The effect of water currents on post-smolt Atlantic salmon, *Salmo salar* (L.)

A welfare approach to exposed aquaculture

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Scientific environment

The work presented in this thesis was conducted at the Institute of Marine Research (Norway) from 2011 through 2015. During this period, I was part of the Animal Welfare research group under the Aquaculture Programme. The laboratory work was performed at the Institute of Marine Research Station in Matre, and field observations were conducted at a commercial salmon sea cage in the Faroe Islands. In my work, I have been supported by my supervisors and their institutes, the Institute of Marine Research and the University of Bergen, in addition to our collaborative partners at the University of Copenhagen.

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Summary

The future development of Atlantic salmon (*Salmo salar* L.) aquaculture is limited by the availability of space and suitable environments along coastal areas. Utilising exposed areas is a possible solution to this problem. The technology for offshore and exposed aquaculture is well developed, but there is a lack of knowledge concerning the effect of fast water current velocities on fish. In some locations, velocities exceed what is considered the critical swimming speed for salmon. Exposing salmon to such fast currents could severely compromise their welfare. Therefore, it is of utmost importance to evaluate the effects of strong water currents on individual fish.

As a first approach, field observations were conducted at an exposed site where salmon behaviour was monitored in relation to current velocity. A behavioural adaptation to fast currents was observed at an ambient current velocity of approximately 0.7 BL s^{-1}. At this velocity, some fish broke their circular schooling behaviour and maintained position against the current, creating a mix of swimming behaviours, i.e., both circular and standing on the current. Further increases in velocity (0.9 BL s^{-1}) resulted in all fish abandoning the circular swimming behaviour to stand on the current.

To explore the effect of a single parameter, water current velocity, a six-week laboratory experiment was conducted. Atlantic salmon post-smolt were exposed to three different velocities in raceways; slow (0.2 BL s^{-1}), moderate (0.8 BL s^{-1}) and fast (1.5 BL s^{-1}). The setup forced the fish to primarily swim at the prevailing velocity. Fish growth, muscle composition and blood chemistry were analysed before and after the experiment. All fish were also examined for fin erosions, external injuries, skeletal morphology and bone composition. The fish behaviour was studied in detail throughout the experiment.

The setup did not allow for circular swimming behaviour, as can be observed in sea cages. However, the plasticity of salmon behaviour was also clear in this setup, and the fish adapted quickly to the prevailing conditions. Slower velocities gave the fish more

opportunities to interact with each other, whereas fish in the fast current velocity mainly swam against the current.

A fast velocity also resulted in isometric growth that was lower than was observed for moderate and slow velocities. Fish kept in a slow or moderate current velocity experienced the same weight gain, but fish at slow velocity gained more fat, whereas fish at moderate velocity gained more protein in white muscle. This suggests that a moderate swimming speed is optimal in terms of production performance. Additionally, individuals at slow velocity had elevated levels of stress hormones, thus indicating that this velocity is stressful. In sea cages, slow velocities are typically related to poor water quality, but in the laboratory experiment, the stress was likely a response to interacting behaviours.

The fin quality of the fish was best at slow and moderate velocity. At fast velocity, an increase in new fin erosions due to unintentional collisions with obstacles and other fish was observed. This should be considered in exposed aquaculture, where fish may have difficulties holding their positions in fast currents.

Water current velocities also affected the vertebrae in terms of morphology, mineral rate and mineral composition. Region-specific effects were observed: mineral rate increased with velocity in the post-cranial and anterior-caudal regions, and relative vertebrae length increased in the post-cranial region and decreased in the ural region. Velocity effects on mineral composition differed between regions and minerals studied. This highlights the importance of considering which region to study.

In conclusion, this thesis represents a first approach for studying how fish are affected by currents in aquaculture. It emphasises the importance of including water current as a parameter in all types of aquaculture. Currents have been demonstrated to cause both positive and negative effects and can be used as an important tool for optimising production and welfare.

List of publications

Paper I

Johansson, D., Laursen, F., Fernö, A., Fosseidengen, J. E., Klebert, P., Stien, L. H., Vågseth, T., & Oppedal, F. (2014): "The interaction between water currents and salmon swimming behaviour in sea cages", PLoS ONE 9:e97635.

Paper II

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Paper III

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Paper IV

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H., & Fernö, A., (2016): "Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon, *Salmo salar*", Aquaculture Environment Interactions, Vol. 8: 339-347.

Contents

1. Introduction

Future expansion of Atlantic salmon (*Salmo salar* L.) aquaculture is limited by available space in easily accessible and sheltered areas. Use of more exposed sites with fast water currents is warranted to further utilise the potential and to optimise production. The technology for exposed sea cages is well developed (Fredheim & Langan, 2009). However, the present scientific knowledge about the physiology, behaviour and swimming capacity of Atlantic salmon at exposed sites is limited and needs to be expanded to ensure efficient production and fish welfare.

1.1 Swimming in salmon

1.1.1 Wild salmonids

Atlantic salmon is an anadromous species with a complex lifecycle. Spawning occurs in fast-flowing rivers. After hatching, salmon are constantly subjected to water currents of varying velocity. The fish must swim against the current or anchor themselves to the bottom to maintain their position in the stream (Arnold et al., 1991). Swimming in this juvenile life stage is typically driven by feeding, predatory avoidance and territorial interactions (McCormick et al., 1998). Juvenile salmon can swim in bursts at more than 5 body length (BL) s^{-1} (Peake & McKinley, 1998). After smoltification, salmon start to swim with the current to reach the sea, where the major growth phase takes place. During this part of their lifecycle, salmon swim on average at 1 BL s^{-1} (reviewed by Drenner et al., 2012). Swimming in the ocean is typically driven by foraging. As they start to mature, salmon migrate back to the river of origin where they spent their juvenile phase.

1.1.2 Salmonids in aquaculture

Salmon in aquaculture are typically subjected to an environment in which few of the motivational drivers in the wild exist. During the freshwater phase, fish are typically kept in indoor tanks with good availability of food, no predators and at densities that minimise territorial interactions (Fernö & Holm, 1986). After smoltification, the fish

are transferred to large sea cages, where food is abundant and predatory attacks are seldom. Even if motivational drivers such as foraging and predator avoidance are limited, salmon in sea cages have been reported to swim at 0.3-3 BL s^{-1}, depending on environmental conditions (Andrew et al., 2002; Blyth et al., 1993; Dempster et al., 2008; Kadri et al., 1991; Korsøen et al., 2009; Korsøen et al., 2012; Pinkiewicz et al., 2011; Sutterlin et al., 1979). Some of these variations in swimming speed can be related to missing data regarding current velocities because swimming speed is rarely studied in relation to the ambient current velocity. At commercial densities, salmon typically form a circular swimming pattern, possibly to avoid collisions with the sea cage and other individuals (Fernö et al., 1995; Føre et al., 2009; Juell, 1995; Juell & Westerberg, 1993). The circular swimming pattern appears to be density-dependent (Juell & Westerberg, 1993) in addition to light-dependent (Oppedal et al., 2001). In some studies, fish have also been observed to hold their position swimming against a current (Oppedal et al., 2001; Smith et al., 1993).

Fish in sea cages cannot migrate to more favourable areas and must cope with the prevailing environment. Environmental preferences in terms of temperature, light and oxygen may act as motivational drivers (Johansson et al., 2006; Oppedal et al., 2007) that results in movements within cages to preferred areas. Johansson et al. (2006) observed that fish in sea cages clustered at high densities, presumably to avoid suboptimal areas. Atlantic cod (*Gadus morhua*) increased their swimming speed as a first response to sudden decreases in oxygen, but at lower oxygen levels, the swimming speed dropped by 41% (Herbert & Steffensen, 2005). The initial response may be linked to a flight response, whereas the subsequent decrease in activity could reduce the metabolic oxygen demand. Light has also been demonstrated to affect swimming speed. Salmon typically swim more slowly at night (Korsøen et al., 2009) when circular swimming behaviour ceases (Fernö et al., 1988; Juell, 1995; Korsøen et al., 2009; Oppedal et al., 2001). This could relate to a difficulty in orientating in the dark because artificial light during the night reinstates a circular swimming behaviour (Oppedal et al., 2001).

Increased water current velocities have been demonstrated to affect behaviour and induce schooling behaviour in rainbow trout (Larsen et al., 2012). Schooling behaviour in sea cages is linked to reduced interactions, increased appetite and a reduced stress level for individual fish (Fernö et al., 1988; Juell, 1995). Additionally, the velocity of the current could affect which behavioural options are available to the fish. Slow current velocities allow the fish to choose their swimming speed, whereas fast currents force the fish to swim at the prevailing water velocity to maintain their position within the cage. Tudorache et al. (2011) demonstrated that adult brook charr had a preferred swimming speed of 0.78-0.95 BL s^{-1}, which is similar to the energetically optimal speed (lowest cost of transportation, COT) of 1.02 BL s^{-1}. This suggests that the preferred swimming speed in aquaculture could be equivalent to the optimal swimming speed in nature when no other limiting or directing factors exist.

1.1.3 Effects of increased water current velocity

Increased swimming speeds at increased water current velocities should contribute to higher exercise levels for the fish. Exercise is known to generate several positive effects in terms of morphology, behaviour, growth and physiology.

Morphological effects
Skeletal growth is often neglected in production performance trials, although increased swimming speed has been demonstrated to enhance bone mineralisation and reduce vertebrae deformations (Deschamps et al., 2009; Totland et al., 2011). Furthermore, studies are often restricted to specific vertebrae regions, which increases the likelihood of missing regions of importance. In mammals, high mechanical loading in a region has been demonstrated to enhance bone formation (Carter et al., 1996; Currey, 1984; 2003; Huang et al., 2003; Notomi et al., 2001; Timlin et al., 2000). A similar effect is likely in the vertebrae column of fish, but this has not been thoroughly investigated.

Behavioural effects
When fish are forced to swim against a current, agonistic behaviours are reduced (Adams et al., 1995; Christiansen & Jobling, 1990; East & Magnan, 1987; Jørgensen & Jobling, 1993). In standing water juvenile salmonids are likely to display territorial

behaviour, resulting in aggressive interactions (Keenleyside & Yamamoto, 1962). These aggressive encounters have a substantial energetic cost that can exceed the cost of swimming and result in reduced growth (Christiansen & Jobling, 1990; East & Magnan, 1987). Agonistic behaviours are also linked to the prevalence and frequency of fin erosion (Turnbull et al., 1998) with an increased susceptibility to pathogen infections (Schneider & Nicholson, 1980; Turnbull et al., 1996). Hence, increased current velocities could lead to reduced aggression and improved fin quality and disease resistance. A more organised group structure could result in a less stressful environment and increased production performance (Adams et al., 1995; Christiansen & Jobling, 1990; East & Magnan, 1987; Jørgensen & Jobling, 1993).

Growth

The optimal current velocity for growth in salmonids varies between 0.45 and 1.75 BL s^{-1} (Castro et al., 2011; Christiansen & Jobling, 1990; East & Magnan, 1987; Houlihan & Laurent, 1987; Jørgensen & Jobling, 1993; Totland et al., 1987). The optimal velocity appears to vary not only with species, size and life stage but also with experimental setup (Table 1). The fastest optimal current velocity, 1.75 BL s^{-1}, was reported by Christiansen & Jobling (1990) for juvenile Arctic charr in circular tanks with no areal restriction (hereafter referred to as open circular tanks). On the other end of the scale is the study by Totland et al. (1987), who reported an optimal velocity of 0.45 BL s^{-1} for adult Atlantic salmon. Their study was conducted in ocean raceways. The reference was a normal cage with a maximum current velocity of less than 0.1 BL s^{-1}. As an average from the studies presented in Table 1, approximately 1 BL s^{-1} appears to be optimal. Castro et al. (2011) observed optimal growth for juvenile Atlantic salmon at this velocity in a setup with a closed off section in a circular tank, i.e., where the fish could not move faster or slower than the prevailing current velocity (closed circular tanks). Rainbow trout kept in the same type of setup at a current velocity of 1 BL s^{-1} doubled their growth compared to controls (Houlihan & Laurent, 1987). The exercise induced growth effects are likely related to increased protein synthesis and stimulation of hypertrophy in the skeletal muscle (Bugeon et al., 2003; Houlihan & Laurent, 1987; Johnston, 1999).

Table 1 Overview of studies in which current velocity is related to optimal growth performance. 'Open circular' refers to circular tanks with no areal restrictions, and 'closed circular' refers to circular tanks in which down- and upstream movements were hindered by closing off a section. All studies had a control velocity close to zero.

Current velocity (BL s^{-1})		Species	Life stage	Experimental setup	References
Optimal	Worst				
1.75	0	Arctic charr	Juvenile	Open circular	Christiansen & Jobling (1990)
1.5	0	Arctic charr	Juvenile	Open circular	Christiansen et al. (1992)
0.8-1.0	0.05	A. salmon	Juvenile	Closed circular	Castro et al. (2011)
1.5-1.3	0.4-0.3	A. salmon	Juvenile	Closed circular	Castro et al. (2013b)
1.5	0	A. salmon	Juvenile	Open circular	Jørgensen & Jobling (1993)
0.45	0.1	A. salmon	Adult	Raceway	Totland et al. (1987)
0.85	2.5	Brook charr	Juvenile	Open circular	East & Magnan (1987)
1.0	0.1	Brown trout	Juvenile	Open circular	Bugeon et al. (2003)
1.5	4.5	Brown trout	Juvenile	Raceway	Davison & Goldspink (1977)
0	1.0-1.4	Rainbow trout	Adult	Raceway	Farrell et al. (1991)
1.0	0	Rainbow trout	Juvenile	Closed circular	Houlihan & Laurent (1987)

Physiological effects

Exercise has been shown to induce several physiological effects in fish. Positive effects include increased muscle energy (McFarlane & McDonald, 2002; Pearson et al., 1990), cardiac output (Farrell et al., 1991), relative ventricular mass (Castro et al., 2013a; Gallaugher et al., 2001), intestinal blood flow (Thorarensen et al., 1993), aerobic capacity (Farrell et al., 1990) and metabolic scope (Larsen et al., 2012). A study by Castro et al. (2011) also suggests that exercise can help fish to better cope with different challenges, such as pathogens.

Exercise increases the energy stores in muscle in terms of glycogen reserves, ATP and CrP (McFarlane & McDonald, 2002; Pearson et al., 1990). An increase in muscle energy due to exercise improves the swimming capacity (McFarlane & McDonald, 2002) and may hence improve the ability to cope with peak velocities of strong currents. Increased lipid stores have also been demonstrated following exercise (East & Magnan, 1987) but only up to a velocity of 0.85 BL s^{-1}, after which increased energy demands reduce the lipid stores. Exercise may also increase lipid metabolism (Farrell et al., 1991; Lauff & Wood, 1996), which results in fish with a low lipid content.

Cardiovascular benefits are another positive effect of exercise. The aerobic capacity can be improved by increased cardiac output, as observed in rainbow trout (Farrell et al., 1991). In some studies, sustained swimming with a corresponding increase in cardiac workload has been correlated with an increase in ventricular mass (Castro et al., 2013a; Gallaugher et al., 2001). The aerobic capacity is also improved by increased capillarisation in muscle (Davie et al., 1986). Increased aerobic capacity has been linked to increased maximum metabolic rate as an effect of increased oxygen extraction by tissues (Gallaugher et al., 2001), which in turn may aid several physiological functions during swimming. This could be beneficial when swimming fish are forced to cope with new challenges.

During intensive swimming, the surface area and permeability in the gills increase to allow for higher oxygen uptake. This could result in loss of water across the gills in sea water fish and an osmotic imbalance (Randall et al., 1972). However, Gallaugher et al. (2001) observed an increased capacity to maintain osmotic balance during intensive swimming in exercised fish. This may be explained by an increase in intestinal blood flow that leads to a better water uptake, which compensates for the loss over the gills (Thorarensen et al., 1993).

Swimming performance

The positive effects of exercise on the cardiovascular system can result in positive effects on swimming performance (Castro et al., 2011; Farrell et al., 1990; Houlihan & Laurent, 1987), but exceptions do exist with no observed effects (Farrell et al., 1991; McKenzie et al., 2012; Thorarensen et al., 1993). Swimming performance is traditionally tested using a swimming respirometer in a critical swimming speed test (U_{crit}). The test was originally developed by Brett (1964; 1967) as a measure of the maximum sustainable swimming speed. An individual fish is usually placed in a respirometer and then forced to swim at current velocities incremented in a stepwise manner until fatigue sets in. Then, U_{crit} can be calculated from the time intervals and increment steps. U_{crit} tests are a well-established measure of swimming performance that can be correlated to several biotic and abiotic factors, such as exercise level, size, temperature, nutritional status, stress, available space in the respirometer and

acclimation time (Beamish, 1978; Brett, 1964; 1967; Hammer, 1995; Martinez et al., 2004; Nelson et al., 2002). It has the potential for providing an absolute upper limit to current velocities in aquaculture systems. However, there is great variability in published U_{crit} values, and few studies have investigated U_{crit} for post-smolt or adult Atlantic salmon. There is also little information about the maximum swimming capacity of schools of salmon, which would be more relevant to exposed aquaculture than studies of individual fish. In schooling fish, hydrodynamic benefits likely increase the swimming speed (Fields, 1990; Herskin & Steffensen, 1998; Svendsen et al., 2003). Reported U_{crit} values vary between 1.6 and 3.0 BL s^{-1} (Cotterell & Wardle, 2004; Deitch et al., 2006; Lijalad & Powell, 2009; McKenzie et al., 2012; Wagner et al., 2003; Wagner et al., 2004; Wilson et al., 2007). The faster swimming speeds are consistent with what have been reported as the maximum voluntary swimming speeds in cages (0.3-3 BL s^{-1}) (Andrew et al., 2002; Blyth et al., 1993; Dempster et al., 2008; Kadri et al., 1991; Korsøen et al., 2009; Korsøen et al., 2012; Pinkiewicz et al., 2011; Sutterlin et al., 1979). However, if the voluntary swimming speed is as high as 3 BL s^{-1}, then the critical swimming speed is likely to be higher. It is possible that the voluntary swimming speed in sea cages has been overestimated due to the ambient current velocity. However, it is also possible that the use of small swimming chambers (chamber/fish length ratio 1:1.6-1:5.2) (Deitch et al., 2006; Lijalad & Powell, 2009; McKenzie et al., 2012; Wagner et al., 2003; Wagner et al., 2004; Wilson et al., 2007) imposes space restrictions that hinder anaerobe swimming behaviours such as bursts and hence cause an underestimate of the maximum swimming speed (Burgetz et al., 1998; Jayne & Lauder, 1996; Peake & Farrell, 2004). Testing swimming performance in a situation in which the swimming behaviour is restricted may not be relevant to commercial situations. For sites with fast water currents, it is of utmost importance to collect reliable data to determine the maximum capacity of fish.

1.2 Exposed aquaculture

Water currents play a crucial role in aquaculture. Slow water currents may generate unfavourable environments for the fish due to low water exchange (Johansson et al.,

2007). Oxygen levels may drop and metabolites increase, thus creating a stressful or harmful environment (D. Solstorm, unpublished data). The lack of suitable environments and conflicting interests in sheltered areas have led to more sea cages being located at exposed sites with fast water currents and high waves. Increased current velocities are often perceived as beneficial because of increased water exchange, which ensures good water quality in the cage (Holmer, 2010). The technology for constructing aquaculture systems that can withstand such external forces are available (Fredheim & Langan, 2009) however, there is a lack in knowledge as to how the fish cope with such conditions. In the Faroe Islands salmon sea cages are located at sites with water currents of up to 80 cm s^{-1} (Øystein Patursson, Aquaculture Research Station at the Faroes, pers. comm.), corresponding to a swimming speed of approximately 4 BL s^{-1} in newly stocked post-smolts. This is far above the documented U_{crit} for post-smolt and adult salmon (2.2-3.0 BL s^{-1}) (Cotterell & Wardle, 2004; Deitch et al., 2006; Lijalad & Powell, 2009; Wagner et al., 2003). Despite this, locations with water currents of up to 120 cm s^{-1} are currently being considered for salmon aquaculture (Øystein Patursson, Aquaculture Research Station at the Faroes, pers. comm.). If available data regarding limitations in salmon swimming speed are correct, such locations would seriously compromise salmon welfare.

Within limits, increased swimming speed is assumed to benefit fish. However, a study of adult rainbow trout did observe a decrease in production performance already at 1.0-1.4 BL s^{-1} (Farrell et al., 1991). U_{crit} is an assessment of the limit to which a fish can perform under given environmental conditions, but this is not the optimal speed for high production performance or welfare. It may be devastating for the fish if water currents as fast as U_{crit} are considered acceptable in aquaculture. Excessive swimming speeds may lead to exhaustion, depletion of energy stores and accumulation of lactate. An increase in intracellular lactate will result in osmotic imbalances, with an increase in plasma electrolytes (Wood, 1991). No possibility for recovery after exhaustive exercise may lead to death. Welfare is most likely compromised long before fatal velocities are reached.

1.2.1 Welfare aspects

When evaluating exposed sites for aquaculture, it is of utmost importance that the welfare aspects are considered. Welfare is as much a production issue as an ethical issue because good welfare will contribute to improved production performance and disease resistance (Read, 2008). Since 1996, the five freedoms for ensuring animal welfare, defined by the UK Farm Animal Welfare Council (1995) have included fish. In recent years, the freedoms have been debated, and among other proposals, a welfare concept based on allostasis has been suggested (Korte et al., 2007). It has been argued that total freedom may contribute to hypostimulation and that welfare should be based on the capacity to change and adapt to internal and external stressors. However, hypostimulation may not be a serious problem in exposed aquaculture because the fish are constantly subjected to a challenging environment. Using the allostasis concept, it may be useful to consider freedoms as freedoms from chronic states in which the fish are no longer able to adapt. Here, the five freedoms are considered in relation to the allostasis concept in an attempt to evaluate how exposed aquaculture could affect fish welfare.

The first freedom, "Freedom from Hunger and Thirst", could be a concern in exposed areas in which fish swimming at high speeds have a higher energy demand. It may also be a challenge to dispense feed without pellets being carried away by the current, resulting in underfeeding.

The second freedom, "Freedom from Discomfort", may be interpreted as assuring a good environment with a high water quality. In exposed areas with a high water exchange rate, waste products are rapidly transported away from the cage and fresh oxygenated water is transported in, which should contribute to a better water quality and better welfare than in areas with a low exchange rate.

Exposed aquaculture could also be beneficial with respect to the third freedom "Freedom from Pain, Injury or Disease", because Castro et al. (2011) observed an increased disease resistance in exercised salmon. However, excessively strong currents

could have the opposite effect, because underfeeding is known to cause fin erosion (Noble et al., 2008).

The fourth freedom, "Freedom to Express Normal Behaviour", is debated in the context of aquaculture because normal in aquaculture is not the same as normal in the wild. The latest report from FAWC (2014) concludes that "greater consideration is needed of how farmed fish express normal behaviour, such as in feeding and social interaction". Currents have been reported to improve group structure and schooling behaviour (Christiansen & Jobling, 1990; Larsen et al., 2012), which may be considered normal behaviour in aquaculture. However, there is a possible risk for abnormal behaviours, such as monotonous and stereotypic behaviour, without social interaction (Ashley, 2007).

The fifth freedom "Freedom from Fear and Distress" can be related to the previous freedom, where abnormal behaviour may result in fear and distress.

To ensure sufficient welfare in exposed areas, all aspects need to be thoroughly investigated.

2. Aim of thesis

The overall aim of this thesis was to investigate the effect of different water currents on Atlantic salmon to acquire knowledge that could ensure acceptable welfare for fish in exposed aquaculture.

Paper I

The objective was to observe and evaluate the behavioural effects of fast water currents on site in exposed sea cages.

Paper II

The aim was to evaluate the effects of three different water current velocities on physiology and production performance in post-smolt salmon. We predicted that there is a limit in velocity below which positive effects are obtained and that excessive velocity has negative physiological effects and reduces production performance.

Paper III

The objective was to determine how three swimming speeds affect different regions along the vertebral column in terms of vertebra morphology, bone mineralisation and mineral substances.

Paper IV

The aim was to perform a detailed study of the behaviour at three different water current velocities and correlate the behaviour with fin erosion. We predicted that faster current velocities would lead to fewer aggressive encounters and less fin erosion.

3. Abstract of papers

Paper I

The interaction between water currents and salmon swimming behaviour in sea cages

David Johansson, Frida Laursen, Anders Fernö, Jan Erik Fosseidengen, Pascal Klebert, Lars Helge Stien, Tone Vågseth, Frode Oppedal

Positioning of sea cages at sites with high water current velocities expose the fish to a largely unknown environmental challenge. In this study we observed the swimming behaviour of Atlantic salmon (*Salmo salar* L.) at a commercial farm with tidal currents altering between low, moderate and high velocities. At high current velocities the salmon switched from the traditional circular polarized group structure, seen at low and moderate current velocities, to a group structure where all fish kept stations at fixed positions swimming against the current. This type of group behaviour has not been described in sea cages previously. The structural changes could be explained by a preferred swimming speed of salmon spatially restricted in a cage in combination with a behavioural plasticity of the fish.

Paper II

Fast water currents reduce production performance of post-smolt Atlantic salmon *Salmo salar*

Frida Solstorm, David Solstorm, Frode Oppedal, Anders Fernö, Thomas William Kenneth Fraser, Rolf-Erik Olsen

In the future, an increasing number of salmon farms may be located in areas with fast water current velocity due to limited availability of more sheltered locations. However, there is little information as to how fast currents affect fish health and welfare. We used raceways to expose Atlantic salmon post-smolts (98.6 g, 22.3 cm) to homogeneous water velocities corresponding to 0.2, 0.8 and 1.5 body lengths s^{-1} (slow, moderate and fast, respectively) over 6 wk. Fish at fast velocity had a 5% lower

weight gain compared to fish at moderate and slow velocities, with a corresponding reduction in length. Fish at moderate and fast velocities had lower lipid content in the muscle compared to fish at slow velocity. Hence, fish at slow and moderate velocities had the same weight gain, but fish at slow velocity gained more fat and fish at moderate velocity more muscle protein. Fish at fast velocity had a higher relative ventricular mass, indicating an increased cardiac workload. At slow velocity, individual fish displayed elevated plasma levels of lactate, osmolality and potassium. Our results suggest that post-smolts had the best growth and welfare at moderate velocity and that a current velocity of 1.5 body lengths s^{-1} could compromise production performance.

Paper III

The vertebral column and exercise in Atlantic salmon – Regional effects

Frida Solstorm, David Solstorm, Frode Oppedal, Per Gunnar Fjelldal

This study investigated the effect of swimming speed on the mineralization (mineral rate, Ca, P, Mg, Zn, K, Mn, Cu) and morphology of vertebra (V) in different anatomical regions of the vertebral column in Atlantic salmon. Triplicate groups of Atlantic salmon post-smolt were forced to swim at slow ($0.2 \pm$ SEM 0.02 BL s^{-1}), moderate ($0.8 \pm$ SEM 0.01 BL s^{-1}) and fast ($1.5 \pm$ SEM 0.02 BL s^{-1}) water current velocities for six weeks in raceways. The specific growth rate (SGR) was lower at fast (1.41 ± 0.04 SEM) compared to moderate (1.49 ± 0.02 SEM) and slow velocities (1.51 ± 0.03 SEM). In the post-cranial region (V1→8) fish at: (*i*) moderate and fast velocities developed higher relative V lengths, and mineral rate (% ash weight of dry weight), compared to fish at slow velocity; (*ii*) moderate velocity developed higher ratio between V length and dorso-ventral diameter (l/d-ratio) – more elongated V – and lower potassium ash content, compared to fish at slow, while those at fast velocity displayed intermediate values for both parameters. In the posterior-truncal region (V9→30), fish at moderate and fast velocity developed lower potassium ash content than those at slow. In the anterior-caudal region (V31→49), fish at: (*i*) fast velocity developed higher mineral rate than those at slow, with those at moderate displaying

intermediate values; (*ii*) moderate velocity developed lower phosphorus and magnesium ash contents than those at slow and fast. In the ural region (V50→ 58), fish at: (*i*) slow velocity developed higher relative V lengths than those at moderate and fast; (*ii*) slow velocity developed higher l/d-ratio than those at fast, with fish at moderate displaying intermediate values. Also, ontological shifts in mineral rate, and Ca and K ash contents along the spine were observed; with time, Ca increased in all regions except the ural, while K decreased in all regions except the ural, where it increased, accompanied with a decrease in mineral rate. The present results show that the positive effects of exercise on vertebrae in farmed Atlantic salmon are region specific.

Paper IV

Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*

Frida Solstorm, David Solstorm, Frode Oppedal, Rolf Erik Olsen, Lars Helge Stien, Anders Fernö

Increased swimming speed of Atlantic salmon is generally considered an improvement to welfare under aquaculture settings, as group structure is improved and agonistic behaviour reduced. As such, establishing fish farms in exposed areas with fast water current velocities should be favourable. However, at some locations, velocities exceed what is known as preferable for salmonids, and this may compromise fish welfare. In this study, behaviour and fin erosion were observed on post-smolt salmon stocked at 39 kg m^{-3} in raceways at 3 water current velocities: fast (1.5 body lengths [BL] s^{-1}), moderate (0.8 BL s^{-1}) and slow (0.2 BL s^{-1}). Movements that affect group structure and interactions between individuals varied by up to 20-fold between velocities. A behavioural change occurred directly after velocities were set. Severe fin erosion decreased over time in all groups, but new injuries increased almost 3-fold in the faster-velocity group. Our results suggest that moderate velocity is ideal from a welfare perspective. At slow velocity, higher frequency of structural movements and between-individual interactions could be stressful for the fish. At faster velocity, the

fish have to focus on swimming, which could increase unintentional collisions with obstacles and other individuals and result in new fin erosion. Our results suggest that management of water currents may be an effective way of controlling behaviour and may thereby improve welfare.

4. Results and discussion

In an exposed sea cage, salmon behaviour underwent a marked change when the ambient water current velocity exceeded 0.7 BL s⁻¹. At slow currents, the fish were schooling with a circular swimming pattern, but at faster currents, the fish faced the current and held position. In the laboratory experiment, the best welfare with regard to low stress levels, high muscle growth and decreased fin erosions was found at a moderate current velocity of 0.8 BL s⁻¹. A water current velocity of 1.5 BL s⁻¹ was too high for salmon in terms of welfare and production performance, and slow water currents of 0.2 BL s⁻¹ induced a less organised group structure and more interactions. Vertebrae morphology and bone mineralisation were also affected by current velocity.

4.1 Research approach

In this project, field observations were combined with laboratory studies to deepen the knowledge of how water currents affect post-smolt salmon. Field studies have the advantage that they are observations in real-life circumstances. The drawbacks are that the results depend on varying and often unforeseen environmental factors. Laboratory experiments have the advantage that single parameters can be manipulated to demonstrate specific effects, but laboratory conditions cannot be directly transferred to real-life conditions. By combining field and laboratory studies, we tried to clarify how currents affect salmon.

The field observations were performed in the Faroe Islands using an exposed commercial sea cage with fast tidal currents (Paper I). Cameras mounted inside the cage recorded fish behaviour over three days while three vertical profilers recorded the oxygen saturation, salinity and temperature inside the cage. An acoustic profiler measured the current velocity at a reference point. Current vectors were located inside the cages to help model the dampening effect of the net cage and to relate the surrounding current velocity to the velocity inside the cage. Due to limitations in profiling water currents inside the cage, values are only presented for the reference

point, and the effect of dampening should be considered when evaluating the velocities experienced by the fish.

Measuring the actual swimming speed of fish has proven to be more challenging than most researchers anticipated. In sea cages, the actual swimming speed is the sum of the current velocity and the distance travelled over time. This information is often missing in publications. In previous experimental setups, it has been difficult to estimate the swimming speed of fish based on the presented current velocities due to the type of setup. In circular tanks, there is a major drop in velocity from the inner to outer wall of the tank, creating a bias. Most published studies have used a variety of circular tanks (Adams et al., 1995; Castro et al., 2011; Christiansen & Jobling, 1990; East & Magnan, 1987; Farrell et al., 1991; Gallaugher et al., 2001; Houlihan & Laurent, 1987; Jørgensen & Jobling, 1993; Kiessling et al., 1994; Larsen et al., 2012; McKenzie et al., 2012; Nielsen et al., 2000) and should be interpreted with caution. For example, in circular tanks, the current velocity can vary more than 3-fold from the inner to the outer circle (Duarte et al., 2011). Depending on where the fish hold position, they will experience different velocities and hence swim at different speeds. In addition, several studies have used a setup in circular tanks with no restrictions (open circular) (Adams et al., 1995; Christiansen & Jobling, 1990; East & Magnan, 1987; Gallaugher et al., 2001; Jørgensen & Jobling, 1993; Kiessling et al., 1994; Larsen et al., 2012; McKenzie et al., 2012). In these tanks, fish can choose to swim faster or slower than the current velocity by moving with or against the current, thus making it difficult to evaluate the actual swimming speed. On the other end of the scale, some exercising regimes, including respiratory swimming chambers, have been conducted in tight confinement, where the fish have limited ability to move around and interact. Such confined environments can impair swimming performance (McFarlane & McDonald, 2002; Peake & Farrell, 2006; Reidy et al., 1995). The underlying reason is unclear, but one explanation is that tight confinement acts as a stressor.

The construction of raceways with constant flows without the stress of confinement is intended to minimise the problems mentioned above (Fig. 1., described in detail in Paper II). Three constant-current velocity regimes were created: slow (4.6 ± 0.02 cm s⁻

^{1}), moderate (20 ± 0.06 cm s^{-1}) and fast (36 ± 0.1 cm s^{-1}) velocities, corresponding to 0.2 ± 0.02, 0.8 ± 0.01 and 1.5 ± 0.02 BL s^{-1} (mean $\pm$ SE), respectively. The raceways (> 7 BL long) allowed the fish to move and interact in a homogenous water current in which they were evenly distributed and held position by swimming. The setup was used to investigate the effect of a single parameter; swimming speed, that corresponded to the water flow velocity.
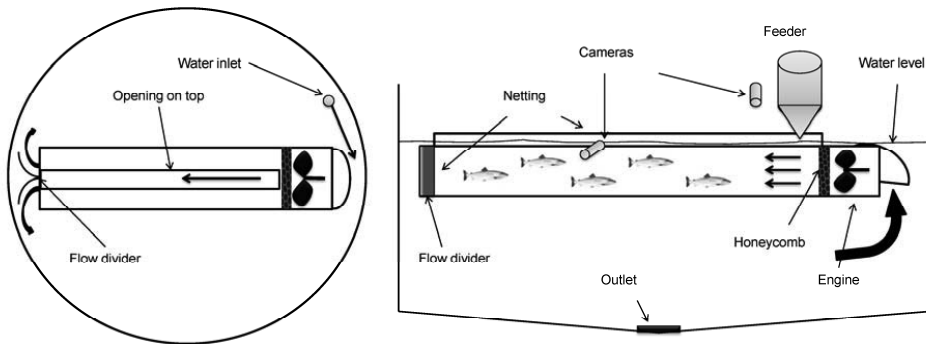

Figure 1. Illustrative sketch of the constructed raceways submerged in tanks of 3 m diameter. The current in the raceway is driven by an electric engine through a honeycomb to create a homogeneous velocity. Thick arrows indicate the direction of water flow in the tank and raceway.

Under commercial aquaculture conditions, fish often aggregate at high densities rather than disperse evenly throughout the sea cage. Consequently, the density experienced by the individual in commercial cages could be much higher than what Norwegian legislation recommends (25 kg m^{-3}) (Johansson et al., 2007; Johansson et al., 2006; Oppedal et al., 2011). The fish in the raceways were stocked at a higher density (39 kg m^{-3}) than the requirements of the Norwegian legislation in an attempt to produce conditions in which social dynamics could be observed.

In the laboratory setup, an environment that aimed to minimise the stress on the fish was created. However, the setup and limited space forced the individuals to mainly swim at the prevailing velocity, which in itself can be perceived as stressful. Johansson et al. (2009) demonstrated that there is a large individual variation in preferences regarding environmental factors. The same most likely holds for swimming speeds

because previous history and internal state could have marked effects on the preferred swimming speed (Claireaux et al., 2005). Hence, one may argue that from a welfare perspective, setups should account for individual differences in capability, allowing fish to choose a swimming speed. Furthermore, from a welfare perspective, the swimming speed of the fastest individuals could be considered irrelevant. Because welfare is an individual experience, the maximum acceptable current velocity should be chosen based on the slowest individuals.

4.2 Effects of slow water currents

Sea cages located in areas with slow current velocities may have low water exchange rates, which results in an unfavourable environment for the fish (Johansson et al., 2007). Under these circumstances waste products will accumulate in the cage and oxygen levels can plunge to below 30% (D. Solstorm, Unpublished data), which is considered lethal for salmon (Stien et al., 2013). This creates highly stressful environments for the fish that are unacceptable from a welfare perspective. In our raceway setup, we could study the effect of slow velocity without affecting oxygen saturation and waste product accumulation. Velocity effects were studied with respect to behaviour, physiology and morphology.

Slow velocities are generally assumed to increase stress and agonistic behaviours between individuals (Adams et al., 1995; Christiansen & Jobling, 1990; East & Magnan, 1987; Jørgensen & Jobling, 1993). This has, in turn, been linked to reduced growth (Adams et al., 1995) and an increased prevalence of fin erosions (Adams et al., 1995; Christiansen & Jobling, 1990; Jørgensen & Jobling, 1993; Turnbull et al., 1998). The reduced growth has been attributed to the elevated energy costs of agonistic behaviours compared with those of undisturbed swimming (Adams et al., 1995; East & Magnan, 1987). Consistent with previous studies, our results demonstrated that at slow velocity, fish exhibited a higher frequency of interactions and movements than those exhibited by fish at moderate and fast velocities (paper IV). However, the increased number of interactions and unstructured movements in our study did not result in reduced growth. In addition, fin erosions were not more frequent at slow velocity than

at the other velocities, where interactions were less frequent. This suggests that the levels of interactions and movements were low compared to previous results (Adams et al., 1995; East & Magnan, 1987). The decreasing frequency of fin erosions during the experiment in the slow velocity group indicates that the level of aggression was low in this setup. This observation could be due to conditions prior to our experiment that resulted in prevalent fin erosions. A naturally higher level of aggression in parr combined with possible feed restrictions (Noble et al., 2008) could account for the prevalent fin erosions at the start of the experiment.

The contradictory results between the present study and previous studies regarding the relationship between agonistic behaviours and fin erosions may also be related to differences in life stages. Aggressive behaviours are common in juvenile salmonids (Fernö & Holm, 1986; Keenleyside & Yamamoto, 1962) but have rarely been documented in post-smolts. The lack of clear negative effects on growth and fin erosion in slow current velocity indicates that the setup created a favourable environment, but in a natural environment, a slow velocity could create an unfavourable environment that would lead to a compromised welfare.

Even though the level of observed interactions in our study was relatively low, it was clear that some individuals were stressed. Because agonistic behaviour did not result in lower growth or higher frequency of fin erosions, the slow velocity in itself may be perceived as stressful. In our setup, the fish had limited possibility to swim at voluntary speed but rather were forced to swim at a leisurely pace to maintain the group structure. Individual variability in swimming capacity could lead to fish with high capacity experiencing the leisurely pace as stressful because they were inhibited from exhibiting their normal behaviour. Stress effects were manifested as increased levels of potassium and lactate in blood plasma in addition to elevated osmolality (Paper II). During stress, the release of stress hormones increases the gill permeability to allow for an increased oxygen uptake, but this also disturbs the ion balance (Gonzalez & McDonald, 1994; Redding & Schreck, 1983) which could partially explain the increased potassium levels. This osmorespiratory compromise leads to an increased osmolality in seawater fish. The increased lactate levels could be an effect of

anaerobic muscle activity due to agonistic behaviours and flight reactions. If the stress becomes chronic, it can impair important physiological functions, such as growth, disease resistance and reproduction (reviewed by Bonga, 1997). Still, in our setup the stress response was not severe (maximum values of; K^+: 4.8 mmol l^{-1}, lactate: 2.8 mmol l^{-1}, osmolality: 369), and few other factors indicate poor welfare.

In conclusion, some of our results may be difficult to relate to commercial situations, in which fish are free to swim faster than the current velocity and low water currents are more likely to generate poor water. However, our observations regarding the fish behaviour could still be relevant for the industry because fish in slow currents are not only free to choose swimming speed but can also, as observed in our study, exhibit interacting behaviours.

4.3 Moderate – voluntary and/or preferred swimming speeds

When fish are free to choose, a daytime swimming speed of 0.3-3 BL s^{-1} has been reported in sea cages (Andrew et al., 2002; Blyth et al., 1993; Dempster et al., 2008; Kadri et al., 1991; Korsøen et al., 2009; Korsøen et al., 2012; Pinkiewicz et al., 2011; Sutterlin et al., 1979). These swimming speeds are rarely correlated with ambient current velocities, which may explain some of the reported variations. During field observations in the Faroe Islands (paper I), a change from circular swimming to holding position against the current was observed in some individuals when the reference current velocity reached 0.7 BL s^{-1} (figure 2). The general circular swimming pattern also became more oval-shaped, which reduced the time fish spent with their lateral side against the current. With a circular swimming pattern, the fish are forced to swim at twice the current velocity in the upstream part of the circle to maintain the pattern. Hence, an observed current velocity of 0.7 BL s^{-1} would generate a minimum swimming speed of 1.4 BL s^{-1} in parts of the circle. When currents increased further (0.9 BL s^{-1}), all fish changed their swimming pattern and held position against the current. In contrast, when current velocities were less than 0.7 BL s^{-1}, the fish chose to swim faster than the current in a circular pattern. The current

velocities were measured at a reference point outside the cage, and due to dampening by the net cage (reviewed by Klebert et al., 2013) fish are likely to experience velocities slower than those measured. Taking this into account, these observations indicate that the preferred swimming speed could be similar to what has been described as preferred for brook charr. Tudorache et al. (2011) found the preferred swimming speed for brook charr to be 0.78-0.95 BL s^{-1}, which is very similar to the optimal swimming speed (1.02 BL s^{-1}) in terms of the cost of transport (COT). The swimming speed at the lowest COT refers to the swimming speed with the lowest energetic cost of traversing a distance and is derived by determining the minimum quota of oxygen consumption and swimming speed (Tucker, 1970). The reported optimal swimming speed also relates well with the average cruising speed of 1 BL s^{-1} observed in wild pelagic salmon (reviewed by Drenner et al., 2012). It is therefore likely that the preferred swimming speed also reflects the lowest COT. In net cages, the fish do not need to go anywhere, but a preferred swimming speed could be an evolutionary remnant from the wild.
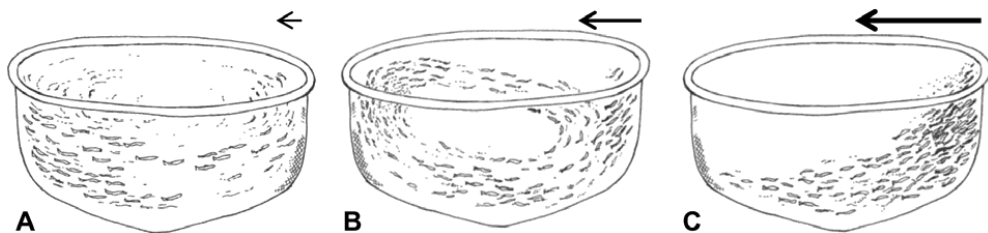

Figure 2 Illustrative sketches of observed swimming patterns: A – circular movement, B – mixed or C – on current. The arrows indicate the strength and direction of the water current. Drawings by Stein Mortensen, Institute of Marine Research.

In the laboratory experiment, a moderate velocity of 0.8 BL s^{-1} was found to result in high muscle growth, minimum stress levels and decreased fin erosion. Increased growth following exercise has been reported in several studies, but the optimal speed for growth varies (table 1). Different species, life stages and stress coping styles (Huntingford & Kadri, 2013) may affect the optimal swimming speed, but when considering different water current regimes, it is clear that the setup also plays a role.

The highest optimal velocities are reported from studies with open circular systems, in which fish can swim both slower and faster than the prevailing current velocity.

One possible benefit of forcing fish to swim against a current is the reduction of aggressive encounters. The cost of agonistic behaviour is considered one of the main reasons for the improved growth in exercised fish (Adams et al., 1995; East & Magnan, 1987). In our study, there was no difference in weight gain between fish exercised at slow or moderate velocities. This indicates that the cost of agonistic behaviours was low throughout the experiment. However, the weight gain in fish at slow velocity was related to a higher lipid deposition whereas the weight gain in fish at moderate velocity was due to a higher protein deposition resulting in a fitter fish. Exercise has also been demonstrated to increase white muscle protein synthesis in rainbow trout (Houlihan & Laurent, 1987). Furthermore, during exercise, lipid is important for fuelling the swimming activity as demonstrated by Lauff & Wood (1996), and this may explain the lower lipid and higher protein content in white muscle. However, a lower level of activity will allow for more energy deposition in the form of lipid (Davison & Goldspink, 1977; East & Magnan, 1987). East & Magnan (1987) found higher lipid deposition in the digestive tract in fish exercised at 0.85 BL s^{-1}, but this could be explained by a high frequency of agonistic behaviours in still water (0.00 BL s^{-1}) being more energetically demanding than swimming activity at moderate speed. With additional current increase, the lipid content and growth was reduced, which suggests that the moderate velocity in their study is similar to our slow velocity.

Reduced interactions and agonistic encounters at moderate swimming speed were expected to result in reduced fin erosions. However, we found that fin erosions also decreased over time even at slow velocity, for which more interactions and agonistic behaviours were observed. Hence, with respect to freedom from injury related to fin erosion, no welfare improvements were observed when current increased to 0.8 BL s^{-1}. Another welfare aspect is the option to swim at preferred speeds (Tudorache et al., 2011) while still being able to interact with other individuals. This could be considered as the possibility to express normal behaviour (UK Farm Animal Welfare (FAWC),

1995) and improve welfare at a moderate velocity. Welfare based on the allostasis concept may also be considered positive at this velocity because the fish appeared to be neither hypo- nor hyperstimulated by the current (Korte et al., 2007). There could still be individual variation in preferred swimming speeds and the limited ability to flee or rest may be stressful for the individual, but this could not be discerned from our results.

When fish are forced to swim, reduced deformities along the vertebrae column have been observed (Deschamps et al., 2009). The posterior-truncal region appeared to be least affected. In our laboratory study (Paper III), no differences in the number of deformities between the different velocity groups were found, but the vertebrae morphology in both the post-cranial and ural regions was affected by swimming speed. The anterior-caudal and ural regions are likely to be affected by profound mechanical loading due to the sub-carangiform swimming mode of salmon, in which the posterior part of the fish is undulating (Sfakiotakis et al., 1999). This is supported by the higher mineral rate observed in the anterior-caudal region with increased swimming speed. However, the same effect was observed in the post-cranial region, where the mechanical loading should be low. The underlying cause for this is unclear. It could be related to buoyancy and the position of the swim bladder in relation to the head, but this question requires further investigation.

Recent research has focused on increased swimming speeds as a method to produce a robust fish with improved cardiovascular functions, disease resistance and osmoregulation (Castro et al., 2013b; Castro et al., 2011; Esbaugh et al., 2014; Takle et al., 2012). An improvement in cardiovascular functions during swimming may aid other physiological functions. Gallaugher et al. (2001) observed increased oxygen uptake in the muscle coupled with higher relative ventricular mass (RVM) and increased maximum oxygen consumption (MO_2 max). The improvement in oxygen consumption was considered beneficial for other systems, such as osmoregulation. This effect may be positive at exposed sites when fish are forced to cope with new challenges while swimming. In our experimental study RVM was highest in fish at fast velocity and similar in fish at moderate or slow velocity (Paper II). Castro et al. (2011)

observed improved disease resistance in interval trained juvenile Atlantic salmon coupled with higher RVM. However, the field of exercise and robustness on salmonids is in its wake and focuses on juvenile stages in an attempt to increase survival following sea transfer (Castro et al., 2013b; Castro et al., 2011; Takle et al., 2012). The effect of water currents on adult Atlantic salmon has not been widely studied.

4.4 What happens when water currents become too fast?

Fast water currents increase water exchange and thereby improve the water quality in exposed aquaculture. The vertical profiles of the cage environment presented in Paper I demonstrated a good homogenous environment in terms of temperature (6.6 °C), salinity (35) and oxygen saturation (>90%). The high oxygen level is an effect of high water exchange, with waste products rapidly being transported away from the cage. Hence, environmental factors at exposed sites are favourable, which should improve fish welfare (reviewed by MacIntyre et al., 2008). However, fish behaviour observed at fast velocities indicates that the fish become restricted in their voluntary swimming behaviour. In our observations, fish clustered at high densities, holding position against the current when the ambient current velocity exceeded 0.9 BL s^{-1} (Paper I). At this velocity, the fish have to swim twice as fast to maintain the traditional circular swimming pattern, and this seemed to exceed their capacity, resulting in a changed swimming pattern. In spite of good water quality, this indicates that welfare could be compromised at ambient current velocities above 0.9 BL s^{-1}.

Individual fish are likely to have different environmental preferences depending on their internal state. Hence, from a welfare perspective, a heterogeneous environment has been suggested to be preferable (FAWC, 2014). Johansson et al. (2009) demonstrated a large variation in the temperature that individual fish experienced at heterogeneous locations. Whether this depends on different individual preferences or limited availability of preferred areas is unknown. There may be a difference in individual preferences for swimming speed that depends on history and internal state. Claireaux et al. (2005) separated poor and good swimmers using a swim challenge and demonstrated that the fish maintained their individual swimming capacity even after 9

months in the same environment. This indicates a strong link to individual capacity. The variation in individual capacity is difficult to consider in exposed areas. Selective breeding for good swimmers could improve fish welfare.

In the laboratory experiment, fast water currents resulted in reduced production performance, restricted behaviour and, contrary to our prediction, an increase in new fin erosion, all of which contribute to reduced welfare.

The lower production performance indicates that fish could not consume or digest as much feed as needed to support the growth rate observed at slower currents. This could be an effect of feeding at fast velocity when the feed is rapidly swept away with the current. In exposed aquaculture with tidal currents, feed is usually delivered during slow current velocities to reduce feed waste and to give the fish more time to feed. In our setup, the water velocity of the currents was constant, but the fish were highly overfed to ensure that they were fed to satiation. It is thus more likely that the fish could not digest enough food for energy to support both swimming and optimum growth, which resulted in a reduced production performance. Grisdale-Helland et al. (2013) demonstrated that exercise increases energy requirements by doubling the energy maintenance and that fish at fast velocity have less dietary energy available for growth. This did not result in reduced growth in their study, which was explained by higher energy efficiency. However, their fast-swimming fish only swam at 1 BL s^{-1}, which is most similar to our moderate velocity. A further increase in velocity might have led to reduced growth if the fish could not further compensate by higher energy efficiency. It would be interesting to investigate the effects of the current on the food conversion rate (FCR), but unfortunately, our setup did not enable feed collection. Previous studies have demonstrated that fish forced to swim became leaner with a lower condition factor (CF), i.e., longer and lighter (Farrell et al., 1991) but in our study, the reduced growth was isometric, with no effect on the CF.

Fish forced to swim at speeds at which growth becomes compromised can be considered both a production problem and a welfare problem. Based on the allostasis concept (Korte et al., 2007) it may be argued that reduced growth indicates that the

fish are not able to maintain stability through change and that fast currents result in a high allostatic load. From a freedom approach (UK Farm Animal Welfare (FAWC), 1995) it can be argued that the high energy demand could lead to constant hunger, which is a violation of the first freedom, i.e., freedom from hunger and thirst.

The fourth freedom, freedom to express normal behaviour, is also compromised at fast velocity. The fish are forced to swim at a constant pace to prevent being swept into the netting with very limited possibilities for interacting. Korte et al. (2007) argues that natural behaviours do not automatically improve welfare and that farmed animals have a modified behaviour due to domestication. In the report by FAWC (2014) it is stated that "greater consideration is needed of how farmed fish express normal behaviour". When domesticating Atlantic salmon, behavioural traits have been neglected (Solberg et al., 2013). This has resulted in a more aggressive fish with a reduced anti-predator response (Einum & Fleming, 1997; Houde et al., 2010) but also in fish with a higher stress tolerance (Solberg et al., 2013). Increased stress tolerance may improve welfare, whereas increased aggression may not. Still, when a dominant fish becomes restricted in its behaviour, due to fast currents, this could be stressful for the individual.

In the fast current velocity, a higher level of unintentional collisions with obstacles and other individuals was observed. The fish appeared to have difficulties holding position against the current. These collisions are most likely the cause for the increase in new fin erosions found on these fish. Fin erosions are common in aquaculture systems (Bosakowski & Wagner, 1994; Turnbull et al., 1996). This can be considered a welfare problem for several reasons. It is a violation of the third freedom, i.e., freedom from pain, injury and disease (UK Farm Animal Welfare (FAWC), 1995). Whether fin erosions cause pain or fish can experience pain is a debated issue, but fins are documented to be nociceptive and hence able to register noxious stimuli (Noble et al., 2012; Roques et al., 2010). Irrespective of the ability to experience pain, fin erosion is definitely an injury that increases susceptibility to pathogens (Turnbull et al., 1996), which may lead to disease and impaired welfare. Fin erosions might also reduce swimming ability, which would be detrimental in exposed aquaculture. At the end of our experiment, new fin erosions were most prevalent in the caudal fin, for which the

effects on swimming performance are likely greatest (reviewed by Sfakiotakis et al., 1999). Although this outcome may be an effect of the setup, abrasion of the caudal fin is a possible outcome also in exposed aquaculture with fast currents. In the field study (Paper I), fish were observed to cluster at high densities trying to hold position against the current already at velocities of 0.9 BL s^{-1}. In faster velocities, the fish may be swept into the cage wall and suffer fin erosions. If caudal fin erosions affect swimming performance, this may create a downward trend in which slower currents lead to new fin erosions. Hence, the maximum swimming speed that the fish can maintain at the beginning may decrease if fin erosions increase. It is therefore important to monitor fin erosions at exposed sites.

4.4.1 Critical current velocities

When water currents exceed a threshold, fish switch from aerobic to anaerobic swimming (Wood, 1991). Anaerobic swimming quickly depletes the energy stores of glycogen, ATP and CrP, increases plasma lactate and may also lead to an impaired ion-balance. In extreme cases, a prolonged energy deficiency could be fatal (Burnett et al., 2014). It is therefore crucial to know the maximum aerobic swimming capacity of the fish. In our experiments, a continuous velocity of 1.5 BL s^{-1} was found to be too high with respect to growth and welfare (Paper II and Paper IV). However, glycogen reserves, ATP and CrP were not depleted, nor was there an accumulation of lactate or an impaired ion-balance, which indicates that this velocity was exhaustive for the fish. However, in areas with strong tidal currents the fish likely experience much faster current velocities during shorter time periods.

A study by Remen et al. (2016) investigated the swimming capacity of salmon schools at commercially relevant stocking densities. The results indicated that post-smolt salmon (29 cm) were capable of swimming up to 91 cm s^{-1} for 20 minutes, and the swimming speed increased with size, such that adult salmon (51 cm) were capable of swimming up to 100 cm s^{-1} under the same conditions. However, when relating the swimming speed to body length, the swimming capacity was considerably lower in the adult fish (2 BL s^{-1}) than in post-smolt salmon (3.1 BL s^{-1}). These results represent an

important step towards providing the industry with acceptable upper limits in water current velocities. In exposed areas, fast currents may be most crucial for newly stocked post-smolt. Although larger salmon were found to have a lower U_{crit} than smaller salmon, expressed in BL s^{-1}, they were still able to swim at faster speeds expressed in cm s^{-1}, which indicates that larger post-smolt salmon are more suitable for exposed areas. However, it is important to consider that the critical swimming speed is only an upper threshold that fish can tolerate under prevailing conditions. The findings should not directly be transferred to standard aquaculture conditions. In addition, swimming at maximum capacity does not result in improved production performance and welfare.

5. Conclusions

This study intended to evaluate how water currents affect post-smolt Atlantic salmon in exposed aquaculture. It has become clear that water currents influence salmon in both positive and negative manners.

Growth is an important factor for the industry and a clear welfare indicator. This study suggests that growth could become impaired already at 1.5 BL s^{-1}. This indicates that to maximise production performance, water current velocities should be less than 1.5 BL s^{-1} for post-smolt salmon. Fish at slow velocity (0.2 BL s^{-1}) became fatter, but with the same weight gain as fish at moderate velocity (0.8 BL s^{-1}). Thus, to optimise muscle growth, a moderate velocity is likely best. When velocities are slow, fish can still swim at faster speeds in commercial setups, but other environmental factors are likely to be detrimental for production performance.

Behavioural responses to currents are clearly plastic, and fish adapt their behaviour to prevailing conditions. At slow velocity, the fish have the possibility to behave in different manners, and in commercial systems, this also includes swimming speed. This possibility decreases with increased velocity, and at fast velocity, the fish can only try to maintain position in the current. This was observed at an exposed site at which fish changed swimming behaviour in response to the current velocity. At slow velocity, increased current velocity can reduce aggressive encounters and increase welfare. However, when currents become too fast, they can lead to restricted monotonous behaviour, which may also impair welfare.

In conclusion, current velocity is an important parameter to be considered in all types of aquaculture because the current can have both positive and negative effects. It has the potential to be used as an important tool when optimising production conditions. In exposed areas, technology may need to focus on reducing the current velocities experienced by the fish, and in sheltered or land based aquaculture, current generators could increase welfare and production performance.

5.1 Future perspectives

This study represents a first approach regarding how water currents can affect fish in aquaculture. It raises several questions that need to be the focus of future research.

In exposed areas, water currents can be present as mostly constant fast currents or as fluctuating tidal currents with peaks in velocities occurring in a regular interval. Threshold values for both types of exposure need to be thoroughly investigated to ensure good welfare. In tidal areas, recovery after fast current velocity peaks needs to be assessed with respect to fish size and peak duration and in combination with other environmental factors. The effect of repetitive high-velocity peaks also needs to be studied.

When fish are exposed to multiple stressors, synergism could influence how well fish cope with strong currents. One of the fast velocity replicates in our study (excluded from the published data) had a technical failure resulting in small air bubbles in the water. This unintentional stressor resulted in increased mortality (unpublished data), thus suggesting that fast current resulted in a high allostatic load and the fish could therefore not cope with an additional stressor. In exposed aquaculture, several stressors, such as handling and environmental changes, may be present. However, exercise could also have positive effects, and exercising regimens should be evaluated in terms of benefits and recovery. Several questions need further investigation to determine adequate policies regarding exposed aquaculture.

Selective breeding for fish with improved aerobic capacity could be an approach to ensure good welfare in areas with fast water currents. In Atlantic salmon, selective breeding has focused on fast growth, delayed maturation and disease resistance (Gjedrem, 2000; Glover et al., 2009; Thodesen & Gjedrem, 2006). When more sea cages are in areas with fast water current velocities, there may be a market for strains selected for high swimming capacity. The inheritance of the variations in individual swimming capacity of fish (Claireaux et al., 2005) is worthy of study. It would also be interesting to investigate the different strains commercially available today in terms of swimming performance to identify strains suitable for exposed aquaculture.

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PLOS ONE

The Interaction between Water Currents and Salmon Swimming Behaviour in Sea Cages

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Abstract

Positioning of sea cages at sites with high water current velocities expose the fish to a largely unknown environmental challenge. In this study we observed the swimming behaviour of Atlantic salmon (Salmo salar L.) at a commercial farm with tidal currents altering between low, moderate and high velocities. At high current velocities the salmon switched from the traditional circular polarized group structure, seen at low and moderate current velocities, to a group structure where all fish kept stations at fixed positions swimming against the current. This type of group behaviour has not been described in sea cages previously. The structural changes could be explained by a preferred swimming speed of salmon spatially restricted in a cage in combination with a behavioural plasticity of the fish.

Introduction

Moving sea cages to exposed sites with strong water currents is an industry-wide trend in Atlantic salmon (Salmo salar L.) farming [1], [2]. This could improve production efficiency through access to high water quality due to rapid transport and dilution of waste products, more stable temperatures, high levels of oxygen and less influence from terrestrial runoff [1], [3]. Other positive effects such as reduction of possible conflicts with other users in the coastal area and avoidance of the ecological carrying capacity limitations of inshore waters have been suggested [3]. One prerequisite for this progress has been the development of strong, resistant farm structures that can withstand the forces produced by strong water currents [4], [5]. However, it is not known how the fish inside the sea cages cope with high water current velocities. The fish has to cope with being forced into an environment that radically differs from the sheltered fjord sites. The question about the amount and type of stress produced by a high-energy environment and the fish capacity to cope is at least as important as the development of new resistant farming platforms. Salmon farms in sheltered localities generally experience current velocities below 20 cm s^{-1} outside the cages [6]. At such velocities the fish will often form a circular, one way directed uniform swimming pattern, possibly as a result of individuals actively avoiding collisions with each other and the cage wall [7]. At these sites salmon typically swim at speeds of 0.3–0.9 BL s^{-1}, with maximum average values of 1.9 BL s^{-1} [8], [9], [10]. The constant swimming of salmon under natural conditions has been associated with an inherent migratory tendency related to optimum cruising speed [10] [11] and in open ocean studies the speed approximates to 1 BL s^{-1}, independent of age [12]. Studies using swim tunnels indicate a critical swimming speed, U$_{crit}$, for

small salmon (400–800 g) of 1.6–2.2 BL s^{-1} [13], [14], although one study reports values as high as 3.0 BL s^{-1} [15].

Although the exact swimming capacity of salmon is uncertain, and will vary with such factors as size, exercise level, degree of satiation [16] and individual fitness, it is evident that salmon inside sea cages must adapt their behaviour to the water current. Hence the objective of this study was to observe the general effects of high water current velocities on fish swimming behaviour at the group level, in an exposed commercial salmon cage.

Materials and Methods

The observations of schooling behaviour were performed from 11th to 13th of February 2012 at a commercial marine salmon farm near Torshavn in the Faroe Islands, Denmark (61.59° N). The farm had 8 circular cages of 41 m diameter, and 2 cages of 50 m diameter, with a depth of 12 m to the bottom ring. The depth below the cages varied from 30 to 40 m, and the total biomass at site was 1320 tonnes. The fish were fed continuously from 08:30 to 16:15 h and were exposed to continuous artificial light at 4 m depth. The observed cage (41 m diameter) was selected based on having the highest probability to be exposed to high water current velocities, due to its position at the south end of the farm. According to farm data, the stocking density in this cage was 6.2 kg m^{-3} and the average fish weight 1.54 kg, corresponding to an approximate fish length of 50 cm. During the observation period, vertical profiles of water characteristics (oxygen, temperature and salinity) showed little spatial and temporal variation: dissolved oxygen saturation levels were at 94.6±2.3% (mean±SD), temperature 6.6±0.1°C and salinity 35.0±0.1 ppt, all of which were within accepted optimal limits [6], [17], [18]. Vertical profiles of water current down to 20 m of depth were
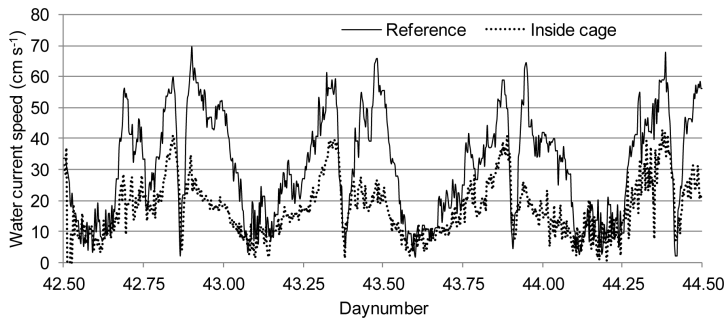
Figure 1. Water current velocity outside the cage (Reference) and inside the cage from 11th to 13th of February, 2012.
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recorded 210 m south of the farm with open sea between the observed cage and the reference point using an Acoustic Wave And Current profiler (AWAC, Nortek, Oslo, Norway). In order to minimize disturbance from the fish, single point measurements were taken at 6.2 m depth in the centre of the cage using a Vector Aquadopp 3D (Nortek, Oslo, Norway). The observed water current velocities varied in a tidal pattern between 0 to 69 cm s^{-1} at the reference point, and between 0 to 42 cm s^{-1} at the single point measured inside the cage (Figure 1). The reduced current velocity inside the cage (Figure 1) is related to dampening by the net and the fish inside the cage and the cages north of the observed cage [6], [19], [20]. The vertical profiles showed little differences in current speeds and directions between 0 and 12 m depth. The tidal nature of the current produced a variable main direction between 120° and 300°. Unless otherwise specified, we refer to the current data collected at the reference point. The schooling behaviour of the salmon was observed with two remotely controlled underwater pan/tilt cameras (Orbit GMT AS, Førresfjord, Norway) connected to a recording DVD player. One camera was positioned next to the net and the other was positioned approximately 15 m from the net at the opposite side of the cage at approximately 6 m of depth to give a good representation of behaviours both up- and downwards. The 48 h period of recordings were divided into four minutes subsamples, which were post-analysed and manually classified for swimming structure (see Results). Recordings of poor quality (e.g. too low light intensity or no fish in picture) were discarded from further analysis. An average of the observed water current velocities between surface and 12 m of depth was used in the analysis. Inherently, this type of time series data produces temporal pseudo replication. The relationships between current velocity and observed swimming structure were therefore investigated using mixed effects models to resolve the non-indecencies

in our data [21], with swimming structure as fixed effect and time as continuous random effect (function lme, the R software system Version 2.15.0, The R Foundation for Statistical Computing, Vienna, Austria). Model checking plots were used to check that the residuals were well behaved (function plot,~fitted(.)) and to check the normality assumption (function qqnorm).

Results and Discussion

A first screening of the videos revealed that the swimming structure could be divided into three main categories: Circle = polarized swimming in a circular movement, On Current = swimming towards the current with no forward movement and Mixed = both Circle and On Current structures present at the same time (Figure 2). Based on data from the more centralised camera (n = 155), the mixed effect model associated the Circle swimming structure with low current velocities (intercept = 22.4 cm s^{-1}, SE = 3.1, p<0.001), the Mixed structure with increased current velocities (+13.7 cm s^{-1}, SE = 2.2, p<0.001), and the On Current structure with an even higher current velocity (+24.3 cm s^{-1}, SE = 1.7, p<0.001). Similarly, for the camera close to the net (n = 347), the Circle structure was associated with low current velocities (intercept = 20.1 cm s^{-1}, SE = 2.6, p<0.001) and the Mixed and On Current swimming structures with increasing current velocities (+13.2 cm s^{-1}, SE = 2.3, p<0.001 and +26.5 cm s^{-1}, SE = 1.4, p<0.001, respectively).

Hence, at low current velocities (≈20 cm s^{-1}) the fish swam in circles (Figure 2A, Table 1) and occupied most of the cage volume. With increasing current velocities (≈35 cm s^{-1}), a shift occurred with some fish seeking a new position facing the net towards the current while other fish continued to swim in elliptic-shaped circles behind the stationary fish at the net (Figure 2B, Table 1). When the circling fish came to a position where they were exposed to
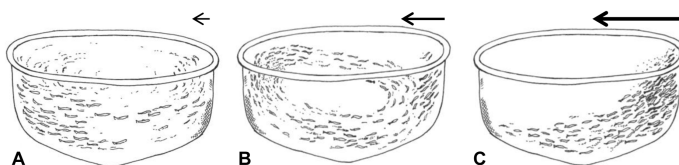

Figure 2. The three observed swimming structures Circle (A, circular movement), Mixed (B, Circle and On Current) or On Current (C, standing on current). The arrows indicate strength and direction of the water current during the different group structures. Drawings by Stein Mortensen, Institute of Marine Research.
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Table 1. Modeled water current speed in cm s^{-1} at the reference point for the observed swimming categories Circle (circular movement), Mixed (Circle and On Current) or On Current (standing on current).

Camera pos.	Swimming Structure		
	Circle	Mixed	On Current
Centralized	22.4 (≈0.45)	36.1 (≈0.72)	46.7 (≈0.93)
Side	20.1 (≈0.40)	33.4 (≈0.67)	46.6 (≈0.93)

Water current velocity is given as BL s^{-1} in brackets.
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current on their sides they turned inwards toward the centre of the cage and drifted with the current to the leeward side of the cage. Following this, they turned towards the current and continued to swim the remainder of the circle's distance. With further increase of current velocity, a larger proportion of the group switched from schooling to swimming towards the current next to the net wall, until all fish stood in a dense group along the side of the cage with no circling fish left (≈47 cm s^{-1}, Figure 2C, Table 1). With sudden changes of current velocities, there was a period of chaos before the fish established a stable structure again.

It is thus clear that the fish experience new challenges when exposed to strong water currents. We have for the first time observed shifts in swimming structure of salmon in sea cages connected to changes in current velocities. The shift from the traditional circular schooling to stationary swimming against the current in a group could reflect energetic optimization as a response to the increased current velocities. Fish swimming behind others have been reported to save energy [22]. However, since previously reported U$_{crit}$ [13], [14] is higher than all the observed current velocities this is probably not the only underlying mechanism. The driving force could instead be a combination of U$_{crit}$ and the large variation in current velocities within the cage, thereby restricting the traditional structure when swimming down-compared to upstream.

Theoretically, if a salmon cage is exposed to an increasing current speed, the typical torus shape of a salmon school within the cage will force the upstream fish to double their swimming speed in order to maintain the group structure. If this pattern is broken up by fish changing to stand on the current, the group structure is probable to collapse and move towards a new stable structure with all fish to hold a constant position against the current.

The On Current structure was observed at water current velocities of approximately 47 cm s^{-1} (Table 1). At such velocities fish in a Circle structure swimming against the current would have to swim at least 94 cm s^{-1} to maintain the group structure, which is close to the U$_{crit}$ for Atlantic salmon [13], [14]. However, the dampening effect of the net [20] suggests that a lower current velocity triggers the shift in group structure. Logically, the swimming speed observed in normal schooling structures, during low current velocity, can be identified as the fishes' preferred speed. This can be termed as such, since fish are able to choose their speed without influence from water current conditions. This chosen speed is assumedly a manifestation of their optimal cruising

speed for minimal energy expenditure, as in migrating salmon [10]. Current velocities for Mixed structures were about 35 cm s^{-1} outside the cage; the fish swam in both Circle and On Current structures at this velocity, and this level could represent the approximate breakpoint when the swimming speed started to exceed the preferred swimming speed for some individuals. This current velocity equates to a swimming speed of 2 * 0.7 BL s^{-1} = 1.4 BL s^{-1} (when fish are swimming towards the current in a circular structure), which is higher than previously reported swimming speeds of 0.3–0.9 BL s^{-1} at more sheltered sites [8], [9], [10]. Taking into account the observed dampening effect, the reduced current speed could result in swimming speeds similar to the previously reported preferred swimming speeds.

From a welfare perspective it could be argued that sites with current velocities that do not exceed the school's preferred swimming speed should provide good welfare since the animal are free to express behaviours within its natural range (item 2 of the Five Freedoms), [23]. Yet, the salmon showed a high degree of plasticity in their behaviour and adapted to the frequent challenges forced upon them by the intermittent and strong water currents. This documented adaptive capacity indicates that conclusions only based on studies performed in laboratories and at unexposed localities could be of limited value due to the different behavioural response to the variable environment. Understanding the effect of water currents on individual fish of different size, as well as on the group as whole, is therefore of utmost importance for the progress of fish farming. High-resolution studies of behaviour in relation to the environment at such sites are needed to ensure environmental conditions acceptable for animal welfare and good production performance.

Author Contributions

Conceived and designed the experiments: FO PK DJ TV JEF FL AF. Performed the experiments: DJ JEF TV. Analyzed the data: DJ FL AF LHS. Wrote the paper: DJ FL AF JEF PK LHS TV FO.

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II

Fast water currents reduce production performance of post-smolt Atlantic salmon *Salmo salar*

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ABSTRACT: In the future, an increasing number of salmon farms may be located in areas with fast water current velocity due to limited availability of more sheltered locations. However, there is little information as to how fast currents affect fish health and welfare. We used raceways to expose Atlantic salmon post-smolts (98.6 g, 22.3 cm) to homogeneous water velocities corresponding to 0.2, 0.8 and 1.5 body lengths s^{-1} (slow, moderate and fast, respectively) over 6 wk. Fish at fast velocity had a 5% lower weight gain compared to fish at moderate and slow velocities, with a corresponding reduction in length. Fish at moderate and fast velocities had lower lipid content in the muscle compared to fish at slow velocity. Hence, fish at slow and moderate velocities had the same weight gain, but fish at slow velocity gained more fat and fish at moderate velocity more muscle protein. Fish at fast velocity had a higher relative ventricular mass, indicating an increased cardiac workload. At slow velocity, individual fish displayed elevated plasma levels of lactate, osmolality and potassium. Our results suggest that post-smolts had the best growth and welfare at moderate velocity and that a current velocity of 1.5 body lengths s^{-1} could compromise production performance.

KEY WORDS: Aquaculture · Welfare · Exercise · Growth · Swimming · Stress

INTRODUCTION

Traditionally, fish farms have been located in sheltered areas. However, environmental aspects and conflicting interests regarding usage of coastal waters in populated areas have contributed to an increased number of farms being located in exposed areas (Stickney & McVey 2002, Benetti et al. 2010, Holmer 2010). Areas with fast current velocity may improve water quality by increased water exchange in cages, and the technology for constructing cages capable of withstanding such external forces is well developed (Fredheim & Langan 2009). However, there is a lack of knowledge when it comes to how fast water currents affect the performance and welfare of caged fish.

In areas with slow currents, fish have the option to vary their swimming speed. In exposed areas with faster currents, fish are forced to swim at the prevailing velocity to maintain their position (Johansson et al. 2014). When the current is not restricting the fish, they may be able to choose their preferred swimming speeds. For adult brook charr *Salvelinus fontinalis*, preferred swimming speeds lie between 0.78 and 0.95 body lengths (BL) s^{-1} (Tudorache et al. 2011). In adult Atlantic salmon *Salmo salar*, daytime swimming speed in cages appears to vary between studies, ranging from 0.4 to 1.5 BL s^{-1} (Sutterlin et al. 1979, Kadri et al. 1991, Blyth et al. 1993, Andrew et al. 2002, Dempster et al. 2008, Korsoen et al. 2009). However, the swimming speeds measured in sea cages are rarely corrected for ambient current veloc-

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ity, which could explain the variation in observed swimming speeds.

Swimming as a form of exercise is often perceived as positive for fish welfare. For example, increased growth and protein deposition following exercise have been reported in several salmonid species at water velocities ranging from 0.80 to 1.75 BL s^{-1} (East & Magnan 1987, Houlihan & Laurent 1987, Totland et al. 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Davison 1997, Castro et al. 2011). For juvenile Atlantic salmon, Jørgensen & Jobling (1993) showed a maximal specific growth rate when fish were exercised at 1.5 BL s^{-1} compared to 0, 1 and 2 BL s^{-1}. For pre-smolts, Castro et al. (2011) reported a 20% increase in growth when salmon were exercised continuously or at intervals (0.8–1.0 BL s^{-1}) compared to controls (0.05 BL s^{-1}). Reduced ATP depletion after fatigue challenge and increased energy stores in muscle occurs in rainbow trout *Oncorhynchus mykiss* as an effect of exercise (Pearson et al. 1990, McFarlane & McDonald 2002). Other positive effects of exercise include increased cardiac output (Farrell et al. 1991), ventricular mass (Gallaugher et al. 2001, Castro et al. 2013), intestinal blood flow (Thorarensen et al. 1993), maximum metabolic rate (McKenzie et al. 2012) and swimming performance (Farrell et al. 1990, Pearson et al. 1990). There are also indications that exercise generates a more robust fish with a higher ability to cope with different challenges, such as pathogens (Castro et al. 2011).

Considering the positive effects of increased swimming speeds, exposed farming should be a favourable choice both in terms of production performance and fish welfare. There are, however, reports of water velocity at some fish farms that exceed the documented critical swimming speed of salmon (Øystein Patursson, Aquaculture Research Station at the Faroes, pers. comm.). Under these circumstances, fish welfare may be compromised. For instance, reduced growth (East & Magnan 1987, Farrell et al. 1991) and decreased muscle energy stores (Jørgensen & Jobling 1993) have been reported in fish forced to swim above optimal velocity. If energy reserves become depleted during intensive swimming, this could limit the fish's ability to cope with new challenges. In such instances, fast velocity may be perceived as a stressor that has a negative influence on fish performance and welfare.

In sea cages exposed to fast currents, fish are not able to choose their swimming speed. When the current increases, the fish must swim against it at the prevailing velocity to avoid being swept into the netting (Johansson et al. 2014).

Here we investigated the effects of slow, moderate and fast swimming speeds in Atlantic salmon post-smolts with regard to growth, muscle composition, blood chemistry and heart morphology. We used raceways to ensure that the fish swam at the same speed as the water current velocity. Our objective was to reveal whether there are any limitations or risks when locating new farms in areas exposed to fast water current velocity.

MATERIALS AND METHODS

Facilities and experimental animals

On 17 January 2012, 1000 Atlantic salmon parr (NLA strain, hatched March 2011) were transferred to 2 circular tanks (∅ 3 m, 5.3 m^3) in the Tank Environmental Laboratory at the Institute of Marine Research, Matre (Norway). Fish were kept in freshwater at 8°C with a continuous flow of 150 l min^{-1} on a constant light regime for 8 wk to complete smoltification. Prior to transfer, the fish had been reared in tanks (∅ 5 m) under natural light and temperature conditions. On 15 March, the water supply was changed to seawater (33 psu) and the temperature was slowly increased to 10°C. Fish were kept under these conditions for 1 wk prior to transfer into the experimental tanks. During the experiment, constant temperature (10°C), water exchange (120 l min^{-1}), salinity (33 psu) and a constant light regime were maintained. Dissolved oxygen levels were always above 80%.

Twelve experimental raceways (Fig. 1) were designed to produce a homogeneous water current through an open cylinder. The trans-sectional area of the cylinder was 0.10 m^2 (∅ 0.36 m) and the length available for the fish was 2.0 m, giving a volume of 0.20 m^3. Raceways were fitted with an electric engine (Minn Kota RT80/EM, Johnson Outdoors Marine Electronics) with adjustable speed followed by a honeycomb (5.0 mm opening and 101.6 mm long, PC 5.0 G4, Plascore) to generate a laminar flow. To keep the fish inside, a plastic-coated wire netting (10 × 10 mm) was placed before a turbulence-reducing flow divider at the outlet and on the opening at the top (1.9 m long and 0.2 m wide). Each raceway was submerged in a circular tank (∅ 3 m, 5.3 m^3) with the water surface just covering the cylinder of the raceway. Two cameras were mounted so that fish in both the front and back of the raceway could be observed. Fish (98.6 ± 20 g, 22.3 ± 1.3 cm, mean ± SD) were transferred to raceways on 22 March 2012, at a mean
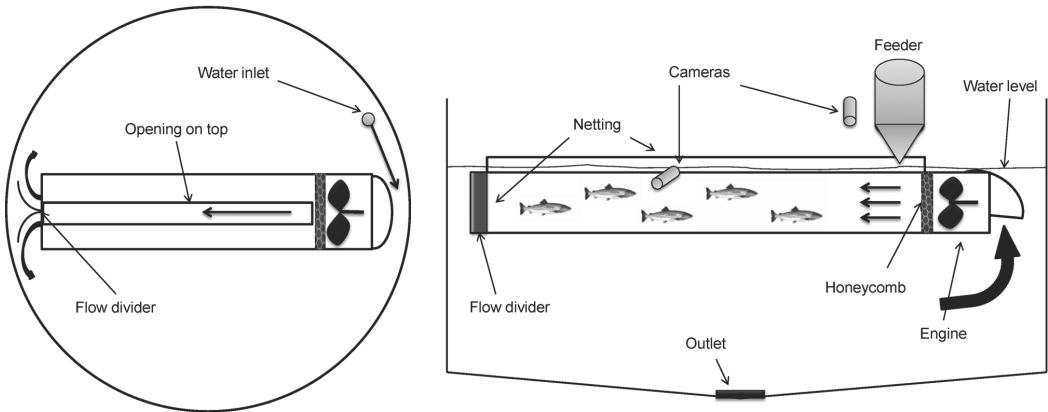
Fig. 1. Illustrative sketch of the constructed raceways submerged in tanks of 3 m diameter. The current in the raceway is driven by an electric engine through a honeycomb to create a homogeneous velocity. Thick arrows show the direction of water flow in the tank and raceway

(±SE) stocking density of 38.7 ± 0.28 kg m^{-3} (n = 80 per raceway). Fish were acclimatized to the raceways for 19 d at a water current velocity of approximately 0.5 BL s^{-1}.

Experimental design

On 10 April 2012, the water currents were adjusted in the raceways and the experiment started (Day 0). Three current regimes were generated in 4 replicate raceways: slow, moderate and fast velocities corresponding to 0.2 ± 0.02, 0.8 ± 0.01 and 1.5 ± 0.02 BL s^{-1} (mean ± SE), respectively. The slow current velocity was chosen to be as close to 0.0 BL s^{-1} as possible, without compromising oxygen levels and the transport of faeces and food waste. The moderate current velocity was selected to be in the range of velocities previously described to have a positive effect in salmonids (East & Magnan 1987, Houlihan & Laurent 1987, Totland et al. 1987, Castro et al. 2011, Tudorache et al. 2011). The fast current velocity was chosen to be twice the amplitude of the moderate velocity but still below critical swimmig speed (U_{crit}) (Tang & Wardle 1992, Stevens et al. 1998). All tanks had the same water exchange, securing good water quality in all treatments and ensuring that velocity was the experimental factor. Prior to the experiment, profiles at 3 horizontal (front, middle and back) and 3 diagonally vertical (top, centre and bottom) points showed negligible variations in velocity. Increased turbulence was measured in close proximity to the

honeycomb. However, daily observations showed that this area was avoided by the fish and hence we assumed that swimming speed would not be affected. During the experiment velocity was only measured at one point (middle, centre) to minimise disturbance for the fish.

Fish were held under these conditions for 6 wk. Halfway into the experiment the currents were adjusted to maintain the same body length per second as the fish grew.

Fish were fed (Skretting Spirit 75) at the front of the raceway. Food was provided in excess every 15 min throughout the day. Video recordings and stomach analysis during sampling demonstrated that fish were feeding at all velocities.

Sampling procedures

Prior to raceway transfer, all fish were anesthetised (100 mg l^{-1} Finquel), weighed (±1 g) and measured (±0.5 cm). Condition factor was calculated according to the equation described below. Five fish from each tank were sampled at the beginning of Weeks 0 and 6. At the beginning of Weeks 2 and 4, 5 fish were removed from each tank to keep density down as the fish gained weight. The fish were rapidly netted and killed with a blow to the head. They were weighed and the total length was measured. Muscle samples were taken behind the dorsal fin and frozen in liquid nitrogen. Muscle samples for glycogen and energy phosphates were taken and frozen within 45 s of

netting. Blood samples were taken from the caudal vein and distributed to heparinised Eppendorf tubes on ice within 5 min. The heart (ventricle, atrium and bulbus arteriosus) was collected at the beginning of Week 6 and was fixed in 4% buffered formaldehyde prior to analysis.

After the final sampling, all remaining fish were anesthetised, weighed and measured.

Analyses

Blood. Blood samples for haematocrit were collected in capillary tubes and centrifuged for 2 min (StatSpin Multipurpose Centrifuge). Blood for haemoglobin analyses (50 µl) was collected from the heparinised Eppendorf tubes and stored at −80°C. The remaining blood in the heparinised tubes was centrifuged at 3220 relative centrifugal force (RCF) at 4°C for 10 min and the resultant plasma was distributed into 2 Eppendorf tubes and stored at −80°C prior to analyses.

Plasma samples were analysed for lactate and glucose using MaxMat PL (MaxMat). Osmolality was measured in 20 µl plasma with Fiske 210 Micro-Sample Osmometer (Advanced Instruments) and ions were analyzed with Cobas c 111 System (Roche Diagnostics).

Haemoglobin samples were diluted 1:150 before analysis with a QuantiChrom Hemoglobin Assay Kit (DIHB-250) from BioAssay Systems.

Muscle. Muscle samples for analysis of protein, lactate, adenosine triphosphate (ATP), adenisone diphosphate (ADP), creatine phosphate (CrP), creatine (Cr), glucose, glycogen and lipids were freeze-dried for 48h. White muscle was dissected free from red muscle, skin and connective tissue and ground to a fine powder and stored at −80°C.

Protein analyses. Protein was extracted from 10 mg of freeze-dried white muscle with 500 µl 1 M NaOH in an ultrasonic bath for 10 min. The samples were neutralised with 500 µl radioimmunoprecipitation assay (RIPA) buffer and centrifuged (5400 RCF, 1 min and 4°C), and the supernatant was diluted 1:10 in RIPA before being analysed using the Pierce BCA Protein Assay Kit (Pierce Biotechnology). Bovine serum albumin was used as protein standard.

Glucose and glycogen analyses. Glycogen was extracted from 10 mg of freeze-dried material with 500 µl 2.0 M HCl and hydrolysed at 90°C for 2 h. After centrifugation (1810 RCF, 15 min and 4°C), the supernatant was transferred to Eppendorf tubes and stored at −80°C until further analysis. Free glucose was extracted from 20 mg of freeze-dried white mus-

cle with 200 µl 2.0 M HCl before centrifugation (1810 RCF, 15 min and 4°C). The supernatant (50 µl) was stored at −80°C until further analyses. After defrosting, samples were neutralised (1:1) with 2.0 M NaOH and diluted in buffer (9:1) (13.8 g $NaH_2PO_4 \cdot H_2O$, 18.0 g $Na_2HPO_4 \cdot 2H_2O$, 100 ml H_2O). Samples were analyzed using the Glucose Colorimetric Assay Kit (Cayman Chemical Company). Results were adjusted for dilution volume and the exact amount of tissue analysed. For glycogen results, the free glucose was subtracted from the results.

ATP and CrP analyses. Freeze-dried white muscle samples of 10 mg were dissolved in 1000 µl 0.4 M perchloric acid by thoroughly mixing the samples and immersing them in an ultrasonic ice bath for 10 min. After mixing, the samples were neutralised with 350 µl 2.0 M KOH, centrifuged (5400 RCF, 1 min and 4°C) and purified by syringe filter (Acrodisc GHP, 0.45 µm, 13 mm). Analyses were performed immediately to prevent samples from decomposing. The injection volume was 20 µl. Results were adjusted for dilution volume and the amount of tissue analysed.

ATP, ADP, CrP and Cr were analysed using ultra high performance liquid chromatography (Nexera UHPLC, Shimadzu Corporation) with an adapted method based on Sellevold et al. (1986) and Volonté et al. (2004).

Isocratic chromatographic separation between substances was performed using a C_{18} HPLC column (250 × 4.6 mm, 5 µm particle size, Supelco Discovery) at 30°C. The flow of buffer (215 mM KH_2PO_4, 3.5% acetonitrile and 2.3 mM TBAHS, pH 6.25) was set at 1.3 ml min^{-1} and detection was performed at a wavelength of 215 nm.

Stock solutions were prepared in 0.4 M perchloric acid and diluted with buffer to standard concentrations: ATP 0.190 µmol ml^{-1}, ADP 0.0353 µmol ml^{-1}, CrP 0.400 µmol ml^{-1} and Cr 2.745 µmol ml^{-1}. The standard was injected in volumes of 10, 20, 30, 40 and 50 µl to create a standard curve. After dilution with buffer, the solution was only stable for 30 min.

Lipids. Lipids were extracted from approximately 100 mg of freeze-dried white muscle according to Folch et al. (1957) and dried under a stream of nitrogen, and total lipids were measured gravimetrically.

Heart morphology

The atrium and the bulbus arteriosus were removed from all the fixed hearts and the remaining ventricle was weighed with 1×10^{-4} g accuracy. The

relative ventricular mass was calculated according to Eq. (2). Images of the caudal surface of the heart ventricle were used to determine the roundness of the ventricle according to Fraser et al. (2014).

Calculations and statistics

Condition factor. Fulton's condition factor (CF) was calculated according to the formula (Busacker et al. 1990):

$$CF = \frac{W}{L^3} \times 100 \qquad (1)$$

where W is the wet weight of the fish (g), and L is the length (cm).

Relative ventricular mass. Relative ventricular mass (RVM) was calculated according to the formula (Gallaugher et al. 2001):

$$RVM = \frac{M_v}{M_b} \times 100 \qquad (2)$$

where M_v is the ventricular mass and M_b is the total body mass of the fish (g).

Statistical analysis. All results were checked for normality and homogeneity of variance. Percentage values were arcsin transformed prior to analyses. Outliers were individually investigated for underlying reasons and the outliers were removed from the data set where methodological causes were detected. Potential tank effects were equalised using nested ANOVA. Data that fulfilled the assumptions of normality and homogeneity were analysed with a 1-way ANOVA to test for significant differences ($p < 0.05$) between treatments. Data that did not meet the assumptions of homogeneity or normality were tested with the non-parametric Kruskal-Wallis test. All analyses were performed with Statistica (version 11, StatSoft). All presented values are shown as means ± SE.

During the experiment, 2 replicates were eliminated due to technical problems — one from the fast current velocity in Week 5 and one from the moderate current velocity in Week 4 — resulting in triplicate treatments for the aforementioned groups at the final sampling.

RESULTS

Growth

At the start of the experiment there were no differences between treatments in weight, length or condi-

Table 1. Growth parameters (mean ± SE) of post-smolt Atlantic salmon *Salmo salar* at the start and end of the experiment for the different water current treatments (slow: 0.2 ± 0.02 body lengths [BL] s^{-1}; moderate: 0.8 ± 0.01 BL s^{-1}; fast: 1.5 ± 0.02 BL s^{-1}). Significant differences ($p < 0.05$) are presented with superscripted letters

	Slow	Moderate	Fast
Weight (g)			
Start	98 ± 1	100 ± 1	98 ± 1
End	250 ± 3[a]	251 ± 3[a]	238 ± 3[b]
Length (cm)			
Start	22.3 ± 0.1	22.3 ± 0.1	22.2 ± 0.1
End	28.4 ± 0.1[a]	28.4 ± 0.1[a]	27.9 ± 0.1[b]
Condition factor			
Start	0.879 ± 0.003	0.885 ± 0.003	0.879 ± 0.004
End	1.079 ± 0.004	1.081 ± 0.005	1.086 ± 0.006

tion factor (Table 1). After 6 wk, fish at all velocities had more than doubled their weight. Salmon at slow 'and moderate water current velocities were approximately 5% heavier and 2% longer compared to fish at fast velocity (Table 1). There was no difference in condition factor at the end of the experiment.

Muscle composition

Muscle composition differed among treatments at the end of the experiment (Table 2). Fish at the slow velocity had a higher lipid content compared to those kept at moderate and fast velocities. This was linked to lower protein content in dry matter (DM) and a 15 and 21% higher lipid content in this group of fish compared to fish in moderate and fast velocities, respectively (Table 2). Glycogen content was also 21% higher in fish kept at slow velocity compared to fast velocity, but not significantly higher compared to fish kept at moderate velocity. There were no effects of exercise on free glucose and ATP content in the muscle. However, CrP was 20 and 21% higher in fish kept at fast velocity compared to those kept at moderate and slow velocities, respectively.

Blood chemistry

At the end of the experiment there were some differences in the clinical blood parameters among the treatment groups (Table 3). There was a trend for reduced plasma osmolality related to exercise, with 1–2% lower values in the fast velocity group compared to the slow and moderate velocity groups. The

Table 2. Muscle composition of post-smolt Atlantic salmon *Salmo salar*. Data are presented as mean ± SE at the start and end of the experiment for the different velocity treatments (see Table 1). Significant differences (p < 0.05) are presented with superscripted letters. DM: dry matter; CrP: creatine phosphate

	Slow	Moderate	Fast
Protein (g g^{-1} DM)			
Start	0.550 ± 0.009	0.544 ± 0.007	0.558 ± 0.009
End	0.543 ± 0.001[b]	0.602 ± 0.001[a]	0.607 ± 0.001[a]
Lipids (g g^{-1} DM)			
Start	0.087 ± 0.007	0.082 ± 0.005	0.092 ± 0.011
End	0.173 ± 0.007[a]	0.151 ± 0.007[b]	0.143 ± 0.008[b]
Glycogen (μmol g^{-1} protein)			
Start	117 ± 9	131 ± 9	126 ± 7
End	156 ± 8[a]	138 ± 6[a,b]	129 ± 4[b]
Glucose (μmol g^{-1} protein)			
Start	4.42 ± 0.42	4.26 ± 0.33	4.44 ± 0.37
End	3.41 ± 0.28	2.61 ± 0.21	2.84 ± 0.19
ATP (μmol g^{-1} protein)			
Start	53.4 ± 2.4	55.0 ± 1.6	53.3 ± 1.1
End	51.6 ± 1.4	48.6 ± 1.0	51.5 ± 1.1
CrP (μmol g^{-1} protein)			
Start	94 ± 11	108 ± 13	101 ± 11
End	107 ± 6[b]	108 ± 5[b]	130 ± 6[a]

Table 3. Blood chemistry of post-smolt Atlantic salmon *Salmo salar*. Data are presented as mean ± SE at the start and end of the experiment for the different velocity treatments (see Table 1). Significant differences (p < 0.05) are presented with superscripted letters

	Slow	Moderate	Fast
Haematocrit			
Start	32.9 ± 0.8	34.0 ± 0.9	30.7 ± 1.0
End	38.1 ± 1.0	36.6 ± 0.8	37.9 ± 1.1
Haemoglobin (g dl^{-1})			
Start	11.8 ± 0.6	11.7 ± 0.6	11.1 ± 0.3
End	11.3 ± 0.2	10.9 ± 0.2	11.4 ± 0.2
Osmolality (mOsm kg^{-1})			
Start	341 ± 3	340 ± 2	343 ± 2
End	351 ± 2[a]	348 ± 1[a]	343 ± 2[b]
Na$^+$ (mmol l^{-1})			
Start	161 ± 1	161 ± 1	161 ± 1
End	162 ± 1	162 ± 1	161 ± 1
K$^+$ (mmol l^{-1})			
Start	0.530 ± 0.039	0.640 ± 0.077	0.512 ± 0.032
End	0.841 ± 0.134[a]	0.486 ± 0.031[b]	0.430 ± 0.016[b]
Cl$^-$ (mmol l^{-1})			
Start	135 ± 1	135 ± 1	134 ± 1
End	131 ± 1	131 ± 1	131 ± 1
Lactate (mmol l^{-1})			
Start	0.750 ± 0.071	0.740 ± 0.045	0.805 ± 0.073
End	0.860 ± 0.16[a]	0.500 ± 0.041[b]	0.380 ± 0.033[b]
Glucose (mmol l^{-1})			
Start	6.33 ± 0.15	6.09 ± 0.15	6.06 ± 0.17
End	6.51 ± 0.15	6.33 ± 0.10	6.37 ± 0.15

reduction may be caused in part by general reductions in K$^+$, but no effects on Na$^+$ and Cl$^-$ were detected, so there may be other factors affecting osmolality. An interesting result was the higher levels of K$^+$ and lactate in the slow velocity group compared to the other groups. No detectable treatment effects were found for haemoglobin, haematocrit or glucose.

Heart morphology

The relative ventricular mass of the fish kept at fast velocity was 15 and 10% higher than fish kept at moderate and slow velocities, respectively (Fig. 2). No effect on heart shape was observed (slow: 58 ± 1.1°, moderate: 57 ± 1.0° and fast: 57 ± 1.5°).

DISCUSSION

Our objective was to determine the effects of water current velocities on physiological and biochemical markers of fish welfare and production quality in post-smolt Atlantic salmon. A slow velocity led to alterations of many physiological characteristics, including indicators of stress and increased lipid deposition. A moderate velocity of 0.8 BL s^{-1} resulted in low values of stress-related parameters and best pro-

duction results, and hence a higher welfare compared to the other groups. At fast velocity (1.5 BL s^{-1}), growth was reduced, while at slow velocity (0.2 BL s^{-1}), some physiological parameters such as lactate had a large individual variation, possibly as an effect of agonistic behaviour.

Growth

Fish swimming at fast water current velocity had a lower growth compared to fish swimming at slow and moderate velocities. Previous studies have shown improved growth when fish have been forced to swim against a current (East & Magnan 1987, Houlihan & Laurent 1987, Totland et al. 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Davison 1997, Castro et al. 2011).

A significant challenge when determining the effects of prolonged swimming at different speeds in fish is the generation of a homogeneous water current within the test environment. In many earlier experiments, the homogeneity of the water current was difficult to evaluate, making interpretation of the
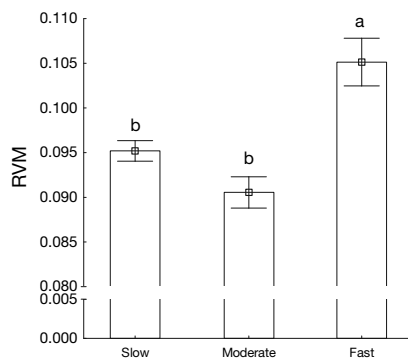
Fig. 2. Relative ventricular mass (RVM, mean ± SE) in Atlantic salmon *Salmo salar* in slow, moderate and fast velocity groups (see Table 1) at the end of the experiment. Significant differences between groups (p < 0.05) are presented with lowercase letters

data difficult. The use of circular tanks without any constraints is a good example of an experiment in which a homogeneous current velocity is difficult to achieve. For instance, within circular tanks the water velocity may be over 3 times faster closer to the outside wall (Duarte et al. 2011) and the fish can change position and thereby vary the experienced current velocity. In addition, if the fish is not restricted to a closed off section in the tank (hereafter referred to as closed/open circular if a closed off section exists/is absent) this may also influence the results as the fish have the ability to move either slower or faster than the generated current and the actual swimming speed of individual fish will be unknown. This seems to be the case in several studies (East & Magnan 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Kiessling et al. 1994, Adams et al. 1995, Gallaugher et al. 2001, Larsen et al. 2012, McKenzie et al. 2012). The difficulties in evaluating current homogeneity and actual swimming speed may explain inconsistencies in results within the literature. However, relative differences of exercised and unexercised fish may still be observable. Constraining the fish may also be stressful if they do not have the possibility to change position or vary swimming speed. In the present study, the available length of space for the fish was 9 to 7 body lengths as the fish grew, allowing the fish to change position.

Castro et al. (2011) observed an increase in growth for pre-smolt Atlantic salmon kept at both continuous and alternating current velocities compared to control fish kept at minimal current velocity. Their continuous current velocity corresponded to our moder-ate velocity, but the study was conducted in a closed area in circular tanks where velocity may differ between the inner and outer tank walls. Jørgensen & Jobling (1993) observed an optimal growth at a water current velocity of 1.5 BL s^{-1} in juvenile salmon, corresponding to our fast velocity, which resulted in suboptimal growth in our study. However, their study was carried out in open circular tanks where fish were free to move around and where actual swimming speeds might have differed from the water current velocity. Furthermore, in several studies (East & Magnan 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Castro et al. 2011), the slow velocities were close to zero and fish then would likely have had the possibility to move around and interact freely. This could also account for the inconsistencies in results within the literature where fish at slow velocity may display more agonistic behaviour than at higher velocities. Agonistic behaviour has been linked to a higher energy demand than swimming in Arctic charr (Adams et al. 1995), resulting in decreased growth at slow velocity.

To our knowledge, no previous studies of post-smolt Atlantic salmon have found clear negative effects on growth from exercise. Jørgensen & Jobling (1993) observed a smaller increase in growth in juvenile fish kept at the fastest velocity (2.0 BL s^{-1}) but only compared to fish kept at 1.0 and 1.5 BL s^{-1}, whereas we observed negative effects already at 1.5 BL s^{-1}. However, as discussed above, the discrepancies may be due to different experimental setups or may be an effect of testing different life stages. In a study on adult rainbow trout using a setup for fast velocity more similar to ours, Farrell et al. (1991) observed decreased growth at swimming speeds of 1.0–1.4 BL s^{-1} compared to fish kept at minimal velocity, which corresponds better with our results. The reduced growth was due to a reduction in feeding for fish at the faster velocity, resulting in less energy for growth. Our setup did not allow control of feed intake, but the stomach contents and visual observations indicated that all fish fed. All fish had also doubled their weight during the experiment, indicating good food accessibility. Kiessling et al. (1994) also observed a higher energy demand for exercised Chinook salmon expressed as an increase in feed intake. But their study was conducted in open circular systems where absolute swimming speed is difficult to evaluate. However, an increase in energy demand may explain the reduced growth at fast velocity in our study, where fish must allocate a large part of their available energy into swimming with less energy left for growth.

Metabolic effects and blood chemistry

We found that at moderate velocity, fish growth was more related to an increase in muscle mass in comparison to slow velocity, where part of the growth was due to increased lipid deposition. Increased lipid content with increased growth at faster velocity has been observed by East & Magnan (1987) in open circular systems. However, both Farrell et al. (1991) and Lauff & Wood (1996) found increased lipid metabolism in aerobically exercised rainbow trout, which could explain our findings with decreased lipid content and increased protein content in fish at moderate and fast velocities. Higher protein content associated with increased swimming speeds has also been observed by Houlihan & Laurent (1987), with white muscle protein synthesis positively correlated to whole animal growth rates. In the present study, intramuscular glycogen and lipids were higher in the fish kept at slow velocity, indicating an accumulation of intramuscular energy. ATP content did not differ between velocities. Depletion of ATP is closely linked to fatigue (McFarlane & McDonald 2002, Richards et al. 2002), which indicates that, irrespective of current velocity, the fish in the present study did not deplete their energy reserves, nor were they close to fatigue. In addition, lactate levels were not elevated in the fast velocity treatment, which may have been expected if there was anaerobic swimming activity. There was however, an increase in CrP in fish kept at fast velocity. This may be explained by an up-regulation of the CrP shuttle, compensating for an increased demand for ATP transport from the mitochondria, as observed in humans (Perry et al. 2012).

In our setup, fish at all velocities were forced to swim at the given velocity as there were limited possibilities to swim faster, slower or stand on the bottom. However, at slow velocity, the fish interacted and displayed more agonistic behaviours compared to fish at moderate and fast velocities (F. Solstorm et al. unpubl.). This likely explains the higher levels of and greater variation in lactate and potassium at slow velocity in comparison to the moderate and fast velocities, since fish displaying dominant behaviour may stress subordinate fish. Yet, this behavioural effect did not result in decreased growth, as previously described by Adams et al. (1995), suggesting that our setup minimised the effects of behaviour on growth. Osmolality could also be affected by behavioural stress, but our lower values in the fast velocity group are more likely caused by a physiological adaptation to the high swimming activity, as described by Gallaugher et al. (2001).

The lack of detectable treatment effects on haematocrit and haemoglobin levels may be affected by sampling time, as Djordjevic et al. (2012) discerned that values over 30 in haematocrit likely reflect sampling stress. However, they used cannulated fish, a method that reduces sampling stress, but unfortunately that was not possible in our setup.

Heart morphology

Relative heart size was greater in the fastest velocity group compared to the slow and moderate velocity groups. This result is in line with previous studies on exercised salmonids (Gallaugher et al. 2001, Castro et al. 2013), even considering differences in setup, although exceptions do exist (Farrell et al. 1990). The increase in heart size is most likely related to an increased cardiac workload during sustained swimming through an increase in aerobic respiration. For instance, improvements in cardiac output and output power (Farrell et al. 1991), increased active metabolic rate (Gallaugher et al. 2001), and increased number of capillaries (Davie et al. 1986) are known to result from exercise training in salmon.

CONCLUSIONS

Fish kept at a water velocity of 1.5 BL s^{-1} had a lower production performance than fish kept at either 0.8 or 0.2 BL s^{-1}. The slow velocity resulted in fish with higher lipid and lower protein content in the muscle compared to fish kept at moderate and fast velocities. Hence, fish kept at moderate velocity grew well and had a high protein content, indicating an efficient energy conversion. The reduction in growth in fish kept at fast velocity should be taken into account when evaluating new fish farm sites with fast water current velocity if the goal is to maximise production performance manifested as increase in total biomass. Based on our study and setup, it may be argued that water velocity over 1.5 BL s^{-1} could become problematic for Atlantic salmon. However, the results are evaluated on the strain, fish size and density that we employed at a temperature of 10°C. The slow velocity in this study is more difficult to relate to commercial farms since the fish in farms normally swim at higher speeds than the water velocity, whereas in the present study they had to swim at given velocity because of space restrictions. To benefit from the positive effects of exposed farming, such as better water quality and fewer conflicting

interests, the fish's swimming limitations need to be taken into account. Cages may need to be modified to create a more optimal environment with regard to currents. Furthermore, it may be worthwhile to study whether salmon from different strains and degrees of domestication perform better in fast velocity and may thereby be more suitable for production in exposed areas.

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III

The vertebral column and exercise in Atlantic salmon — Regional effects

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ABSTRACT

This study investigated the effect of swimming speed on the mineralization (mineral rate, Ca, P, Mg, Zn, K, Mn, Cu) and morphology of vertebra (V) in different anatomical regions of the vertebral column in Atlantic salmon. Triplicate groups of Atlantic salmon post-smolt were forced to swim at slow ($0.2 \pm$ SEM 0.02 BL s^{-1}), moderate ($0.8 \pm$ SEM 0.01 BL s^{-1}) and fast ($1.5 \pm$ SEM 0.02 BL s^{-1}) water current velocities for six weeks in raceways. The specific growth rate (SGR) was lower at fast (1.41 ± 0.04 SEM) compared to moderate (1.49 ± 0.02 SEM) and slow velocities (1.51 ± 0.03 SEM). In the post-cranial region (V1 → 8) fish at: (*i*) moderate and fast velocities developed higher relative V lengths, and mineral rate (% ash weight of dry weight), compared to fish at slow velocity; (*ii*) moderate velocity developed higher ratio between V length and dorso-ventral diameter (l/d-ratio) – more elongated V – and lower potassium ash content, compared to fish at slow, while those at fast velocity displayed intermediate values for both parameters. In the posterior-truncal region (V9 → 30), fish at moderate and fast velocity developed lower potassium ash content than those at slow. In the anterior-caudal region (V31 → 49), fish at: (*i*) fast velocity developed higher mineral rate than those at slow, with those at moderate displaying intermediate values; (*ii*) moderate velocity developed lower phosphorus and magnesium ash contents than those at slow and fast. In the ural region (V50 → 58), fish at: (*i*) slow velocity developed higher relative V lengths than those at moderate and fast; (*ii*) slow velocity developed higher l/d-ratio than those at fast, with fish at moderate displaying intermediate values. Also, ontological shifts in mineral rate, and Ca and K ash contents along the spine were observed; with time, Ca increased in all regions except the ural, while K decreased in all regions except the ural, where it increased, accompanied with a decrease in mineral rate.

The present results show that the positive effects of exercise on vertebrae in farmed Atlantic salmon are region specific.

Statement of relevance

Exercise promotes vertebra mineralization in Atlantic salmon.

1. Introduction

The vertebral column of Atlantic salmon (*Salmo salar*) consists of on average 58 amphicoelous vertebrae. These can be sub-divided into 4 distinct anatomical regions: (*i*) post-cranial region (R1) with vertebra nos. 1 to 8; (*ii*) posterior-truncal region (R2) with vertebra nos. 9 to 30; (*iii*) anterior-caudal region (R3) with vertebra nos. 31 to 49; (*iv*) ural region (R4) with vertebra nos. 50 to 58 (Kacem et al., 1998, Fig. 1). The vertebrae in R3 have the largest cranial to caudal length, dorso-ventral diameter, lateral diameter, and dry weight, but there is also an increase in the three latter parameters towards the head within R1 (Kacem et al., 1998). The shape of the vertebra, measured as the ratio between the cranial to caudal length and the dorso-ventral diameter (l/d ratio), also changes along the vertebral column, and there is a general increase

with age i.e. the vertebrae gets more elongated in post-smolts (Fjelldal et al., 2005). Vertebrae in the anterior-caudal region have the highest l/d ratio, mineral rate, and mechanical strength throughout life (Fjelldal et al., 2005; Fjelldal et al., 2006; Fjelldal et al., 2009). Ontologically, the vertebrae in the anterior-caudal and ural regions increases in relative size during parr-smolt transformation (Fjelldal et al., 2006), probably as an adaption to increased swimming activity in post-smolts compared to parr. The teleost vertebral column is not weight bearing, and mechanical loading is restricted to muscular contractions imposed by the large lateral muscle during swimming and locomotion. Swimming in salmonids is classified as sub-carangiform swimming where the posterior part of the body is undulating to generate a forward force (Sfakiotakis et al., 1999). Considering this swimming mode, the mechanical load is likely greatest in the anterior-caudal and ural regions of the vertebral column.

Exercise has a positive effect on vertebra in salmonids, shown by enhanced mineralization of anterior-caudal vertebrae with increasing swimming speed (Deschamps et al., 2009; Totland et al., 2011). The
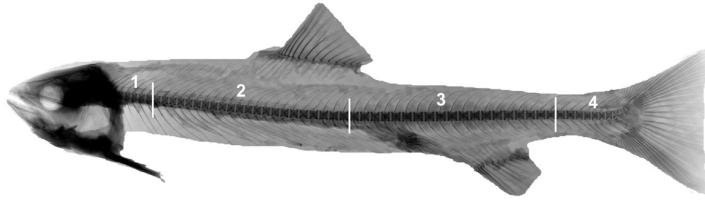
Fig. 1. Lateral radiograph of the Atlantic salmon. The vertebral (V) regions are shown: (*i*) post-cranial region (R1) with vertebra nos. 1 to 8; (*ii*) posterior-truncal region (R2) with vertebra nos. 9 to 30; (*iii*) anterior-caudal region (R3) with vertebra nos. 31 to 49; (*iv*) ural region (R4) with vertebra nos. 50 to 58.

two major bone minerals are Ca and P, but also other elements like Zn, Mn, K, Mg and Cu are present in lower amounts (Roy and Lall, 2003). There are no teleost studies on how exercise affects vertebra mineralization and morphology in different regions of the vertebral column, or how mineral composition varies accordingly.

Swimming speed in the marine phase of wild salmonids is on average 1 body length (BL) s^{-1} (Drenner et al., 2012), which correspond well with what has been documented for farmed salmon (Blyth et al., 1993; Juell and Westerberg, 1993; Kadri et al., 1991; Sutterlin et al., 1979). Swimming speeds of 0.8 to 1.0 BL s^{-1} has also been reported as the preferred swimming speed in brook charr (*Salvelinus fontinalis*) when the fish was free to choose (Tudorache et al., 2011). However, following the trend of more farms being located in exposed areas with fast water current velocities fish may have to swim faster than what is considered optimal. In some areas there are reports of water current velocity up to 80 cm s^{-1} and areas with current velocity of 150 cm s^{-1} are being considered for salmon farming (Pers. Comm. Øystein Patursson Ph.D. Fiskaaling, Aquaculture Research Station at the Faroes). The effect that these fast water currents have on fish is scarcely investigated. In sheltered areas with slow water current velocity the fish have the possibility to choose their swimming speed. But, in exposed areas the fish must swim at the prevailing velocity (Johansson et al., 2014). Increased swimming speeds are often conceived as positive for farmed fish with increases in growth, protein deposition, cardiac output, energy stores, metabolic rate, swimming performance etc. (Castro et al., 2011; Christiansen and Jobling, 1990; Davison, 1997; East and Magnan, 1987; Farrell et al., 1991; Farrell et al., 1990; Houlihan and Laurent, 1987; Jørgensen and Jobling, 1993; McFarlane and McDonald, 2002; McKenzie et al., 2012; Pearson et al., 1990; Totland et al., 1987).

In this study we examined the effects of slow (0.2 BL s^{-1}), moderate (0.8 BL s^{-1}), and fast (1.5 BL s^{-1}) swimming speeds on vertebra in different regions of the vertebral column in Atlantic salmon post-smolts, with emphasize on vertebrae size and morphology, mineralization and mineral composition.

2. Materials and methods

2.1. Experimental design and animals

Postsmolt Atlantic salmon, 98.6 ± 20 g, 22.3 ± 1.3 cm, mean ± SD, (AquaGen strain, hatched March 2011) were forced to swim at different water current velocities during a six week trial from April till May 2012, in the Tank Environmental Laboratory at the Institute of Marine Research, Matre (Norway). Fish were kept at three different velocities in triplicate raceways (n = 80 per raceway) submerged in tanks (∅ 3 m, 5.3 m^3). The raceways were designed as an open cylinder creating homogenous water current (Solstorm et al., 2015). The trans-sectional area of the cylinder was 0.10 m^2 (∅ 0.36 m) and the length available for the fish was 2.0 m, giving a volume of 0.20 m^3. Slow, moderate and fast (0.2 ± 0.02, 0.8 ± 0.01 and 1.5 ± 0.02 BL s^{-1} (mean ± SEM)) water currents were generated with an electric engine (Minn Kota RT80/EM, Johnson Outdoors Marine Electronics, Inc., Racine, WI,

USA) with adjustable speed. A honeycomb (5.0 mm opening and 101.6 mm long, PC 5.0 G4, Plascore GmbH & Co KG, Waldlaubersheim, Germany) assured a laminar flow. A plastic coated wire netting (10 mm × 10 mm) was placed at the outlet and on the top to keep the fish inside. Fish were transferred to raceways at a stocking density of 38.7 ± SEM 0.28 kg m^{-3} (n = 80 per raceway). Five fish from each replicate were removed every second week during the trial to minimize increases in density. During the trial, constant temperature (10 °C), water exchange (120 l min^{-1}), salinity (33 psu) and continuous light were maintained. Dissolved oxygen (DO) levels were always above 80%. Fish were fed commercial feed (Skretting Spirit 75) in excess throughout the day (24 h) to ensure sufficient food supply.

All fish were anesthetized (100 mg/l Finquel®), weighed (±1 g) and measured (±0.5 cm) before and after the trial.

2.2. Bone analysis

Fish were rapidly killed and frozen prior to and after the trial for vertebra analysis. 30 fish were collected before the trial and 10 from each of the 9 replicate after the trial. All the frozen fish were thawed and carefully filleted to remove surrounding flesh around the vertebral column. This was done in order to increase the quality of the radiograph images. Subsequently, the vertebral columns were radiographed (Porta 100 HF; Eickemeyer Medizintechnik für Tierärzte KG, Tuttlingen, Germany) onto a 35 × 43 cm image plate in a rigid cassette (Dürr Medical, Bietigheim-Bissingen, Germany) with 40 kV and 10 mAs with a distance of 70 cm. The image plate was scanned (CR 35 VET; Dürr Medical) and the resulting image converted into a TIFF file (Vet-Exam Plus Software, version 4.14.0). The software program Adobe Photoshop CS2 was used for the evaluation of vertebral deformities, and Image J was used to measure vertebrae cranial-caudal lengths and dorso-ventral diameters. Five random fish from each replicate was selected for further analysis of mineral rate (% ash weight of dry weight) and mineral composition. Analyses were performed on three vertebrae in each of the four anatomical regions; post-cranial (R1) – vertebra numbers (V nos) 3–5, posterior-truncal (R2) – V nos 18–20, anterior-caudal (R3) – V nos 40–42 and ural (R4) – V nos 53–55. Mineral rate was measured according to Grini et al. (2011). For analysis of mineral composition, ash samples were pooled per replicate and region and sent to ALS Laboratory Group Norway AS (Oslo, Norway) for analysis of calcium (Ca), phosphorus (P), zinc (Zn), magnesium (Mg), potassium (K), manganese (Mn) and cupper (Cu) content in mg kg^{-1} ash.

2.3. Statistics

Statistical analysis were performed in STATISTICA (StatSoft, Inc. 2012. data analysis software system, version 11. www.statsoft.com). Data were analysed with nested ANOVA to check for possible tank effects. Significant nested ANOVAs ($p < 0.05$) were followed up by one-way ANOVAs, followed by Fisher LSD-test. All values are presented per region and are shown as mean ± standard error of mean (SEM).

3. Results

3.1. Growth

During the experiment fish at all velocities had a good growth in both length and weight (0.91–0.99 mm day^{-1} and SGR 1.41–1.51). Fish at fast velocity grew slower compared to fish at moderate and slow velocities with respect to both weight and length (Slow: 0.99 mm day^{-1} ± 0.03 SEM, SGR 1.51 ± 0.03 SEM, Moderate: 0.98 mm day^{-1} ± 0.02 SEM, SGR 1.49 ± 0.02 SEM, Fast: 0.91 mm day^{-1} ± 0.02 SEM, SGR 1.41 ± 0.04 SEM).

3.2. Vertebra size and morphology

The relative length of the vertebra differed between regions with the anterior-caudal region having the longest vertebrae and the post-cranial region the shortest (Fig. 2). The posterior-truncal region had the second longest vertebras and the ural region had the second shortest vertebrae. In the post-cranial region, the relative vertebral length decreased during the 6 week experiment. There was also an effect of exercise in this region at the end of the experiment, where fish kept at slow velocity had shorter vertebrae compared to fish at moderate and fast velocities. The effect of time could also be seen in the anterior-caudal region, but with the opposite effect. Here, vertebrae were longer after the 6 week experiment. In the posterior-truncal region, no effect of time or treatment was observed. In the ural region fish from moderate and fast velocities had the same relative length as the start fish, but fish from slow velocity had increased their vertebra length.

At the start of the experiment the ratio between the length and the dorso-ventral diameter (l/d ratio) differed between regions, with the anterior-caudal region having the most elongated vertebra (Fig. 3). The posterior-truncal region had the second most elongated vertebrae and the post-cranial and ural region had the least elongated vertebrae. The corresponding differences were seen at the end of the experiment.

But a difference between the post-cranial and ural region for fish kept at slow velocity was also observed with vertebrae in the ural region being more elongated. In the post-cranial region there was a decrease in the l/d ratio over time. There was also an effect of swimming speed in this region where fish at moderate velocity had a higher l/d ratio compared to fish at slow velocity. In the posterior-truncal and ural regions, only the fish in the fast group displayed a reduced l/d ratio with time. A reduction in l/d ratio for fish at fast velocity was also observed compared to fish at slow velocity at the end of experiment in the ural region.

3.3. Vertebra deformities

There were no difference in the occurrence of fish with deformed vertebra on radiographs between current velocities (Slow: 15.2%, Moderate: 15.2%, Fast: 13.3%), and no difference in average number of deformed vertebra among deformed individuals (Slow: 3.0 ± 0.6SEM, Moderate: 3.0 ± 0.7SEM, Fast: 3.75 ± 1.1SEM).

3.4. Mineral rate

At the start of the experiment mineral rate was higher in the anterior-caudal region compared to the other regions (Fig. 4). In fish kept at slow and fast velocities, mineral rate was higher in the anterior-caudal region than in the post-cranial and ural region, and the posterior-truncal region was higher than the ural region. At moderate velocity, the mineral rate in the ural region was lower than the other regions. In the post-cranial region, there was an effect of treatment with higher values in fish at moderate and high velocities compared to slow velocity, and over time in fish kept at moderate and fast velocities, but not at slow velocity. The posterior-truncal region showed no effect over time or between treatments. In the anterior-caudal region, there was an effect of treatment with a higher mineral rate in fish kept at fast velocity compared to fish at slow velocity. There was an effect over time in the ural region with a higher mineral rate at the start of the experiment.
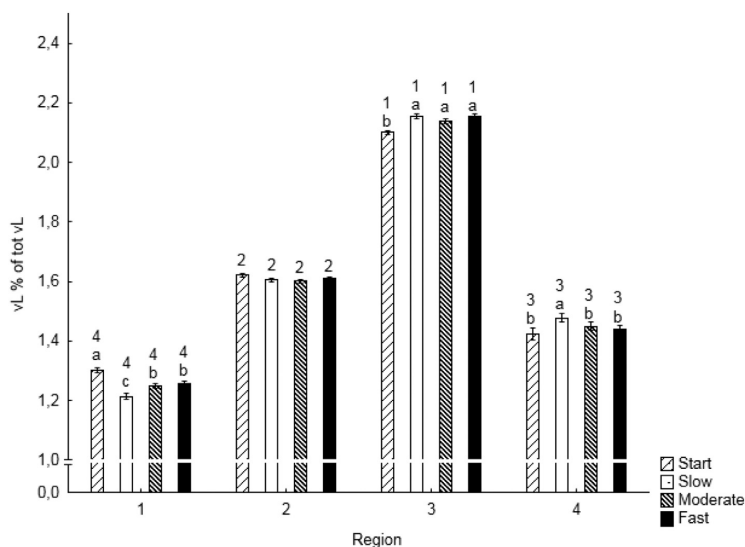

Fig. 2. Vertebra length (as % of total vertebra length, mean ± S.E.M.) in the post-cranial (Region 1), posterior truncal (Region 2), anterior caudal (Region 3), and ural regions (Region 4) of the vertebral column in triplicate groups of Atlantic salmon post-smolt that were maintained at 3 different water current speeds (Slow; 0.2 body lengths (BL) s^{-1}; Moderate; 0.8 BL s^{-1}; Fast; 1.5 BL s^{-1}) for six weeks. The data is based on mean values per region per individual (n = 10 per replicate tank; 30 per group). Different numbers denote significant difference ($p < 0.05$) between regions within treatment, and different lowercase letters denote significant difference between treatments within region.
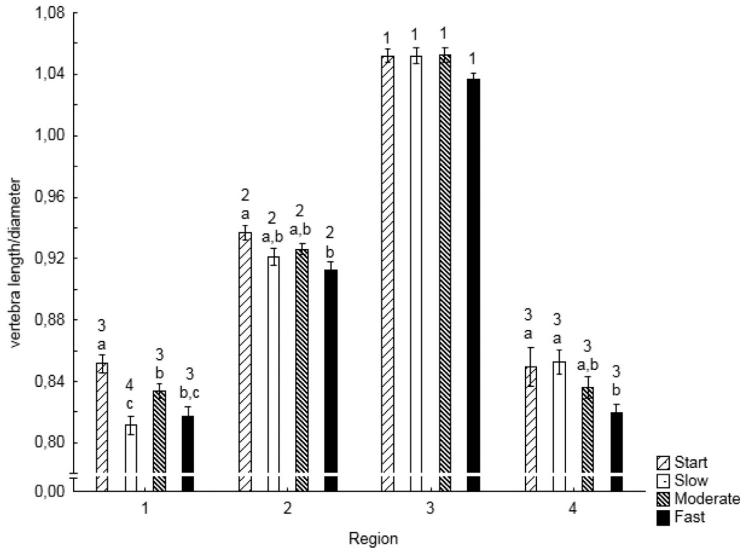
Fig. 3. Vertebra length/dorso-ventral diameter (l/d-ratio) (mean ± S.E.M.) in the post-cranial (Region 1), posterior truncal (Region 2), anterior caudal (Region 3), and ural regions (Region 4) of the vertebral column in triplicate groups of Atlantic salmon post-smolt that were maintained at 3 different water current velocities (Slow; 0.2 body lengths (BL) s^{-1}; Moderate; 0.8 BL s^{-1}; Fast; 1.5 BL s^{-1}) for six weeks. The data is based on mean values per region per individual (n = 10 per replicate tank; 30 per group). Different numbers denote significant difference ($p < 0.05$) between regions within treatment, and different lowercase letters denote significant difference between treatments within region.

3.5. Vertebra ash composition

3.5.1. Calcium

There were no differences between regions at the start of the experiment (Fig. 5). In fish kept at slow velocity the post-cranial region had higher levels of ash calcium compared to the anterior-caudal and ural regions, and the anterior-caudal had higher levels than the ural region. In fish at moderate and fast velocities, the ural region differed from the other regions with lower calcium content. In the post-cranial and anterior-caudal region, there was an effect of time in fish at slow and moderate velocities with a higher level at the end of the experiment in these groups. Fish at moderate velocity had a higher calcium content
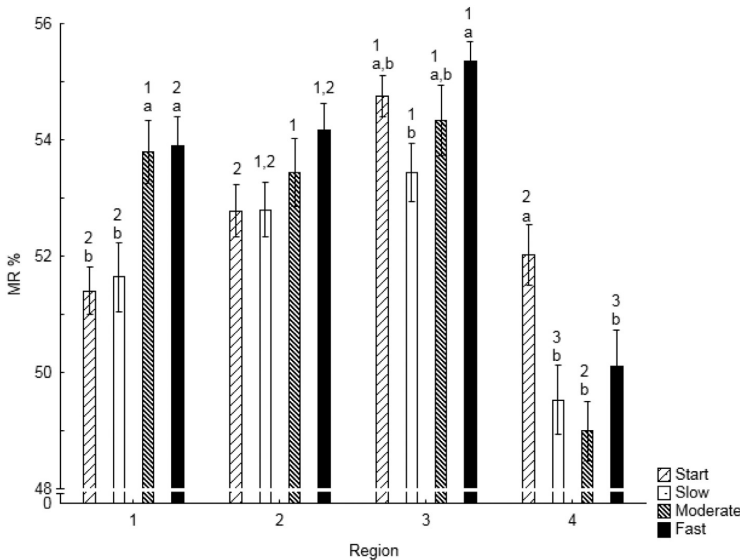

Fig. 4. Vertebrae mineral rate (MR%, percentage ash weight of defatted dry weight, mean ± S.E.M.) in the post-cranial (Region 1), posterior truncal (Region 2), anterior caudal (Region 3), and ural regions (Region 4) of the vertebral column in triplicate groups of Atlantic salmon post-smolt that were kept at 3 different water current velocities (Slow; 0.2 body lengths (BL) s^{-1}; Moderate; 0.8 BL s^{-1}; Fast; 1.5 BL s^{-1}) for six weeks. The data is based on mean values per region per individual (n = 5 per replicate tank; 15 per group). Different numbers denote significant difference ($p < 0.05$) between regions within treatment, and different lowercase letters denote significant difference between treatments within region.
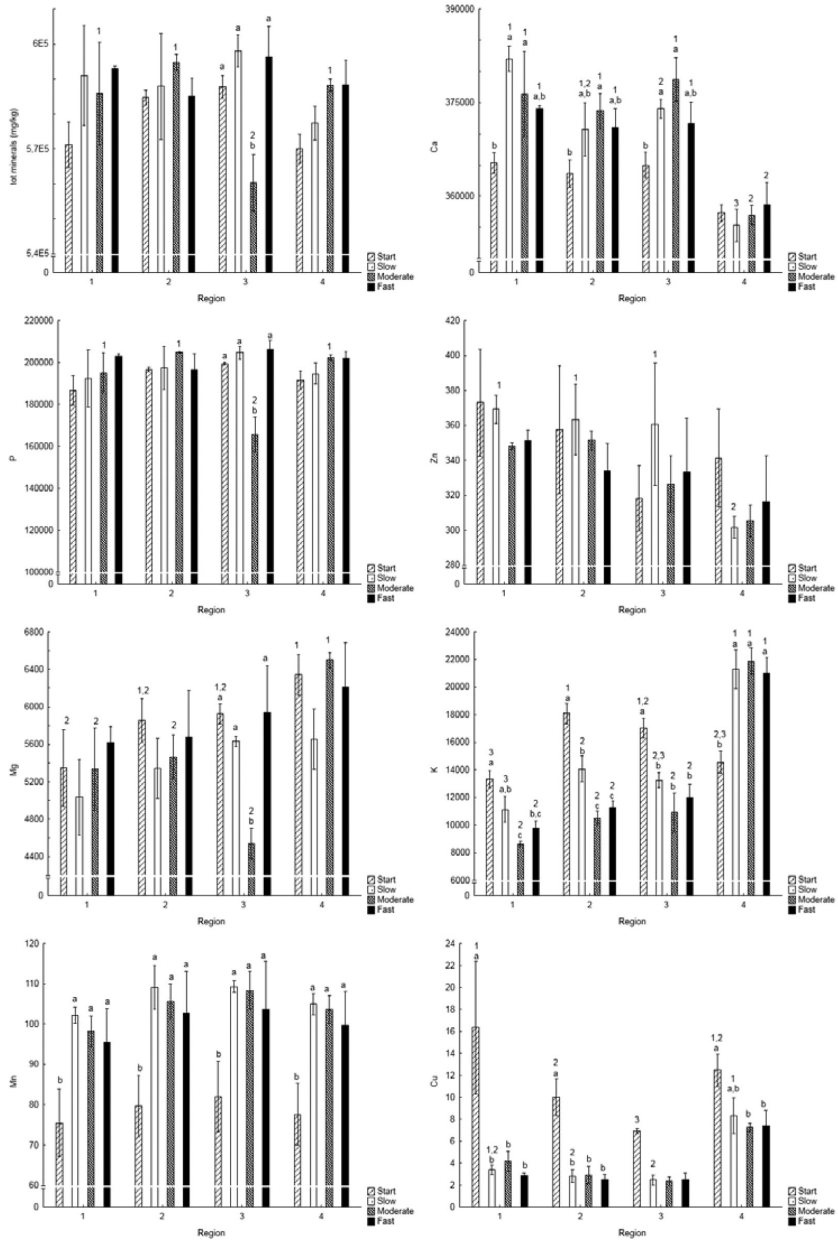
Fig. 5. Vertebrae minerals (mg kg^{-1} ash) presented as mean ± S.E.M. in different regions of the vertebral column in the post-cranial (Region 1), posterior-truncal (Region 2), anterior-caudal (Region 3), and ural regions (Region 4) of the vertebral column in triplicate groups of Atlantic salmon post-smolt that were kept at 3 different water current velocities (Slow; 0.2 body lengths (BL) s^{-1}; Moderate; 0.8 BL s^{-1}; Fast; 1.5 BL s^{-1}) for six weeks. The data is based on mean values per region pooled per replicate (n = 1, 5 polled per replicate tank; 3 per group). Different numbers denote significant difference ($p < 0.05$) between regions within treatment, and different lowercase letters denote significant difference between treatments within region.

compared to start levels in the posterior-truncal region. No effects of time could be seen in the ural region and there were no effects between treatments in any regions.

3.5.2. Phosphorus

Phosphorus ash content was similar in all regions except for fish at moderate velocity in the anterior-caudal region where a lower level

was observed both compared to other regions and treatments as well as over time (Fig. 5).

3.5.3. Zinc

In fish kept at slow velocity, there was an effect of vertebra region, with lower zinc ash content in the ural region compared to the other regions (Fig. 5). No effects over time or between treatments were discernible.

3.5.4. Magnesium

At the start of the experiment the ural region had higher levels of magnesium in ash compared to the post-cranial region (Fig. 5). At the end of the experiment fish at moderate velocity had a higher magnesium level in the ural region compared to all the other regions. There was an effect over time in the anterior-caudal region where fish at moderate velocity had lower magnesium content at the end of the experiment. This magnesium content in the anterior-caudal region for fish at moderate velocity was also lower compared to fish at fast and slow velocities in the same region.

3.5.5. Potassium

The posterior-truncal region had higher levels of potassium in ash compared to the post-cranial and ural region at the start of the experiment, and the anterior-caudal region was higher than the post-cranial (Fig. 5). At slow velocity fish had higher levels in the ural region compared to all other regions, and the posterior-truncal region was also higher than the post-cranial. In fish at moderate and fast velocities, the ural region had higher levels than all the other regions. In the post-cranial region, there was an effect over time in fish at moderate and fast velocities with a decrease at the end of the experiment. There was also a treatment effect with higher levels of potassium in fish at slow velocity compared to fish at moderate velocity. In the posterior-truncal and anterior-caudal regions there was a similar effect over time, but with decreasing levels in all the treatments. Potassium level in fish at slow velocity was also higher compared to fish at moderate and fast velocities in the posterior-truncal region. A reverse effect was seen in the ural region with increasing levels of potassium in all treatments over time.

3.5.6. Manganese

Manganese ash content was lower at the start when compared to at the termination of the experiment in all regions (Fig. 5). No effects between regions or treatments were discernible.

3.5.7. Cupper

There was an effect of region at the start of the experiment with higher levels of cupper ash content in the post-cranial region compared to the posterior-truncal and anterior-caudal regions (Fig. 5). The posterior-truncal region was also higher than the anterior-caudal. At the end of the experiment fish at slow velocity had a higher level in the ural region compared to the posterior-truncal and anterior-caudal regions. In the post-cranial and posterior-truncal regions, there was an effect of time with higher start levels compared to all the treatments. In the ural region, there was decreasing effect over time in fish at moderate and fast velocities.

4. Discussion

Our main findings are that swimming speed has region-specific effects on vertebra size and morphology, and mineral rate and composition. Increased swimming speed resulted in longer post-cranial vertebrae and more compressed ural vertebrae, increased mineral rate in post-cranial and anterior-caudal vertebrae, and decreased potassium ash content in post-cranial and posterior-truncal vertebrae. Further, the mineral rate and composition changed over time: (*i*) ash content of Ca increased and K decreased in post-cranial, posterior-truncal and anterior-caudal

vertebrae; (*ii*) mineral rate decreased and ash K content increased in ural vertebrae; (*iii*) Mn decreased and Cu increased throughout the vertebral column.

The fish growth rate (SGR 0.91–0.98 mm day^{-1}) during the experimental period was comparable to what has previously been reported in Atlantic salmon post-smolts reared in tanks at the same temperature (Fjelldal et al., 2011: SGR 0.92–0.97; Ørnsrud et al., 2013: SGR 1.09–1.27). This implies that the system used did not impair the ability to feed, or the appetite of the fish.

Fish from slow and moderate velocities grew better than fish at fast velocity both with respect to weight and length. The physiology of the fish presented in Solstorm et al. 2015 showed that fish at moderate velocity had the best muscular growth while fish at slow velocity gained more fat. This indicates that fish at moderate velocity had the best exercise effect with respect to muscular growth. Exercise has in most previous studies been demonstrated to have a positive effect on growth (East & Magnan 1987, Houlihan & Laurent 1987, Totland et al. 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Davison 1997, Castro et al. 2011), but there are exceptions where fish at fast velocity have displayed a decrease in growth similar to our result (Farrell et al. 1991). In the present study, the lower growth rate in fish at fast velocity did not seem to affect exercise effect on the vertebrae.

In fish at slow current velocity, the posterior-caudal and ural vertebrae showed an increase in relative lengths over the 6 week experimental period. This growth pattern has been shown in post-smolts in an earlier study (Fjelldal et al., 2006). The fish at moderate and fast velocities developed relatively longer anterior-caudal vertebrae over time, but not in the ural region. Indeed, in the present study, increased current velocity resulted in relatively longer post-cranial vertebrae and relatively shorter ural vertebrae, accompanied by a development of more elongated post-cranial vertebrae, and more compressed ural vertebrae. In Atlantic salmon post-smolt, photoperiod is known to affect the growth pattern along the vertebral column (Fjelldal et al., 2005). This may have been an effect of different swimming speeds under continuous light when compared to natural light in sea-cages (Hansen et al., unpublished data). It may be that the mechanical loading on the vertebrae is different in different regions as swimming speed increases, and that this gives site specific effect convoyed through the regulation of growth in individual vertebrae or sets of vertebrae. Indeed, in mammals, bone formation is stimulated at anatomical sites of high strain energy (Carter et al., 1996; Currey, 1984, 2003; Huang et al., 2003; Notomi et al., 2001; Timlin et al., 2000). Further, exercise is known to promote bone mineralization in mammals (Turner, 2006; Wallace et al., 2007; Warden et al., 2007), and teleost (Deschamps et al., 2009; Totland et al., 2011). Totland et al. (2011), suggested the bone lining osteoblast as the most obvious mechanosensory cell candidates in Atlantic salmon, while the osteocytes that are located within the bone matrix are believed to have this role in mammals (Bonewald and Johnson, 2008). The presently observed positive effect of exercise on mineral rate, orchestrated by the increase in relative vertebra length, and development of more elongated (increased l/d ratio) post-cranial vertebrae indicates a stimulatory effect of exercise on osteblasts in this region. Conversely, the general decrease over time and lack of exercise effects on mineral rate, lack of the earlier documented increase in relative vertebra length at this life stage, and the development of more compressed shaped (decreased l/d ratio) in ural vertebrae with increasing velocity speed may indicate that the function of the osteblasts is different there. The reduced mineral rate over time in the ural vertebrae probably reflects that there were less minerals incorporated into the bone matrix that was laid down in the 6 week period from experimental start to termination, as discussed by Totland et al. (2011). That the radiological examination showed no pre-clinical signs of vertebra deformity in the ural vertebra indicates that the observed alternations in vertebra shape are morphological adaptations to increased swimming speed. Ca incorporation in the vertebra over time was generally lower in ural compared to post-cranial, posterior-truncal and anterior-caudal vertebrae; this may

explain the general decrease over time in mineral rate in the ural vertebrae. Since ash content of Ca was ~15 fold higher than that of K, the general increase in ash K content in ural vertebra was not sufficient to prevent the drop in mineral rate. The regional opposite changes of Ca and K over time shows that these changes are ontological. Changes over time in ash Mn and Cu, however, are pronounced, but with no regional differences. Although the fish were fed the same type of commercial diet during the experimental period, the possibility of an altered diet composition of Cu and Mn with time cannot be excluded. Ontological differences in growth between different vertebra regions have been shown for Atlantic cod (*Gadus morhua*) (Fjelldal et al., 2013) and Atlantic salmon (Fjelldal et al., 2006), but such differences has not been reported before with respect to mineral rate or composition. That there are different regional responses to exercise and regional differences in mineral rate and composition during ontogeny can be related to distinct developmental differences between the trunk and tail. For instance, in zebrafish (*Danio rerio*) embryos, Hox genes are expressed only in the regions that originate solely from the Spemann organizer – here the postcranial and posterior-truncal regions – where they are expressed in different combinations in numeric order in a specified segment and may participate in determining the regional identity of the vertebrae (Agathon et al., 2003; Ahn and Gibson, 1999; Kimmel et al., 1995; Morin-Kensicki et al., 2002; Prince et al., 1998; van der Hoeven et al., 1996). Regional differences in expression of the two mineralization-related genes *bgp* and *alp* caused by regional differences in mechanical loading may be responsible for the presently observed effects on exercise on vertebra mineralization (Totland et al., 2011).

Also, anterior-caudal vertebrae responded with increased mineral rate to exercise in the present study. This finding has been reported before (Totland et al., 2011). Since the anterior-caudal vertebrae are largest and most highly mineralized, earlier studies have suggested the mechanical loading during swimming to be largest in these vertebrae (Fjelldal et al., 2004, 2005, 2006). The fish sampled at the start, and the fish at slow current velocity in the present study displayed the earlier documented pattern in mineral rate along the vertebral column, with steadily increasing mineral rate from the post-cranial to the anterior-caudal region, followed by decreased mineral content in the ural region (Kacem et al., 2000; Fjelldal et al., 2005). In the present study, both P and Mg ash contents were lower in fish at moderate compared to slow and fast current velocities in the large anterior-caudal vertebra. The lack of response on P and Mg to exercise at high velocity may be coupled to the slower growth in this group. Also the total content of all measured elements was lowest in the anterior-caudal vertebrae at moderate velocity, while mineral rate was intermediate between the slow and fast velocities. The gap between the total content of measured elements and mineral rate indicates that the mineral fraction in the large anterior-caudal vertebra of the moderate current velocity group consisted of a more calcium rich apatite with a higher carbonate content (Pellegrino and Biltz, 1968; Mkukuma et al., 2004). A rationale explaining why P and Mg should be partly replaced by carbonate during formation of new bone tissue, under exercise conditions with fast growth, is not clear. The present study, confirms the earlier studies on the size, mineral rate and effect of exercise on mineralization in the anterior-caudal vertebrae, but brings forward the post-cranial vertebrae as being most heavily affected by exercise in Atlantic salmon post-smolts. Indeed, in farmed Atlantic salmon, deformities occasionally develop in this region with un-known causes (reviewed in Fjelldal et al. (2012)). Development of deformities in post-cranial vertebrae during the parr stage (Fjelldal et al., 2007), may be linked to that farmed salmon are forced to constant swimming in tanks during a life stage where they naturally are bottom dwelling and not constantly swimming. Although posterior-truncal vertebrae showed no effect of exercise on mineral rate, the ash K content in these vertebrae was lower in the fish at moderate and fast current velocities compared to fish at slow current velocity. Why exercise reduces incorporation of K especially in posterior-truncal vertebrae is not clear. Liang et al. (2014) found that

vertebra K content increased with increasing dietary K content in grass carp (*Ctenopharyngodon idella*). If increased dietary K would prevent an exercise effect on bone K content remains to be studied.

With regard to practical fish farming, the present study shows that the vertebral column of farmed Atlantic salmon post-smolt adapts to increased current velocity by morphological changes and increased mineralization. The increased mineral rate in the bone of the vertebra with increased swimming speed shows that exercise has a positive effect on bone health. However, the reduced SGR at $1.5 \pm SEM\ 0.02\ BL\ s^{-1}$ shows the potential down-side of high current velocity.

Acknowledgement

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IV

Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*

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ABSTRACT: Increased swimming speed of Atlantic salmon is generally considered an improvement to welfare under aquaculture settings, as group structure is improved and agonistic behaviour reduced. As such, establishing fish farms in exposed areas with fast water current velocities should be favourable. However, at some locations, velocities exceed what is known as preferable for salmonids, and this may compromise fish welfare. In this study, behaviour and fin erosion were observed on post-smolt salmon stocked at 39 kg m^{-3} in raceways at 3 water current velocities: fast (1.5 body lengths [BL] s^{-1}), moderate (0.8 BL s^{-1}) and slow (0.2 BL s^{-1}). Movements that affect group structure and interactions between individuals varied by up to 20-fold between velocities. A behavioural change occurred directly after velocities were set. Severe fin erosion decreased over time in all groups, but new injuries increased almost 3-fold in the faster-velocity group. Our results suggest that moderate velocity is ideal from a welfare perspective. At slow velocity, higher frequency of structural movements and between-individual interactions could be stressful for the fish. At faster velocity, the fish have to focus on swimming, which could increase unintentional collisions with obstacles and other individuals and result in new fin erosion. Our results suggest that management of water currents may be an effective way of controlling behaviour and may thereby improve welfare.

KEY WORDS: Exposed farming · Swim speed · Environmental variability · Swimming behaviour

INTRODUCTION

Motivational drivers for swimming in wild Atlantic salmon include migration, feeding, predatory avoidance and mating. For fish in aquaculture systems, few of these drivers are functional. Farmed fish cannot move to another habitat, they do not need to hunt food or avoid predators, and they usually do not mature sexually. In the marine life stages, wild salmonids swim on average at 1 body length (BL) s^{-1} (Drenner et al. 2012). This is similar to observed swimming speeds in salmon farms (Sutterlin et al. 1979, Kadri et al. 1991, Blyth et al. 1993, Juell & Westerberg 1993). In the

wild, this cruising speed is suggested to be the energetic optimum with lowest cost of transport (Drenner et al. 2012). Similarly, a laboratory study by Tudorache et al. (2011) showed that the optimal swimming speed for brook charr *Salvelinus fontinalis* was 1 BL s^{-1} and that this corresponded to the preferred swimming speed (0.8 and 1.0 BL s^{-1}) when the fish was free to choose. Swimming at a preferred speed may be considered positive for welfare. One approach to fish welfare is the Five Freedoms (defined by the UK's Farm Animal Welfare Council [FAWC 1995]). One of the freedoms concludes that the animal should be free to express normal behaviour; swimming at a preferred

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speed could be considered a normal behaviour and, hence, positive for welfare.

An increasing number of salmon farms are now located in exposed areas with fast water current velocities, where the fish are forced to swim faster than their preferred speed. Previous laboratory studies have, to our knowledge, only demonstrated positive behavioural effects of increased swimming speeds, which would imply improved welfare (e.g. Korte et al. 2007). Slow current velocities may result in more interactions between individuals and increased aggression that could result in fin erosion (Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Adams et al. 1995, Turnbull et al. 1998) and promote secondary infection (Schneider & Nicholson 1980), reducing production performance and welfare (Stien et al. 2013). Faster current velocities have been reported to reduce agonistic behaviour and create a more ordered group structure with higher production performance (East & Magnan 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Adams et al. 1995). Based on this, fast current velocities in exposed areas could be considered positive for the fish up to an unknown critical level. However, at water velocities above 0.7 BL s^{-1}, Johansson et al. (2014) observed a breakdown of the circular group structure in Atlantic salmon *Salmo salar* in net cages. When the current increased further to 0.9 BL s^{-1}, all fish abandoned the circular school structure and maintained a position facing the current. As the current shifted, there was a chaotic and challenging transition before a new group structure was established. This indicates that the fish strive for structure to cope with the dynamic environment and the high densities in the cage (see also Ashley 2007). Farms located in areas with strong tidal currents will expose salmon to repeatedly changing current conditions. At more extreme current velocities than Johansson et al. (2014) observed, it is unclear how the group structure and behaviour of the fish change. As the fish approach their maximum sustainable swimming capacity, all available energy would assumedly be allocated to swimming. This may result in decreased energy stores and thus reduced growth (East & Magnan 1987, Farrell et al. 1991, Jørgensen & Jobling 1993) and welfare (e.g. Ashley 2007).

In previous studies, no negative effects of fast water current velocities were found on behaviour and fin erosion, and positive effects of swimming speeds up to 2.5 BL s^{-1} have been reported (East & Magnan 1987). However, negative effects of fast current velocity on physiology and production performance have been demonstrated in Solstorm et al.

(2015) and thus are hypothesised to also have impacts at the behavioural level. This study performed a detailed analysis of behaviour and fin erosion when Atlantic salmon post-smolts were exposed to water currents of slower and faster velocities than their preferred range, with the latter prevailing in farms located at exposed sites.

MATERIALS AND METHODS

Facilities and experimental animals

A behavioural study of post-smolt Atlantic salmon (AquaGen strain, hatched March 2011) exposed to water currents was conducted at the Tank Environmental Laboratory at the Institute of Marine Research, Matre (Norway), during 6 wk starting April 2012. The study was part of a larger experiment also investigating physiological effects, where a more detailed description of setup and experimental design can be found (Solstorm et al. 2015). Fish (98.6 ± 20 g, 22.3 ± 1.3 cm, mean ± SD) were transferred to raceways at a mean (±SE) stocking density of 38.7 ± 0.28 kg m^{-3} (n = 80 per raceway) as smolts. This density was chosen as representative for typically observed swimming densities in sea cages, where salmon are known to trade off variable environmental drivers, including temperature and water currents (Oppedal et al. 2011a,b, Johansson et al. 2014). Prior to smoltification, fish had been reared in tanks (∅ 5 m) under natural light and temperature conditions. To finalise smoltification, fish were kept on a constant light regime for 8 wk at 8°C with a flow of 150 l min^{-1} until all fish had smoltified.

Raceways (trans-sectional area 0.10 m^2, ∅ 0.36 m, length 2.0 m, giving a volume of 0.20 m^3) were submerged in circular tanks (∅ 3 m, 5.3 m^3). A laminar water current velocity was produced by an electric engine (Minn Kota RT80/EM, Johnson Outdoors Marine Electronics) with adjustable speed followed by a honeycomb (5.0 mm opening, 101.6 mm thickness, PC 5.0 G4, Plascore). Water temperature was 10°C, water exchange was 120 l min^{-1}, salinity was 33 psu and dissolved oxygen levels were above 80%. A constant light regime was maintained during the experiment, and the fish were fed (Skretting Spirit 75) in excess every 15 min throughout the day (24 h) to ensure that food would not be a limiting factor. Feed was distributed by automatic feeding units (Arvo-Tec T Drum 2000, http://www.arvotec.fi) controlled from custom-made computer software (SD Matre, Normatic AS). An underwater video camera (SV27, SeaVision) was

mounted in the middle of each raceway, with the field of view covering the posterior area of the chamber. Video recordings were stored on a central PC by a video capture card (GV-800, GeoVision) and multi-camera surveillance system (CV-800, GeoVison).

Experimental design

Fish were kept at a velocity of 0.5 BL s^{-1} in the raceways to acclimatise for 19 d prior to the experiment. This velocity was chosen as it is the velocity typically used for acclimation prior to swimming performance tests in salmonids, and all fish were to experience a change when the trial commenced. During the experiment, fish were kept at 3 different water current velocities for 6 wk in 4 replicate raceways: slow, moderate and fast velocities corresponding to 0.2 ± 0.02, 0.8 ± 0.01 and 1.5 ± 0.02 BL s^{-1} (mean ± SE), respectively. In this setup, water current velocity was considered the same as swimming speed since the fish had to hold station against the current not to be swept back into the netting and had limited space (9 to 7 BL, upstream–downstream direction) to move around freely. Slow current velocity was adjusted to be close to zero without compromising oxygen levels and the transport of faeces and food waste out of the raceway. Moderate current velocity was selected to be in the range of the preferred swimming speed (Tudorache et al. 2011). Fast current velocity was chosen to be twice the amplitude of the moderate but still below the critical swimming speed (Tang & Wardle 1992, Stevens et al. 1998), although actual velocity was slightly lower. After 3 wk, the currents were adjusted to the increased fish length (due to growth) to maintain the same velocity in body length per second.

The experiment was conducted in accordance with laws and regulations of the Norwegian Regulation on Animal Experimentation (application ID 4146).

Behavioural observations

Video recordings of fish behaviour were made without disturbance in Weeks 0, 2, 4 and 6 by the GeoVision system pre-programmed to record for 12 min and 30 s during 08:00 to 12:00 h simultaneously in 2 tanks at a time to increase the video quality. Recordings in Week 0 were done 1 d after the current velocities

were set; the other recordings were done at the beginning of the week. Fish behaviour was classified as interactions or movements affecting the group structure (structural movements) and ranked according to the assumed intention (Table 1). Ranking was classified from 1 to 6, with 1 as the highest ranking. Biting was given the highest ranking based on an assumed impact scale. When one type of behaviour was documented, other behaviours were assumed a consequence of the first behaviour, and thus lower-ranked behaviours were not documented. For instance, a biting attempt could be preceded by bursts and collisions, but since these behaviours were of lower ranking, only the biting was documented. Structural behaviours were analyzed according to a Cartesian coordinate system, where X and Y are movements in the cross-sectional plane, and –Z movements are movements with the flow. Behaviours were analysed for 12 min and 30 s, and the number of behavioural occurrences per minute was calculated.

Fin erosion

External injuries and fin erosion were recorded on all fish before and after the experiment. Fin erosion was observed on the pectoral, dorsal, pelvic, anal and caudal fins and divided into 3 categories: fins with 0 to 10% of the fin missing (eroded) were classified as uninjured due to difficulties in assessing a perfect fin (0% erosion), fins with 10 to 50% erosion were classified as moderate fin erosion and fins with >50% erosion were classified as severe fin erosion. This

Table 1. Definitions of the behavioural categories used. Behaviours were ranked from 1 to 6, with 1 as the highest ranking

Behaviour	Definition	Ranking
Interaction		
Biting	Active attempt to bite another fish	1
Displacement	A fish forces another out of position, with or without close contact	2
Intentional collision	A fish directs its swimming and bumps into another fish intentionally, not resulting in a bite attack or displacement of the attacked fish	3
Structural movement		
Burst	Fast acceleration against the current	4
Move –Z	Turning and swimming with the current instead of against the current	5
Move XY	Vertical movements in X or Y direction, with a relocation of at least 0.5 body length not followed by other higher-ranked behaviour	6

classification was based on an index used by Swedish governmental agencies and chosen as the index based on the observers' experience. The index is a modified version based on Person-Le Ruyet et al. (2007), where the 0 group has been judged irrelevant for farmed fish and is thus removed from the index. Difficulties distinguishing between their levels 2 and 3 were simplified by adding them to the same group (here, moderate fin erosion). Fin erosions were also classified as either new damage with bleeding or older damage that may have healed.

Statistics

Data analyses were performed using R software Version 3.1.0 (Copyright 2009, The R Foundation for Statistical Computing, Vienna). Counts are reported as mean number of counts $min^{-1} \pm$ SE. Deviance in behaviour counts was modelled using generalized linear models with quasi-Poisson errors, as recommended for count data with over-dispersion (Crawley 2012). Week numbers (0, 2, 4 and 6) and treatments were set as explanatory factors in the models (function glm, R). Model simplification was performed in cases where the simpler model was not significantly different from the more complex model (function ANOVA, R, test = Chi; Crawley 2012). Fin erosion data were analyzed in a similar way; however, as data were proportional (percentage fish with damage), the error distribution was set as binomial and, in the case of over-dispersion, quasi-binomial (Crawley 2012). F-tests were used to compare the original to simplified models (function ANOVA, R, test = F; Crawley 2012). Significance level was set at p < 0.05.

During the experiment, 2 replicates were eliminated due to technical problems—one from the fast current velocity in Week 5 and one from the moderate current velocity in Week 4—resulting in triplicate treatments for the aforementioned groups at the final sampling.

RESULTS

Behavioural observations

Group structure

Current velocity had a large influence on structural movements, with the highest frequency of behaviours observed in fish kept at

the slower velocity and decreasing levels of movement with the moderate and faster velocities (Fig. 1). When the treatment velocities were applied, behaviours immediately changed. Similar differences were observed in the following 4 wk. In the final week, there was an increase of structural movements at moderate velocity compared to the second week, due to increased horizontal movements (Move XY). Even so, fish at both moderate and faster velocities had fewer movements than fish at slower velocity. Fish at faster velocity also showed significantly fewer movements than fish at moderate velocity in the final week. Except for an increase in horizontal movements from Week 2 until Week 6, at moderate velocity, there were no changes over time within treatments in the different types of structural behaviours (Fig. 1).

Interactions

More interactions were observed in fish kept at slow velocity compared to both moderate and faster velocities (Fig. 2). Fish at fast velocity did not differ significantly compared to moderate velocity, but a decreasing trend of interactions was observed. At slow velocity, fewer collisions were observed in Week 4 compared to Weeks 0 and 2, resulting in no treatment effect in Week 4. In the other interacting behaviours, no effects were observed over time.
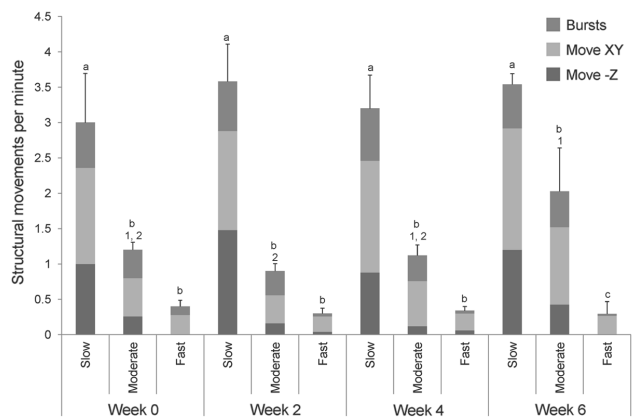

Fig. 1. Mean of structural behaviours per minute in fish at 3 different water current velocities (slow, 0.2 body length [BL] s^{-1}; moderate, 0.8 BL s^{-1}; and fast, 1.5 BL s^{-1}) over 6 wk. Error bars represent SE of total occurrences. Lowercase letters denote significant differences between current velocities, and numbers denote significant differences between weeks based on the cumulated behaviours (p < 0.05)
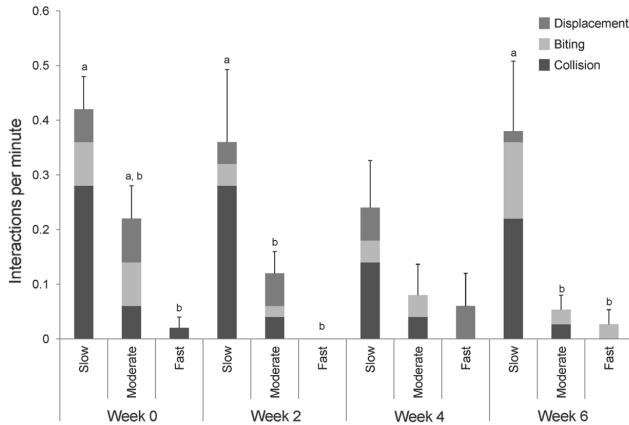
Fig. 2. Mean of interacting behaviours per minute in fish at 3 different water current velocities (slow, 0.2 body length [BL] s^{-1}; moderate, 0.8 BL s^{-1}; and fast, 1.5 BL s^{-1}) over 6 wk. Error bars represent SE of total occurrences. Lowercase letters denote significant difference between current velocities based on cumulated interactions (p < 0.05)
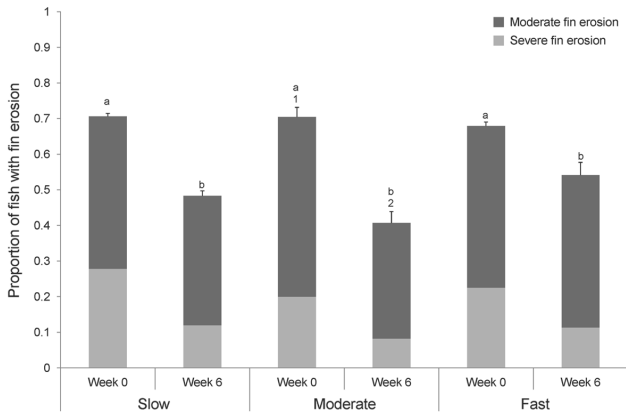

Fig. 3. Mean of cumulated fin erosions at start and end of experiment in fish kept at slow (0.2 body length [BL] s^{-1}), moderate (0.8 BL s^{-1}) and fast (1.5 BL s^{-1}) water current velocities. The columns are separated into fish with moderate fin erosion and fish with severe fin erosion. Error bars show SE of cumulated fin erosions. Lowercase letters denote significant differences between current velocities, and numbers denote significant differences between weeks (p < 0.05)

Fin erosion and other injuries

The proportion of fish with fin erosion decreased from Weeks 0 to 6 in all groups (Fig. 3). Severe fin erosion decreased in fish at all 3 velocities over time, while moderate fin erosion only decreased at the moderate velocity. However, at the faster velocity,

there was an increase in new fin erosion from the start to the end of the experiment (Fig. 4). New fin erosion was also more prevalent in fish at faster velocity than at moderate and slower velocities.

The increase in new fin erosion was caused by an increase in caudal fin erosion (Fig. 5). Damage on the dorsal fin was significantly less frequent at the end, with no effect of velocity. Fish at faster velocity had a significantly higher frequency of caudal fin erosion than fish at slower velocity at the end of the experiment. No effects could be seen for the other fin erosions.

DISCUSSION

The water current velocities had an effect on both behaviour and fin erosion in post-smolt salmon in raceways. Increasing current velocities induced a higher level of organised swimming in salmon, while agonistic behaviour decreased. However, the prevalence of new fin erosion increased at the fast current velocity. These findings suggest that increased current is positive but that welfare may be compromised above a certain velocity.

When the fish are not forced to swim against a current, they may choose various behavioural strategies within the tank. Some fish move at random, while others are stationary. Some may display territorial behaviour, while others are subordinate (Fernö & Holm 1986, Adams et al. 1995). The higher frequency of movements affecting group structure (structural movements) and agonistic behaviour in fish kept at slow current velocity has also been observed in previous studies on salmonids, although these studies did not observe the group structure in detail (East & Magnan 1987, Christiansen & Jobling 1990, Christiansen et al. 1992, Jørgensen & Jobling 1993, Adams et al. 1995). It is assumed that aggression is energetically costly and will result in decreased growth (East & Magnan 1987, Christiansen & Jobling 1990). Solstorm et al. (2015) found, based on physiological parameters, that some individuals at slow velocity experienced elevated
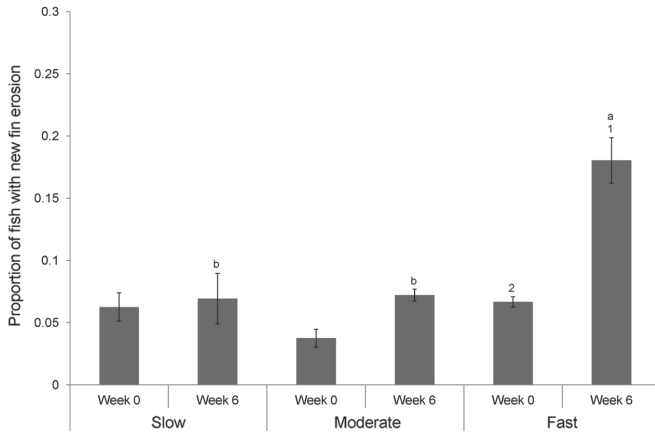
Fig. 4. Mean proportion of fish with new fin erosion at start and end of experiment in fish kept at slow (0.2 body length [BL] s^{-1}), moderate (0.8 BL s^{-1}) and fast (1.5 BL s^{-1}) water current velocities. Error bars represent SE. Lowercase letters denote significant differences between current velocities, and numbers denote significant differences between weeks (p < 0.05)
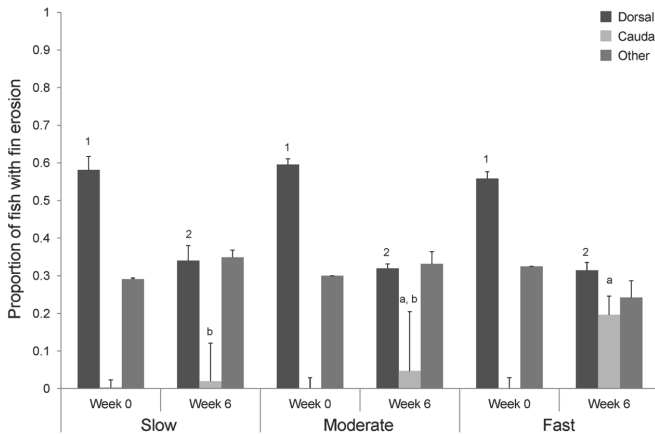

Fig. 5. Mean proportion of fin erosions separated into dorsal, caudal or other, with other including pectoral, pelvic and anal fins. Columns are presented for fish kept at slow (0.2 body length [BL] s^{-1}), moderate (0.8 BL s^{-1}) and fast (1.5 BL s^{-1}) water current velocities at the start and end of a 6 wk experiment. Error bars represent SE. Lowercase letters denote significant differences between water current velocities, and numbers denote significant differences between weeks (p < 0.05)

stress levels. This may suggest that agonistic behaviour and structural movements displayed at slow velocity are stressful for the individual, resulting in compromised welfare for the individual. However, fish at slow velocity had the same growth as fish at moderate velocity (Solstorm et al. 2015), indicating that the level of interactions at slow velocity was not severe enough to result in decreased growth. Also, when relating the level of agonistic behaviour to previous studies, these values are low (East & Magnan 1987, Adams et al. 1995). Adams et al. (1995) observed approximately the same number of agonistic behaviours in exercised Arctic charr as was seen at slow velocity in this study, but in their setup, 10 fish were observed, whereas in this study, up to 80 fish were studied. In our setup, there is a possible risk that the slow velocity induced structural movements and interactions as an effect of the possibility to swim at voluntary speed and thereby create an organised group structure. In contrast to raceways, salmon in cages are largely free to choose their swimming speed and create an organized structure, making results from our slow velocity difficult to relate to commercial situations, where fish are commonly held in sea cages. Farmed salmon are raised in high densities and are thus habituated to social organisation; rapid changes in current velocity can break down their schooling structure, and this could cause brief disorder. Johansson et al. (2014) observed that after this type of disorder, salmon strive to organise a new structure.

Fin erosion is a welfare problem that can arise from increased agonistic behaviour. Damage to the tissue is a direct violation of one of the Five Freedoms, i.e. freedom from pain and injury (defined by FAWC [1995]) and may also lead to health problems, with increased susceptibility to pathogens due to damaged tissue (Turnbull et al. 1996). Even though a fish's ability to experience pain is widely debated, the fins contain nociceptors, and adverse behaviour after fin clipping has been demonstrated, suggesting that fin erosion contributes to negative welfare (Roques et al. 2010, Noble et al. 2012). Causes for fin erosion are nipping from others, abrasion and bacterial infection (Latremouille 2003). Fin nipping mostly targets the dorsal fin in salmonid parr (Turnbull et al. 1998). The high frequency of dorsal fin erosions at the start of our experiment

could be explained by the fish coming directly from the freshwater life stage potentially combined with periodic restrictions in feed availability, as often seen under commercial parr production. At the parr stage in fresh water, wild salmon are territorial and frequently show agonistic behaviours (Keenleyside & Yamamoto 1962), while fish stressed by feed restrictions in aquaculture production units may show increased fin nipping and thus erosion (Noble et al. 2008). The reduction of cumulated fin erosions over time in our experiment may therefore be explained by a life stage-related decrease in agonistic behaviour, where salmon go from being territorial on riverbeds to becoming non-territorial pelagic swimmers in the open ocean (e.g. McCormick et al. 1998). Lending evidence to this is the fact that we observed the highest rate of agonistic behaviours at slower velocity, yet fin erosion declined over time. This suggests that the conditions before the experiment promoted fin erosion.

Previously documented behavioural effects with respect to reduced aggression, increased group structure and decreased fin erosion (East & Magnan 1987, Christiansen & Jobling 1990, Christiansen et al. 1992, Jørgensen & Jobling 1993, Adams et al. 1995) are in line with the results of the present study. Moderate current velocity forced the fish to swim against the current to prevent being swept back into the netting or colliding with other fish, which reduced interactions and structural movements. However, even though agonistic behaviours decreased at moderate compared to slow velocity, the fish displayed the same low level of fin erosion at the end of the experiment.

In previous studies, current velocities far exceeding our fastest velocity caused reduced aggression and improved fin quality in juvenile Arctic charr, brook charr and Atlantic salmon (East & Magnan 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Adams et al. 1995). At our fast velocity (1.5 BL s^{-1}), fish focused on swimming against the current to avoid being swept back into the netting; thus, structural movements and agonistic behaviour were almost absent. Yet, caudal fin erosion increased at the end of the experiment in the fast velocity. During daily maintenance, fish were observed to have difficulties in maintaining their position without unintentionally colliding with each other and the back netting. Hence, abrasion is the most likely cause for the observed caudal fin erosion, and this is supported by the increase in new fin erosion over time at the fast current velocity. Our experimental setup differed from previous studies and could explain the different outcome that we observed. As described in Solstorm et al. 2015, it is difficult to compare swimming speed to earlier studies where homogeneity of the current and absolute swimming speed is difficult to evaluate. Previous studies have mostly been conducted in open circular tanks (East & Magnan 1987, Christiansen & Jobling 1990, Jørgensen & Jobling 1993, Adams et al. 1995) with no back netting where the fish can attain abrasive fin erosion, and therefore our experimental setup may have unintentionally caused further fin erosion. Yet, if this is the case, our setup is relevant for fish in sea cages, where fast current velocities could force the fish into the cage netting and thereby cause the same abrasion. With the trend of having land-based post-smolt production, these aspects should not constitute a problem. Abrasion to other fins could also arise from the use of raceways (Arndt et al. 2001), but in our setup, the rough fin index could not detect any clear differences. It is possible that the rounded bottom in our raceways had a positive effect, since no fish were observed to stand on the bottom.

Previous studies have assumed that reduced interactions between fish are always positive and have not focused on the potential need for fish to express different types of behaviour. It could be that fast current velocity does not permit the fish to move around and interact in the environment, which could be seen as a violation of one of the Five Freedoms (defined by FAWC [1995]), i.e. the possibility to perform normal behaviour. In salmon feeding areas in the wild, such as the Nordic seas, individuals experience mean water velocities of 20 to 35 cm s^{-1}, with considerably higher maximum velocities exceeding 100 cm s^{-1} (Orvik et al. 2001). From an evolutionary perspective, salmon should therefore be adapted to a range of velocities, and normal behaviour therefore needs to have a wide range. Even so, wild salmon may drift along when velocities exceed limits in swimming performance, while farmed salmon are forced to maintain position within the cage. This may lead to welfare acceptable limits being breached in aquaculture settings. At the slower velocity, frequent movements and interactions could create a stressful environment for some individuals (Solstorm et al. 2015). Yet, this does not apply to wild salmon, as they do not typically swim in schools in the open sea, and even if interactions occur, they may quickly escape each other in an infinite water volume. As mentioned above, farmed salmon in cages with slow water velocities choose their own swimming speed, making results from slow velocity difficult to relate to fish held in sea cages and therefore welfare evaluation

from this group is not commercially relevant. Fish at moderate velocity display a lower degree of interactions and movements, but the current does not seem to be restrictive. The moderate velocity in our study is also within the range considered as the preferable swimming speed for brook charr (Tudorache et al. 2011), as well as in the range of swimming speeds for salmonids in the wild (Drenner et al. 2012). Swimming speeds that are preferred or chosen should be expected to optimise welfare. In view of the welfare concept based on allostasis (Korte et al. 2007), the fish at moderate velocity seemed to uphold stability through change the best (see also Solstorm et al. 2015), resulting in the highest welfare. Altogether, from a welfare perspective, our study showed that a moderate water current velocity likely contributed to the highest welfare with regard to social interactions, swimming speed and fin erosion.

CONCLUSIONS

Our results indicate that there is an upper limit in current velocity where positive effects are gained. An increase in water current velocity and the resulting swimming speed is initially positive by reducing structural movements and agonistic behaviour, but if the current is too strong, it can result in negative effects like increases in new fin damage and a reduced possibility to express different behaviours.

The fish in the present study displayed a behavioural plasticity when rapidly adapting their behaviour to different water current velocities at the start of the experiment, analogous to the response to changing currents in the field (Johansson et al. 2014). This suggests that currents are an effective way of controlling behaviours that may compromise fish welfare. If the fish experience water currents that are too fast, the netting could be modified to decrease the currents (Klebert et al. 2013), or more sheltered areas may be selected. Further studies on both fish behaviour and physiology under different water current velocities are needed to identify optimal current velocities for salmon net cages.

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