud, D., Mørkøre, T., Østbye, T.-K., Veiseth-Kent, E., Thomassen, M.S., Ofstad, R., 2010**.** Muscle
561 structure responses and lysosomal cathepsins B and L in farmed Atlantic salmon (*Salmo salar* L.) pre-
562 and post-rigor fillets exposed to short and long-term crowding stress. Food Chem. 118, 602-615.

563 Bernardi, D.C., Máralém, E.T., de Freitas, M.Q., 2013. Quality Index Method (QIM) to assess the
564 freshness and shelf life of fish. Braz. Arch. Biol. Technol. 56, 587-598.

565 Berry, F., Iversen, E.S., 1967. Pompano: Biology fisheries and farming potential. Proc. Gulf Carib. Fish.
566 Inst. 19, 116-128.

567 Borderias, A.J., Sánchez-Alonso, I., 2011. First processing steps and the quality of wild and farmed fish.
568 J. Food Sci. 76, R1-R5.

569 Bosworth, B.G., Small, B.C., Gregory, D., Kim, J., Black, S., Jerrett, A., 2007. Effects of rested-harvest
570 using the anesthetic AQUI-S^{TM} on channel catfish, *Ictalurus punctatus*, physiology and fillet quality.
571 Aquaculture 262, 302-318.

572 Cakli, S., Kilinic, B., Cadun, A., Dincer, T., Tolasa, S., 2006. Effect of ungutting on microbiological,
573 chemical and sensory properties of aquacultured sea bream (*Sparus aurata*) and sea bass
574 (*Dicentrarchus labrax*) stored in ice. Eur. Food Res. Technol. 222, 719-726.

575 Cakli, S., Kilinc, B., Dincer, T., Tolasa, S., 2006. Effects of using slurry ice during transportation on the
576 microbiological, chemical, and sensory assessment of aquacultured sea bass (*Dicentrarchus labrax*)
577 stored at 4 ^{o}C. Crit. Rev. Food Sci. Nutr. 46, 453-458.

578 Curran, C.A., Poulter, R.G., Brueton, A., Jones, N.S.D., 1986. Cold shock reactions in iced tropical fish. J.
579 Food Technol. 21, 289-299.

580 Dawood, A.A., Roy, R.N., Williams, C.S., 1986. Effect of delayed icing on the storage life of rainbow
581 trout. J. Food Technol. 21, 159 – 166.

582 Donaldson, M.R., Cooke, S.J., Patterson, D.A., MacDonald, J.S., 2008. Review paper – Cold shock and
583 fish. J. Fish Biol. 73, 1491-1530.

584 Dowlati, M., Mohtasebi, S.S., Omid, M., Razavi, S.H., M. Jamzad, M., de la Guardia, M., 2013. Freshness
585 assessment of gilthead sea bream (*Sparus aurata*) by machine vision based on gill and eye color
586 changes. J. Food Eng. 119, 277-287.

587 Erikson, U., 2001. Rigor measurements. In: Kestin, S.C., Warriss, P.D. (Eds.), Farmed Fish Quality.
588 Blackwell Science, Oxford, UK, pp. 283-297.

589 Erikson, U., 2011. Assessment of different stunning methods and recovery of farmed Atlantic salmon:
590 isoeugenol, nitrogen, and three levels of carbon dioxide. Anim. Welf. 20, 365-375.

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

593 Erikson, U., Lambooij, B., Digre, H., Reimert, H.G.M., Bondø, M., van der Vis, H., 2012. Conditions for
594 instant electrical stunning of farmed Atlantic cod after de-watering, maintenance of unconsciousness,
595 effects of stress, and fillet quality – A comparison with AQUI-S^{TM}. Aquaculture 324-325, 135-144.

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

598 Erkan, N., Özden, Ö., 2006. Gutted and un-gutted sea bass (*Dicentrarchus labrax*) stored in ice:
599 influence on fish quality and shelf life. Int. J. Food Properties 9, 331-345.

Fletcher, G.C., Corrigan, V.K., Summers, G., Leonard, M.J., Jerrett, A.R., Black, S.E. 2003. Spoilage of rested harvested king salmon (*Oncorhynchus tshawytscha*). J. Food Sci. 68, 2810-2816.

Fogaca, F.H.D.S., Junior, M.A.G., Vieira, S.G.A., Araujo, T.D.S., Farias, E.A., Ferreira-Bravo, I.A., Silva, T.F.A., Calvet, R.M., Pereira, A.L.M., Prentice-Hernández, C., 2017. Appraising shelf life of farmed cobia, *Rachycentron canadum*, by application of a Quality Index Method. J. World Aquacult. Soc. 48, 70-82.

Fujii, R., 2000. Review – the regulation of motile activity in fish chromatophores. Pigment Cell Res. 13, 300-319.

Gao, M., Feng, L., Jiang, T., Zhu, J., Fu, L., Yuan, D., Li, J., 2014. The use of rosemary extract in combination with nisin to extend the shelf life of pompano (*Tranchius ovatus*) fillets during chilled storage. Food Control 37, 1-8.

Hansen, A.Å., Mørkøre, T., Rudi, K., Langsrud, Ø., Eie, T., 2009. The combined effect of superchilling and modified atmosphere packaging using CO_2 emitter on quality during chilled storage of pre-rigor salmon fillets (*Salmo salar*). J. Sci. Food Agric 89, 1625-1633.

Huidobro, A., Pastor, A., Tejada, M., 2000. Quality index method developed for raw gilthead seabream (*Sparus aurata*). J. Food Sci. 65, 1202-1205.

Huidobro, A., Mendes, R., Nunes, M.L., 2001. Slaughtering of gilthead seabream (*Sparus aurata*) in liquid ice: influence of fish quality. Eur. Food Res. Technol. 213, 267-272.

Huss, H.H. (Ed.), 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper, No. 348, 195p. FAO, Rome, Italy.

Hylding, G., Green-Petersen, D.M.B., 2004. Quality Index Method – An objective tool for determination of sensory quality. J. Aquatic Food Prod. Technol. 13, 71-80.

621 Iger, Y., Abraham, M., Zhang, L., Stoumboudi, M., Alexis, M., Tsangaris, K., Wendelaar Bonga, S., van
622 Ham, E., 2001. Fish skin alterations as indicators of stress for stress in fresh- and seawater aquaculture.
623 Eur. Aquacult. Soc. Special Publ. No. 29, 109-110.

624 International Commission on Microbiological Specifications for Foods (ICMSF), 1986. Sampling plans
625 for fish and shellfish. In: Microorganisms in Foods, Sampling for Microbiological Analysis: Principles and
626 Specific Applications, 2nd ed., Vol. 2. University of Toronto Press, Toronto, Canada.

627 Jerrett, A.R., Stevens, J., Holland, A.J., 1996. Tensile properties of white muscle in rested and exhausted
628 chinook salmon (*Oncorhynchus tshawytscha*). J. Food Sci. 61, 527-532.

629 Kestin, S.C., van de Vis, J.W., Robb, D.H.F., 2002. Protocol for assessing brain function in fish and the
630 effectiveness of methods used to stun and kill them. Vet. Rec. 150, 302-307.

631 Kildea, M.A., Allan, G.L., Kearney, R.E., 2004. Accumulation and clearance of anaesthetics clove oil and
632 AQUI-S™ from the edible tissues of silver perch (*Bidyanus bidyanus)*. Aquaculture 232, 265-277.

633 Knowles, T.G., Brown, S.N., Warriss, P.D., Lines, J., Tinarwo, A., Bravo, A., Carvalho, H., Goncalves, A.,
634 2007. Effect of electrical stunning at slaughter on the carcass, flesh and eating quality of farmed sea
635 bass (*Dicentrarchus labrax*). Aquacult. Res. 38, 1732-1741.

636 Kolakowska, A., Zienkowicz, L., Domiszewski, Z., Bienkiewicz, G., 2006. Lipid changes and sensory
637 quality of whole- and gutted rainbow trout during ice storage. Acta Ichthyol. Pisc. 36, 39 – 47.

638 Lambooij, E., Gerritzen, M.A., Reimert, H., Burggraaf, D., van de Vis, J.W., 2008. A humane protocol for
639 electrostunning and killing of Nile tilapia in fresh water. Aquaculture 275, 88-95.

640 Lambooij, E., Grimsbø, E., van de Vis, J.W., Reimert, H.G.M., Nortvedt, R., B. Roth, B., 2010. Percussion
641 and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on
642 brain and heart activities. Aquaculture 300, 107 – 112.

643 Lazo, J.P., Allen Davis, D., Arnold, C.R., 1998. The effects of dietary protein level on growth, feed
644 efficiency and survival of juvenile Florida pompano (*Tranchinotus carolinus*). Aquaculture 169, 225-
645 232.

646 Liston, J., 1980. Microbiology in fishery science. In: Connell, J.J. (Ed.), Advances in Fish Science and
647 Technology, Fishing News Books Ltd, Farnham, England, pp. 138-157.

648 Lowe, T.E., Ryder, J.M., Carragher, J.F., Wells, R.M.G., 1993. Flesh quality in snapper, *Pagrus auratus*,
649 affected by capture stress. J. Food Sci. 58, 770-773, 796.

650 Martin, R.E., Gray, R.J.H., Pierson, M.D., 1978. Quality assessment of fresh fish and the role of the
651 naturally occurring microflora. Food Technol., May, 188-192, 198.

652 Matos, E., Silva, T.S., Tiago, T., Aureliano, M., Dinis, M.T., Dias, J., 2011. Effect of harvesting stress and
653 storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): A
654 differential scanning calorimetry study. Food Chem. 126, 270-276.

655 McMaster, M.F., Gopakumar, G., 2016. Fisheries and Aquaculture Resources Use and Conservation
656 Division Aquaculture Team (FIAA) 2016-2017. Cultured Aquatic Species Information Programme.
657 *Trachinotus carolinus*. Cultured Aquatic Species Information Programme (18 p.).
658 www.fao.org/fishery/culturedspecies/Trachinotus_spp/en

659 Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V., 2013. Effect of essential oils on
660 pathogenic bacteria. Pharmaceuticals 6, 1451-1474.

661 Papadopoulos, V., Chouliara, I., Badeka, A., Savvaidis, I.N., Kontominas, M.G., 2003. Effect of gutting
662 on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*)
663 stored in ice. Food Microbiol. 20, 411-420.

664 Pavlidis, M., Papandroulakis, N., Divanach, P., 2006. A method for the comparison of chromaticity
665 parameters in fish skin: Preliminary results for coloration pattern of red skin Sparidae. Aquaculture
666 258, 211-219.

667 Poli, B.M., Parisi, G., Scappini, F., Zampacavallo, G., 2005. Fish welfare and quality as affected by pre-
668 slaughter and slaughter management. Aquaculture Int. 13, 29-49.

669 Rodriguez, C.J., Besteiro, I., Pascual, C., 1999. Biochemical changes in freshwater rainbow trout
670 (*Oncorhynchus mykiss*) during chilled storage. J. Sci. Food Agric. 79, 1473-1480.

671 Roth, B., Birkeland, S., Oyarzun, F., 2009. Stunning, pre slaughter and filleting conditions of Atlantic
672 salmon and subsequent effect on flesh quality on fresh and smoked fillets. Aquaculture 289, 350 – 356.

673 Sattari, A., Lambooij, E., Sharifi, H., Abbink, W., Reimert, H., van de Vis, J.W., 2010. Industrial dry
674 electro-stunning followed by chilling and decapitation as a slaughter method in Claresse®
675 (*Heteroclarias* sp.) and African catfish (*Clarias gariepinus*). Aquaculture 302, 100-105.

676 Scott, D.N., Fletcher, G.C., Hogg, M.G., Ryder, J.M., 1986. Comparison of whole with headed and gutted
677 orange roughy stored in ice: sensory, microbiology and chemical assessment. J. Food Sci. 51, 79-83, 86.

678 Seafish, 2010. Sensory assessment scoresheets for fish and shellfish – Torry & QIM, 58 p., SeaFish, UK.

679 Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T.S., Seland, A., 1997. Handling stress and
680 storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). J. Food Sci.
681 62, 898-905.

682 Tejada, M., Huidobro, A., 2002. Quality of farmed gilthead seabream (*Sparus aurata*) during ice storage
683 related to slaughter method and gutting. Eur. Food Res. Technol. 215, 1-7.

684 Townley, R.R., Lanier, T.C., 1981. Effect of early evisceration on the keeping quality of Atlantic croaker
685 (*Micropogon undulatus*) and grey trout (*Cynoscion regalis*) as determined by subjective and objective
686 methodology. J. Food Sci. 46, 863-867.

687 Tsuchimoto, M., Misima, T., Utsugi, T., Kitajima, S., Yada, S., Senta, T., Yasuda, M., 1986. The speed of
688 lowering in freshness of fishes in several waters and the effect of the habitat temperature on the
689 speed. Bull. Jap. Soc. Sci. Fish. 52, 1431-1441.

690 Tufts, B.L., Tang, Y., Tufts, K., Boutilier, R.G., 1991. Exhaustive exercise in 'wild' Atlantic salmon (*Salmo*
691 *salar*): acid-base regulation and blood gas transport. Can. J. Fish. Aquat. Sci. 54, 868-874.

692 Tutman, P., Glavic, N., Kozul, V., Skaramuca, B., Glamuzina, B., 2004. Preliminary information and
693 growth of pompano, *Trichinotus ovatus* (Linnaeus 1758) (Pisces; Carangidae) in captivity. Aquaculture
694 Int. 12, 387-393.

695

696

697

698

699

700

701

702

703

704

705

706

707

708

709

710

711

712

TABLES

Table 1

White muscle pH, core temperature and development of rigor mortis during ice storage of shortfin pompano. On Day 0, initial pH values reflected anaerobic, excessive swimming during harvesting.

Group	Day						
	0	1	3	7	10	14	18
				Muscle pH			
AQUI-S™	6.55 ± 0.05[a]	6.11 ± 0.03[a]	6.15 ± 0.04[a]	6.53 ± 0.04[a]	6.42 ± 0.04[a]	6.51 ± 0.03[a]	6.54 ± 0.05[a]
Gutted	NA	6.26 ± 0.06[b]	6.23 ± 0.06[a]	6.37 ± 0.03[a]	6.44 ± 0.06[a]	6.52 ± 0.02[a]	6.60 ± 0.02[a]
Ungutted	6.55 ± 0.03[a]	6.30 ± 0.04[b]	6.26 ± 0.04[a]	6.55 ± 0.03[a]	6.51± 0.04[a]	6.52 ± 0.06[a]	6.65 ± 0.02[a]
				Core temperature (°C)			
AQUI-S™	29.2 ± 0.1[a]	4.0 ± 1.4[a]	1.9 ± 0.3[a]	1.2 ± 0.2[a]	2.4 ± 0.4[a]	4.0 ± 0.8[a]	1.2 ± 0.1[a]
Gutted	NA	1.4 ± 0.2[a]	2.3 ± 0.3[a]	1.4 ± 0.3[a]	2.8 ± 0.3[a]	2.0 ± 0.4[b]	1.2 ± 0.2[a]
Ungutted	9.7 ± 0.8[b]	1.6 ± 0.2[a]	1.9 ± 0.2[a]	2.0 ± 0.3[a]	2.1 ± 0.3[a]	1.3 ± 0.2[b]	1.6 ± 0.0[a]
				Rigor status (0 – 5)			
AQUI-S™	rigor*	3.9 ± 0.3[a]	2.1 ± 0.5[a]	1.0 ± 0.2[a]	0	0	0
Gutted	rigor*	3.3 ± 0.3[a]	2.9 ± 0.2[a]	0.9 ± 0.1[a]	0	0	0
Ungutted	rigor*	3.7 ± 0.1[a]	1.6 ± 0.6[a]	0.8 ± 0.1[a]	0	0	0

*Mean values ± SEM (n = 10 on Day 0, otherwise n = 6); *stiffness occurred shortly after killing (Rigor Status was not determined on Day 0). Different letter, a or b, means significant differences among treatments (P<0.05).*

730 **Table 2**

731 Comparison of skin and eye color characteristics of shortfin pompano shortly after harvesting (Day 0). The AQUI-
732 S™ fish were euthanised by an anesthetic overdose whereas the ungutted fish were killed by exposure to ice-
733 slurry.

Group	L*	a*	b*	H° (°)	C*
			Dorsal anterior		
AQUI-S™	62.0 ± 2.9[a]	1.7 ± 0.3[a]	3.5 ± 0.7[a]	1.0 ± 0.1[a]	4.1 ± 0.6[a]
Ungutted	61.4 ± 3.9[a]	0.2 ± 0.5[b]	-0.6 ± 0.4[b]	4.4 ± 0.7[b]	1.6 ± 0.4[b]
			Dorsal posterior		
AQUI-S™	77.4 ± 1.2[a]	-0.1 ± 0.3[a]	6.9 ± 0.7[a]	1.6 ± 0.1[a]	6.8 ± 0.6[a]
Ungutted	70.6 ± 1.3[b]	0.0 ± 0.2[a]	0.5 ± 0.6[b]	3.2 ± 0.8[b]	1.8 ± 0.3[b]
			Belly		
AQUI-S™	92.5 ± 0.9[a]	-0.9 ± 0.2[a]	6.1 ± 0.4[a]	1.7 ± 0.0[a]	6.2 ± 0.4[a]
Ungutted	91.7 ± 0.5[a]	-0.7 ± 0.1[a]	1.5 ± 0.3[b]	2.1 ± 0.1[a]	1.7 ± 0.2[b]
			Eyes		
AQUI-S™	44.2 ± 3.2[a]	1.1 ± 0.5[a]	4.2 ± 0.9[a]	1.4 ± 0.3[a]	4.8 ± 0.8[a]
Ungutted	27.1 ± 0.8[b]	4.3 ± 0.2[b]	-2.3 ± 0.4[b]	6.8 ± 0.1[b]	5.0 ± 0.3[a]

734 *Mean ± SEM (n = 10). Different letter, a or b, indicates differences between treatments (P<0.05).*

735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750

Table 3

Skin and eye color of shortfin pompano after ice storage for one day post mortem.

Group	L*	a*	b*	H^o (^{o})	C*
			Dorsal anterior		
AQUI-S™	59.9 ± 4.1[a]	- 0.5 ± 0.7[a]	-2.0 ± 0.8[a]	4.3 ± 0.6[a]	2.8 ± 0.6[a]
Gutted	64.3 ± 1.0[a]	- 1.1 ± 0.4[a]	-4.6 ± 0.5[b]	4.5 ± 0.1[a]	4.7 ± 0.5[b]
Ungutted	64.5 ± 1.6[a]	- 1.2 ± 0.2[a]	-5.7± 0.5[b]	4.5 ± 0.0[a]	5.9 ± 0.5[b]
			Dorsal posterior		
AQUI-S™	68.7 ± 1.6[a]	-1.6 ± 0.2[a]	-1.9 ± 0.4[a]	4.0 ± 0.1[a]	2.5 ± 0.4[a]
Gutted	70.2 ± 0.6[a]	-1.4 ± 0.3[a]	-4.2 ± 0.4[b]	4.4 ± 0.1[b]	4.4 ± 0.4[b]
Ungutted	70.4 ± 1.3[a]	-1.2 ± 0.2[a]	-3.8 ± 0.6[b]	4.4 ± 0.1[b]	4.0 ± 0.5[ab]
			Belly		
AQUI-S™	91.4 ± 0.7[a]	-1.4 ± 0.2[a]	1.3 ± 0.4[a]	2.5 ± 0.1[a]	1.9 ± 0.3[a]
Gutted	92.0 ± 0.7[a]	-0.5 ± 0.1[b]	-1.7 ± 1.1[b]	4.2 ± 0.3[b]	1.8 ± 0.4[a]
Ungutted	92.7 ± 0.9[a]	-0.7 ± 0.0[ab]	-0.8 ± 0.2[b]	3.9 ± 0.2[ab]	1.1 ± 0.2[a]
			Eyes		
AQUI-S™	32.8 ± 1.8[a]	3.0 ± 0.1[a]	-6.0 ± 1.2[a]	7.3 ± 0.1[a]	6.9 ± 1.0[a]
Gutted	33.3 ± 0.9[a]	2.7 ± 0.1[a]	-7.8 ± 0.6[a]	7.5 ± 0.0[a]	8.3 ± 0.5[a]
Ungutted	34.5 ± 1.8[a]	2.8 ± 0.2[a]	-7.5 ± 1.2[a]	7.4 ± 0.1[a]	8.1 ± 1.1[a]

Mean ± SEM (n = 6). Different letter, a or b, indicates differences between treatments (P<0.05).

Table 4

Skin and eye color of shortfin pompano after ice storage for 18 days.

Group	L*	a*	b*	H^o (^{o})	C*
			Dorsal anterior		
AQUI-S™	59.6 ± 1.4[a]	0.4 ± 0.2[a]	-3.4 ± 0.6[a]	7.1 ± 0.5[a]	3.5 ± 0.5[a]
Gutted	61.4 ± 2.0[a]	- 0.5 ± 0.6[a]	-5.9 ± 0.3[b]	6.1 ± 0.7[a]	6.0 ± 0.3[b]
Ungutted	63.2 ± 1.2[a]	0.1 ± 0.2[a]	-3.7 ± 0.3[a]	6.1 ± 0.7[a]	3.8 ± 0.3[a]
			Dorsal posterior		
AQUI-S™	67.3 ± 2.1[a]	0.0 ± 0.2[a]	-3.2 ± 0.7[a]	6.6 ± 0.7[a]	3.3 ± 0.7[a]
Gutted	64.8 ± 1.8[a]	-0.2 ± 0.3[a]	-4.9 ± 0.5[a]	5.6 ± 0.6[a]	4.9 ± 0.8[a]
Ungutted	65.9 ± 2.6[a]	-0.5 ± 0.4[a]	-4.9 ± 0.8[a]	5.6 ± 0.7[a]	5.0 ± 0.5[a]
			Belly		
AQUI-S™	92.5 ± 0.3[a]	0.1 ± 0.1[a]	0.5 ± 0.4[a]	2.6 ± 1.0[a]	0.9 ± 0.2[a]
Gutted	92.0 ± 0.4[a]	0.0 ± 0.1[a]	0.3 ± 0.6[a]	4.1 ± 1.2[a]	1.3 ± 0.2[a]
Ungutted	91.4 ± 0.6[a]	0.3 ± 0.1[a]	-0.5 ± 0.2[a]	6.4 ± 0.9[a]	0.7 ± 0.2[a]
			Eyes		
AQUI-S™	42.8 ± 2.1[a]	9.5 ± 1.5[a]	1.1 ± 1.1[a]	3.4 ± 1.4[a]	9.8 ± 1.6[a]
Gutted	43.9 ± 1.5[a]	4.7 ± 2.2[a]	-1.1 ± 1.3[a]	4.8 ± 1.4[a]	5.8 ± 0.8[a]
Ungutted	43.1 ± 1.5[a]	5.8 ± 1.5[a]	-1.6 ± 0.8[a]	5.7 ± 1.1[a]	6.5 ± 1.1[a]

Mean ± SEM (n = 6). Different letter, a or b, indicates differences between treatments (P<0.05).

782 FIGURE CAPTIONS

783

784 **Fig. 1.** Skin and eye color were measured at the four indicated locations during ice storage. A shortfin pompano
785 (*Trachinotus falcatus*) from the present study is shown as assessed shortly after harvesting (Day 0). Color was
786 determined in the CIE L*a*b* color space using the Minolta Chroma Meter where the diameter of the probe was
787 8 mm.

788

789 **Fig. 2.** Total bacterial counts (TBC) in the flesh of shortfin pompano during ice storage. The effects of (i) exposure
790 to AQUI-S^{TM} during harvest and of (ii) gutting on TBC are both demonstrated. The AQUI-S^{TM} fish were not gutted.
791 Different letters (a, b or c) mean significant differences among treatments on each storage day ($P<0.05$).
792 Significant differences within each treatment, due to storage time only, are expressed as differences ($P<0.05$)
793 compared to the preceding day of storage: x, y and z denote changes related to AQUI-S^{TM}, gutted, and ungutted
794 fish, respectively. NS – no significant differences among treatments.

795

796 **Fig. 3.** Development of skin, eye and gill demerit scores during ice storage for each quality attribute of whole
797 shortfin pompano. Pooled data from ungutted and AQUI-S^{TM} (ungutted) fish are shown (mean values ± SEM, n =
798 12).

799

800 **Fig. 4.** Quality Index of shortfin pompano during ice storage (linear regressions). The maximum attainable QI
801 score was 18 (AQUI-S^{TM} and ungutted fish). Traditional gutting of pompano in Vietnam involves removal of gills.
802 Hence, the maximum attainable QI score of gutted fish is 11. Mean values ± SEM (n=6). An asterisk (*) indicates
803 storage days where AQUI-S^{TM} and ungutted fish were different ($P<0.05$).

804

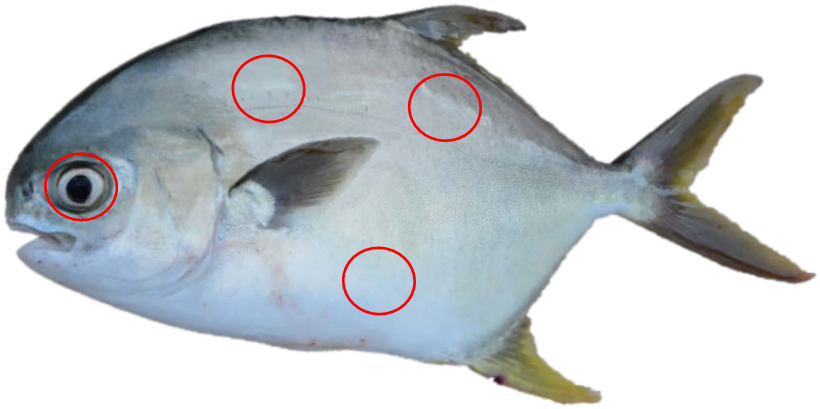
805

806

807

808 FIGURES

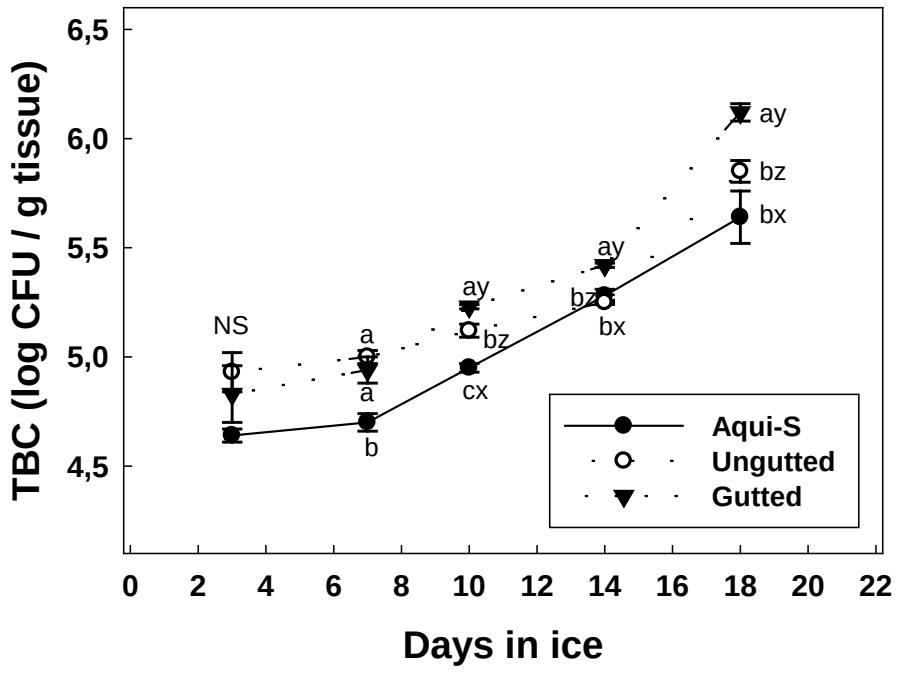
809



810

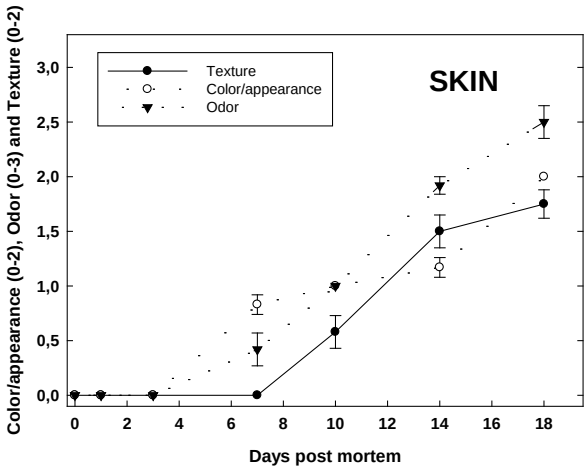
811 *Fig. 1*

812

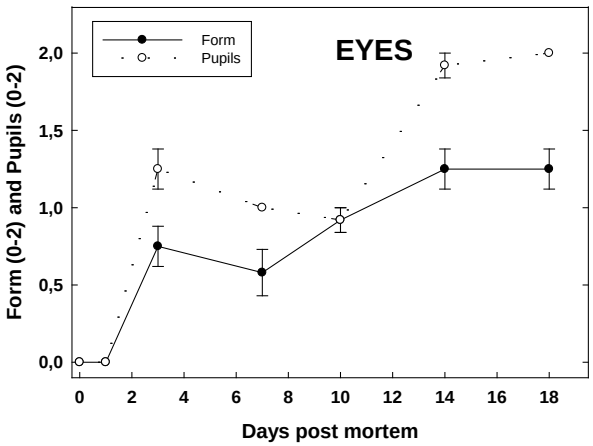


813

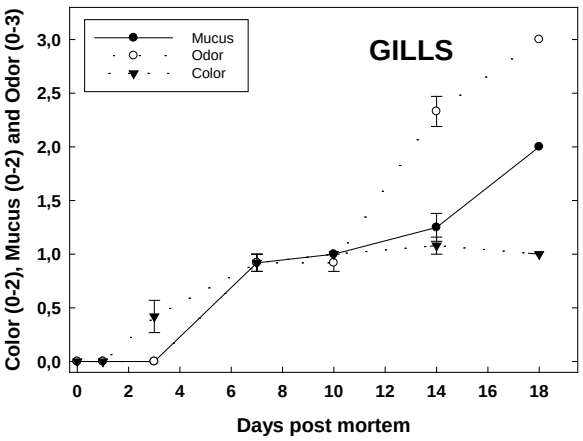
814 *Fig. 2*



815



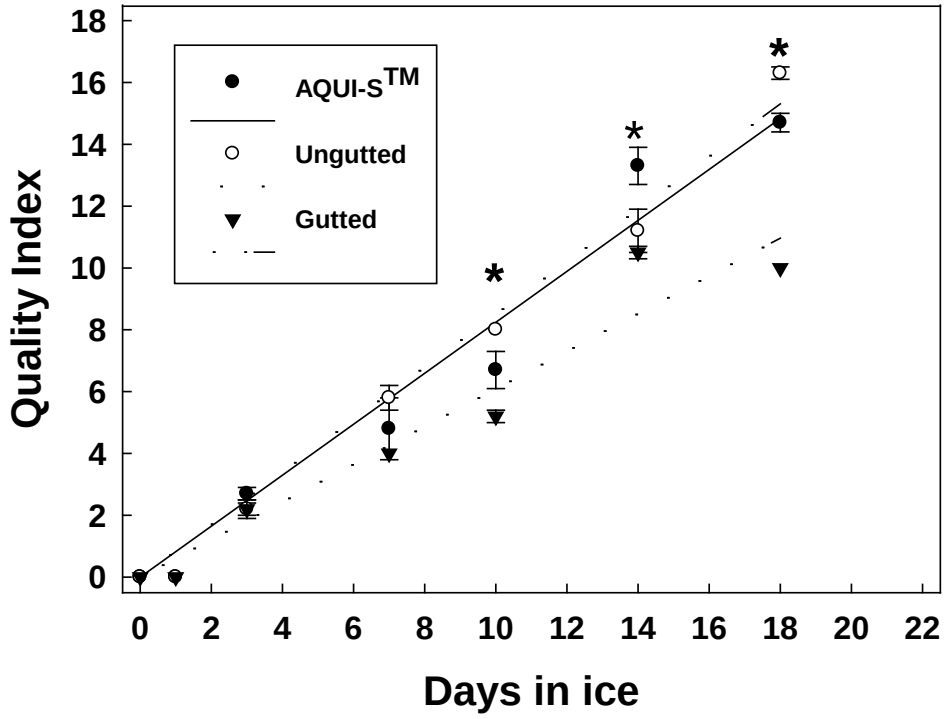
816



817

818 *Fig. 3*

819



820

821 *Fig. 4*

822

823

824

825