Performance of Atlantic salmon following simulated thermal delousing with AQUI-S® sedation

FINAL REPORT

Dr Mark Adams

February 2019

Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49,

Hobart TAS 7001

Enquires should be directed to:

Dr Mark Adams

Institute for Marine and Antarctic Studies

University of Tasmania

Locked bag 1370, Launceston, Tasmania 7250, Australia

Mark.Adams@utas.edu.au

Ph. (03) 6324 3467

Table of Contents

OBJECTIVE **1**

NON-TECHNICAL SUMMARY **1**

KEYWORDS **2**

ACKNOWLEDGMENTS **2**

BACKGROUND & AIMS **3**

MATERIALS AND METHODS **4**

HOUSING AND HUSBANDRY: 4

EXPERIMENTAL DESIGN AND PROCEDURES 4

Design Overview & Statistical Analysis *4*

Delousing treatment simulation: *6*

Behavioural observations: *6*

Analytical procedures: *6*

Fish Performance: *7*

RESULTS & DISCUSSION **8**

BEHAVIOURAL OBSERVATIONS: 8

Effect of sedation during crowding *8*

Effect of sedation upon treatment at 15°C and 34°C *8*

Effect of sedation upon recovery *8*

PHYSIOLOGICAL RESPONSES TO TREATMENT SIMULATION: 10

Plasma cortisol *10*

Plasma lactate *11*

Plasma osmolality *12*

FISH PERFORMANCE FOLLOWING TREATMENT: 13

CONCLUSION: **17**

Performance of Atlantic salmon following simulated thermal delousing with AQUI-S® sedation

PRINCIPAL INVESTIGATOR: Dr Mark Adams

ADDRESS: IMAS - Launceston

University of Tasmania

Locked Bag 1370

Launceston Tasmania 7250

Telephone: 03 63243467 Fax: 03 63243804

Objective

Conduct a simulated experiment *in vivo* that examines the effect of sedation with AQUI-S® during crowding immediately prior to simulated thermal delousing treatment upon stress physiology, performance and behaviour of Atlantic salmon.

Non-Technical Summary

Atlantic salmon were grown to 300g in freshwater and then allocated to a 12 tank RAS (350L/tank). Fish were fed twice daily to satiation and feed intake recorded. Four groups in triplicate were randomly allocated and following habituation, acclimation and a stabilisation period (49 days) the fish in each tank were crowded for 30 mins (at 60kg/m^3) and subsequently "treated" at either 15°C or 34°C with half of the replicates at each temperature sedated with AQUI-S during crowding. Following treatment the fish were fed to satiation three times daily for two days and then twice daily for 26 days. Growth metrics (SGR, FCR, weights, K) were analysed using ANOVA . Stress parameters measured included cortisol, glucose, lactate and osmolality. Qualitative behavioural observations such as response to feed, reactivity to intrusion, schooling, position in water column, general activity and ventilation rates were made during anaesthetic induction, crowding, immersion and recovery.

This project has provided fundamental information regarding the impact of exposure of salmon to brief thermal extremes in physiological, behavioural and performance contexts. Collectively, the results from this trial suggest that AQUI-S® had a measurable benefit toward stress reduction and adverse behaviour before, during

and after a brief immersion in water at 33-34°C. Key stress parameters were lower; the fish were calmer and recovered more rapidly when fish had been sedated during the crowding stage prior to treatment. We were unable to detect any significant differences in performance indicators under the conditions tested here; however repeat treatments scheduled within a standard growth trial would be warranted. Regardless, it is likely that a welfare benefit would be achievable and further testing under commercial conditions is recommended.

Keywords

Atlantic salmon, sea lice, freshwater, thermal delicing, parasitic disease

Acknowledgments

Md. Nurul Amin and Heindrick Petero for husbandry, sampling assistance and RAS maintenance.

Background & Aims

The sea lice *Lepeophtheirus salmonis* and *Caligus rogercresseyi* are ectoparasitic copepods of salmonids in the Atlantic and Pacific oceans which are able to rapidly reproduce, colonize and feed on cutaneous mucus, epithelium and blood of cultured salmonids. Despite implementation of integrated treatment strategies Atlantic salmon producers in countries such as Norway, Scotland, Canada and Chile (>95% of world production) remain significantly impacted by lice infestations. Commercial treatments currently include a range of bath/in-feed treatments with various chemotherapeutants, however, widespread and rapid resistance to treatment (Jansen etal 2016; Helgensen etal 2017) is driving substantial effort toward the development of alternative approaches to lice mitigation. Recently, thermal de-lousing has emerged as an option to delouse salmon with the technology now commercially available (eg Steinsvik Thermolicer).

Thermal delousing relies on the inability of lice to remain attached to their host when exposed to higher temperatures whilst at the same time exploiting the salmon's tolerance to short term temperature increase (Elliot 1981). In the commercial setting fish are crowded by a sweep net and pumped through a dewatering station, briefly immersed in warm water (30-35 °C, 30-35 seconds) and returned to the cage via another dewatering station that reclaims the treatment water for reheating and filtration of lice and other particulates. This technology is used routinely under commercial conditions, however there is little published information regarding the physiological and welfare effects of briefly exposing fish to extreme water temperatures.

This project aimed to investigate whether a simulated thermal delousing event (where fish are briefly exposed to high temperature without a louse infection) will impact upon stress physiology, behaviour and performance indicators subsequent to treatment and whether any impacts can be ameliorated by sedation with AQUI-S®. The response will be measured by collecting sample for analysis of key stress hormones and metabolites as well as documenting behaviour, feed intake and growth for a period after simulated treatment.

Materials and Methods

Housing and husbandry:

Atlantic salmon (≈150g) were transferred from Huon Aquaculture's Lonnavale hatchery to the IMAS Aquaculture facility (University of Tasmania) during October 2017. The fish were maintained in 3500L Rathbun tanks within a 40000L freshwater recirculating aquaculture system and hand fed at 1 -1.5% of body weight with a commercial diet (Spectra, Skretting Australia). On February 2nd 2017 fish were then allocated (under sedation 7.5 µL/L) to a 12 tank RAS (vol. ≈ 6000L). This system was comprised of a filter sump, reservoir, submerged biofilter, heat chill unit (15kw), foam fractionator and UV disinfection. Each tank was a circular "Reln" tank (350L) fitted with de-watering feed collectors on each outlet. The initial stocking density of each tank was ≈ 18 kg/m3 for each tank (n=25, calculated average individual weight ≈ 250g).

Water quality parameters were maintained within normal water quality safety limits for Atlantic salmon during both the freshwater (temp. 15°C ± 0.5°C, DO > 85 mg/L, TA-N <2 mg/L, nitrite < 2mg/L, pH ≈7.0) and saltwater stages (temp. 15°C ± 0.5°C, DO >80 mg/L, FA-N <0.025 mg/L, nitrite < 5 mg/L, pH ≈ 8.0). Prior to seawater acclimation, RAS water was exchanged continuously at 10%/day. Seawater was subsequently batch exchanged every two to three days (≈20% of RAS volume/exchange). RAS salinity was raised incrementally over 14 days to 25 PSU by isolating and draining the reservoir and seawater (35 PSU). Fish were fed twice daily to satiation (Spirit Suprme – Skretting Australia) with uneaten feed captured on a dewatering screen which were cleared within an hour after the food ration had been supplied. Feed intake was recorded daily from four days prior to acclimation until the end of the experiment.

Experimental Design and Procedures

Design Overview & Statistical Analysis

Four groups in triplicate were randomly allocated (by RNG) across the experimental RAS. Following habituation, acclimation and a stabilisation period the fish were starved (24 h) before the first measure (weight, length, general condition) and blood samples were collected. These data provided a baseline for comparison with samples collected after simulated treatment. Post-treatment samples and measure were made after the fish had been fed for a further two days and again starved (24 h) then crowded, transferred and immersed (hereafter referred to as a treatment or

immersion) in order to simulate a de-lousing scenario either with or without sedation at either 15°C or 34°C as follows:

- Un-sedated crowding (30 min) and transfer to a 35 second bath @ 15°C
- Sedated crowding (30 min) and transfer to a 35 second bath @ 15°C
- Un-sedated crowding & transfer to a 35 second bath @ 34°C
- Sedated crowding (30 min) and transfer to a 35 second bath @ 34°C

Following treatment the fish were fed to satiation three times daily for two days and then twice daily for 26 days. Stress parameters measured included cortisol, glucose, lactate and osmolality. Individual fish weights were measured during collection of plasma concurrent with weight, length and condition observations three days before treatment and after the post treatment feeding period.

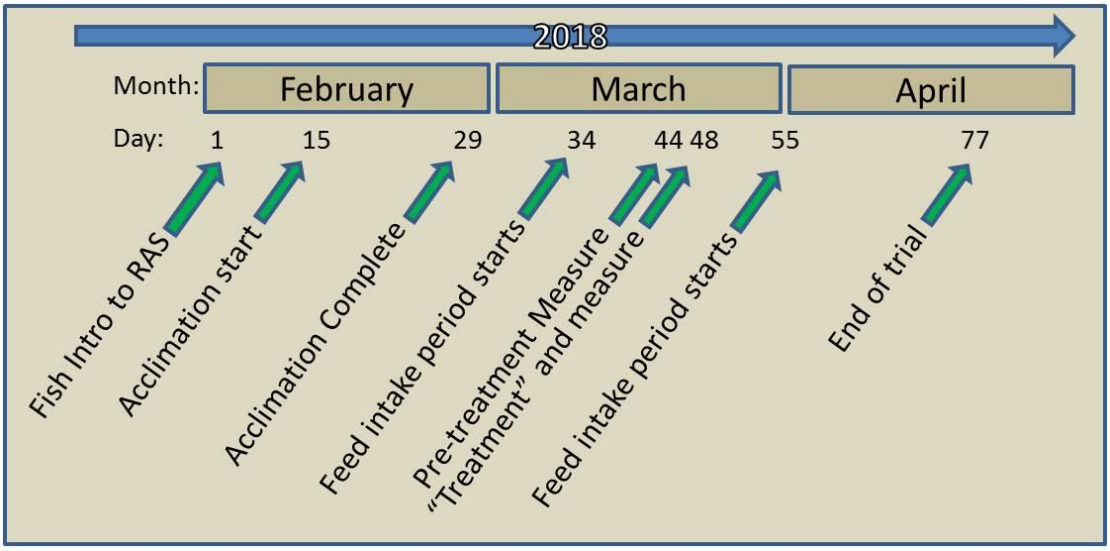

Figure 1. Experimental timeline detailing the major experimental phases and sampling points.

Data generated from measurements of cortisol, glucose, lactate, osmolality and feed intake (%bw dry feed/day) were analysed by three way ANOVA with sampling time (pre or post treatment simulation), sedation (no sedation/AQUI-S® sedation) and temperature (15°C or 34°C) as fixed factors. Growth metrics (SGR, FCR, weights) were analysed by two-way ANOVA with temperature and treatment as fixed factors. Tukey's post hoc test (HSD) was used to examine whether a significant difference ($P<0.05$) was apparent for the main effects and their interactions. All data were

analysed for normality (Shapiro-Wilk test) and homogeneity (Levene's test) and data transformed as appropriate. SPSS v22.0 software was used for all statistical analysis.

Delousing treatment simulation:

Following starvation (24 h) the fish in each tank were in turn crowded by lowering the water level to 100 L for 30 min. For half of the tanks, the previous step was preceded by the introduction of a working solution of AQUI-S® (5 min prior to crowd) to attain a nominal concentration of 4.5 mg/L. The fish were then quickly captured by dip net by two operators (<1 min) and transferred to a 100L tub fitted with a dewatering cage constructed from 25 mm PVC pipe and 6mm oyster mesh. The assembly was mounted on a flatbed trolley and the fish wheeled to an adjoining room. The dewatering cage was then lifted leaving the water behind and the fish immersed into the treatment tank (850 L) containing seawater (25ppt) at either 15 or 34°C without anaesthetic. Following immersion (35 s) the fish were returned to their tanks and their behaviour monitored intermittently. A sample of each tank's population (n = 5) were then captured, anaesthetised (80 mg/L AQUI-S®) until easily handled (1-2 min), killed using an ike jime tool, bled with a 3ml heparinised syringe and 22g needle and then measured (weight, length & condition).

Behavioural observations:

The dewatering cage was fitted with a video camera (GoPro Hero 3 or 4) both at the base of the cage (upward view) and above the cage (downward view). A separate camera was mounted on a tripod to the side of the treatment tank. Qualitative behavioural observations such as reactivity to intrusion, schooling, position in water column, general activity and ventilation rates were made during anaesthetic induction, crowding, immersion and recovery. An initial recovery feed response was tested for fish that had been crowded either with or without anaesthetic and "treated" at 34°C was undertaken 6 h after treatment. Forty pellets were introduced a few at a time and the uneaten pellets counted after recovery from the tank's outlet.

Analytical procedures:

Collected blood was immediately spun down for 7 min at 15000g, stored on ice until frozen later that day (-80 °C). Plasma cortisol was *determined by* using a commercially available enzyme-linked immuno-sorbent assay (ELISA) kit as per manufacturers instructions. This kit has been previously used for salmonids and many other fish species (ADI-900-071, Enzo, United States) (McCain etal 2015). Plasma glucose and lactate were determined spectrophotometrically using an Accutrend point of care reader as per the manufacturer's instructions (Stoot etal

2014). Plasma osmolality was determined by the use of a Vapro vapor-pressure osmometer (Model number 5520) likewise in accordance with the manufacturer's instructions.

Fish Performance:

Individual fish weight, length, condition were initially measured three days prior to treatment simulation and again 28 d following the treatment. Following distribution of daily feed rations any uneaten pellets were collected and counted. Each ration was weighed before and after feeding and individual pellet weights calculated from the weight of 1000 pellets/1000 in order to calculate uneaten feed weights and net daily feed intake. From feed intake and growth data the following parameters were calculated:

- Condition Factor K = 10^s x weight (g) / L $(mm)^3$
- Weight gain = final average weight – initial average weight
- % body weight increase = initial average weight/final average weight x 100
- Fish days = cumulative fish alive each day of experiment (fish x days)
- Feed Intake (g/fish) = total feed consumed/survival
- FCR = total feed consumed (as is) per fish day/weight gain per day
- SGR (%.d-1) = (ln final wt - ln initial wt)*100/days
- Individual average feed intake/7 days pre initial weight check and immediately post treatment (as % predicted BW/day and actual pellet intake g / tank biomass)

During individual weight check each fish was examined for any gross anomalies (eg active fin/tail erosion, lesions, contusions etc) and mortality was recorded on a daily basis.

Results & Discussion

Behavioural observations:

Effect of sedation during crowding

During crowding of un-sedated fish in each tank, the fish were clearly unsettled and swimming intermittently in short bursts with the occasional individual leaping or skipping across the water surface. Once the water level stabilised, this behaviour abated after ~10 minutes and the fish aligned to the aeration flow emanating from the centre of the tank. However during capture and transfer the fish again were highly reactive and struggled vigorously when dewatered either by dip net or when transferred to the treatment tank. In contrast, fish that were sedated appeared to continue in a calm manner generally swimming in an aimless manner with some fish aligned to aeration flows. During capture and transfer the act of dewatering did invoke some reaction although with less vigour than seen in un-sedated fish.

Effect of sedation upon treatment at 15°C and 34°C

Distinctly different behavioural patterns were noted for each treatment which were consistent across each group's corresponding replicates (figure 2). Fish immersed at 15° without prior sedation tended to stay at the base of the cage with little to no movement for the majority of the immersion period. Conversely, sedated fish immersed at the same temperature continued to behave in the manner described above during the crowding period (generally swimming in an aimless manner). At 34°C, un-sedated fish almost immediately began to seek escape from the treatment area by banging into the sides and/or broaching the surface which occurred with increasing frequency throughout the immersion period. Similar, albeit less vigorous behaviour was observed in fish sedated prior to immersion however the onset was delayed in comparison to un-sedated fish.

Effect of sedation upon recovery

Sedation had a marked effect on fish behaviour once they had been returned to the tank. Sedated fish (at both temperatures) quickly returned to normal swimming behaviour defined as the population holding station against the current with an apparently random distribution (figure 3a). Un-sedated fish at 34°C remained crowded together in groups or singularly on the bottom of each tank for approximately 30 minutes (figure 3b)(. During this time fish were noted to be venting rapidly and counts of 2-3 fish per tank indicated a rate of approximately 100 vents/min for fish un-sedated after immersion at 34°C. Typical ventilation counts for Atlantic salmon at this life stage are approximately 60 vents/min, however no direct

counts were made upon other fish sedated after 34°C or 15°C immersion. A significant difference (P = 0.005 – Students t test) was observed in feed acceptance between sedated and un-sedated fish immersed at 34°C with the sedated group consuming 50% more of the pellets offered 6 h post-treatment (figure 4).

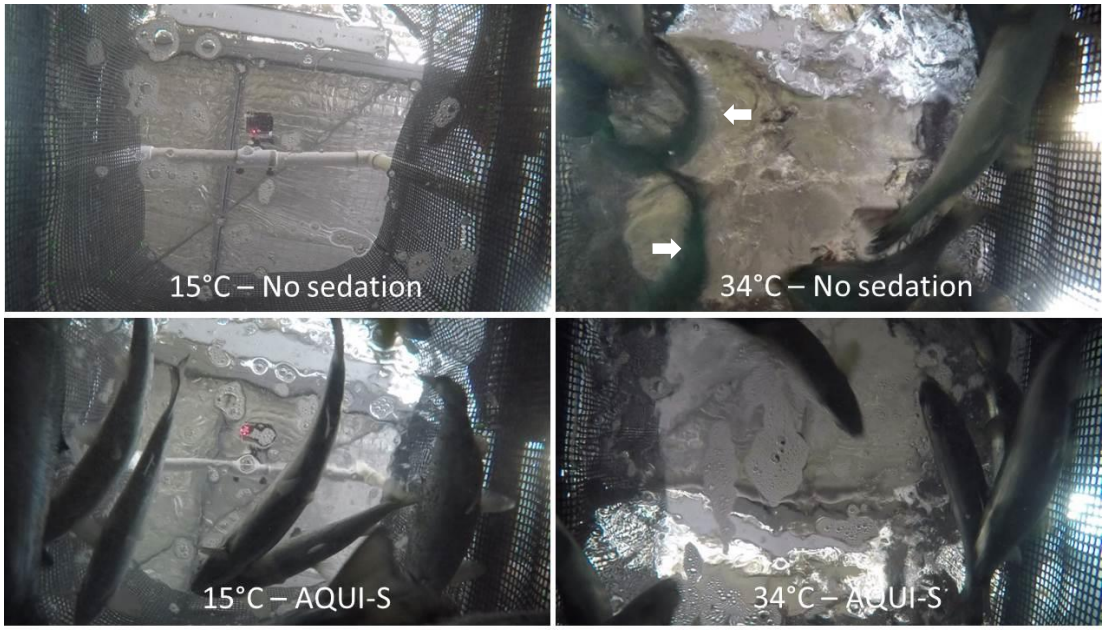

Figure 2. Screen grabs from video captured from the base of the de-watering cage during treatment simulations (30 seconds after immersion). Top right - Fish immersed at 15° without prior sedation tended to stay at the base of the cage. Sedated fish immersed at the same temperature (bottom right) were dispersed throughout the cage and appeared to be calm. At 34°C, un-sedated fish (top left) can be seen contorting markedly, the water surface is clearly agitated due to fish movement in comparison to other plates. Less vigorous activity was noted in fish sedated prior to immersion at 34°C (bottom left).

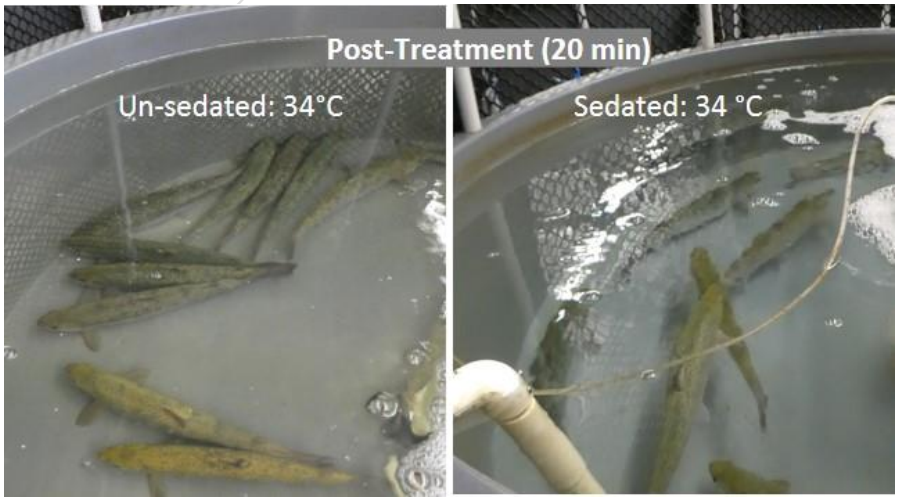

Figure 3. Fish behaviour observed following treatment (20 min) at 34°C that were either un-sedated (right) or sedated (left) during crowding. Un-sedated fish were

grouped together whilst venting rapidly at the bottom of each tank. Sedated fish (left) were swimming normally throughout the water column.

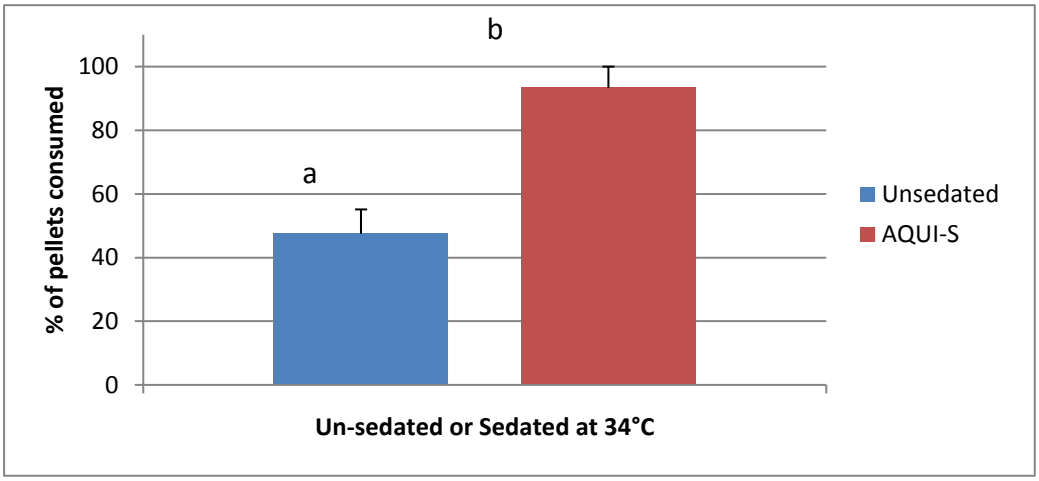

Figure 4. The average percentage of forty pellets consumed by three replicate tanks of fish (either un-sedated or sedated) prior to immersion for 35 s at 34 C approximately 6 h post treatment. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

Physiological responses to treatment simulation:

Plasma cortisol

No significant differences were found for plasma cortisol between groups of fish sampled three days prior to treatment. Following treatment, plasma cortisol was significantly elevated in fish that were un-sedated and exposed to 34°C (P=0.02) than those sedated at the same temperature (figure 5). Mean plasma cortisol values of fish un-sedated at 15°C were not significantly different to those treated at 34°C or sedated at 15°C. (P = 0.99 & 0.71 respectively).

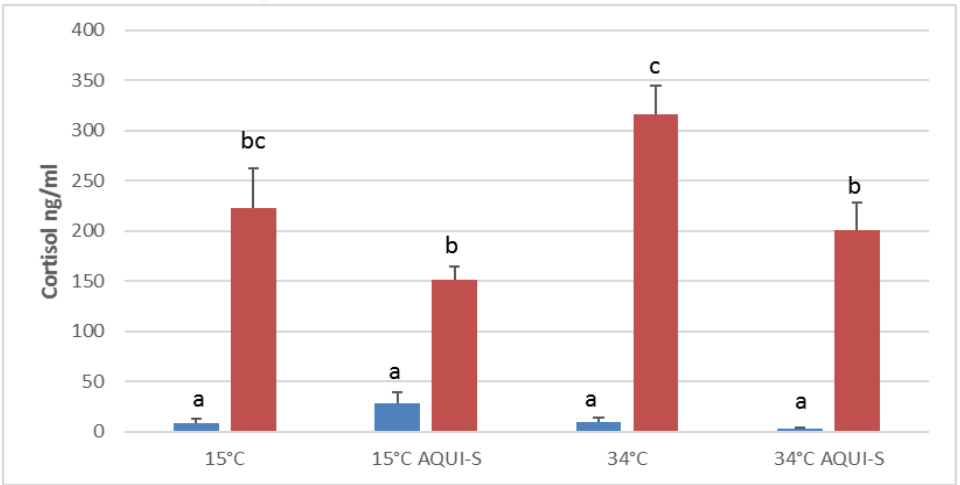

Figure 5. Plasma cortisol concentrations in fish prior to and after treatment. Fish un-sedated and immersed at 3418/02/2019C for 35 s had significantly higher levels

compared to fish treated at the same temperature and sedated with AQUI-S®. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

Baseline plasma glucose values were not significantly different between groups. Significantly higher (P=0.02) values were found post-treatment in sedated and non-sedated fish at 34°C (figure 6). Post hoc analysis indicated a significantly higher mean plasma glucose level in fish that were treated at 34°C without sedation, 40% greater than fish sedated at the same temperature. Mean plasma glucose values of fish sedated at 34°C were not significantly different to either group treated at 15°C but were significantly different to all groups sampled before treatment (P=0.043 or less). Significantly higher (P=0.01) mean glucose values were observed for fish treated at 15°C without sedation compared baseline values for that group.

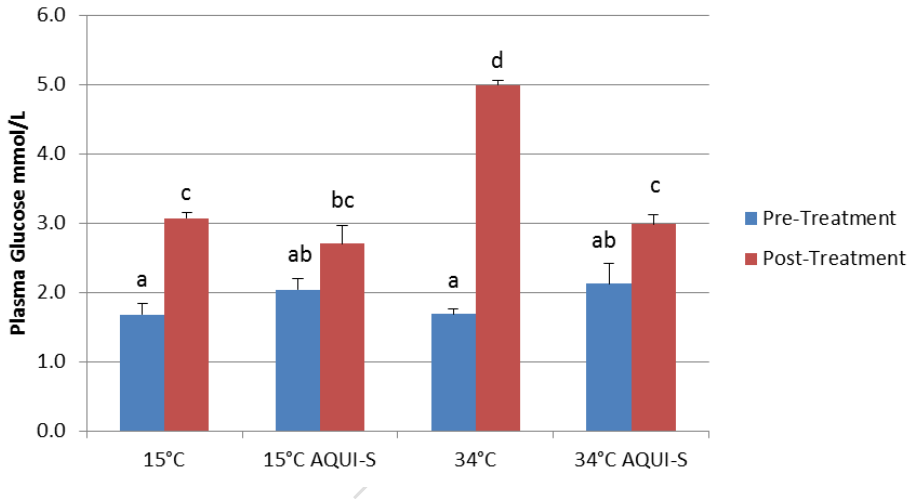

Figure 6 (previous page). Plasma glucose concentrations in fish prior to and after treatment. Fish un-sedated and immersed at 34C for 35 s had significantly higher levels compared to fish treated at the same temperature and sedated with AQUI-S®. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

Plasma lactate

Plasma lactate was significantly elevated in un-sedated fish treated at 34°C in comparison to all other post-treatment groups and nearly three times the value for fish sedated at the same temperature (figure 7). Interestingly the mean lactate value for fish treated without prior sedation at 15°C was also significantly higher than all other values except those un-sedated at 34°C.

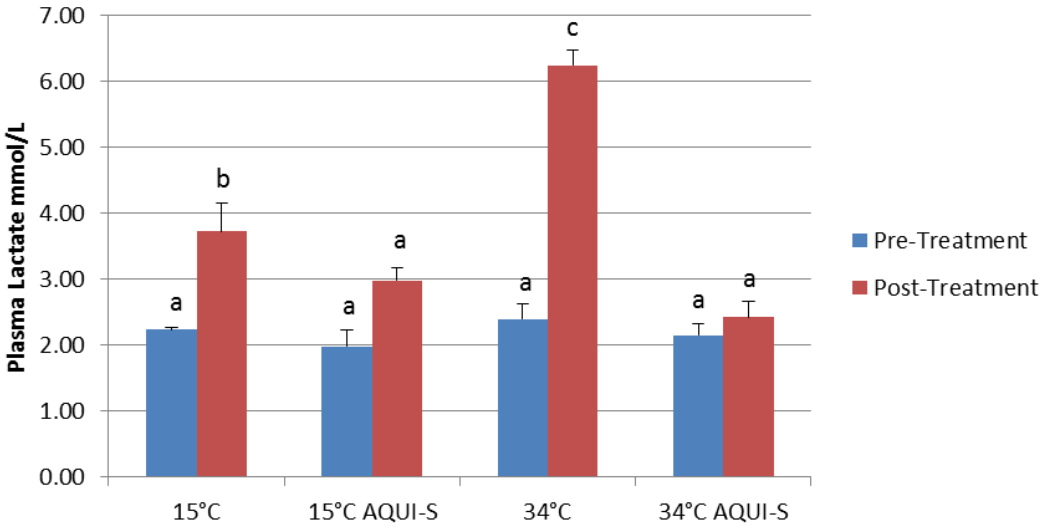

Figure 7. Plasma glucose concentrations in fish prior to and after treatment. Fish sedated with AQUI-S® and immersed at 34C for 35 s showed no significant difference to any replicate means before treatment. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

Plasma osmolality

There were no significant interactions between baseline groups, sedation and temperature for plasma osmolality data by three-way ANOVA (figure 8). However, the interactions of treatment time and temperature, treatment time and sedation and temperature and sedation were all significant ($P<0.036$ and lower). Although interactions in the model were statistically significant it would seem unlikely that the observed differences were biologically significant in terms of ion balance at the time samples were collected.

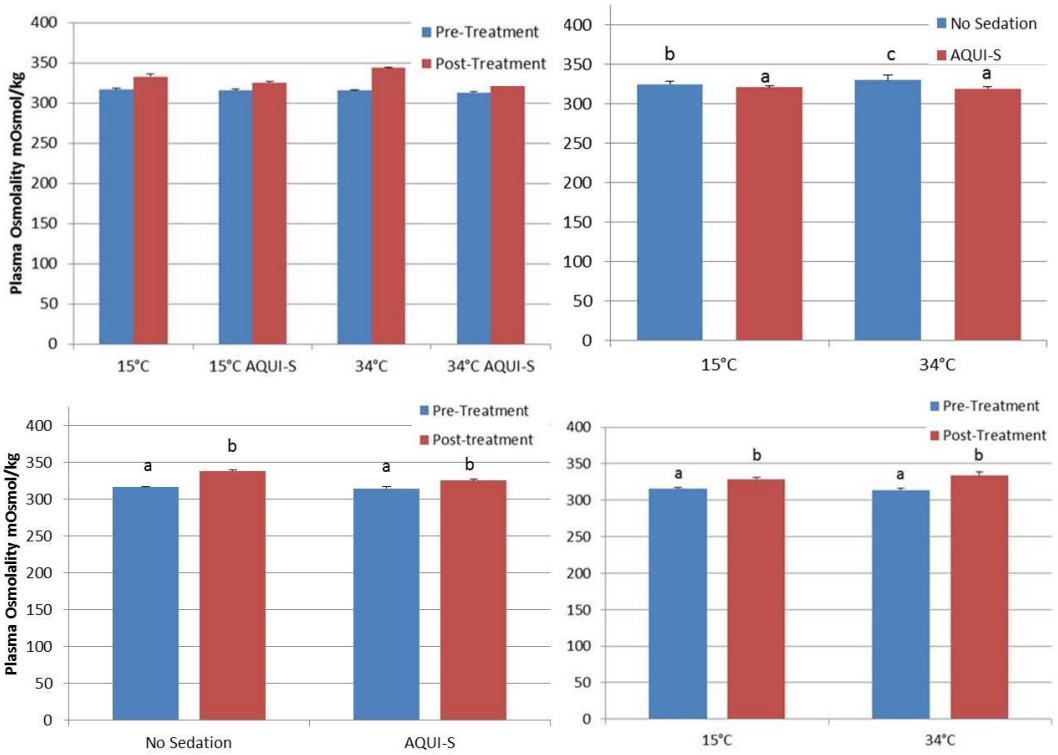

Figure 8. Plasma osmolality values in fish prior to and after treatment. Three way ANOVA did not detect significant differences for three way interactions although mean values are displayed top right (n=3 tanks / treatment). Two way interactions are displayed in the remaining plates (n=6 tanks / interaction). Different letters indicate significantly different means, bars indicate one standard error.

Fish Performance following treatment:

A single mortality occurred shortly after immersion of fish from a single replicate tank treated at 34°C without sedation. The gills were haemorrhaging from a rupture located on the ventral area of the junction between the filaments and the arch. It was not possible to determine a causal factor and no other mortalities were recorded at any other point throughout the trial's entirety.

There was a significant increase in weight and condition factor for all treatment groups after 28 days of feeding however the model indicated only the main effect of treatment time was significant (P = 0.0001 and 0.002 respectively) (figure 9). No significant differences were found within the model for temperature or sedation imparting an effect upon weight and condition of fish.

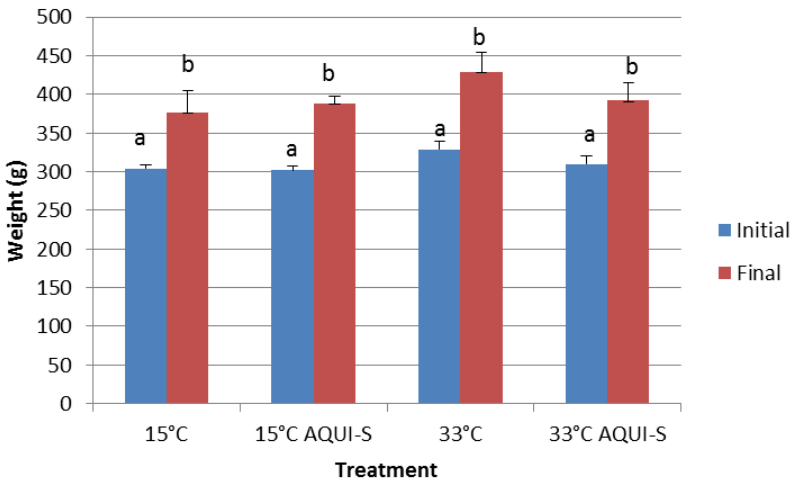

Figure 9. Mean individual weight of fish groups at initial and final sampling points. There were significant increases for all groups after the post treatment feeding period of 28 days. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

General body and fin condition was excellent for the majority of fish inspected during baseline sampling (figure 10). Fish inspected at final sampling were also in good condition although initial signs of erosion of the tail (ventrally) and right pelvic fins were noted (prevelance 15% & 20% respectively). These anomalies were not specific to any group but were variably prevalent amongst tanks.

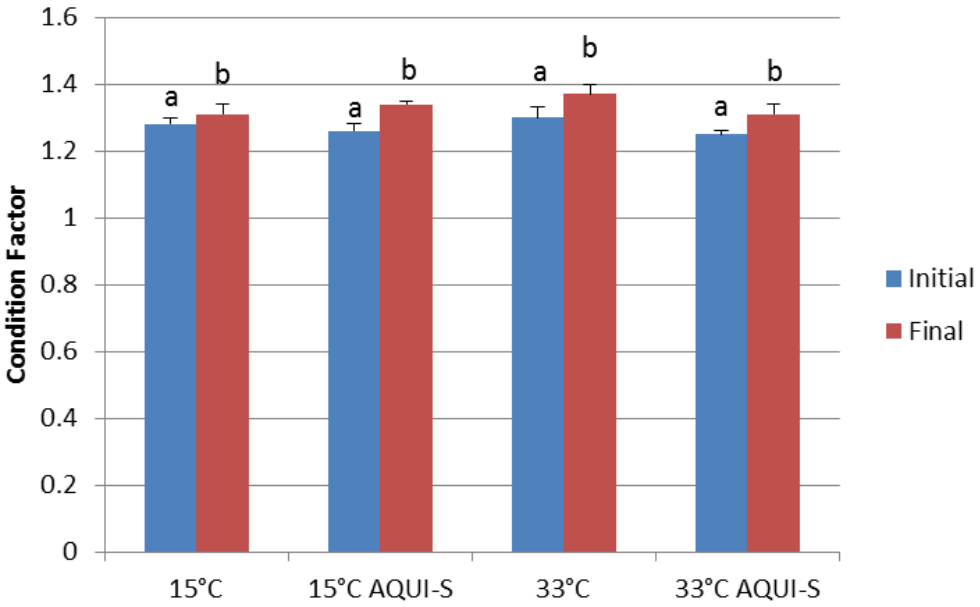

Figure 10. Mean condition factor of fish groups at initial and final sampling points. There were significant increases for all groups after the post treatment feeding

period of 28 days. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

There were no significant differences in % weight gained, FCR, or SGR between groups following 28 days of feeding (figures 11, 12 & 13).

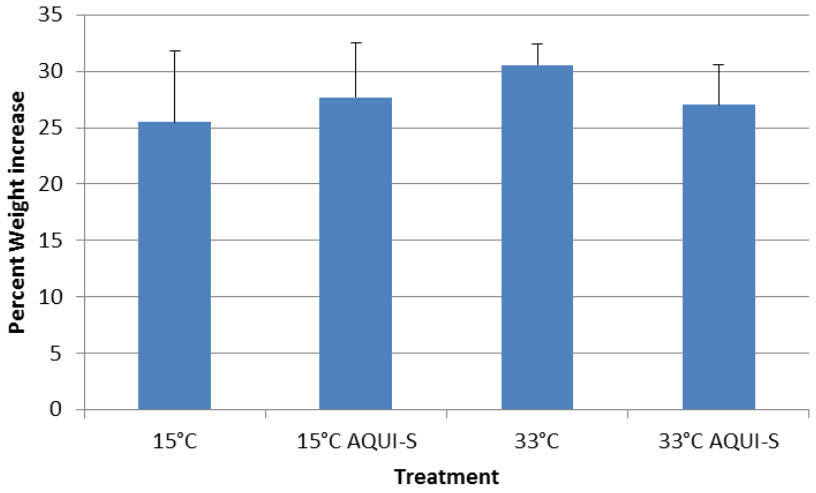

Figure 11. Mean percentage weight increase at final sampling. There were no significant differences detected between groups after the post treatment feeding period of 28 days. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

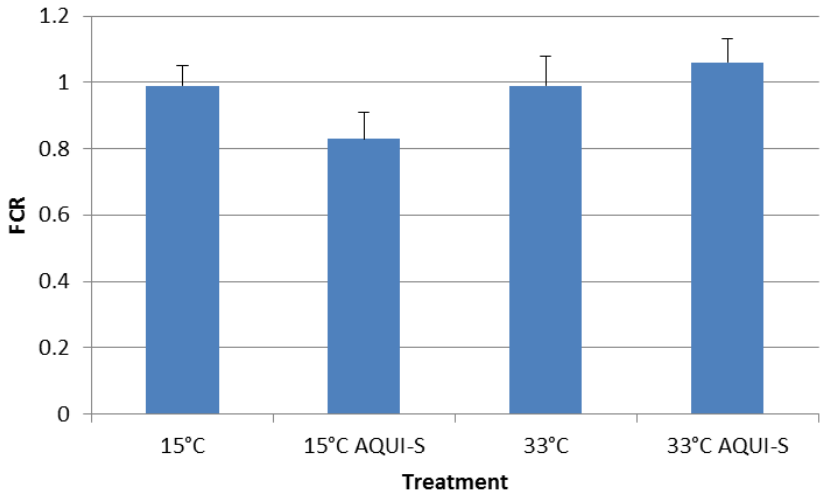

Figure 12. Mean FCR calculated over the duration of the post treatment feeding period. There were no significant differences detected between groups after the post treatment feeding period of 28 days. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

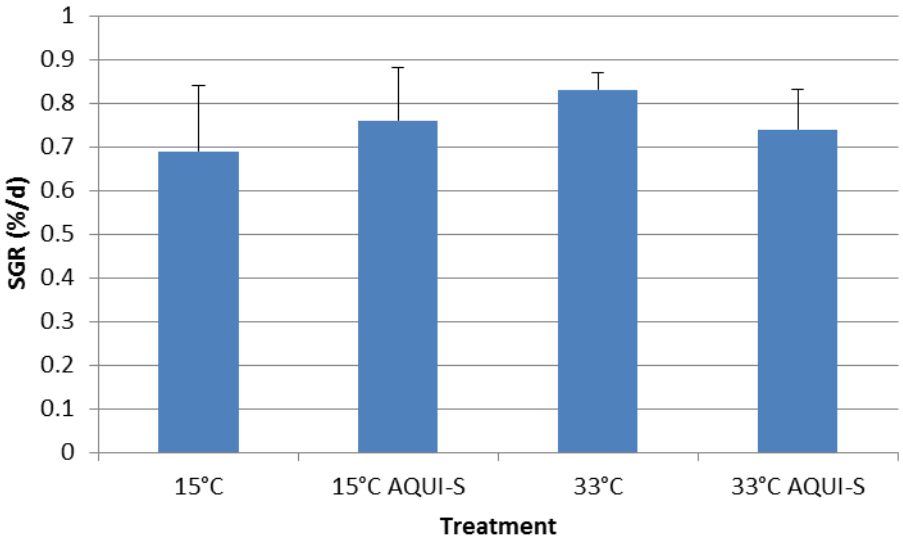

Figure 13. Mean SGR calculated over the duration of the post treatment feeding period. There were no significant differences detected between groups after the post treatment feeding period of 28 days. Different letters indicate significantly different means, bars indicate one standard error (n=3 tanks / treatment).

Observed growth trends suggested collectively that replicates immersed at 34°C without sedation were the best performers, however an associated higher feed intake (both as raw intake [g/day] and normalised feed intake [BW dry feed/day] was also evident during habituation and acclimation (figure 14). Given the tank allocations were random it appeared that the growth trend was likewise a random event (or tank effect that replication was unable to supress) that influenced the observed growth trend.

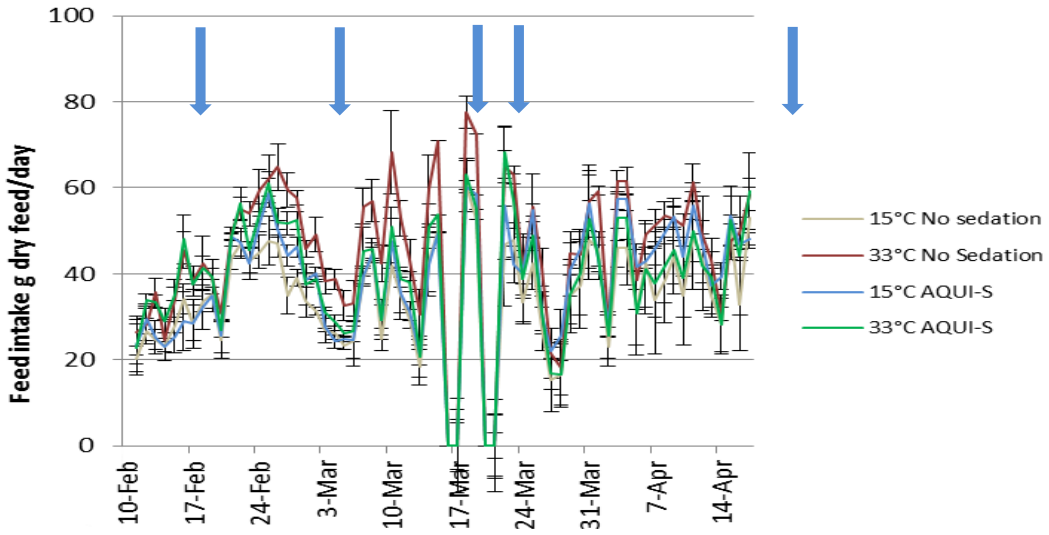

Figure 14. Mean feed intake (pellet wt g/day)for each group from prior to acclimation and through until final sampling. From right to left the arrows indicate the times for

acclimation start, acclimation end, baseline sampling, treatment and final sampling. Note the a generally higher mean intake evident for replicates in the group of fish treated at 34°C without sedation both before and after treatment.

Given there was evidence of behavioural differences in terms of appetite between pre-sedated and un-sedated groups (at 34°C, figure 3) on the day of treatment, normalised feed intake data (feed consumed/biomass) means/variance were determined over 7 days before baseline measurements were taken and again 7 days after treatment and compared. The model suggested no significant difference for any of the factors (figure 15).

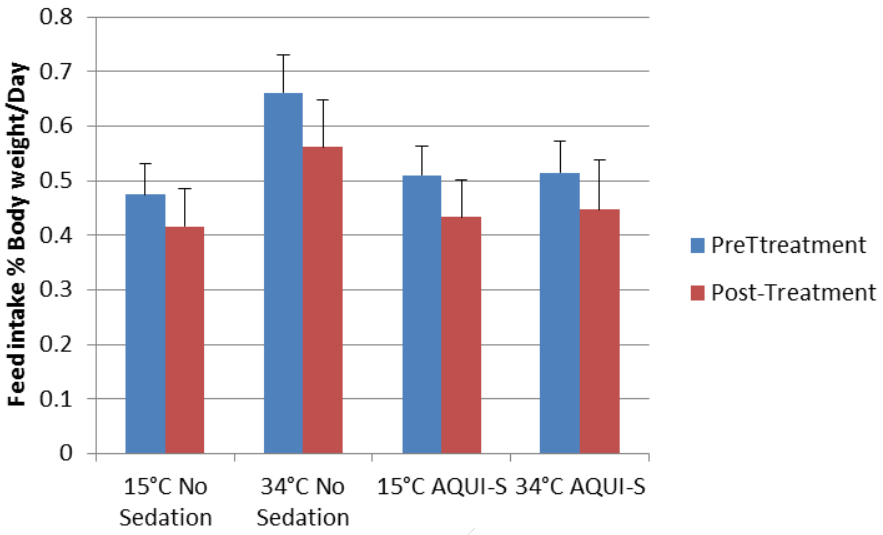

Figure 15. Mean feed intake (normalised with predicted biomass) averaged over seven days prior to baseline sampling and 7 days after treatment.No significant differences were detected.

A further focus on feed intake (normalised for biomass) for the first two days of feeding (where fish were fed three times) similarly found no significant differences between groups.

Conclusion:

This project has provided fundamental information regarding the impact of exposure of salmon to brief thermal extremes in physiological, behavioural and performance contexts. Collectively, the results from this trial suggest that AQUI-S® sedation had a measurable benefit towards stress reduction and notably mitigated visible adverse behaviour before, during and after a brief immersion in water at 33-34°C. Key stress parameters were lower; the fish were calmer and recovered more rapidly when they had been AQUI-S® sedated during the crowding stage prior to treatment. We were unable to detect any significant differences in performance indicators under the

conditions tested here; however repeat treatments scheduled within a standard growth trial would be warranted. Regardless, it is likely that a welfare benefit would be achievable and further testing under commercial conditions is recommended.