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# Report

## Evaluation of the Whooshh Fish Transport System for transfer of Atlantic salmon broodstock between two tanks

Ulf Erikson, Guro Tveit and Marte Schei



# Report

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## Evaluation of the Whooshh Fish Transport System for transfer of Atlantic salmon broodstock between two tanks

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**ABSTRACT**

A new system for gentle transfer of fish (Whooshh Fish Transfer System, WFTS) has been evaluated at the AquaGen Atlantic salmon broodstock premises. Transfer of broodstock between tanks was carried out by WFTS and the traditional hand-carry method which includes a MS-222 anaesthesia step. Transfer of fish by WFTS was carried out by using sedated (AQUI-S) and not sedated fish. The state of the fish were evaluated in terms of stress, behaviour, welfare, possible post-transfer delayed mortalities or other irregularities for up to one week after transfer. Generally, only modest stress reactions were determined and these were mainly related to fish handling operations before transfer, particularly when not sedated fish were loaded into the WFTS. The WFTS transfer itself did not impose an additional stress load. The hand-carry method was equally good, although the method is more labour-intensive. The use of the WFTS seems to eliminate the need for the MS-222 step. No mortalities or other irregularities were observed throughout the experimental period. We were not able to identify particular issues where fish welfare was clearly compromised. For safer and less cumbersome feeding of fish into the WFTS, it is recommended that the fish are sedated before transfer.

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## 1 INTRODUCTION

Whooshh Innovations (Seattle, USA) has developed a new system for transfer of fish - the Whooshh Fish Transfer System (WFTS). During development, particular focus was put on providing a system for gentle transfer where the welfare of the fish is not compromised. WFTS can be used in a number of different applications for wild fish, or in aquaculture and fisheries sectors. The system is already in use, for example as a means to transfer Pacific salmon in rivers around various obstacles such as river dams, or for internal transport of slaughtered Atlantic salmon in a processing plant as a faster alternative to conventional conveyor belts. In the present context, the WFTS was assessed in connection with internal transfer of broodstock at the AquaGen facilities located at Kyrksæterøra in Norway. The distributor of the WFTS is Optimar AS (Valderøya, Norway).

According to regulations in Norway ("Slakteriforskrift § 12, Akvakulturforskriften § 20, Dyrevelferdsloven §§ 3, 8, 19 & IK-akva §§ 4,5"), new methods or equipment intended to be used for live fish in the aquaculture industry must be assessed to verify whether their usage comply with what can be regarded as basic welfare requirements for the fish.

As an independent research institute, SINTEF Fisheries and Aquaculture (SFA) located in Trondheim, Norway, was contacted by Optimar AS to carry out an assessment of the WFTS at the AquaGen facilities. Hence, SFA prepared an application (FOTS) to the Norwegian Food Safety Authority (NFSA) for permission to carry out such a test according to the experimental design shown in this report. Originally, the design also included a test of a system – *Fish Handling Gloves* – intended for easy manual feeding of fish into the WFTS. Due to a technical failure, the system did not perform as expected. Therefore, the planned test including the use of these gloves was excluded from the original experimental design and is therefore not reported herein. It should be emphasized that these gloves are made by another company and that they are not a part of the WFTS.

## 2 OBJECTIVES

- Provide an independent assessment of the Whoosh Fish Transfer System in terms of stress and fish welfare.
- Compare WFTS with current method at AquaGen for internal transfer of broodstock between tanks.

## 3 MATERIALS AND METHODS

### 3.1 Experimental design

The experimental design is shown in Table 1. Briefly summarized, the fish groups to be compared were:

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1. **Control** – State of the fish before transport.
2. **Hand carry** – Current method of transferring fish between tanks as used by AquaGen. The fish are sedated and then anaesthetized before they are carried one by one by hand to the target tank.
3. **WFTS** – Fish sampled immediately (0 min) after transfer by the WFTS as well as 30 min later. Both sedated and not sedated fish were fed manually into the WFTS.

Fish from all groups were transferred to an observation tank where behaviour and factors related to fish welfare were monitored for a week. After the test day, personnel at AquaGen monitored the fish in terms of behaviour, possible loss of mucus and scales and mortalities.

**Table 1** – Experimental design. Control fish (before transfer) and comparison between transfer of Atlantic salmon broodstock between two tanks by traditional method (Hand Carry) and by the Whooshh Fish Transfer System (WFTS). AQUI-S™ was used for sedation whereas MS-222 was used for anaesthetization.

Group	n	Transport length (m)	Anaesthetized before transport	Sampled immediately **/***(n)	Sampled 30 min post transport** (n)	Observed for one week* (n)
<b>WFTS (not sedated)</b>	20	31	No	5 (killed)	5	15
<b>Control (sedated)</b>	20	0	-	5 (killed) 5 (live)	0	15
<b>WFTS (sedated)</b>	20	31	No	5 (killed)	5	15
<b>Hand Carry (sedated)</b>	20	31	Yes	5 (killed)	5	15

\*Ten fish in each group were transferred without any kind of sampling (for one-week observation only). The other five fish in each group were fish that had been subjected to blood sampling 30 min post transfer; \*\*Blood sampling. The fish sampled after 30 min were anaesthetized (MS-222) in a tub before sampling of blood; \*\*\*white muscle sampling.

The rationale behind sampling procedures as shown in Table 1 was as follows:

- Ten fish from each treatment were not subjected to sampling at all. They were directly transferred to the observation tank for further observation (one week)
- Five fish from each treatment were killed immediately after sampling to assess muscle pH and blood chemistry.

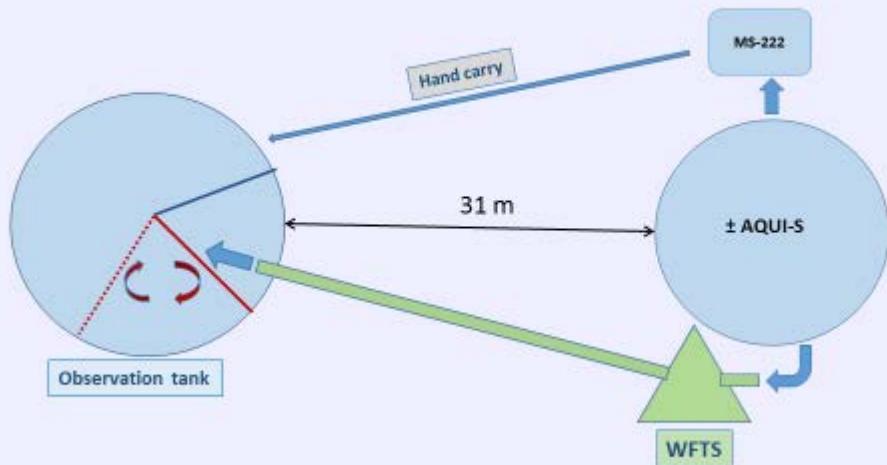
- Another five fish from each transport treatment were anaesthetized 30 min after transport to the holding tank. One fish at a time was quickly netted from the holding tank compartment and put into a tub containing MS-222 for about 3 min. After blood sampling, the fish were transferred back to the bulk volume of the holding tank.
- Thus, for each treatment, five fish were subjected to muscle measurements and ten fish were subjected to blood chemistry measurements.
- *0 min vs 30 min sampling:* Due to the very short fish transfer time when WFTS is used (few seconds), we decided to do a second sampling after 30 min. Since the response time for most of the different stress indicators are longer than a few seconds, we expected *a priori* a second blood sampling would be necessary to reveal a possible stress effect.

### 3.2 Experimental set-up

Two of AquaGen's 60 m<sup>3</sup> indoor tanks, Tank B1 ("holding tank") and Tank B5 ("observation tank") located 31 m apart, were chosen for the current test. Recirculated (max. 85 %) seawater is routinely supplied to the tanks. Outlet water treatment includes passage through heat exchanger, aerator, filter (60 µm), and UV units. Initially, 106 fish were held in Tank B1, corresponding to a fish density of approximately 14 kg/m<sup>3</sup>, whereas Tank B5 contained no fish.

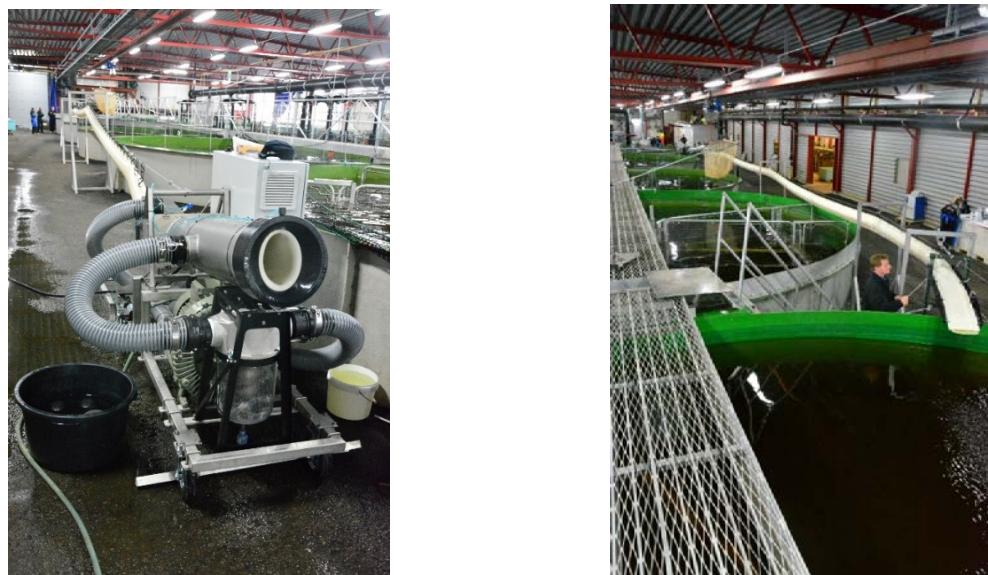
The experimental set-up is shown and explained in Figure 1. Not sedated or sedated single fish was netted and lifted out of the holding tank for feeding into the WFTS. Sedated fish were also netted to a tub containing MS-222 (Hand Carry group only). Hand-carried fish were put into the bulk volume of the observation tank. In case of WFTS, fish for immediate sampling (0 min) were caught one by one directly in a hand-hold net in front of the outlet side of the WFTS. For fish to be sampled after 30 min, all fish in the group were transferred one by one into a compartment of the observation tank in order to keep the fish separate from fish in the bulk volume. After the blood sampling carried out after 30 min was completed, the fish were put in the bulk volume of the observation tank. When all groups of fish had been transferred to the observation tank, the device for dividing the tank into two compartments was removed.

## Transfer of broodstock between two tanks

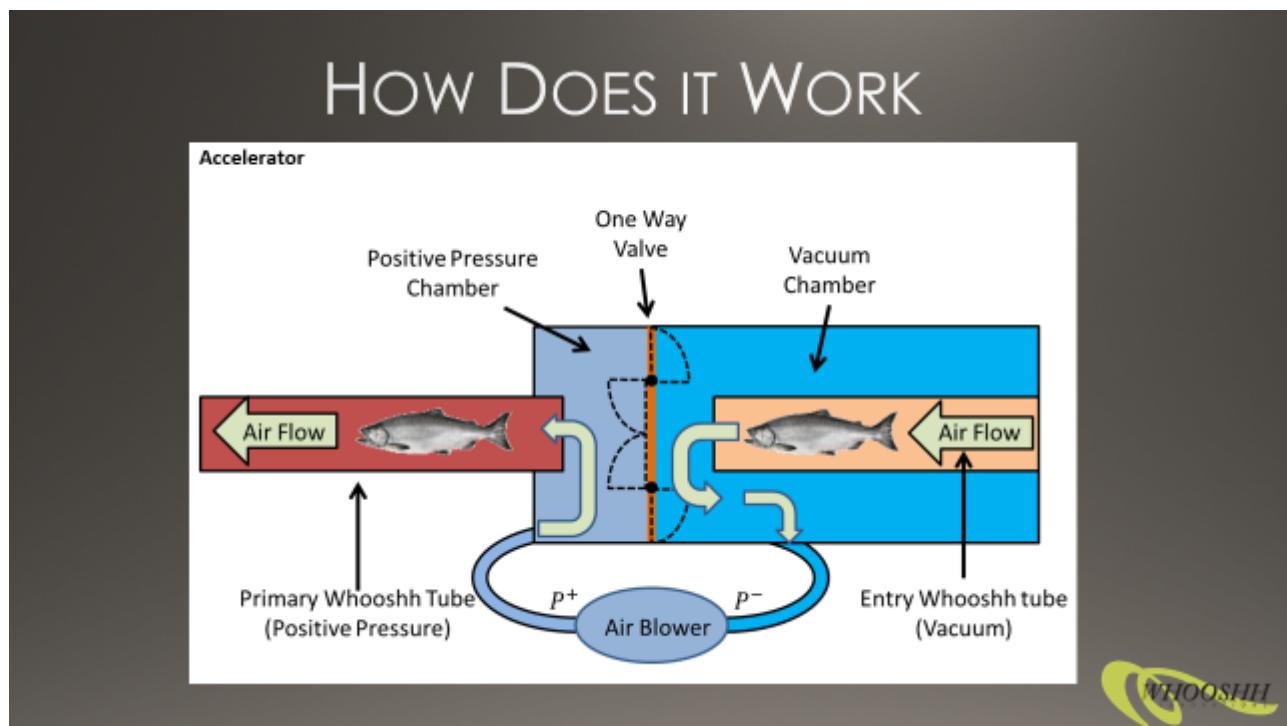


**Figure 1** – Atlantic salmon broodstock in a holding tank (right side) were transferred to another tank (observation tank, left side) by (i) AquaGen's standard procedure which involves sedation by AQUI-S™ followed by anaesthesia (MS-222 in an adjacent tub) before single fish are carried (in air) by hand to the observation tank. Alternatively, (ii) single fish was feed into the Whooshh Fish Transfer System (WFTS) showed in green colour. Both sedated and not sedated fish were transferred. During the test, a movable enclosure (in red) was used to divide the tank into two separate compartments to accommodate (a) the bulk of the transferred fish in one compartment, and in a smaller compartment, (b) fish awaiting sampling after 30 min post transfer, or (c) as a safety precaution to avoid mixing each transferred fish, intended for sampling, with the rest of the fish. The fish were kept in the observation tank until the test was terminated after one week. The blue arrows show the flow direction of the fish.

The WFTS is shown in Figure 2 and the operating principle is shown in Figure 3. One fish at a time was quickly transferred 2-3 m, from the holding tank to the WFTS, where the fish was put head first into the inlet (Figure 4) located at a height of about 1.5 m. The "speed" setting was set to position "3/4". The system is continuously wetted ("lubricated") by a small flow of water (0.5 l/min) on the inlet side. According to Whooshh Innovations, the average pressure around each fish in the system is 0.069 bar (1 psi) and the maximum pressure is 0.275 bar (4 psi).



**Figure 2**– The WFTS set-up at AquaGen's broodstock facilities. Left photo: Inlet side next to the holding tank. Right photo: WFTS outlet to the observation tank.



**Figure 3** – The working principle of the Whooshh Fish Transfer System. Source: *Whooshh Innovations*.



**Figure 4** – Feeding of Atlantic salmon broodstock into the Whooshh Fish Transfer System entry tube. Sedated and not sedated fish were transferred from the holding tank (shown in grey in the background) to the observation tank. *Source: Optimar AS.*

### 3.3 The fish

Sexually mature Atlantic salmon (*Salmo salar*) broodstock were used for the present test. Round weight, fork length and circumference (mean  $\pm$  SD) were  $8 \pm 1$  kg ( $n = 20$ ), and  $85 \pm 5$  cm ( $n = 40$ )  $47 \pm 4$  cm ( $n = 40$ ), respectively. The water temperature had been fairly constant at 7.7 - 7.8 °C (Table 2) for several weeks before the test was carried out meaning the fish were acclimated to this temperature level. Since all fish at AquaGen are routinely fitted with PIT tags, we were able to use the unique PIT tag code to identify each experimental fish.

### 3.4 Sedation and anaesthesia

As shown in Table 1, several fish were subjected to sedation (AQUI-S™) and/or anaesthesia (MS-222). For sedation, AQUI-S™ was added to holding tank to minimize stress before netting and feeding of fish into the WFTS. Since Tank B1 was part of an open system, additional amounts of AQUI-S™ were periodically added to the tank to maintain an adequate concentration throughout the test. Fish were anaesthetized in connection with the Hand Carry group, and where the fish were subjected to blood sampling after 30 min (live fish). These fish were netted into a tub containing seawater and MS-222. After 2-3 min, the fish were apparently unconscious and they then lifted out and carried to the observation tank, or to blood sampling before transfer back to the observation tank. Sedation and anaesthesia were carried out according to AquaGen's normal routines, that is, using concentrations of 0.08 L AQUI-S™ stock solution/L and 0.27 g MS-222/L.

### 3.5 Test protocol

The test was carried out on the 12 September 2016. As measured at start-up, the water quality (dissolved oxygen, carbon dioxide, pH and temperature) of Tank 1 and 5 was, according to logged data on the control system display, as shown in Table 2. The values showed in the table stayed fairly constant during the day according to sporadic readings.

**Table 2 – Basic water quality parameters in the two experimental tanks.**

Tank	Dissolved oxygen (% saturation)	Carbon dioxide* (mg/l)	pH*	Temperature (°C)
Holding tank (B1)	103	13.8	6.7	7.8
Observation tank (B5)	113	13.8	6.7	7.7

\*measured in common pipe system (i.e. mean value of all tanks)

The order of which each fish group was tested was as shown in Table 1, starting with the "WFTS not sedated" group since the other groups were studied after AQUI-S™ had been added to the tank. Fish were sampled before and after transfer to the observation tank, except from the control fish which were sampled only directly from the holding tank. Where muscle biochemistry (initial pH) was measured, the fish were killed by a sharp cranial blow within 5-10 sec after they were netted. Blood samples, for analysis of cortisol, chloride, glucose, lactate and pH, were instantly drawn from the caudal peduncle before the initial pH of the white muscle was measured directly in the muscle, between the lateral line and the dorsal fin. Body temperature was then determined in the same location. Fork length, round weight and maximum circumference (measured in front of the dorsal fin) were then recorded. To identify individual fish, each fish was scanned with the portable PIT tag transceiver whereby the PIT tag code was recorded. In cases where muscle biochemistry and temperature were not determined, the same procedure was followed except from that the fish were anaesthetized (see above) before blood sampling was carried out. These fish were subsequently transferred back to the observation tank. Fish behaviour was studied for all fish just after transfer to the observation tank as well as sporadically up to 7 days after the transfers were carried out. In addition, ten more fish from each treatment were transferred directly to the observation tank without being subjected to any kind of sampling or measurements.

### 3.6 Analytical methods

#### 3.6.1 Blood chemistry and white muscle biochemistry

*Plasma cortisol* - Blood was sampled with heparinized syringes and centrifuged (6 000 rpm, 5 min) with a Galaxy Mini Star Silverline C1413-VWR230 centrifuge (Radnor, USA) to enable extraction of blood plasma. The plasma was subsequently stored at -20 °C for later analysis of cortisol. Cortisol was determined by using a radioimmunoassay method as described by Iversen et al. (1998).

*Plasma chloride* – The chloride concentration was determined from the same extract as the one intended for analysis of cortisol. Analysis was carried out by using a Radiometer CMT 10 chloride titrator (Radiometer AS, Copenhagen, Denmark).

*pH in blood and white muscle* - Whole blood pH was measured immediately after sampling of blood. Subsequently, initial pH in muscle was measured with another, similar instrument. A shielded glass electrode (WTW SenTix 41, WTW, Weilheim, Germany) connected to a portable pH meter (model WTW 315i) was used.

*Blood glucose* - The tip of a glucose test strip was dipped briefly in whole blood immediately after the blood was sampled with the syringe. The strip was then inserted into an Ascensia Contour, meter (Bayer HealthCare LLC, Mishawaka, USA) and the glucose concentration read in mmol/l.

*Blood lactate* - Whole blood lactate was assessed with a Lactate Scout+ meter (EKF Diagnostics GmbH, Magdeburg, Germany). A test strip was briefly soaked in whole blood immediately after the withdrawal from caudal vessels. The strip was inserted into the instrument and the lactate concentration was read in mmol/l directly on the display.

*Body temperature* - Fish body temperature was measured by using a Testo 110 thermometer (Testo AG, Lenzkirch, Germany).

#### 3.6.2 Fish behaviour

Fish in the observation tank were monitored from just after transfer up to seven days post transfer. In particular, we looked for signs of swimming impairments such as loss of equilibrium, lethargy and disorientation. Furthermore, loss of mucus, descaling, as well as delayed mortalities were considered.

### 3.6.3 Statistics

To test possible differences in stress-related data, that is, between 0 and 30 min sampling of fish within each group, pairwise Student t-test were run. Since it turned out that there were no significant differences between the two sampling times (with one exception), the data were pooled and taken as mean values of each group. The different groups were then compared using a one-way ANOVA. When significance was indicated, and depending on whether the data passed Normality and Equal Variance tests, the following post-test methods were used: Holm-Sidak's Method for All Pairwise Multiple Comparisons, Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Tukey's or Dunn's method for pairwise comparisons. Significant differences were defined as P<0.05.

## 4 RESULTS AND DISCUSSION

Since the variation in stress was modest or not significant, and since no particular detrimental incidents occurred during the whole test period of one week, we did not consider it necessary to record the history of individual fish within each treatment. For simplicity, the PIT tag codes for the fish are therefore not shown in this report.

Tank-to-tank transfer times for fish carried by hand and by WFTS were recorded and the results are shown in Table 3. The corresponding WFTS transfer speed was about 5 m/s (distance 31 m). The fish exposure time to air was about five times shorter by WFTS transfer (6 sec) than by the traditional method (Hand Carry) at 29 sec.

**Table 3** – Fish transfer times from holding tank to observation tank, a distance of 31 m. Comparison between the traditional method (Hand Carry) and Whooshh Fish Transfer System (WFTS).

Hand Carry (sec)	WFTS (sec)
29 ± 2 (n = 5)	6 ± 3 (n = 9)

### 4.1 Blood chemistry

The blood chemistry of the various treatments are shown in Table 4. Blood samples were withdrawn immediately after the fish were exposed to the different treatments (0 min) as well as after 30 min. The latter sampling point was included to make sure a potential stress response was not missed as the treatment periods were very short in this study (few seconds).

Most of the significant differences in blood chemistry observed in this study were related to the stress hormone cortisol. The mean values are shown in Table 4. Significant increases of cortisol levels from 0 to 30 min were only observed for the Whooshh sedated and Hand Carry treatments. Higher cortisol levels were observed both at 0 and 30 min for fish that were not sedated. These fish struggled during transfer from tank to the WFTS entry tube. Typically, the levels of cortisol ranges from  $25 \pm 25$  nmol/l in rested salmon (Einarsdottir and Nilssen, 1996) to more than 1000 nmol/l in extreme cases. For example, during commercial crowding of Atlantic salmon for 1 – 2 h in a net-pen, the cortisol levels ranged from 608 to 736 nmol/l (Erikson et al., 2016). In the light of these data, the stress response was moderate in the present study. Notably, the cortisol levels before transport (control) and after transport to the holding tank, either by sedated fish in the WFTS, or by the traditional method (Hand Carry), were similar ( $P>0.05$ ). This indicates the main stressor in this study was related to handling of fish before transport. The differences between treatments become clearer when the data are plotted as a Box-plot where the distribution of the cortisol values within each treatment is shown (Figure 5). The main features that can be seen are that there is a general tendency for higher values after 30 min and that some fish that were not sedated exhibited high cortisol levels. It is therefore recommended that the fish are sedated or immobilized before transfer by WFTS to reduce stress and to facilitate easy fish handling.

The plasma chloride values were within the normal range that can be expected for Atlantic salmon. No significant differences were observed, both in terms of immediate versus 30 min sampling within each treatment as well as between treatments.

Similarly, no differences were observed in case of blood glucose. Since it is known that the stress response of glucose is slower than the other blood chemistry variables used here, it could theoretically be possible that a significant effect of stress was not yet seen within the 30 min time frame used here. The observed values (3 to 4 mmol/l) are within the range of unstressed fish.

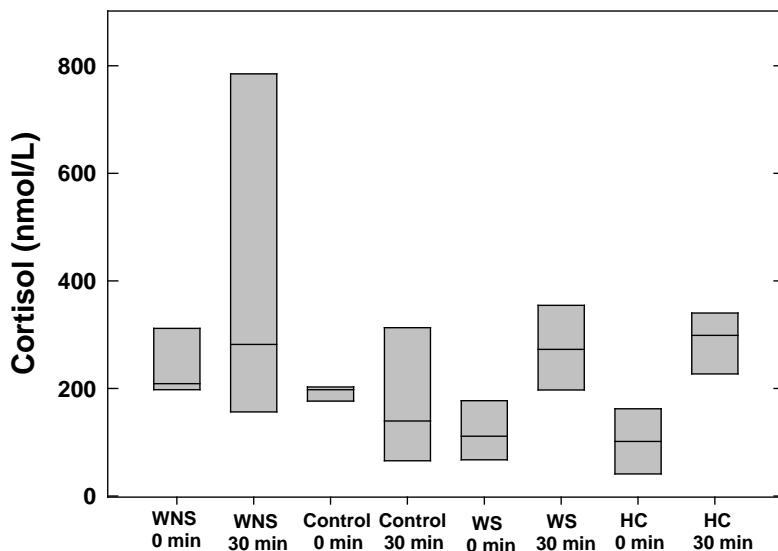
The blood lactate levels were very low, near the detection limit of the instrument used for analysis (0.5 to 0.8 mmol/l for "Control", "Whooshh sedated" and "Hand Carry" groups). Fish from the "Whooshh not sedated" group exhibited slightly higher values ( $P<0.05$ ). By comparison, it could be mentioned that typical lactate levels of rested and exhausted rainbow trout are about 0.6 and 9 – 11 mmol/l, respectively (Milligan and Wood, 1986). Since lactate originates from the white muscle, the results show that minimal (short duration) muscle activity (escape behaviour) took place. This was in line with our visual observations during the course of the study (little struggling for extended periods of time took place).

Regarding blood pH, only the fish that were not sedated before transfer by the WFTS exhibited a clear stress effect (pH 7.44). In rested salmon, the blood pH has been determined as pH 7.848. Exercise to exhaustion causes a significant acidosis and the pH is reduced to pH 7.316 (Tufts et al., 1991). Thus, a slight to moderate drop in pH was observed in this study. When fish are sedated or anaesthetized, respiration rates decrease. This may increase the carbon dioxide levels in blood causing a certain drop in pH. The larger drop in pH for the "Whooshh not sedated" group was probably caused by another mechanism, namely some degree of struggling before transfer to the observation tank.

**Table 4–** Blood chemistry in Atlantic salmon broodstock as determined after 0 and 30 min in fish from holding tank before transport (Control), after transfer to observation tank of not sedated fish by WFTS (Whooshh not sedated), after transfer of sedated fish by WFTS (Whooshh sedated), and after transfer by the traditional method (Hand Carry).

Parameter	Sampling time (min)	Control	Whooshh not sedated	Whooshh sedated	Hand Carry
Plasma cortisol (nmol/l)	0	191 ± 6 <sup>a<sub>x</sub></sup>	246 ± 28 <sup>b<sub>x</sub></sup>	120 ± 34 <sup>a<sub>x</sub></sup>	102 ± 28 <sup>a<sub>x</sub></sup>
	30	179 ± 61 <sup>a<sub>x</sub></sup>	433 ± 161 <sup>a<sub>x</sub></sup>	275 ± 46 <sup>a<sub>y</sub></sup>	287 ± 28 <sup>a<sub>y</sub></sup>
Plasma chloride (mmol/l)	0	133 ± 1 <sup>x</sup>	140 ± 10 <sup>x</sup>	131 ± 5 <sup>x</sup>	126 ± 2 <sup>x</sup>
	30	146 ± 7 <sup>x</sup>	144 ± 4 <sup>x</sup>	132 ± 1 <sup>x</sup>	131 ± 4 <sup>x</sup>
	Pooled	140 ± 4 <sup>a</sup>	142 ± 5 <sup>a</sup>	132 ± 3 <sup>a</sup>	129 ± 2 <sup>a</sup>
Whole blood glucose (mmol/l)	0	4.4 ± 0.6 <sup>x</sup>	4.1 ± 0.6 <sup>x</sup>	4.0 ± 0.4 <sup>x</sup>	3.0 ± 0.2 <sup>x</sup>
	30	3.1 ± 0.2 <sup>x</sup>	3.7 ± 0.4 <sup>x</sup>	3.7 ± 0.3 <sup>x</sup>	3.4 ± 0.5 <sup>x</sup>
	Pooled	3.8 ± 0.4 <sup>a</sup>	3.9 ± 0.3 <sup>a</sup>	3.9 ± 0.2 <sup>a</sup>	3.2 ± 0.3 <sup>a</sup>
Whole blood lactate* (mmol/l)	0	0.7 ± 0.2 <sup>x</sup>	1.4 ± 0.1 <sup>x</sup>	0.7 ± 0.1 <sup>x</sup>	0.8 ± 0.1 <sup>x</sup>
	30	0.6 ± 0.1 <sup>x</sup>	1.8 ± 0.5 <sup>x</sup>	0.8 ± 0.1 <sup>x</sup>	0.5 ± 0.1 <sup>x</sup>
	Pooled	0.7 ± 0.1 <sup>a</sup>	1.6 ± 0.3 <sup>b</sup>	0.7 ± 0.1 <sup>a</sup>	0.6 ± 0.1 <sup>a</sup>
Whole blood pH	0**	7.77 ± 0.05 <sup>x</sup>	7.44 ± 0.04 <sup>x</sup>	7.64 ± 0.08 <sup>x</sup>	7.54 ± 0.06 <sup>x</sup>
	30	7.52 ± 0.08 <sup>y</sup>	7.44 ± 0.05 <sup>x</sup>	7.56 ± 0.04 <sup>x</sup>	7.65 ± 0.06 <sup>x</sup>
	Pooled**	7.65 ± 0.06 <sup>a</sup>	7.44 ± 0.03 <sup>b</sup>	7.60 ± 0.04 <sup>ab</sup>	7.60 ± 0.04 <sup>ab</sup>

Mean values ± SEM (0 and 30 min: n = 5; pooled values: n = 10); different letter x and y: significant difference between 0 and 30 min groups within each treatment ( $P<0.05$ ); different letter a and b: significant differences between treatments for each parameter (cortisol, chloride, glucose, lactate or pH); \*In five cases, the lactate concentrations were lower than the detection limit of the instrument (<0.5 mmol/l). In these cases, the lactate concentrations were set to: (0 + 0.5)/2 = 0.25 mmol/l. \*\*The blood pH of the 0 min fish (n=5) resembled typical pH of rested fish. The outcome of the statistical analyses between treatments were identical regardless of using the 0 min group (comparison with rested fish) or the pooled values for this treatment (Control).



**Figure 5** – The cortisol stress response of Atlantic salmon broodstock as subjected to different treatments. Fish were sampled immediately after treatment (0 min) and after 30 min to ensure the expected slow post-stress increase in cortisol levels was detected. WNS: not sedated fish netted from holding tank followed by Whooshh transfer; Control: fish sedated and netted from holding tank (no transport); WS: sedated fish netted from tank followed by Whooshh transfer; HC: sedated fish netted from tank to a tub containing MS-222. Single anaesthetized (unconscious) fish were then carried by hand to the observation tank (traditional method for inter-tank transfer). The figure shows a Box Plot where the upper and lower limits of the box represent the 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively. The bar inside the box represents the median.

#### 4.2 Muscle biochemistry

The body temperature (mean  $\pm$  SD, n = 20), as measured in the white muscle was  $8.2 \pm 0.2^\circ\text{C}$ . In terms of muscle activity (escape swimming) the results from the various treatments are shown in Table 5. In live Atlantic salmon, the reference frame for pH in white muscle is pH  $7.5 \pm 0.1$  for rested fish whereas the values typical of exhausted fish are within the range of pH  $6.7 \pm 0.1$  (Erikson and Misimi, 2008). Thus, all groups, except the "Whooshh not sedated" group, fell within the "rested" category ( $P < 0.05$ ). The data also indicate that it was struggling related to transfer of not sedated fish from the holding tank to the WFTS inlet that limited the performance of that group. Not surprisingly, this observation was in line with our visual observation/experience of the rather cumbersome feeding of not sedated fish into the WFTS. The traditional method of carrying the fish by hand also yielded a good result, probably since the fish were both sedated and anaesthetized.

**Table 5 – Initial pH in Atlantic salmon white muscle as indices of muscle activity during handling.**

Muscle activity	Control	Whooshh not sedated	Whooshh sedated	Hand Carry
Initial pH	7.45 ± 0.05 <sup>a</sup>	7.09 ± 0.09 <sup>b</sup>	7.37 ± 0.09 <sup>ab</sup>	7.53 ± 0.06 <sup>a</sup>

Mean ± SEM (n=5). Different letter, a and b, means statistical differences between treatments (P<0.05).

#### 4.3 Behaviour

When not-sedated and sedated fish entered the observation tank after WFTS transfer, they quickly attained a normal upright position and swam away at a leisurely pace. The anaesthetized fish (Hand Carry and 30 min sampled fish) rested belly down on the bottom of the tank for several minutes before they slowly regained normal swimming behaviour. During the next week, no clear evidence of loss of mucus or scales was observed. Nor were there any mortalities. Thus, there were no differences between groups, nor as to whether the fish had been sampled for blood (after 30 min) or not on the first day of the test period. All fish appeared to be in a normal state throughout the one-week observation period.

#### 4.4 Welfare considerations

As described above, the stress effect of was modest in all treatments. Transport did not impose an additional stress load. Since water quality was good and no injuries or signs of abnormal behaviour or other irregularities were observed, it was difficult to identify particular issues related to the current set-up that resulted in compromised fish welfare. Thus, we conclude that fish welfare was good throughout the execution of the present study.

## 5 CONCLUSIONS AND RECOMMENDATIONS

Under the conditions studied in the present test, the main conclusions and recommendations related to fish handling practices and technology are:

- Both the traditional broodstock transfer method by hand and the WFTS performed well in terms of maintaining low stress levels and providing for good fish welfare.
- For safer and easier handling/feeding of broodstock into the WFTS, it is recommended that the fish are sedated prior to netting and handling.
- Another advantage of using sedation was that rested fish could be feed into the WFTS. No significant effects of stress were observed as a result of transfer by WFTS.
- Transfer by WFTS reduced the fish exposure time to air compared with the traditional hand carry method.
- When WFTS is used, our data suggest that the step involving anaesthesia by MS-222 can be eliminated.
- Transfer of fish by WFTS was quicker, safer (lower risk of dropping the fish) and less labour-intensive than by hand carry (traditional method).

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