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# Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The aim of this study was to investigate the effects of pre-slaughter procedures on stress responses and flesh quality in sea-farmed rainbow trout (*Oncorhynchus mykiss*). A total of 114 fish were slaughtered 1) at the farm, 2) after transport, 3) after 4 days in the holding cage and then 30–50 min crowding, 4) after pumping, 5) after pumping and electrical stunning, 6) after 4 h crowding and 7) after 4 h crowding and pumping. Blood samples were collected from the fish at consecutive pre-slaughter stages. The time course of *rigor mortis* was evaluated for 72 h post mortem. Fillet cuts were evaluated for such quality parameters as gaping and weight loss after ice or frozen storage. The results show that, in most cases, blood cortisol, lactate and $pCO_2$ levels increased while blood pH and $pO_2$ decreased according to the number of events added to the slaughter process. A significant increase in hematocrit and blood glucose after transportation was observed. Both crowding and pumping led to acceleration in onset of *rigor mortis*, whereas transportation and electrical stunning did not show any significant effect on rigor development. The severity of gaping and the degree of weight loss were not significantly affected by fish pumping and electrical stunning. We conclude that pre-slaughter procedures such as crowding and pumping cause stress and affect flesh quality accelerating the onset of rigor mortis in sea-farmed rainbow trout.



## 1. Introduction

The pre-slaughter procedures for salmonids in Norway generally include fasting and transport by wellboat followed by transfer to the processing line or holding cage located near the slaughter house. The transport itself consists of a sequence of different events including crowding in the production cage, onloading to the wellboat, shipment and offloading. Vacuum pumping is the most common way to move fish through a pipe during onloading, offloading and transfer to a stunning table at the slaughter house. In order to facilitate transfer by pumping, fish are normally crowded prior to pumping, which may last from a few minutes up to several hours. At the end of the harvest process fish are usually subjected to stunning and phlebotomization. The most common stunning methods for salmonids are percussive, electrical or carbon dioxide ($CO_2$) stunning (Azam et al., 1989; Duran et al., 2008; Robb et al., 2000; Roth et al., 2002). After stunning the application of an additional killing method such as exsanguination is necessary, to prevent recovery of consciousness and avoid deterioration in quality (Robb, 2001).

Present pre-slaughter procedures might have adverse effect on welfare and flesh quality in salmonids (Barton, 2000; Barton and Peter, 1982; EFSA, 2009; Iversen et al., 1998; Iversen et al., 2005; Nomura et al., 2009; Sandodden et al., 2001). Crowding in the cage and pumping are stressful operations which affect welfare and quality in Atlantic salmon (*Salmo salar*) (EFSA, 2009; Gatica et al., 2008; Skjervold et al., 1999; Skjervold et al., 2001a). However, shipment by wellboat followed by resting in a holding cage is known to have an important function in recovery from loading stress for salmon smolts (Iversen et al., 1998; Iversen et al., 2005) and adults (Farrell, 2006; Tang et al., 2009). Furthermore, no dramatic effect of transport on the flesh quality of Atlantic salmon was observed (Erikson, 2000; Erikson et al., 1997). A similar stress recovery was demonstrated on rainbow trout (*Oncorhynchus mykiss*) subjected to loading with hand nets and truck transportation (Barton, 2000). Among the stunning methods $CO_2$ stunning was shown to be a severe stressor for salmonids (Robb, 2001) as compared with less stressful electrical and percussive stunning (Roth et al., 2002). Electrical stunning, in particular combining AC with DC currents (AC + DC), has been shown to reduce incidence of fish carcass damages (Roth et al., 2009).

Packing, evisceration and handling the fish in-rigor can cause damage to the flesh and hence be destructive for fillet quality (Robb, 2001). In accordance to that in-rigor fillets were found to have higher gaping score compared to the pre-rigor group (Skjervold et al., 2001b). Therefore, common practise in Norway is immediate fish bleeding and gutting after harvest followed by storage on ice while in rigor and then processing further after rigor resolution.

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Pre-slaughter procedures for sea-farmed rainbow trout are generally the same as for Atlantic salmon (EFSA, 2009). Meanwhile, because of major physiological variations in the stress response between salmonids (Barton, 2000), it is important to exercise caution when applying methods developed for one specific species to another. In a similar way, results of studies developed on farmed rainbow trout from freshwater systems are not directly applicable for sea-farmed rainbow trout, since sea-farmed trout express higher growth rates, are much larger and in much resemblance with Atlantic salmon, slaughtered in a more industrialized way.

The aim of this study was to evaluate the stress effects of different stages along a current commercial pre-slaughter line in sea-farmed rainbow trout.

## 2. Materials and methods

### 2.1. Sampling

In October 2007 a total of 114 farmed rainbow trout (*O. mykiss*) average (SD) weight of 4.3 (0.94) kg were commercially slaughtered at Lerøy Fossen slaughter house or at the production site (Kvamme fish farm), both located at Osterøy, Hordaland, Norway. These fish, originating from the Ilsvåg strain, were stocked in sea-water on 25th of June 2006, farmed for 15 months in sea-cages (20 kg/m$^3$) and fasted for 13 days prior to transport. Before the slaughter, the fish were crowded (200–300 kg/m$^3$) and pumped onboard the wellboat (Movi Star, Viking Shipbrokers AS ®), the water quality was monitored continuously. The transportation between the farm and the slaughter house took approximately 15 min, and loading and offloading took around 2 h and 1 h respectively. The fish were then kept in a holding cage for 4 days (19 kg/m$^3$) prior to crowding (200–300 kg/m$^3$) and pumping by a single pump (Ryco Equipment Inc ®) into the slaughter house. The fish were pumped through a 20 m pipe, exiting 6.5 m above sea level onto a conveyer belt and transported through an electrical dry stunner (Stansas#01, Seaside AS, Stranda, Norway) using 60 $V_{rms}$, 100 Hz AC + DC for approximately 6 s.

Fish were sampled at consecutive pre-slaughter steps: 1) at the production site prior to transport (Farm), 2) after transportation process, including loading, transportation and unloading procedures (Transport), 3) after 4 days in the holding cage followed by short term (30–50 min) crowding (Crowd), 4) after short term crowding followed by pumping (Pump) and 5) after short term crowding followed by pumping and electrical stunning (El-stun). Two additional groups of fish were sampled after long term (4 h) crowding (Supercrowd) and after long term crowding followed by pumping (Superpump). All fish were killed by a percussive blow to the head. Blood was collected from the *ductus Cuvier* (v. *cardinalis communis*) in heparinised syringes. For fillet quality analysis, after cutting the gills fish were exsanguinated in ice bath for 30 min, gutted and manually filleted. Due to practical reasons the number of samples and analysis varied between groups.

### 2.2. Blood analysis

Blood samples were analyzed using i-STAT® 300 Portable Clinical Analyzer (I-stat, Abbott, Princeton, NY, USA). The analyzers were used in conjunction with EC8+ and CG4+ disposable cartridges. Blood was automatically heated to 37 °C and analyzed for pH, sodium ($Na^+$), potassium ($K^+$), hematocrit (Hct), hemoglobin (Hb), glucose (Glu), lactate (Lac), partial pressures of carbon dioxide ($pCO_2$) and oxygen ($pO_2$). The blood gases and pH values were corrected to seawater temperature ($T$), where $\Delta T = (37\ ^\circ C - T)$ (Ashwood et al., 1983; Mandelman and Skomal, 2009).

$$pO_{2corr} = pO_2 \times 10^{-0.0058\times\Delta T}$$

$$pCO_{2corr} = pCO_2 \times 10^{-0.019\times\Delta T}$$

$$pH_{corr} = pH - 0.015 \times (T - 37)$$

All $pO_2$ measurements below the sensitivity threshold of the instrument (3.5 mm Hg) were taken into analysis as 3.5 mm Hg. For cortisol measurements blood plasma was collected after 10 min of centrifugation at 5500 rpm and stored at −80°C. Cortisol was analyzed using the ELISA Cortisol EIA kit (Cayman Chemical®) as provided by manufacturer's protocol. Coefficient of variation (CV) was estimated for four replicates of each sample. When the threshold of 10% was exceeded, a replicate that was the most different from the average was discarded (Hill and Lewicki, 2007).

### 2.3. Quality evaluation

All fish sampled for rigor evaluation were individually tagged and stored ungutted in ice filled polysterene boxes (EPS). Rigor was evaluated according to Cutting's method (tail drop) at 0, 6, 16, 28, 36, 48, 60 and 72 h post mortem for fish sampled on the 1st of October and at 0, 3, 6, 12, 24, 36, 48, 60 and 72 h post mortem for fish sampled on the 5th of October. The rigor index (Ir) was calculated from Ir = [(L0 − Lt) / L0] • 100 (Bito et al., 1983); L represents the vertical drop (cm) of the tail, when half of the fish fork length is placed beyond the edge of a table. L0 is the lowest measured drop of the tail, while Lt represents measurements throughout the experiment.

Immediately after filleting, the fillets were weighted ($W_0$) and a descriptive scale from 0 to 3 was used to evaluate the state of rigor development during filleting, where 0) indicates pre-rigor fillet, 1) onset of rigor, 2) in rigor represented by a clearly stiffened fillet and 3) full rigor represented by a very stiff fillet (Erikson et al., 1999). Both fillets of each fish were wrapped separately in aluminium foil and placed into EPS. One fillet was stored on ice in a cold room (0–4°C) for 10 days, while the other fillet was placed in a freezer (−20 °C) for 3 days and then stored for 10 days in the cold room. After the storage period, fillet gaping was evaluated visually by counting numbers of slits along the epaxial muscle (Andersen et al., 1994). The weight loss (WL) was estimated after the following formula $WL = [(W_0 - W_t) / W_0] * 100$; where $W_t$ represents the fillet weight at the end of storage.

### 2.4. Statistical analysis

Statistical analysis was performed by Statistica™ version 7 (Hill and Lewicki, 2007). One-way ANOVA was performed to test for differences in blood parameters (pH, $pCO_2$, cortisol and lactate levels) among the groups sampled along the pre-slaughter line. Student's $t$ test was used to determine whether individual procedure within the pre-slaughter process had a significant effect on measured blood parameters. Kruskal–Wallis ANOVA by ranks was used to discover difference in blood $pO_2$ levels among the groups in the pre-slaughter process. Mann–Whitney $U$ test was applied to check whether particular procedure had a significant effect on blood $pO_2$. The results of the tail-drop test were evaluated with Scheffe's post hoc test. Nonparametric Kruskal–Wallis one-way ANOVA and Mann–Whitney $U$ test were applied in the evaluation of descriptive variables such as fillet rigor and gaping. Weight loss was evaluated by ANCOVA using initial fillet weight taken as a covariate. All results in text represent mean (±SEM).

## 3. Results

### 3.1. Blood parameters

There was a 22-fold increase in blood cortisol ($P<0.01$, one-way ANOVA, Fig. 1), a 15-fold in lactate ($P<0.001$, one-way ANOVA, Fig. 1),

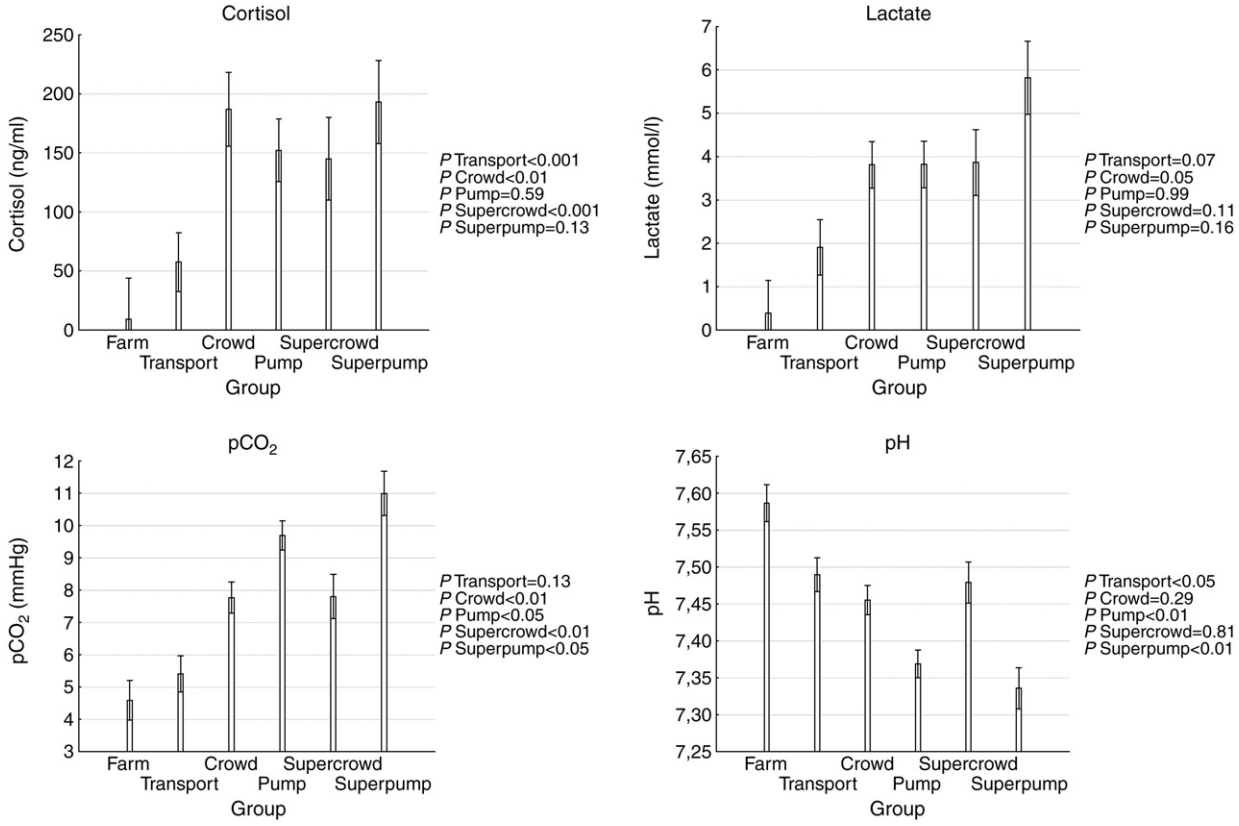


**Fig. 1.** Mean ± SE plasma cortisol, lactate, $pCO_2$ and pH levels in rainbow trout at 1) at the farm (Farm), 2) after transport (Transport), 3) after 4 days exposure to the holding cage followed by 30–50 min crowding (Crowd), 4) after pumping (Pump), 5) after 4 h crowding (Supercrowd) and 6) after 4 h crowding and pumping (Superpump) ($n = 5$–10). *P* transport, *P* Crowd, *P* pump, *P* supercrowd and *P* Superpump are the significance levels for the effects of transportation, short term crowding, short term crowding followed by pumping, long term crowding and long term crowding followed by pumping respectively.

a 2-fold in $pCO_2$ ($P<0.001$, one-way ANOVA, Fig. 1) and stepwise decrease in pH ($P<0.001$, one-way ANOVA, Fig. 1) levels across the pre-slaughter line.

Cortisol levels significantly increased from 9 (±35) ng/ml at farm to 57 (±25) ng/ml in response to transportation, 187 (±31) ng/ml in response to short term crowding and 145 (±35) ng/ml in response to long term crowding (Fig. 1). Similarly, there was a tendency of increase in lactate levels from 0.4 (±0.8) mmol/l at the farm to 1.9 (±0.6) mmol/l in response to transportation, 3.8 (±0.5) mmol/l in response to short term crowding or 3.9 (±0.8) mmol/l in response to long term crowding (Fig. 1). Cortisol and lactate values were not significantly influenced by pumping after short or long term crowding (Fig. 1).

Plasma $pCO_2$ concentration showed a tendency to increase due to transportation (Fig. 1). The levels of $pCO_2$ significantly increased from 5.4 (±0.6) mm Hg (post-transportation) to 7.8 (±0.7) mm Hg in response to short term crowding and 7.8 (±0.7) mm Hg in response to long term crowding (Fig. 1). Pumping significantly increased $pCO_2$ levels to 9.7 (±0.5) mm Hg after short term or 11 (±0.7) mm Hg after long term crowding (Fig. 1). In accordance with an increase in $pCO_2$ levels, pH levels significantly decreased from 7.6 (±0.02) at the farm to 7.5 (±0.02) in response transportation, 7.4 (±0.02) in response to pumping after short term crowding and 7.3 (±0.03) in response to pumping after long term crowding (Fig. 1). There was no significant decrease in pH values after short term crowding or long term crowding (Fig. 1). While transportation did not affect $pO_2$ levels, there was a decrease in $pO_2$ levels after short term and long term crowding (Fig. 2). Decrease in $pO_2$ levels caused by pumping resulted in 30% and 75% of measurements below the sensitivity threshold of the instrument, in case of fish pumped after short and long term crowding respectively.

The transportation led to significant increase in hematocrit and in blood glucose levels (Table 1). There was no change in blood sodium and potassium concentrations (Table 1).

### 3.2. Quality evaluation

The fish sampled at the farm had later onset of rigor in comparison with fish subjected to crowding at the holding cage (Fig. 3). There was no significant difference in rigor development in fish groups sampled before and after wellboat transportation as well as fish groups sampled before and after electrical stunning (Fig. 3). While the average peak for *rigor mortis* in fish sampled at the farm and after transportation was in 28 h, the average peak for the fish sampled after pumping was achieved in 3–6 h. Pumping shortened pre-rigor time, whereas no additional effect of electrical stunning after pumping was found (Table 2).

Pre-slaughter treatments did not affect weight loss in different groups of fillets stored on ice ($P>0.7$, ANCOVA, Table 2) and in freezer ($P>0.5$, ANCOVA, Table 2). No effects of pre-slaughter procedures on gaping were found for fillets stored on ice ($P>0.7$, Kruskal–Wallis ANOVA by ranks, Table 2) and in freezer ($P>0.9$, Kruskal–Wallis ANOVA by ranks, Table 2). Fillet gaping ($P<0.05$, Mann–Whitney *U* test) and weight loss ($P<0.0001$, ANCOVA) were more severe for fish fillets subjected to freezing and thawing in comparison to the untreated control.

## 4. Discussion

Current studies have shown the loading process to be a more severe stressor to juvenile Atlantic salmon than the transport itself (Iversen et al., 1998; Iversen et al., 2005). Though a tendency to return

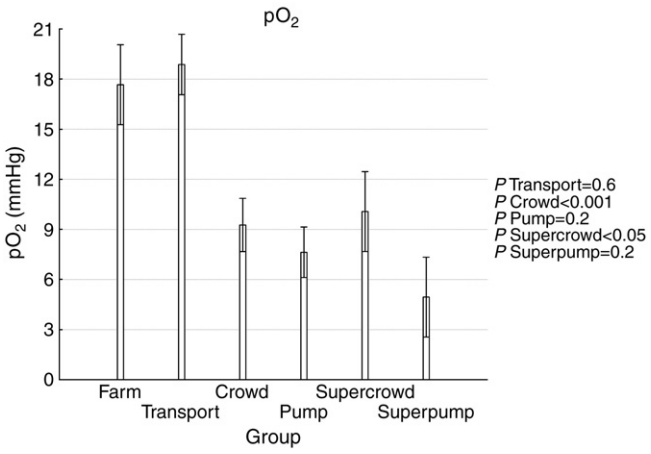


**Fig. 2.** Mean ± SE plasma $pO_2$ levels in rainbow trout at 1) at the farm (Farm), 2) after transport (Transport), 3) after 4 days exposure to the holding cage followed by 30–50 min crowding (Crowd), 4) after pumping (Pump) 5) after 4 h crowding (Supercrowd) and 6) after 4 h crowding and pumping (Superpump) ($n = 5$–10). *P* transport, *P* Crowd, *P* pump, *P* supercrowd and *P* Superpump are the significance levels for the effects of transportation, short term crowding, short term crowding followed by pumping, long term crowding and long term crowding followed by pumping respectively. The percentage of fish with $pO_2$ levels below 3.5 mm Hg (%) was 0% (Farm), 0% (Transport), 11%(Crowd), 30% (Pump), 25% (Supercrowd) and 75% (Superpump).

to pre-transport stress levels during transportation has previously been demonstrated in Atlantic salmon (Iversen et al., 2005; Nomura et al., 2009), the stress effect of the transportation process (loading, followed by transport by wellboat and unloading) was found in this study (Fig. 1, Table 1). This result was, however, expected considering that the transportation time (15 min) was not long enough for recovery from the stress effect of the loading procedure. These findings are in accordance with previous studies in Atlantic salmon, in which better recovery from loading stress was shown after longer rather than shorter periods of transport (Iversen et al., 2005).

Although a post-transportation period leads to stress recovery for rainbow trout juveniles (Barton, 2000) and Atlantic salmon (Iversen et al., 1998). In the current study, a significant increase in stress responses and decrease in $pO_2$ still occurred despite a long post-transportation period (Figs. 1 and 2). A reason for this discrepancy may lay in the stress brought by high fish density during crowding prior to sampling. The present results are therefore in line with the previous studies on Atlantic salmon where crowding during 2 h (300 kg/m$^3$) and 24 h (200–500 kg/m$^3$) led to significant increase in lactate levels (Skjervold et al., 1999; Skjervold et al., 2001a). Moreover, early rigor mortis onset after crowding in this study (Fig. 3) corresponds with previous findings describing the effect of high fish density on rigor development in Atlantic salmon (Skjervold et al., 2001a).

Counter-current swimming behaviour of salmon has been observed in experiments where a transparent tube with fish was connected to the pump (EFSA, 2009). Most salmon after being pumped to the stunning area were exhausted (EFSA, 2009; Erikson and Misimi, 2008) and demonstrated fast onset of rigor after death (Roth et al., 2009) caused by depletion of ATP. However, rainbow trout is another species than Atlantic salmon and could therefore be differently affected by pumping. Indeed blood cortisol and lactate levels were not significantly affected by pumping in the present study (Fig. 1). Meanwhile, these results may be explained by the relatively short pumping time (about 1–2 min) in accordance with the results developed on Atlantic salmon (EFSA, 2009). This time period may not be enough to change these parameters significantly. Moreover, the lack of increase in cortisol and lactate due to pumping might be interpreted in terms of reaching maximal cortisol levels during the crowding stress prior to pumping. On the other hand $pCO_2$ levels increased while $pO_2$ and pH levels decreased in response to pumping (Figs. 1 and 2). These facts together with acceleration in rigor onset in pumped fish (Table 2) demonstrate a stressful effect of pumping on rainbow trout.

It has previously been shown that rigor onset for percussively stunned fish was delayed compared with electrically stunned rainbow trout (Azam et al., 1989). Moreover, similar investigations in Atlantic salmon showed that the current duration (using 1000 Hz AC) prolonged to 12 s led to an early onset of *rigor mortis*, whereas the current duration within 6 s did not affect rigor development (Roth et al., 2002). In accordance with these results, in this study no significant difference in rigor development was found between fish that were percussively or electrically stunned (Fig. 3, Table 2).

Pre-slaughter stress and activity in fish shorten pre-rigor time, increasing the possibility of damage to the flesh during processing (Robb, 2001). In the current study, pre-slaughter procedures such as crowding and pumping affected flesh quality by an early onset of rigor mortis in rainbow trout. Consequently, fish sampled after pumping were in rigor before further processing which potentially could lead to increase in mechanical gaping during in-rigor fish handling.

Although an increase in fillet gaping and weight loss due to crowding stress has been documented in Atlantic salmon (Roth et al., 2006), not all studies come to the same conclusion (Skjervold et al., 2001a). Our study showed no significant effects of pre-slaughter procedures on fillet gaping and weight loss (Table 2). These contradictory results could be explained by the difference in pre-slaughter process between studies and hence distinction in the magnitude of stress experienced by the fish in these studies.

**Table 1**
Plasma glucose (mg/dl), sodium (mmol/l), potassium (mmol/l) and hematocrit levels (%) at the farm (Farm) ($n = 5$) and after transportation (Transport) ($n = 10$) in rainbow trout.

| Blood parameter | Farm | Transport | *P*-value (*t*-test) |
|---|---|---|---|
| Glucose | 61.4 ± 10.5 | 96.9 ± 8.3 | $P<0.05$ |
| Hematocrit | 20.7 ± 3.2 | 30.5 ± 2.3 | $P<0.05$ |
| Sodium | 139.5 ± 1.7 | 141.4 ± 1.2 | $P=0.395$ |
| Potassium | 4.1 ± 0.3 | 4.5 ± 0.3 | $P=0.34$ |

Values are means ± SEM.

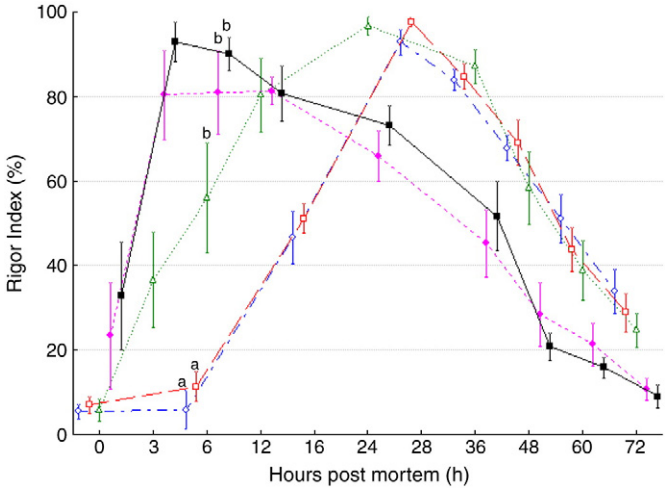


**Fig. 3.** Measurement of *rigor mortis* (tail drop test) for fish ($n = 8$–15) from Farm (▽), Transport (□), Crowd (△), Pump (◇), and El-stun (■) group. The bars and error bars denote mean and standard error of means, respectively. Different superscript letters (a, b) indicate significant differences of ($P<0.05$).

**Table 2**
Effect of pre-slaughter procedures and the storage conditions on the quality of fish fillets ($n = 5–6$) 1) after 4 days exposure to the holding cage followed by 30–50 min crowding (Crowd), 2) after pumping (Pump) and 3) after pumping and electrical stunning (El-stun). Different superscript letters a, b represent a significant difference of $P<0.05$.

| Fish group | Storage | Rigor Median | Gaping Median | Weight loss % (SEM) |
|---|---|---|---|---|
| Crowd | Freezer | $0^a$ | 2 | 1.6 (0.18) |
| Pump | Freezer | $2^b$ | 1.5 | 1.8 (0.17) |
| El-stun | Freezer | $2^b$ | 1 | 1.8 (0.18) |
| Crowd | Ice | $0^a$ | 0 | 1.1 (0.18) |
| Pump | Ice | $2^b$ | 0 | 1.2 (0.17) |
| El-stun | Ice | $2^b$ | 0 | 1.0 (0.18) |

Weight loss and gaping in fillets are influenced by the methods of storage (Einen et al., 2002). As provided by the previous studies, a significant increase in fillet weight loss and gaping after freezing-thawing as opposed to the untreated control was observed in this study.

In line with previous studies on Atlantic salmon, these results on trout demonstrated that crowding and pumping led to stress responses followed by an earlier onset of *rigor mortis* (Erikson et al., 1997; Iversen et al., 1998; Iversen et al., 2005). At the same time, an increase in stress levels along the pre-slaughter line was demonstrated, with fish exposed to the maximum number of pre-slaughter events exhibiting maximum stress levels. Therefore minimizing stress is a necessary measure for keeping fish welfare at reliable and recommendable levels during pre-slaughter procedures.

## 5. Conclusions

The results of this project showed that pre-slaughter procedures affected blood cortisol, lactate, pH, $pCO_2$ and $pO_2$ levels and rigor development in sea-farmed rainbow trout. Long term crowding of fish followed by pumping led to the highest stress levels among the pre-slaughter procedures. In this study pre-slaughter procedures affected rainbow trout flesh quality by accelerating the onset of rigor mortis.

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