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Comparing the effects of swimming exercise and dissolved oxygen on important performance parameters of early-rearing Atlantic salmon *Salmo salar* and Rainbow Trout *Oncorhynchus mykiss*.

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Comparing the effects of swimming exercise and dissolved oxygen on important performance parameters of early-rearing Atlantic salmon *Salmo salar* and Rainbow Trout *Oncorhynchus mykiss*.

Thomas B. Waldrop

THESIS

Submitted to the Davis College of Agriculture, Natural Resources, and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

WILDLIFE AND FISHERIES RESOURCES

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2019

Keywords: Atlantic salmon, Rainbow Trout, swimming speed, dissolved oxygen

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Abstract:

Comparing the effects of swimming exercise and dissolved oxygen on important performance parameters of early-rearing Atlantic salmon *Salmo salar* and Rainbow Trout *Oncorhynchus mykiss*.

Thomas Waldrop

Swimming exercise (bodylengths/sec), and dissolved oxygen (DO), are important environmental variables in aquaculture. While there is an obvious physiological association between these two parameters, their interaction has not been adequately studied in Atlantic salmon, *Salmo salar* and Rainbow trout, *Oncorhynchus mykiss*. Since both variables can be easily manipulated in modern aquaculture systems, we sought to assess the impact of these parameters, alone and in combination, on the performance, health, and welfare parameters of the two species. Atlantic salmon and Rainbow trout were stocked into twelve circular 0.5 m³ tanks and exposed to either high (1.5-2 BL/s) or low (<0.5 BL/s) swimming speed and high (100% saturation) or low (70% saturation) DO while being raised from 10g to approximately 350g in weight for Atlantic salmon and 1 kg in weight for Rainbow trout. Throughout the study period, we assessed the impacts of both variables on important production performance parameters. By study's end, both increased swimming speed and higher DO were independently associated with a statistically significant increase in growth performance ($p < 0.05$) in Atlantic salmon, while only higher DO was independently associated with growth performance in Rainbow trout. No significant differences were noted in survival and feed conversion. Experimental results suggest that providing exercise and dissolved oxygen at saturation during early rearing can result in improved growth performance in both Atlantic salmon and Rainbow trout.

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Foreword

This thesis contains two chapters. The first Chapter, "Comparing the effects of swimming exercise and dissolved oxygen on the performance, health, and welfare of early-rearing Atlantic salmon *Salmo salar*" was published in the journal, Aquaculture Research. The chapter was written following the "Aquaculture Research" journal guidelines for authors. The complete citation is listed below:

Waldrop, Thomas & Summerfelt, Steven & Mazik, Patricia & Good, Christopher. (2017). The effects of swimming exercise and dissolved oxygen on growth performance, fin condition and precocious maturation of early-rearing Atlantic salmon Salmo salar. Aquaculture Research. 49. 10.1111/are.13511.

The second chapter has already been submitted for publication into Aquaculture Research (ARE-OA-19-Oct-1015), and is entitled, "The effects of swimming exercise and dissolved oxygen on growth performance, fin condition, and survival of rainbow trout *Oncorhynchus mykiss*". The chapter was also written following "Aquaculture Research" journal guidelines for authors and is in the review process. The expected citation is below:

Waldrop, Thomas & Summerfelt, Steven & Mazik, Patricia & Kenney, Brett & Good, Christopher. (2019). The effects of swimming exercise and dissolved oxygen on growth performance, fin condition, and survival of Rainbow trout. Aquaculture Research.

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Chapter 1. Comparing the effects of swimming exercise and dissolved oxygen on the performance, health, and welfare of early-rearing Atlantic salmon *Salmo salar*

Abstract: Swimming exercise, typically measured in body-lengths per second (BL/s), and dissolved oxygen (DO), are important environmental variables in fish culture. While there is an obvious physiological association between these two parameters, their interaction has not been adequately studied in Atlantic salmon *Salmo salar*. Because exercise and DO are variables that can be easily manipulated in modern aquaculture systems, we sought to assess the impact of these parameters, alone and in combination, on the performance, health, and welfare of juvenile Atlantic salmon. In our study, Atlantic salmon fry were stocked into twelve circular 0.5 m³ tanks in a flow-through system and exposed to either high (1.5-2 BL/s) or low (<0.5 BL/s) swimming speed and high (100% saturation) or low (70% saturation) DO while being raised from 10g to approximately 350g in weight. Throughout the study period, we assessed the impacts of exercise and DO concentration on growth, feed conversion, survival, and fin condition. By study's end, both increased swimming speed and higher DO were independently associated with a statistically significant increase in growth performance ($p < 0.05$); however, no significant differences were noted in survival and feed conversion. Caudal fin damage was associated with low DO, while right pectoral fin damage was associated with higher swimming speed. Finally, precocious male sexual maturation was associated with low swimming speed. These results suggest that providing exercise and dissolved oxygen at saturation during Atlantic salmon early rearing can result in improved growth performance and a lower incidence of precocious parr.

Keywords: Atlantic salmon, swimming speed, dissolved oxygen, circular tanks

1. Introduction

The culture environment of farmed aquatic animals has an enormous influence on the growth performance, health, and welfare of these organisms. Ongoing research is necessary to identify and refine environmental variables in aquaculture settings in order to enhance production while maintaining optimal fish health and well-being. Important variables impacting fish performance include exercise (i.e., creating water currents to stimulate swimming) (Josse et al., 1989) and dissolved oxygen (DO) (Fischer, 1963); these two variables interact to the extent that increased metabolism increases the consumption of dissolved oxygen by the exercising fish (Lauff and Wood, 1996). While numerous studies have been published demonstrating the independent effects of swimming speed and dissolved oxygen on a range of outcomes, very little research has assessed these variables in combination.

Increased growth performance has been demonstrated in various salmonid species exposed to prolonged moderate exercise, including rainbow trout *Oncorhynchus mykiss* (Josse et al., 1989), masu salmon *Oncorhynchus masou* (Azuma, 2001; Azuma et al., 2002), brook trout *Salvelinus fontinalis* (Leon, 1986), arctic charr *Salvelinus alpinus* (Christiansen and Jobling, 1990), and atlantic salmon *Salmo salar* (Jorgensen, 1993). Other reported benefits associated with sustained, moderate exercise include improved feed conversion (Leon, 1986; Jobling et al., 1993), improved fin quality and decreased aggression (Jorgensen et al, 1993; Jobling 1993), increased muscular endurance (Besner and Smith, 1983), and increased survival following pathogen challenge (Castro et al., 2011). Modern aquaculture facilities with properly designed and operated circular tanks can provide rotational water velocities directly promoting fish exercise while operating in

a self-cleaning manner (solids are rapidly flushed to the center drain and do not significantly dissolve in the culture tank water). Larmoyeux et al. (1973) noted that fish tend to be more evenly distributed in circular tanks, and described how rotational velocities could be managed independent of flow rate. Through adjusting these water velocities over time as fish grow, a sustained exercise of approximately 0.5-1.75 body-lengths per second (BL/s) has been shown to enhance fish growth, primarily through increased feed intake and/or improved feed conversion (Jobling et al, 1993; Losordo and Westers, 1994; Davison, 1997; Palastas and Planas, 2011).

Dissolved oxygen is a critical variable in aquaculture systems and needs to be maintained at sufficient levels to support fish performance, welfare, and survival. In particular, salmonids are strong and active swimmers and require highly oxygenated environments (Spence et al., 1996). Svobodova et al. (1993) suggest that DO concentrations be maintained at a minimum of 6 mg/l for cold-water species such as rainbow trout and Atlantic salmon; however, higher DO levels (i.e., 80-100% saturation) have long been recommended for various salmonids in order to maximize growth and avoid the consequences of prolonged hypoxia – namely, stress, slower growth, decreased tissue repair, susceptibility to disease, and mortality (Herrmann, 1962; Fischer, 1963; Itazawa, 1970; Cameron, 1971). Many intensive aquaculture systems use some form of pure oxygen supplementation to keep DO at levels optimizing fish production, because as feed loading and biomasses increase, DO can quickly become a limiting factor. The use of pure oxygen can increase carrying capacity without increases in water flow rates, but it can also substantially increase production costs (Timmons 2002).

Given the recent focus of raising pre- and post-smolt Atlantic salmon in land-based, closed containment systems prior to their transfer to sea cages, research is necessary to optimize environmental conditions in these settings. To support this endeavor, we sought in the present study to assess the relative impacts of exercise and DO on a range of performance, health, and welfare outcomes of first-year Atlantic salmon.

2. Materials and Methods

Atlantic salmon eggs were obtained from a commercial supplier and hatched in vertical hatch tray stack columns. Fry (<1g) were stocked into several rearing units in a flow-through system, which contained a total of twelve 0.5 m³ circular tanks. Experimental conditions began when fry were approximately 10g in mean weight, at which point the fish were distributed evenly among the twelve tanks (approximately 300 fish per tank). The study concluded at 440 days post-hatch when fish were approximately 350g in overall mean weight among treatment groups. The following summarizes experimental materials and methodologies employed.

2.1 Experimental Conditions

Utilizing all twelve flow-through circular tanks, a 2x2 factorial study was carried out wherein juvenile Atlantic salmon were exposed to either high or low DO (100% vs. 70% saturation, respectively) and high or low swimming speed (1.5-2.0 BL/s vs <0.5 BL/s, respectively). Tanks were assigned to a particular treatment regime based on random number selection.

To provide swimming exercise, each tank was retrofitted with a magnetic pump connected to an inlet and discharge manifold. These pumps created rotational velocities by removing water from the tank and pumping it back into the same tank through the discharge manifold. All pumped water was injected beneath the surface to reduce the likelihood that surface agitation would increase oxygen levels to atmospheric saturation in the low DO tanks, or strip oxygen from the high DO tanks. Velocity manifolds were composed of 2-in (5 cm) schedule 40 pipe with holes drilled and spaced approximately 1 in (2.5 cm) apart. The manifolds in the 2.0 BL/s treatment tanks were positioned approximately parallel to the tank wall to create the strong rotational velocity, whereas the manifolds in the 0.5 BL/s tanks directed the pumped water flow toward the tank wall. The maximum output of the pumps was fixed and could not be increased as fish grew; however, the angle of the discharge manifolds could be manipulated to increase or decrease velocities as needed to maintain the target swimming speeds for each treatment regime, and rotational velocities were adjusted accordingly as fish grew in length (measured at each fish sampling event - see below). Rotational velocities were measured using a floating velocity sphere timed around various points of the tanks' perimeters, and well as by using an underwater flow meter.

As the fish grew over the study period, exercise treatment rotational velocities were harder to maintain at 2.0 BL/s; however, swimming speeds in these groups were maintained at 1.5 BL/s or greater up to the end of the experiment. Each of the twelve tanks was also fitted with a water manifold (i.e., independent of the velocity manifold), providing incoming spring water. The water manifold could also be manipulated by angling the manifold in a more parallel or perpendicular direction relative to the tank

wall, in order to supplement water velocity control if required. The combination of flow from the velocity and water manifolds provided the exercise groups with the required currents as fish grew over the study period, such that these fish swam at 1.5-2.0 BL/s throughout the experiment.

To provide high or low DO environments, tank effluent DO levels were monitored and maintained at approximately 70% and 100% of atmospheric oxygen saturation. Target DO levels were derived using oxygen solubility tables (Colt, 1984), which take into account water temperature and barometric pressure, and from these tables the DO levels were set at 10.5 mg/l and 7.3 mg/l for the 100% and 70% saturation tanks, respectively. Dissolved oxygen levels were measured and recorded twice weekly in mg/l and % saturation levels using a Hach portable dissolved oxygen Flexi HQ 30D meter (Hach, Loveland, Colorado, USA). Dissolved oxygen levels were adjusted each time overall tank feed was increased or decreased, by two primary means: (i) increasing or decreasing oxygen gas flow, and/or (ii) increasing or decreasing total water flows to each experimental tank. As the tank biomass grew and feeding increased, DO levels were adjusted appropriately. Under normal operating conditions, all 12 flow-through system tanks were operated with degassed water that entered tanks after passing through a modified packed column oxygen vessel adding pure oxygen to the incoming water. For this experiment, however, it was not possible to utilize this column for the 70% dissolved oxygen treatment tanks, as pure oxygen would result in undesirably high DO levels; therefore, a water line that bypassed the packed column oxygen vessel was installed, and this supplied spring water (referred to as “bypass” water) directly to the six low DO treatment tanks. For the high DO tanks, no modifications were necessary, as the modified

oxygen pressure vessel added the necessary supplemental oxygen to maintain the higher oxygen regime.

As salmon grew and biomasses increased, the densities in each tank were allowed to reach a maximum of 80 kg/m^3 . As fish grew to this limit, tanks were harvested and densities were reduced to approximately 40 kg/m^3 , which provided comparable densities among all study tanks. Study salmon received the same commercially available diet throughout the experiment, and feed was administered by a computer-controlled program that was identical for all twelve experimental tanks. Daily feed levels were determined using established feed charts for Atlantic salmon; however, daily feeding amounts were adjusted based on feeding activity, via daily observation of individual feed sumps, i.e. cylindrical vessels receiving bottom drain flow from each tank for the purpose of collecting uneaten feed or excess feces as an ongoing guide to adjusting feeding rates.

2.2 Data collection and analysis

2.2.1 Water quality monitoring

In addition to the twice weekly dissolved oxygen measurements, carbon dioxide, total gas pressure (TGP), pH, alkalinity, total suspended solids (TSS), nitrite nitrogen ($\text{NO}_2\text{-N}$), and total ammonia nitrogen (TAN) were taken weekly to ensure that water quality was not negatively affecting experimental fish and treatments. Total ammonia nitrogen was determined using Hach method 8038; $\text{NO}_2\text{-N}$ was determined using Hach method 8507. Both TAN and $\text{NO}_2\text{-N}$ were measured using a Hach spectrophotometer (Model DR/4000). Total gas pressure was determined using dissolved oxygen measurements in combination with an In-Situ Model 300E tensionometer (In-Situ, Inc.,

Fort Collins, Colorado, USA). The remaining water quality parameters were determined using standard methods (APHA, 2005): TSS was measured using standard method 2540, carbon dioxide readings were obtained using standard method 4500-CO₂, and alkalinity was determined via standard method 2320.

2.2.2 Fish performance and welfare

Data were collected throughout the study in order to assess fish growth performance, feed conversion, survival, and fin condition. Monthly length and weight sampling was carried out using anesthesia (75 mg/L) with tricaine methanesulfonate (Tricaine-S; Western Chemical Inc., Ferndale, Washington, USA), and sample sizes for these data collection events were determined using the formula:

$$n = (Z * (\text{stdev.}_{\text{grams}} / \text{accepted error}_{\text{grams}}))^2$$

where $Z = 1.95$ (relative to a 95% confidence interval), assuming an accepted error of 5 grams. Condition factor (K) could then be calculated using the following formula:

$$K = 100 * W(\text{g}) / L(\text{cm})^3$$

Feed amounts were measured and recorded for each feeder; feed conversion rate (FCR) was calculated for each treatment tank by dividing the total amount of feed administered (kg) by the total weight gain (kg) exhibited by that tank. Daily mortality data were collected to assess within-treatment survival. Fin erosion, an established indicator of fish

welfare (Ellis et al., 2008), was assessed qualitatively by a single reviewer during the final length and weight sampling based on a three-point visual scale (i.e., 1-3, representing none/mild, moderate, or severe fin erosion) for dorsal, caudal, and pectoral fins. During the final length and weight sampling, fish were also observed for the occurrence of precocious males, based on characteristic changes in coloration and confirmed via post-euthanasia gonadal examination.

Statistical analyses were carried out using STATA 9 software (StataCorp LP, College Station, Texas, USA). Performance data were analyzed using multivariable ANOVA with DO and swimming speed as covariates, as well as an interaction term for these independent variables. Non-normally distributed outcome variables that were resilient to transformation were assessed non-parametrically for association with each treatment variable via the Kruskal-Wallis rank test. Fin erosion data were assessed using multivariable ordered logit regression; male maturation was assessed using multivariable logistic regression. A significance level of $p \leq 0.05$ was used to determine relationships among the variables assessed.

3. Results

3.1 Water quality

Mean values for measured water quality parameters are summarized in Table 1. Total suspended solids and $\text{NO}_2\text{-N}$ were significantly associated with decreased and increased rotational water velocities, respectively, while CO_2 was significantly associated with reduced DO. Despite statistical associations, it is likely that these differences were

not biologically significant, i.e. that they did not impact fish performance in any meaningful way.

3.2 Fish performance

Both DO and swimming speed had a significant, positive influence on final weight (Table 2; Figure 1). There were no significant differences among treatment groups for the other performance outcomes, including condition factor, cardio- and viscerosomatic indices, feed conversion rate, or survival.

3.3 Fish welfare

By study's end, moderate dorsal fin erosion was noted in all treatment populations (Table 2), while very minor erosion of the caudal and right and left pectoral fins was also noted in most groups. Caudal fin erosion, while minor overall, was significantly greater in the low DO groups; right pectoral fin erosion, likewise very minor overall, was significantly associated with increased swimming speed.

3.4 Sexual maturation

Maturation assessments during final sampling indicated that early maturing males represented 6.5% and 11.5% of the exercised and unexercised treatment groups, respectively; statistical analysis revealed that the odds of maturation in the unexercised groups were significantly higher than in the exercised group (Table 3). No association was determined between early maturation and dissolved oxygen levels.

4. Discussion

The major finding of our study was that Atlantic salmon, raised up to 350g, demonstrate increased growth performance when provided moderate sustained swimming exercise and/or DO at saturation. Feed conversion was not statistically different between treatment groups, suggesting that the treatment conditions related to superior growth performance were also associated with an overall increase in feed consumption. This finding is in agreement with results obtained by Jorgensen and Jobling (1993). They found that increased feed intake, including improved FCR, has been reported as significantly associated with swimming exercise (Jobling et al., 1993; Davison, 1997; Palastas and Planas, 2011). Leon (1986) reported that brook trout exercised at 1.5-2 BL/s showed statistically significant gains in growth and swimming stamina compared to unexercised satiated cohorts as well as exercised unsatiated cohorts. Although not statistically significant, the exercised satiated fish had a higher intake of feed with a lower feed conversion, suggesting exercised fish were more metabolically efficient than unexercised cohorts. Grisdale-Helland et al. (2013) concluded that post-smolt Atlantic salmon continuously exercised were able to respond to higher activity costs by utilizing energy and protein more efficiently for growth. Among non-salmonid studies, juvenile gilthead sea bream *Sparus aurata* exercised at 1.5 BL/s over a 4-week period grew faster, but did not consume a greater amount of feed, than controls (Ibarz et al., 2011), suggesting that feed consumed was utilized more efficiently. The highest feed amount fed (221 kg) over the course of the present experiment was to the non-exercised, low DO treatment group. In contrast, the second highest amount of feed administered (218 kg)

was given to those fish in the exercise, high DO tanks; however, FCR was considerably, though not statistically, lower in this group compared to the “worst case” treatment regime.

Swimming exercise can indirectly enhance growth performance through ram ventilation utilized by fish in faster currents. Ram ventilation permits increased water flow directly over the gills, while reducing the need for active ventilation through the branchial pump, and therefore allows the countercurrent oxygen process to work with less energy cost. Farrell et al. (1987) showed that ram ventilation can lead to a substantial reduction in oxygen costs as the fish open mouths in current and use swimming tail muscles to force water over the gills; oxygen consumption decreased by 2 -10 % when rainbow trout shifted from active to ram ventilation. Another benefit of providing swimming exercise is a reduction of aggressive behaviors, and likewise, reduced dominance behaviors that may be too energetically costly to sustain for the aggressor. It is reported that salmon benefit from maintaining positions in sustained currents; Fausch (1984) summarized the behavior of wild juvenile Atlantic salmon as “holding static” positions while swimming and competing for food in the natural environment. Adams et al. (1998) reported consistent behavior with salmon parr in aquaculture systems as fish hold stationary positions in current and compete for food as well. East and Magnan (1987) demonstrated that Brook char forced to swim at higher currents had higher energy costs and therefore tried to conserve energy by reducing aggressive behaviors. Grant (1977) demonstrated that fish will defend a space for food, but only if the benefits exceed the cost. Therefore, in theory, fish held in faster velocities may be less likely to expend energy to defend tank space, which reduces aggression. Thus, managing water velocities

may be a viable tool for salmonid farmers aiming to reduce aggressive behaviors and, consequently, health and welfare issues such as fin erosion, skin damage, and secondary infections by opportunistic pathogens.

The present study did not demonstrate a significant statistical interaction between swimming speed and dissolved oxygen; however, oxygen conditions clearly have an impact on swimming performance. Dahlberg et al. (1968) reported that as water temperatures approached 20°C, the subsequent decreases in available oxygen resulted in decreased swimming performance among coho salmon *Oncorhynchus kisutch*. Davis et al. (1963) also concluded that coho salmon showed decreases in maximum sustainable swimming as dissolved oxygen values fell below saturation levels for 10°C, 15°C and 20°C. This is in agreement with Bjornn and Riser (1991), who reported that swimming performance of migrating salmonids can be negatively impacted by reduced dissolved oxygen concentrations. It is important to note that swimming speeds should be optimal and not exhaustive to the animal, as fish that are continually exercised at excessive swimming speeds can become exhausted and/or perform poorly and demonstrate signs of stress. Tufts et al (2011) reported extremely high blood lactate levels for at least 8 hours in wild Atlantic salmon exposed to exhaustive exercise in laboratory studies, while Wood et al. (1983) reported that exhaustive exercise stress can be severe enough to cause death due to intracellular acidosis. Growth performance in relation to exercise can only be maximized when salmonids swim at, or close to, their optimal speed (Davison, 1997). As such, rearing vessel water velocities should be checked on a regular basis to ensure that animals are not being exhausted, or conversely, that swimming speeds are not inappropriately low. Losordo and Westers (1994) suggested that water velocities be

maintained between 0.5 and 2.0 BL/s for optimal fish health, performance, and respiration in order to avoid the reduced growth and higher stress observed at high swimming speeds and the increased aggressive behavior and dominance exhibition facilitated by lower swimming speeds. Reduced swimming exercise can have additional deleterious fish health outcomes: Castro et al. (2011) described how non-exercised Atlantic salmon demonstrated reduced disease resistance compared to those exercised either continuously or through interval training. These authors also demonstrated that heart health was improved with aerobic exercise, through reducing inflammation and increased removal of free radicals. It is likely that the reduced heart health observed in unexercised fish could be further exacerbated by feeding fish a high fat and high energy diet in low rotational velocity environments.

Finally, our study found that exercise decreased the prevalence of male precocity in the study population, which may be important as sexually developing fish present numerous problems for farmers, including decreased growth and FCR (McClure et al., 2007), reduced product quality (Aksnes et al., 1986), and an increased susceptibility disease caused by opportunistic pathogens (St-Hilaire et al., 1998). Exercise in general has been shown to influence maturation in aquatic species, as review by Palstra and Planas (2011); however, this influence can work in a positive or negative direction depending on numerous host and environmental factors. Early maturation has been shown to be influenced by whole body lipid content (Shearer and Swanson, 2000; Shearer et al., 2006); however, no consensus currently exists as to the relationship between exercise and whole body composition (fat, protein, etc.) and energy deposition in fish (Jobling et al., 1993; Rasmussen et al., 2011). Hence, any observed preventive effect

of exercise on early maturation is likely via unknown metabolic avenues rather than through reduced adiposity. Unfortunately, we did not carry out whole body proximate analyses in this study, and further research focusing on exercise and maturation would benefit from such investigation. Our results, however, are encouraging and warrant further study to confirm the association between exercise and reduced precocious maturation in early rearing Atlantic salmon.

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Table 1. Environmental conditions (mean $\pm$ SE) during the study period.

Condition	<u>DO 100% saturation</u>		<u>DO 70% saturation</u>	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
<i>Treatments</i>				
Velocity (BL/s)	1.83 $\pm$.017	0.524 $\pm$.004	1.82 $\pm$.018	0.552 $\pm$ 0.03
DO (mg/l)	10.2 $\pm$.075	10.5 $\pm$.072	7.20 $\pm$.065	7.40 $\pm$.060
<i>Water quality</i> [*]				
TAN (mg/l)	0.165 $\pm$ 0.026	0.159 $\pm$ 0.021	0.154 $\pm$ 0.020	0.142 $\pm$ 0.018
NO ₂ -N (mg/l) ^a	0.00446 $\pm$ 0.000606	0.00389 $\pm$ 0.000544	0.00475 $\pm$ 0.000556	0.00395 $\pm$ 0.000531
Alkalinity (mg/l)	265 $\pm$ 2.18	265 $\pm$ 3.04	263 $\pm$ 2.97	264 $\pm$ 2.77
CO ₂ (mg/l) ^b	18.0 $\pm$ 0.650	18.0 $\pm$ 0.573	18.6 $\pm$ 0.605	18.2 $\pm$ 0.461
TSS (mg/l) ^a	1.13 $\pm$ 0.192	1.81 $\pm$ 0.587	1.00 $\pm$ 0.152	1.75 $\pm$ 0.653
TGP (%)	95.3 $\pm$ 0.657	96.2 $\pm$ 0.483	95.6 $\pm$ 0.523	95.8 $\pm$ 0.508
Temperature (°C)	13.4 $\pm$ 0.133	13.4 $\pm$ 0.130	13.4 $\pm$ 0.148	13.4 $\pm$ 0.140

^{*} Superscripts indicate parameters significantly ($p < 0.05$) impacted by swimming speed (a) and/or dissolved oxygen (b)

Table 2. Final growth performance results (mean weight $\pm$ SE), with summary of multivariable ANOVA model results.

Outcome [*]	<u>DO 100% saturation</u>		<u>DO 70% saturation</u>	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
Final weight (g) ^{a,b}	344 $\pm$ 6.27	316 $\pm$ 7.53	306 $\pm$ 6.87	292 $\pm$ 6.96
K	1.26 $\pm$ 0.0218	1.26 $\pm$ 0.0190	1.21 $\pm$ 0.0108	1.27 $\pm$ 0.0245
CSI (%)	0.158 $\pm$ 0.008	0.131 $\pm$ 0.0115	0.125 $\pm$ 0.012	0.125 $\pm$ 0.00851
VSI (%)	7.71 $\pm$ 0.260	8.28 $\pm$ 0.673	8.44 $\pm$ 0.641	8.73 $\pm$ 0.267
FCR	1.08 $\pm$ 0.004	1.14 $\pm$ 0.026	1.19 $\pm$ 0.072	1.20 $\pm$ 0.062
Survival (%)	97.4 $\pm$ 0.074	96.8 $\pm$ 0.068	97.2 $\pm$ 0.060	98.3 $\pm$ 0.020
<i>Fin erosion score</i>				
Dorsal	2.18 $\pm$ 0.050	2.18 $\pm$ 0.051	2.27 $\pm$ 0.055	2.27 $\pm$ 0.056
Caudal ^b	1.08 $\pm$ 0.021	1.13 $\pm$ 0.027	1.24 $\pm$ 0.033	1.21 $\pm$ 0.031
Right pectoral ^a	1.03 $\pm$ 0.012	1.02 $\pm$ 0.010	1.10 $\pm$ 0.023	1.01 $\pm$ 0.008
Left pectoral	1.02 $\pm$ 0.010	1.02 $\pm$ 0.010	1.08 $\pm$ 0.020	1.01 $\pm$ 0.008

* Superscripts indicate parameters significantly ($p < 0.05$) impacted by swimming speed (a) and/or dissolved oxygen (b)

Table 3. Logistic regression model reporting odds ratios for the probability of precocious males within the specified level of each treatment group.

Treatment	Odds ratio	(95% conf. int.)	p-value
0.5 BL/s	1.896	(1.121, 3.208)	0.017
70% DO	0.945	(0.546, 1.636)	0.839

Figure 1. Comparison of growth performance for the four treatment groups up to 440 days-post hatch. Different letters beside the final mean weight values indicate significant ($p < 0.05$) differences between treatment groups.

The effects of swimming exercise and dissolved oxygen on growth performance, fin condition, and survival of rainbow trout *Oncorhynchus mykiss*

Abstract: Swimming exercise and dissolved oxygen (DO) are important parameters to consider when operating intensive salmonid aquaculture facilities. While previous research has focused on each of these two variables in rainbow trout *Oncorhynchus mykiss*, studies examining both variables in combination, and their potential interaction, are largely absent from the scientific literature. Both swimming exercise (usually measured in body-lengths per second, or BL/s) and DO can be readily controlled in modern aquaculture systems; therefore, we sought to evaluate the effects of these variables, separately and combined, on several outcomes in rainbow trout including growth performance, fin health, and survival. Rainbow trout fry (18 g) were stocked into 12 circular 0.5 m³ tanks, provided with either high (1.5 - 2 BL/s) or low (<0.5 BL/s) swimming speeds and high (100% saturation) or low (70% saturation) DO, and grown to approximately 1 kg. By the conclusion of the study, higher DO was independently associated with significantly ($p<0.05$) better growth performance. Significant differences were not noted in other outcomes, namely feed conversion, condition factor, and mortality, although caudal and right pectoral fin damage was associated with low oxygen and low swimming speed treatments, respectively. Cardiosomatic index was significantly higher among exercised fish. These results suggest that swimming exercise and DO at saturation during the culture of rainbow trout can be beneficial to producers through improved growth performance and cardiac health.

Keywords: Rainbow trout, swimming speed, dissolved oxygen, circular tanks

1. Introduction

Optimizing environmental quality can provide significant benefits for cultured fish performance and welfare, as well as enhancing the overall profitability of commercial aquaculture operations. Increasing operational productivity without compromising fish health and welfare is a constant challenge faced by aquaculturists, and ongoing research is necessary to continue identifying and refining important environmental variables that can be manipulated to improve farmed fish production. Two such variables are swimming speed, which can be regulated by creating water currents to stimulate swimming exercise (Josse et al., 1989), and dissolved oxygen (DO) (Fischer, 1963). Swimming speed and DO interact to the extent that increased metabolic rate during exercise increases the consumption of dissolved oxygen (Lauff and Wood, 1996). The independent effects of swimming speed and dissolved oxygen on a range of outcomes have been previously studied; however, little research has examined these parameters in combination. Hence, we sought to assess the relative impacts of exercise and DO on a range of performance, health, and welfare outcomes with rainbow trout *Oncorhynchus mykiss* grown from fry to a harvest size of 1 kg.

Several salmonid species have demonstrated improved growth performance with prolonged moderate exercise; these include rainbow trout (Greer-Walker and Emerson, 1978; Josse et al., 1989; Houlihan and Laurent, 1987), Atlantic salmon *Salmo salar* (Totland et al., 1987; Castro et al., 2011; Waldrop et al., 2018), brook trout *Salvelinus fontinalis* (Leon, 1986; East and Magnan 1987), brown trout *Salmo trutta* (Davison and Goldspink, 1977) and Arctic char *Salvelinus alpinus* (Jorgensen, 1993; Grünbaum et al., 2008). Sustained, moderate exercise has also been shown to improve feed conversion

(Leon, 1986; Jobling et al., 1993), improve fin quality (Jorgensen and Jobling, 1993), decrease aggression (Postlethwaite and McDonald, 1995), increase endurance (Besner and Smith, 1983), and increase disease resistance and survival during pathogen challenge studies (Castro et al., 2013).

Appropriately designed circular tanks can be self-cleaning if rotational water velocities are maintained between 15 to 30 cm/s (Burrows and Chenoweth, 1970; Makinen et al., 1988), such that solids are quickly flushed to the center drain and do not remain in the tank to dissolve and deteriorate water quality. Rotational water velocities can also provide swimming exercise and can be managed independent of tank exchange rate. Fish in circular tanks tend to be more evenly distributed (Larmoyeux et al., 1973), although rotational velocities must be kept at appropriate levels so as to not be exhaustive; Wood et al. (1983) reported that exhaustive exercise stress can be severe enough to cause death due to intracellular acidosis. Parker and Barnes (2015) demonstrated that well-fed rainbow trout can be safely cultured in velocities high enough to maintain self-cleaning characteristics of circular tanks. As such, rearing vessel water velocities should be checked on a regular basis to ensure that animals are not being exhausted, or conversely, that swimming speeds are not inappropriately low.

Growth performance in relation to exercise may be optimized when salmonids swim at, or close to, their optimal speed (U_{opt}) (Davison, 1997). Rainbow trout U_{opt} has been reported ranging from 0.9 body-lengths per second (BL/s) to 1.4 BL/s (Webb, 1971; Bushnell et al, 1984; Shingles et al, 2001). Other studies have noted maximum growth at swimming speeds ranging from 0.85 - 1.8 BL/s (Greer-Walker and Emerson, 1978; East and Magnan, 1987). Conversely, Farrell et al (1991) noted that some rainbow trout

exercised continuously at 1.6 BL/s demonstrated signs of fatigue; however, these studies were conducted in swim tubes, wherein a range of water velocities is not present. Fish in circular tanks, by comparison, can self-select a range of water velocities across a given tank's radius, with the highest velocities occurring immediately beside the tank wall (Davidson and Summerfelt, 2004).

Dissolved oxygen must be sufficient to support fish performance, welfare, and survival, and in intensive production pure oxygen is often employed to sustain high feed loadings and stocking densities. Pure oxygen can increase an aquaculture system's carrying capacity without an increase in water flow rate; however, use of pure oxygen can also substantially increase production costs (Timmons, 2002). Salmonids in general are strong, active swimmers, and perform best in well-oxygenated environments (Spence et al., 1996). Dissolved oxygen saturation levels between 80 – 100 % have been recommended for many salmonid species to support maximum growth and feed conversion efficiency, whereas lower DO saturation levels have been associated with stress, reduced growth performance, decreased wound healing, increased disease susceptibility, and mortality (Herrmann et al., 1962; Fischer, 1963; Itazawa, 1970; Cameron, 1971).

Land-based, closed containment salmonid production is a growing industry sector (Summerfelt and Christianson, 2014), and research is necessary to more fully understand and optimize conditions in these relatively novel environments. As such, we sought to evaluate the relative impacts of exercise and DO on the performance, health, and welfare of rainbow trout raised from juveniles to approximately market size, in a similar approach to our previously published research on Atlantic salmon (Waldrop et al., 2018). In the

present study, we assessed the individual and combined effects of high versus low swimming speed (1.5 – 2.0 vs. < 0.5 BL/s, respectively) and high versus low DO levels (100 % vs. 70 % saturation, respectively), on multiple outcomes, including growth performance, fin condition, and survival, as juvenile rainbow trout were raised from approximately 18 g up to a maximum of 1,020 g mean weight. Our maximum swimming speed was selected based on previous studies (Jorgensen and Jobling, 1993; Jobling et al., 1993; Davison, 1997; Castro et al., 2011); lower ranges of both swimming speed and DO were meant to be comparable to typical conditions in raceway-based culture.

2. Materials and Methods

All-female diploid rainbow trout eggs were obtained from a commercial supplier and hatched in a vertical heath tray stack incubator. At approximately 17 days post-hatch, fry (<1 g) were transferred to a flow-through system containing twelve 0.5 m³ circular tanks. Study treatments (see below) began at 18.04 g $\pm$ 0.47 g mean fry weight and concluded at 341 days post-hatch (approximately 950 g $\pm$ 11.9 g mean weight among all treatment groups).

2.1 Experimental Conditions

A 2 x 2 factorial study design (n = 3) was utilized with all 12 flow-through circular tanks, with juvenile rainbow trout being exposed to either high or low DO (100% vs. 70% saturation, respectively) and high or low swimming speed (1.5 - 2.0 BL/s vs <0.5 BL/s, respectively), and with tanks being assigned a particular treatment regime based on random number selection. Methodologies to induce the experimental conditions are

described in detail by Waldrop et al. (2018). Briefly, each tank was retrofitted with a magnetic pump connected to an inlet and discharge manifold, inducing rotational water velocities for swimming exercise by removing water from the tank and pumping it back through the discharge manifold. Water was injected beneath the surface to reduce surface agitation and the potential for tank dissolved gas conditions being disrupted. Maximum pump output was constant, and therefore the discharge manifold angle was altered to increase or decrease velocities to achieve targeted BL/s swimming speeds as fish length increased over time. Each tank was also fitted with a water manifold, providing incoming spring water and additional rotational flow; likewise, the water manifold angle could also be adjusted to supplement water velocity control if necessary. Combined flows provided exercised groups with at least 1.5 BL/s swimming speed for the duration of the experiment, while non-exercised fish were exposed to water velocities of <0.5 BL/s via perpendicular angle placements of the manifolds. Rotational velocities were measured regularly by timing floating velocity spheres at various points along tank perimeters, as well as with an underwater flow meter.

For the high and low DO treatments, tank effluents were maintained at approximately 100% and 70% saturation, respectively, with twice-weekly measurements carried out using a Hach portable dissolved oxygen Flexi HQ 30D meter (Hach, Loveland, Colorado, USA). Oxygen solubility tables (Colt, 1984) were consulted to set target DOs of 10.5 mg/l and 7.3 mg/l for the 100% and 70% saturation tanks, respectively. Dissolved oxygen levels were adjusted with each feeding rate change, as well as in response to tank biomass increase over time, through either increasing or decreasing oxygen gas flow and/or increasing or decreasing total influent water flow

rates. Normally, degassed water entering tanks in this particular system first passes through a modified packed column oxygen vessel adding pure oxygen; however, in this experiment a water line bypassing the packed column oxygen vessel was installed to supply degassed spring water (referred to as “bypass” water) to the six 70% DO treatment tanks, while 100% DO tanks received water as per usual through the modified oxygen pressure vessel.

Tank densities were allowed to reach a maximum of 80 kg/m³, at which point densities were reduced to approximately 40 kg/m³ through culling. A standard commercial feed was administered to all tanks by a computer-controlled program, with daily feed levels being determined via established rainbow trout feed charts fine-tuned through daily observations of feeding activity as well as tank-side triple sumps that settled out feed and feces leaving each tank.

2.2 Data collection and analysis

2.2.1 Water quality monitoring

In addition to DO measurements described above, carbon dioxide (CO₂), total gas pressure (TGP), pH, alkalinity, total suspended solids (TSS), nitrite nitrogen (NO₂-N), and total ammonia nitrogen (TAN) measurements were taken on a weekly basis. Total ammonia nitrogen and NO₂-N were determined using Hach methods 8038 and 8507, respectively and employing a Hach spectrophotometer (Model DR/4000). Total gas pressure was determined via DO measurements in combination with an In-Situ Model 300E tensionometer (In-Situ, Inc., Fort Collins, Colorado, USA). Standard methods

(APHA, 2005) were employed for quantifying TSS (method 2540), CO₂ (method 4500-CO₂), and alkalinity (method 2320).

2.2.2 Fish performance and welfare

Fish growth performance, feed conversion, survival, and fin condition were measured or calculated at regular intervals throughout the study. Monthly length and weight sampling was carried out after sedating the fish with 75 mg/L tricaine methanesulfonate (Tricaine-S; Western Chemical Inc., Ferndale, WA, USA). Sample sizes were determined using the formula:

$$n = (Z * (\text{stdev.}_{\text{grams}} / \text{accepted error}_{\text{grams}}))^2$$

where $Z = 1.95$ (relative to a 95% confidence interval), assuming an accepted error of 5 grams. Condition factor (K) was calculated using the formula:

$$K = 100 * W \text{ (g)} / L \text{ (cm)}^3$$

Feed conversion ratio (FCR) was calculated by dividing the total amount of feed administered (kg) by the total weight gain (kg) observed in each tank. Mortality data were collected daily, while fin erosion, an established indicator of fish welfare (Ellis et al, 2008), was assessed qualitatively during the final length and weight sampling based on a three-point visual scale (none/mild, moderate, or severe) for dorsal, caudal, and pectoral fins. Cardiosomatic indices were calculated from data collected during the final

performance sampling event, wherein 5 fish per tank were humanely euthanized using 200 mg/L tricaine methanesulfonate followed by carefully removing and weighing the hearts, in order to assess heart weight as a proportion of total body weight.

Statistical analyses were performed using STATA 9 software (Stata Corp LP, College Station, Texas, USA) using multivariable ANOVA models with DO, swimming speed, and an interaction term serving as independent variables. Non-normally distributed outcome variables were assessed non-parametrically for association with each treatment variable via the Kruskal-Wallis rank test. A level of $p \leq 0.05$ was used to determine significant relationships between independent and dependent variables.

2.2.3 Product Quality

At the end of the experiment, fish from each treatment were either butterfly filleted or processed as boneless/skinless fillets. Each of these fillet portions types were assessed for overall fillet yield (percentage fillet as a proportion of total body weight) and analyzed for cook yield % and texture (Kramer g/gwt). To determine cook yield %, fillet sections were thermally processed in a microprocessor-controlled smoked oven (Model CVU-490; Enviro-Pak, Clackamas, Oregon., U.S.A.) set at 82 °C; fillets were considered cooked once the internal fillet temperature reached 65.5 °C, with total cooking time being approximately 45 min. Once the product was cooked and had reached room temperature, weight was determined for cooked sections. The cook yield was calculated by expressing cooked weight as a percent of raw weight (Aussanasuwannakul et al, 2010). Texture was determined from the cooked fillet sections as measured using a 5-blade, Allo-Kramer shear attachment mounted to a TA-HDi[®] Texture Analyzer (Texture Technologies Corp.,

Scarsdale, NY, U.S.A.) which was equipped with a 50 kg load cell. Tests were performed at a crosshead speed of 127 mm/min and shear force was applied perpendicular to muscle fiber orientation. Force-deformation graphs were recorded and maximum shear force (g/g sample) was determined using the Texture Expert Exceed software (version 2.60; Stable Micro Systems Ltd., Surrey, U.K.). (Aussanasuwannakul et al, 2010).

3. Results

3.1 Water quality

Weekly measurements for each water quality parameter were averaged over the entire study period and summarized in Table 1. All measured water quality parameters were not statistically different ($p>0.05$) among treatment groups, and were within typical concentration ranges for normal fish culture. Mean total ammonia nitrogen (TAN) was slightly higher in the high DO groups, presumably due to higher feed consumption.

3.2 Fish performance and welfare

Dissolved oxygen had a significant, positive influence on final weight (Table 2, Figure 1). Swimming speed also had a positive effect on cardiosomatic index (Table 2). There were no significant differences among treatment groups for the other performance outcomes, namely condition factor, mortality, and feed conversion; however, at study's end, the higher caudal fin damage was associated with the low oxygen groups, while right pectoral fin damage was associated with the low swimming speed groups (Table 2).

3.3 Product quality

No significant differences in butterfly fillet yield (%), boneless/skinless fillet yield (%), cook yield (%) and texture (Kramer g/gwt) were observed among all treatment groups at study's end.

4. Discussion

Our results indicate that rainbow trout raised to a harvest size of 1 kg show increased growth performance when provided dissolved oxygen at 100% saturation, independent of whether the fish were exercised or not. Our previous study with Atlantic salmon (Waldrop et al. 2018) demonstrated that both exercise and 100% DO were significantly associated, independently, with increased growth performance. One explanation for the discrepancy between the results from Waldrop et al. (2018) and the present study, aside from the species examined, is the final fish size at study termination: the salmon in the previous study were grown to approximately 350 grams, while the rainbow trout were grown to over a mean weight of 1 kg. As the trout grew in size it became more difficult to maintain the higher BL/s swimming speed, and this may have influenced why swimming exercise had less of an impact on rainbow trout growth compared to Atlantic salmon. Swimming exercise, however, did have a significant effect on cardiosomatic index, an indicator of cardiac performance (Helland et al, 2009). Farrell et al. (1991) reported that exercised rainbow trout had an 18% increase in cardiac output and a 25% increase in maximum power output, while Poppe et al. (2003) determined differences in cardiac morphology between domesticated and wild salmonids, with domesticated individuals having more rounded ventricles in comparison with the triangular shaped ventricles and pointed apex of wild salmonids. Poppe et al. (2003) also

reported a strong positive correlation between the triangular shape and optimal cardiac performance, and that the abnormal rounded cardiac morphology was associated with higher mortality during stressful handling and sampling events. Therefore, providing fish with exercise could help aquaculture facilities reduce mortalities during times of stress. Castro et al. (2011) demonstrated that non-exercised Atlantic salmon had reduced disease resistance compared to those exercised either continuously or through interval training, and that heart health was improved with aerobic exercise through reducing inflammation and increased removal of free radicals.

Condition factor (K) is another fish performance and welfare parameter potentially linked to cardiac health. Claireaux et al. (2005) reported a correlation between rounded ventricles and higher condition factors. As condition factor is an index of fish stoutness as it relates to overall length and weight, higher than normal condition factor could be indicative of a lower exercise environment and the consequent higher deposition of intracoelomic fat. The highest condition factor ($K=1.82$) for any treatment group occurred in the lowest swimming velocity and highest oxygen treatment (Table 2); therefore, the higher growth associated with a 100% saturated oxygen environment could be counterproductive if no swimming exercise is provided.

Fish survival is another important performance and welfare parameter that can greatly affect the profitability and success of any aquaculture operation. In our study, no statistical difference in mortality was observed among treatment groups; however, the study had a final minimum harvest size of 1 kg, whereas some salmonids species have higher market size target weights. In such cases, it is possible that longer culture periods in the low swimming speed and DO environments could result in lower survival rates,

although further research is necessary to investigate this possibility. Problems such as increased fat deposition, hepatic lipidosis, and decreased cardiac performance over longer durations could result in higher mortalities and decreased fish performance.

Damaged or frayed fins can decrease fish welfare, aesthetics, and performance, and the causes and consequences of fin erosion have been reviewed by Latremouille (2003). Frayed fins can provide a portal for opportunistic pathogen entry and subsequent systemic infection (Turnbull et al., 1998), while market-size fish with poor fin quality can lead to challenges in selling whole fish (Klima et al., 2013). Cvetkovikj et al. (2013) reported that fin erosion was prevalent to some degree on all fins among cultured rainbow trout, and that the dorsal and pectoral fins were the most susceptible for erosion. Findings in the present study were similar; however, the higher dissolved oxygen treatments resulted in significantly less caudal fin erosion, while the higher swimming speed treatments results in significantly less pectoral fin erosion. This finding was expected, to an extent, as other studies have documented increased antagonistic behaviors and fin biting in low swimming environments (Christiansen & Jobling, 1990; Postlethwaite and McDonald, 1995); however, it was interesting that higher dorsal and caudal fin erosion was observed in the high swimming and low oxygen treatment. Higher oxygen may play an important role in allowing more growth and energy to be devoted to fin quality, but it is likely also important to maintain optimal swimming speed to prevent fish-to-fish contact (abrasion, biting) that can lead to fin erosion. Overall, the higher swimming speed and dissolved oxygen regimes reduced antagonistic behaviors amongst fish resulting in better fin condition, which is in agreement with Solstorm et al. (2015) who suggested that swimming speeds be kept at moderate speeds of 0.8 – 1.0 BL/s to minimize the effects of

antagonistic behaviors but also keep the fish from being stressed and exhausted from faster water velocities.

No statistical significances among product quality parameters were noted between treatment groups; proximate analyses, butterfly fillet yield, boneless fillet yield, cook yield and texture were all not associated with one or more specific treatments. One major criterion of flesh quality is texture, which is determined by muscle cellularity and connective tissue characteristics (Johnston, 1999; Kiessling et al., 2004). Previous exercise studies with Atlantic salmon (Totland et al., 1987) and brown trout (Bugeon et al., 2003) have shown improvements to the textural characteristics of flesh quality when fish are exercised. Totland et al. (1987) reported a 38% growth increase in exercised Atlantic salmon, mainly as increased white muscle growth. Given that Sanger and Stoiber (2001) determined that white skeletal muscle accounts for up to 98% of the edible fillet, with a minimum value of 70%, this suggests a likely mechanism for how texture can be affected by swimming exercise. No significant differences were found in product quality parameters during the present experiment, however, but it should be noted that these product quality parameters, especially fillet texture, can be strongly influenced by a variety of factors at harvesting time, such as feeding, fish handling, processing, and storage temperatures of slaughtered fish. It is therefore possible that one or more of these additional factors served to obscure a relationship between swimming exercise and improve fillet texture.

Lastly, fish growth is perhaps the most important performance outcome measured by aquaculture producers, and our findings confirm better growth performance in oxygen saturated environments, with trout in the high DO treatment having a 17% growth

advantage compared to those raised in low DO. Faster growth rates over the life of a production cohort can increase operational profitability by shortening production cycles and allowing for extra harvests over time. There are numerous possible explanations for the differences in growth rate observed in our study. Although not significantly different in this study, feed conversion rates were generally lower in the high DO regimes, and given that exercised fish had a higher intake of feed with a lower feed conversion, exercised fish were likely more metabolically efficient than unexercised cohorts.

Grisdale-Helland et al. (2013) concluded that continuously exercised post-smolt Atlantic salmon were able to respond to higher activity costs by utilizing energy and protein more efficiently for growth. Houlihan and Laurent (1987) demonstrated that rainbow trout forced to swim at 1.0 BL/s grew almost twice as fast and converted protein more efficiently than unexercised counterparts over a 6-week period. The faster growth yet similar feed conversion rates observed in the present study could be from exercised fish offsetting the increased energetic costs of swimming, i.e., protein utilization was more efficient when converting the feed to flesh. A similar trend (higher oxygen, slightly better feed conversion rates, and increased growth) was observed in our previous exercise and DO study with Atlantic salmon (Waldrop et al., 2018). Additional previous research has also supported these findings: Lovell (1989) reported that fish convert food less efficiently as DO decreases; Pedersen (1987) suggested that oxygen levels should be at least 7 mg/L for optimized feed conversion and growth in juvenile rainbow trout; and Saravanan et al. (2013) reported that rainbow trout kept in hypoxic conditions (6.0 mg/L) demonstrated substantially lower feed intake than those fed under normoxic conditions (Saravanan et al, 2013). This lower feed intake during hypoxic conditions is likely

associated with the consequent reduction in metabolic scope of aerobic activities and metabolism (Glencross, 2009).

In conclusion, the results of our study suggest that swimming exercise and DO at saturation during the culture of rainbow trout can be beneficial to producers through improved growth performance and cardiac health. Facilities utilizing salmonids and other species with a high metabolic scope and positive rheotaxis may benefit by maintaining dissolved oxygen at saturation and providing moderate swimming exercise in the right balances.

6. References

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Table 1. Mean ($\pm$ SE) water quality parameters and conditions during the study period.

Condition	<u>DO 100% saturation</u>		<u>DO 70% saturation</u>	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
<i>Treatments</i>				
Velocity (BL/s)	1.66 $\pm$.015	0.55 $\pm$.012	1.65 $\pm$.015	0.522 $\pm$ 0.10
DO (mg/l)	10.2 $\pm$.071	10.4 $\pm$.079	7.14 $\pm$.043	7.29 $\pm$.033
<i>Water quality</i>				
TAN (mg/l)	0.231 $\pm$ 0.018	0.210 $\pm$ 0.018	0.174 $\pm$ 0.010	0.166 $\pm$ 0.009
NO ₂ -N (mg/l)	0.00405 $\pm$ 0.000468	0.00322 $\pm$ 0.000289	0.00340 $\pm$ 0.000376	0.00332 $\pm$ 0.001037
Alkalinity (mg/l)	277 $\pm$ 2.12	274 $\pm$ 2.30	273 $\pm$ 2.06	272 $\pm$ 2.52
CO ₂ (mg/l)	18.3 $\pm$ 0.401	18.8 $\pm$ 0.372	18.8 $\pm$ 0.312	18.4 $\pm$ 0.303
TSS (mg/l)	1.58 $\pm$ 0.150	1.69 $\pm$ 0.133	1.78 $\pm$ 0.241	1.71 $\pm$ 0.209
TGP (%)	95.4 $\pm$ 0.336	95.8 $\pm$ 0.341	95.7 $\pm$ 0.322	95.6 $\pm$ 0.256
Temperature (°C)	13.6 $\pm$ 0.043	13.5 $\pm$ 0.035	13.6 $\pm$ 0.038	13.6 $\pm$ 0.036

Table 2. Final rainbow trout performance, health and welfare results (means $\pm$ SEs).

Outcome [*]	<u>DO 100% saturation</u>		<u>DO 70% saturation</u>	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
Final weight (g) ^b	1048 $\pm$ 41	990 $\pm$ 56	868 $\pm$ 9	887 $\pm$ 15
K	1.75 $\pm$ 0.08	1.82 $\pm$ 0.02	1.68 $\pm$ 0.06	1.65 $\pm$ 0.12
FCR	1.25 $\pm$ 0.02	1.24 $\pm$ 0.06	1.31 $\pm$ 0.02	1.26 $\pm$ 0.02
Mortality (%)	2.12 $\pm$ 0.82	1.78 $\pm$ <0.01	0.94 $\pm$ 0.31	1.65 $\pm$ 0.24
Cardiosomatic index ^a	0.00122 $\pm$.00015	0.00109 $\pm$.00011	0.00124 $\pm$ 0.00021	0.00111 $\pm$ 0.00012
<i>Fin score</i>				
Dorsal	0.413 $\pm$ 0.081	0.693 $\pm$ 0.082	0.813 $\pm$ 0.090	0.573 $\pm$ 0.083
Caudal ^b	0.253 $\pm$ 0.054	0.267 $\pm$ 0.061	0.653 $\pm$ 0.087	0.427 $\pm$ 0.081
Right pectoral ^a	0.066 $\pm$ 0.044	0.227 $\pm$ 0.065	0.067 $\pm$ 0.035	0.253 $\pm$ 0.071
Left pectoral	0.013 $\pm$ 0.013	0.081 $\pm$ 0.037	0.053 $\pm$ 0.033	0.120 $\pm$ 0.058

^{*} Superscripts indicate parameters significantly ($p < 0.05$) impacted by swimming speed (a) and/or dissolved oxygen (b); fin score based on 0=None/Mild, 1=Moderate, 2=Severe

Table 3. Fillet quality attribute summary for processed rainbow trout at study conclusion.

Outcome	<u>DO 100% saturation</u>		<u>DO 70% saturation</u>	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
Butterfly Fillet Yield (%)	66.30 ± 2.923	66.20 ± 2.442	66.76 ± 2.125	67.24 ± 3.622
Boneless/Skinless Fillet Yield (%)	46.20 ± 3.157	46.59 ± 2.756	46.68 ± 2.791	48.81 ± 2.203
Cook Yield (%)	88.04 ± 2.878	89.09 ± 1.804	88.07 ± 1.349	88.77 ± 2.852
Texture (Kramer g/gwt)	309.3 ± 115.5	272.2 ± 76.74	284.1 ± 81.20	330.1 ± 91.04

Figure 1. Rainbow trout growth performance among treatment groups up to 338 days post-hatch. Error bars represent standard errors.

Appendix:

Figure 1. Experimental tanks used for both the Rainbow trout and Atlantic salmon portions experiments.

Figure 2. Individual tank used in the experiment. A total of 12 experimental tanks were utilized.

Figure 3. A single feed controller for all experimental tanks. All feeders were controlled by this unit.

Figure 4. Automatic feeder on each experimental tank.

Figure 5. Each experimental tank utilized a separate waste sump tank. The sump tank may it very easy to distinguish between over feeding (presence of extra feed) and underfeeding (little feed present).

Figure 6. Velocity control pump for each experimental tank.

Figure 7. Velocities in each tank were easily manipulated by valves and positioning of tank manifolds.

Figure 8. Rotational velocity in high swim speed treatment tanks.