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Effects of Pumping Height and Repeated Pumping in Atlantic Salmon *Salmo salar*

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Abstract

The aim of this study was to evaluate the effects of pumping height and repeated pumping on the generalized stress response and gross injuries in harvest sized Atlantic salmon. Fish pumped from a net pen at either high (5.2 m) or low (3.6 m) pumping heights showed an elevated, but not severe physiological stress response (pH, pCO_2, lactate, potassium, haematocrit, and sodium) compared to fish netted (not pumped), while effects of different pumping heights were overall not demonstrated. Repeated pumping (either 3 or 6 times) also caused an increase in stress response (pH, pCO_2, pO_2, lactate, potassium and sodium) compared to control fish, and a positive dose-response relationship was found for lactate. No fish died as a result of pumping, nor were injuries observed that could exclusively be attributed to pumping. In conclusion, although elevated from the control groups, the stress response following increasing pumping height and repeated pumping as conducted in these experiments were not indicative of causing severe stress or injuries.

Keywords

Handling, Pumping Height, Repeated Pumping, Atlantic Salmon, Stress, Welfare

1. Introduction

In the period before slaughter, salmon experience intense and repeated handling when they are crowded, pumped and transported. There is concern that this handling may affect fish welfare and product quality [1]. Previous studies have documented variable degree of stress and its subsequent effects on fish quality during the

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complete slaughter processes e.g. [2] [3], during transport [4]-[6] and during crowding [7]-[10]. Repeated handling may stress fish more than single handling events, as the fish are not allowed to recover between each event [11] [12]. Although knowledge about effects of the whole slaughter process is important, knowledge about the effects of each stage of the process is needed to define where improvement may be necessary.

Farmed Atlantic salmon are mostly moved with vacuum pumps that suck water and fish into a pipe and up to a pump chamber, before compressed air push them out from the chamber into a new pipe that leads the fish to the receiving unit. This operation may cause injuries from contact with equipment and conspecifics, in addition to being stressful for the fish. The effects of pumping isolated from other handling events are however poorly described for salmonids.

Farmers report that blood in water and injuries on fish are sometimes seen after pumping, especially after repeated pumping and with high pumping height. According to Norwegian regulations, pumping height should be regulated to avoid injuries [13]. However, hitherto no studies on salmon are available to support that larger pumping heights lead to higher occurrence, or more severe injuries.

To provide a better understanding of pumping and its effects on overall salmon stress response during slaughter, we conducted two separate experiments where the aims were to investigate effects of i) pumping heights and ii) repeated pumping.

2. Material and Methods

2.1. Effects of Pumping Height (Experiment 1)

In Experiment 1, salmon of the AquaGen strain (AquaGen AS, 7462 Trondheim, Norway) held in a seawater cage (7 × 7 × 7 m) at Averøy (mid-west part of Norway) for 12 months before experimental start (November 2010) were used. One week prior to the experiment the fish were transferred to one out of two cages (3 × 3 × 3 m) that were placed close up to each other and close to the pumping site (**Figure 1**). Water temperature during Experiment 1 was 8.0˚C ± 1.0˚C.

Sixty salmon (65 ± 3.8 cm, mean ± S.D), divided between three groups were compared. Pumping heights 5.2 m (Group 1, N = 20) and 3.6 m (Group 2, N = 20) from sea level to pump inlet were manipulated with high and low tide, while control fish (Group 3, N = 20) were not pumped, but netted directly from the cage.

A vacuum pump (Stranda Prolog; tank = 2000 litre; capacity = 500 m^3/h; pipe diameter = 305 mm) was used (**Figure 1**). Each pumping sequence involved pumping one fish at the time from the cage. With a landing net

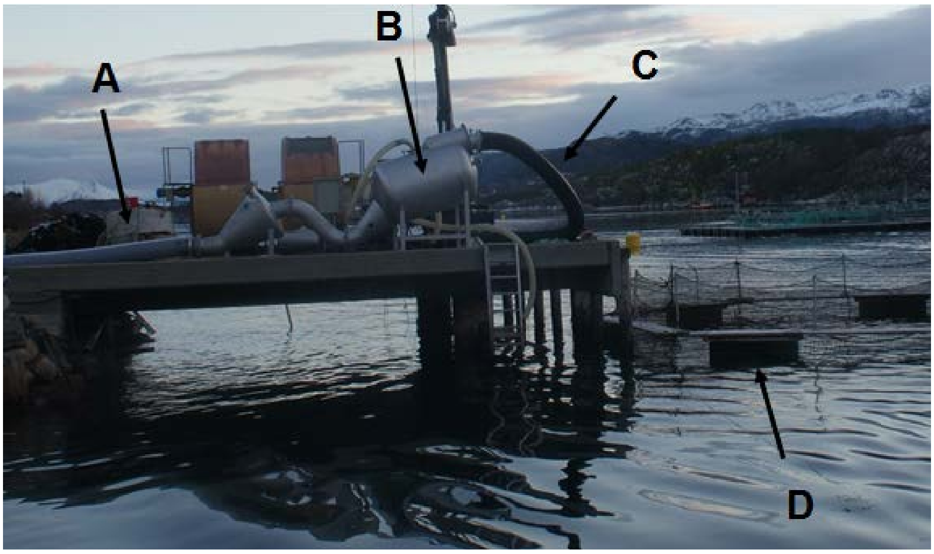

Figure 1. The vacuum pump from Stranda Prolog used in the experiment 1 and 2. Tank = 2000 liter; capacity = 500 m^3/h; pipe diameter = 305 mm. The evacuation of gas from the tank generates a vacuum that sucks water and fish through a 10 m length pipe (C) from the cage (D) into the pressure tank (B), and then by pressurization of the tank using compressed air, fish and water are pushed out through the 20 m outlet pipe (A).

single fish was carefully moved from the stock cage (containing all 60 experimental fish) to the empty neighbour cage with the suction inlet, from where it was pumped. All fish were moved in the exact same way to avoid netting effects. The control fish were moved similarly, but not pumped. One pumping sequence lasted for 25 - 30 seconds. Each fish was collected at the outlet pipe with a net immediately after pumping, visually inspected for bleedings, and killed with a sharp blow to the head dorsally using a metal priest. Immediately after stunning, each fish was sampled for blood withdrawn from the caudal vein in the tail region using heparinised vacutainers (Heco Laboratorieutstyr AS, Norway). The fish were then photographed from both sides. The pictures were analysed for pectoral and pelvic fin wounds, but were unsuited for analyses of dorsal fin. The injuries were classified as present or absent.

2.2. Effects of Repeated Pumping (Experiment 2)

In Experiment 2, the stock population was held in one cage (5 × 5 × 5 m) for 16 months before experimental start (March 2011). One week before the experiment, the fish were moved to one experimental cage (7 × 7 × 7 m) close to the pumping site (**Figure 1**). The water temperature during Experiment 2 was 3.5˚C ± 0.3˚C. Sixty Atlantic salmon (74 ± 4.4 cm) were divided into three groups of twenty fish each, being pumped either 3 (Group 1 = 3 × Pumping) or 6 (Group 2 = 6 × Pumping) consecutive times, and a control group (Group 3) netted directly from the cage (no pumping). The experimental cage was divided in two with a net; one compartment contained all experimental fish while the other was empty. Prior to pumping single experimental fish was carefully moved from the first to the second and empty compartment without being exposed to air. During pumping, this single fish was carefully directed to the pipe inlet, as in Experiment 1, and using the same pump. One pumping sequence lasted for 25 - 30 seconds. To repeat the pumping (3 or 6 times), the outlet pipe and the suction inlet were placed in the same net pen compartment, so that the fish was pumped from and pushed out to the same cage. The fish was gently redirected to the inlet pipe when it returned to the cage, a few seconds after its previous pumping. After the last pumping, the fish was inspected visually for gill bleedings, killed and blood samples obtained as in Experiment 1. The total time including pumping, killing and sampling lasted no longer than 10 minutes. To prevent bias from high and low tide (with a maximum difference of 113 cm), the treatment with the three different groups were randomised in time.

2.3. Blood Analyses

Blood partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), sodium (Na^+), potassium (K^+), glucose (Glu) and haematocrit (Hct) were analyzed from whole blood with i-STAT® portable clinical analyser (i-STAT, Abbott, Princeton, NY, USA) [14] in conjunction with CG8+ disposable cartridges at 20˚C. The blood gases were corrected for actual seawater temperature [15] [16]. Lactate was analyzed with Arkray LactatePro portable test meter (Shiga, Japan) and LactatePro Test Strips [17], also in whole blood. In Experiment 1, plasma was prepared from the remaining blood sample by immediate centrifugation at 3000 rpm for 10 min and stored at −20˚C until analyzed for cortisol with Spectria® Cortisol RIA coated tube radioimmunoassay (Cat. No. 06119) (detection limit = 2.0 ng/ml).

pH in muscle was obtained by making an incision with a scalpel at the fish left side above the lateral line where a pH electrode (Hamilton model 238400/06) and temperature probe (WTW TFK 325) were inserted and measurements recorded with a pH meter (WTW pH 330/SET-1) [2]. pH in blood was measured by opening the pericardium with a scalpel, punctuating the heart and measurements made directly with the same pH meter inserted into accumulate blood in the pericardial cavity. The instrument was frequently calibrated using pH 4.01 and pH 7.00 buffers and the electrode was cleaned with distilled water between each measurement.

2.4. Statistical Analyses

Data were analysed with StatsDirect software (version 2.7.8). Physiological stress variables were tested with one-way ANOVA analyses with subsequent multiple comparisons (Tukey). Injuries (presence/absence) data were on nominal categorical level and thus presented as frequencies and tested with Chi-square. Data are presented with means ± SD. The level of significance was set to 0.05.

3. Results

For both Experiment 1 and 2 simple regression analyses showed no significant time dependent stress responses during the time that the experiments lasted.

3.1. Effects of Pumping Height (Experiment 1)

All fish were alive and bleedings were not observed before stunning was administered. There were no significant differences between pumped fish and control fish, or between pumping height groups, regarding any injuries. The percentage of injuries in pumped ($n_{pumped\ pooled}$ = 40) vs. control (n_{total} = 20) fish were: pectoral fin damages 5% vs. 5%, split pelvic fins 45% vs. 25%.

Pumped fish had significantly lower levels of pH in blood and muscle, higher levels of pCO_2, and higher levels of lactate than control fish (**Table 1**). Potassium and Hct were higher in fish pumped at 5.2 m compared to control (**Table 1**). With the exception of sodium concentration that was higher in fish pumped at 5.2 m than in fish pumped at 3.6 m and control fish there were no significant differences in blood parameters between the different pumping height groups (**Table 1**). Cortisol levels were relatively high in all groups, including the control, and did not differ between treatments (**Table 1**).

3.2. Effects of Repeated Pumping (Experiment 2)

All fish were alive and bleedings were not observed before stunning was administered.

Lactate increased significantly with treatment load and was the only variable that differed between the two pumping groups (3× and 6× pumping, **Table 2**). pH in blood and muscle, and pO_2 were lower and potassium was higher in pumped fish compared to control fish, while sodium was only higher when pumped 6 times compared to controls (**Table 2**). pCO_2 was also higher in fish pumped 6 times compared to the control fish (**Table 2**), however results of pCO_2 for fish pumped 3 times were recorded as not valid by the i-STAT, and are therefore missing from the data set.

4. Discussion

The present paper aimed at investigating stress and injuries caused by commercially relevant pumping practice. Two separate experiments were conducted at different times of the year, hence different temperatures, and under slightly different conditions. During the interpretation of the data no attempts to compare the results between Experiment 1 and 2 will therefore be done.

The results show that pumping had effects on the physiological status of the fish as the pumping treatments pumping heights (Experiment 1), and repeated pumping (Experiment 2) caused differences between the pumped fish and the not pumped controls. This accounts for pH in blood and muscle, pCO_2, pO_2, Na^+ and K^+, Hct and lactate. Increased exercise caused by pumping leads to increased metabolism that results in increased pCO_2 and decreased pO_2 in the blood [18] [19]. Stress may cause elevated concentrations in blood erythrocytes shown as

Table 1. Physiological stress variables in Experiment 1.

	Pump height 5.2 m	Pump height 3.6 m	Control	Statistics
pH blood	7.18 ± 0.12^a	7.25 ± 0.15^a	7.36 ± 0.08^b	F = 10.86; p = 0.0001
pH muscle	6.92 ± 0.21^a	6.95 ± 0.21^a	7.11 ± 0.17^b	F = 5.84; p = 0.005
pCO_2 (mmHg)	8.40 ± 1.00^a	9.17 ± 1.13^a	6.30 ± 0.90^b	F = 12.52; p = 0.0003
pO_2 (mmHg)	10.07 ± 2.93	12.32 ± 0.57	12.97 ± 6.08	NS
Na (mmol/l)	157.42 ± 2.99^a	152.89 ± 5.08^b	151.53 ± 3.39^b	F = 11.23; p < 0.0001
K (mmol/l)	5.16 ± 0.77^a	5.04 ± 0.57^{ab}	4.55 ± 0.63^b	F = 4.28; p = 0.02
Glu (mmol/l)	5.28 ± 0.69	4.86 ± 1.15	4.67 ± 0.7	NS
Hct (%)	34.89 ± 9.74^a	33.37 ± 3.39^{ab}	28.59 ± 6.01^b	F = 4.0; p = 0.02
Cortisol (ng/ml)	170.7 ± 55.6	168.4 ± 55.2	174.3 ± 42.7	NS
Lactate (mmol/l)	4.92 ± 1.03^a	4.74 ± 2.08^a	3.08 ± 0.8^b	F = 10.2; p = 0.0002

Blood samples were taken immediately after death. Differences between groups are tested with one-way ANOVA, unequal super-script letters indicate statistical differences.

Table 2. Physiological stress variables in Experiment 2.

	6 × Pumping	3 × Pumping	Control	Statistics
pH blood	7.36 ± 0.14^a	7.44 ± 0.15^a	7.70 ± 0.11^b	F = 33.31; p < 0.0001
pH muscle	7.10 ± 0.17^a	7.15 ± 0.15^a	7.40 ± 0.18^b	F = 17.42; p < 0.0001
pCO_2 (mmHg)	32.15 ± 10.82^a	No valid data	13.90 ± 0.50^b	F = 10.21; p = 0.05
pO_2 (mmHg)	9.36 ± 3.74^a	11.33 ± 4.56^a	37.01 ± 22.26^b	F = 26.40; p < 0.0001
Na (mmol/l)	159.32 ± 3.38^a	156.70 ± 4.97^{ab}	155 ± 6.59^b	F = 3.40; p = 0.04
K (mmol/l)	5.05 ± 0.69^a	4.56 ± 0.67^a	3.62 ± 0.98^b	F = 16.50; p < 0.0001
Glu (mmol/l)	6.41 ± 1.20	6.53 ± 0.80	6.05 ± 0.79	NS
Hct (%)	29.05 ± 5.24	30.25 ± 2.86	27.60 ± 4.60	NS
Lactate (mmol/l)	4.02 ± 0.56^a	2.64 ± 0.56^b	0.82 ± 0.25^c	F = 221.73; p < 0.0001

Blood samples were taken immediately after death. Differences between groups are tested with one-way ANOVA, unequal super-script letters indicate statistical differences.

increased levels of Hct [20]. Also, changes in Na^+ and K^+ indicate osmotic disturbances caused by stress. It is suggested that osmotic disturbances is a result of stress, but of relatively short duration [21]. This is further supported in this study by the lack of changes in glucose that is a result of more long lasting aerobic muscle activity than the fish experienced in this study. On the contrary, lactate is a result of shorter and/or intense anaerobic muscle activity, and is indicative of increased activity resulting from stress [5] [8] [22]. Associated with the increased lactate in the blood, both pH in muscle and blood decreased. Both Experiment 1 and 2 lasted for more than eight hours, which could potentially have accumulated stress level before pumping due to repeated netting in the experimental cages. However, regression analyses showed no changes in stress responses with time in neither experiments, suggesting that accumulation of stress before pumping during the experiments was low.

4.1. Effects of Pumping Height (Experiment 1)

Except for Na^+ levels, we did not demonstrate differences between pumping heights for any variable, only effects of pumping vs not pumping were documented. Whole blood analyses, done with portable hand held apparatuses, such as i-STAT and LactatePro enabled stress evaluation through secondary stress variables (**Table 1**). The differences between the two pumping height groups were small, which could be because manipulating pumping heights with tide water gave small differences in heights (5.2 vs 3.6 m). The large size of the pump made it impossible to lift to increase the height differences. However, the experiment was designed to mimic commercial and relevant heights, and pumping height of 5.2 meters is considered to be high in commercial salmon farming today, as most pumps used during harvest are placed very close to the cages at sea level. Within the range used in the present study, there is no supportive evidence to the supposition from the legislation that height alone may cause severe physiological stress. However, pumping *per se* caused increased physiological stress.

4.2. Effects of Repeated Pumping (Experiment 2)

For repeated pumping (Experiment 2), the only variable that had a clear dose-response relationship was lactate (**Table 2**). The highest levels of lactate measured in this study were between 4 - 5 mmol/l, and is not high compared to stressed fish in other studies (e.g. 7, 10). Visual observations of the pumped fish showed that they did not struggle or swim intensely inside the transparent pipe, however they did struggle inside the vacuum tank and this may explain the higher levels of lactate in repeatedly pumped fish. Cortisol was not analysed in Experiment 2. Since the analyses of cortisol in Experiment 1 did not show any differences between the pumping groups, it was decided not to include cortisol in Experiment 2, but rather concentrate on secondary stress variables that are expected to change because of exhaustion.

Compared to commercial farming the fish in both Experiments 1 and 2 were handled gently as they were not crowded or otherwise handled prior to the experiments. Other studies have documented effects of repeated handling [11] [23], and recorded elevated values of cortisol, lactate, pCO_2 and lower values of pH and pO_2 in blood as the number of handling events were added up [3]. Gatica *et al.* [6] followed a commercial slaughter process and documented elevated levels of cortisol, lactate, Na^+, Cl^- and osmolality after pumping, and elevated levels of glucose after transport. As the pumped fish in these mentioned studies had experienced series of accumulated events before pumping, they did not estimate the effect of pumping *per se*. In the present Experiment 1, there were no differences in cortisol levels between the groups, but the levels among the control fish were quite high. This likely originates from some stress prior to sampling, even though effort was made not to disturb the fish more than necessary.

No significant differences in injuries between pumped and control fish were demonstrated for any of the experiments, even though all groups had some injuries. Our sample of 120 fish in total, of which 80 were subjected to pumping may be insufficient to conclude that bleedings and injuries do not happen during pumping, but still our results indicates that the proportion of fish that are injured is not high. Injuries and bleedings sometimes reported by farmers are likely results of suboptimal construction of the pump and pipes [24] (closing valves and sharp bends), and/or through collisions with constructions or other fish [25]. We only pumped single fish and thus we do not cover injuries resulting from collisions with other fish. Also, in our study, pipes and bends were optimized to avoid injuries from suboptimal construction.

5. Conclusion

In conclusion, this study shows that pumping *per se*, isolated from other harvesting procedures, causes elevations in physiological measures reflecting stress responses, even though there were no obvious differences between groups with different pumping heights or groups pumped 3 or 6 times. It is suggested that when pumping is done with caution and when there are no physical conditions in the pump that injure the fish, pumping does not harm the fish at a high degree.

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