FIN DAMAGE IN JUVENILE ATLANTIC SALMON: FARM AND EXPERIMENTAL, CAUSES AND CONSEQUENCES

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Declaration

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree.

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Abstract

Fin damage in farmed salmonids is an important topic from both a welfare and economic perspective. Fin damage is perceived as a welfare issue as fish fins are living tissue and any damage can potentially be a source of pain and route for infections. The onset and prevalence of fin damage is complex and multifactorial and controlling fin damage is extremely challenging as triggering factors are difficult to identify. The aims of this thesis were to investigate food withdrawal as a major risk factor associated with the development of fin damage and strategies to reduce on-farm fin damage, including reducing industry-standard food withdrawal periods and providing environmental enrichment. The potential consequences of reducing fin damage is an improvement in farm productivity, fish health and welfare. Chapter 1 outlines our current understanding of fish welfare and fin damage. Chapter 2 details a study on the effect of food withdrawal on fin damage. Chapter 3 compares invasive and non-invasive methodologies used to assess fin damage. Chapters 4 and 5 detail a study carried out on a commercial Atlantic salmon farm to investigate the effect of enrichment at the farm-level. Chapter 6 details a novel, on-farm fish welfare assessment method. Chapter 7 summarises the findings of the thesis and directions for future fin damage related research and potential management strategies. The results of this thesis show that food withdrawal is a major risk factor in the development of fin damage. Results also indicated that environmental enrichment reduced the occurrence of fin damage and stress levels, improved growth in the early developmental stages and fish were more spatially distributed, suggesting an overall improvement in general welfare. The significance of the effect of food withdrawal on fin damage has not previously been reported and this is the first report of enrichment effects at the farm-level.

Keywords: Fish welfare, fin damage, enrichment, behaviour, QBA.

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1 General Introduction

1.1 The proposed study

Fin damage continues to be a widespread problem amongst most farmed fish, which not only detrimentally effects productivity, but also presents fish health and welfare issues. It is considered that there is potential to prevent fin damage through improvements in management practices (Moring, 1982). A number of risk factors are under the control of the farm such as water quality, stocking density, feed distribution and quality of food. Food restriction has been identified as a major risk factor in the onset of fin damage due to fin biting. However, the relationship between fin damage and food restriction has only been investigated under small scale experimental conditions and not on-farm. Restricting food has led to abnormal biting in terrestrial farm animals that have been mitigated against by various forms of enrichment. Currently there are no studies of the effect of enrichment at the farm level.

1.2 Aims and objectives of this thesis

The overall aim of this study was to investigate food withdrawal as a major risk factor associated with the development of fin damage in freshwater salmon populations. A secondary aim was to assess the effect of mitigation strategies to reduce fin damage, including reducing food withdrawal periods and environmental enrichment.

There were three main objectives; 1) to conduct a large -scale experiment to investigate the effect of food withdrawal on fin damage using industry standard food withdrawal periods and stocking densities; 2) to investigate the use of enrichment and reduced food withdrawal times on reducing on-farm fin damage; 3) to assess the effect of enrichment on production parameters and behaviour.

1.2.1 Project Outline

The remaining sections in Chapter 1 form a literature review relating to aquaculture, fin damage and welfare. Chapter 2 details a study on the effect of food withdrawal on fin damage, carried out in experimental tanks units with stocking densities at near industry level. The experimental design of this study included two methods to assess fin damage. One method required fish to be removed from the water (invasive method) and have their fins photographed for future digital analysis. The other method used underwater video and proprietary written software to analyse fin damage (non-invasive method). A comparison of the invasive and non-invasive methodologies used to assess fin damage is detailed in Chapter 3.

Chapter 4 details a study carried out on a commercial farm to investigate the effect of enrichment on fin damage, growth parameters and stress on Atlantic salmon parr. The effects of environmental enrichment on behaviour is the topic of Chapter 5.

Chapter 6 details a study which looks at qualitative behaviour assessment (QBA) as a novel on-farm tool to assess fish welfare. QBA has been used in assessing terrestrial animal welfare but this is the first study to use QBA to assess the effect of using environmental enrichment on-farm.

Chapter 7 is a general discussion, which summarises the outcomes of this thesis, proposes directions for future fin damage related research and potential management and mitigation strategies.

1.3 Introduction

Globally, aquaculture is the fastest growing animal-sourced food producing sector, reaching a milestone in 2014 when the aquaculture sector's contribution to the supply of fish for human consumption overtook that of wild-caught fish for the first time (FAO, 2016). In the past four decades, aquaculture development has outpaced human population growth, resulting in increased per capita aquaculture production (Figure 1-1). Hence on a global scale, aquaculture has the potential to meet the food supply demands of an increasing human population. Global consumption of fish per capita is approximately 20kg per year (2013-2015) and expected to rise to 22kg per capita by 2025 (FAO, 2016).

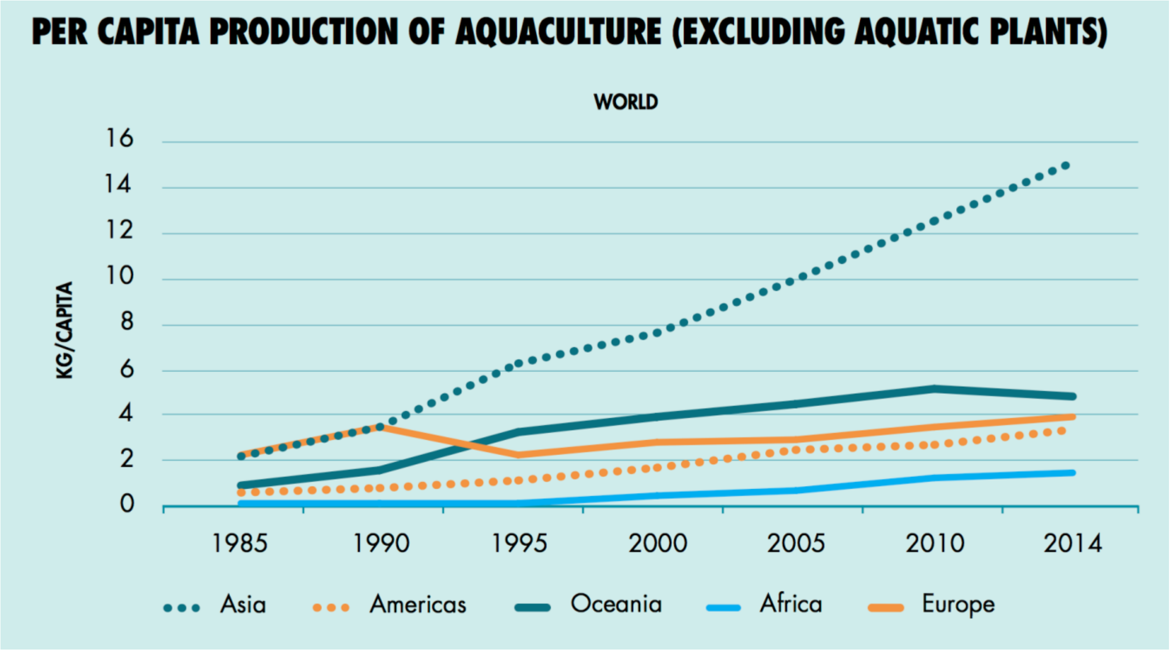

Figure 1-1 *Per capita production of aquaculture per year (excluding aquatic plants) source: FAO 2016*

Asia in particular has seen a large increase in aquaculture production with China the biggest producer in terms of volume (58.8% of production in Asia). The European Union (EU) only accounts for 1.25% of global aquaculture production but is one of the major producers of salmonids, with the United Kingdom (UK) being the top EU exporter of salmonids (EU, 2016). Globally the UK is one of the top three producers of Atlantic salmon

(*Salmo salar*) commanding a market share of 7%, compared with the top producers Norway (53.8%) and Chile (25.9%) (Globefish, 2015).

Within the UK the majority of the aquaculture industry, particularly finfish aquaculture, is based in Scotland and is a vital contributor to the local and national economy and employment. Based on data from 2012 (Marine Scotland Science, 2014), the Scottish aquaculture industry contributes a turnover of at least £550m and over 2800 jobs from direct production alone. The Scottish supply chain (including suppliers, farm production, processing and retail) generated over £800m annually and provided employment for over 4800 people. It is estimated that the added income generated by aquaculture contributes as much as £1.4bn and employment for 8000 people across the wider Scottish economy, not only in isolated rural communities but also in the central belt of Scotland. The total value of Scottish aquaculture to the UK is £1.8bn and 8800 jobs. Should Scotland achieve its aspirations to sustainably increase production by 28% towards 2020 then the economic value, based on current projections would be estimated at £1.1bn and provide 7000 jobs for Scotland, across the aquaculture supply chain. However, the sustainability of the industry has been challenged on two aspects: environmental impacts and the welfare of stocks (Fishcount, 2019). As a result, the husbandry practices and the level of welfare experienced by these animals are emerging issues in national and international science programs, organisations concerned with the treatment of animals, and amongst consumers (FAO, 1996). Despite progress in addressing many welfare concerns, fin damage is an issue that has persisted in both the salmon and trout sectors, and with UK hatcheries incubating around 180 million salmon and trout eggs each year (FAWC, 2014) has the potential to be a welfare issue for a very large number of animals. Fin damage is perceived as a welfare issue as fish fins are living tissue with a nerve and blood supply (Becerra et al., 1983) and can potentially be the source of pain, fin damage provides a secondary route for infections and affects swimming ability (Ellis et al., 2008). Damaged fins reflect poorly on hatchery production techniques yet are commonly found in farmed trout and salmon (Moring 1982; Jobling et al., 1993; Turnbull et al., 1996).

This introduction is a review of the literature investigating the risk factors identified to date, and the possible aetiology of fin damage in intensively farmed salmonids.

Salmonids

The term salmonid is derived from the family name *Salmonidae* and the members of this family are the only extant members of the order *Salmoniformes*. The species of salmonids of most interest to aquaculture are those in the subfamily *Salmoninae* which includes the genera *Oncorhynchus, Salmo* and *Salvelinus*, more commonly referred to as trout, salmon and charr, respectively (Barton, 1996).

External Morphology

All species of salmonids are similar in appearance and are characterised by a fusiform shape with fin placement consistent among species and genera. Salmonids are soft-rayed fishes with a single dorsal and anal fin, a homocercal caudal (tail) fin and paired pectoral and pelvic fins (Barton, 1996). A distinctive feature of salmonids is a fleshy adipose fin, located dorsally between the main dorsal fin and the tail fin (See Figure 1-2).

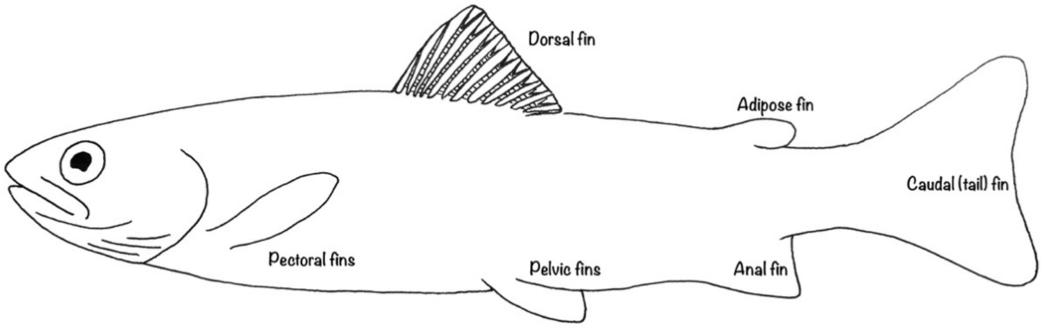

Figure 1-2 *Salmon fins layout (Image courtesy of J.F. Turnbull)*

Most salmonid fishes are anadromous, which means that they migrate from salt water to fresh water to spawn. Freshwater juveniles are generally dark backed with parr markings or body spotting dependent on species. Mature marine individuals are usually silvery however, purely river or lake stocks have the dark back without the intense silvering, in common with freshwater juveniles (Barton, 1996).

1.4 Understanding the welfare of fish

In this section I will define what is meant by the term animal welfare, and then explore the various aspects of biology that contribute to welfare and how it relates to fish.

1.4.1 What is meant by animal welfare?

The term animal welfare is being used increasingly by businesses, consumers, veterinarians, politicians and scientists. However, it can mean different things to different people, making animal welfare a difficult concept to define and is often culturally value-dependent. Farmers and veterinarians tend to focus on the physical well-being of an animal whereas consumers are more interested in ensuring a lack of cruelty and providing more natural environments, especially in intensively farmed species (Fraser, 2003). Dawkins (2008) proposes that to determine the welfare state of an animal, two questions need to be answered: whether an animal is healthy, and does it have what it wants? In contrast, Broom (1991) has defined animal welfare as the state of an animal in relation to its ability to cope with its environment.

To properly assess welfare, we need to agree on what it is. Fraser (2003) outlines three different views to which scientists may subscribe to when trying to identify when an animal is in a good state of welfare:

(1) *function-based definition*- that animals should be raised under conditions that promote good biological functioning in the sense of health, growth and reproduction. Pain, fear and hunger would not be seen as relevant to welfare unless health or survival was threatened (McGlone, 1993).

(2*) feelings-based definition* - that animals should be raised in ways that minimise suffering and promote positive states such as comfort, contentment and pleasure. Good welfare requires that the animal feels well, be free from negative experiences such as pain and fear and have access to positive experiences (Duncan, 1993).

(3) *nature-based definition* - that animals should be allowed to lead relatively natural lives (Kiley-Worthington, 1989).

However, pursuing one of these definitions to the detriment of the other two does not necessarily lead to good welfare. For example, a social animal can have good biological functioning but deprived of companionship, may have poor welfare according to a feelings-based definition (Huntingford & Kadri, 2008). Prey species would naturally be exposed to predators in the wild, however it is unlikely that anyone would advocate exposing a captive animal to predation and describe it as good welfare. A caveat to that would be where an animal was being bred to re-stock a depleted wild population in which case learning natural prey avoidance behaviour would be beneficial. Good welfare should encompass all three definitions with varying overlap depending on the species.

Animal welfare is not only about the absence of negative experiences; it is also about ensuring that an animal has positive experiences. Historically, animal welfare has focussed more on avoiding negative welfare experiences such as reducing the risks of poor physical health outcomes, mainly due to the legal requirement to reduce unnecessary suffering (FAWC, 2009). Recently, however, there has been a shift in this paradigm to recognise the importance of positive welfare experiences where animals have a 'life-worth-living' (FAWC, 2009; Yeates, 2011; Mellor, 2016); a quality of life which exceeds that which is necessary for immediate survival. Animals kept in barren, cramped or isolating conditions can be negatively affected by anxiety, fear, frustration, boredom and anger (Mellor, 2016), potentially leading to abnormal behaviours such as bar biting in dairy cows (Krohn, 1994) and feather pecking in chickens (Dixon et al., 2010). However, these negative effects can be replaced with positive effects by providing safe, enriched enclosures that provide opportunities to engage in more natural and rewarding behaviours such as the presence of play, curiosity driven exploration and some types of vocalisations (Boissy et al., 2007). There are challenges in identifying positive experiences in fish, partly due to the difficulties in observing in the aquatic environment and the lack of facial expressions and vocalisations. To be able to have negative and positive experiences implies the capacity to experience pain and there is still scientific uncertainty with regards to whether fish have the capacity to suffer.

Recent studies with teleost fish suggest physiological and behavioural responses to external stressors and painful stimuli are indicative of a pain response (Sneddon et al., 2003; Braithwaite & Huntingford, 2004; Chandroo et al., 2004). However, as pain is a subjective experience, others contend that fish lack the necessary neurobiology to consciously experience pain (Rose et al., 2014; Key, 2015) or related emotions. While there continues to be a lack of consensus on whether fish feel pain, the working hypothesis for organisations involved in fish welfare is that fish probably do have the capacity to perceive painful stimuli although are unlikely to experience conscious suffering in the same way as humans do (Braithwaite & Huntingford, 2004). Also, in the absence of conclusive evidence one way or the other, society should take the ethical stance that we should give fish the benefit of the doubt i.e. the precautionary principle. Many research funding bodies, e.g. Norwegian Research Council, have adopted a cautionary approach (since ca. 2005).

There is a growing body of evidence indicating cognitive abilities in fish and evidence for pain and stress responses. This is discussed in sections 1.4.3 – 1.4.5 below.

1.4.2 Welfare Indicators

A priority for the fish farming industry is to be able to objectively assess the welfare of farmed fish and to develop and validate a checklist of practical indices of poor welfare (FAWC, 1996; Berrill et al., 2012). The identification of operational welfare indicators (OWI) is a key development in being able to assess not only the current state of farmed fish but also to assess and compare the costs and benefits of any interventions designed to improve welfare.

Figure 1-3 outlines a range of OWI's for potential use by fish farmers as a means to monitor fish welfare (Huntingford & Kadri, 2014; Noble et al., 2018). OWIs can be categorised into direct welfare indicators such as animal-based measures and indirect measures such as environmental and these can be further divided into whether the effect is predominantly at the individual animal-based level (physiology and physical health) or group level (environmental and behaviour), see Figure 1-3. Examples of indicators that are directly observable include feed intake, ventilation rate, swimming patterns, body coloration (as a proxy for stress levels), body and fin condition. Fish with damaged fins are perceived as having poor welfare in comparison with fish that have intact fins, therefore fin damage has the potential to provide the basis of a non-invasive OWI under practical farming conditions. The value of fin damage as an OWI is that it is easily observable, does not require expensive equipment, on-site analytical methods or the killing of fish (Hoyle et al., 2007). A difficulty in using fin damage as an OWI is that to pinpoint what action to take requires a knowledge of when the damage occurred (i.e. is it historical or active) to determine the underlying conditions at the time.

A further category of laboratory-based welfare indicators (LABWI's) are WI's that need to be processed in a laboratory rather than being directly available on-farm (Noble et al., 2018). Figure 1-3 outlines some LABWI's which are mainly based on physiological measurements. Hand held instruments are now available for lactate and glucose measurements, so those physiological measurements can be made available on-farm.

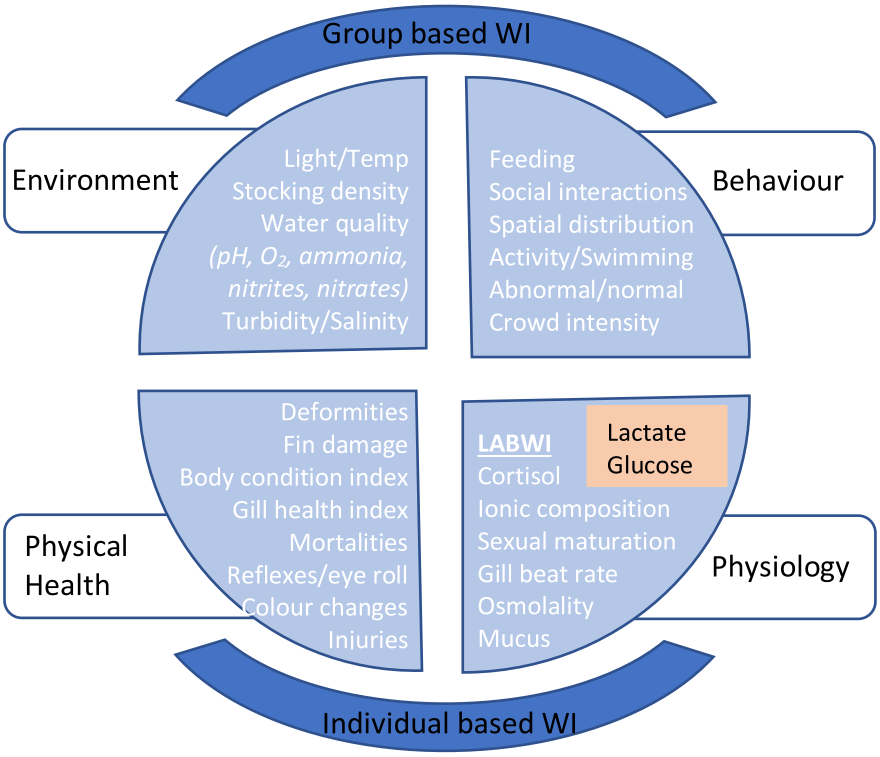

Figure 1-3 *Overview of potential on-farm operational welfare indicators (OWIs) and welfare indicators that require processing in a laboratory (LABWI).*

1.4.3 Fish cognition

The function-based definition of animal welfare is the avoidance of suffering which is an underlying principle of animal welfare legislation. The topic of whether fish are capable of suffering is still controversial as it implies a level of consciousness not normally associated with fish and suffering is difficult to define and measure (Dawkins, 1998). An alternative view is that what an animal 'feels' is related to its cognitive abilities and concern for an animal's welfare should be related to its level of cognition, thus setting aside the debate on consciousness (Duncan & Petherick, 1992; Dawkins, 2001). Animal cognition is defined as the process by which animals acquire, process, store and use information gathered from the environment to guide their behaviour (Shettleworth, 2010). It involves perception (detecting and interpreting signals from external events), learning and memory, and allows an animal to detect its current environment and be aware of any changes (Braithwaite et al., 2013). Cognitive abilities are involved in many fitness-related activities, including foraging, avoiding predators, conspecific competition and the finding of reproductive partners (Shettleworth, 2010). Recent reviews of fish cognition show a diversity of sophisticated behaviours indicative of advanced cognitive ability (Brown et al., 2011). Tasks that can be applied to understanding animal cognitive abilities include classical and operant conditioning, reversal learning, social learning, and spatial learning tasks (Brown, 2015). The following sections will discuss the application of these techniques to fish.

Conditioning and reversal learning

Complex associative learning in fish has been studied using classical and operant conditioning as has reversal learning, a measure of cognitive flexibility characterised by the ability to adapt quickly to changed rules. Early studies of conditioning in fish demonstrated shock avoidance learning (Gleason et al., 1977). More recent applications of positive rewards and reinforcement have been shown to be effective at modifying fish behaviour. For example, zebrafish (*Danio rerio*) are capable of learning colour discrimination using a food reward. Upon reaching a set criterion (i.e. *n* consecutive correct choices) the reinforced alternative is switched with the previously unreinforced alternative (reversal). The number of trials required to reach criterion is a measure of how quickly the fish learn the new rule (Colwill et al., 2005; Parker et al., 2012). On successive trials the fish took fewer trials to reach criterion and took less time to apply the rule to two different colours, compared with the initial training sessions, thereby also demonstrating attentional set shifting capability (Parker et al., 2012).

Social learning

The ability to learn new skills by copying a conspecific is a key aspect of social learning. Hatchery-reared Atlantic salmon parr were taught to recognise and find live prey by observing other fish (Brown & Laland, 2002). A number of fishes were pre-trained to accept either live prey or food pellets to serve as either a demonstrator (live prey trained) or as a sham-demonstrator (food pellet trained). Naïve fish were then paired with either a demonstrator or a sham-demonstrator and fed live prey. The fish could visually see each other but were physically separated by a clear perspex partition. Naïve observer fish paired with a demonstrator learned to accept live prey at a significantly faster rate than naïve observer fish paired with a sham-demonstrator and consumed more prey. The suggestion is that the direction of the strike made by demonstrators at prey items provided information to the naïve observer fish about food availability and location. The performance of naïve fish when paired with another naïve fish (visually connected but physically separated) decreased in comparison to fish housed singly, suggesting social inhibition. Singletons had a shorter latency to strike and consumed more prey than paired naïve fish. Social learning of foraging skills is particularly applicable to hatchery-reared fish destined for re-stocking in the wild. The survival of hatchery-reared fish in the wild is poor, mainly due to predation but also starvation which leads to poor condition and hence increased vulnerability to predation (Ersbak & Haase, 1983). Hatchery-reared fish are fed a commercial pellet diet at predictable intervals and location; therefore, have poor foraging skills in the wild. Seeding the hatchery tanks with a few individuals trained in foraging on live prey prior to release may increase survival on release (Brown & Laland, 2002).

Spatial learning

The classic example of spatial learning is that of rock-pool dwelling gobies (reviewed in Odling-Smee & Braithwaite, 2003). The fish can form complex navigational maps involving cognitive learning and memory abilities. Gobies build up a mental representation of their environment during high tide, when everything is underwater. During periods of low-tide, when much of the environment is out of water, they are able to recognise and use the resultant rock pools, leaping into neighbouring pools if disturbed (Aronson, 1971) and can find their way back to their home pool after being displaced (White & Brown, 2013). There is a strong selection advantage for fish to be able to learn and recall the location of important features, such as predators, refuge, food and mates (Bshary & Brown, 2014). There is also evidence of sex differences in spatial abilities. For example, male guppies (*Poecilia reticulata*) successfully navigated a complex maze to achieve a social reward after 1 trial whereas female guppies failed to do so after 5 trials (Lucon-Xiccato & Bisazza, 2017). Differences in cognitive abilities have also been shown to be influenced by the environment. Juvenile wild-strain Atlantic salmon were raised in standard hatchery conditions until the age of 10 months, when half were transferred to enriched tanks and half to control tanks. At 1 year old the fish in the enriched tanks had superior spatial learning ability, exiting a maze task faster than fish reared in barren tanks (Salvanes et al., 2013). Enriched fish had increased neural plasticity in the telencephelon, with upregulated NeuroD1 mRNA expression, a transcription factor involved in the development of the central nervous system, hippocampal neurogenesis and dendritic spine stability (Salvanes et al., 2013).

Neural plasticity

Neural plasticity is an important aspect in the development and function of cognitive processes and refers to the brain's ability to reorganise itself by forming new neural connections in response to the environment and life experiences (Knudson, 2004). In particular, neurogenesis in the hippocampal region affects learning and memory and can be regulated in response to stress in fishes, giving rise to differences in cognition and behaviour (Sørensen et al., 2013).

1.4.4 Pain in Fish

There is currently a major unresolved issue as to what extent an animal can feel pain and experience suffering or emotional distress (Huntingford et al., 2006; Rey et al., 2015). Partly this is due to the way pain is defined as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage,” (IASP). This definition was meant for humans and creates problems trying to apply the same definition to non-verbal animals, such as fish. The problem is that this definition requires that we scientifically demonstrate that fish have emotional experience. The experience of emotion requires consciousness (Stevens, 2013). Consciousness has a variety of meanings but in this context refers to the “general awareness of place (where I am relative to where I have been), time, self and an abstract ability to represent the perceptual, emotional, motivational, cognitive and motor states being processed moment by moment” (Nussbaum & Ibrahim, 2012). The complexity of this definition of consciousness underlines the challenge in identifying consciousness in fish, and other non-human animals (Stevens, 2013).

Comparative psychologists often use the ‘mark and mirror test’ (Gallup, 1970) to assess the level of consciousness in non-human animals. In the ‘mark and mirror test’ an animal is marked in a position only visible in a mirror and how the animal interacts with the mirror reflection and the mark on itself (e.g. trying to remove the mark) is a measure of the capacity for self-recognition. Self-recognition is a component of self-awareness, which is a dimension of consciousness, as described above. If an animal possesses the capacity to self-recognise then it could potentially have some level of self-awareness (i.e. knowledge of its mental states, like emotions) (de Waal, 2019).

The majority of human infants pass the mark test before the age of 24 months (Bard et al., 2006). However, very few non-human animals have been found to pass the mark test, notably chimpanzees (Gallup, 1970), Asian elephants (*Elephas maximus*) (Plotnik et al., 2006), dolphins (*Tursiops truncatus*) (Reiss & Marino, 2001) and the Eurasian magpie (*Picus picus)* (Prior et al., 2008). Critics of claims that Asian elephants, dolphins and magpies pass the mark test point to methodological problems with these studies and a lack of replication (Gallup & Anderson, 2020). A recent study with cleaner wrasse

(*Labroides dimidiatus*) claimed to have demonstrated that fish potentially have the capacity to self-recognise (Kohda et al., 2019). Cleaner wrasse showed atypical swimming behaviour in response to the mirror such as upside-down swimming and tried to remove a mark on its throat by scraping against the substrate, then seemingly checking with the mirror reflection to see if it was removed. However, Gallup & Anderson (2020) point out that cleaner wrasse evolved to remove ectoparasites from themselves as well as other fish and in the process developed behaviours to manipulate the movement of other fish to gain better access to the ectoparasite. The atypical behaviours may also be in response to the reflection not reacting in the socially normal way as it would not be able to react independently of what the cleaner wrasse is doing. Both of these interpretations may explain the atypical behaviours rather than being interpreted as an indication of self-recognition. The scraping of the mark on the substrate could possibly indicate irritation at the site of the mark rather than having any visual knowledge of the mark (de Waal, 2019; Gallup & Anderson, 2020).

The traditional view of self-awareness is binary, either you are self-aware or not and Gallup & Anderson (2020) contend that that cleaner wrasse did not pass the mirror self-recognition test and hence cannot be self-aware. In comparison, de Waal (2019) proposes a gradualist approach to self-awareness in which humans are assigned the highest level of self-awareness and other species lie along a continuum below this. Although the cleaner wrasse did not pass the mirror self-recognition test based on visual information alone, however, by combining information (visual and physical irritation) they demonstrated an intermediate level of self-awareness (de Waal, 2019). The difficulties in assigning self-awareness in non-human animals further illustrates the challenges in assessing whether non-human animals can have the emotional experience of pain.

There is a degree of difficulty in assessing pain even in humans. Pain is subjective and can vary widely between and within individuals (Bateson, 1991). However, learning to avoid damage, or conditions previously associated with potentially harmful situations and limit activities that would delay recovery from disease or injury would likely confer a fitness advantage; not only for humans but other animals (Bateson, 1991; Brown, 2016). Non-verbal behavioural and physiological signs used for recognising pain in humans can be considered when assessing animals, where animals possess the physical capacity to express them.

Behavioural studies in pain mechanisms recognise a distinction between nociception and pain (National Research Council 2009). Nociception refers to the process through which information about peripheral stimuli is transmitted by primary afferent nociceptors to the spinal cord dorsal horn (or its trigeminal homologue, the nucleus caudalis), brainstem, thalamus, and subcortical structures. The result is a reflex movement away from a noxious stimulus, to limit damage. Pain is a subjective experience that requires higher brain centre processing whereas nociception can occur in the absence of pain.

To demonstrate that animals can perceive pain it has been proposed that two main criteria need to be met; that the animal possesses the necessary receptors (nociceptors) for detecting noxious stimuli and that behavioural responses to potentially painful stimuli reflect both an immediate avoidance and learning to associate the unpleasant experience as something to be avoided in the future (Sneddon et al., 2002). Evidence of motivation to avoid a painful experience and protect injured tissue means that the behaviour is not just a simple reflex response but involves learning and memory, fear, anxiety and stress; an indication of central processing within brain structures analogous to the human neocortex (Sneddon et al., 2002). A third criteria is that analgesics modify the response to the noxious stimuli, however information on appropriate dosage for many animals is currently lacking (Bateson, 1991) and not all animals have appropriate opioid receptors (Barr & Elwood, 2011).

Nociceptors, similar to those found in higher vertebrates have been found in teleost fish (Sneddon et al., 2002). Nociceptors are sensory receptors consisting of free nerve endings that detect tissue damaging stimuli that evoke a response to stimulation from mechanical, thermal or chemical irritation. Nociceptors are not “pain receptors” but detect the same sensations as other touch receptors in the body but they have a higher threshold, and they only activate when the stimulus is strong enough to threaten injury. There are two main types of nerve fibres (axons) whose free endings form nociceptors and connect the peripheral organs to the spinal cord; classified as A-delta fibres and C-fibres. The two types of fibres differ in their diameter and in the thickness of the myelin sheath. The initial sharp, acute pain felt in response to an injury is transmitted by nerve impulses along the A- delta fibres which gives way to a slower, more poorly localised dull pain conducted along the C-fibres. Rainbow trout have been shown to possess both A-delta and C fibres (Sneddon et al., 2002) although the nociceptors are not responsive to cold temperatures below 4 ºC, which for rainbow trout is likely adaptive (Ashley et al., 2007). In mammals, A-delta and C fibres convey nociceptive information to the thalamus (Lyn, 1994). A recent study (Dunlop & Lamming, 2005) has demonstrated A-delta and C fibres activated in the higher central nervous system (telencephalon) in fish, in response to a noxious stimulus. If responses were confined to the dorsal root ganglion, that would suggest simple, reflexive nociception. However, activity in higher brain centres such as the telencephelon would suggest the possibility of pain perception, with the telencephalon co-ordinating pain information in fish, as the cortex performs this in mammals (Dunlop & Lamming, 2005).

Much of the argument against fish pain is based on the perspective that an animal must be conscious to respond emotionally to damage. Key (2015) contends that studies of behavioural nociceptive responses are purely reflexes and fish lack the neuroanatomical structures responsible for pain perception in humans, namely a neocortex (Rose et al., 2014; Key, 2015). However, although fish brains are smaller and organized differently there is structural homology and functional equivalency between forebrain structure in fishes and mammals. In particular, the lateral and medial pallia in fishes are homologous to the tetrapod hippocampus (involved in learning and spatial memory) and amygdala (involved in the generation of emotions), respectively (Mueller, 2012). Several fish species are capable of learning complex spatial relationships and forming mental maps (reviewed in Odling_Smee & Braithwaite, 2003) which is disrupted by lesions to the lateral pallia, and lesions to the medial pallia disrupt fear conditioning and avoidance learning (Broglio et al., 2005) and is further evidence that the lateral pallia is analogous to the hippocampus.

The counter argument does not disagree that fishes, like all vertebrates have a rapid reflexive withdrawal response to a noxious stimulus. This view argues that from an evolutionary perspective there is an obvious long-term fitness benefit for an animal to associate painful stimuli within specific contexts to avoid it in the future (Brown, 2016).

Fish have been shown to avoid an adverse stimulus such as an electric shock (Ehrensing et al., 1982) and carp (*Cyprinus carpio*) become hook shy for up to 3 years after being hooked once (Beukema, 1970). A majority of animals do learn this association, and recent studies suggest physiological and behavioural responses to external stressors and painful stimuli are indicative of a pain response in teleost fish (Sneddon et al., 2003a; Braithwaite & Huntingford 2004; Chandroo et al., 2004) and that these responses can be blocked by the use of appropriate analgesics (Sneddon 2003b; Jones et al., 2012). However, carp did not show a pain response, suggesting responses to noxious stimuli is either species-specific (Reilly et al., 2008) or the stimuli was not considered painful. Self-medication in animals can be an indication of pain. Lame rats and broiler chickens consumed more food laced with pain-relief than controls (Colpaert et al., 1980; Danbury et al., 2000). Fish do not eat when in pain (Sneddon, 2009) therefore the self-selection of drugged water or food to reduce pain is not an appropriate test for fish. However, fish will change their preferred choice for a lesser choice if it reduces pain. When given a choice of an enriched versus barren area of tank, zebrafish preferentially chose the enriched area. After being given a painful treatment they chose the analgesic laden barren area over the enriched area. Controls who were not given the painful treatment but also had access to the analgesic laden barren area continued to prefer the enriched area (Sneddon, 2012).

The weight of evidence of complex cognitive abilities in fish, possessing the necessary receptors and similar behavioural responses that indicate pain in mammals show that fish are highly likely to be capable of feeling pain.

1.4.5 Stress in fish

As the functioning-based approach to animal welfare has tended to dominate in fish welfare, previous studies have tended to focus on fish stress mechanisms and responses to a wide variety of conditions and procedures (Huntingford et al., 2006).

Stress has been defined as “the nonspecific response of the body to any demand made upon it” (Selye, 1973) but is not necessarily detrimental to fish welfare. A stressor is any event, experience or external environmental stimulus that triggers a stress response in an organism. The stressors can be either physical or psychological and be a positive or negative experience. The response to stress is an adaptive mechanism that allows the fish to cope with real or perceived stressors in order to maintain its normal or homeostatic state See Figure 1-4.

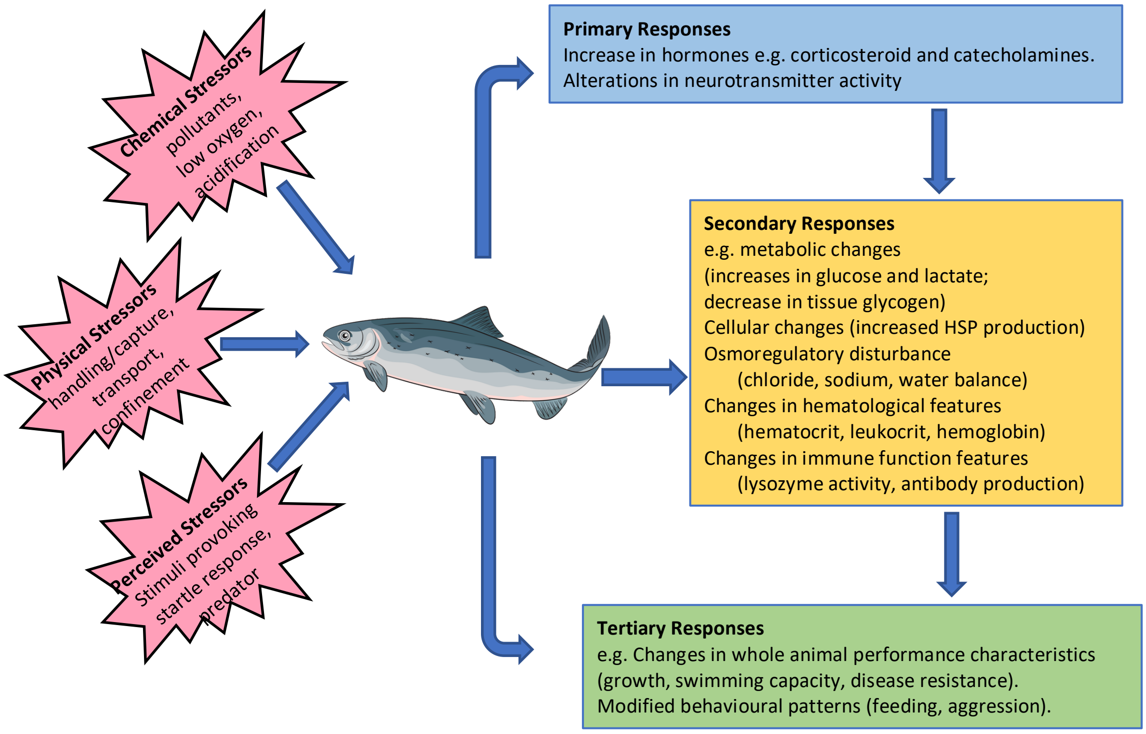

Figure 1-4 *Physical, chemical and other perceived stressors act on fish to evoke physiological and related responses, which are categorised as primary, secondary and tertiary or whole-animal responses. In many instances, the primary and secondary responses, in turn, may directly affect secondary and tertiary responses, respectively, as indicated by the arrows. Adapted from Barton (2002)*

The stress response in fish is very similar to that of other vertebrates and can be described as occurring in three stages (Figure 1-4), the primary, secondary and tertiary responses (Barton et al., 2002). The primary response involves the activation of two neuroendocrine axes; the hypothalamus-sympathetic-chromaffin (HSC) cell axis and the hypothalamic-pituitary-interrenal (HPI) axis. The HSC produces catecholamines (adrenaline and noradrenaline) from the chromaffin cells, the equivalent of the adrenal medulla in mammals. The release of catecholamines prepares the body for the ‘fight or flight’ response, accelerating heart and respiration rates, increasing blood flow to muscles and stimulating glucose release for the increased metabolic requirements (Wendelaar, 1997).

The HPI controls the production of corticosteroids from the interrenal cells, the equivalent of the adrenal cortex in mammals. The interrenal cells and the chromaffin cells are located primarily in the head kidney (Wendelaar, 1997).

As is typical of many vertebrates, the hypothalamus and the pituitary gland control the secretion of corticotrophin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) which are the primary hormones for stimulating the release of cortisol from the interrenal tissues in teleost fish (Wendelaar, 1997). Elevated plasma cortisol is the most widely used indicator of stress in fish (Pickering & Pottinger, 1989). Plasma cortisol levels increase dramatically in the few minutes after exposure to an acute stressor and can remain elevated for one to two hours before returning to normal levels (Wendelaar, 1997). If the stressor is chronic then plasma cortisol levels may remain elevated for longer although well below the peak levels seen after an acute stressor (Barton & Iwama, 1991).

The secondary stress response involves physiological changes in response to the stress condition and involves changes in metabolic and immune functioning. The release of cortisol mainly affects the regulation of the hydromineral balance and energy metabolism (Wendelaar, 1997).

The tertiary response is evoked by prolonged exposure to a stressor and refers to the changes in the whole organism (Iwama, 2007). If the intensity of the stressor is excessive, repetitive, persistent or mismanaged, physiological response mechanisms may be compromised and can become detrimental to the fish's health and well-being. Indicators of tertiary stress response are impaired growth rate, low body condition score, increased incidence of infection and low reproductive status. This is usually linked to chronic stress situations likely to be due to social stress (i.e. aggression).

For a subordinate fish, the experience of defeat can cause stress, as can the experience of repeated threats from more dominant fish (Zayan, 1991). Previous work in rainbow trout (*Oncorhynchus mykiss*) has shown also that individuals in the middle of the hierarchy exhibit higher stress levels (Noakes & Leatherland, 1977) and have increased susceptibility to disease, compared with the dominant and subordinate fish in the group.

1.4.6 Monitoring behaviour to assess fish welfare

The monitoring of behaviour has many advantages as a non-invasive welfare indicator. Potential behavioural indicators of compromised conditions linked with acute and chronic stressors include loss of appetite, increased ventilator activity, erratic swimming, shelter seeking, aggression, and performance of stereotypies or other abnormal behaviour (Huntingford & Kadri 2014; Schrek et al., 1997). For instance, brown trout (*Salmo trutta*) showed a reduction in food intake for 3 days in response to an acute handling stress (Pickering et al., 1982). Erratic swimming was observed in Atlantic salmon on transfer to experimental tanks, characterised by vigorous swimming against the tank walls that suggests they are attempting to escape from the tank, and this response is believed to be an expression of emotional stress (Fenderson & Carpenter, 1971). Øverli et al., (2004b) demonstrated that rainbow trout subjected to a short-term social stressor (a large, aggressive conspecific) reacted with increased aggression towards smaller fish. Moreover, the presence of fish that were socially subordinate to the subject fish decreased the levels of plasma cortisol and increased brain serotonin suggesting that subordinate fish play a stress-reducing role by providing an aggressive outlet.

A stereotypy is a behavioural pattern that is performed in response to stress and can be defined as repetitive, invariant and with no obvious goal or function (Mason, 1991). Stereotypical behaviours are thought to be caused ultimately by artificial environments that do not allow animals to satisfy their normal behavioural needs; which in this case is defined as a behaviour normally found in the natural habitat of the species (Keeling & Jensen, 2002). The performance of stereotypical behaviour reveals underlying conflict. Frustration and disturbance may be soothing or stimulating, providing a coping mechanism for the animal (Mench & Mason, 1997). There is a paucity of studies related to stereotypies in fish. Kristiansen et al. (2004) suggest that vertical swimming behaviour found with a flatfish Atlantic halibut (*Hippoglossus hippoglossus*) kept at high stocking densities could be stereotypical.

Nieuwegiessen et al., (2009) defined ‘continuous and compulsive swimming in a fixed repetitive pattern for at least 10 sec’ as a stereotypical swimming behaviour in African catfish (*Clarias gariepinus*). However, no justification was given for its inclusion as a welfare indicator. It has also been suggested that the circular shoaling seen in Atlantic salmon tanks can be described as abnormal. Wild juvenile parr Atlantic salmon are territorial and do not normally shoal until they are preparing to migrate back to their home river to spawn (Lymbery, 2002). When deciding whether a behaviour is abnormal or not we have to establish parameters of normal behavioural repertoire, in order to use behaviour as an indicator of welfare.

The previous behavioural indicators are examples of a functional-based approach to fish welfare and focuses on identifying poor welfare. However, more recently, research has started to focus on identifying fish being in a good welfare state. As animals are motivated to seek rewards, not merely to avoid pain and suffering, then measurements of exploratory behaviour, feed anticipatory activity and reward-related operant behaviour has been suggested as indicators of positive emotion and welfare (Galhardo, 2010; Galhardo et al., 2011). These measures are associated with a feelings-based approach.

Underwater cameras are extensively used to monitor behaviour however, in intensively farmed systems there are limitations to their usefulness to observe individuals. Interpreting the behaviours as good or poor welfare can be problematic unless linked to other physiological measures (Dawkins, 1998).

1.4.7 Stress coping styles

Individuals within a population often have variable behavioural responses to challenges, giving rise to the concept of stress-coping styles; defined as a suite of ‘behavioural and physiological responses to challenge that is consistent over time and across context and that is characteristic of a certain group of individuals’ (Koolhaas et al., 1999; Koolhaas & Van Reenen, 2016). The term coping styles is similar to personality (Gosling, 2001), behavioural syndromes (Sih et al., 2004) and temperament (Francis, 1990).

The behavioural responses to a challenge vary along a proactive-reactive continuum (Koolhaas et al., 1999). Reactive fish usually display a more intense activation of the HPI axis compared to proactive fish. Proactive individuals are often characterised by a ‘flight or fight’ reaction involving active avoidance or high levels of aggression, are more likely to take risks (boldness), establish dominance and are quick to develop fixed pattern routines. In contrast the reactive individual responds with immobility when challenged, has low levels of aggression, is more risk averse (shyness), and tends to occupy a subordinate role although has a more flexible behavioural profile (Øverli et al., 2007; Ruiz-Gomez et al., 2011).

Comparative research has shown that differences in behavioural and physiological responses to challenge exist in a wide range of vertebrate species, including fish (Øverli et al., 2007; Mehta & Gosling, 2008). In one study, rainbow trout were bred to be either highly responsive (HR) to cortisol or low responding (LR) to cortisol (Pottinger & Carrick, 1999). In subsequent staged fights between pairs, fish from the LR line tended to win more fights than fish in the HR line (Pottinger & Carrick, 2001), indicative of contrasting stress coping styles. There were also differences between the strains in the extent to which they followed learned routines. LR and HR fish were trained to find food in a particular location after which the food source was moved. LR fish swam directly to the previously rewarded position, bypassing the new food location to do so, whereas HR fish found and consumed food at the new location before going to the previously rewarded position. The food source was then relocated back to the original position and a novel object was placed between the shelter and food source. LR fish swam over the novel object to access the food whereas HR fish were more hesitant and avoided that area (Ruiz-Gomez et al., 2011). The authors concluded that the HR fish were more aware of changes in the environment and exhibited a reactive coping style with a higher degree of behavioural flexibility. In contrast, the lack of behavioural responsiveness in LR fish appeared proactive, relying on routines in response to changes in the environment. In this study both strains were equally capable of learning a task but differed in their subsequent response to environmental changes. Similar proactive and reactive responses to learning have been found in rodents, pigs and birds (Benus et al., 1991; Bolhuis et al., 2004; Dingemanse & de Goede, 2004). However, the coping style may also be context dependent. Basic et al. (2012) found that introducing a novel object inhibited the LR fish more than the HR fish when no shelter was available, and it was suggested that the novel object presented in this manner constituted an inescapable challenge. When placed within an unfamiliar social group, fish originally identified as bold became shyer although still showed lower levels of plasma cortisol than fish initially labelled shy. In contrast, the behavioural response of shy fish did not change (Thomson et al., 2016). Bold rainbow trout observing other bold individuals interacting with a novel object retained their boldness when tested later with a novel object. However, when a bold fish observed a shy fish interacting with a novel object they in turn became more cautious in their reaction to novelty, suggesting a degree of social learning in bold individuals (Frost et al., 2007). Shy individuals observing bold fish interacting with a novel object did not change their response. This lack of consistency in individual differences between contexts may present a challenge in characterising coping styles for fish in the production environment (for a review see Castanheira et al., 2017).

The majority of studies into coping styles characteristics have been performed on selected HR-LR fish lines, however practical methods for assessing coping styles in non-selected populations are also required to determine the distribution within populations. Øverli et al., (2004b) found that social dominance in rainbow trout could be predicted from the latency to resume feeding after exposure to a stressful event (confinement). Within pairs of fish, the individual which resumed feeding the fastest nearly always won a subsequent contest to assess social dominance in the pair. Inhibition of feeding after experience of a stressor is mediated as part of the physiological stress response and the resumption of food intake possibly reflects a reduction in this response (Øverli et al., 1998). In contrast with the HR-LR fish lines, initially there was no difference in plasma cortisol levels between fish subsequently identified as dominant and subordinate, although post-bout plasma cortisol levels were negatively correlated with aggression levels in dominant individuals. Fish that exhibited a lower cortisol response (LR) during confinement prior to the bout were more aggressive once dominance was obtained post-bout (Øverli et al*.,* 2004). Ventilation rates in Nile tilapia (*Oreochromis niloticus*) and skin pigmentation in Atlantic salmon and rainbow trout have also been suggested as predictors of coping styles. The skin of individuals with a proactive coping style (LR) were more densely populated with black spots (Barreto & Volpato, 2011; Kittilsen et al., 2009). Gesto et al. (2017) did not find any relationship between cortisol levels and melanin spots in rainbow trout. This study used a group-based chasing challenge as the stressor whereas in the Kittilsen et al. (2009) study fish were individually confined. We know from the Thomson et al. (2016) study that proactive fish in particular can modify their coping style depending on the presence of conspecifics. The proactive and reactive behavioural strategies represent two different, but equivalent, coping styles and the success of one over the other will depend on different environmental conditions (Benus et al., 1991).

1.5 Fin Damage in Salmonids

Historically the terms ‘fin erosion’ (in trout) or ‘fin rot’ (in salmon) were used to describe damage to the fins of farmed fish (Larmoyeux & Piper, 1971; Turnbull et al., 1996, 1998). These terms implied that the abrasion or bacterial infections were the cause of the damage. However, in the late 80’s and early 90’s it was shown that some of the damage was due to fish biting each other (Abbott & Dill, 1985) as demonstrated by scanning electron micrographs of damaged fins from fish-farms clearly showing tooth marks and an absence of bacterial infection (Turnbull et al., 1996). In the absence of any clear evidence of disease or abrasion a more appropriate term to use is fin damage; this would cover the multitude of injuries seen on fins without inferring cause (Abbott & Dill, 1985). Damage to fins can result in decreased size, splitting and fraying of the fins and exposure of the fin rays with underlying damage to the epidermis (Sharples & Evans, 1996; Latremouille, 2003). Inflammatory and proliferative processes and secondary infections are also involved.

Fin damage due to fin biting is an abnormal behaviour more prevalent in hatcheries than in the wild (Latremouille, 2003). However, an abnormal behaviour in a production environment maybe a normal reaction to an unnatural environment and expressed by the majority of the animals. For the purposes of this introduction an abnormal behaviour will be one that is not normally performed by a species in its natural habitat (Keeling & Jensen, 2002). The onset and prevalence of fin damage is complex and multifactorial. Controlling fin damage is extremely challenging as triggering factors are difficult to identify. There is a delay between the onset of fish biting and lesions on fins becoming visible, which is complicated by healing which happens quicker at higher temperatures.

This delay causes difficulty in identifying common factors present at the time fin biting was initiated but there is some evidence that slow water flow rates (Jobling et al., 1993), crowding (Cañon-Jones et al., 2011), degraded water quality (Hosfeld et al., 2009), poor feed quality (Ellis et al., 2008) and food distribution (Ryer & Olla, 1996) contribute to the problem. Some factors are under the control of the farmer such as stocking density, water quality, feed quality and distribution. However, a number of normal farming procedures such as size grading and vaccinations disturb density levels, water quality and normal feeding regimes which can lead to the onset of fin damage (Ellis et al., 2008).

Fin damage is perceived as a welfare issue as fish fins are living tissue with a nerve and blood supply (Becerra et al., 1983) and can potentially be the source of pain, fin damage provides a secondary route for infections and affects swimming ability (Ellis et al., 2008). Damaged fins reflect poorly on hatchery production techniques yet are commonly found in farmed trout and salmon (Moring, 1982; Jobling et al., 1993; Turnbull et al., 1996).

1.6 How can fin damage be measured?

1.6.1 Scoring Indices

A priority for UK stakeholders interested in fish welfare is to develop methods that can be simple and rapid indicators of how well fish are coping with their environment (FAWC, 1996). The aim is to develop and validate a checklist of practical indices to indicate poor welfare, the risk factors for poor welfare and good welfare to be used on all fish farms. The presence of fin damage could be used as a welfare indicator as it known to occur under poor welfare conditions, it is visible and easily identified by farmers and auditors and potentially simple to quantify.

A number of different assessment methods to quantify fin damage have been proposed and used by different researchers, quality and welfare schemes. They either describe the state of the fin or attempt to quantify the reduction in surface area due to fin damage (Latremouille, 2003). Indices that describe fin condition include: (i) the Health Condition Profile fin condition Index (Goede & Barton, 1990) (ii) Dorsal fin damage on a 7-point scale

(Turnbull et al., 1996) (iii) (MacLean et al., 2000) used a 3-point scale (mild, absent or severe) to describe fraying and splitting as well as thickening of the dorsal fin (iv) The frequency of fin fraying/splitting within a population (Bosakowski & Wagner, 1994).

Various indices attempted to quantify the amount of tissue loss using; (v) A 3-point scale (Bosakowski & Wagner, 1994), a 4-point scale (Moutou et al., 1998) and a 5-point scale (MacLean et al., 2000) (vi) Fin length expressed as a percentage of total fish length (Kindschi, 1987) (vii) Fin length expressed as a percentage of standard fish length and correcting for allometric fin growth (Ellis et al., 2009) (viii) Using digital photography and image analysis techniques to quantify fin area (North et al., 2006).

Goede & Barton's (1990) method of assessing fin condition, as part of a larger organosomatic index of fish health, is based on classifying the state of the different fins into several categories as follows: 0- All fins or other extremities intact; 1- previous fin damage that has healed over; 2- current fin damage but relatively mild degeneration with possible slight haemorrhaging; 3- extensive active tissue degradation, possibly accompanied by haemorrhaging and secondary infection (Latremouille, 2003). Turnbull's et al. (1996) classification system has seven categories for dorsal fin damage based upon appearance and tissue loss: 1-peripheral damage and ray splitting; 2-peripheral damage with some nodularity; 3- severe nodularity with tissue loss; 4-extensive dorsal fin loss; 5-smooth thickening of the fin; 6- haemorrhagic lesions, and 7- healed lesions. Bosakowski & Wagner's (1994) 3-point scale classifies fins as perfect, slightly eroded or severely eroded; whereas Moutou's et al. (1998) 4-point scale includes no damage, minor damage (<30% missing), severe damage (30%-70% missing) and very severe damage (>70%). The 5-point scale (MacLean et al., 2000) assessed the amount of fin remaining: undamaged (>90% fin remaining), 60-90%, 30-60%, 10-30% and <10% of fin remaining.

Indices that rely on observers to describe the state of the fin or the amount of fin loss ((i) - (v)) are attractive because they are simple and quick to use, however, they are also inherently subjective. Kindschi (1987) proposed using fin length expressed as a percentage of fish total length (LT) to generate a "Fin Index", as a way to quantify the degree of fin damage; fish total length may be substituted with standard length if the degree of damage to the caudal fin is too great. However, the equation relies on the assumption that the relative length of the fins remains constant as fish length changes i.e. fin growth is isometric. Subsequent studies appear to support this approach in wild rainbow trout (Bosakowski & Wagner, 1994), however their statistical analysis may be in error as it differs from that recommended when allometry is involved (Ellis et al., 2009).

More recent studies have shown that fin growth is isometric in the pectoral, pelvic and anal fin but negatively allometric in the dorsal and caudal fins, indicating that relative fin size decreases with increasing fish size for the latter two fins (Hoyle et al., 2007; Ellis et al., 2009). The erroneous results from the Bosakowski & Wagner (1994) study may have been influenced by including the caudal fin in the calculations, which is subjected to allometric growth and fin damage (Ellis et al., 2009). Another potential flaw with the Fin Index is that the natural fin shape of salmonids may be affected by strain and environment (Dynes et al., 1999; Pelis & McCormick, 2003), invalidating the assumption that the fin length-LT relationship is constant for all populations of rainbow trout. A study by Ellis et al., (2009) developed predictive equations from fin and body length measurements obtained from a wild population of rainbow trout in the UK to allow comparisons with farmed rainbow trout. To test for isometric (or allometric) growth a method developed by Warton et al., (2006) was adopted which fits major axis (MA) regression lines to fin data.

There is also a need to differentiate between active and healed fin damage as fin size reflects both historical and current environmental conditions (Ellis et al., 2008) to which end Noble et al. (2018) proposes a 3-point scale (mild, active or severe) including whether active or healed fin damage. The main drawback for scoring indices is the need for a reference fin against which to quantify fin loss, and quantitative measurements can be impractical for on-farm assessment due to time and effort requirements (Hoyle et al., 2007).

1.7 Risk factors

The following section outlines risk factors that can affect fin damage and fish welfare that are mainly under the control of farmers.

Stocking density

Animal welfare pressure groups continue to link high stocking density to poor welfare (Stevenson, 2007; FAWC, 2014). Previously the emphasis was on specifying maximum stocking densities, but more recent research has identified that water quality is more important. A number of studies have suggested that increased stocking density will not necessarily have a negative impact on salmonid fish welfare as long as high-water quality is maintained (Soderberg & Meade 1987; Ellis et al., 2002; North et al., 2006; Person-LeRuyet et al., 2008; Hosfeld et al., 2009). High stocking densities generally causes deterioration in water quality due to the reduction in dissolved oxygen, a build-up of fish metabolites and carbon dioxide, and a reduction in pH levels (Hosfeld et al., 2009). Increasing oxygenation (Person-LeRuyet et al., 2008) and water flow (Ellis et al., 2002) would allow stocking densities to be increased. As long as water quality, specific flow rates and feeding requirements can be met then rearing densities of up to 86kg.m-3 can be achieved without compromising fish welfare and production (Hosfeld et al., 2009; Calabrese et al., 2016). However, where the evaluation of fin damage was included in the study, the majority of studies reported that increasing density had an adverse effect on fin damage (Ellis et al., 2002) even though growth rates and overall condition were favourable (Cañon-Jones et al., 2011).

High stocking density may still contribute to welfare issues such as fin damage, disease transmission and problems with social behaviour (e.g. feed competition, displacement of subordinate fish), irrespective of whether high water quality is maintained. At high densities, fin damage can occur by abrasion against tank surfaces and collisions with other fish especially during feeding. Poor water quality can impair healing (Ellis et al., 2008). However, there is increasing evidence that the primary cause of fin damage is due to fin biting. The majority of attacks are aimed at the dorsal fin, as seen in juvenile steelhead trout (*Salmo gairdneri*) and Atlantic salmon parr (Abbott & Dill, 1985; Turnbull et al., 1998). Hatchery-reared Atlantic salmon parr are selectively bred for high growth rates which appears to have increased social aggression in these fish compared to wild salmon (Fenderson & Carpenter, 1971; Huntingford et al., 2004). Salmonids defend territories in the wild (Gibson, 1981) and wild Atlantic salmon parr frequently show agonistic behaviours (Keenleyside & Yamamoto, 1962). In farm conditions it has been reported that there is a high incidence of aggressive interactions in Atlantic salmon parr (Wedermeyer, 1997) and Arctic charr (*Salvelinus alpinus*) (Brown et al., 1992) when held at low densities. Experimentally, Cañon-Jones et al. (2011) found that Atlantic salmon kept at high density had more dorsal fin damage due to fin biting than fish kept at low densities. However, fish kept at low densities were subjected to more overall aggression resulting in poorer growth rates and body condition therefore both high densities and low densities can have a detrimental effect on fish welfare, although this was a small-scale study compared to farming conditions. This study manipulated the stocking density by inserting a structure into the tanks to reduce volume, which could potentially have affected behaviour.

Water flow rate

Water flow rate can affect fish behaviour. For example, Arctic charr responded to an increase in water flow rates by increasing their swimming speed which in turn reduces agonistic interactions (Adams et al., 1995). An increase in water flow can also cause a change from territorial to shoaling behaviour (Christiansen & Jobling, 1990). It has previously been shown that experimentally increasing water flow rates increased rates of weight gain, improved food utilisation and decreased fin damage in Arctic charr and Atlantic salmon (Jobling et al., 1993). Highest growth rates were achieved when water flow rates allowed Atlantic salmon parr to swim at their preferred speed of approximately 1 - 1.5 body lengths per second (Huntingford et al., 1998). Solstorm et al. (2016) exposed Atlantic salmon to water speeds outwith their preferred range and found a similar decrease in agnostic interactions at high water speeds (1.5 body lengths s^{-1}) compared to slow speeds (0.2 body lengths s^{-1}) although caudal fin damage increased at the higher velocities. In the Solstorm et al. (2016) study, dorsal fin damage reduced over time at all velocities. However, in other studies it has been noted that dorsal fin deterioration continues over time in juvenile rainbow trout (Kindschi & Barrows, 2009; Person Le-Ruyet et al., 2008) and Atlantic salmon parr (Pelis & McCormick, 2003). The salmon in the Solstorm et al. (2016) study were post smolts sourced from a freshwater parr facility subjected to periods of feed restriction (such as during vaccination, grading and transport), as often found in commercial facilities. Smolting is the physiological change necessary for salmon to move from freshwater to salt water and also involves behavioural changes such as lower levels of territorial aggression. Aggressive behaviour in Atlantic salmon parr has been shown to increase during periods of food withdrawal (Symons, 1968; Cañon-Jones et al., 2010) and restricting rations can cause an increase in dorsal fin damage (Noble et al., 2008; Cañon-Jones et al., 2010). This suggests that the behavioural differences between aggressively territorial parr and non-territorial smolts may account for the reduction in dorsal fin damage over the course of the Solstorm study. This is significant in that the majority of studies on stocking densities of Atlantic salmon have focussed on post smolts. Therefore, when comparing studies of fin damage in relation to stocking density, the life stage of the fish should be considered.

Spatial distribution

It has been suggested that how fish are spatially distributed within a rearing environment can be an indicator of the relationship between them (Turnbull et al., 2008). Therefore, an analysis of the spatial distribution of the fish in the rearing environment and any changes from normal may be an indicator of a welfare issue. Distribution will also be affected by the preferred environmental conditions. At the high stocking densities found in production systems there are likely to be localized sub-optimal areas into which subordinate fish may be forced (Juell et al., 1994); Johansson et al., 2006). When presented with a novel stimulus, fish rapidly migrate to the bottom of the tank before returning to pre-stimulus levels (Bui et al., 2013) and this latency to return may be an indication of stress. Fish held at a high enough density will tend to shoal around the perimeter of tanks and sea cages and generally avoid the surface of the water until feeding time (Juell et al., 1994). Atlantic salmon in sea cages were observed to have a bimodal distribution when fed a restricted diet suggesting that subgroups were formed with different motivations to feed or approach the surface (Juell et al., 1994).

Feed distribution

Feed is one of the few resources that farmed fish can fight over. It is important that feed is widely and regularly distributed to ensure that all fish have easy access to feed. When food is dispersed in a spatially localised fashion, highly competitive individuals can defend the resource excluding less competitive conspecifics (Ryer & Olla, 1996). On-demand feeding has the capacity to reduce incidences of dorsal fin damage (Noble et al., 2007) although fin damage was more prevalent in the on-demand fed group after size grading for a short period of time, with the smallest fishes suffering the most fin damage. Fish fed to satiation are less likely to bite each other (Larmoyeux & Piper, 1971). Neither daily nor intermittent feeding had any impact on fin damage (Klontz et al., 1991) although a more recent study by Noble et al. (2007) showed that feeding once a day compared to 3 times a day increased aggressive interactions and reduced healing of the dorsal fin. In juvenile Atlantic salmon, aggressive nipping tends to increase after feeding (Fenderson & Carpenter, 1971; Keenleyside & Yamamoto, 1962).

Food Withdrawal

Any periods of feed withdrawal should be kept to a minimum and where possible avoided however, withdrawing food for up to 72 hours is a common husbandry technique used to void the fishes' digestive tract prior to any procedures that necessitates crowding, such as vaccination, grading, transport and slaughter. The combination of waste food, fish excrement and crowding stress decreases water quality. As fish are cold-blooded, unlike terrestrial animals, fish can go for long periods of time without food without being detrimental to welfare. Fasting for 1-5 days (prior to slaughter) is unlikely to be detrimental (Lines & Spence, 2012). There was no significant effect of a 14 day fast on the live weight of rainbow trout after a period of refeeding, compared with 0, 2, 4 and 8 days of fasting, due to hyperphagia (Nikki et al., 2014).

Feed restriction

Food restriction has been found to have an impact on fin damage. Atlantic salmon subjected to 10 days of reduced food (a third less than control) developed significantly more severe fin damage than control fish (Cañon-Jones et al., 2017). The formation of dominance hierarchies resulted from the food restriction which led to attacks on subordinate fish, which continued even after the resumption of full rations (Cañon-Jones et al., 2017).

A study by Moutou et al. (1998) found that feeding different ration sizes (0.25%, 0.5%, 1.0%, 1.5% of body weight per day) to juvenile rainbow trout also led to the formation of feeding hierarchies with aggression level and hierarchy strength decreasing with increasing ration size. The severity of dorsal fin damage correlated with ration size with the strongest hierarchies established where the food was most restricted (0.25% and 0.5% classes). The distribution of food and growth rates was also the most heterogeneous in groups fed these rations. No dorsal fin damage was noted in the 1.0% and 1.5% ration groups. Social rank appeared to be the determining factor of the severity of dorsal fin damage within the restricted ration groups. In each ration group, the fish were ranked by their individual food consumption into four feeding classes, bottom, lower middle, upper middle and top. In the 0.25% ration group the majority of fish suffered severe fin damage. The top ranked class suffered minor damage compared to the bottom class, but the upper middle class had the lowest fin damage score. In the 0.5% group, the top and upper middle-class group had the lowest fin damage score however fin damage in all classes was minor compared to that seen in the 0.25% group. This data suggests that there is a cost to low social dominance when resources are scarce (Moutou et al., 1998).

Bergman et al. (2013) subjected brown trout to either their normal daily food ration or 50% of their daily food ration. This study found that overall fin damage was low (scoring 2 on a 6-point scale) and damage was restricted to the dorsal and pectoral fins. Both studies recorded that fin damage increased over time, however neither of these studies provided treatment replicates. The Bergman et al. (2013) study had 5 sample points between November 2009 to April 2010 whereas the Moutou et al. (1998) study sampled between March and May.

Central control of hunger

In mammals, the feeding centres appear to be restricted to the hypothalamus which is a key player in the control of food intake and energy balance in vertebrates. Some of the key neuropeptides involved in this regulation have been identified including: agouti-related protein (AgRP), cocaine and amphetamine regulated transcript (CART) and neuropeptide Y (NPY) (Berthoud & Morrison, 2008). In Atlantic salmon AgRP-1 brain mRNA levels decrease after 6 days of fasting (Murashita et al., 2009) and increase after feeding (Valen et al., 2011) pointing to a possible anorexigenic role in fish compared to the orexigenic role it plays in mammals. Individual differences in the expression of these genes or hunger itself may be a mechanism for redirected foraging to other fishes' fins when food is not available.

Nutritional Deficiency

Historically, nutritional deficiency in the composition of commercial fish feeds was known to affect levels of fin damage (Ellis et al., 2008) with varying levels of fatty acids (e.g. Castell et al., 1972), amino acids (e.g. Ketola, 1983), vitamins (e.g. Woodward, 1984) and minerals (e.g. Ogino & Yang, 1980) contributing to the problem.

Data from a study by Barrow and Lellis (1999) suggested that dorsal fin erosion among rainbow trout was influenced by dietary protein source. They fed rainbow trout either a krill-based diet (mainly marine invertebrates) or an anchovy-based diet (mainly marine teleosts); the latter of which contained lower amounts of calcium, iron, copper, magnesium sodium and strontium. Each diet had an equal nutrient content. Fish on the krill-based diet had dorsal fin heights comparable to wild fish (rainbow trout) compared with those fed on the anchovy-based diet, however on the anchovy-based diet the fish grew faster. Rainbow trout fed on krill-based diet had a soft and supple dorsal fin with black spots throughout and an orange band along the outer edge. Fish on the anchovy-based diet had dorsal fins that were darker, no spots, often frayed and exhibited a white band of necrotic tissue along the leading edge. Supplementing the anchovy meal-based diet with sodium, magnesium and copper decreased the level of fin damage but not to the levels seen in the krill-based diet. The results suggest that the micro-nutrient content of the protein source in the diet has an effect on fin damage

In rainbow trout excess dietary copper has been shown to reduce aggression and competition for food, leading to less fin damage (Campbell et al., 2005). However, nutritional deficits are unlikely to be the primary cause of fin damage as fish held in isolation but fed the same diet as group-held fish had less fin damage (Kindschi et al., 1991) and not all fish are subjected to agonistic interactions.

In terrestrial animals, a nutrient-deficient diet has been identified as one of the risk factors in severe feather pecking in laying hens and tail-biting in pigs. Diets deficient in crude protein, amino acids and minerals, such as sodium and calcium, have been shown to elicit feather pecking (for a review see Brunberg et al. 2016) and a sodium deficiency in pigs has been shown to increase the likelihood of tail biting (Fraser, 1987). The mechanism by which suboptimal feeding tends to increase injury in these animals appears to act through an increase in exploratory behaviours triggered by nutritional deficiency. Damage caused by these exploratory behaviours attracts conspecifics to further attack the victims, especially when coupled with the presence of other negative environmental factors, such as a lack of suitable foraging material, leading to more severe feather pecking (McAdie & Keeling, 2000) and tail biting (BPEX, 2004).

1.8 Comparable Systems

1.8.1 Terrestrial Animals

There are fundamental differences between fish and terrestrial farm animals which mean that assuming that factors that affect welfare in terrestrial farm animals will affect fish is very risky. Fish live in water, so water quality is very important especially to the delivery of oxygen. Removing fish from the water is a massive stressor. Fish are particularly vulnerable to skin damage, especially when handled or in overcrowded conditions, which can make them more susceptible to diseases. They also move about in three dimensions making observations more challenging. They are poikilothermic (cold blooded) so are more likely to be affected by ambient temperature but less likely to be affected by periods of food deprivation than homeotherms (warm blooded animals). Despite these differences, fin biting has similarities to abnormal behaviours seen in other intensively farmed species such as feather pecking in laying hens (*Gallus gallus*) and tail biting in domestic pigs (*Sus scrofa domesticus or Sus domesticus*).

Historically, the management of feather pecking, and tail biting has involved beak trimming and tail docking. However, new legislation from the EU seeks to outlaw these practices as they can cause unnecessary suffering (EU directive 1999/74/EC and 2008/120/EC). In common with fin damage, the research on severe feather pecking and tail biting has focussed on how housing and management can affect the development of these abnormal behaviours. The causes are multifactorial, and despite sharing the same environment we know that not all animals in a group are equally likely to develop harmful behaviours; some individuals are more likely to become victims, while others seem to avoid becoming a victim or a perpetrator (Brunberg et al., 2016). The underlying mechanisms underpinning these abnormal behaviours are difficult to study due to the unpredictable nature of outbreaks, difficulty in pinpointing triggers and the fact that for many of the identified risk factors it is unclear what the role is in the development of tail biting (D'Eath et al., 2014). However, examining the mechanisms used to investigate feather pecking and tail biting behaviours and the underlying causes may help identify comparable mechanisms in fish.

Feather Pecking in laying hens

Feather pecking is considered a significant risk to animal welfare within the poultry industry and is defined as a detrimental behaviour whereby birds forcefully peck and remove feathers from other birds, which can cause severe bleeding, and in some instances, stimulates cannibalism (Savory, 1995). Severe feather pecking is distinct from gentle feather pecking which does not cause injury and needs to be distinguished during observations as they are considered distinct behaviours with different underlying neural mechanisms and motivations (Hughes & Buitenhuis, 2010). Neither should feather pecking be confused with aggressive pecking which is directed at the head and neck usually resulting in little or no damage (Savory, 1995).

There are two main theories about the causes of severe feather pecking, both relating to ground pecking behaviour; one hypothesis is that it is related to foraging motivation

(Blokhuis & Arkes, 1984; Blokhuis, 1986) and the second hypothesis is that it is linked to dust bathing. Huber-Eicher and Wechsler (1997) found that access to straw (a foraging substrate) decreased severe feather pecking but access to sand (dust bathing substrate) did not significantly decrease severe feather pecking. This is supported by a study which identified distinct 'fixed action patterns', with severe feather pecks being similar to foraging pecks but significantly different from novel object pecking, drinking or dust bathing indicating that severe feather pecking likely evolved from frustrated motivation to forage rather than dust bathing (Dixon et al., 2008).

Severe feather pecking has also been related to dietary factors such as eating loose feathers from the litter, which has a positive effect on gut motility similar to that of roughage (Harlander-Matauschek et al., 2006) and may be related to the relatively low fibre content of commercial laying hen diets (Rodenburg et al., 2013). Once feather eating has been established and the loose feathers depleted, the feather eating behaviour may be re-directed to feather pecking at other birds (McKeegan & Savory, 1999). It has been shown that removing loose feathers from the litter during the rearing period suppresses the development of feather eating thereby reducing feather pecking during the laying period (Ramadan & Von Borell, 2008).

EU Council directive 1999/74/EC (July 1999) banned the use of conventional battery cages for laying hens from 1st January 2012. Enriched cage systems can still be used but must provide a nest, perching space, litter to allow pecking and scratching and unrestricted access to a feed trough. Feather pecking is a considerable problem in free-range systems compared to cage systems, especially when hens are not beak trimmed and has been shown to be associated with breed type (Weeks et al., 2016). Beak trimming by infra-red technology is still allowed in the UK on birds under 10 days old, to help reduce feather pecking outbreaks while research continues to better understand the trigger points for an outbreak. A comparison of barn, enriched and conventional housing systems showed that hens in enriched cages had the best overall welfare score in terms of gentle feather peck given, feather damage score, proportion of hens with feather damage, proportion of the flock using perches and faecal corticosterone (Sherwin et al., 2010).

Although severe feather pecking seems to be more clearly related to foraging behaviour rather than dust bathing, its development and underlying causes are not yet fully understood and remains unpredictable in commercial situations (Hartcher et al., 2016). The causes of severe feather pecking are considered to be multi-factorial including early life experiences, the environment, genetics, individual differences (fearfulness/social motivation), diet and feeding behaviour. However, research in these areas is contradictory and little consensus exists in the literature as to the specific contributions of these factors. (reviewed in Rodenburg et al., 2013).

Tail biting in pigs

In the literature tail biting is categorised as being anything from oral manipulation to severe injury, possibly leading to cannibalism and is a major welfare problem in weaned and growing pigs (D'Eath et al., 2014). Tail injuries may indicate pain and suffering in the bitten animal and can be a site for further infection. It may be stressful to the group and may indicate frustration experienced by the biting animal (Schroder-Petersen & Simonsen, 2001). Tail biting continues to persist as a problem as outbreaks tends to occur sporadically making it difficult to observe and pinpoint specific triggers (D'Eath et al., 2014).

Risk factors for tail biting are considered to be multifactorial including environmental factors (such as feed, season, climate control, availability of foraging material), individual factors (age, sex, breed, genetics, neurobiology) and social factors such as group dynamics (reviewed in Schroder-Petersen & Simonsen, 2001; Brunberg et al., 2011; D'Eath et al., 2014). Tail biting has also been correlated with the selection for production traits such as increased back fat which led to an increase in tail biting (Moinard et al., 2003; Brunberg et al., 2011). Differential expression of the PDK4 gene was found between neutral pigs and those involved in tail biting (whether victim or perpetrator) (Brunberg et al., 2013) and this gene is known to be associated with back fat depth (Lan et al., 2009). The mechanism by which each of these risk factors influence the development of tail biting, is, in many cases, currently unknown (D'Eath et al., 2014).

Recent work has focussed on identifying early warning signs to help predict an outbreak of tail biting. Four main types of early warning signs have been identified that may indicate the onset of tail biting before any lesions are visible: increases in general restlessness, increases in non-damaging mouthing of tails, tails are held down or tucked under and, changes in feeding patterns (reviewed in D'eath et al., 2014). Changes in general restlessness and feeding patterns are indications used by fish farmers that something is amiss. Operationalising these factors as part of a welfare assessment on fish farm may be useful as would be investigating whether there is a correlation between general restlessness and the onset of fin biting.

1.9 Mitigation Measures

1.9.1 Environmental Enrichment

Most studies to date have viewed fin biting as an abnormal behaviour brought about by the unnatural conditions inherent in intense fish farming. Recent research is starting to focus on providing some form of enrichment to mimic salmonid's natural environments in an effort to reduce fin biting. FAWC (1996) calls for salmon environmental enrichment to be investigated and recommends that the industry should endeavour to develop better methods of inspecting fish to recognise those which are diseased or distressed.

The provision of in-tank environmental enrichment has been shown to increase behavioural flexibility and, social learning in fish and produce fish better able to adapt to novel situations (Näslund & Johnsson, 2014).

For financial reasons intensive fish farming practices requires stocking densities which exceed the density of fish normally found in the wild. This can lead to an increased frequency in social encounters and hence potentially aggressive encounters (Huntingford, 2004). Fish are kept in barren tanks for ease of cleaning, removal and transfer of fish and the reduction in the spread of disease. The farm environment is less challenging in that food is readily available, so there is no need for fish to forage, and it is free from predators (Huntingford, 2004). However, there is little evidence for the benefits or otherwise of enriched environments for farmed fish.

To ensure increased production and promotion of good welfare, producers and regulators should aim for a low-stress rearing environment. This is characterised by the fish appearing “settled”. There are a few studies that have assessed the effect of enrichment on fish. Pounder et al., (2016) found that in rainbow trout the opercular beat rate (an indicator of stress) recovered faster from a standard stressor (1 min air emersion) when kept in an enriched environment versus a barren environment. The enriched tanks in this study had the addition of gravel, plastic plant and overhead cover. In the same study it was found that there was no treatment effect of environment on the recovery time from experiencing a noxious stimulus (subcutaneous injection of 1% acetic acid into frontal lips). There was also no difference in plasma cortisol levels over any of the treatment groups. However, this may have been due to the 3-hr delay in taking the samples. Post-stress plasma cortisol levels in rainbow trout tend to be greatest approximately 1 hr following an acute stressor and then decline (Pickering & Pottinger, 1989). In another study, no post-stressor difference was found in plasma cortisol in Atlantic salmon following a 30 min confinement period for fish housed in enriched and barren environments (Näslund et al., 2013). However, the basal levels of plasma cortisol were on average two to three times higher in the barren tanks compared to the enriched tanks; with levels similar to that found in Atlantic salmon exposed to chronic stress (Fridell et al., 2007). Atlantic salmon parr without access to shelter had a higher resting metabolic rate compared to those which did have access to shelter, probably due to increased vigilance and maintenance of a flight response (Millidine et al., 2006). In the Näslund study there were two types of enrichment; plastic tubes or shredded plastic bundled on the tank floor as well as a barren tank. These results suggest that enrichment can potentially provide a lower stress environment by ameliorating the effects of chronic stress as opposed to acute stress.

Effect of enrichment on fin damage

It has been shown that the majority of fin damage sustained in farmed fish is due to fish biting each other (Abbott & Hill 1985; Turnbull et al., 1998), especially the dorsal fin. An enrichment study by Näslund et al. (2013) demonstrated that dorsal fin damage on Atlantic salmon deteriorated more over the winter months in barren tanks compared with enriched tanks. This contrasts with a reduction in fin damage over the winter period found by MacLean et al. (2000), who suggest reduced appetite over the winter months reduced aggression. Berejikian (2005) compared the dorsal fins of rainbow trout reared in barren tanks, enriched tanks and a natural stream. The enriched tanks contained submerged dried tree branches from the tops of Douglas firs and overhead netting to provide 60% shade covering. At the end of the study period, the dorsal fins of fish in the barren tanks sustained significantly more fin damage than in the enriched tanks, and fish in the enriched tanks exhibited fin quality similar to that of naturally reared fish. Although fin biting was not measured in this study, it was hypothesised that the visual isolation provided by the submerged structure served to reduce the frequency of fin biting. The use of structural enrichment in experimental tanks has been shown to reduce fin damage (Rosengren et al., 2017), however there are few if any studies showing the effects of enrichment under commercial conditions.

The salmonid dorsal fin plays an important role in aggressive and submissive posturing during territorial contests with juvenile Atlantic salmon raising its dorsal fin as it charges (Keenleyside & Yamamoto, 1962) and a fully depressed dorsal fin signifying submission in rainbow trout (Berejikian et al., 1996). Berejikian (2005) found that fish having an undamaged dorsal fin over a lower quality damaged fin did not necessarily gain an advantage in agonistic encounters and that other factors such as behavioural development likely plays a more important role in determine the outcome of agonistic encounters.

Structural enrichment and density

Stocking densities of salmonid fishes in production systems is unnaturally high compared to that found in the wild (Latremouille, 2003). However, under natural stream-dwelling conditions, the density of juvenile salmonid fish increases with habitat complexity. Areas with an abundance of coarse woody debris (Roni & Quinn, 2001) or augmented with artificial vegetation (Eklov & Greenberg, 1998) support a higher density of fish than those without. Many models used to predict salmonid biomass correlate fish abundance with measures of habitat complexity (reviewed in Fausch et al., 1988). The use of structural enrichment in production tanks could potentially allow a more natural environment for high stocking densities than barren tanks. Three hypotheses are commonly invoked to explain the increase in salmonid density with habitat complexity (Huntingford et al., 1988).

1. Territory-size hypothesis

This hypothesis assumes that visual isolation increases with habitat complexity, which impedes detection and expulsion of intruders and food items leading to smaller territories and higher density –assumes territorial fish do not prefer complex habitats.

2. The predator-refuge hypothesis

Fish near cover perceive reduced risk of predation, exhibit decreased wariness to predators; often quantified as the reactive distance to the approach of a predator or novel stimulus.

3. Foraging benefits hypothesis

Increase in complexity leads to variance in current velocity allowing fish to reduce energy expenditure by holding position in low velocity locations whilst obtaining a foraging benefit from adjacent high velocity locations that provide a drift-funnelling effect.

Most studies assume habitat complexity is positive and ignore possible negative aspects. Structural complexity can provide greater refuge for the prey to the detriment of the predator (Warfe & Barmuta, 2004). There is also the potential interaction between the size of fish and structures in being able to access refuges and prey. Bilhete & Grant (2015) exposed Atlantic salmon to low and high complexity habitats and found that the addition of structures to habitats may be beneficial at the population level, in that it allows an increase population density and lower rates of aggression. However, at the individual level there was a cost to bear with smaller territories and lower foraging rates. Rosengren et al. (2017) found fish reared in complex environments had decreased growth and suggest this may have been the result of risk-sensitive behaviour with fish preferring to remain sheltered rather than forage.

1.9.2 Selection for coping style

Research into characterising coping styles has relevance for aquaculture as some behavioural and physiological aspects of the fish stress response are heritable (Pottinger & Carrick, 1999; Millot et al., 2014). This brings the possibility to select for fish with low stress responsiveness to husbandry practices therefore improving welfare. The selection for fast growth in farmed fish has inadvertently selected for the behavioural traits of high aggression and risk taking (Huntingford & Adams, 2005), associated with a proactive (bold) coping style. The behavioural traits of this coping style are advantageous in that it is characterised by a low stress response and increased risk-taking behaviour, for example making it more likely that the fish will explore new environments to find food when moved between tanks. Stable, predictable environments, such as those found in fish farms should benefit proactive animals. However, when these proactive animals are confronted with any variation in the environment, disease resistance may be compromised, leading to impaired welfare (Fevolden et al., 1992, 1993). High aggression in farmed fish can also compromise welfare by causing injuries such as fin damage, especially during competition for food (Noble et al., 2007) and overall growth may be suppressed as subordinate fish may be prevented from feeding (Christiansen & Jobling, 1990).

The practicality of characterising coping styles for individuals in large groups and the lack of consistency in those methods currently make it impractical to pre-screen populations for fish farming. Huntingford & Adams (2005) suggest modifying husbandry systems to ameliorate the effect of aggression such as by increasing water flow rate to increase the energetic cost of fighting and modifying food distribution to reduce competition.

1.9.3 Husbandry procedures

A number of risk factors for fin damage can be mitigated against by monitoring and changing farming practices. Fish fed to satiation are less likely to nip each other and fin damage was much reduced by the addition of non-digestible bulk to the diet, although at the expense of increased waste products (Larmoyeux & Piper, 1971). Widely dispersed food and a high density of food is less defensible leading to reduced competition (Abbott & Dill, 1985; Cañon-Jones et al., 2010; Ryer & Olla, 1996). Optimising water current (Jobling et al., 1993) and stocking densities (North et al., 2006; Siikavuopio & Jobling, 1995) reduces aggression.

The Scottish Salmon Producers' Organisation (SSPO), the British Trout Association and marine flatfish producers all subscribe to the Code of Good Practice (CoGP). Other species-specific voluntary codes of practice include the RSPCA Welfare Standard for Farmed Atlantic Salmon, under its RSPCA assured scheme (formerly Freedom Foods). The voluntary codes of good practice are based on the principles of the five freedoms (FAWC, 1996): freedom from hunger and malnutrition, freedom from discomfort, freedom from pain injury and disease, freedom to express normal behaviour and freedom from fear and distress. The CoGP details good practice criteria in fish welfare; fish health and biosecurity; management and protection of the environment; fish feed and feeding practice. There is a high uptake, with 95% of salmon and 90% of trout production farmed by UK fish farmers registered as working to the code (https://www.seafoodscotland.org).

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2 The effect of food withdrawal on the development of fin damage in freshwater reared Atlantic salmon (*Salmo salar*) juveniles.

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Preface

Fin damage has been studied for many years as conclusively defining risk factors has been challenging. The results of this study showed that food withdrawal was a major contributory factor to fin damage, the significance of which had not previously been reported. The project required the design of instrumentation and proprietary software to non-invasively monitor fin damage and an analysis of non-invasive and invasive sampling of fin damage is described in Chapter 3. The results from this chapter contributed to the design of an on-farm experiment to investigate the effects of enrichment on food withdrawal and fin damage (Chapter 4 and 5).

This study was part of a Defra-funded project AW1209 investigating risk factors for fin damage, that was in progress when I started my PhD. The experimental design of this chapter was carried out by my co-authors. Prior to my participation all samples had been collected except the final sample. I collected the final sample along with my supervisors Dr. Sonia Rey and Professor Jimmy Turnbull and processed all the samples from the whole duration of the experiment. The data analysis was conducted by me with support from Professor Toby Knowles at Bristol University. I prepared this manuscript with edits from my supervisors. This chapter is designed in the format of a publication to be submitted to the journal Aquaculture and the results were presented at a World Aquaculture Society (WAS) conference in Montpellier, France in 2018.

2 The effect of food withdrawal on the development of fin damage in freshwater reared Atlantic salmon (*Salmo salar*) juveniles.

Abstract

Fin damage in farmed salmonids is a welfare issue and costly to farmers. Despite decades of research, conclusively identifying risk factors has proved difficult, partly due to a delay between initiating events and the development of visible lesions; with the delay varying depending on water temperature. There is some evidence to suggest that feed withdrawal may be an initiating event or trigger. This study examines the effect of fasting, a normal husbandry technique on fish-farms, on dorsal fin damage and how this is affected by developmental stage. Six tanks (415 fish per tank) were randomly assigned to a control or treatment group. In phase I of the experiment three tanks had food withdrawn for 3 days (treatment) while three tanks remained on a normal feeding regime (control). Tanks were sampled ten days after normal feeding had resumed. Samples were weighed, photographed and the condition of the dorsal fin recorded, using a fin condition index developed previously. In phase II the three treatment tanks were allowed to recover and each of the previous control tanks were exposed in turn to a three-day period of food withdrawal and sampled as previous, to have more replicates of the same treatment at a different developmental stage of the fish. Food withdrawal initiated more dorsal fin damage and lower growth than controls in phase I of the experiment. There was no difference in fin damage in phase II. However, growth in phase II was higher in food withdrawal tanks than control tanks. The significance of the effect of food withdrawal on fin damage has not previously been reported. In conclusion, as food withdrawal has been identified as a trigger for dorsal fin damage, there is the potential for farmers to explore ways to avoid withdrawing food or reducing the duration of food withdrawal to reduce the prevalence of fin damage and consequently improve fish welfare.

2.1 Introduction

Globally, aquaculture growth rates continue to outpace other food production sectors, producing 80 million tonnes of food fish in 2016 (FAO, 2018). While many species of carp predominate the top ten of global production by volume of finfish, Atlantic salmon (*Salmo salar*) accounts for only 7% of the top ten species yet represents 17% of economic value (FAO, 2018). Over 2 million tonnes of salmon were produced globally in 2016 and depending on size at harvest could represent in the region of 450 million to 750 million individual fish (assuming 3-5 kg at harvest). The welfare of farmed fish has only recently become of concern compared with the welfare of other intensively farmed terrestrial species and it can be seen that any welfare issues have the potential to affect a great number of individuals. Stressful farming conditions, such as handling, crowding, poor water quality and the presence of pathogens may impair the welfare of fish and possibly reduce their health status (Huntingford et al., 2006). This inherent link between welfare conditions and health of fish translates directly into the economic sustainability of fish farms through losses suffered from poor health and disease and the increased use of treatments (Segner et al., 2012).

Despite progress in addressing many welfare concerns, fin damage is an issue that has persisted in the salmon sector and many others globally. The prevalence of fin damage in farmed populations is higher than natural populations (Hansen et al., 1987). It is so widespread, within many species of fresh and sea water farmed fish, that it can be used to differentiate between escaped farmed fish and wild fish. For example, cod (*Gadus morhua*) (Uglem et al., 2011), gilthead sea bream (*Sparus aurata*) (Arechavala-Lopez et al., 2013), Atlantic salmon (*Salmo salar*) (Lund et al., 1989, Fiske et al., 2005) and rainbow trout (*Oncorhynchus mykiss*) (Bosakowski & Wagner, 1994) can all be differentiated from farmed conspecifics by the presence of fin damage. However, there was no differentiation in fin damage between escaped farmed and wild European sea bass (*Dicentrarchus labrax*) (Arechavala-Lopez et al., 2013).

Fin damage can appear as splitting of the fin rays, tissue loss and pale nodular thickening of the distal portion of the fin (Turnbull et al., 1996; Winfree et al., 1998). Unlike feathers and hair, fish fins are living tissue with nerve and blood supply. Damage to fins can affect swimming ability, increase susceptibility to infections (Ellis et al., 2008) and potentially be a source of pain (Sneddon et al., 2003; Braithwaite & Huntingford, 2004; Chandroo et al., 2004). Damaged fins reduce the value of the fish particularly when sold as whole fish and are an indicator of problems in rearing conditions (Klíma et al., 2013). There is some evidence that slow water flow rates (Jobling et al., 1993; Solstorm et al., 2016), stocking density (Ellis et al., 2002; Cañon-Jones et al., 2011), poor water quality (Hosfeld et al., 2009), poor feed quality (Ellis et al., 2008) food distribution methods (Ryer & Olla, 1996; Winfree et al., 1998; Cañon-Jones et al., 2012) and feed restriction (Moutou et al., 1998; Noble et al., 2007a, 2007b; Cañon-Jones et al., 2017) contribute to the problem. However, nutritional deficiencies and water quality are unlikely to be primary factors, as isolated fish held in the same tanks as fish showing signs of fin damage and with the same feeding regime, did not have fin damage (Kindschi et al., 1991).

Previous research has demonstrated that most fin damage is the result of biting by conspecifics (Abbott & Dill, 1985; Turnbull et al., 1996) with the dorsal fin most likely to receive damage (Turnbull et al., 1998). Using a scanning electron micrograph, tooth marks were clearly visible on damaged fins of farmed Atlantic salmon and with the absence of bacterial infection indicated that damage was mainly due to biting (Turnbull et al., 1996). However, risk factors triggering this behaviour have been difficult to identify and is complicated by the difficulty in observing and quantifying biting behaviour and a delay between the potential initiating events and the lesions becoming visible. Healing is also temperature dependent, resolving faster at higher temperatures, further confounding any association between initiating conditions and observable lesions (Anderson & Roberts, 1975; Andrews et al., 2015). Biting between individuals can also imply the formation of social hierarchies. Strong social hierarchies develop during food deprivation and increases in aggression, such as lateral and frontal displays, were observed more than charging and nipping (Symons, 1968; Damsgård et al., 1997). Moutou et al. (1998) found that restricting food led to the formation of feeding hierarchies with a corresponding increase in aggression levels and hierarchy strength with decreasing ration size. Social network analysis (Cañon-Jones et al., 2017) identified that there were clusters of fish that initiated aggression during a 10-day food restriction period (fish subjected to 30% of satiation ration for 10 days), which is far longer than the 3 days normally observed by commercial companies. Fin damage was more severe in in the food restricted group and only evident on the receivers but not the aggressors.

Feeding to satiation is a method used to control fin damage on fish-farms (Larmoyeux & Piper, 1971) suggesting that changes in feeding behaviour is a primary factor in the development of fin damage. Food withdrawal is a normal husbandry procedure used to empty the fishes' digestive tract prior to any procedures that necessitates crowding, such as vaccination, grading and transport. Food withdrawal is used to maintain water quality during these procedures as the combination of waste food, fish excrement and crowding can decrease water quality (Carmichael et al., 2001; Harmon, 2009). However, reducing food rations can result in increasing fin damage. Studies by Moutou et al., (1998) and Gregory & Wood (1999) found that feeding different ration sizes to juvenile rainbow trout resulted in increased fin damage with decreasing ration sizes and Atlantic salmon subjected to 10 days of reduced food developed significantly more severe fin damage than control fish (Cañon-Jones et al., 2017). Reducing food rations also resulted in fin damage in Atlantic cod (Hatlen et al., 2006) and arctic charr (*Salvelinus alpinus)* (Damsgård et al., 1997).

Atlantic salmon parr are known to be aggressive (Keenleyside & Yamamoto, 1962) resulting in high levels of fin damage especially to the dorsal fin (Turnbull et al., 1998). In previous studies the conditions that would normally prevail on farm sites such as high stocking density and complete food withdrawal were not recreated. The aim of this study was to investigate the effect of food withdrawal on dorsal fin condition in large groups of Atlantic salmon parr kept under near commercial culture conditions. In addition, the impact of food withdrawal on fin damage at different developmental stages was also investigated.

2.2 Materials and Methods

2.2.1 Animals and experimental set up

This study was conducted between July and November 2015 at the Niall Bromage Freshwater Research Unit at Buckieburn, central Scotland. Ethical approval was obtained from the University of Stirling's Animal Welfare and Ethical Review Body (IoA AWERB April 15 interim ASPA 04.docx).

Fish used in the study were of the '*Salmobreed*' strain, sourced commercially from Howietoun Fishery, Stirling (UK) with initial weight (mean ± SD) 8.21 ± 1.58g and standard length 80.2 ± 5.04mm. Six experimental groups were used in the study; initially three control groups and three treatment groups. At the start of the study each group consisted of 500 juvenile fish. However, one week prior to the treatment being applied (day 49) a problem developed in one of the tanks (T8) due to a suspected feeder malfunction and all the fish in that tank were euthanised for welfare reasons. Approximately 83 fish from each of the other tanks were re-distributed into tank 8, to give 415 fish in each experimental group.

The experiment was run in two phases (Figure 2-1). In the first experimental phase three tanks were randomly allocated to the treatment group (T2, T8, T9), where food was withdrawn for three consecutive days. The three control tanks (C3, C4, C7) remained on the standard feeding regime, which comprised of feeding EWOS Micro 5PLR pellets every 15 mins over 24 hours from calibrated feeders (Arvotec) located above each tank.

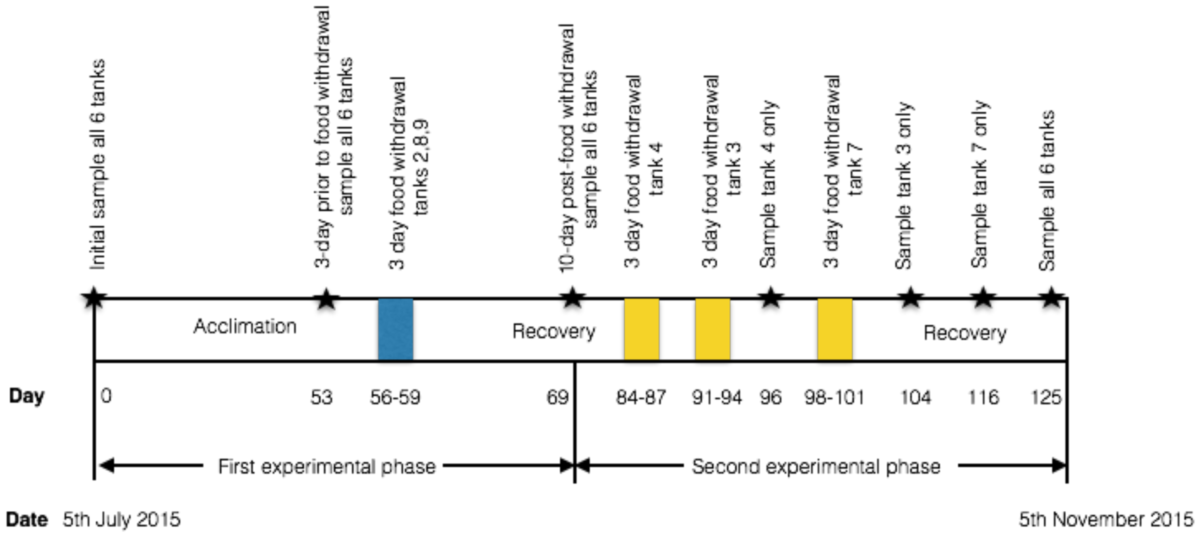

Figure 2-1 *Experimental timeline showing sampling points (star) and food withdrawal periods (coloured areas).*

All fish were allowed to acclimatise in the experimental tanks for 55 days prior to feed being withdrawn in the treatment tanks on day 56. The standard feeding regime was used during the acclimation and recovery phases in both the treatment and control tanks. In the second phase the original three treatment tanks (2, 8, 9) were kept on the standard feeding regime and left to recover whereas each of the previous control tanks were exposed in turn to a three-day period of food withdrawal (3, 4, 7), see Figure 2-1. Using this experimental design, the power of the experiment was maximised without increasing the number of fish and allowed each tank to act as its own partial control. By the final sample (day 124) tanks 2, 8 and 9 had 65 days to recover from the end of their food withdrawal period whereas recovery time at the final sample for tank 3 = 30 days, tank 4 = 37 days and tank 7 = 23 days.

2.2.2 Housing, water quality and environmental conditions

Fish were housed in 3000L dark green fibreglass circular tanks (1m high x 2m diameter) with a centrally hinged, fitted lid. Tanks were filled to a depth of 0.7m (2000L) achieving stocking densities of ca. 0.5kg/m^3 at the beginning of the experiment increasing to ca. 7kg/m^3, immediately prior to treatment being applied. Two fixed lights mounted on the lid and two fluorescent tubes, mounted cross-wise on a PVC base floating on the water surface, produced a light intensity of 0.6 W/ m^2 at the surface and 0.2 W/m^2 at the bottom of the tank. Tanks were kept on a 24-hr photoperiod regime for the duration of the experiment, to avoid smoltification of the stock. A single automated feeder (ArvoTec, Huutokoski, Finland) was mounted on the lid with the food hopper located downstream of the water inflow pipe. Water was gravity fed, from a local freshwater reservoir, into a flow-through system. Water temperature ranged from 13.75°C ± 0.4°C at the start of the experiment to 10.3°C ± 0.9°C at the conclusion to the experiment.

2.2.3 Sampling procedure and physical measurements

Twenty fish were netted from each of the six tanks at each sampling point; at the start of the experiment, three days prior (day 53) to the first feed withdrawal (n=120) and ten days after (day 69) feeding had resumed (n=120), see Figure 2-1. During the second experimental phase 20 fish were netted from each of the treatment tanks only, ten days after feeding had resumed on day 96, day 104 and day 116 (n=60) and a final sample of 60 fish from each of the six tanks (n=360). Fish samples were euthanised by an overdose bath of MS222 and onset of rigor mortis confirmed. All the fish were euthanised at the end of the experiment. Individual fish taken for sampling were weighed and photographed. A camera (Pentax K-30 SRL digital camera) was mounted on a vertical stand over a board with a ruler (Figure 2-2)

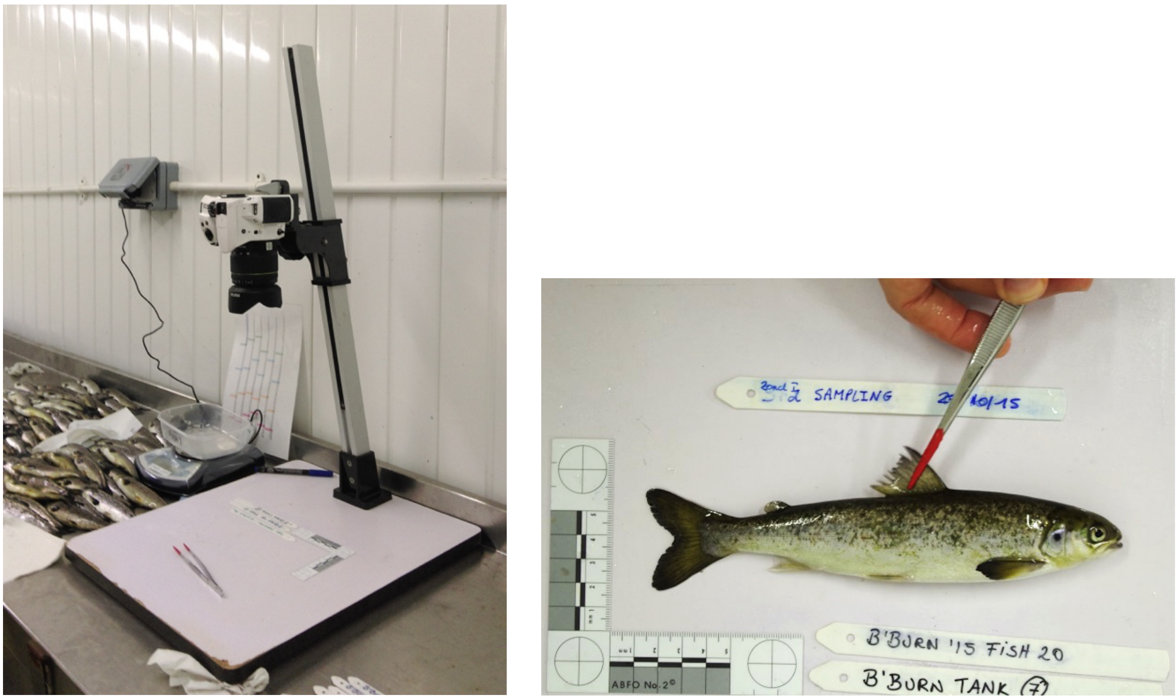

Figure 2-2 *Photographs of the sampling set up showing camera affixed to an adjustable mount and an example of an image used to obtain physical measurements, see text for details.*

Physical measurements were made from the digital photographs using ImageJ software (Schneider *et al*., 2012). Measurements taken were standard length (SL), fork length (FL), and height of the leading edge of the dorsal fin (HLE) as shown in Figure 2-3.

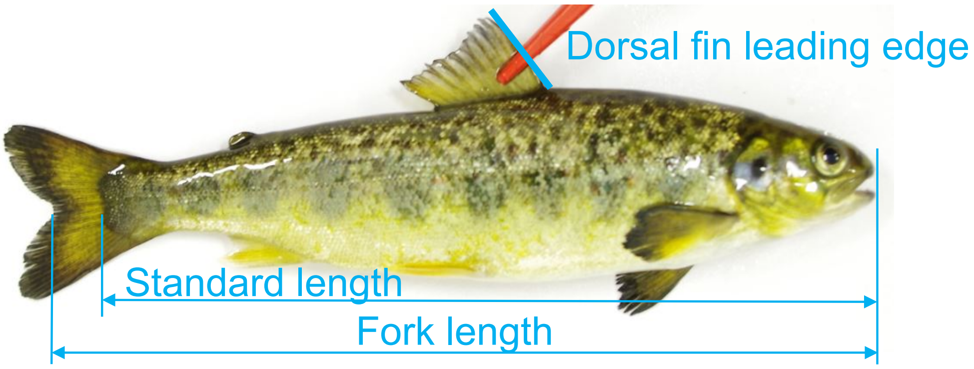

Figure 2-3 *Physical measurements recorded for each sample*

2.2.4 Quantification of fin damage

A fin damage score was recorded for each sampled fish. Damage to the dorsal fin was categorised on a 5-point ordinal scale adapted from Maclean et al. (2000): 0 (0-10% loss, good condition), 1 (10-20% loss, frayed or mild damage), 2 (20-30% loss, moderate damage), 3 (30-60% loss, severe damage), 4 (> 60% losses, very severe damage/no fin), see photographic key in Figure 2-4. Hoyle et al., (2007) demonstrated good inter- and intra- reliability between observers when using photographic keys to assess fin damage. Inter-rater reliability in this study was assessed between two observers using samples from three random tanks (n=60). Weighted kappa analysis showed substantial agreement between observers (0.78) which agrees with that found by Hoyle et al., (2007). Scoring for all samples used in the following analysis was done by the author using the photographic key.

Figure 2-4 *Dorsal fin condition score from 0 (perfect fin) to 4 (severe damage)*

2.2.5 Statistical analysis

Multi-level modelling was performed using MLWin (Rasbash et al., 2009). The log-likelihood method was used to gauge the fit of the model. Normality and homoscedasticity of the residuals, for the models, were checked using Q-Q plots and standardised residuals vs. fitted values. In all statistical tests, p= 0.05 was taken as the level of significance.

Dorsal fin condition at Phase I final sample (day 69)

To give an overview of fin damage at the last sample in phase I (day69) fin condition scores were combined into three categories ‘good’ (Fin Score 0 or 1), ‘medium’ (Fin Score 2) or having severe damage ‘poor’ (Fin Score 3 or 4). The percentage of fins scoring as good, medium or poor in each tank was calculated.

Phase I Modelling the effect of food withdrawal on the dorsal fin (day 69)

Separate two- level linear models (TankID and FishID) were constructed to investigate the relationship between response variables (Fin condition and HLE) and treatment (3-day food withdrawal). The value for Fin Condition at day 53 was used as a covariable in the model so that the model was comparing the change in fin condition pre- and post-food withdrawal and similar for HLE.

Phase I Modelling the effect of food withdrawal on growth parameters at day 69

Separate two-level linear models (TankID and FishID) were constructed to investigate the relationship between the response variables (Weight, FL, SL) and treatment (3-day food withdrawal). The model for weight at day 69 included weight and FL at day 53 (prior to food withdrawal) as covariables. The models for FL and SL included weight and FL or SL at day 53 as covariables.

Phase II Fin condition and growth parameters at final sample day 124

Separate 2 level linear model (TankID and FishID) was used to investigate the relationship between the response variables (Weight, Fin condition, FL, SL and HLE) and treatment (3-day food withdrawal) between day 69 and day 124. The outcome measure is the value at day 124 (final sample of Phase II), with a fixed effect of withdrawal (or not) and the average measurement at day 69 sample (final sample of Phase I) used as a co-variate in the model. Weight was also used as a co-variate in SL and FL models and FL was used as co-variate in weight and HLE models.

Phase II Modelling the staged effect of time of treatment

A two- level model (TankID, FishID) was used to investigate the interaction of the time treatment was applied in the individual tanks (Tanks T3, T4, T7) in Phase II. A separate model was constructed for each response variables weight, Fin Condition, SL and FL. Fixed effects were the measures at day 69 and 10 days after food withdrawal. The model was constructed by fitting a 2nd order polynomial to the changes across time for each individual tank by means of a Day x Tank interaction.

2.3 Results

Experimental phase I

At the start of the experiment (day 0) there were no significant differences in weight or fin score between control tanks (C3, C4, C7 = 7.95g ± 1.36g, 0.9 ± 0.57, mean ± SD), and treatment tanks (T2, T8, T9 = 8.46g ± 1.760, 0.8 ±0.49, mean ± SD). At day 0, fish sampled from each tank had good dorsal fin condition scores with 90% - 100% of fish scoring either 0 or 1. However, at day 53 (3 days prior to food withdrawal), there were significant differences in weight between tanks; control C3 = 18.4g ± 7.93g, C4 = 34.9g ± 8.24g, C7= 36.4g ± 8.2g; and treatment T2 = 27.3g ± 8.15g, T8 = 22.9g ± 8.42g, T9 = 33.2g ± 8.04g (mean ± SD).

2.3.1 Dorsal fin condition at Phase I final sample (day 69)

The percentage of fins scoring as good, medium or poor at the final sample of phase I (day 69) is shown in Figure 2-5. As over 90% of fish in all tanks had good fin condition at day0, fin condition has deteriorated in food withdrawal tanks (T2, T8, T9) as well as control tank C3.

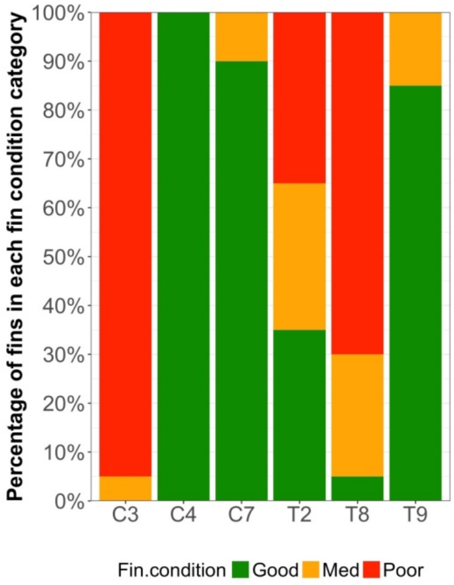

Figure 2-5 *Percentage of different categories of fin damage in each tank at day 69 (end of phase I) in treatment tanks which had a 3-day food withdrawal period (T2, T8, T9) compared with control tanks on the normal feeding regime (C3, C4, C7).*

2.3.2 Modelling the effect of food withdrawal on the dorsal fin (day 69)

The linear model predicted output is shown overlying the raw data for each of the tanks in Figure 2-6. Prior to food being withdrawn, fin condition in the control tanks C4 and C7 is good whereas C3 is very poor. However, fin condition in C3 improved between day 53 and day 69 (lower fin condition score means fins are in better condition). Fin condition in the treatment tanks T2 and T8 is medium whereas T9 is good. However, fin condition deteriorates between day 53 and day 69 in tanks T2 and T8 but not in T9. There was a statistically significant effect ($p = 0.028$) of treatment (food withdrawal) on fin condition with fish in food withdrawal group having higher levels of fin damage than the control group (Table 2-1). The height of the leading edge (HLE) of the dorsal fin was significantly shorter (-1.54mm, $p<0.01$) in the food withdrawal group than in the control group.

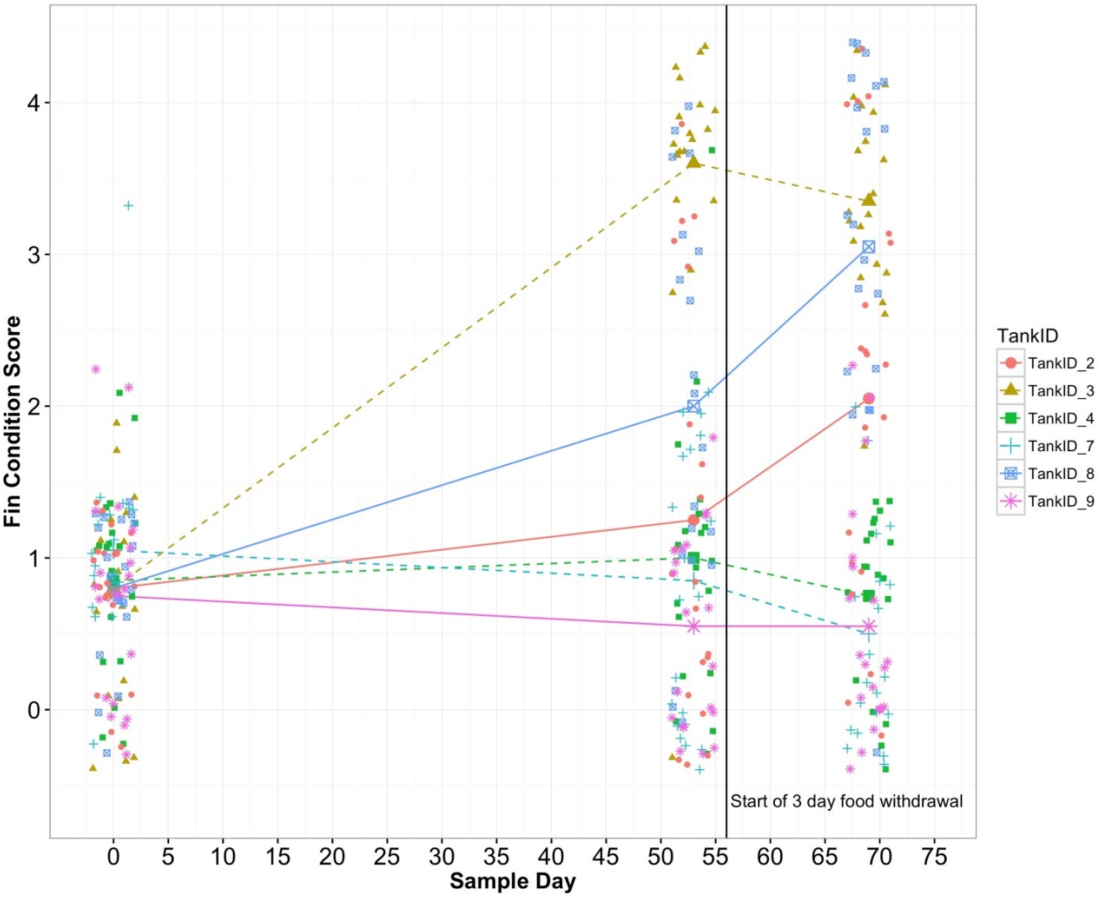

Figure 2-6 *Linear model predictions for control tanks (dashed lines) and treatment tanks (solid lines) overlaid on observed data points. Samples taken at day 0, day 53 (3-days prior to food withdrawal) and day 69 (10-days post food withdrawal). An increase in fin condition score means more fin damage. (Raw data points jittered slightly to aid visual interpretation).*

Table 2-1 *Phase I output coefficients for two-level linear models for the effect of treatment (food withdrawal or not) on weight, standard length, fork length, fin condition and height of the leading edge of the dorsal fin on the final sample (day 69). A -ve coefficient for the explanatory variable of treatment would indicate that the response variable was a lower value in the food withdrawal tanks than the control tanks and the converse for a +ve coefficient. A positive value for fin condition would indicate worsening fin condition.*

Response Variables	**Fin Condition (FC)**			**Height of leading edge of dorsal fin (HLE)**			**Weight (Wgt)**			**Standard Length (SL)**			**Fork Length (FL)**		
	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value
Explanatory Variables															
constant	1.533	0.113	**< 0.001**	14.12	0.36	**< 0.001**	37.77	1.059	**< 0.001**	134.37	0.99	**< 0.001**	142.79	1.056	**< 0.001**
Treatment	0.35	0.159	**0.028**	-1.54	0.51	**0.002**	-4.17	1.497	**0.005**	-2.9	1.4	**0.038**	-3.27	1.49	**0.028**
FL (Treatment)				0.112	0.017	**< 0.001**									
Wgt-(Treatment)										1.56	0.048	**< 0.001**	1.615	0.052	**< 0.001**
Covariables															
FC-day53	-2.525	1.263	**0.046**												
HLE-day53				0.918	0.115	**< 0.001**									
Wgt-day53	-0.56	0.192	**0.004**				0.986	0.115	**< 0.001**						
SL-day53										-0.105	0.058	0.07			
FL-day53							0.552	0.018	**< 0.001**				-0.1	0.06	0.096

2.3.3 Change in fin condition pre- and post-food withdrawal

There was a greater change in fin condition in the treatment (food withdrawal) group and this change resulted in worse fins compared with the control group. The means and 95% CI of the difference in fin condition between treatment and control tanks from the observed data is shown in Figure 2-7. It can be seen that there was a tendency for fin condition scores to reduce (lower score=better fin condition) between day 53 and day 69 in control tanks so that the difference is negative (-0.28 ± 1.4, mean ± SD) and increase in treatment tanks (0.62 ± 1.2, mean ± SD).

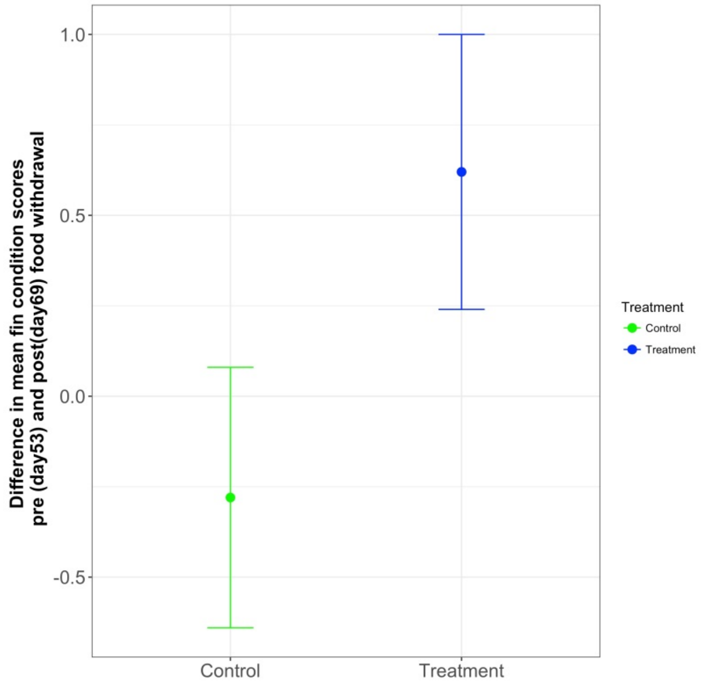

Figure 2-7 *The mean at day 69 (10 days post food withdrawal) was subtracted from the mean at day 53 (3 days prior to food withdrawal) to give the change in fin condition scores (n= 60) after a period of 3- day food withdrawal in treatment tanks (mean ± 95% CI). A higher score signifies more fin damage.*

2.3.4 Relationship between weight and fin condition

Weight was correlated with fin damage with smaller fish having more severe fin damage (Figure 2-8) which was more evident in control tanks ($R^2 = 0.539$) than treatment tanks ($R^2 = 0.163$).

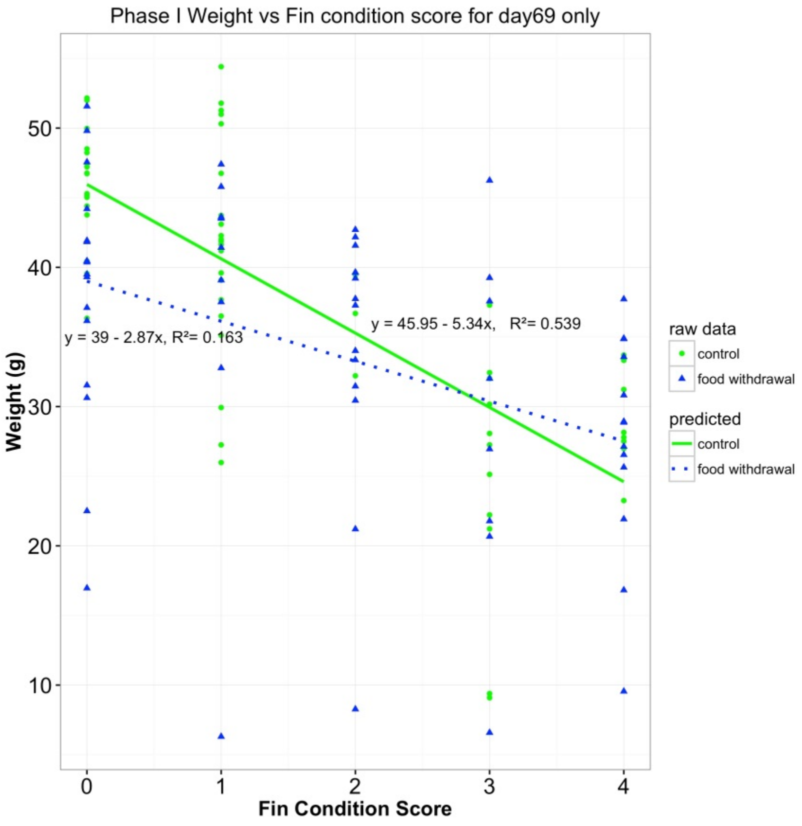

Figure 2-8 *Phase I weight vs fin condition score for sample 10 days after food withdrawal (day 69). Raw data is shown as individual points and fitted with a linear regression line for the control group and the treatment (food withdrawal) group.*

2.3.5 Modelling the effect of food withdrawal on growth parameters (day 69)

There was a statically significant difference in weight ($p < 0.01$) between control and treatment tanks at the final sample in Phase I (day 69), see Table 2-1. Fish in control tanks were on average 4.17g heavier than fish in food restricted tanks. There was also a statistically significant difference in fish length (FL, $p = 0.038$ and SL, $p = 0.028$) where the fork length of fish in control tanks were on average 3.27 mm longer than fish in the food restricted tanks. Table 2-1 lists the model output coefficients. A positive coefficient indicates that the value of that variable was higher in food restricted tanks compared with the control tanks and a negative coefficient would indicate the converse. Figure 2-9 plots the difference in weight of observed values at day 69 compared with day 53 for control and treatment tanks.

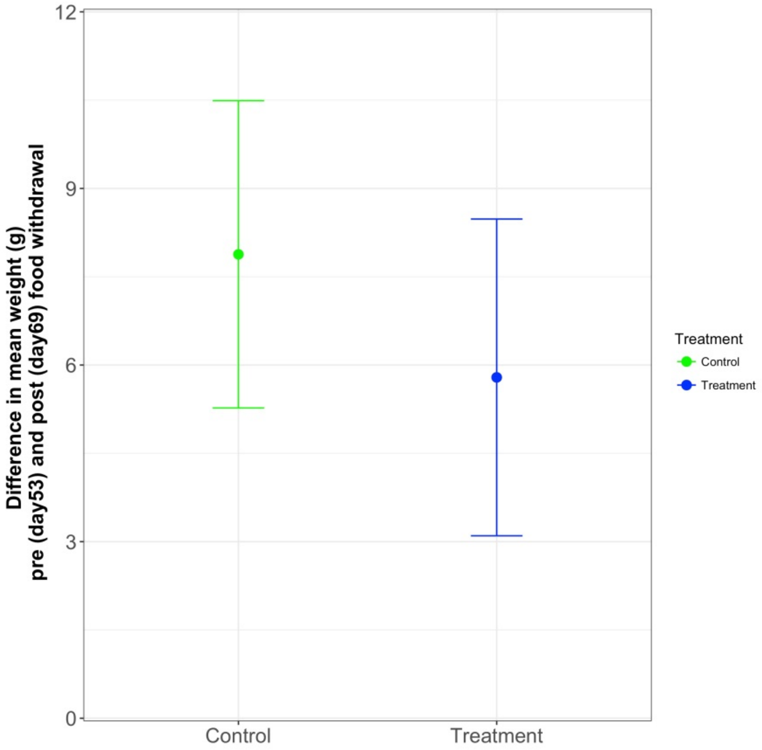

Figure 2-9 *The mean at day 69 (10 days post food withdrawal) was subtracted from the mean at day 53 (3 days prior to food withdrawal) to give the difference in weight (n= 60) after a period of 3- day food withdrawal in treatment tanks (mean ± 95% CI).*

2.3.6 Experimental Phase II

At the end of the experiment all remaining fish were euthanised and measurements recorded (Table 2-2).

Table 2-2 Phase II *measurements (mean ± s.d.) at final sample (day 124) of treatment tanks (T3, T4, T7) and control groups (C2, C8, C9).*

	Tank ID	
	Treatment	Control
Weight (g)	66.6 ± 17.3	62 ± 14.8
FL (mm)	178.3 ± 19.1	175.9 ± 16.7
SL (mm)	168.5 ± 18.4	165.8 ± 16.4
HLE (mm)	16.8 ± 6.5	16 ± 6.31
Fin Score	1.5 ± 0.84	1.8 ± 0.84

2.3.7 Modelling the effects of food withdrawal on fin condition and growth

Table 2-3 shows the results of the modelling of the effects of food withdrawal on treatment and control tanks between day 69 and the final sample at day 124. There was no significant difference in Fin Condition score or HLE between treatment tanks (T3, T4, T7) and control tanks (C2, C8, C9) therefore the condition of the dorsal fin was similar in each group. Fish in the treatment group were significantly larger and longer than those in the control group. On average fish were 4.6g heavier and 2.5mm longer in fork length in the treatment group than the control group.

2.3.8 The effect of staged food withdrawal across time in tanks 3, 4 and 7

The effect of staging food withdrawal across time in each of the treatment tanks is shown in Figure 2-10. There was a significant interaction of sample day x tank for each of the outcome measures. The staged effect of treatment is evident in tanks 3 and 7 (the second and third tanks to have the treatment applied at day 91 in tank 3 and day 98 in tank 7). The curves show that the effect of food withdrawal is not noticeable at the time of next sampling (day 104 in tank 3 and 116 in tank 7) as the curves continue on the same trajectory up the curve but is measurable by the next sampling point at the end of the study (day 124). The curves for tank 4 behave differently in that the effect of the treatment is not evident at the next sample (day 96) nor at the end of the study (day 124). This result is interesting in that it appears that the temporal effect of treatment can be picked up in the analysis and provides some useful information about the time response to feed withdrawal. However, caution should be applied in interpreting the data as there is only one tank per sample.

Table 2-3 *Phase II output coefficients for 2-level linear models. Control tanks (T3, T4, T7) from Phase I were subjected to food withdrawal (treatment). Response variables were measured at day 124 (final sample). A -ve coefficient for the explanatory variable of treatment would indicate that the response variable was a lower value in the food withdrawal tanks than the control tanks and the converse for a +ve coefficient. A positive value for fin condition would indicate worsening fin condition.*

Response Variables	**Fin Condition (FC)**			**Height of leading edge of dorsal fin (HLE)**			**Weight (Wgt)**			**Standard Length (SL)**			**Fork Length (FL)**		
	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value	coeff	S.E.	p-value
Explanatory Variables															
constant	1.8	0.146	**< 0.001**	16	0.302	**< 0.001**	62.04	0.448	**< 0.001**	165.82	0.512	**< 0.001**	175.79	0.597	**< 0.001**
Treatment	-0.328	0.207	**0.114**	0.832	0.427	**0.052**	4.561	0.634	**< 0.001**	2.71	0.724	**< 0.001**	2.485	0.844	**0.003**
FL				0.114	0.014	**< 0.001**									
Wgt										0.965	0.022	**< 0.001**	0.986	0.022	**< 0.001**
Covariables															
FC-day69	0.316	0.136	**0.02**												
HLE-day69				0.819	0.059	**< 0.001**									
Wgt-day69	0.011	0.027	**0.679**				-0.126	0.061	**0.039**						
SL-day69										0.184	0.043	**< 0.001**			
FL-day69							0.86	0.019	**< 0.001**				0.206	0.047	**< 0.001**

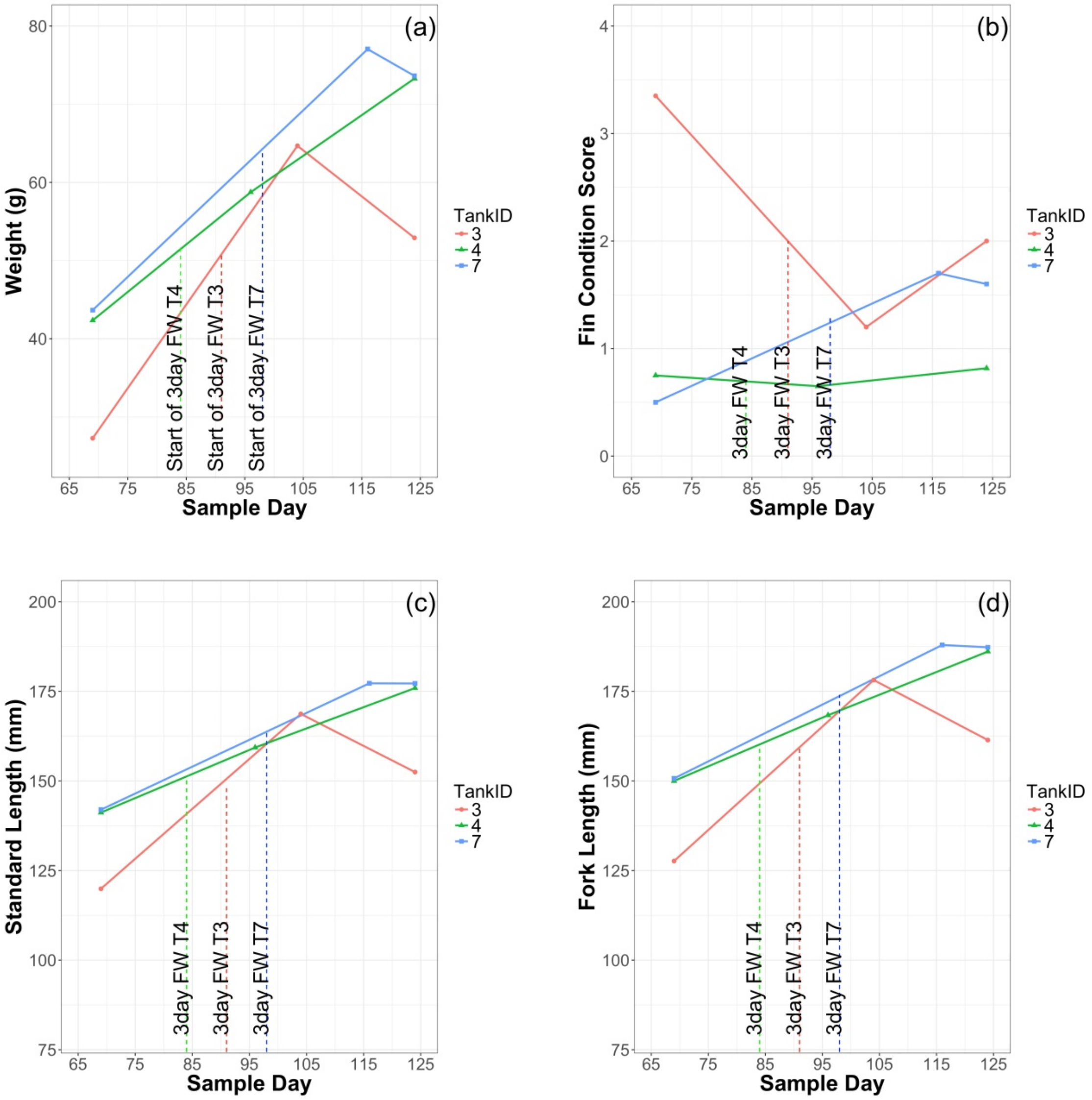

Figure 2-10 *Model of Phase II treatment tanks for weight (a), fin condition (b), SL (c) and FL (d). Food withdrawal (FW) was staggered across time for each tank (n=3). All tanks were sampled at the start (day69) and end (day124). A sample was taken from individual tanks 10 days after FW (T3 at day 104, T4 at day 96 and T7 at day 116). The effect of food withdrawal is not significant at the sample point immediately post food withdrawal although there is a noticeable effect at the final sample point in tanks 3 and 7 but not in tank 4*

2.4 Discussion

Periods of food deprivation appears to have had a negative effect on growth and be a primary initiating factor in the development of fin damage, in phase I. Fin damage was more severe and fins shorter in tanks that had been food deprived. Growth was also reduced in food deprived tanks as fish in control tanks were heavier and longer. At the end of phase I, food deprived tanks were reassigned as control tanks and vice versa for phase II. Food withdrawal was staged across time in the newly assigned treatment tanks. While fin damage was evidently associated with food deprivation in the first phase of the experiment it was not so obvious in phase II. At the end of phase II, fin condition was similar in treatment and control tanks. However, fish were on average larger and heavier in the treatment group. Fish were potentially further along in their development during phase II and could feasibly be more advanced towards smolting; a physiological change necessary for salmon to move from freshwater to salt water and which also involves behavioural changes such as less territorial aggression (Mork et al., 1989). However, caution should be applied to the interpretation of the data in phase II as there was only one tank per sample time.

In this present study there was some indication that smaller fish had more severe fin damage which would be consistent with other studies (Abott & Dill, 1989; Damsgård et al., 1997; Moutou et al., 1998; Symons, 1968). In contrast, McLean et al. (2000) found that fin damage was more prevalent in larger fish when kept in big groups, although the food ration was not manipulated in that study. Size heterogeneity can have an effect on the level of aggression, with the presence of larger fish reducing overall aggression and promoting better growth among smaller fish. However, removing larger fish can disrupt stable social hierarchies leading to more aggression (Adams et al., 2000). This is a potential mechanism for the increase in fin damage after fish were re-distributed to re-populate tank 8 prior to the food withdrawal event.

The size of fish in the treatment tanks at the end of phase I was significantly smaller than fish in the control tanks. This size discrepancy remained through to the final sample at the end of phase II, despite the control and treatment tanks being swapped over. This again could be due to the small sample size in phase II or perhaps due to compensatory growth, which is a widespread phenomenon in the animal kingdom, seen in birds and mammals (Wilson & Osbourn, 1960; Lawrence & Fowler, 1997). When fish resume feeding after a period of fasting they can become hyperphagic (Jobling & Koskela, 1996; Nikki et al., 2004) leading to high rates of growth. Feeding hierarchies break down when food becomes more available (Jobling & Koskela, 1996) resulting in previously suppressed fish gaining access to food culminating in rapid growth. There is some support for this theory as after fish were re-distributed the average weight in tank 3 decreased, signifying the non-random removal of the largest fish. This would have allowed the previously suppressed smaller fish easier access to food and as a result tank 3 had twice the growth spurt after the fish were re-distributed, compared to the other tanks. As the food withdrawal tanks in phase II had a longer feeding period between resumption of food and the final sample the hyperphagic phase may have been extended compared to phase I. In theory having the capacity to compensate for growth loss during fasting should allow the fasted group to catch up to the un-fasted group however social factors may prevent full access to food suppressing any growth spurt (Maclean et al., 2001; Nikki et al., 2004). Another unknown is what the effect of smolting would be in this case. Overall, fish in the treatment tanks at the start of phase II were larger than controls therefore likely to reach smolting status earlier.

Aggression is often proposed as the motivation for fin biting by conspecifics. If the function of aggression is to displace other fish from feeding territories (Keenleyside & Yamamoto, 1962; Symons, 1968) then charging or nipping should cease once the interloper has moved on. To achieve the level of fin damage apparent after fasting would require that certain individuals are continually targeted or are continually straying into defended areas. It is possible that in tanks of comparable production densities there are a number of mini-territories being defended that the hapless subordinate fish is continually straying into as it navigates the tank.

A significant portion of a fishes' time budget is spent in relation to food, so the main question is what motivates fishes to fin biting when food is removed. Previous studies have noted an increase in aggression (although fin damage was not measured) when expected rewards were not forthcoming (Vindas et al., 2012). This would imply that it is not simply aggression that lies at the core of fin biting behaviour but that the behaviour is similar to abnormal behaviours seen in terrestrial animals, such as feather-pecking in laying hens (Blokhuis & Arkles, 1984) or tail-biting in pigs (Brunberg et al., 2011). Studies of abnormal behaviour on intensively farmed terrestrial animals may provide other methodologies that could be adapted to investigate the motivation for fin biting in fish.

The ability to consistently instigate fin biting by fasting the fish will allow studies to be designed to investigate other motivations to bite fins such as boredom, frustration, hunger, re-directed foraging or whether related to genetics. An understanding of the underlying motivations would help to identify methods to possibly reduce the occurrence of fin damage at the farm level. In the meantime, an achievable management technique to reduce fin damage would be for farmers to reduce fasting time.

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3 Comparison of invasive and non-invasive methods to assess fin damage in farmed Atlantic salmon (*Salmo salar*)

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Preface

This chapter compares invasive and non-invasive methods for assessing fin damage used in the experiment detailed in chapter 2. Sampling methods to measure fin damage normally require removing fish from the water which is a major stressor for fish. Non-invasive measurements would be a major improvement for fish welfare. This study was part of a Defra-funded project AW1209 to study fin damage that was in progress when I started my PhD and my co-authors carried out the initial experimental design, as discussed in Chapter 2. Continually monitoring fish over a long period of time generates a vast amount of video data requiring specialist equipment and proprietary software which was designed and built for the project by Dr. Jeff Lines and Ronan Turner of Silsoe Research Ltd. My contribution to the project consisted of processing all the video data from the duration of the experiment using the proprietary software, deciding upon and undertaking the statistical analysis and preparation of this manuscript, with edits from my supervisors. This chapter is designed for publication in the journal Aquaculture hence there is some repetition in the methods section with Chapter 2.

3 Comparison of invasive and non-invasive methods to assess fin damage in farmed Atlantic salmon (*Salmo salar*)

Abstract

Fin damage in farmed salmonids is an important topic from both a welfare and economic perspective. A number of different assessment methods to quantify fin damage have been proposed either describing the state of the fin or attempting to quantify the reduction in surface area due to fin damage. Assessment normally requires removing fish from the water, a stressful and invasive procedure for fish and time consuming. This study compared the assessment of fin damage using invasive and non-invasive techniques used during an experiment of food withdrawal on fin damage. The invasive procedure required twenty fish to be removed from each tank and euthanised at each sample point. Each fish was given a fin condition score (5-point scale) and photographed for future morphometric measurements using image analysis software. Underwater video cameras were used to monitor fish non-invasively and recordings stored. The underwater cameras were fitted with a laser calibration to facilitate extracting measurements from the fish, using proprietary software written for the project. Fish length versus dorsal fin base length measurements differed between methods by < 14% for the smallest fish and < 2% for larger fish and followed the same slope and growth curve. This level of difference is acceptable in measuring fin damage. Growth of the leading edge of the dorsal fin (HLE) was isometric with standard length (SL) and used to calculate a Fin Index ((HLE/SL)*100). Fin Index measurements were able to discriminate between the different 5-point fin condition score, except between ‘very good’ and ‘good’ fins. A Fin Index was calculated for each tank, using data from photographs and video recordings, and showed no significant difference between methods in four out five tanks. The findings from this study would indicate that measurement of fin damage using non-invasive video monitoring is comparable with those using invasive methods. Non-invasive video monitoring has the potential to be an efficient, welfare-friendly method for assessing on-farm fin damage over time and at sites which are difficult to access such as open water pens.

3.1 Introduction

Fin damage is common in intensively farmed fish, less so in wild populations (Hansen et al., 1987) and is considered an important topic from both a welfare and economic perspective. Fin damage is an injury to live tissue, potentially causing pain (Sneddon et al., 2003; Braithwaite & Huntingford 2004; Chandroo et al., 2004) and increased susceptibility to disease (Ellis et al., 2008) and has been identified as a useful welfare indicator. Damaged fins can potentially reduce the value of the fish and are an indication of problems in rearing conditions, reflecting poorly on the rearing environment (St-Hilaire et al., 2006). Fin damage can appear as frayed rays, tissue loss, pale nodular thickening of the distal portion of the fin and necrosis (Turnbull et al., 1996; Winfree et al., 1998; Latremouille, 2003).

It has been identified that biting by conspecifics (Abbott & Dill, 1985; Turnbull et al., 1996) is a major cause of fin damage, with the dorsal fin most likely to receive damage in Atlantic salmon (Turnbull et al., 1998; Pelis & McCormick, 2003) and trout (Bosakowski & Wagner, 1994; Hoyle et al., 2007). Although risk factors triggering this behaviour have been difficult to identify, inappropriate feeding regimes have led to an increase in fin damage (Ellis et al., 2008; Latremouille, 2003; Ryer & Olla, 1996; Winfree et al., 1998; Cañon-Jones et al., 2012) and restricting food appears to play a major role (Moutou et al., 1998; Noble et al., 2007a, 2007b; Cañon-Jones et al., 2017).

A number of different assessment methods to quantify fin damage have been proposed and used by different researchers, quality and welfare schemes, either describing the state of the fin or attempting to quantify the reduction in surface area due to fin damage (Latremouille, 2003). Indices that describe fin condition include: (i) the Health Condition Profile fin condition Index (Goede & Barton, 1990) (ii) dorsal fin damage on a 7-point scale (Turnbull et al., 1996) (iii) a 3-point scale (mild, absent or severe) to describe fraying and splitting as well as thickening of the dorsal fin (MacLean et al., 2000) (iv) the frequency of fin fraying/splitting within a population (Bosakowski & Wagner, 1994) or (v) the absence/presence of fin damage (Suzuki et al., 2008). Various indices attempt to quantify the amount of tissue loss and reduce subjectivity: (vi) a 3-point scale (Bosakowski &

Wagner, 1994), a 4-point scale (Moutou et al., 1998) and a 5-point scale (MacLean et al., 2000) (vii) fin length expressed as a percentage of total fish length (Kindschi, 1987) (viii) fin length expressed as a percentage of standard fish length and correcting for allometric fin growth (Ellis et al., 2009) (ix) using digital photography and image analysis techniques to quantify fin area (North et al., 2006).

Measuring fins is time consuming and difficult, especially on farms and in open water pens. Also, the proliferation of the different assessment schemes makes comparison between different studies difficult. Examination of the fins to measure and score condition or take photographs for later measuring requires handling the fish, a stressful and invasive procedure. Quite often the samples of fish taken are killed, prior to measuring and photographing. A non-invasive and practical method of quantifying fin damage would reduce the need to kill or sedate fish, which could potentially lead to an increase in sample sizes. This could lead to a better uptake among the different stakeholders researching and monitoring fish welfare.

The aim of this study was to compare the results of invasive and non-invasive quantification of dorsal fin damage in Atlantic salmon parr (*Salmo salar*), by measuring key morphometrics from digital still photography (invasive method) with that obtained non-invasively by underwater videos.

3.2 Materials and Methods

3.2.1 Animals and experimental set up

This study was conducted between July and November 2015 at the Niall Bromage Freshwater Research Unit at Buckieburn, central Scotland. Ethical approval was obtained from the University of Stirling's Animal Welfare and Ethical Review Body (IoA AWERB April 15 interim ASPA 04.docx).

Fish used in the study were Atlantic salmon (*Salmo salar*) of the '*Salmobreed*' strain, sourced commercially from Howietoun Fishery, Stirling (UK). Fish were housed in 3000 litre, dark green fibreglass circular tanks (1m high x 2m diameter) filled to a depth of 0.7m (2000 litre) covered with an opaque fiberglass lid that could be half opened with a hinge. Tanks were gravity-fed with water from a local freshwater reservoir, into a flow-through system.

This methodology was developed for an intervention experiment, designed to test the effect of food withdrawal on fin damage (Chapter 2) which comprised of six experimental groups; three control tanks and three treatment tanks, with 415 fish in each tank at first sample. Tanks were randomly assigned to control (Tanks 3, 4, 7) or treatment (Tanks 2, 8, 9). During the food withdrawal experiment, after a period of acclimatisation, food was withdrawn for three days in the three treatment tanks whereas the normal feeding regime continued in the control tanks. Normal feeding was resumed in all tanks, after the three-day food withdrawal, until the end of the experiment. This part of the experiment ran from 3rd July 2015 (day 0) until 21st September 2015 (day 80), with the start of a three-day food withdrawal on the 28th August 2015 (day 56).

3.2.2 Quantification of Fin Damage

Fin damage was examined using two methods:

a) Photographic Samples: the fish were schedule 1 killed and photographed (section 3.2.3). Morphometric measurements and a fin damage score were recorded (section 3.2.4).

b) Video Samples: measurements were taken from footage, collected non-invasively, from underwater video recordings of live fish (section 3.2.5).

3.2.3 Photographic Samples - Post mortem measurement of fin damage

3.2.3.1 Sampling procedure and physical measurements

Twenty fish were netted from each of the six tanks (n=120) at the start of the experiment (day 0), three days prior to food withdrawal (day 53) and fourteen days after food resumed (day 69). Fish were killed using an overdose of MS222 and observation to confirm lack of recovery. Fish were weighed and photographed. A camera (Pentax K-30 SRL digital camera) was mounted on a vertical stand over a board with a ruler for calibration purposes (Figure 3-1).

Figure 3-1 *Photographic setup*

Measurements were made from the digital photographs using ImageJ software (Schneider et al., 2012). The measurements taken were fork length (FL) measured from snout to fork; standard length (SL) measured from snout to caudal peduncle; height of the leading edge of the dorsal fin (HLE) and the dorsal fin base length (DFBL) (Figure 3-2).

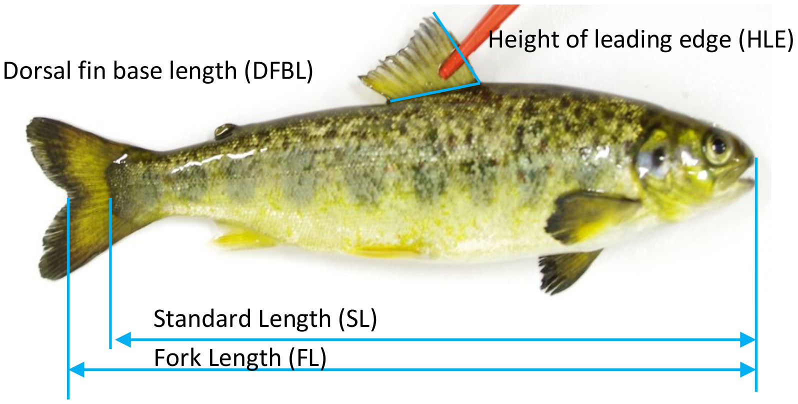

Figure 3-2 *Physical measurements recorded from digital image*

3.2.4 Fin condition measurements

A fin damage score was recorded for each sampled fish. Damage to the dorsal fin was categorised on a 5-point ordinal scale adapted from Maclean et. al. (2000): 0 (0-10% loss, good condition), 1 (10-20% loss, frayed or mild damage), 2 (20-30% loss, moderate damage), 3 (30-60% loss, severe damage), 4 (> 60% losses, very severe damage/no fin), see Figure 3-3. Inter-rater validity was assessed between two observers using samples from three random tanks (n=60). Weighted kappa analysis showed substantial agreement between observers (0.78) which is in line with that previously found with photographic keys in assessing fin damage (Hoyle et al., 2007). Scoring for all samples used in the following analysis was done by the author using the photographic key (Figure 3-.3)

Figure 3-3 *Dorsal fin condition score from 0 (perfect fin) to 4 (severe damage)*

3.2.5 Video measurement of fin damage

Video cameras (Go-Pro Hero 4©) were used to monitor fish behaviour and to non-invasively quantify fin damage, without removing fish from the water. Each of the six experimental tanks were fitted with two cameras; one mounted at the water surface providing a "birds eye view" of fish behaviour in a small section of the tank and one mounted underwater with a horizontal view of the fish to monitor fin condition (Figure 3-4 and 3-5). See below for description of tank lighting, laser calibrator and pods.

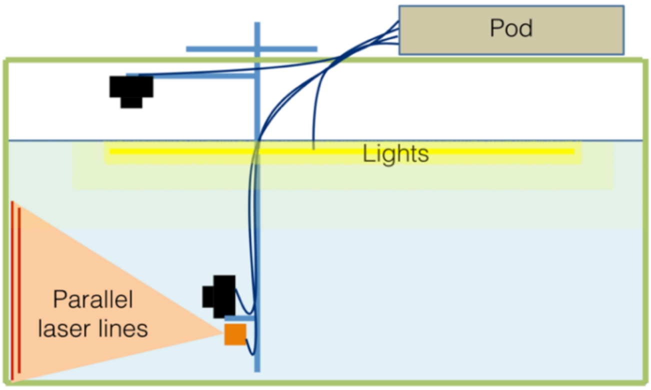

Figure 3-4 *Diagram of the arrangement within a single tank*

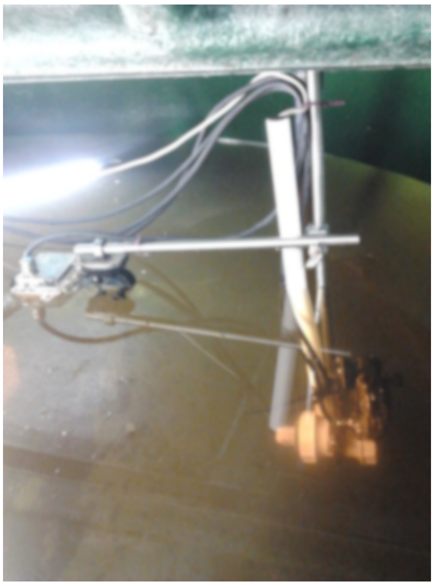

Figure 3-5 *Image of Go Pro cameras mounted in a tank. (Photo courtesy of Sonia Rey)*

Two fixed lights mounted on the lid and two fluorescent tubes, mounted cross-wise on a PVC base floating on the water surface (Figure 3-6), produced a light intensity of 0.6 W/m^2 at the surface and 0.2 W/m^2 at the bottom of the tank. Tanks were kept on a 24-hr photoperiod regime for the duration of the experiment, to avoid smoltification of the stock and to provide enough light for video recording.

Figure 3-6 *Fluorescent lights floating on the surface of the water, in the tank. Two further lights attached to tank lid (Photo courtesy of Sonia Rey)*

Parallel laser lights, 2.6 cm ± 0.15 apart, were projected onto the side wall of the tank as a calibration source while these images were being collected to allow linear measurements to be extracted from the video recordings (Figure 3-7). Only the underwater camera was used for fin damage analysis as the purpose of the top view camera was for behavioural analysis and did not provide a good view of the dorsal fin to monitor fin damage.

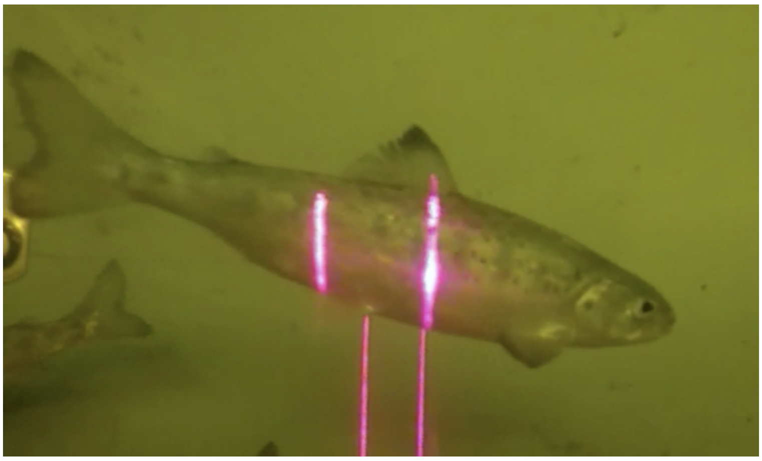

Figure 3-7 *Laser lines projected onto tanks sidewall to allow calibration of measurements*

A scan sampling regime was used to collect short (4 secs) high-speed underwater sequences four times per hour, for 24 hours a day for a period of several months (July-November 2015).

Instrumentation pods (Figure 3-8) were specially built for the project to control; record and store data; with one pod handling two tanks. Each pod was provided with mains power and controlled the cameras, laser module and lighting. A Raspberry Pi ® (mini-computer board) provided USB interfaces to wireless adaptors and a USB flash-drive (Figure 3-9).

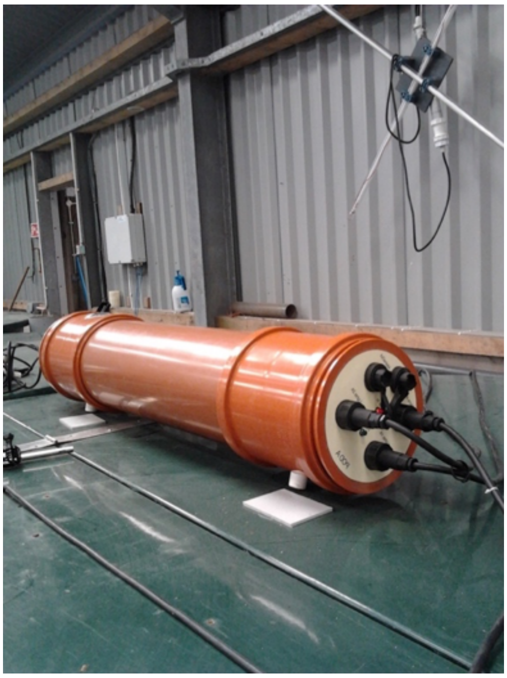

Figure 3-8 *Instrumentation pods, mounted on the lid of a tank, each pod controlled two tanks*

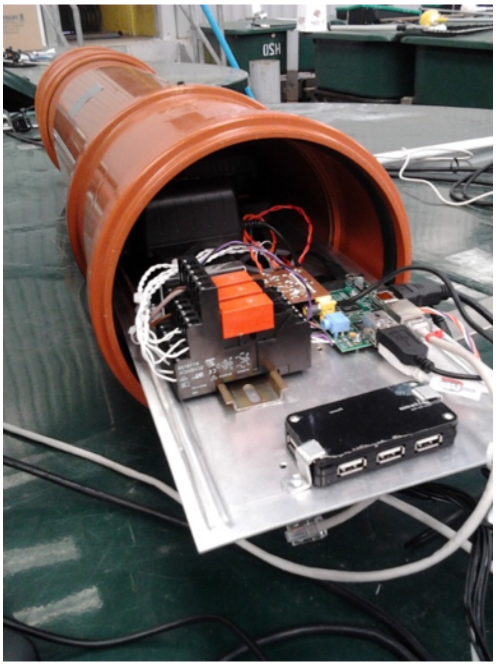

Figure 3-9 *Raspberry pi and associated electronics for controlling cameras, lighting, laser module and storing data*

One wireless adaptor communicated with the surface cameras; a further two adaptors communicated with underwater wireless adaptors mounted near the underwater cameras. The USB flash-drive was removed weekly and the data transferred to external storage. Watchdog circuitry forced a reboot of the Raspberry Pi if no signal was detected within a pre-set time, thereby minimising any data loss.

3.2.6 Video analysis software

Due to the large amount of video data generated, proprietary software was written (external contractor) to facilitate the processing and analysing of the video data. The software used the Python programming language V2.7 (Python Software Foundation, https://www.python.org/) making extensive use of libraries OpenCV, NumPy, Tkinter and PIL. Three software programs were written called Waster Mover, Fins Filter and Fins Player (Table 3-1). Installation instructions and user guides can be found at link http://www.fins.stir.ac.uk/nav-hwsetup.html.

Table 3-1 *Software programs written to facilitate processing videos recorded in tanks*

Software Program	**Description**
Waste Mover	Filtering program to remove defective files i.e. files < 1MB)
Fins Filter	Program to pre-select videos for future analysis (i.e. discard videos with no visible fish). View four frames from video then select to keep video for future analysis or move rejected videos to a sub-directory.
Fins Player	Program that displays randomly selected videos in a directory. Video can be viewed, frames skipped, jumped and paused. When paused the following measurements can be recorded: Standard length (SL) Dorsal fin base length (DFBL) Dorsal fin height of the leading edge (HLE) Calibration laser line width

Fins Player is the program which was used to take measurements from fish (Table 3-1). The laser line width was measured in each frame to act a reference calibration. The software uses the laser calibration line measurement to calculate SL, DFBL and HLE. Measurements were made by selecting left and right endpoints, by left-clicking/right-clicking a mouse, on the area to be measured. A red line is drawn between the two points to highlight the measurement and hitting <RETURN> stores the measurements to a text file along with the video file name, which is not visible on the screen when viewing the videos. Multiple fish can be measured in each frame provided they are covered by the laser calibration lines. The programme did not have the option of recording a fin condition score and as the video is randomly selected and the filename is not visible during viewing a fin condition score could not be retrospectively added to the text file.

3.2.7 Data analysis

Initial data analysis assessed the strength of the relationships between standard length (SL) and dorsal fin base length (DFBL) as well as between SL and height of the leading edge (HLE) of the dorsal fin. As data were not normally distributed Spearman rank correlation coefficient was used to test the degree to which the two variables are related. Tests were performed on data from photographed samples, presumed to be the standard to compare the video samples against.

SL and DFBL were chosen as variables to compare the two measurement systems (photographic or video) as they are not subjected to fin damage. A one-way analysis of covariance (ANCOVA) was performed on DFBL measurements to evaluate the effect of the source (photos or videos) on the images being measured. SL was used as the covariate as the length of the dorsal fin base (DFBL) is dependent on fish size. Model residuals were checked for normality and homoscedasticity by visual inspection of residual plots. The assumption of homogeneity of regression slope was examined by testing the interaction effect of the independent variable (source) on the covariate (SL).

To assess fin damage between the two measurement systems the percentage fin loss was quantified as a Fin Index based on the method originally proposed by Kindschi (1987). This method involves measuring the longest part of the remaining fin and standardising for the size of the fish by expressing as a percentage of the total length, to calculate 'relative fin length' (i.e. (fin length/total length)*100). However, as the caudal fin can also be affected by fin damage, reducing overall length, a refinement to the original equation was proposed by Ellis et. al. (2009) to use standard length instead of total length. For this study on dorsal fin damage, Fin Index (%) = (HLE/SL)*100 was used. Fin Index inherently assumes that relative fin length is a constant proportion of body length i.e. fin growth is isometric. To assess whether fin growth is isometric or allometric, the method of Major axis (MA) regression of $\log_{10}$ transformed data is recommended (Warton et al., 2006; Ellis et al., 2009). MA regression is useful where the aim is how one variable scales against another (i.e. slope of the regression line) rather than being able to predict one value from another, where OLS (ordinary least square) regression would be more appropriate. MA regression and OLS regression differ in how the regression line is estimated. MA regression minimises residuals perpendicular to the regression line whereas OLS regression minimises residuals in the direction of the Y-axis. To test for isometry, where fins would be a constant proportion of body length, the slope of the MA regression line should not be significantly different from 1 (one variable is directly proportional to another because data have been log-transformed). A value < 1 indicates negative allometry i.e. the fin was relatively shorter in larger fish. A value > 1 indicates positive allometry, i.e. the fin was relatively longer in larger fish (Ellis et al., 2009). MA regression lines were fitted for $\log_{10}$ DFBL against $\log_{10}$ SL and $\log_{10}$ HLE against $\log_{10}$ SL. The MA regression and tests of slope were obtained using the SMATR package in R (Warton et al., 2012).

Fin Index was used to compare the extent of fin damage, in each tank, between the two measurement systems. A Mann-Whitney test was used as data were not normally distributed. Fin Index was also used to compare levels of the ‘subjective’ fin condition scores (Figure 3-3). A one-way ANOVA was conducted with post-hoc comparisons using the Tukey HSD test. Model residuals were checked for normality and homoscedasticity by visual inspection of residual plots. Fish weight was only available for photographic samples therefore this data was used to calculate the fish length-weight equation

$$W = aL^b$$

Where W is weight (g), L is total length (cm) and factors a (intercept) and b (slope) were estimated using OLS regression through log transformation $\log W = \log(a) + b*\log(L)$. When the value of the slope (b) = 3, growth is isometric. When $b \neq 3$ weight growth is allometric, which can be positive ($b > 3$) where fish are heavier relative to their length or negative ($b < 3$) where fish are lighter relative to their length (Wootton, 1998). In this study total length is replaced by standard length as standard length is the only measurement available for video samples. The resulting equation was then used to estimate fish weight for video samples. All data analysis was carried out using R software (version 3.5.3) (R Development Core Team, 2019).

3.3 Results

3.3.1 Photographic Measurements

3.3.1.1 Growth parameters

Over the time period the mean weight was 24.5g (5g to 54 g range), mean SL was 114mm (69mm to 167mm range). The weight-length relationship is shown in Figure 3-10. For this sample, growth is negatively allometric (b=2.7) and within the expected range for fish 2.5 < b > 3.5 (Froese, 2006).

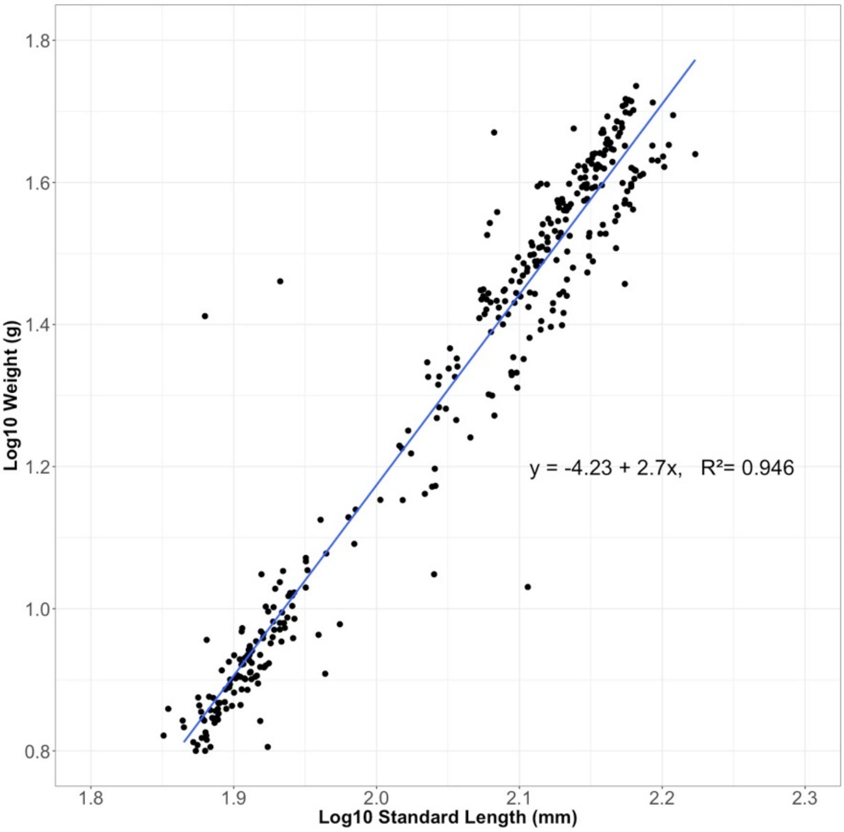

***Figure 3-10** Log transformed weight versus standard length for photographic samples from day 0 to day 69 (n=360).*

Major axis (MA) regression lines and OLS regression lines are plotted for DFBL against SL (Figure 3-11) and HLE against SL (Figure 3-12), to check whether growth is isometric (Warton et al., 2006). The data plotted in Figure 3-12 are HLE data for samples where fin condition was scored as 0 (very good condition i.e. no fin damage). DFBL and SL are positively linearly related (rho=0.97) as is HLE(0) and SL (rho=0.94). Test of slope shows that DFBL/SL is positively allometric with slope=1.04 (CI 1.02-1.06), $p < 0.001$ and HLE(0)/SL is isometric with slope=0.978 (CI 0.93-1.03), p=0.398. Therefore, for this sample, the assumption of isometric growth for Fin Index (HLE/SL *100) is met. Although the slope test shows slight allometric growth, the difference in predicting measurement of DFBL over the range of SL (69mm to 167mm range) using the MA regression equation (-0.93 + 1.04*SL) or OLS equation (-0.86 + 1.01*SL) is less than 2%, for this size range of fish. This result indicates that using DFBL against SL to compare photographed samples and video samples (see section 3.3.3) is valid.

3.3.1.2 Fin Index and Fin Condition Score

A Fin Index was calculated from the physical measurements (day 0 to day 69, n=360) and compared with the dorsal fin condition score for each of the fish (Table 3-2 and Figure 3-3).

Table 3-2 *Fin condition score versus Fin Index*

Fin Score	Fin Index	±S.D.
0	12.6	1.01
1	12.2	1.38
2	9.8	1.79
3	7.2	1.47
4	5.2	1.98

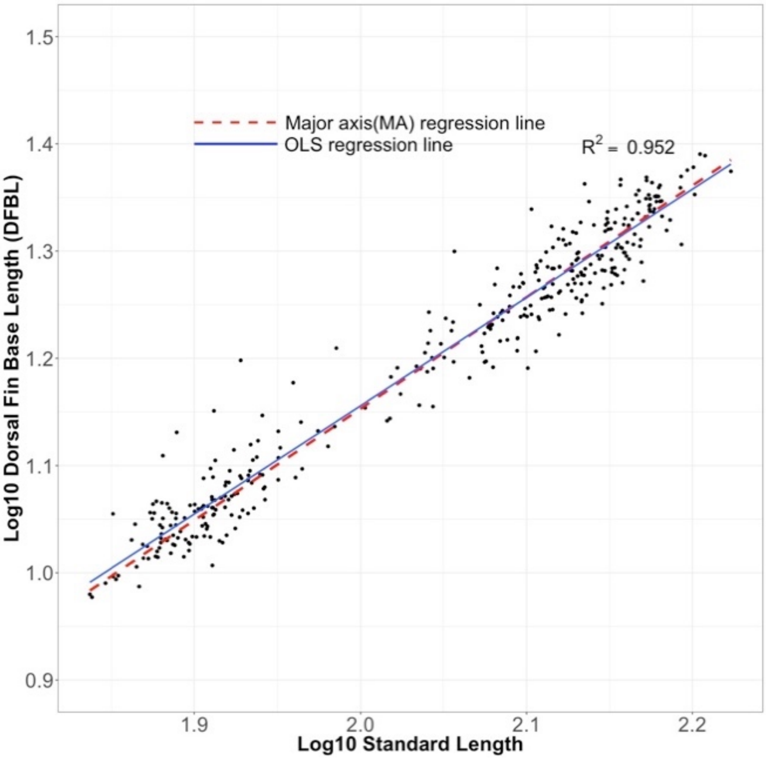

Figure 3-11 *Linear regression (OLS, solid line) and Major Axis (MA, dashed line) regression plots of* $\log_{10}$ *DFBL versus* $\log_{10}$ *standard body length for Atlantic salmon (n=360), from photographic measurements.*

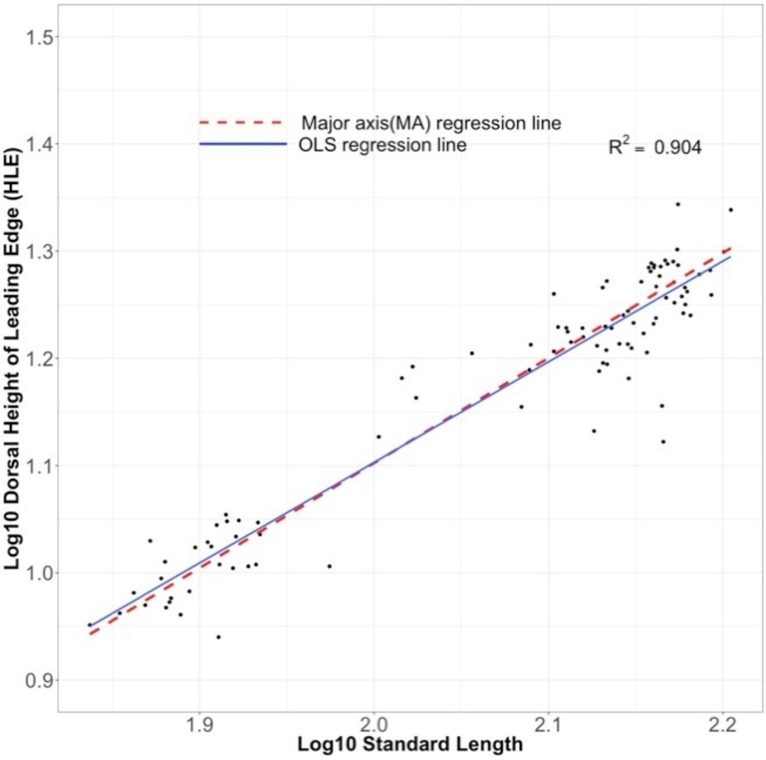

Figure 3-12 *Linear regression (OLS, solid line) and Major Axis(MA, dashed line) regression plots of* $\log_{10}$ *HLE versus* $\log_{10}$ *standard body length for Atlantic salmon (n=96), from photographic measurements condition was scored as 0 (very good condition). The* R^2 *value is the same for OLS and MA regression line.*

There was a significant effect of fin condition score on FinIndex, F=280.2, df=4, p < 0.001. Post hoc comparisons using the Tukey HSD test indicated that the mean Fin Index at fin condition score level 0 was not significantly different from level 1. However, there were significant differences between each of the other levels (Figure 3-13). Fin Index decreases with increasing severity of fin damage and are comparable with the fin condition scoring system (Figure 3-13).

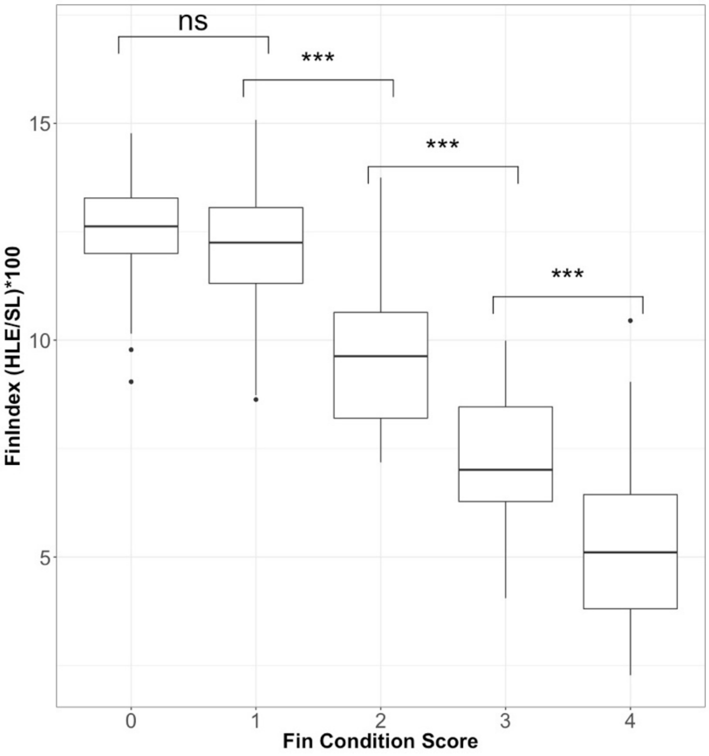

Figure 3-13 Fin Index calculated for each of the Fin Condition (FC) Scores where very good condition = 0, n=96; FC1, n= 153; FC2, n=37; FC3, n=32; up to severe fin damage FC4, n=42. Significance levels indicate non-significant (ns) difference between levels (p > 0.05) and highly significant differences (***, p <0.001). (Box plot shows median*, first and third quartile)*

3.3.2 Video samples

The number of fish available to be measured on the videos was greatly reduced from that expected, due to fish not being visible in front of the camera or lighting too dark to be able to discriminate fish from the background. In total only n=123 fish were measured from the videos. As fish weight could not be measured in the videos, it was calculated using the regression line equation generated for photographic measurements (see Figure 3-10). Over the time period the mean weight was 32g (2.4g-115g range); mean SL was 126mm (51mm to 215mm range).

3.3.3 Comparison of measurements obtained from video recordings and photographic images

Measurements of standard length (SL) and dorsal fin base length (DFBL) obtained from video recordings (n=123) and photographic images (n=360) were plotted for comparison (Figure 3-14). The test of the interaction effect of the independent variable (source) on the covariate (SL) was not significant ($p = 0.165$). Therefore, the assumption of homogeneity of regression slope, a requirement of ANCOVA, was satisfied. Figure 3-14 also shows that the two regression lines are approximately parallel suggesting the homogeneity of regression slope is satisfactory, therefore a common slope value can be assumed. The result from the ANCOVA shows that the intercepts for each regression line are significantly different, $F_{1,480} = 89.98$ $p < 0.001$. The unadjusted and adjusted means as well as the ANCOVA output are presented in Table 3-3. The measurements of DFBL recorded from the videos had a significantly higher adjusted mean (19.32 mm ± 5.3; mean ± sd) relative to the measurements of DFBL from the photographs (17.46 mm ± 4.3; mean ± sd). This result suggests that for similar lengths of fish, DFBL measurements taken from video are 1.86mm longer than that obtained from measurements taken from photographs. The difference in measurements between photographic and video samples is 14% to 1.35% over the SL range in this sample (75mm to 215mm).

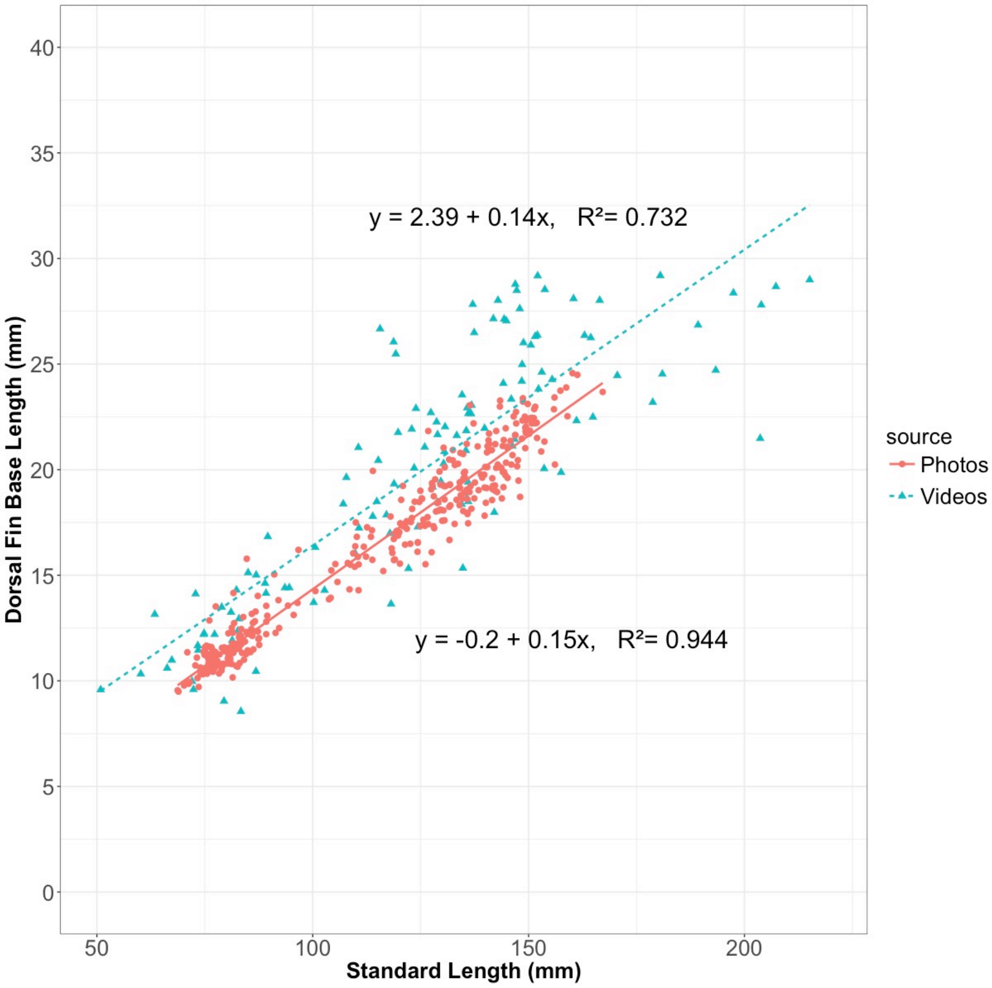

***Figure 3-14** Linear regressions for DFBL vs SL measurements recorded from photographic (n=360) and video samples (n=123). The equations for the individual regression lines from the raw data (unadjusted) are shown.*

***Table 3-3** Adjusted and unadjusted means of DFBL measurements from video or photographs*

	DFBL (mm)		
Group	Unadjusted mean (sd)	Adjusted* mean (sd)	Adjusted regression line coefficients from ANCOVA
Video measurements (n=123)	20.01 (5.8)	19.32 (5.3)	1.73 + 0.145x
Photo measurements (n=360)	16.50 (4.4)	17.46 (4.3)	-0.13 + 0.145x

**The adjusted mean is the predicted value for the y variable when the x variable is the mean of all the observations using the regression equation with the common slope*

3.3.4 Compare Fin Index between photographic and video measurements

A comparison was made of Fin Index, as an indication of fin damage, between measurements taken from photographs and videos for each of the tanks (Figure 3-15). The samples used for this analysis were recorded from measurements after the three-day food withdrawal treatment (day 56), an event that is a risk factor for fin damage. Photographic samples were from day 69 (n=100) and video samples were from day 61 to day 80 (n=38) to ensure enough video samples for comparison. There were no usable videos for Tank2 over this period, so no comparison could be made for that tank. Tanks 3, 4 and 7 were control tanks and tanks 2, 8, 9 were treatment tanks (food withdrawal). Data were not normally distributed therefore a non-parametric analysis (Mann-Whitney test) was performed. No significant differences were found in Fin Index between measurements obtained from videos and photographs in each of the tanks except for Tank 8; (FinIndex= 9.95 (video) compared with 7.34 (photos), p = 0.046).

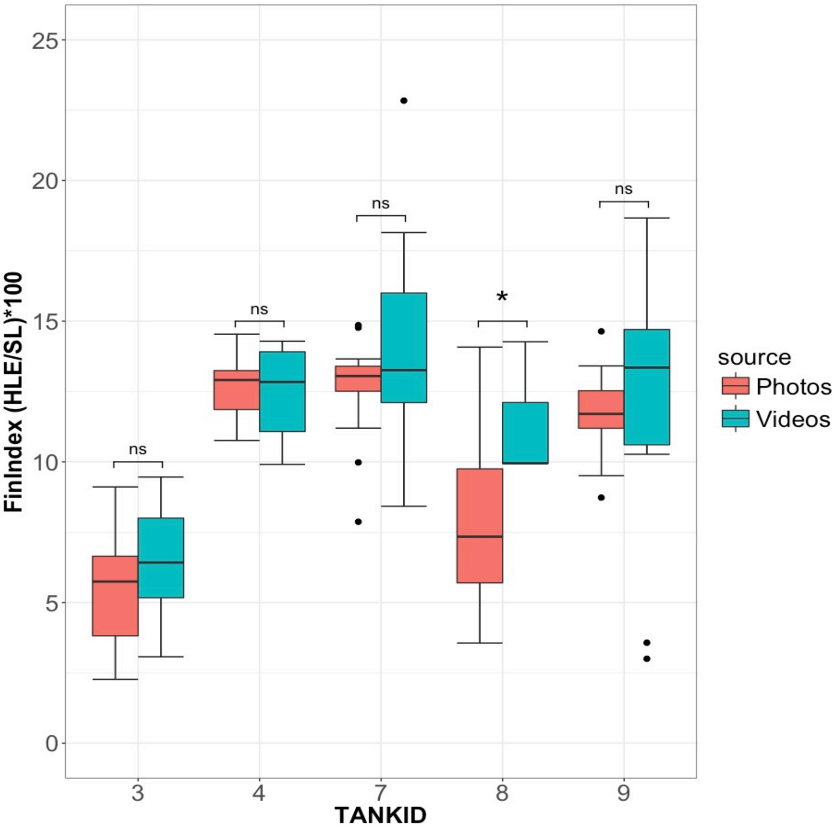

Figure 3-15 *Comparison of FinIndex in each tank, from measurements taken from photos (p) and videos (v) from samples post food withdrawal. For all photo samples in each tank n (p)=20. For video samples - Tank 3 n(v)=8; Tank 4 n(v)=6; Tank 7 n(v)=10; Tank 8 n(v)=3; Tank 9 n(v)=11. There is no comparison of Tank 2 as there are no video measurements for Tank 2. Significant differences (* p < 0.05, ns = not significant).*

3.4 Discussion

Within this size range, fish growth was negatively allometric which is consistent with wild Atlantic salmon of comparable size (Sutton et al., 2000). It suggests large fish are more elongated or that small fish are in better nutritional condition (Froese, 2006). The most likely explanation is that fish are heading towards smolting.

The measurement difference between photograph and video samples (DFBL against SL) was less than 14% for the smallest fishes and less than 2% as the fish size increased. The difference between measurements suggests the laser calibration scale for the video samples was perhaps inaccurate. Another source of measurement error is the measurement of SL, particularly where the light is poor causing difficulty in picking out the edge of caudal peduncle, this is true of both video and photographed samples. Fish in video samples may not be fully parallel to the camera so that SL is measured shorter than it is in reality. However, in terms of measuring fin damage this measurement difference is acceptable and follows the same slope and growth curve.

Fin damage was assessed using Fin Index, which assumes isometric fin growth of the dorsal fin. A comparison of Fin Index among fishes of different size would be biased if the relationship was not linear. MA regression is the statistical method recommended when assessing whether the relative size of fish fins stays constant as body size changes (Warton et al., 2006). Using MA regression of fin length with standard length, Ellis et al., (2009) did not find isometric growth of the dorsal fin in wild rainbow trout (*Oncorhynchus mykiss*). This disagrees with Bosakowski & Wagner (1994), who compared the slope of linear regression of 'relative fin length' with total body length of wild rainbow trout to a slope = 0 and did find isometric growth in the dorsal fin. Ellis et al., (2009) conducted an MA analysis on the Bosakowski & Wagner (1994) data and agreed with their conclusions of isometry in the dorsal fin. They suggested that including the caudal fin in the measurement may have masked the effect of allometry, however, Pelis & McCormick (2003) found that dorsal fin growth of stream-reared Atlantic salmon parr was linearly related with fork length. These studies investigated the growth of wild fish fins to use as a reference to compare fin loss of hatchery reared fish. This study's main focus was to compare the different measurement systems (video as a non-invasive method versus photographs that require fish handling) to assess fin damage in hatchery-reared Atlantic salmon parr, therefore a wild fish reference was not required. Dorsal fin growth was found to be isometric with standard length, using photographed fish samples assessed as having fins with no damage. Fin Index correlated well with each of the fin condition scores and was able to discriminate between the separate levels, except for levels 0 and 1. This perhaps is not surprising as fin damage at level 1 is assessed as having some splitting and/or slight fraying along the top edge which would leave the leading edge of the dorsal fin relatively untouched. This could be a weakness in the use of fin indices to quantify fin damage, particularly if a single fin ray of near normal length was present but tissue severely eroded around it (Hoyle et al., 2007). In this study the height of the leading edge of the dorsal fin was used to calculate Fin Index, regardless if there were longer rays behind it, which would only be the case if the leading edge was eroded. In general, the severity of fin damage is reflected in the height of the leading edge, as can be seen in the fin condition scoring category.

A comparison of Fin Index within each tank showed that fin damage measured was similar between the two measurement systems, except for tank 8, which was likely due to small numbers (n=3). The number of video samples overall was short of that expected. The background colour of the experimental tanks was darker than ideal, due to being painted dark green for a different experiment and would have benefited by using a lighter coloured insert. To counteract this lighting was increased; however, this seemed to have had the effect of keeping the fish to the bottom of the tank reducing the number of fishes which swam past the cameras. The average size of fish, caught on video, was larger than that of the samples removed for photographing. Larger fish tend to be bolder individuals which are more likely to be active in the presence of a stressor than shy fish, who remain on the bottom (Sneddon, 2003), which could potentially bias the results. A further complication of live video data is determining if the fish swimming past are all different individuals, which may be alleviated by moving the camera within the water column.

Storing digital images of fish samples has the advantage of being able to go back and re-measure or re-assess the sample. Photographing samples for future morphometric analysis allows for faster sampling. Measurements are optimised by good lighting, fish being prone on the measuring board and fins splayed for photographing. However, this method requires the fish to be removed from the water, anaesthetised and in some instances sacrificed. The environment is not always suitable for collecting and photographing samples, for example open water pens. When collecting video samples, lighting is very important to be able to discriminate the fish from the background and especially so when trying to measure the standard length (caudal peduncle). Fish also need to be held at a sufficient stocking density to be distributed in the water column, swim past the camera, side on, and preferably have fins splayed. The splaying of fins is not totally necessary when measuring the length of the leading edge as even when the fin is folded this edge is available. This method is limited to measuring the dorsal and caudal fins as the other fins are more difficult to observe under farming conditions. However, there is the potential to increase the sample size with video monitoring as there is no need to sacrifice fish.

To conclude, subject to optimising lighting and stocking density, live video has potential as a non-invasive method to measure fish and characterise fin damage, reducing the need for handling, stressing and killing of fish.

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4 Effects of environmental enrichment on growth, fin damage and stress in Atlantic salmon (*Salmo salar*) on a freshwater commercial farm

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Preface

This chapter investigates enrichment before and after a stressful period during the freshwater stage in Atlantic salmon production. There is a lack of knowledge on the effect of enrichment at farm-level and to my knowledge this is one of the first studies to investigate enrichment on a fish farm, during a normal production period. Food withdrawal is a common husbandry technique on fish farms and identified as a major contributor to fin damage in Chapter 2. This study found that enrichment did have a positive effect on fish welfare and that it was important to reduce unplanned food withdrawal periods such as feeder malfunctions. Monitoring behaviour as a welfare indicator is ranked highly by farmers although considered difficult to quantify. Chapter 5 looks at the effect of the environmental enrichment on behaviour and Chapter 6 explores a novel method of measuring behaviour using data collected in Chapter 5. Many thanks must go to the staff and management of Loch Duart Hatchery, Scotland, UK. In particular the farm manager David Roadknight who had the original concept of using the artificial kelp as enrichment. Staff at the farm provided support in collecting the fish samples from the tanks for me to process. Dr. Sonia Rey, Dr. Susan Jarvis and PhD students Bernat Morro and Savitree Ritchuary (Nui) provided additional support during the two occasions to take tissue and blood samples. Fiona Strachan at Stirling University was an immense help in supervising plasma cortisol preparation and running the liquid chromatography tandem mass spectrometer. The experimental design was done by myself in consultation with my supervisors (co-authors) and farm staff. I prepared the manuscript and did the statistical analysis with the support from my supervisors and Prof Toby Knowles at Bristol University.

4 Effects of environmental enrichment on growth, fin damage and stress in Atlantic salmon (*Salmo salar*) on a freshwater commercial farm

Abstract

The use of structural enrichment under laboratory conditions has been shown to reduce fin damage and potentially increase growth and reduce stress in some fishes. However, there are no studies of the effects of structural enrichment at the commercial farm level under normal production conditions. This study compared Atlantic salmon parr reared in enriched tanks (artificial kelp) and standard production tanks (non-enriched) with regards to growth, dorsal fin damage, plasma cortisol levels and hepato-somatic index (HSI). Data was collected pre- and post-vaccination and grading with the last sample acquired just before fish were transferred to sea. Food was withdrawn, for either 24hrs or 48hrs prior to vaccination and grading, and the subsequent effect of enrichment and food withdrawal on fin damage was also investigated. There was no effect of enrichment on growth parameters prior to vaccination and grading. However, there was significantly more fin damage in non-enriched tanks compared with enriched tanks, with smaller fish showing more severe fin damage. Results from samples taken 4.5 weeks after post-vaccination and grading showed a tendency for fish to be larger (p=0.052) and have less fin damage (p=0.057) in enriched tanks, although not significant. This result indicates that perhaps fish in enriched tanks resumed eating earlier than fish in non-enriched tanks. After 8.5 weeks post vaccination and grading there was no difference in growth, but fin damage was significantly lower in enriched tanks with a 24hr feed withdrawal period compared with a 48hr period. Plasma cortisol levels and HSI were significantly lower in the non-enriched tanks although plasma cortisol levels were consistent with unstressed fish in both enriched and non-enriched environments. However, by the end of the study period (17 weeks post vaccination and grading) there was no difference in growth and fin damage between enriched and non-enriched tanks. The main results indicate that enrichment did not affect fish growth but had a positive effect on dorsal fin damage, potentially leading to improved welfare on-farm.

4.1 Introduction

Growing public demands for aquaculture sustainability and environmental concerns have led to an increased interest in fish welfare. One of the concerns of European consumers is a reduction in the use of hormones and drugs in food production (Grimsrud et al., 2013; Olesen et al., 2010; Zander & Feucht, 2018). Concern for animal welfare, measured by the willingness-to-pay a premium for products farmed with a higher welfare standard, was highest in countries such as the UK, Finland, Germany and Spain (Zander & Feucht, 2018). However, fish welfare ranked lower than terrestrial farmed animals in Finland (Kupsala et al., 2013).

National and international legislation and guidelines for welfare are increasingly incorporating fish welfare. Environmental enrichment has been characterised as a "deliberate increase in environmental complexity with the aim to reduce maladaptive and aberrant traits in fish reared in otherwise stimuli-deprived environments", (Näslund & Johnsson, 2014). A common issue in intensively reared farmed animals is the development of maladaptive behaviours leading to injuries from attacks on conspecifics such as feather pecking in laying hens (*Gallus gallus*) (Lambton et al., 2010) and tail biting in pigs (*Sus scrofa domesticus*) (D'Eath et al., 2017). These attacks reduce welfare, lead to reduced carcass weight and decreased profitability (Harley et al., 2014). Environmental enrichment that stimulates natural foraging behaviour reduces feather pecking in laying hens (Dixon et al., 2014) and adding rooting substrates has been shown to reduce tail biting in pigs (Beattie et al., 2001). Within aquaculture, a major contributor to dorsal fin damage is fin biting by conspecifics (Abbott & Dill, 1985; Turnbull et al., 1998) and is exacerbated by periods of food restriction (Cañon-Jones et al., 2017) or unpredictable feed delivery (Cañon-Jones et al., 2012). Fin damage is a well-known problem in many species of farmed fish, with a negative effect on fish health, farm productivity and is considered to be an important indication of poor fish welfare (Noble et al., 2007). Fish fins are innervated living tissue and therefore fin damage can potentially cause pain, provides a route of entry for infections and affect swimming ability (Ellis et al., 2008). Fin damage is typically fins shortened in size due to contact with solid objects, pathogenic infections or attacks by conspecifics (fin biting). If fin damage is not too severe fins are able to heal and regenerate (Akimenko et al., 2003).

For financial reasons intensive fish farming practices requires stocking densities which exceed the density of fish normally found in the wild. This can lead to an increased frequency of social encounters and hence potentially aggressive encounters (Huntingford, 2004). Aggression and territoriality are common among Atlantic salmon parr (*Salmo salar*) (Keenleyside & Yamamoto, 1962), an important commercial fish, and can increase stress levels in both subordinate (Abbott & Dill, 1985; Cañon Jones et al., 2010; Moutou et al., 1998) and dominant fish (McLean et al., 2000). Stress causing events are inevitable on fish farms as fish need to be confined, handled, vaccinated, graded and transported (Barton, 1997). However, stress responses are adaptive, allowing animals to maintain homeostasis in response to real or perceived stressors (Barton, 2002). Stress is only detrimental to fish health and welfare when a stressor is excessive, repetitive, prolonged or mismanaged. Chronic stress is associated with low body condition score, negative effects on growth (Pickering, 1993) and the immune system (Pickering & Pottinger, 1989).

Change in blood cortisol levels is routinely used as a measure of the stress response (Barton & Iwama, 1991). Cortisol is released from the interrenal cells of the head kidney into the bloodstream stimulated by the hypothalamus-pituitary-interrenal (HPI) axis. The neuroendocrine stress response (HPI) is activated by the perception of a stressful stimulus. Blood sampling is relatively simple however, being handled and removed from the water is a major stressor for fish which triggers an acute stress response. As cortisol needs to be produced within the body, there is a latency of a few minutes before blood cortisol levels begin to rise in response to an acute stressor (Molinero et al., 1997). Therefore, it is important to restrict the collection of blood samples to within a few minutes after handling fish, to minimise sampling effects. Basal plasma cortisol levels vary widely between and within a species hence sampling at a single time point is meaningless; measurements should be taken between treatment and control fish (Ellis et al., 2012). Interpretation of the results of cortisol measurements require context as plasma cortisol levels in fish have natural seasonal and diurnal variations (Nichols & Weisbart, 1984; Thorpe et al., 1987) and can indicate positive as well as negative experiences (Boissy et al., 2007). Plasma cortisol analysis requires processing in a laboratory meaning the results are not immediately available for on-farm assessment.

Fish are kept in barren tanks for ease of cleaning, removal and transfer of fish and the reduction in the spread of disease (Pounder et al., 2016). Physical structures can provide shelter for subordinates within tanks and can potentially reduce aggression by limiting visual contact and reducing general activity levels (Eason & Stamps, 1992). Thus, potentially saving energy through decreased metabolism and reduced stress (Näslund et al., 2013). Structural enrichment in tanks has been shown to reduce dorsal fin damage in Atlantic salmon (Näslund et al., 2013) and rainbow trout (*Oncorrhynchus mykiss*) (Arndt et al., 2001; Berejikian, 2005; Bosakowski & Wagner, 1995). However, others have reported no effect on fin damage in Atlantic salmon from enrichment (Brockmark et al., 2007) as well as detrimental effects due to suspected increased territoriality (Persson & Alanärä, 2014). The presence of shelters reduced basal plasma cortisol levels in Atlantic salmon indicating that fish in enriched environments were less stressed than fish in non-enriched environments (Näslund et al., 2013). However, cortisol levels did not differ between zebrafish (*Danio rerio*) with or without shelter (Wilkes et al., 2012) or were slightly elevated in the enriched tanks (von Krogh et al., 2010). This may indicate that some forms of enrichment are more effective than others. The effect of environmental enrichment on salmonid growth parameters is varied and appears to be species and developmental stage specific. There are reports of salmonids growing better in structurally enriched environments (Brockmark et al., 2007; Hyvarinen et al., 2011), or the environment having no effect (Arndt et al., 2001; Näslund et al., 2013; Brockmark et al., 2010) or growing less well in structurally enriched environments (Bosakowski & Wagner, 1995). Providing shelter or a visual barrier may potentially reduce time spent foraging or restrict view of food, leading to poor growth, hence the selection of the type of enrichment needs to be tailored to the species and life stage (Killen et al., 2013; Näslund et al., 2013).

This study investigated the effect of environmental enrichment on growth rates, stress levels and fin damage in Atlantic salmon parr rearing tanks on a commercial farm under normal production conditions. The use of structural enrichment in experimental tanks has been shown to reduce fin damage and has the potential to reduce stress but its effect on growth is less clear. This is the first study to show the effects of structural environmental enrichment at the commercial farm level.

4.2 Materials and Methods

The study was conducted at a fresh water site owned by Loch Duart Salmon Ltd; Duartmore hatchery, near Badcall Bay, Sutherland in Scotland, UK. The site had an indoor heated hatchery and outdoor on-growing tanks which were split into two areas, Northside and Southside. The Northside area had 20 tanks where fish are grown to ~ 5g before being graded and moved to the larger southside tanks (Figure 4-1). The Southside had 23 tanks, 5m diameter x 2m depth that were the main focus of the study. All outside tanks were on natural photoperiod and ambient temperature; water supply to all tanks was via a flow-through system from a nearby Loch Duart. Food was provided to each tank using a compressed air system with a spinning arm (Figure 4-2) to distribute food around the tank (Arvo-tec, Finland). The provision of food was controlled at an individual tank level by a central computer and varied across the season between daylight hours.

Figure 4-1 *Duartmore site, Southside 23 x freshwater tanks*

Figure 4-2 *Rotating arm for distributing feed from the centre of tank*

The study was in two parts, Pre-vaccination and Post-vaccination, see Figure 4-3 for timeline and sampling points. Vaccination and grading are extremely stressful for fish as they have food withdrawn, are transported between tanks, sedated, handled and injected with vaccine.

The first phase (Pre-vaccination) started when fish were moved to the Southside tanks (10th Aug 2016) until one week prior to food withdrawal for vaccination and grading. The second phase (Post-vaccination) started after vaccination until one week prior to fish being transported to sea (March 2017). Normal farm husbandry practices were followed.

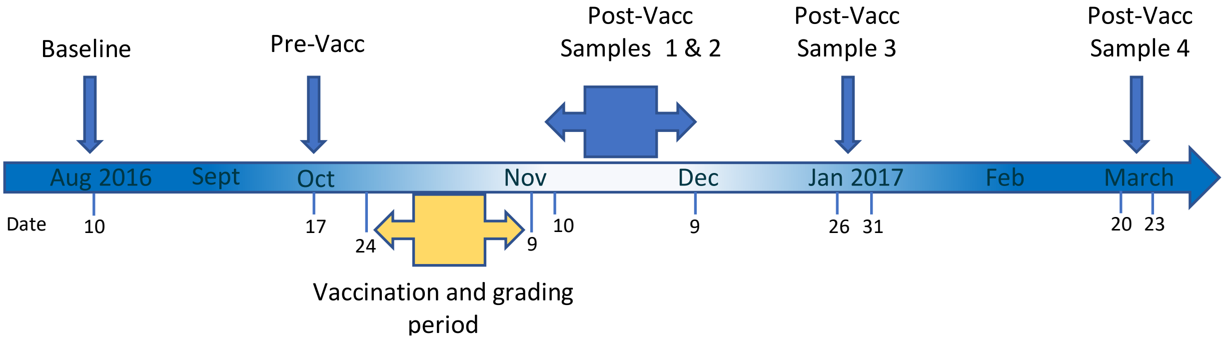


Figure 4-3 *Timeline of study on-farm enrichment*

Phase 1 (Pre-vacc) started on the 10th August 2016 and ran until 17th October 2016. Vaccination and grading commenced on 24th October 2016 until 9th November 2016. Sampling occurred at baseline and one week prior to the start of food withdrawal (Pre-vacc sampling). Post-vaccination sampling started 17 days after the start of vaccination and grading (Post-vacc sample 1) with Post-vacc sample 2 starting 14 days after sample 1. Post-vacc samples 1 and 2 were staggered to coincide with tanks being processed, see section 4.2.2. Post-vacc sample 3 was 42 days after final sample 2 and Post-vacc sample 4 was 1 week prior to fish being moved to sea, in March 2017.

4.2.1 Pre-Vaccination

Fish from the Northside were graded large and medium when moved to the Southside tanks in August 2016. Grading was by a mechanical grader using rollers with a variable gap. The definition of large and medium was relative to the average size of the originating tank with large being either > 10g and medium 6 - 10g or large > 6 g and medium 3 - 6g. Any fish below 3g were culled. Initially each tank had approximately 32,000 fish. After fish were moved to the Southside, enrichment was randomly added to nine out of the 17 tanks. The enrichment consisted of artificial kelp (plastic) with dimensions 150mm (w) x 1500mm (L) (Figure 4-4) and weighted at the bottom. Six ropes were evenly strung across the top of the tank, three on each side of the central pillar. Each rope held three strips of artificial kelp which were staggered on adjacent ropes. All the production fish were of the same origin, but they were divided according to their eventual destination (Figure 4-5). Tanks A1-A5, B1-B5 plus C3 were the main tanks in the study and fish were destined to be transported to sea in March 2017. Tanks D1-3 and E1-3 were destined for a fresh water cages in Loch Na Thull in September 2016, prior to being moved to sea cages in March 2017. Moving fish to Loch Na Thull freed up the tanks for vaccination and grading at which point each remaining stocked tank would be split into two tanks. Enrichment was added to the tanks as shown in Figure 4-5. Sampling took place at baseline and one week prior to vaccination and grading. See section 4.2.3 for sampling protocol.

Prior to vaccinating and grading fish, feed was withdrawn. Tanks were randomly selected to have food withdrawn for 24 hrs or 48 hrs (normal farm practice) to give a 2 x2 factorial design of enrichment/non-enrichment and 24 hr/48 hr food restriction (Figure 4-6A).

After vaccinating and grading, the remaining 11 stocked tanks were to be split between two tanks, in a randomised 5 x 4 block design (Figure 4-6B).

Figure 4-4 *Artificial kelp enrichment in tank with Atlantic salmon*

4.2.2 Post Vaccination

As a commercial site, post vaccination protocols were under the control of the fish farm and not the researchers. Fish were vaccinated for furunculosis (*Aeromonas salmonicida*), infectious pancreatic necrosis virus (IPNv) and *Moratella viscosus* (winter ulcer). Fish were pumped from each tank through a grading machine. After grading, fish passed through into separate holding tanks for the different sizes where they were sedated before being passed over to the vaccination table. A vaccinating team injected each individual fish by hand with a multidose gun then directed fish through a water channel to a counting machine and back into the main tanks to recover.

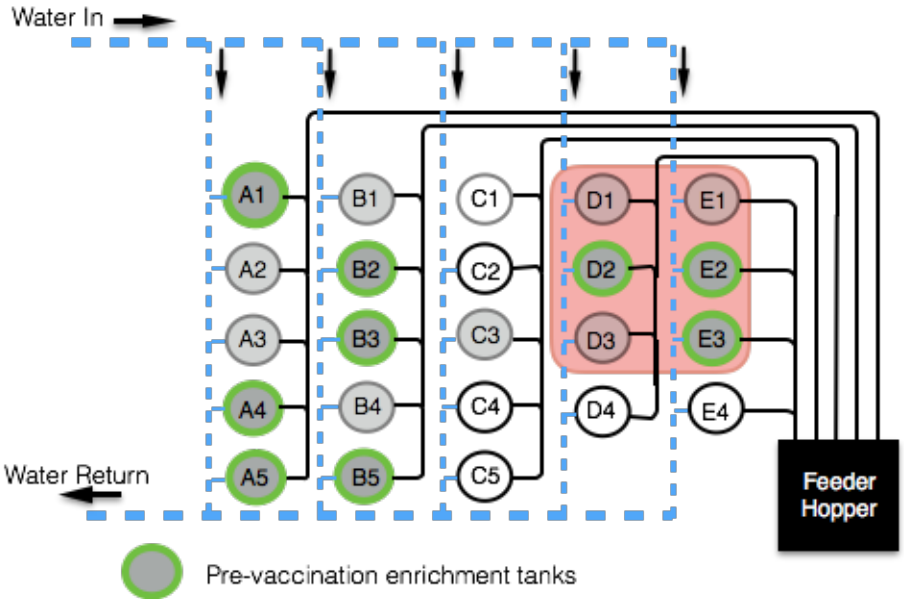


Figure 4-5 *Pre-vaccination tanks and enrichment*

Tanks A1-A5, B1-B5, C3 were experimental tanks. Tanks D1-D3 and E1-E3 were vaccinated first and moved to a freshwater loch (Loch Na Thull) freeing up these tanks to move fish from experimental tanks after vaccinating and grading, when each tank would be split between two tanks to go from the initial 11 tanks to 22 tanks.

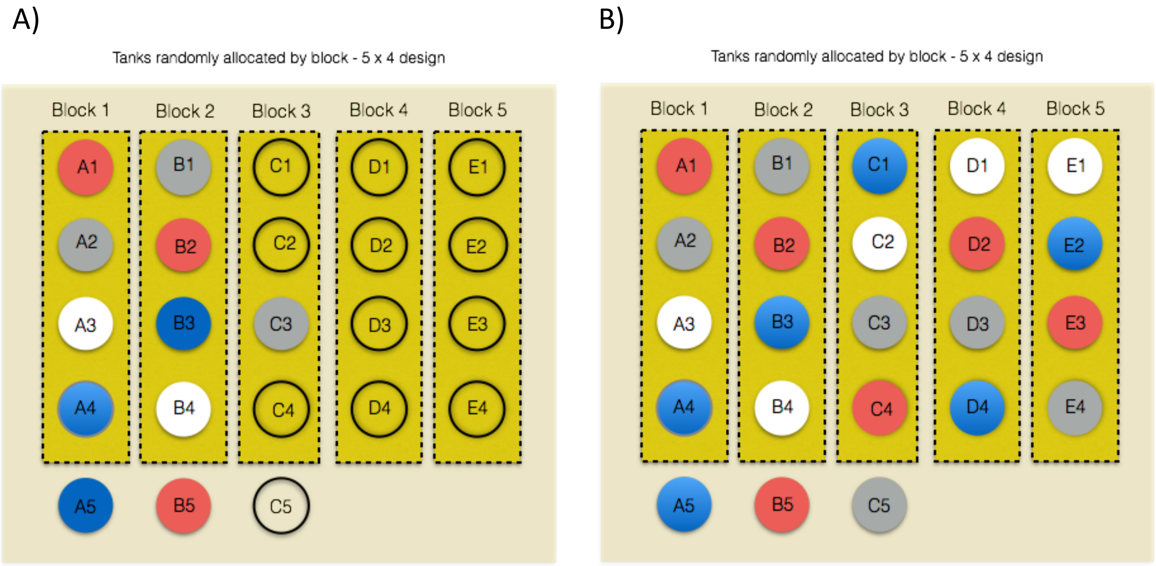


Figure 4-6 *A) Pre-vaccination layout of tanks B) Ideal layout*

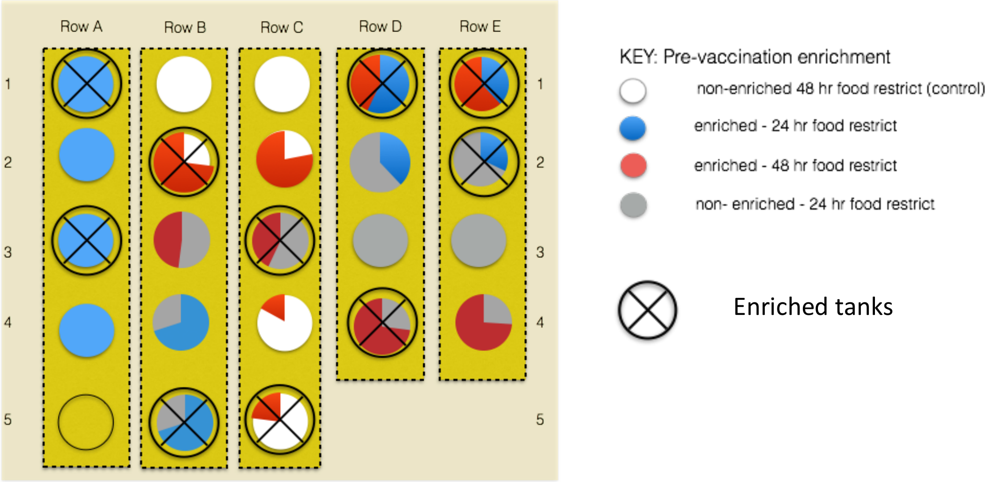


***Figure 4-7** Tank layout post-vaccinating and grading*

Only tanks A1, A3, B1, C1, D3, E3 remained in the same treatment pre and post vaccination. All other tanks were mixed treatments as shown. The colours signify the amount of mixing of each of the treatments i.e. tank C2 had more fish originating from enriched/48hr food restriction tanks (red) than non-enriched/48hr food restriction tanks (white). Enrichment was randomly re-assigned to the mixed tanks as shown. A5 remained empty.

Approximately 15,000 fish were re-distributed into each tank. Distribution of fish into the 5 x 4 block design after vaccination and grading was discussed with farm management and agreed; the required layout shown in Figure 4-6B was provided. However, farm practicalities meant that the ideal distribution was not achieved resulting in treatments being mixed in tanks as shown in Figure 4-7. Enrichment was therefore randomly reallocated after fish movement keeping those tanks that remained in treatment pre- and post-vaccination. A randomisation tool (https://www.randomizer.org) was used to randomise the placement of tank enrichment.

4.2.3 Sampling

Sampling occurred at time points shown in Figure 4-3 to measure welfare indicators growth, fin damage, cortisol and hepato-somatic index, further described below.

Twenty fish were netted from each tank under investigation (Pre-vacc n= 220; Post-vacc n = 440 for each sample point). A single person (farm staff) collected the fish samples from each tank using a consistent method of passing the net in the same direction around the perimeter of the tank and transferring twenty fish into a pre-prepared anaesthetic bath. Fish were euthanized by anaesthetic overdose of tricaine methanesulfonate (MS-222, buffered with sodium bicarbonate, Pharmaq UK Ltd) (Carter et al., 2011) and onset of rigor mortis confirmed. The author weighed and scored fin condition (see Figure 4-8, Table 4-1) for all samples. Blood and tissue sampling are described in section 4.2.3.1.

Figure 4-8 *Fin condition score from 0 (very good condition) to 4 (very severe damage)*

Table 4-1 *Fin condition scoring key*

Fin Condition Scoring Key	
%loss	*Score*
0-10%	0-Very good condition
10-20%	1-Frayed or Mild damage
20-30%	2-Moderate damage
30%-60%	3-Severe damage
>60%	4-Very severe damage/no fin

The fish were photographed (Nikon D3300 camera) for later analysis. Physical measurements were made from the digital photographs using ImageJ software (Schneider *et al*. 2012). Measurements taken were standard length (SL), fork length (FL), height of the leading edge of the dorsal fin (HLE) and dorsal fin base length (DFBL), as shown in Figure 4-9.

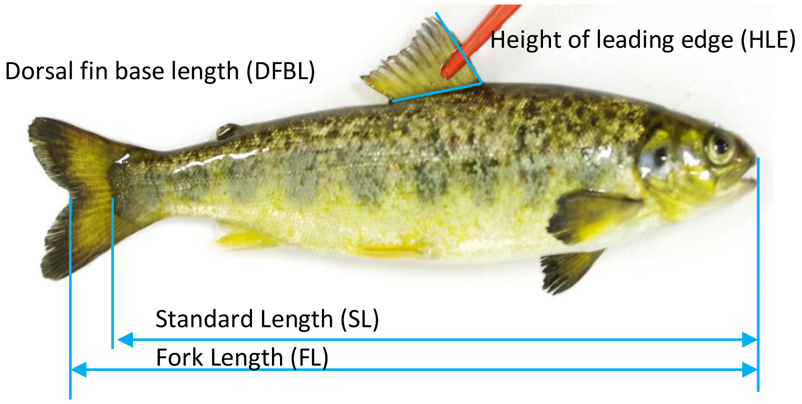


Figure 4-9 *Physical measurements recorded from digital image*

The sampling procedure during Post-vacc samples 1 & 2 was slightly different in timing. During vaccination and grading only one tank was processed per day, Monday to Thursday, therefore it took 17 days to complete vaccination and grading of all tanks. The first tank was processed on 24th Oct 2016 and the last tank on the 9th November 2016. From previous unpublished MSc thesis (Stoltz, 2014), fin damage was evident 14 days after food withdrawal and as water temperature decreased over this period sampling was timed in degree days. This ensured that timing between each tank being processed and sampled would be biologically similar. Post-vaccination sample 1 occurred 145-degree days after vaccination, based on the 17 days between the first and last tank processed, and Post-vaccination sample 2 occurred 85-degree days (14 days) after Post-vaccination sample1.

4.2.3.1 Blood and tissue sampling

Blood and tissue sampling occurred at Post-vacc sample 3 and final sample 4 (Figure 4-3). It was found that blood and tissue sampling all 20 sampled fish from each tank was not practical in the time allotted therefore a random sub-sample of 8-12 fish were taken from the six tanks that had remained in treatment pre- and post- vaccination (Figure 4-7). A heparinised syringe was inserted into the caudal vein withdrawing up to 100μL of blood, where possible. After removal of the needle, blood was dispensed into a 1mL Eppendorf and kept chilled on dry ice. After all the fish samples in each tank were processed the blood sample was centrifuged for 10 min at 3500 rpm. The plasma was transferred to a labelled cryogenic vial then chilled in dry ice. The liver of each fish was removed, weighed, placed on a piece of foil and stored in dry ice. Samples were transported back to laboratory (University of Stirling) the following day and stored in -80°C freezer until analysis.

4.2.3.2 Plasma sample preparation

For plasma sample preparation, liquid-liquid phase extraction (LLE) was used before liquid chromatography, tandem mass spectrometry (LC/MS/MS) analysis. An internal standard (IS) of 100ng/ml d4-cortisol in LC/MS/MS grade methanol (MeOH) was added to 100 µl of plasma in a conical glass test tube then 2ml of ethyl acetate was added before stopping off the test tube. The mixture was vortexed for 20 secs, ensuring that the liquid did not reach the top of the test tube, then left to settle for 5 mins before adding 0.5ml of 0.88% potassium chloride (KCl), mixed well before centrifuging at 1400 rpm for 5 mins. The top organic layer was then removed into a clean conical test tube and evaporated to dryness under nitrogen before being re-suspended in 45% methanol and transferred to a total recovery vial. Samples were allowed to stand for a minimum of 2 hrs in 4°C fridge before analysis. Standard curve samples were prepared by the same method. Stock solutions of cortisol at 10µg/ml were diluted in methanol to 1µg/ml and 10ng/ml and used to prepare dilutions (in methanol) of 1ng/ml, 10ng/ml, 50ng/ml, 75ng/ml and 100ng/ml each spiked with 50 µl of internal standard.

4.2.3.3 Liquid chromatography tandem mass spectrometry

The LC/MS/MS system was a Waters Xevo TQS coupled to an Acquity I Class UPLC. Chromatographic separation was achieved on a Polaris C18-A column (3µ, 150 × 4.6 mm, Varian Inc.). The guard column was a MetaGuard Polaris C18-A (5 µ, 10 × 4.6 mm, Varian Inc.). Autosampler injections of 20 µl onto the LC/MS/MS system were made with the autosampler needle placement 3mm from the bottom of recovery vial. The mobile phase comprised a binary solvent system: MillieQ water (solvent A) and methanol (solvent B), both containing 2 mmol/l ammonium acetate with 0.1% formic acid. The initial solvent composition was 55% A and 45% B. The mobile phase gradient profile involved two steps; increasing from the initial conditions to 98% B within 5 mins holding for 1 min before returning to the initial state at 7 mins, allowing 1 min for column re-equilibration. The total run time was 8 min, injection-to-injection. The flow rate was 0.6 ml/min and the column was maintained at 60°C. The instrument was operated in positive electrospray ionization (ESI) mode using MassLynx V4.1 Software (Waters). Electrospray ionization and tandem mass spectrometry parameters were individually optimized for cortisol and cortisol-d 4. Using the optimized transitions 363 > 121 (cortisol) and 367 > 121 (cortisol-d 4) limits of detection (LOD) and quantification (LOQ) of 0.02 and 0.06 ng/mL respectively were achieved in standard solution. The coefficient of variation (CV) for the sample inter-assay was 7.1% (n=5) and the intra-assay average %CV was 1.1% (n=9).

4.2.4 Statistical Analysis

Two-level linear models (TankID and FishID) using MLWin (Rasbash et al., 2009) were constructed to investigate the relationship between the response variables (Weight, Fin condition, SL and HLE) and treatment (enrichment/food restriction). Initial values for each response variable was calculated as the average values in each tank at the first sample point post-vaccination and used as a covariate in the model. Model simplification was carried out using a stepwise approach and likelihood ratio test. Normality and homoscedasticity of the residuals were checked using Q-Q plots and standardised residuals vs. fitted values.

All other data analysis was carried out using R software (version 3.5.3) (R Development Core Team, 2019).

To compare the mean weight between each fin condition score the function ‘compare_means’ in R package ggpubr v0.2 was used with Bonferroni correction. Variances were tested using Bartlett’s test for homogeneity and found not equal (K-squared=12.17, df = 4, p=0.016) therefore the Wilcoxon signed rank test was used within the function compare_means.

Plasma cortisol concentrations and the hepatosomatic index (HSI) were log-transformed to meet the assumptions of normality and homoscedasticity of the residuals. Changes in plasma cortisol concentrations were compared between treatment (enrichment) and across fish size (weight) using a generalised linear model with weight and treatment as fixed effects and an interaction term weight*treatment. HSI was compared between the factors of treatment, weight and plasma cortisol concentrations. Model simplification utilised the Maximum Likelihood approach. In all statistical tests, p= 0.05 was taken as the level of significance.

4.3 Results

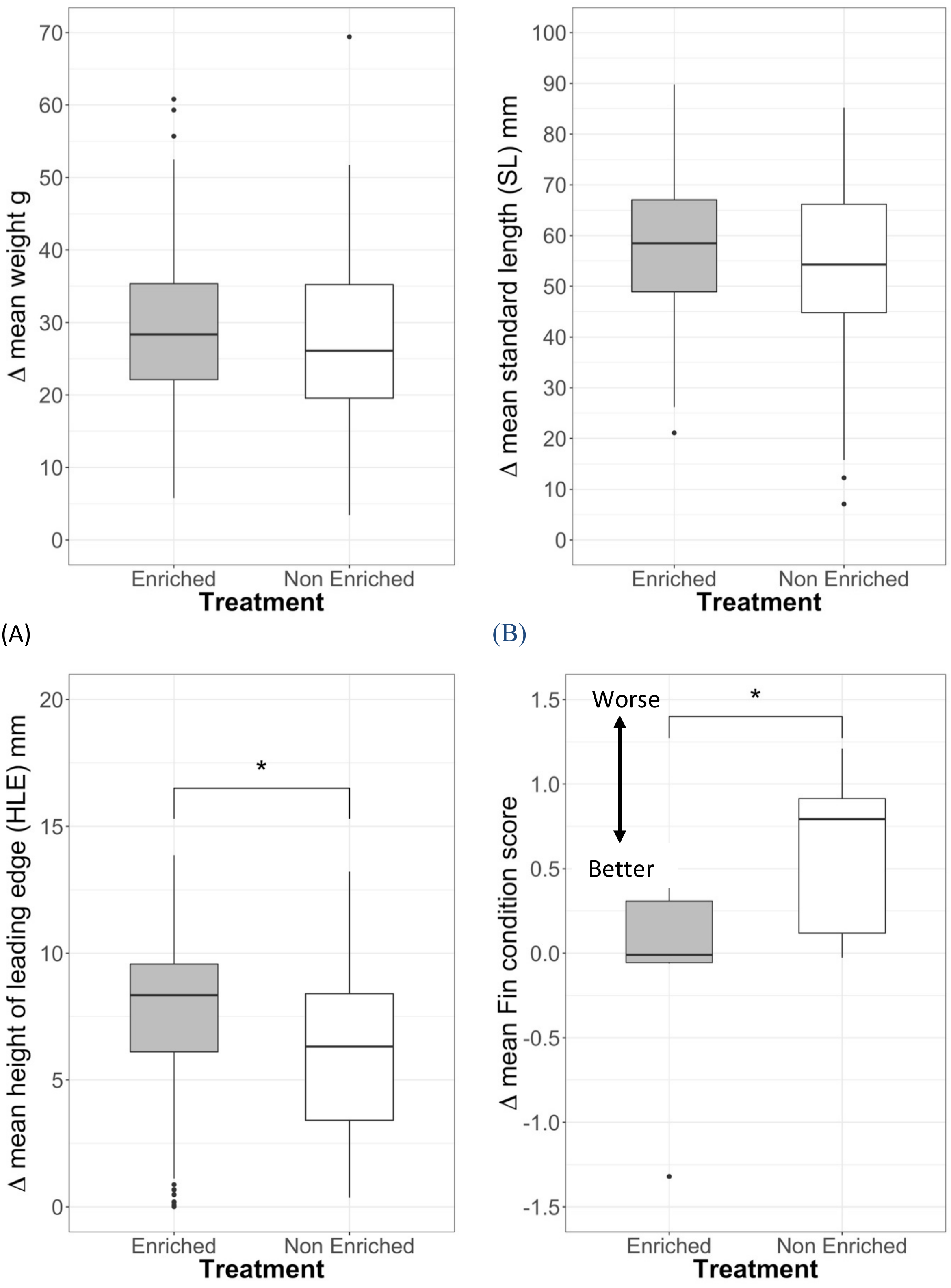
4.3.1 Pre-vaccination results

4.3.1.1 Effect of Enrichment

A two-level linear model (TankID/FishID) showed that, over the 10-week period between baseline sample and Pre-vacc sample 1, there was no effect of enrichment on fish growth, either weight (p = 0.259) or standard length (p = 0.785). Model output is shown in Table 4-2. Although growth parameters were higher in enriched tanks this was not significant, see Figures 4-10 (A) and (B), which show baseline values subtracted from pre-vacc sample 1. There was a significant effect of enrichment on fin damage with dorsal fins, on average, 2.2mm longer (HLE, p = 0.046) in enriched tanks (Figure 4-10(C)). Fin Condition scores were also lower (-0.370) in tanks with enrichment (p = 0.039) indicating less fin damage, see Figure 4-10(D).

Table 4-2 *Model coefficients showing covariates included in the model. Initial starting values for weight (Wgt0), standard length (SL0), height of the leading edge (HLE0), fin condition score (FC0) and size grade). Covariates are the mean values in each tank measured at baseline. Where the effect of enrichment was significant p values are shown in bold.*

Variable	constant	coeff	S.E.	p-value	covariates
Weight	32.86	2.303	2.038	0.259	Wgt0 Size
SL	131.73	1.07	3.291	0.785	SL0
Fin Condition Score	1.38	-0.403	0.195	**0.039**	FC0
HLE	13.59	1.36	0.682	**0.046**	HLE0
FinIndex	11.09	0.81	0.391	**0.038**	



(A) (B)

(C) (D)

Figure 4-10 *Comparison between enriched and non-enriched tanks of the mean of the difference between baseline and pre-vacc sample.*

A) weight, B) standard length (SL), C) Height of leading edge (HLE) of the dorsal fin and D) dorsal Fin condition score. Note the higher the fin condition scores the worse condition of fins. (represents p < 0.5, see Table 4-2).*

4.3.1.2 Pre-vaccination Fin Condition

Prior to vaccination and grading 61.2% of dorsal fins were scored as very good condition (Fin score 0 and 1) and 26.7% scored as being severely damaged (Fin scores 3 and 4). Table 4-3 shows the breakdown of percentages of fin scores and corresponding mean weight ± S.D. Comparison of the mean weights (Table 4-4) show that there was no significant difference in mean weight between Fin Scores 0, 1 and 2. However, there was a significant difference in mean weight between Fin Scores 0-2 and 3-4. This result indicates fish with more severe fin damage are smaller in size than fish with better fins.

Table 4-3 *Summary of weight (mean ± S.D.) for each of the fin condition scores, the number of occurrences of each score (counts) and the percentage*

Fin Condition Score	Weight (g)	S.D.	counts each score	% each score
0	38.73	11.34	117	47.4
1	40.16	10.45	34	13.8
2	36.11	9.29	30	12.1
3	31.95	7.26	48	19.4
4	30.95	11.14	18	7.3

Table 4-4 *Results of comparisons of mean weight between each fin condition score where ns=not significant, * = P <0.05, ** = p < 0.02, *** = P< 0.001.*

	Fin condition Score			
	1	2	3	4
0	ns	ns	***	**
1	-	ns	***	**
2	-	-	*	*
3	-	-	-	ns

4.3.2 Post vaccination

The mixing of treatments post-vaccination complicated the analysis therefore it has been broken down into: 1) the effect of post-vaccination enrichment only, 2) the interaction effect of pre- and post-vaccination enrichment and 3) interaction effect of food restriction and post-vaccination enrichment. Twelve days prior to the final sample in March 2017 there was malfunction in the automatic feeder resulting in a period with no food for 2 days then food being distributed by hand for 4 days, in all tanks. As food restriction was part of the treatment sample periods 1-3 were analysed together and sample period 4 analysed separately.

4.3.2.1 Effect of post-vaccination enrichment only

The two-level linear model, looking at the effect of post-vaccination enrichment only, showed that there was no overall main effect of post-vaccination enrichment (Table 4-5) but by sample 2 there was a tendency for weight and standard length to be higher in enriched tanks than non-enriched tanks (Figures 4-11, 4-12). The interaction effect of sample time x enrichment showed a small positive coefficient (Table 4-5), however, the effect was not highly significant for weight (p=0.052) or standard length (p=0.075). This would imply that there was a tendency for fish to be an average of 2g heavier and 2 mm longer in enriched tanks compared with non-enriched tanks for a period after vaccination and grading. However, by sample 3, fish in the non-enriched tanks had caught up. There was a tendency for less fin damage in enriched tanks compared to non-enriched tanks (Figure 4-13). small negative coefficient (Table 4-5) shows that enriched tanks had a lower fin condition score than non-enriched tanks, although not at a significant level (p=0.057). Between samples 3 and 4 there was a feeder malfunction and fin damage increased in both enriched and non-enriched tanks (Figure 4-14).

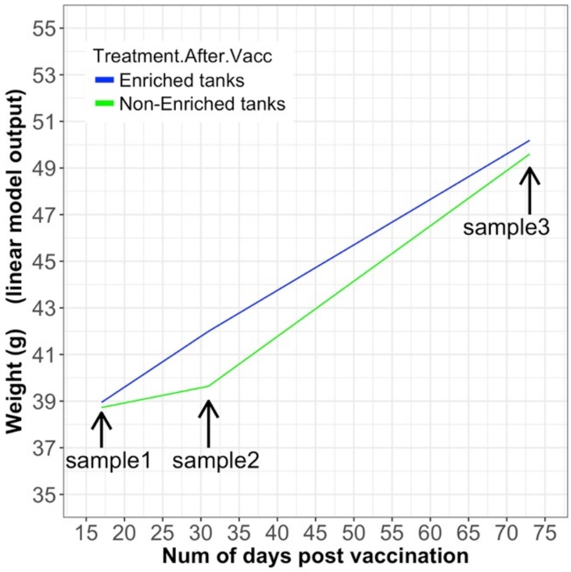


Figure 4-11 ***Figure 4-11*** *Linear model output showing the effect of post-vaccination enrichment on weight.*

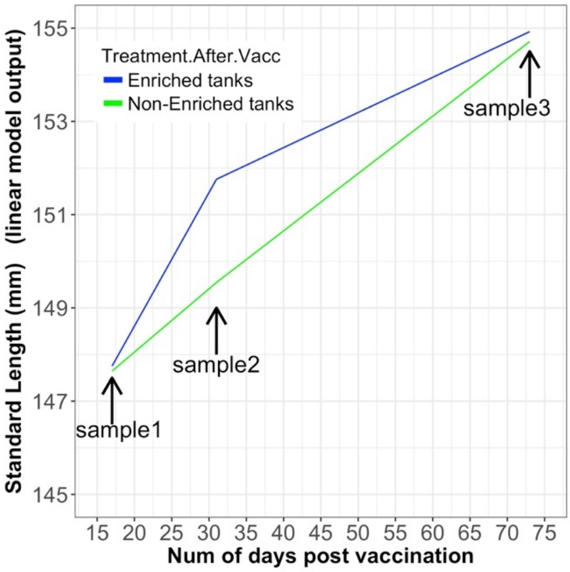


Figure 4-12 *Linear model output showing the effect of post-vaccination enrichment on standard length (SL).*

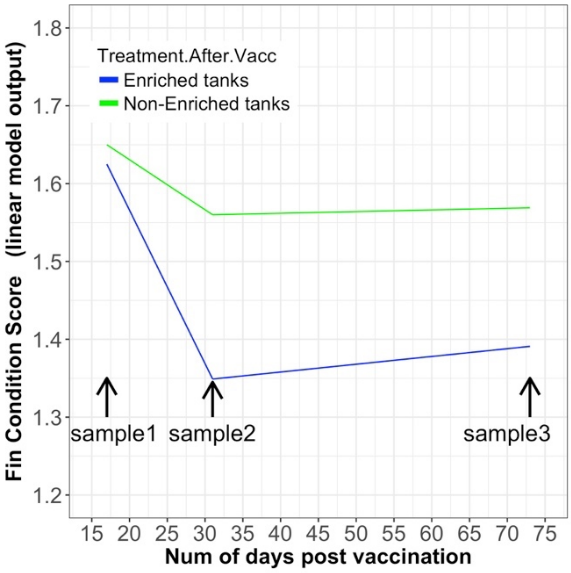


Figure 4-13 *Linear model output showing the effect of post-vaccination on fin condition scores.*

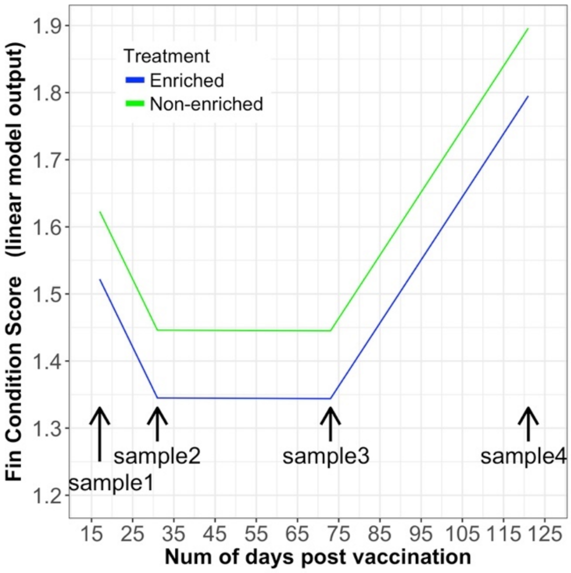


Figure 4-14 *Linear model output showing the effect of post-vaccination on fin condition scores after feeder malfunction between samples 3 and 4.*

Table 4-5 *Coefficient estimates (± SE) with associated p values for all terms retained in the simplified models for the first 3 sample periods for weight, standard length and fin condition score on the effect of only post-vaccination enrichment. Covariables (Wgt0, SL0, FC0) are the mean values in each tank measured at sample 1.*

	Response variable	Weight (g)			Standard Length (mm)			Fin condition score		
Exploratory variables		Coeff	SE	p-value	Coeff	SE	p-value	Coeff	SE	p-value
constant		5.965	3.697	0.107	22.069	13.398	0.100	0.529	0.189	<0.001
PostVaccEnrichment	Enriched	0.222	1.033	0.830	0.110	1.159	0.925	-0.136	0.072	0.057
Size grade (med/large)		3.595	1.385	0.009	3.138	1.500	0.036	-0.143	0.077	0.063
Sample time	S2	0.914	0.731	0.211	1.924	0.790	0.015	-0.179	0.084	0.033
	S3	10.875	0.759	<0.001	7.066	0.821	<0.001	-0.147	0.086	0.088
Interactions	S2 x Enriched	2.100	1.079	0.052	2.076	1.167	0.075			
	S3 x Enriched	0.364	1.119	0.745	0.097	1.210	0.936			
Initial values	Wgt0	0.806	0.102	<0.001						
	SL0				0.841	0.093	<0.001			
	FC0							0.751	0.096	<0.001

4.3.2.2 Effect of pre- and post-enrichment on fin condition

The number of combinations of treatment are listed in Table 4-6. The output of a 2-level linear model output, looking at the interaction effect of pre- and post-vaccination enrichment on fin condition is shown in Figure 4-15. Fish movement after vaccination and grading resulted in some tanks not remaining in the same treatment pre- and post-vaccination. This resulted in some post-vaccination tanks having fish that originated from tanks that were from enriched and non-enriched pre-vaccination (Figure 4-7).

Table 4-6 *Treatments pre- and post- vaccination and grading*

Pre-Vacc enrichment	**Post-Vacc enrichment**	**No. of tanks**
Enriched	Non-enriched	2
Non-enriched	Non-enriched	4
Mixed (Enriched + non-enriched)	Non-enriched	6
Mixed (Enriched + non-enriched)	Enriched	6
Enriched	Enriched	4

Note there is no combination of pre-vacc non-enriched and post-vacc enriched.

There was a significant main effect of post-vaccination enrichment (Table 4-7) with a negative coefficient (-0.255) indicating that tanks with post-vaccination enrichment had significantly lower fin condition (p=0.038) than non-enriched tanks. A lower fin condition score indicates better fin condition. There were no significant interaction terms indicating that post-vaccination fin condition was primarily due to post-vaccination enrichment than any effect of pre-vaccination enrichment, although tanks that remained in enrichment pre- and post-vaccination had the lowest levels of fin damage. However, fin condition had deteriorated in all tanks by the end of the sampling period (Figure 4-15).

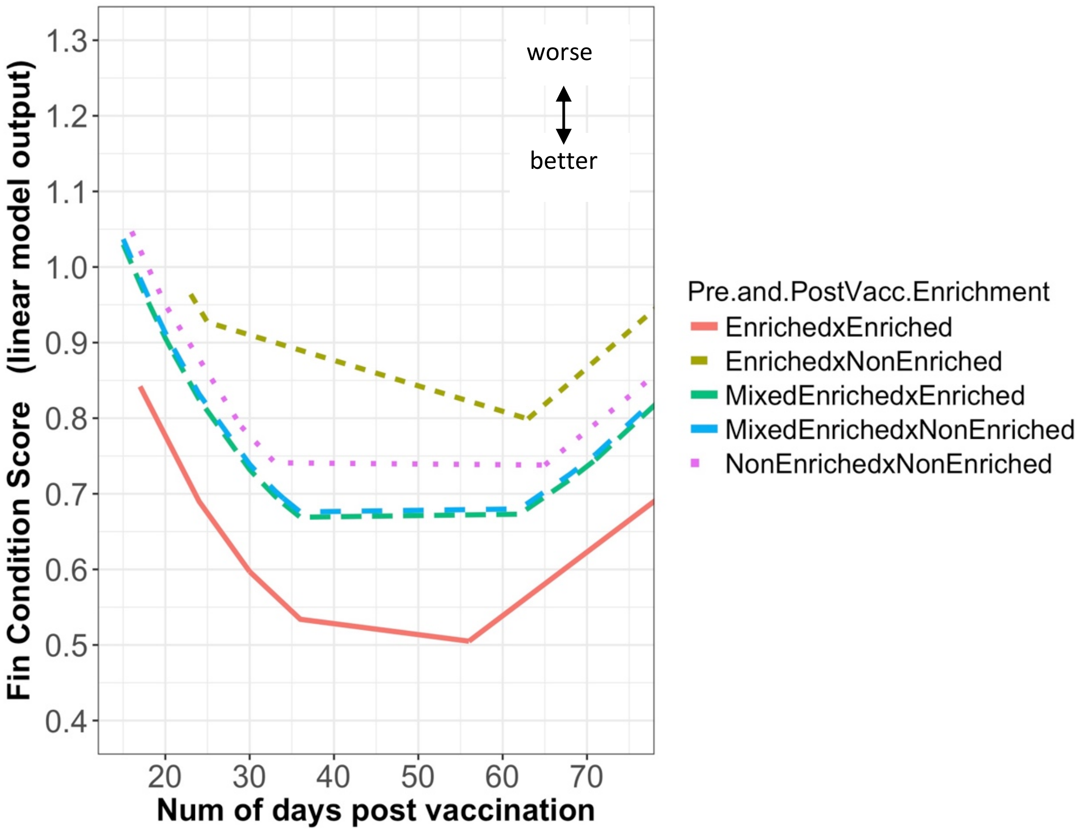


Figure 4-15 *Linear model output of the effect of pre- and post-vaccination enrichment on fin condition during the first 3 sample periods. The key lists the status of enrichment post-vaccination and the enrichment status of the originating tanks (pre-vaccination) after fish size graded, during vaccination, into new tanks e.g. MixedEnriched x Enriched are tanks that had enrichment post-vaccination, but the fish originated from enriched tanks and non-enriched tanks. Fin damage was lower in tanks with post- vaccination enrichment compared with non-enriched tanks.*

Table 4-7 *Coefficient estimates (± SE) with associated p values for all terms retained in the simplified models for the first 3 sample periods of fin condition scores, looking at the effect of pre- and post-vaccination enrichment. Negative coefficients signify lower fin scores in enriched tanks compared to non-enriched tanks. Covariables (FC0) are the mean values in each tank measured at Sample 1. Significant terms are in bold.*

Fin condition

Independent Variables		Coefficient	S.E.	*p*
constant		1.691	0.293	<0.001
Environment	Post-Enriched	-0.255	0.123	**0.038**
	Pre-Enriched	-0.076	0.127	0.549
	Pre-Mixed	-0.113	0.125	0.367
Time		-0.045	0.014	**<0.01**
Size grade	Large	-0.118	0.069	0.085
Initial value (FC0)		0.469	0.091	<0.001
Interactions	Pre-Mixed x Post-Enriched	0.248	0.152	0.103

4.3.2.3 Effect of food restriction and post-vaccination enrichment on fin condition

Treatments were mixed in some tanks leading to the combinations in Table 4-8. The output of a two-level linear model, looking at the interaction effect of treatments (food restriction and post-vaccination enrichment) on fin condition is shown in Figure 4-16 and Table 4-9.

Table 4-8 *Treatment combinations post vaccination and grading*

Feed restriction	Post Vacc Enrichment	Number of Tanks
48 hr	enriched	2
48 hr	non-enriched	4
24 hr	enriched	4
24 hr	non-enriched	6
Mixed (24 hr + 48 hr)	enriched	4
Mixed (24 hr + 48 hr)	non-enriched	2

Tanks that underwent 48 hr food withdrawal initially had increased fin damage post-vaccination, regardless of post-vaccination enrichment (Figure 4-16). Tanks with Mixed and 24 hr food withdrawal initially had reduced fin condition scores (less fin damage). There was a main effect of post-vaccination (Table 4-9), however since the interaction non-enriched x 48 hr was significant (p=0.047), the main effect is ignored. The small positive coefficient (0.332) indicates that the interaction non-enriched x 48 hrs environments had more fin damage than enriched x 24 hrs environments. However, by the 3rd sample period all tanks experienced an increase in fin damage.

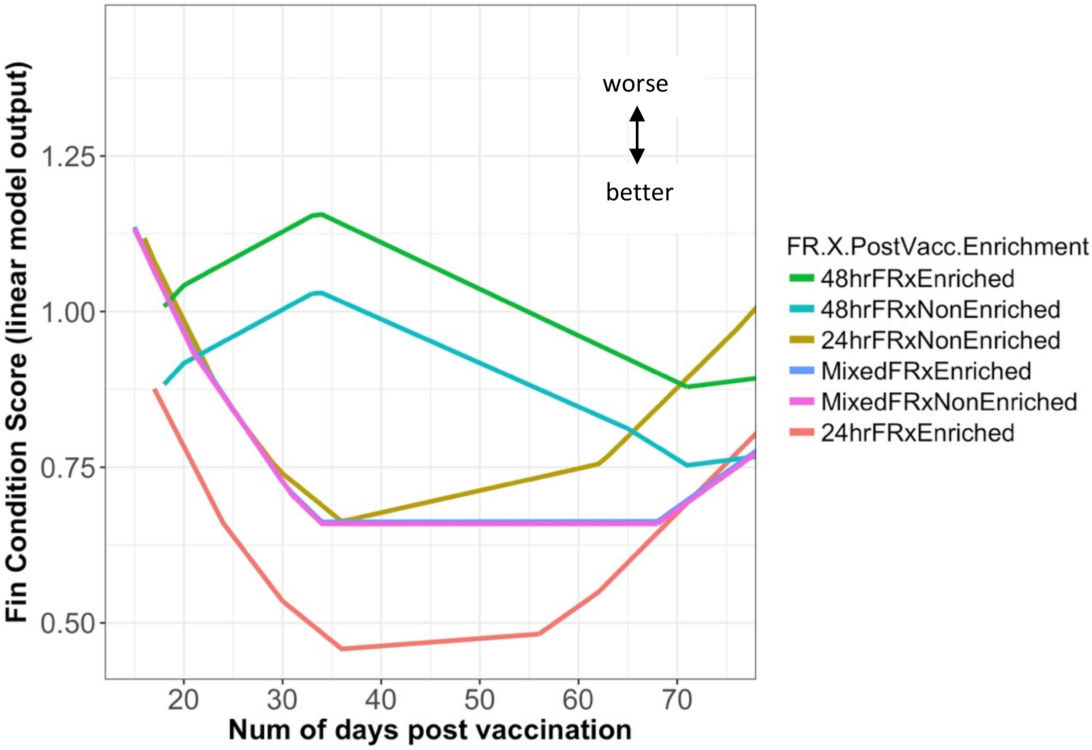


Figure 4-16 *Linear model output of the interaction effect of pre-vaccination food withdrawal and post-vaccination enrichment on fin condition, for the first 3 sample periods. Fin damage initially increased in tanks with 48 hr food withdrawal, regardless of post-vaccination enrichment before reducing.*

Table 4-9 *Coefficient estimates (± SE) with associated p values for all terms retained in the simplified models for the first 3 sample periods for fin condition scores looking at the effect of pre-vaccination food withdrawal period and post-vaccination enrichment. Positive coefficients signify higher fin scores in non-enriched tanks compared with enriched tanks. Covariable (FC0) is the mean values in each tank measured at Sample 1. Significant terms are in bold.*

Fin condition

Independent Variables		Coefficient	S.E.	*p*
Constant		1.692	0.295	**<0.001**
Environment	Non-Enriched	-0.223	0.093	**0.016**
Food withdrawal (FW)	24 hr / 48 hr (mixed)	-0.137	0.118	0.247
	48 hr	-0.054	0.093	0.565
Time		-0.044	0.014	**<0.01**
Initial value (FC0)		0.429	0.09	**<0.001**
Size grade	Large	-0.111	0.069	0.105
Interactions	Non-Enriched x (24hr/48hr)	0.228	0.152	0.133
	Non-Enriched x 48 hr	0.332	0.167	**0.047**

4.3.2.4 Cortisol

Samples taken from January 2017 and March 2017 were analysed separately. Using the maximum likelihood method, the linear model for the January data (S3) was simplified to treatment only and the full model retained for the March data (S4) (Table 4-10A, B).

Mean cortisol level (± s.d.) in the enriched group was 3.22 ± 2.44 (n=17) and in the non-enriched group was 2.05 ± 2.69 (n=20). There was a significant difference in plasma cortisol concentrations with treatment (Figure 4-17, Table 4-10A) in January (S3). Cortisol levels were 57% lower ($\exp^{-0.849}$) in the non-enriched environments compared with the enriched environments (Figure 4-17), indicating fish in non-enriched environments were potentially less stressed. Figure 4-18 shows no interaction with weight.

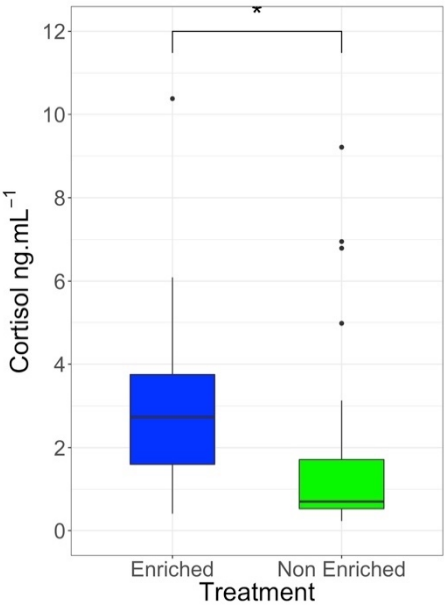


Figure 4-17 *January data (S3) of cortisol in Atlantic salmon held in enriched or non-enriched environments. (line = median, * = $p < 0.05$)*

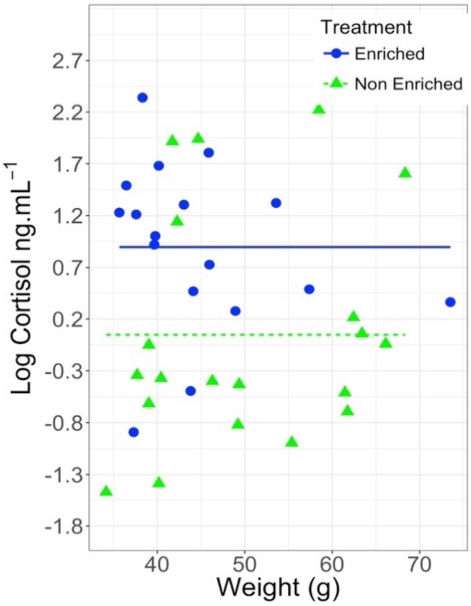


Figure 4-18 *January 2017 (S3) plot of log-transformed cortisol against weight, with model prediction lines*

March (S4) data for plasma cortisol concentrations versus treatment is shown in Figure 4-19. Mean cortisol level (± s.d.) in the enriched group was 2.44 ± 2.63 (n=30) and in the non-enriched group was 3.14 ± 2.74 (n=25). There was a significant main effect of treatment (Table 4-10B), however since the interaction of treatment x weight was significant, the main effect was ignored. The interaction between treatment and weight showed that in the non-enriched environment, plasma cortisol concentrations significantly increased as fish weight increased whereas in the enriched environment cortisol levels decreased as fish weight increased (Figure 4-20, Table 4-10B). This indicated that bigger fish were more stressed in the non-enriched environment compared with the enriched environment. Over this weight range, plasma cortisol concentrations in larger fish increased by up to 3% ($\exp^{0.032}$) more in non-enriched environments.

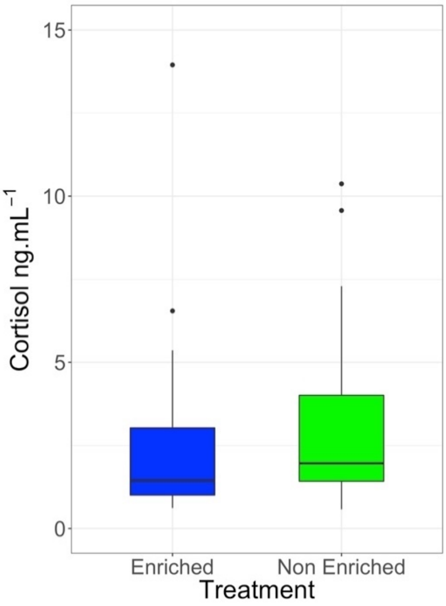


Figure 4-19 March data (S4) of cortisol in Atlantic salmon held in enriched or non-enriched environments. (line=median)

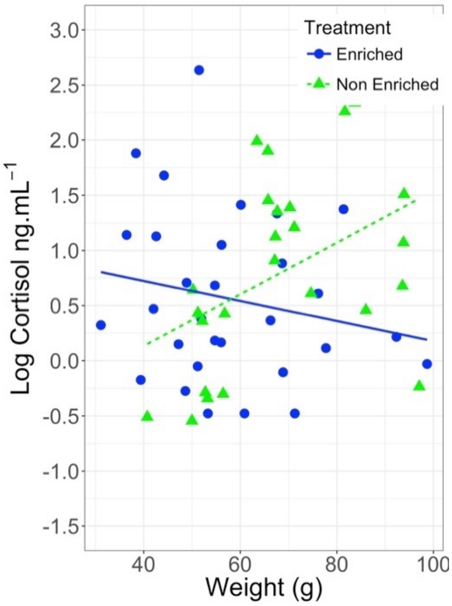


Figure 4-20 March 2017 (S4) plot of log-transformed cortisol against weight, with model prediction lines.

***Table 4-10**. Coefficient (log) estimates (± SE) with associated t and p values for all terms retained in the simplified models for cortisol measurements for (A) January 2017 data (sample 3) and (B) March 2017 data (sample 4). Significant terms are in bold.*

A: Cortisol levels January 2017 (sample 3)

Independent Variables		Coeff (log)	S.E.	*t*	*p*
Intercept		0.897	0.24	3.735	**< 0.001**
Treatment	Non-enriched	-0.849	0.327	-2.598	**< 0.013**

B: Cortisol levels March 2017 (sample 4)

Independent Variables		Coeff (log)	S.E.	*t*	*p*
Intercept		1.088	0.53	2.049	**0.046**
Treatment	Non-Enriched	-1.89	0.866	-2.182	**0.034**
Weight (g)		-0.009	0.008	-1.031	0.307
Treatment x Weight		0.032	0.013	2.469	**0.017**

4.3.2.5 Hepato-somatic Index

The hepato-somatic index (HSI) is calculated by (Liver weight/Body weight) * 100. Plasma cortisol was not available for all fish samples with corresponding liver data therefore only data with both liver weight and cortisol measurements were used in the analysis. Samples taken from January 2017 (S3) and March 2017 (S4) have been analysed separately. Utilising the maximum likelihood method, the model for the January data (S3) was simplified to the effect of treatment only (enrichment) i.e. there was no effect of body weight, cortisol or any interactions. There was a significant difference in HSI with treatment (Figure 4-21, Table 4-11A) in January (S3). HSI values were 18% lower ($\exp^{-0.195}$) in the non-enriched environments compared to the enriched environments (Figure 4-21, 22).

There was no effect of treatment on HSI levels in March (S4) (Figure 4-23). The main effects of body weight and cortisol levels were significant with a small negative coefficient (Table 4-11B), indicating that HSI levels decreased slightly with increasing body weight (Figure 4-24) and cortisol levels (Figure 4-25). For example, a 10% increase in cortisol reduces the HSI by 0.3% ($\exp^{-0.031*\log(1.1)}$) and a 10% increase in weight reduces the HSI by 0.07%.

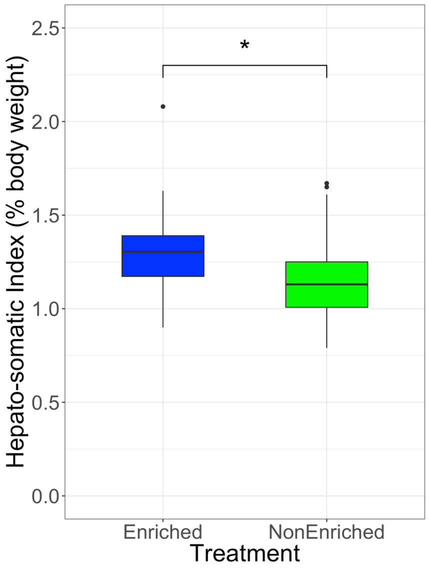


Figure 4-21 *January data(S3) of HSI in Atlantic salmon held in enriched or non-enriched environment. (line=median, * = p<0.05)*

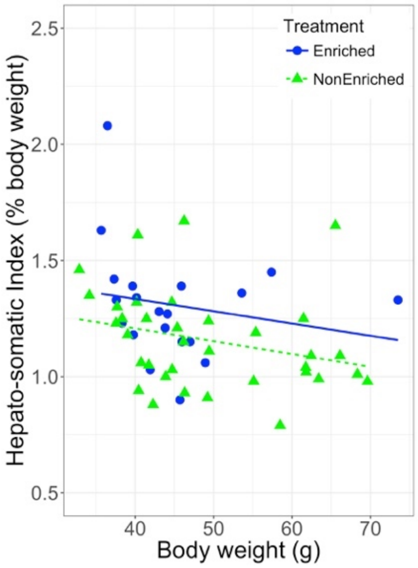


Figure 4-22 *January data(S3) of change in HSI over body weight in Atlantic salmon.*

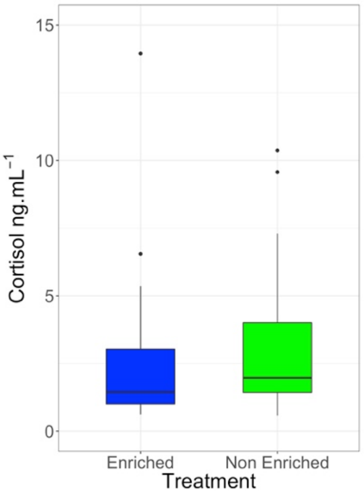


Figure 4-23 *March data(S4) of HSI in Atlantic salmon held in enriched or non-enriched environments. (line=median).*

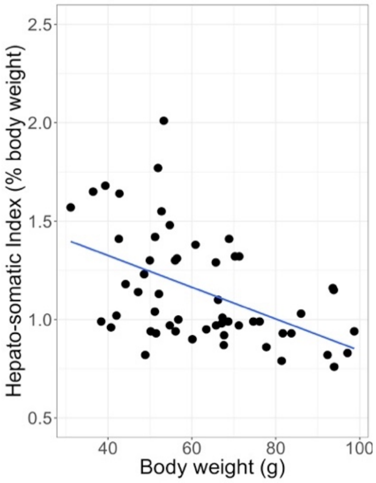


Figure 4-24 *March data(S4) of change in HSI over body weight in Atlantic salmon. HSI reduces with increasing body weight.*

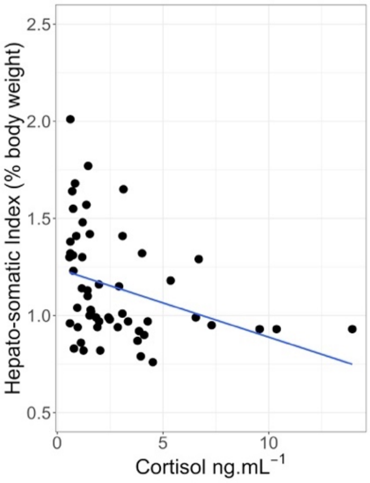


Figure 4-25 *March data(S4) % change in HSI over cortisol levels in Atlantic salmon. HSI reduces with increasing cortisol.*

Table 4-11 *Coefficient(log) estimates (± SE) with associated t and p values for all terms retained in the simplified models for hepato-somatic Index (HSI) for (A) January 2017 data (sample 3) and (B) March 2017 data (sample 4) Significant terms are in bold.*

A: Hepato-somatic Index (HSI) January2017 (sample 3)

Independent Variables		Coeff (log)	S.E.	*t*	*p*
Intercept		0.299	0.06	4.931	**< 0.001**
Treatment	Non-Enriched	-0.195	0.07	-2.778	**< 0.05**

B: Hepato-somatic Index (HSI) March 2017 (sample 4)

Independent Variable	Coeff (log)	S.E.	*t*	*p*
Intercept	0.638	0.103	6.15	**< 0.001**
Cortisol(ng)	-0.031	0.012	-2.469	**0.013**
Body weight (g)	-0.007	0.002	-4.028	**< 0.001**

4.4 Discussion

This study investigated the effects of simple environmental enrichment on growth, fin damage and recovery from stress after a routine major stressor (vaccination and grading) on Atlantic salmon on a commercial fish farm.

Pre-vaccination

The period prior to vaccination and grading showed no significant effect on growth due to enrichment. However, there was less dorsal fin damage and longer dorsal fins in enriched tanks compared with non-enriched tanks and fish with the most severe fin damage tended to be smaller in size. Previous studies have shown that recipients of aggressive attacks tend to smaller than the aggressor (Abbott & Dill, 1985; Cañon Jones et al., 2010; Moutou et al., 1998). One study (McLean et al., 2000) found that larger fish had more fin damage and suggested that the larger fish were fighting amongst themselves for access to territory and food.

Post-vaccination – growth

Working on a commercial fish farm has many challenges resulting in post-vaccination and grading experimental conditions varying from those originally planned. Despite the majority of tanks not remaining in treatment (food withdrawal and enrichment), pre- and post-vaccination, it was still possible to discern some effects. Although not significant there was a tendency for fish to be longer and heavier in enriched tanks compared with non-enriched tanks, when comparing the effects of post-vaccination enrichment only. There was a significant difference in growth at the period just after vaccination and grading, with fish in enriched tanks growing better than those in non-enriched tanks. This may indicate that fish in enriched tanks recovered better and resumed eating faster after vaccination and grading. Pounder et.al. (2016) found that rainbow trout recovered better following a noxious stimulus when provided with structural enrichment. However, by the end of the experimental period there was no difference in growth parameters.

Post-vaccination fin damage

There was less fin damage in enriched tanks compared with non-enriched, although not significant. Fin damage increased in all tanks by the end of the hatchery production run, possibly due to a malfunction of the automatic feeder system prior to the final sample, before fish were transported to sea pens. Fin damage had started to increase prior to the feeder malfunction and it is likely that individual tanks suffered food blockages for short periods, in the spinner arm of the feed delivery system, when food got damp. These instances were not recorded on-farm. Unpredictable feeding is known to increase aggressive interactions (Cañon Jones et al., 2012; Vindas et al., 2014) resulting in increased fin damage (Cañon Jones et al., 2012; Shi et al., 2017). Alternatively, appetite is suppressed during the winter months and the increase in fin damage in March 2017 could be due to the resumption of feeding as water temperatures and day length would be increasing. However, water temperatures only increased from 4°C to 6°C between January 2017 and March 2017. An enrichment study by Näslund et al. (2013) found Atlantic salmon in non-enriched tanks had greater fin deterioration over winter compared with enriched tanks, suggesting that the faulty feeder in the present study played a major role in increasing fin damage in March. Post vaccination enrichment appeared to have a more positive effect on fin damage than pre-vaccination enrichment and tanks that remained in enriched treatment pre- and post-vaccination had the least fin damage. A similar result was found in a study of the effect of enrichment on a spatial learning task. Bergendahl et al. (2016) maintained rainbow trout either without enrichment, exposed to an early period of enrichment then returned to a barren environment, maintained in barren environments then exposed to enrichment near the end or maintained in enriched throughout the rearing period. Fish which had been maintained in the enriched environment, throughout the rearing period, had superior spatial learning abilities compared to the other three treatments. However, fish which only experienced enrichment near the end of the rearing period, performed better compared with no enrichment or fish exposed to early enrichment.

Post-vaccination food withdrawal

Fish farms routinely withdraw food for 48 hrs prior to any major stressful activity such as transporting or vaccination and grading. Results from this study indicate that a period of 48 hr food withdrawal had a worse effect on fin damage than 24 hrs, in the period immediately after vaccination and grading.

Post-vaccination cortisol and hepato-somatic index

The plasma cortisol levels (< 5ng ml^{-1}) are consistent with that found in other studies for unstressed fish. Plasma cortisol levels were < 5ng ml^{-1} in unstressed Atlantic salmon (Mes et al., 2018) and rainbow trout (Pickering & Pottinger, 1989; Pounder et al., 2016). Plasma cortisol levels can vary widely, depending on the stressor. Mild stressors (confinement test) can raise plasma cortisol levels between 25ng – 90ng ml^{-1} (Mes et al., 2018; Näslund et al., 2013) in Atlantic salmon and can be double that after a more stressful stimulus such as a 5-min chase (Barton & Iwama, 1991). The plasma cortisol levels in this study indicate that at the time of sampling there was no lasting effect of the vaccination and grading process and fish were in an unstressed state regardless of treatment and the increase in fin damage in March 2017. Although plasma cortisol levels indicate fish were unstressed at the sample points in this study, there were still significant differences between enrichment. In January 2017 plasma cortisol levels were higher in the enriched tanks compared with the non-enriched tanks. Cortisol levels change with the time of day (Thorpe et al., 1987) so may reflect time of sampling or be related to inherent variability within basal plasma cortisol levels (Ellis et al., 2012). Similar slight elevations in plasma cortisol in enriched environments have been recorded for zebrafish (*Danio rerio*) (von Krogh et al., 2010) and in glucocorticoid levels in mice (Haemisch et al., 1994) and rats (Moncek et al., 2004) and perhaps indicate positive stressors (e.g. eustress (Selye, 1975)). In March 2017 plasma cortisol levels were significantly higher in larger fish in the non-enriched tanks. A previous study (Rosengren et al., 2017), providing shelter as environmental enrichment, noted the same effect of higher cortisol values in larger fish in the non-enriched environment (no shelter) and during a similar time period (February). Elevated cortisol levels are known to suppress appetite in fish and hence reduce growth

(Pankhurst et al., 2008; Pickering, 1993). However, at this time period Atlantic salmon are beginning to smolt and it is known that basal plasma cortisol levels are higher during the parr-smolt transformation and that pre-smolts exhibit a heightened stress response compared with parr (Carey & McCormick, 1998). The lower hepato-somatic index in non-enriched tanks, in January 2017, indicate fish were in poorer condition than fish in enriched tanks although by March 2017 fish were in similar condition, regardless of enrichment. Increasing cortisol levels showed an expected reduction in the hepato-somatic index (Barton et al., 1986) but with such low levels is unlikely to be biologically significant.

By the end of the study the artificial kelp had a thin biofilm which was not of concern to the farmer. As the farmer was heavily involved in the design of the kelp that meant it was practical with minimum disruption to day-day tasks and relatively inexpensive for the farm to implement. It did not interfere with routine maintenance. It only needed to be moved out of the tank when the fish were being pumped out of the tank prior to vaccination and grading. The lack of no real difference in growth at the end of the production cycle may be a deterrent to implementing structural enrichment however, overall general fish welfare was improved and that may translate into a robustness e.g. better response to disease outbreaks. Further investigation is required into this area. Further research is also required on what any effect of prior enrichment has on fish once they go to sea. This would entail following individuals over their lifecycle.

In conclusion, this relatively simple strategy of supplying artificial kelp in tanks appears to have significant benefits in relation to fin damage but no real benefit in growth. Fish seem to recover better from an extreme stressful event such as vaccination and grading. The mechanism by which this occurs may be through reduced energy expenditure. Either by providing a place to escape aggressive conspecifics, decreasing stress levels or as a holding station to catch drifting food; a natural behaviour in Atlantic salmon. The addition of this environmental enrichment may have, through facilitating more natural behaviours, improved fish welfare and consequently reduced the incidence of fin biting.

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5 Effect of environmental enrichment on spatial distribution, swimming behaviour and fin damage of Atlantic salmon (*Salmo salar*) on a freshwater commercial farm

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Preface

There is a huge knowledge gap in our understanding of environmental enrichment at the farm-level. Whereas Chapter 4 investigated the effect of enrichment on some health and physiology welfare indicators, this chapter focuses on the effect of enrichment on behaviour. Monitoring behaviour is a non-invasive welfare indicator often used by farmers. Good behavioural welfare indicators for fish can include spatial distribution within the tank, swimming speed, motivation to feed and the number of aggressive incidents. Using these indicators, the results of this study suggests that fish reared in tanks with structural enrichment had better welfare than fish reared in conventional hatchery tanks. Quantitively analysing behavioural data is laborious therefore methods to streamline this process, especially for on-farm use, is desirable. Chapter 6 details a novel method to assess the effect of enrichment on fish behaviour.

This chapter was designed for publication in the journal Aquaculture hence there is some duplication in the method section with Chapter 4. Many thanks must go to the staff and management of Loch Duart Hatchery, Scotland, UK who allowed us to run the experiment at their farm site. The experimental design was in consultation with myself, my supervisors and farm staff. Laura Dunn (SRUC MSc student) and I collected the videos. Timothy Wiese (Stirling University PhD student) helped to extract feeding events from the videos. The data analysis and manuscript preparation were done by me with edits from my supervisors.

5 Effect of environmental enrichment on spatial distribution, swimming behaviour and fin damage of Atlantic salmon (*Salmo salar*) on a freshwater commercial farm

Abstract

There is increasing awareness of the need to provide complex, enriched environments for captive animals to ensure good welfare. The welfare of farmed fish is coming under increasing scrutiny by the public and consumer. Farmed fish, particularly in land-based tank systems are held in barren tanks for ease of cleaning, maintenance and to reduce the spread of disease. Results from laboratory studies show that enriched environments can have positive effects on physiology and health which can lead to improved welfare. However, there is huge knowledge gap in our understanding of the effects of environmental enrichment at the farm-level. This study investigated structural environment enrichment on a commercial farm. Atlantic salmon parr were held in standard production tanks at normal industry stocking levels or in tanks with structural enrichment. The study took place in a commercial farm under the normal husbandry procedures for that facility. Video was recorded in all tanks and analysed to investigate the spatial distribution of fish within tanks, dorsal fin damage and behaviour before, during and after a feeding event. Fish were less crowded in enriched tanks and more vertically distributed compared with the non-enriched standard tanks. There was less dorsal fin damage and a more settled appearance in swimming behaviours, indicating that fish were calmer in enriched tanks. These results have important implications for the husbandry and welfare of farmed Atlantic salmon.

5.1 Introduction

For financial reasons, intensive fish farming practices involving salmonids requires stocking densities which exceed the density of fish normally found in the wild. This can lead to an increased frequency of social encounters and hence potentially aggressive encounters (Huntingford, 2004) compromising welfare. Fish do not utilise all of the space in tanks, preferring to stay away from the surface until feeding time, for example. This can result in localised areas which are very crowded, leading to reduced oxygen levels and creating sub-optimal environments for some fish (Johansson et al., 2006). Fish are kept in barren tanks for ease of cleaning, removal and transfer of fish and the reduction in the spread of disease. The farm environment is less challenging compared with in the wild in that food is readily available and is usually free from predators (Huntingford, 2004). However, the evidence for the benefits or otherwise of enriched environments for farmed fish is currently lacking. Most studies to date have viewed fin biting as a harmful, undesirable behaviour exacerbated by the somewhat artificial conditions inherent to fish farming. Research is starting to focus on providing some form of enrichment to mimic salmonid's natural environments in an effort to reduce fin biting. It has been shown that the majority of fin damage sustained in farmed fish is due to fish biting each other (Abbott and Hill, 1985; Turnbull et al., 1998), especially the dorsal fin. Laboratory enrichment studies have shown reduction in fin damage in Atlantic salmon and rainbow trout (two commercially important species) when provided with structural enrichment (Berejikian, 2005; Näslund et al., 2013; Rosengren et al., 2017).

The provision of structural environmental enrichment has been to be shown to increase behavioural flexibility and social learning in fish and produce fish better able to adapt to novel situations (Näslund & Johnsson, 2014). Fish recovered faster from a stressor such as being removed from the water (Pounder et al., 2016). Basal levels of plasma cortisol were 2-3x higher in barren tanks compared with enriched (Näslund et al., 2013); with levels similar to that found in Atlantic salmon exposed to chronic stress (Fridell et al., 2007). Fish without access to shelter were found to have a higher resting metabolic rate compared to those with shelter which the authors suggest is due to increased vigilance and maintenance of a flight response (Millidine et al., 2006). These results suggest that enrichment can potentially provide a lower stress environment by ameliorating the effects of chronic stress as opposed to acute stress.

The use of structural enrichment in experimental tanks has been shown to reduce fin damage and has the potential to reduce stress. The study presented here investigated the effect of structural enrichment on a commercial fish farm with regards to behaviour and fin damage. We focus on damage to the dorsal fin as it has been shown previously that the dorsal fin is the main target for fin biting (Turnbull et al., 1998).

5.2 Method and materials

5.2.1 Ethical Review

Approval for the enrichment experiment was obtained from the University of Stirling's Animal Welfare and Ethical Review Body (AWERB) as part of a PhD project investigating fin damage in farmed Atlantic salmon (IoA AWERB April 15 interim ASPA 04.docx).

5.2.2 Animals, Housing and Husbandry

Atlantic salmon (*Salmo salar*) of 12 months old from a hatchery and rearing site owned by Loch Duart Salmon Ltd, near Badcall Bay, Sutherland in Scotland, UK was used in the study (Figure 5-1). The site had 23 tanks, 5m diameter x 2m depth supplied with fresh-water via a flow-through system from nearby Loch Duart (Figure 5-1). All tanks were outside exposed to a natural photoperiod all year long and ambient temperatures. At time of video recording water temperature was 5°C ± 0.1°C. The experiment was conducted under the standard husbandry practices for the Loch Duart hatchery. Fish were fed standard salmonid pelleted rations (Skretting Nutra Advance/Supreme©) every 10-20 minutes, controlled by a central computer at an individual tank level and varied across the season between daylight hours. Food was provided to each tank using a compressed air system with a spinning arm to distribute food around the tank (Figure 5-2) (Arvo-tec, Finland). Tanks were cleaned daily and mortalities removed between 9am-11am except on days when video recording was taking place, at which time cleaning was done after recordings were complete. At the time of recording there was approximately 15,000 salmon in each tank with an average stocking density of $20 kg/m^3$.

Structural enrichment was provided in ten tanks while twelve tanks were left as standard production tanks (Figure 5-3). Enrichment consisted of green artificial (plastic) kelp with dimensions 150mm (w) x 1500mm (L) (Figure 5-4). Six ropes were evenly strung across the top of the tank, three on each side of the central pillar. Each rope held three strips of artificial kelp in position, which were weighed down to keep them vertical (Figure 5-4 and Figure 5-5).

Figure 5-1 *Layout of tanks on farm*

Figure-5-2 *Spinner arm for distributing food*

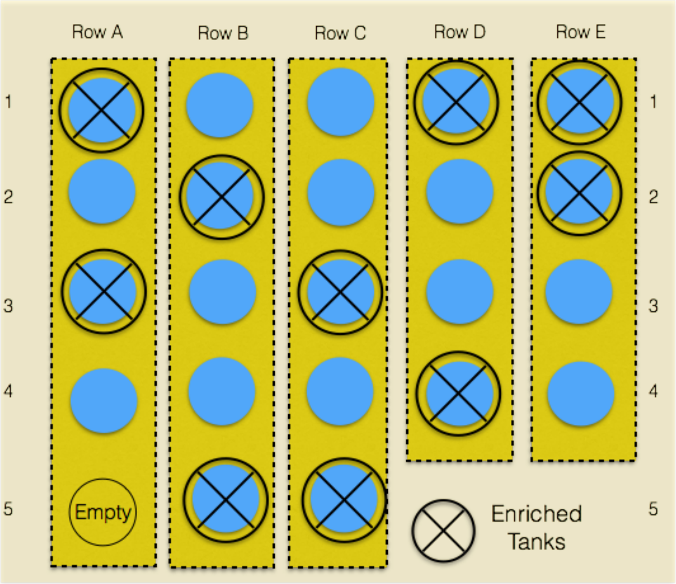

Figure 5-3 *Farm layout of tanks, showing tanks with enrichment and tanks without enrichment. Tank A5 was empty for the duration of the study*

Figure 5-4 *Artificial kelp suspended in tank and kept vertical by a weight at the bottom*

Figure-5-5 *Artificial kelp in tank with Atlantic salmon*

5.2.3 Behaviour assessment

Videos were recorded in each tank on the 22nd March 2017, using six GoPro® cameras in waterproof casings (Figure 5-6). Prior to March, the turbidity of the water made observing fish behaviour difficult. Each camera was attached to a pole, which was clamped to the top of the tank, to record at a depth of 1.6m (Figure 5-7). Video was recorded in each of the 22 tanks between 10am and 12 noon for approximately 17 minutes, to catch at least one feeding event. Video clips were extracted from the main recordings to cover 30 seconds prior to a feeding event up to 2 minutes post feeding event. Videos from seven of the tanks were not utilised, either due to some disturbance near the feeding event, not being able to detect a feeding event or turbidity in the water making observation of the fish difficult. One video was used to assess the range of behaviours observable in the tanks and to develop an ethogram to analyse fish behaviour (Table 5-1). Swimming behaviours were the dominant observable behaviour. Data from this recording was not used in subsequent analyses. The final analysis included video clips from seven enriched tanks and seven non-enriched tanks.

Table 5-1 *Ethogram for Atlantic salmon*

Behaviour	Description
Active	Continuous smooth propulsion over at least 0.5x the camera field of view. Active swimming can be fast or slow, defined below.
Stationary	Stationary /holding position. Can be swimming in place with fewer than 2 tail beats per second or move less than half the body length in distance. Should not involve significant forward propulsion.
Swim Fast	Continuous smooth propulsion across screen with tail beats > 3 per second
Swim Slow	Continuous smooth propulsion across screen with tail beats < 3 per second
Face clockwise direction	Fish orientated in clockwise direction. Same direction as water flow but in opposition to the orientation (anti-clockwise) of the majority of fish in the production tank.
Change direction	Fish turn 90° to face camera or towards the centre of the tank. Can be actively swimming or holding station
Reverse direction	Fish reverses swimming direction (180° turn)
Aggression	Fish charges, nips or chases, causing conspecific to flee
Feeding	Fish observed to eat food pellet. Note whether actively approach food or hold position waiting for food.

The occurrence of aggressive and feeding behaviour was rare therefore the frequency of these behaviours was recorded for the original 17-minute recordings. The remaining behaviour categories in the ethogram were recorded in the extracted clips with the feeding event. Each of these clips was sectioned into 5 second segments. For each segment, one fish (the focal fish) was followed and the presence of any of the behaviours in the ethogram recorded. This gave 24 individual observations of behaviour from the feed event video clip. The focal fish was selected from the first fish to emerge from either the left or right of the screen, from a group nearest to the camera. To be considered as being near the camera, the body length of the fish was set at a minimum of 0.25 x the width of the viewing screen. Dorsal fin damage for each focal fish was assessed as being present or absent.

Figure 5-6 *Go Pro® camera and waterproof housing*

Figure-5-7 *Camera in waterproof housing attached to the bottom mounting pole*

5.2.4 Spatial distribution in tanks after the introduction of a novel object

Additional recordings were made by attaching three cameras to two poles, with cameras placed at 0.3m, 0.7m and 1.6m below the top of the water. There were two poles with three cameras attached. Videos were recorded in three enriched and three non-enriched tanks for approximately 15 minutes. The behavioural response of fish to immersing a novel object (the camera pole in the instance) into the tank is to flee to the bottom of the tank. To assess the vertical distribution of the fish, the latency to appear in each of the cameras after the cameras were inserted into the tank was recorded. For each video a record was made of the time the camera was inserted and when the first ten fish appeared in the viewing screen of each camera. The cameras faced in towards the centre of the tank therefore multiple fish were always visible in the background. To be considered as being near the camera the body length of the fish was set at a minimum of a 0.25 x the width of the viewing screen.

5.2.5 Statistical analysis

Data analysis was carried out using R software (version 3.5.3) (R Development Core Team, 2019). In all statistical tests, p= 0.05 was taken as the level of significance.

Spatial distribution and response to a novel object

A mixed-effect linear model (lme4 package) was used to analyse the effect of enrichment on the response time to approach the vicinity of cameras with enrichment type as a fixed factor and tank as a random factor. As the *lmer* function in the lme4 package does not provide p-values, the *Anova* function in the car package was used to calculate p-values for the fixed effect in the model. The *Anova* function provides an Analysis of Deviance Table (Type II Wald chisquare tests). A separate model was constructed for cameras situated at 1.6m and 0.7m. No fish in non-enriched tanks approached the top camera at 0.3m. Normality and homoscedasticity of the residuals, for the models, were checked using Q-Q plots and standardised residuals vs. fitted values.

Behaviour

Individual fish were not externally marked or tagged therefore data is presented for each tank. Principal components analysis (PCA, covariance matrix, no rotation) (FactoMineR package in R) was used to examine the relationship among the number of events of observed behaviours and combined into a single behaviour score. The PCA output score was subsequently used to analyse the effect of enrichment on swimming behaviour using a general linear model (R Stats package) with PCA output score as the dependent variable and enrichment type as the independent variable. The likelihood ratio test was used to test the significance of the overall model. Normality and homoscedasticity of the residuals, for the models, were checked using Q-Q plots and standardised residuals vs. fitted values.

Dorsal fin damage

A generalised linear model (GLM – R Stats package) was constructed to investigate the effect of the independent variable enrichment on dorsal fin damage (dependent variable). Dorsal fin damage was scored as either present or absent. The proportion of sampled fish with and without dorsal fin damage was then calculated for each tank. Logistic regression with a binomial distribution and logit link function was employed for the GLM. The Wald statistic was used to check the significance of the regression coefficient and the likelihood ratio test was used to test the significance of the overall model.

5.3 Results

5.3.1 Aggressive incidents

The number of aggressive incidents observed was low with only six incidents being recorded over all the videos (Table 5-2). Incidents were recorded in two enriched tanks (B5, C5) and two non-enriched tanks (A4, E4) and did not appear to be related to feeding time. In all cases the attacked fish quickly fled the area and in 4 out of 6 incidents the perpetrator took the vacated position. Most of the attacks came from below with bites to the pelvic region observed. The dorsal region was the focus of the majority of the attacks. Prior to the attacks the perpetrator was in close proximity (~ 1.5 body lengths) to the victim in 5 out of 6 incidents. In tank E4 the attacker approached from off screen, orthogonal to the direction of the other fish and bypassed other fish to access its target. The rate of incidents is 1.5 per 17 minute which equates to 127 incidents per day. This result illustrates that over the lifetime that fish are in the production system aggressive incidents can be substantial.

Table 5-2 *Description of aggressive incidents recorded.*

	Attack direction					
TankID	Behind	Below	Above	Contact Position	Bite	Took position
C5	x	x		Pelvic fin	x	x
C5		x		Dorsal fin		x
B5		x		Caudal fin		x
A4		x		Pelvic area	x	x
B5	x		x	Dorsal fin		
E4			x	Dorsal area		

5.3.2 Spatial distribution and response to a novel object

In all tanks fish fled towards the bottom of the tank and to the opposite side from where the camera was inserted. Initially there were no fish near the lower camera at 1.6m. Fish appeared at the 1.6m camera in all tanks (Figure 5-8) but there was no significant difference in approach times between enriched and non-enriched tanks. Fish in four out of six tanks appeared at the camera at a depth of 0.7m. Fish in one non-enriched tank (E3) and one enriched tank (B2) did not approach the camera at 0.7m. Fish in enriched tanks were 3.7 minutes faster in returning to a depth of 0.7m and this result was statistically significant [χ^2=4.02, df=1, p=0.045]. By the end of the observation period fish appeared at a depth of 0.3m in two enriched tanks but in none of the non-enriched tanks. This result suggests in enriched tanks fish are more spatially distributed and quicker to approach a novel object.

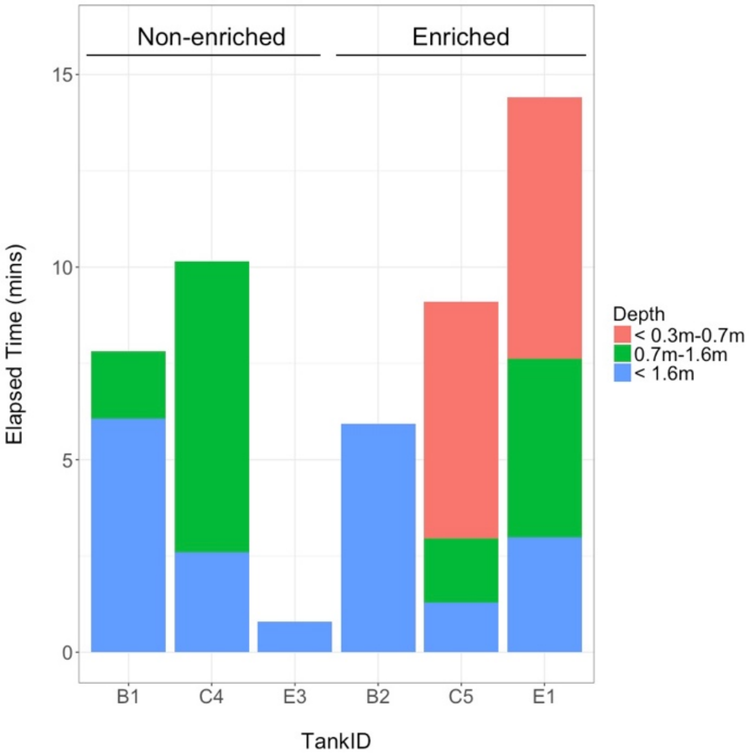

Figure 5-8 *Time taken for fish to distribute upwards in the water column, after presentation of a novel object (camera) into the tank. No fish in the three non-enriched tanks reached 0.3m whereas fish in two out of the three enriched tanks reached 0.3m. Transition points are at 1.6m, 0.7m and 0.3m below water level.*

5.3.3 Behaviour before during and after a feeding event

Fish tended to distribute around the perimeter of the tank in an anti-clockwise direction, facing against the direction of water flow.

5.3.4 Food anticipation

On food delivery, all fish moved away from the surface in nine out of fourteen tanks before resuming their initial depth a few seconds later. There was no noticeable reaction to food delivery in tanks E1-E3 (nearest the food delivery hopper) or D4 and C5. Very few fish approached the surface for food pellets in any tank (< 5). Fish actively feeding was only observed in seven tanks (4 x non-enriched and 3 x enriched) with an average of 4 pellets per tank. Fish actively moving towards a food pellet was only observed 5 times.

The majority of fish, that fed, held station while taking food. This result suggests there was no food anticipatory activity and few fish feeding during this observation period.

5.3.5 Effect of enrichment on swimming behaviour

The first two components from the PCA accounts for 84% of total variance with PC1 accounting for 70% of total variance. Only PC1 had an eigenvalue > 1 (Table 5-3).

Table 5-3 *Percentage variation and eigenvalues of principal components (PC1-PC4)*

	PC1	**PC2**	**PC3**	**PC4**
Eigenvalue	2.186	0.439	0.276	0.181
% variation explained	70	14	9	6
% cumulative variation	70	84	93	99

As only PC1 had an eigenvalue >1 therefore only PC1 output score was used to test the effect of enrichment. PC1 generated positive loadings for stationary swimming behaviours and negative loadings for active swimming behaviours (Table 5-4). Active swimming was strongly associated with fast swimming, swimming against the normal direction and with frequent changes of direction (Figure 5-9) and fish in non-enriched tanks were significantly more likely to be active and swimming chaotically compared with enriched tanks (Coeff=2.29, s.e. ±0.48, t-value=4.73, $p < 0.001$).

Table 5-4 *Loadings on the first two principal components (PC). 'Reverse direction' is a complete 180° turn whereas 'Change direction' fish turned towards the camera or towards the tank centre. Face CW is fish facing clockwise in opposition to the majority of the fish in the tank.*

Variable	PC1	PC2
Active	-0.454	0.046
Stationary	**0.416**	-0.018
Swim Fast	**-0.669**	**0.426**
Swim Slow	-0.077	-0.218
Face CW	-0.344	-0.416
Reverse direction	-0.070	-0.524
Change direction	-0.208	**-0.565**

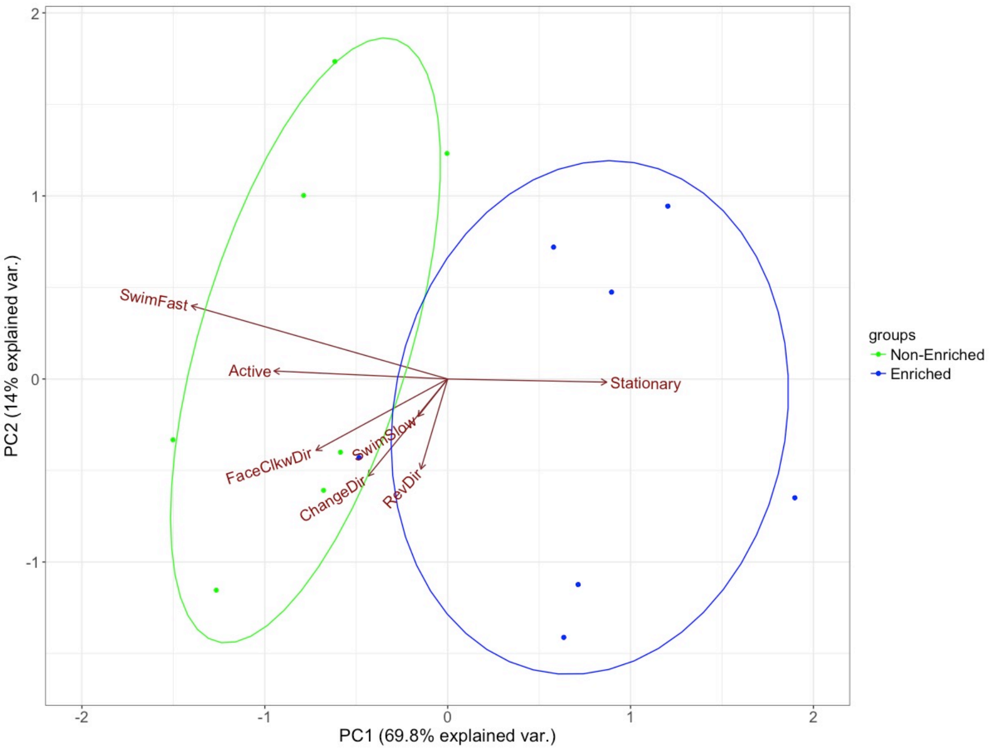

Figure 5-9 *Plot of PCA output and behaviours variables grouped by treatment (non-enriched and enriched). Circles= 95% confidence ellipses. Swimming behaviour in enriched tanks is characterised by holding station or slow swimming. In non-enriched tanks swimming behaviour is characterised by more activity with fast swimming, more changes of direction and more likely to be facing in opposite direction to the majority.*

5.3.6 Dorsal fin damage

The results of a logistic regression suggest the presence of enrichment in tanks had a protective factor with a 0.49 decrease in the odds ratio of sustaining dorsal fin damage in enriched tanks compared with non-enriched tanks [odds ratio (β) =0.49, se ±0.11, 95% CI: 0.27 – 0.72, Wald = 4.29, $p < 0.001$]. Figure 5-10 shows the proportion of fish sustaining dorsal fin damage for non-enriched and enriched tanks.

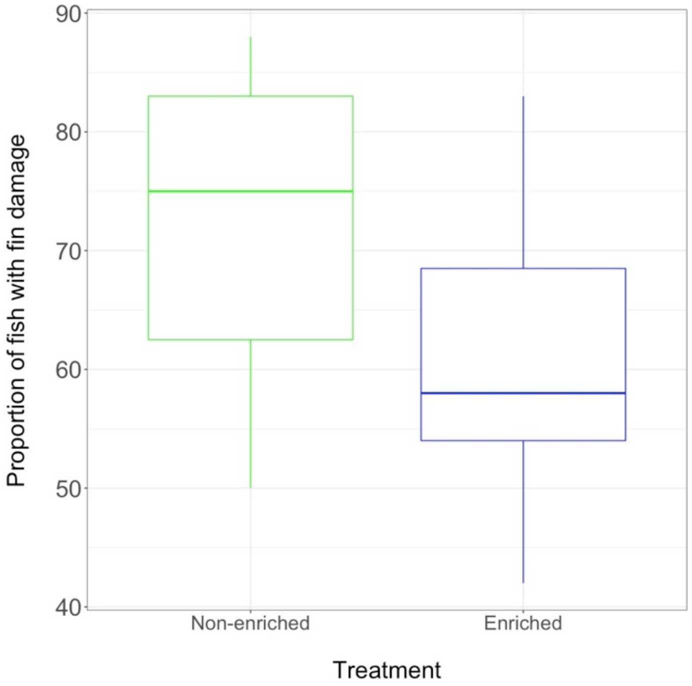

Figure 5-10 *Proportion of fish with damaged fins. There was a higher proportion of fin damage in non-enriched tanks compared with enriched (centre line =median).*

5.4 Discussion

On inserting the camera into the water fish fled to the bottom of the tanks. There was no difference in the time taken for the fish to return back to 1.6m depth however, fish returned faster to a depth of 0.7m in the enriched tanks. The latency to return after the presentation of a novel object may be an indication of stress (Bui et al., 2013). No fish in any of the non-enriched tanks moved into the vicinity of the camera at 0.3m whereas fish in two out of three enriched tanks moved up to within 0.3m of the water surface. This suggests that fish in enriched tanks were more spatially distributed within the tanks. Observation of fish at 1.6m in the enriched tanks showed more space between fish compared to non-enriched tanks which looked very crowded in comparison. There is little in the literature with regards to spatial distribution of fish in freshwater tanks. The localised density of fish is likely to be higher in non-enriched tanks which can lead to sub-optimal environments and an adverse effect on fin damage, in general (Ellis et al., 2008).

There was no difference in feeding anticipatory behaviour between enriched and non-enriched tanks. Fish did not respond to food delivery by going to the surface and instead momentarily moved downwards in the tank. This is in marked contrast with that seen in Atlantic salmon sea cages where fish swam towards the surface with subsequent movement downwards and towards the periphery as hunger was reduced (Juell et al., 1994). However, Atlantic salmon post-smolts actively seek food (Haugland et al., 2006) whereas salmon parr have a “sit-and wait” approach (Metcalfe et al., 1997). They maintain a stationary position and catch drifting food particles or occasionally dart towards food particles from a stationary position. Previous studies have used a conditioned response to light as a measure of food anticipation. The latencies to respond to the light was used to gauge motivation to feed after a stressor (Folkedal et el., 2012). Conditioning responses in fish would not be practical on-farm therefore the lack of food anticipation does not appear to be a useful behaviour to measure on-farm to assess welfare. Current farm practices use feed conversion ratios (FCR) and specific growth rate (SGR) to monitor feeding. Any changes from normal alerts farmers to potential problems very quickly. It is likely that the fish were not hungry during the observation period especially as few fish were seen to take food pellets. Observations were carried out in

March at a time when fish were smolting which may account for the reduced eating (Pankhurst et al., 2008). Feeding response was similar in all tanks therefore the presence of enrichment was unlikely to have impeded access to food.

There were few aggressive incidents recorded. However, the occurrence rate over the life time of the fish in the production system could still be substantial. Fish were not fed overnight, so it would be of interest to observe behaviour in the tanks overnight especially during the first feed of the morning as salmonids are known for aggressive behaviour for a period during sunrise (Kadri et al., 1997; Gregory & Griffiths, 1996).

It would appear that fish in enriched tanks were less affected by the introduction of the camera as the latency to return to the middle camera was a lot less than that for similar fish in conventional non-enriched environments. Exposure to structural enrichment in the rearing environment has been shown to affect neural plasticity and cognitive abilities (Ebbesson & Braithwaite, 2012), producing fish better able to adapt to varying and novel situations (Näslund & Johnsson, 2014). Environmental condition has an impact on fish development as a fish's brain remains plastic throughout its life (Kihslinger & Nevitt, 2006). Atlantic cod (*Gadus morhua*) reared in an enriched environment were faster to explore a novel environment (Braithwaite & Salvanes, 2005). Atlantic salmon were able to find the exit from a maze more efficiently than similar fish reared in conventional tanks (Salvanes et al., 2013) and juvenile steelhead trout (*Oncorhynchus mykiss*) grew larger cerebella when reared in enriched tanks compared with similar fish in conventional tanks (Kihslinger & Nevitt, 2006). Enriched environments can potentially provide a lower stress environment. Fish recovered faster from a stressor such as being removed from the water (Pounder et al., 2016) or chased by a net (Braithwaite & Salvanes, 2005). Basal levels of plasma cortisol were higher in conventional tanks compared with enriched tanks (Näslund et al., 2013; Cogliati et al., 2019); with levels similar to that found in Atlantic salmon exposed to chronic stress (Fridell et. al., 2007).

The swimming behaviour of fish indicated that fish in enriched tanks moved about less; they were more 'settled'. Juvenile Atlantic salmon are territorial, sit-and-wait predators (Metcalfe et al., 1997) and visibility may affect their ability to defend a territory and locate food. Increasing environmental complexity reduces visibility for visually oriented species such as Atlantic salmon, thereby reducing movement between territories and limiting the need to chase off conspecifics. Previous studies have shown that environmental complexity reduces territory sizes and increases density of fish within an area (Kalleberg, 1958; Eason & Stamps, 1992; Roni & Quinn, 2001; Dolinsek et al., 2007) with a reduction in fin damage (Näslund et al., 2013; Rosengren et al., 2017). However, habitat complexity did not increase the density of juvenile rainbow trout (*Oncorhynchus mykiss*) as they aggressively defended smaller territories (Imre et al., 2002).

Farmed salmon are normally kept in a homogenous environment at stocking densities far in excess of that found in the wild. When exposed to moderate water flows salmonids tend to orientate into the current and adopt a schooling swimming pattern which reduces agonistic behaviour (Jobling et al., 1993) and minimises the risk of collision with neighbouring fish and the enclosure walls (Føre et al., 2009). The addition of artificial kelp to the environment appears to have disrupted this schooling behaviour. The vertically-suspended artificial kelp potentially reduces the in-tank water velocity (Moine et al., 2016) and allows fish to reduce energy expenditure by holding positions in low velocity locations (Huntingford et al., 1988). Low water velocity can disrupt the schooling activity with fish swimming about in all directions (Nilsen et al., 2019) however in the present study fish remained orientated against the current flow with minimal movement. Water velocity can affect fish growth and many studies have shown positive effects on growth on several species of salmonids when they have been forced to swim against currents of between 0.75-1.5 body lengths^{-1} (Houlihan & Laurent, 1987; Jobling et al., 1993; Castro et al. 2011; Solstorm et al., 2015). Increasing water velocities (> 1.5 body lengths^{-1}) has been shown to reduce growth and impair fish welfare (Solstorm et al., 2015). Rosengren et al. (2017) found that environmental enrichment reduced growth in Atlantic salmon suggesting a lack of motivation to forage due to risk-averse behavior with fish preferring to remain in shelters, although changes in water velocity was not investigated. This present study did not show a positive effect of environmental enrichment on growth (Chapter 4). However, studies using vertically-suspended enrichment found increased weight gain in rainbow trout compared to no enrichment but did not report behaviour activity (Kientz et al., 2018; Crank et al., 2019).

Recording fish at one point in the day may potentially bias results as fish behaviour changes throughout the day. Fish were recorded mid-morning when there was enough visibility without having to introduce artificial lighting (another potential source of bias) and fish will have been fed after a night of no feeding. Low visibility during the night may set up smaller territories in much the same way as the environmental enrichment. As light increases it forces a change in the social hierarchy and changes in spatial distribution which coupled with the start of feeding time can lead to increases in aggression (Gregory & Griffith, 1996). However, monitoring behaviour at one point in time, after behaviour has stabilised, allows comparisons to be made between tanks with different treatments.

The reduction in visibility and water velocity through the use of environmental enrichment may have set up more areas within the tank that are optimal for fish to remain stationary within, leading to fish being more spatially distributed. However, environmental enrichment may compromise growth therefore further investigation is required to determine the effects of environmental enrichment on water velocity, fish movement and growth parameters.

In conclusion, the provision of structural enrichment appears to have had a positive effect of fish welfare. Dorsal fin damage was reduced in enriched tanks and this may be due to fish being more spatially distributed so there is less contact between them and consequently density is reduced so fewer sub-optimal areas within the tank. Behaviour in enriched tanks appeared more settled indicating less stress although further validation is required by comparing with physiological measures by tagging and following a number of individuals.

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6 Novel use of qualitative behaviour assessment to monitor welfare and environmental enrichment in farmed Atlantic salmon (*Salmo salar*)

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Preface

This chapter describes how qualitative behaviour assessment (QBA) was used to assess whether changes in behaviour were detectable between fish reared in enriched versus non-enriched environments. Data was collected during the experiment described in Chapters 4 and 5. Behaviour (i.e. body language) is often used by experienced farmers to alert them to deviations from normal behaviour, indicating potential welfare issues. However, they often find it difficult to articulate exactly what that entails making it difficult to train other people. Quantitative measures of behaviour such as that undertaken in Chapter 5 are laborious to collect on-farm and results are generally not immediately available. QBA is a novel method for assessing fish behaviour which can potentially give immediate feedback of results and provide a tool with which to train staff.

This chapter was designed for publication (Applied Animal Behaviour Science) hence there is some duplication in the method section with Chapters 4 and 5. Thanks go to Dr. Susan Jarvis who first suggested the possible use of QBA with fish. Co-authors and I contributed to the experimental design. An MSc student from SRUC (Laura Dunn) and I recorded the videos. Laura's MSc thesis investigated inter and intra reliability of observers using QBA to assess fish and generated a fixed list of descriptors which I subsequently used in my analysis of QBA and enrichment. I prepared and presented the information and training session to collect the data, with guidance from Marianne Farish at SRUC. I analysed the data with support from Prof. Francoise Wemelsfelder at SRUC and prepared the manuscript with edits from my co-authors.

6 Novel use of qualitative behaviour assessment to monitor welfare and environmental enrichment in farmed Atlantic salmon (*Salmo salar*)

Abstract

Fish welfare is an important issue within aquaculture and animal-based welfare monitoring tools are required. Qualitative Behavioural Assessment (QBA) is a scientific method for assessing the subjective experience of animals through the expressive qualities of behaviour. It is not necessarily *what* the animal is doing but *how* it is doing it, often referred to as 'body language'. Body language can therefore be used to infer an animal's physical or physiological state. This is the first study to use QBA techniques to study the behavioural effects of structural environmental enrichment on fish. A list of twenty descriptive terms previously used to describe expression in Atlantic salmon was used. Eleven observers used a visual analogue scale to score each term from zero to maximum expression while viewing 20 video clips of the fish. The scores were then analysed using Principal Component Analysis (PCA) however, there was poor inter-observer agreement (Kendall's W < 0.4) between the eleven observers. A group of four observers had acceptable inter-observer (Kendall's W > 0.7) and were used for subsequent analysis. The scores of the four observers were analysed using PCA and identified two main principal components (PC) that accounted for 58% of the variation with PC1 accounting 45% of the variation. PC1 was characterised as tense/stressed versus content/tranquil and PC2 characterised as startled versus listless. An ethogram was used to quantify fish behaviour in the same 20 video clips (swimming speed and frequency of chaotic, calm and inquisitive behaviours). Only chaotic behaviour correlated strongly with the qualitative assessment of tense/ stressed expressivity (PC1). A linear model showed a significant affect (p < 0.001) of enrichment with enriched tanks having higher scores for terms describing positive valence (content/calm) and non-enriched tanks having higher scores for terms describing negative valence (tense/stressed). Results showed that 4 observers had good agreement and are able to consistently observe differences between tense and calm fish. This showed that QBA has potential for use in welfare assessment within aquaculture.

6.1 Introduction

Fish welfare is an important issue within aquaculture. Validated and practical methods to assess the welfare of the fish within the farm environment are needed. However, assessing the welfare of fish in aquatic environment is difficult. Physiological indicators, such as levels of stress hormones, are often used as welfare indicators. However, physiological measures of welfare normally require invasive techniques and can vary in a complex fashion and require context as they can vary with positive experiences as well as negative. The stress response is often used as an indicator of animal welfare (Dawkins, 2003) but it only becomes a problem when a stressor is excessive, prolonged (repeated) or mismanaged.

Fish have to be handled and removed from the water, which is extremely stressful for fish. Some measures require the animal to be killed. Physiological measures of stress also require controlled environments which gives rise to practical issues with assessing welfare on farms, especially in remote locations and at open water pens and cages. Physiological responses can also vary with time of day, temperature and season (Pickering & Pottinger, 1983; Ebbesson et al., 2008; Isorna et al., 2017). The interpretation of physiological responses can be difficult as they only indicate levels of arousal but not the valence of the animal’s experience. For example, cortisol levels are elevated after an aggressive interaction in the fish being the aggressor as well as the fish on the receiving end of the aggression (Øverli et al., 1999); however, the welfare outcome would be different for each fish. Context is usually provided by quantitively assessing behaviour such as the number of aggressive interactions, changes of direction of swimming or duration of swimming event. However, linking physiological responses retrospectively with behaviour is difficult when there is a low incidence of that behaviour or it is difficult to quantify (Rousing & Wemelsfelder, 2006). This is especially true in fish tanks, with upwards of 15,000 fish making it impossible to follow individual fish for any length of time.

An alternative qualitative approach, which looks at the whole animal or group as a whole, may provide a more useful tool to provide a rapid, reliable and valid way to monitor changes in the welfare of fish over time. Qualitative behaviour assessment (QBA) is used extensively in the social sciences and has been adapted for use in animal welfare science. QBA does not describe the behaviour (e.g. slowly, darting) but describes the expression of that behaviour (e.g. calmly, anxiously), better known as an animal's body language. It is not *what* an animal is doing but *how* it is doing it. Body language expresses an animal's mood or emotional state which we can use to interpret an animal's welfare state i.e. whether it is generally content or distressed (Wemelsfelder, 2007). QBA links subjective judgements about body language with quantitative measurement approaches (Wemelsfelder et al., 2000; Wemelsfelder, 2007). In QBA analysis, observers assess an animal's body language using descriptive terms which describe an animal's behavioural repertoire. These terms have an expressive, emotional connotation (e.g. relaxed, tense, irritated, calm) and can be individually generated by observers, as in the case of free-choice profiling (FCP) or observers can be provided with a fixed list, generated and validated by the researchers and tested on-farm (Wemelsfelder, 2007). Observers can watch animals either live or on pre-recorded video and score each of the items on a visual analogue scale (VAS), which can be then be used to generate quantitative data for subsequent statistical analysis.

QBA has been used to assess welfare concerns in a variety of animals. Good agreement between observers has been found in assessments of pigs (Wemelsfelder et al., 2000, Wemelsfelder et al., 2012), dairy buffalo (Napolitano et al., 2012), sheep (Phythian et al., 2013; Stockman et al., 2014), cattle (Stockman et al., 2013; De Boyer des Roches et al., 2018), goats (Muri et al., 2013; Grosso et al., 2016) and donkeys (Minero et al., 2016). However, large intra-observer variation has been reported with dairy cows (Bokkers et al., 2012). QBA can potentially be used to assess positive welfare as well as negative welfare. A study of mastitis in dairy cows (De Boyer des Roches et al., 2018) found that cows were assessed as more lethargic and dejected in the acute sickness phase whereas during the remission phase they were assessed as calm and relaxed. The use of qualitative descriptors is what makes QBA an efficient assessment tool allowing a single scale to capture many characteristics of animal behaviour (Meagher, 2009). Individual standardised behavioural tests are normally required to capture different behaviour characteristics. For example, an elevated plus-maze is used to measure anxiety in rodents (Brown, 2007) or an open field test to measure fear in a number of animals.

Traditionally, the view of fish is that they do not have facial or body expressions that are recognisable by humans and usually do not make any sounds which would help the farmer or observer in assessing the welfare state of the animals within the holding tanks. However, anecdotally, tanks of fish have been described as either calm or chaotic, terms which indicate that QBA has the potential to be a tool to assess fish body language. Research into the use of structural environmental enrichment to improve the welfare of fish is in its infancy compared with terrestrial captive animals. However, interest is increasing within aquaculture particularly if it leads to improved yield, growth and quality (Näslund & Johnsson, 2014). Environmental enrichment is also being studied as a means of increasing welfare by reducing injuries and fin damage (Näslund & Johnsson, 2014; Persson & Alanärä, 2014; Torrezani et al., 2013). In addition, fish can be less affected and recover faster from stressors with environmental enrichment (Pounder et al., 2015).

There has been one previous unpublished study of qualitative behaviour assessment of fish (Dunn, 2017), which developed a fixed list of descriptors which had moderate to good inter-observer agreement (Dunn, 2017). Observers were able to consistently detect differences in body language between tense and calm fish. The study presented here will use the fixed list developed by Dunn (2017) and is the first to use QBA techniques to study the behavioural effects of structural environmental enrichment in a commercial Atlantic salmon fish-farm. The previously developed fixed list was used to determine if there were any detectable differences in body language between fish in enriched and non-enriched environments. Agreement between different observers was evaluated and the qualitative judgement was validated with quantitative behaviour measurements.

6.2 Material and Methods

6.2.1 Ethical Review

Approval for the enrichment experiment was obtained from the University of Stirling's Animal Welfare and Ethical Review Body (AWERB) as part of a PhD project investigating fin damage in farmed Atlantic salmon (IoA AWERB April 15 interim ASPA 04.docx). Approval for observer participation in the QBA study was obtained from the University of Stirling's General University Ethics Panel (GUEP 383(A)).

6.2.2 Animals, Housing and Husbandry

The part of the study involving animals was conducted between August 2016 and March 2017 at an Atlantic salmon (*Salmo salar*) hatchery and rearing site owned by Loch Duart Salmon Ltd, near Badcall Bay, Sutherland in Scotland, UK (Figure 6-1). The site had 23 tanks, 5m diameter x 2m depth supplied with fresh-water via a flow-through system from nearby Loch Duart. All tanks were outside, exposed to a natural photo period and ambient temperatures all year round. Fish were fed standard salmonid pelleted rations (Skretting Nutra Advance/Supreme©) controlled by a central computer at an individual tank level and. The amount of food and the frequency of food varied (between every 10-20 minutes) across the season according to daylight hours. Food was provided to each tank using a compressed air system with a spinning arm to distribute food around the tank (Arvo-tec, Finland). Tanks were cleaned daily and mortalities removed between 9am-11am except on days when video recording was taking place, at which time cleaning was done after recordings were complete. At the time of recording there were approximately 15,000 juvenile salmon in each tank with an average stocking density of 20kg/m^3. Structural enrichment was provided in nine tanks while ten tanks were left as standard production tanks (Figure 6-2). Enrichment consisted of green artificial (plastic) kelp with dimensions 150mm (w) x 1500mm (L) (Figure 6-3) and weighted at the bottom. Six ropes were evenly strung across the top of the tank, three on each side of the central pillar. Each rope held three strips of artificial kelp in different locations on adjacent ropes to provide an even spread.

Figure 6-1 *Loch Duart fish farm*

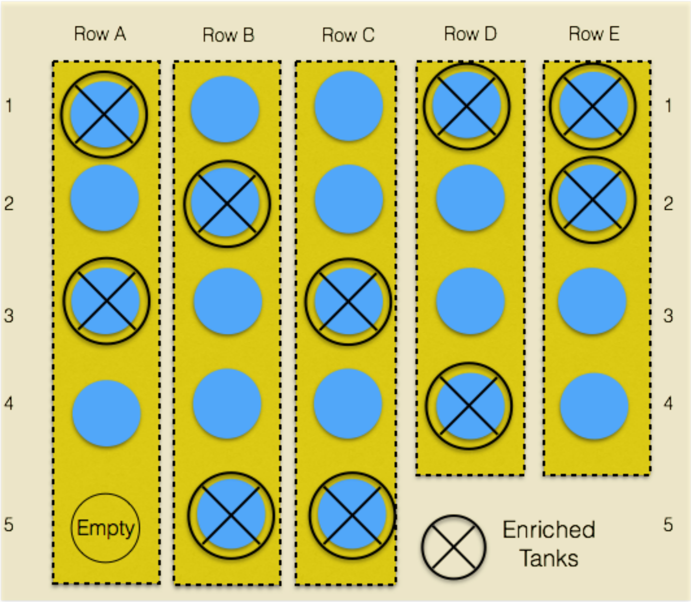

Figure 6-2 *Tank layout showing tanks with enrichment X and tanks without enrichment. Video recordings from tanks A1, A2 and B1 were not used due to poor visibility and tank A5 was empty for the duration of the study.*

6.2.3 Video clips and clip selection

Pre-recorded videos were used in the QBA analysis. Videos were recorded using a Go-Pro© camera within a waterproof housing suspended on a pole which was clamped to the rim of the tank. Video recordings, approximately 17 minutes long, were obtained from the 22 fish tanks with cameras suspended the same distance down in each tank. However, the visibility was poor in three tanks, so the recordings were not used, therefore clips from nine enriched (Figure 6-3) and nine non-enriched tanks (Figure 6-4) were utilised. Short, 45 second video-clips were extracted from the main recordings in a standardised approach. Clips were extracted 3 minutes after the most recent disturbance. A disturbance was classed as any of the following: feeding event (sound/spinner visible), startle event (unknown or known cause) or insertion of the camera into tank. The enrichment was visible in the clips; however, the observers were not made aware of the purpose of the enrichment. The video clips were arranged in random order for viewing and included two duplicates (one enriched and one non-enriched clip) to check intra-reliability of observers.

__Figure 6-3__ Artificial kelp in tank with Atlantic salmon

__Figure 6-4__ Atlantic salmon in standard production tank, no enrichment

6.2.4 Development of a rating scale

A fixed list of descriptors was generated using a focus group of four experienced fish farmers, as part of a MSc project (Dunn, 2017), using clips from videos recorded at the same time and group of tanks as the present study.

The fish farmers were introduced to QBA concepts and given basic guidance in how to generate qualitative terms to describe the expressive quality of body language. Examples from mammalian species were used so as not to influence their word choice in describing fish body language.

Twelve video clips were selected by the researcher to provide a range of overt and contrasting behaviours; e.g. darting, drifting, startle responses or interacting with conspecifics or any structure within the tank. The fish farmers viewed each of the 45 second duration videos and recorded their observations. The farmers were not allowed to confer during this process to reduce any influence from their colleagues. Twenty-six terms resulted from this session. A subsequent communal discussion excluded some descriptors e.g. for being more representative of physical behaviours or having multiple terms with similar meanings and identified other relevant descriptors that may not have been evident in the videos. At the end of the discussion a fixed list of twenty descriptive terms were agreed and is shown in Table 6-1.

A separate session was held to use the twenty descriptors in a QBA scoring exercise to validate the method for assessing fish welfare. Ten students from the Dick Vet Behaviour Society (University of Edinburgh) observed twenty-five clips of one-minute duration. Each video clip was assessed for each of the twenty descriptors with each term scored on a 125 mm horizontal line presented as a Visual Analogue Scale (VAS). Participants scored each term by marking a vertical line along the VAS corresponding with how intensely they felt a particular expressive quality was seen in the behaviour of the fish. The distance of the vertical mark along the VAS was measured and used in the subsequent data analysis. A second session was held ten days later, using the same participants and video clips (but in a different order) to collect data for intra-observer reliability. Principal Component Analysis of the VAS score revealed four dimensions (PC1-PC4) explaining 79% of variation in the data. Only PC1 had strong to moderate inter- and intra-observer reliability based on Kendall's coefficient of concordance (W= 0.68, χ^2= 335.31, p <0.001) and partial correlation (r=0.65, p=<0.001) respectively. The fixed list of twenty descriptors generated from the Dunn (2017) study was used in this present study as was the scoring and measurement protocol which is further described below.

Table 6-1 *List of descriptors and agreed synonyms.*

List of QBA descriptors	Agreed synonyms
Content	Satisfied, at peace, restful
Stressed	Summary term -disturbed, upset, under pressure, mix of anxious and tense
Energetic	Actively, lively, dynamic
Anxious	Worried, apprehensive
Mellow	Easy going, tolerant, unphased
Skittish	Excitable, easy frightened
Irritated	Annoyed, frustrated
Tranquil	Still, quiet, serene
Fearful	Afraid, frightened
Aggressive	Hostile, assertive (violent)
Calm	Peaceful, undisturbed
Crowded	Claustrophobic, overwhelmed
Tense	On edge, strained
Startled	Spooked, surprised
Listless	Lethargic, lifeless
Flighty	Erratic, volatile, unpredictable
Relaxed	At ease, no urgency (not necessarily motionless)
Agitated	Disturbed, unsettled
Unsure	Cautious
Inquisitive	Interested, curious, engaged

6.2.5 Observers and training session

All of the Institute of Aquaculture MSc students received an email asking for participation in a study of QBA in fish. A short explanation of the QBA method was included and the reason given for doing the study was that QBA had not been applied to fish. No mention was made of enrichment. A subsequent request for participation was made at a lecture where the researchers were introduced and the importance of fish welfare and the requirement for reliable, valid tools to assess welfare was discussed. QBA was introduced and an indication of what participating in the study entailed and the duration was given, stressing that participation was voluntary. Eleven MSc students volunteered to take part

(8 females, 3 males) with various levels of prior experience with fish, varying from none at all (n=5), some experience (n=3) to very experienced (n=3).

The eleven volunteers then took part in the QBA session, which consisted of a training session, followed by an observation session. During the training session, the participants were given a further introductory lecture on QBA (mainly from terrestrial farmed animal perspective). The fixed list of twenty descriptors was introduced and participants invited to discuss the terms to ensure they were familiar with the meaning of the different terms or required more clarification. Observers were provided with a paper copy of the fixed list and agreed synonyms, for reference during scoring. A video clip of a group of Atlantic salmon was shown and a demonstration on how to use the terms and score using the visual analogue scale (VAS) was given. The QBA procedure was developed to use a set length of 125mm for the VAS score line for each descriptor. The left end of the scale corresponded to the minimum score (0 mm), meaning the expressive quality indicated by the descriptor term was entirely absent in that group of fish, whereas the right end represented the maximum score (125mm), meaning that the expressive quality indicated by the term was strongly dominant in that group of fish (Figure 6-5). Participants were instructed to draw a vertical line across the VAS at the point they felt was appropriate and to use all the terms for each video clip. The score sheet for the full list of terms is shown in Appendix A.

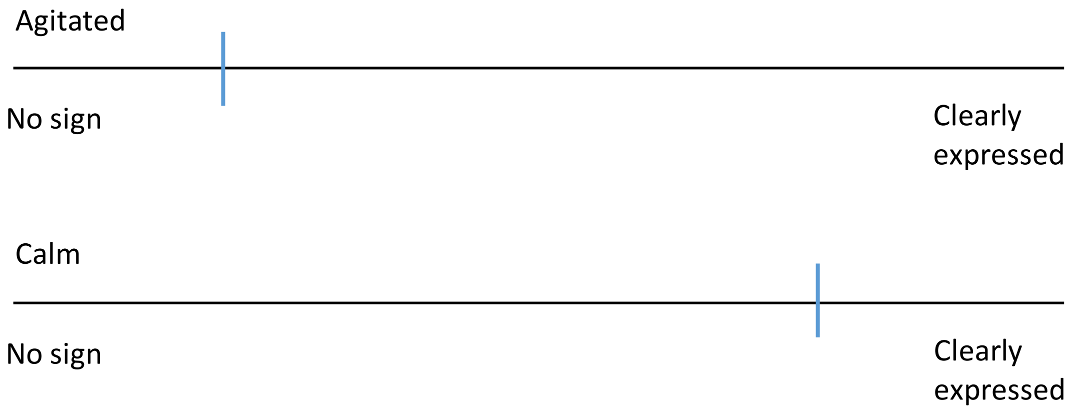

Figure 6-5 *Example of visual analogue scale (VAS)*

6.2.6 Observer QBA scoring session

The QBA scoring session followed on from the training session. Each participant was provided with a scoring sheet, one for each video clip, on which a Visual Analogue Scale (VAS) of 125mm in length were placed next to each of the 20 descriptors. Participants were instructed to concentrate on what the fish were doing and ignore the background within the tanks as the videos were taken in different directions, thus giving different views. No mention was made of enrichment. Participants were also instructed not to confer amongst themselves. Each video was 45 seconds long with a 10 second lead in to remind observers which clip number was being viewed. After each video finished playing, participants were given approximately 2 minutes per clip to score each of the twenty fixed terms on the VAS before the next video clip was played. Twenty video clips were shown with a 20-minute break after the first ten videos, where participants were again instructed to not discuss the session during the break.

6.2.7 QBA scoring

A score was assigned to each term for each clip by measuring the distance (in millimetres) between the minimum point of the VAS to where the observer marked the line (Figure 6-5). Each observer scoring sheet was scanned into a pdf and the measuring tool in Adobe acrobat used to measure the lines and automatically download the measurement to a spreadsheet. An Excel macro was then used to assign the measurements to the correct descriptor and organise the data for analysis.

6.2.8 Quantitative analysis of behaviour

An ethogram was developed to quantitatively analyse the behaviour in the 18 video clips used in the QBA study (Table 6-2). Only 18 clips needed to be analysed out of the 20 clips as two clips were repeats. Each video clip was sectioned into 10 second segments. For each segment, one fish (the focal fish) was followed and the presence of any of the behaviours in the ethogram recorded. The focal fish was selected as the first fish to emerge onto the screen from the lower left quadrant in each segment. The focal fish were selected from those nearest to the camera and body length estimated for each clip to give the width of the viewing area in body lengths (BL) to estimate how fast fish were swimming in BL s^{-1}.

Table 6-2 *Ethogram for Atlantic salmon for quantitative analysis*

Behavior	Description
Active - Speed (BL s^{-1})	Continuous propulsion over at least 0.5x the camera view
Calm - Stationary (counts)	Stationary/holding position can be swimming in place but should not involve significant forward propulsion.
Inquisitive - FaceCamera (counts)	Fish orientated towards camera, swimming in place with no evidence of forward movement being impeded.
Chaotic - Change of direction (counts)	Fish changes direction by more than 90deg and continues forward motion

6.2.9 Statistical analysis

To determine inter-observer reliability for each of the twenty descriptors, VAS scores (distance measurement) were correlated using Kendall’s coefficient of concordance (W). Kendall’s W values can vary from 0 (no agreement at all) to 1 (complete agreement). For coefficients of concordance (W) and correlation coefficients (*r*) five categories were used as defined in Martin & Bateson (2007) i.e. slight correlation 0-0.19; low correlation 0.2-0.39; moderate correlation 0.4-0.69; high correlation 0.7-0.89 and very high correlation 0.9-1.

QBA data were analysed using a Principal Component Analysis (PCA, correlation matrix, no rotation) using the FactoMineR package in R. The output scores of the main dimensions extracted by the PCA were then used to test the inter-observer reliability, using Kendall’s W coefficient of concordance. Where Kendall’s W coefficient of concordance indicated a poor level of agreement, Spearman’s rank correlations were used to evaluate observer pairs. Intra-observer reliability was assessed by Pearson correlations between the main principal component (PC) output scores of the two repeated video clips (video 1 repeated as video 13 and video 2 repeated as video 10).

A mixed effect linear model (lme4 package) was used to analyse the effect of enrichment on the main principal components (*qualitative* behaviour) with enrichment type as fixed factor and clip number and observer number as random factors. A separate model for each of the main principal components was constructed.

The effect of enrichment on *quantitative* behaviour measurements was analysed using a generalised linear model (quasi-Poisson in R Stats package) to compensate for over and under-dispersion in the count data.

To analyse the relationship between the PCA output scores (*qualitative*) and *quantitative* behaviour with the enrichment treatment, a general linear model (R Stats package) was used. For the four *quantitative* behavioural categories a value was assigned to each video clip. The count data were square root transformed, to meet the assumptions of normality. For each video clip the mean PCA score for each dimension was also calculated. The linear model was used to analyse the fixed effects of *quantitative* behaviour and treatment on the mean PCA score. A separate model for each principal component and quantitative behaviour category was constructed. Normality and homoscedasticity of the residuals, for the models, were checked using Q-Q plots and standardised residuals vs. fitted values. Supcol (Ade4 package) was used to analyse the supplementary quantitative data to determine which PC dimensions are associated with each of the quantitative variables and visualise on the same correlation plot as the QBA variables.

Data analysis was carried out using R software (version 3.5.3) (R Development Core Team, 2019). In all statistical tests, p= 0.05 was taken as the level of significance.

6.3 Results

6.3.1 Principal component analysis (PCA) with eleven observers

A PCA was conducted using the QBA scoring from the eleven observers. The PCA combined the 20 descriptors into four dimensions with eigenvalues > 1, explaining 68% of the variation with the first dimension (PC1) accounting for 39% of the variation (Table 6-3). The loadings for each dimension are given in Table 6-4 with the corresponding loading plot for each descriptor along the first two PCA dimensions shown in Figure 6-6.

Table 6-3 *PCA output for the QBA scores for the eleven observers with eigenvalues >1*

	PC1	PC2	PC3	PC4
Eigenvalue	7.785	3.173	1.344	1.121
% variation explained	39	16	7	6
% cumulative variation	39	55	62	68

Table 6-4 *Principal components analysis (PCA) of VAS scores for all participants (n=11). Values in bold and underlined are the top highest positive and negative scores which are used as the summary terms for each PC (PC1 tense-calm, PC2 mellow-stressed, PC3 listless-inquisitive, PC4 listless-skittish). The last column gives the mean QBA score (SE ± 2, for all terms) with the range of the visual analogue scale (VAS) in mm.*

variables	PC1	PC2	PC3	PC4	VAS score
Content	-0.593	0.667	-0.128	0.019	58 (0-125)
Stressed	0.837	**0.057**	0.022	0.180	32 (0-104)
Energetic	0.537	0.176	-0.474	-0.229	58 (1-122)
Anxious	0.825	0.181	0.064	0.090	30 (0-117)
Mellow	-0.477	**0.743**	-0.017	0.048	55 (0-124)
Skittish	0.699	0.220	0.173	**-0.282**	30 (0-112)
Irritated	0.576	0.354	0.329	-0.209	21 (0-112)
Tranquil	-0.640	0.603	0.067	0.013	57 (0-123)
Fearful	0.729	0.322	0.065	0.047	21 (0-119)
Aggressive	0.508	0.293	-0.079	0.094	18 (0-120)
Calm	**-0.645**	0.638	0.043	-0.088	66 (0-125)
Crowded	0.366	0.390	-0.065	0.442	54 (0-124)
Tense	**0.845**	0.116	-0.001	0.099	29 (0-121)
Startled	0.561	0.449	0.154	-0.277	21 (0-116)
Listless	0.007	0.145	**0.574**	**0.631**	17 (0-111)
Flighty	0.708	0.140	-0.039	-0.054	28 (0-112)
Relaxed	-0.632	0.622	0.113	-0.205	67 (0-125)
Agitated	0.779	0.099	0.166	**-0.261**	35 (0-121)
Unsure	0.637	0.304	-0.206	0.148	27 (0-121)
Inquisitive	0.159	0.279	**-0.708**	**0.263**	42 (0-118)

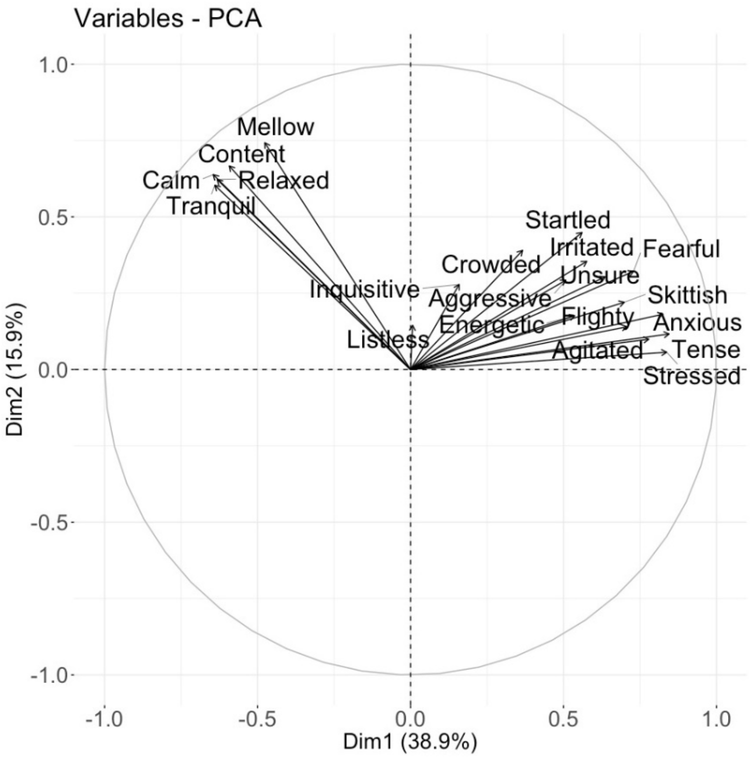

Figure 6-6 *PCA analysis of the 20 descriptors and 11 observers, along the first two main PCA factors (Dim 1 and Dim 2). Correlation circle with an arrow per variable where the length of the arrow indicates the strength of the correlation with the dimension. For example, 'content' and 'stressed' are strongly correlated with the first dimension.*

6.3.2 Inter and intra reliability of observers

Inter-observer reliability was calculated for each dimension, using Kendall's coefficient of concordance (W). As a general guideline a Kendall's W of > 0.7 would be considered acceptable as this indicates a substantial level of agreement between scoring for the different observers (Martin & Bateson, 2007). As can be seen from Table 6-5 none of the principal components (PC1-PC4) achieved good inter-observer reliability for all eleven observers. Spearman's rank correlations were used to investigate the correlation between the different observers (Table 6-6). As can be seen from Table 6-6 the scoring from observer 11 does not correlate with any other observers. Removing observer 11 data and re-calculating gives a Kendall's W =0.476 for PC1 (Table 6-5). Only data from observers 1,2,6 and 10 could be used. They were in good agreement and showed a concordance of W=0.735 (χ^2 =55.9, $p < 0.001$) but only for PC1. There was also very strong intra-observer reliability for PC1 shown by a high correlation between the two repeated videos (r_p=0.949; p <0.001) for the 10 observers and a moderate correlation for PC2 for the same video clips (r_p=0.689; p <0.001). There was only a weak correlation for PC3 (r_p=0.284; p =0.22) and PC4 (r_p=0.063; p =0.79) for the same video clips. Further analysis will be conducted using data from observers 1, 2, 6 and 10 only.

Table 6-5 _Kendall's coefficient of concordance for different number of observers. All 11 observers, observer 11 removed (n=10) and combinations that give Kendall’s W > 0.6 [observers 1, 2, 6, 9, 10 (n=5) and observers 1, 2, 6, 10 (n=4)]. P-values in brackets with *< 0.05, ** < 0.01, *** < 0.001. A p-value <0.05 rejects null hypothesis that there is no agreement among observers._

	PC1	**PC2**	**PC3**	**PC4**
Kendall’s W (n=11)	0.394***	0.196**	0.363***	0.131 (0.09)
Kendall’s W (n=10)	0.476***	0.206**	0.368***	0.126 (0.198)
Kendall’s W (n=5)	0.643***	0.373*	0.465***	0.202 (0.441)
Kendall's W (n=4)	0.735***	0.504**	0.56**	0.233 (0.541)

Table 6-6 _Spearman rank correlations between pairs of observers’ PC1 scoring. Values in bold > 0.4_

	Obs1	Obs2	Obs3	Obs4	Obs5	Obs6	Obs7	Obs8	Obs9	Obs10	Obs11
Obs1	1	**0.646**	**0.568**	0.374	0.23	**0.738**	**0.504**	**0.412**	0.362	**0.649**	0.12
Obs2		1	0.393	**0.475**	0.318	**0.709**	**0.652**	0.357	0.351	**0.566**	0.151
Obs3			1	**0.527**	0.225	**0.578**	0.117	0.156	**0.404**	**0.568**	-0.049
Obs4				1	0.299	**0.491**	0.27	0.182	0.08	**0.616**	0.123
Obs5					1	**0.416**	0.215	**0.649**	0.372	0.246	-0.06
Obs6						1	**0.481**	0.356	**0.538**	**0.574**	-0.036
Obs7							1	0.212	**0.44**	**0.488**	0.021
Obs8								1	0.261	0.33	-0.221
Obs9									1	**0.404**	-0.533
Obs10										1	-0.021
Obs11											1

6.3.3 Principal component analysis with four observers

A principal component analysis (PCA) was conducted using the data from four observers (1, 2, 6 and 10), identified in section 6.3.2 as having good inter-observer reliability. The fish experience level of these four observers ranged from no experience (n=1), some experience (n=1) to very experienced (n=2). The PCA combined the 20 descriptors into four dimensions with eigenvalues > 1. The first four dimensions account for 73% of variance (Table 6-7) with the first dimension (PC1) accounting for 45% of the variation. Only the first two dimensions (eigenvalues >1) were retained for further analysis. The loadings for the first two dimensions are given in Table 6-8 and for comparison the loading factors for all eleven observers are also included. The corresponding loading plot for each descriptor along the first two PCA dimensions, from the four-observer data, is shown in Figure 6-7. The dimension of the x-axis (PC1) ranged from content/tranquil to tense/stressed. As many of the terms load strongly on the first principal component, accounting for 45% of the total variance, this suggests that this component is important in the description of the valence (how positive or negative an event is) with a positive valence characterised by more negative score. The dimension on the y-axis (PC2) ranged from listless to startled accounting for 13% of variance and would appear to be more related to the level of arousal (whether an event is exciting or calming).

Table 6-7 *PCA output for the QBA ratings scale and inter-observer reliability (Kendall's W) for observers 1, 2, 6 and 10. P-value in brackets with *p<0.05, **p<0.01, ***p<0.001*

	PC1	**PC2**	**PC3**	**PC4**
Eigenvalue	8.9401	2.709	1.9138	1.0609
% variation explained	45	13	10	5
% cumulative variation	45	58	68	73
Kendall's W	0.725***	0.42*	0.509**	0.28 (0.33)

Table 6-8 *Principal components analysis (observers n=4 and n=11). Values in bold and underlined are the top highest positive and negative scores which are used as the summary terms for each PC (PC1 tense-content, PC2 startled-listless). A further 1-3 additional highly scoring terms are in bold to further characterise each PC. Inter-rater reliability (Kendall's W) of VAS scores for each term (Kendall's; ***p<0.001, **p<0.01, *p<0.05).*

	4 Observers					**11 Observers**	
	VAS score Mean	**±SD**	**Kendall's W**	**PC1** Tense-content	**PC2** Startled-listless	**PC1** Tense-calm	**PC2** Mellow-stressed
Content	66	37	**0.645*****	**-0.785**	0.433	-0.593	**0.667**
Stressed	28	27	**0.651*****	**0.896**	0.003	**0.837**	0.057
Energetic	53	33	0.462*	0.474	0.349	0.537	0.176
Anxious	31	32	0.562**	**0.878**	0.098	**0.825**	0.181
Mellow	60	38	**0.649*****	-0.668	0.528	-0.477	**0.743**
Skittish	27	28	0.416*	0.803	0.187	0.699	0.220
Irritated	17	22	0.438*	0.523	0.436	0.576	0.354
Tranquil	64	38	0.592***	**-0.780**	0.414	**-0.640**	0.603
Fearful	19	25	0.487**	0.773	0.198	0.729	0.322
Aggressive	14	19	0.148	0.473	0.245	0.508	0.293
Calm	73	36	**0.651*****	-0.725	**0.564**	**-0.645**	**0.638**
Crowded	46	38	0.291	0.233	**0.577**	0.366	0.390
Tense	27	30	0.592***	**0.904**	0.065	**0.845**	0.116
Startled	15	21	0.477**	0.432	**0.578**	0.561	0.449
Listless	16	22	0.428*	-0.163	**-0.372**	0.007	0.145
Flighty	23	24	0.362	0.780	0.068	0.708	0.140
Relaxed	76	36	**0.602*****	**-0.732**	0.560	**-0.632**	0.622
Agitated	32	30	0.574***	0.779	0.208	0.779	0.099
Unsure	26	28	0.461*	0.612	0.342	0.637	0.304
Inquisitive	36	33	0.549**	0.064	0.099	0.159	0.279

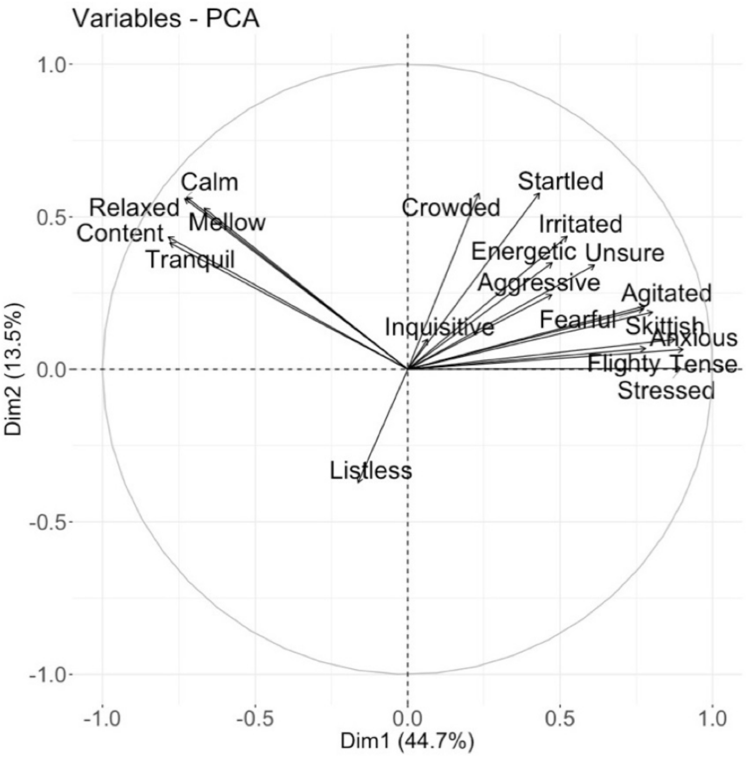

Figure 6-7 *PCA analysis of the 20 descriptors and 4 observers (Obs 1, 2, 6 and 10), along the first two main PCA factors (Dim 1 and Dim 2). Correlation circle with an arrow per variable where the length of the arrow indicates the strength of the correlation with the dimension. For example, 'content' and 'stressed' are strongly correlated with the first dimension whereas 'listless' has low correlation and 'startled' moderate correlation with the second dimension.*

6.3.4 Inter and intra observer reliability with four observers

Inter-observer reliability was calculated, for each dimension, using Kendall's coefficient of concordance (W). Only PC1 had good inter-observer reliability (W=0.725, χ^2=55.14, p < 0.001), see Table 6-7. There was very good intra-observer reliability, shown by strong correlations between the two repeated videos for the 4 observers; PC1 (r_p=0.963; p <0.001), PC3 (r_p=0.83; p = 0.01) and PC4 (r_p=0.914; p < 0.001) and moderate correlation for PC2 (r_p=0.678; p=0.06), indicating that observers were consistent in scoring the same video clip. Inter-observer reliability (Kendall's W) was also calculated for the VAS scores for each descriptor (Table 6-8). Three descriptors (aggressive, crowded and flighty) had poor W-coefficients with p-values > 0.15. The remaining 17 descriptors all had statistical significance with W-coefficients = 0.416 to 0.651.

6.3.5 Quantitative and qualitative data correlation

Pearson correlations between the quantitative data PCA output and the QBA score PCA output are shown in Table 6-9 for the two main PCs. There was a strong correlation between chaotic behaviour with fish changing direction more where tense behaviour was expressed (+ve correlation). Moderate correlation between inquisitive behaviour and listless term had a high loading in the QBA.

There was moderate relationship with inquisitive behaviour with fish turning towards the camera when listless behaviour was expressed (-ve correlation). The coordinates of a given quantitative variable is calculated as the correlation between the quantitative variable and the QBA principal components so that the quantitative variables can be overlaid on the QBA variable plot (Figure 6-8).

Table 6-9 *Pearson correlation between quantitative behaviours PCA output and QBA score PCA output. Values in bold show significant correlations. P-values in brackets.*

Behaviour	**PC1** Tense-content	**PC2** Startled-listless
Active (Speed BL s^{-1})	0.048 (0.84)	- 0.11 (0.64)
Calm (Stationary)	0.031(0.89)	-0.30 (0.19)
Inquisitive (FaceCamera)	-0.24 (0.313)	**-0.47 (0.03)**
Chaotic (ChangeDir)	**0.65 (< 0.01)**	0.01 (0.95)

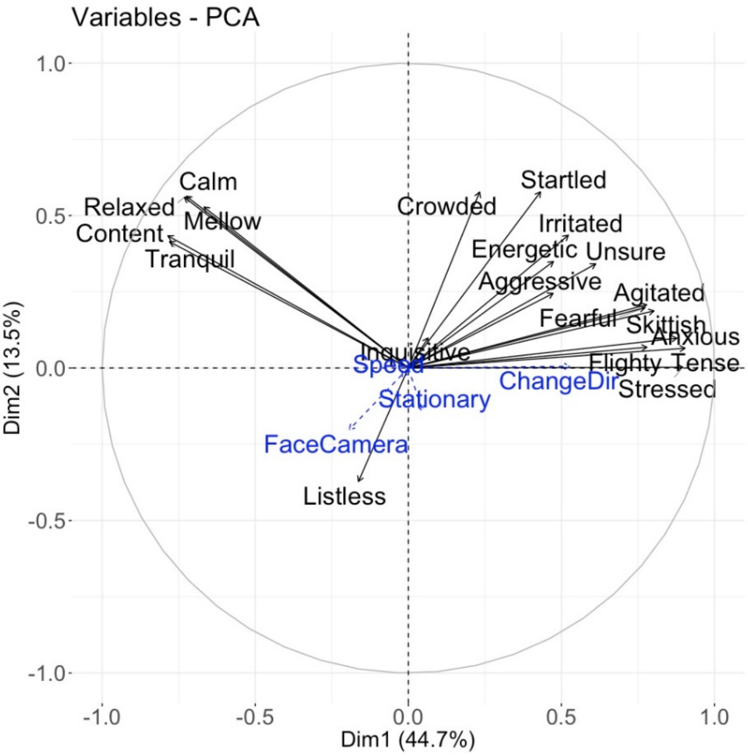

Figure 6-8 *PCA analysis of the 20 descriptors and 4 observers (Obs 1, 2, 6 and 10), along the first two main PCA factors (Dim 1 and Dim 2). Correlation circle with an arrow per variable where the length of the arrow indicates the strength of the correlation with the dimension. For example, 'content' and 'stressed' are strongly correlated with the first dimension. The correlation of the quantitative behavioural variables with each dimension are overlaid on the PCA analysis (Speed, Stationary, FaceCamera, ChangDir).*

6.3.6 Effect of enrichment

There was a significant effect of enrichment on PC1 (χ^2= 6.1, df=1, p=0.013) with enriched tanks having higher loadings on content/tranquil and non-enriched tanks having higher loadings on tense/stressed (Figure 6-9). When all 11 observers were included in the analysis enriched tanks also had higher loadings on content/tranquil and non-enriched had higher loadings on tense /stressed. There was no significant effect of enrichment type on PC2.

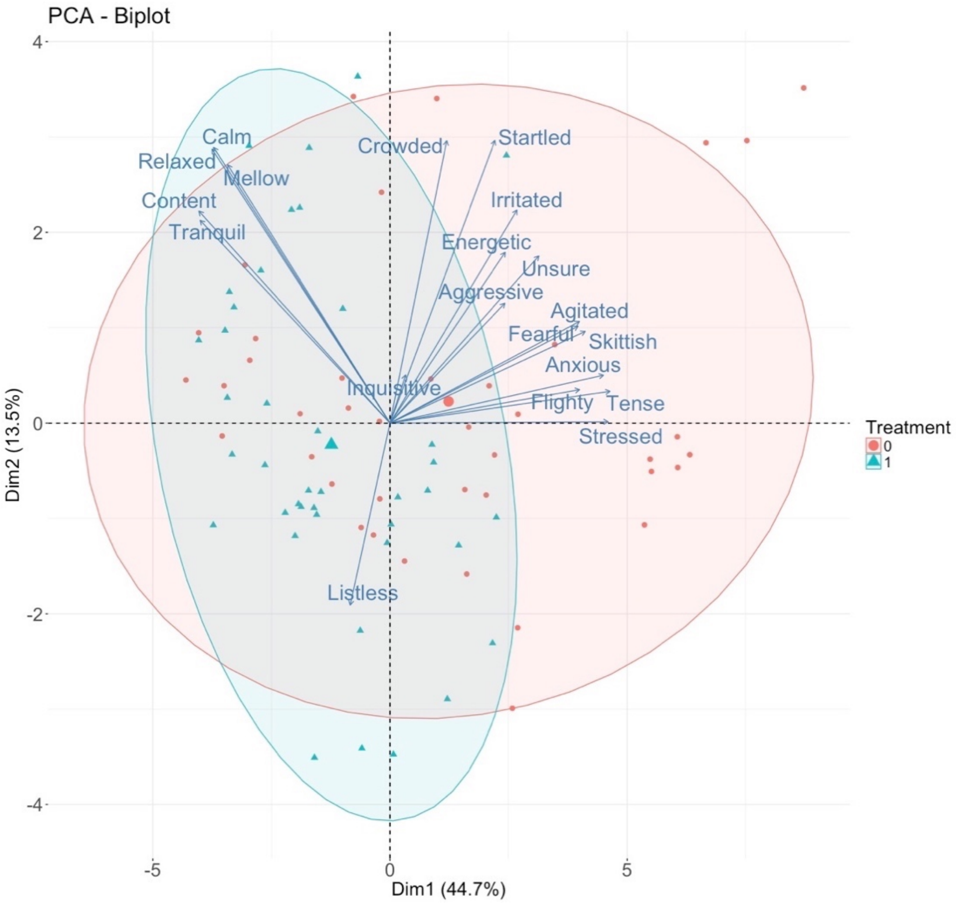

Figure 6-9 *Plot of PCA output of 4 observers (points) and descriptor variables grouped by treatment where 0=non-enriched and 1=enriched. Circles= 95% confidence ellipses. Enriched tanks load more towards content/tranquil whereas non-enriched tanks load more towards stressed/tense.*

6.3.7 Quantitative behaviour and the effect of enrichment

The results of the analysis of quantitative behaviours and enrichment is shown in Table 6-10A. The analysis suggests that fish in enriched tanks had significantly fewer changes of direction (p=0.028) and were significantly more likely to be stationary (p=0.029) compared with fish in non-enriched tanks. Fish in enriched tanks were also more likely to turn and face the camera, however the result was not significant (p=0.064). This result suggests that enriched tanks had more calm behaviours and a tendency to be more inquisitive compared with non-enriched tanks whereas non-enriched tanks had more chaotic behaviour.

6.3.8 Comparison of principal component scores with respect to quantitative behaviour measurements and enrichment type.

The result of the analysis of the effect of qualitative behaviour measurements and enrichment on PC1 loading scores (Table 6-10B) indicate that there was no effect of treatment on how quickly fish moved within a tank or how often they turned to face the camera. Fish changed swimming direction significantly more often in non-enriched tanks compared with enriched (p=0.047) and an examination of the interaction plot of ChangeDir* Treatment showed that the PC loading score became more positive (tense/stressed) with increasing numbers of changes in direction (see Figure 6-8). In summary, the calm behaviour (Stationary) measured quantitively in the video clips was not reflected in the QBA PCA scoring whereas chaotic behaviour (ChangeDir) was strongly recognised as being a tense/stressed behaviour.

The effect of the quantitative behaviours and treatment on PC2 was also analysed using linear models; however, there were no significant results to report.

Table 6-10 *Coefficient estimates (± SE) with associated p values of linear models with quantitative behaviour and treatment (A) and mean PC1 score with quantitative behaviour and treatment (B). Significant p values are in bold.*

		Coeff	**Std.error**	**t**	**p-value**
A. Model outputs (quasi-Poisson) - quantitative behaviours (counts) vs treatment (non-enriched/enriched)					
Intercept1	(**ChangeDir**)	0.59	0.23	2.56	0.198
	Treatment (Enriched)	-1.1	0.46	-2.39	**0.028**
Intercept2	(**FaceCamera**)	-1.61	0.74	-2.16	0.045
	Treatment (Enriched)	1.61	0.81	1.97	0.064
Intercept3	(**Stationary**)	0.18	0.25	0.72	0.48
	Treatment (Enriched)	0.74	0.31	2.37	**0.029**
B. Model outputs - mean PC1 score per video vs each of the quantitative behaviours (sqrt transformed) + treatment					
Intercept1		0.81	0.69	1.17	0.24
	Speed	0.16	0.2	0.79	0.43
	Treatment (Enriched)	-1.88	1.03	-1.88	0.064
Interaction	Speed*Treatment	-0.21	0.27	-0.78	0.44
Intercept2		-3.38	0.97	-3.47	**< 0.001**
	ChangeDir	3.63	0.71	5.15	**< 0.001**
	Treatment (Enriched)	1.38	1.62	0.85	0.4
Interaction	ChangeDir*Treatment	-2.87	1.42	-2.02	**0.047**
Intercept3		2.82	1.8	1.56	0.12
	FaceCamera	-1.953	2.15	-0.91	0.37
	Treatment (Enriched)	-4.23	2.16	-1.96	0.054
Interaction	FaceCamera*Treatment	2.11	2.36	0.89	0.37
Intercept4		-5.18	1.33	-3.88	**< 0.001**
	Stationary	4.86	0.97	5.01	**< 0.001**
	Treatment (Enriched)	8.32	3.03	2.74	**< 0.01**
Interaction	Stationary*Treatment	-7.41	1.85	-4.01	**< 0.001**

6.4 Discussion

The aim of this study was to evaluate the potential of using QBA to monitor the effect of structural enrichment on Atlantic salmon, in a commercial farm setting. The use of QBA techniques could enable a non-invasive, low cost and rapid assessment of welfare, if it proved to be reliable. There was poor agreement between all eleven observers in scoring the descriptive terms. One observer was consistently different in their scoring compared with the remaining ten observers. A group of four observers reached an acceptable level of inter-reliability agreement and were used for further analysis. Further research is required into the descriptive terms used and better training. One main dimension (PC1) was identified characterised by the terms tense/stressed versus content/tranquil, explaining 45% of the variation and with both good inter- and intra-reliability. The description of PC1 is consistent with what has been found previously (Dunn, 2017) and it may be the most diverse expression that can be scored reliably for fish. PC2 (startled/listless) had poor inter-reliability, strongly dependent on individual observer scores. An important finding of this study is that the observers' behavioural expression scores differed between enriched and non-enriched groups. The behavioural expression of fish in enriched tanks was described qualitatively as content/tranquil/relaxed and in the non-enriched tanks as stressed/anxious/tense. This effect was detectable with the four observers who had good inter-reliability agreement as well as with all eleven observers. The good correlation between chaotic behaviour in the tense /stressed direction provides further validation of PC1 on a quantitative basis. The poor correlation of calm behaviour with PC1 can perhaps be attributed to the normal group movement in tanks with high stocking density. When Atlantic salmon are stocked at high density there is a tendency for fish to school. Observers assessing each video clip at a group level would likely see similar fish movements in all tanks whereas changes of direction in swimming (chaotic) are obvious at a group or individual level. Lack of movement (stationary) as a proxy for calm behaviours in this instance was likely a poor choice. The moderate correlation of inquisitiveness on PC2 suggests a level of arousal, that despite poor agreement between observers' behavioural expression scores may benefit from further study. Quantitative behaviour measurements differed by enrichment, with non-enriched tanks being more chaotic and enriched tanks calmer. However, only chaotic behaviours were reflected in the comparison of quantitative behaviours and enrichment on PC1 behavioural expression scores, in all likelihood for the same reason as above. Dunn (2017) also found good correlations between quantitative behaviour and the first principal component in that study. However, video clips for that study were selected on the basis of a number of overt behaviours whereas the video clips in this study were selected to be disturbance-free.

Dimension characteristics and observer agreement

The main dimension PC1, with summary terms tense/anxious/stressed to content/tranquil/relaxed, appears to have more easily recognisable qualities compared with other dimensions. As many of the terms correlate strongly on the first principal component, accounting for 45% of variance, this suggests that this component is important in the description of the valence (how positive or negative an event is). PC2 ranged from listless to startled, accounting for 13% of variance and would appear to be more related to the level of arousal (whether an event is exciting or calming), however there was poor observer agreement in behavioural expression scoring. The lack of terms scoring on PC2 would suggest that levels of arousal in fish were difficult to recognise by observers, were not overtly displayed by fish or that the terms used did not adequately describe arousal levels in fish. QBA studies with terrestrial animals often focus on individual animals or small groups (< 100) of animals (Wielebnowski, 1999; Wemelsfelder et al., 2000; Rutherford et al., 2012; Minero et al., 2016). In contrast, tanks in this study had a large number of fishes in the field of view potentially masking any subtle visual cues relating to arousal levels, from the general background.

Observers' ratings are assigned relative to norms for a species or population, which means that they will likely depend on the observer's range of experience, opinions and backgrounds (Meagher, 2009). Wemelsfelder (2012) found that there was no difference in the ability to judge pig behaviour and welfare between experienced animal keepers, veterinarians, animal rights activists and naïve observers (Wemelsfelder et al., 2001). However, pig farmers were found to assess welfare more positively than animal scientists and townspeople (Duijvesteijn et al., 2014). It was suggested that this was due to farmers being more concerned about health whereas animal scientists and townspeople were more concerned about the pigs being able to express natural behaviours. Large intra-observer variations have been reported with dairy cows (Bokkers et al., 2012) although there was consistency of scoring over time i.e. observers who scored low (or high) in the first session scored low (or high) in the second session (Clarke et al., 2016). The coefficient of concordance (Kendall's W) for a number of terms increased 2x in a subsequent session (Bokkers et al., 2012), highlighting the importance of experience and training with dairy cows (Clarke et al., 2016). People are not as familiar with fish as they are with terrestrial animals although good observer agreement was achieved in the first study of fish (Dunn, 2017) using naïve observers and a range of overt behaviours in the video clips. The present study was focussed on enrichment therefore overt behaviours would not necessarily be available within the videos selected for this study. Having observers watch videos of the full range of a species' behavioural repertoire and what is considered normal or extreme behaviour during the training session would likely be beneficial in improving observer agreement (Fleming et al., 2016). Future studies should concentrate on smaller numbers of fish and better training of observers to evaluate whether inter-observer reliability can be improved, and further dimensions identified which could extend our understanding of behavioural expressivity in fish.

Fixed term list

The majority of the descriptors had moderate agreement between observer scores for each of the items on the fixed list. Three items (aggressive, flighty and crowded) had poor levels of agreement. Direct fish aggression can be difficult to detect in tanks with large numbers of fish as the incidences of aggression can be relatively few and happen too quickly for most people to spot. Recognising aggressive behaviour in fish may also require some experience. At the time of recording, the fish would have been getting ready to smolt and Atlantic salmon are less aggressive as smolts than as parr (Keenleyside & Yamamoto, 1962). All of these factors could have contributed to the poor scoring on aggression. Examining the individual scores, the two observers who identified as being experienced scored aggression very low whereas the observers with low or medium experience scored moderately. The term crowded has general physical connotations, however an experienced fish person would categorise crowding from vigorous activity within the crowd along with gasping and burrowing behaviour. It is likely that inexperienced observers consider the high density of fish found in fish farms as being crowded. Experienced observers scored crowded as moderate whereas the observer with no experience scored very high and medium experience scored low. The observer scoring for the term flighty showed no pattern suggesting poor understanding of that term or not meaningful for fish.

Free Choice Profiling (FCP) versus Fixed lists

FCP allows observers to develop their own descriptive terms to interpret what they see. Inherent in this process is that observers understand the meaning that they attribute to each term. However, FCP can fail to capture important qualitative behavioural descriptors (Fleming et al., 2013) especially if the observers lack experience with the species under assessment. In contrast, fixed lists may have some terms whose meaning is unclear to some observers particularly if translated into different languages (Fleming et al., 2016). This requires that observers undergo training and instruction on how to use fixed lists. There is evidence of good agreement between observers in scoring sow behaviour whether using FCP or the fixed list methods (Clarke et al., 2016).

The poor observer agreement between all eleven observers suggests that the training in this study, with this group, was not sufficient to establish effective understanding of the terms. Not all members of the group had English as their first language, although first language was not recorded with observer number, but may account for some of the poor observer agreement. Fixed lists may also fail to capture subtle variations in behaviour which may have contributed to the poor observer agreement on dimensions other than PC1. The twenty descriptors used in this study were generated by Dunn (2017), in conjunction with experienced fish farmers, using video clips taken from the same recordings as the present study. The fixed list had good inter- and intra-observer reliability in the Dunn (2017) study therefore was used in the present study for comparison. Future studies may benefit from exploring the use of FCP with fewer fish, which would also allow for direct comparison with physiological measurements.

However, fixed lists are more appropriate for on-farm assessment, particularly if comparing assessments over time and between farms.

Context

The environmental enrichment was visible in some of the videos although observers were naïve to the purpose of the enrichment. Qualitative measures can be sensitive to environmental context. This has advantages in extracting information, but also has the risk of observer bias in relation to that context (Fleming et al., 2016; Tuyttens et al., 2014), particularly if the context is judged from a moral standpoint i.e. judged as 'good' or 'bad'. For example, if observers consider outdoor pig pens to be superior to indoor pig pens then pigs lying down in a barren pen may be assessed as 'bored' whereas the same pig lying down in an outdoor pen may be assessed as 'content'. Wemelsfelder et al., (2009) conducted a study with observers viewing the same pig digitally projected onto an indoor or outdoor background. Scoring of the individual pigs retained the same pattern independent of background although observers assessed pigs as more confident/content and less cautious/nervous in outdoor compared with indoor clips. In the present study the quantitative behaviour measurements identified differences in enriched and non-enriched tanks therefore the separation of behavioural expression along the main dimension (PC1) depending on enrichment was likely not biased by the visibility of the enrichment.

Monitoring behaviour as a welfare indicator is ranked highly by farmers although they have expressed opinion that it is difficult to quantify and must be interpreted in the context of 'normal' behaviour (North et al., 2008). QBA potentially addresses these concerns. Quantifying behaviour is difficult and time consuming therefore unsuitable for day-day welfare assessment on-farm. Subtle differences in behaviour may be missed due to the time it takes to quantify individual behaviours and analyse the data. In comparison, QBA is relatively quick (a list of 20 descriptive items can be assessed in < 5 minutes). This means it is capable of capturing dynamic changes in body language which may be important for welfare assessment (Fleming et al., 2016). A priority for the fish farming industry is to develop and validate a checklist of practical indices to assess fish welfare. A key development to realising this checklist is the identification of objectively defined operational welfare indicators (OWIs) e.g. fin damage, mortalities, water quality, cortisol, swimming behaviour (see section 1.4.2). As noted previously, objectively quantifying behaviour is challenging in a farm environment therefore QBA combined with OWIs could potentially make a valuable contribution to on-farm welfare assessment.

The results of this study are encouraging in that observers with varied fish-based experience can consistently judge the tense/stressed versus content/tranquil dimension of expressivity as represented by PC1 and that differences in body language were detectable between enriched and non-enriched environments. QBA has the potential to provide a rapid, reliable tool to monitor changes in the welfare of fish over time and provide evidence of improvement (or otherwise) in interventions designed to improve welfare. Future studies should improve training in the use of fixed lists for assessment and focus on fewer fish in tanks, allowing direct comparison with an individual's physiology to further validate QBA of fish.

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6.6 Appendix A

Visual analogue score sheet for qualitative behaviour assessment

No sign
Clearly expressed

Inquisitive
Unsure
Agitated
Relaxed
Flighty
Listless
Startled
Tense
Crowded
Calm
Aggressive
Fearful
Tranquil
Irritated
Skittish
Mellow
Anxious
Energetic
Stressed
Content

7 General Discussion

7.1 Introduction

The principal aim of this thesis was to investigate food withdrawal as a major risk factor associated with the development of fin damage in fish and the effect of mitigation strategies to reduce fin damage including reducing industry standard food withdrawal periods and environmental enrichment. The potential consequences of reducing fin damage is an improvement in farm productivity, fish health as well as in fish welfare. The relationship between fin damage and food withdrawal has only been examined under small scale experimental conditions (e.g. Cañon Jones et al., 2011). Other studies have examined the effects of food withdrawal but only on short term stress related parameters and consequently would not have detected a relationship with fin damage (e.g. López-Luna et al., 2016). In this thesis fin damage has been investigated under large-scale experimental conditions (Chapter 2 and 3) and on-farm (Chapter 4, 5, and 6). The benefits of environmental enrichment have been demonstrated mainly under laboratory conditions and investigations into up-scaling to farm level was required (Huntingford et al., 2012). Chapter 4 and 5 details the effects of environmental enrichment on fin damage, growth and stress levels at the farm-level and what behaviours can be encouraged that may reduce fin damage. To accurately assess fin damage on-farm and the results of any interventions to improve fish welfare, methods which are easy to apply and reproducible are required. An important requirement for any on-farm assessment method is for immediate feedback, for example reducing the need to send samples off-site for testing. Chapters 2 and 6 details novel assessment methods for fin damage and fish welfare generally. The remainder of this chapter discusses the outcomes of the studies.

7.2 Fin Damage

The food withdrawal experiment using industry level feed withdrawal times and stocking densities (Chapter 2) demonstrated that lack of access to food was a significant risk factor for the development of fin damage. The significance of the effect of food withdrawal on fin damage has not previously been reported. The RSPCA (2018) standard is for fasting periods to not exceed 72 hours. Within the fish-farming industry it is normal to implement 48 hours fasting prior to any stressful husbandry procedures such as vaccination and grading, handling or transport. A subsequent on-farm experiment indicated that a food withdrawal period of 48 hours had significantly more fin damage than 24 hours (Chapter 4). An achievable management technique to reduce fin damage would be for farmers to reduce fasting time. Fin damage assessments would be enhanced by including whether fin damage was active or healed. Active fin damage would indicate that immediate action is required to address the problem (Noble et al., 2018). Healed fins are evidence of historical damage and using a video monitoring system such as that detailed in Chapter 3 may provide information to identify the risk factor prevailing at that time. Food deprivation is not uncommon in teleost fishes. Periods of limited food intake due to the unavailability of food resources are common in wild animals. However, farmed fish live in barren environments and removing the food source which comes at regular intervals essentially removes their main activity. Lack of an expected reward can lead to increased aggression in Atlantic salmon (Vindas et al., 2012; Shi et al., 2017). Lack of stimulation may also lead to boredom or re-directed foraging behaviour. Stereotypical biting behaviour has been observed in pigs and laying hens, with food deprivation identified as a major risk factor (Brunberg et al., 2016). Feather pecking in laying hens is considered a re-directed foraging behaviour and this behaviour is reduced through providing adequate substrate to peck at (Huber-Eicher & Wechsler, 1998). There has been a wealth of research dedicated to preventing tail biting in pigs (D'Eath et al., 2017) and feather pecking (Hartcher et al., 2016). This could be a potential resource for identifying further risk factors and mitigation measures.

7.3 Fin damage and enrichment

In the on-farm enrichment experiment (Chapter 4) fin damage was significantly reduced in enriched tanks prior to and following a major stressor (vaccination and grading). This is the first time that the effect of enrichment on fin damage at farm level has been reported. Fin damage in the period just after vaccination and grading was less in enriched tanks but not significantly so. However, 2 months post vaccination and grading fin damage was significantly less in enriched tanks. Prior to the fish being transported to sea fin damage had increased in both enriched and non-enriched tanks and levels were not different between enriched and non-enriched tanks. There had been a feeder malfunction in the main hopper that resulted in an unplanned food withdrawal period prior to the final sample. Observations on farm also showed that intermittently the spinner arms would get clogged with food due to dampness and individual tanks could be food deprived for short periods. The results of food deprivation on fin damage highlights the need for farmers to ensure reliable food delivery and regular monitoring of fin damage. Systems exist to automatically monitor food delivery in individual tanks and farmers should be encouraged to implement such systems as appropriate.

The enrichment type in this study affected the spatial distribution with the fish being more dispersed within the tank (Chapter 5). This may be the mechanism by which fin damage was reduced. More space equals better access to food and oxygen and water quality generally. Less competition and hypothetically less damage. Discussions with farmers, during the on-farm enrichment experiment (Chapter 4), identified that encouraging first-feeders to distribute vertically in the water column is an aim for farmers to improve growth and mortality. Therefore, further investigations are required into the effects of enrichment on water quality and food access at earlier developmental stages of salmon. There have been some studies on spatial distribution in sea cages (Juell et al., 1994; Juell & Fosseidengen, 2004). but appears to be little research into spatial distribution in freshwater production tanks.

7.4 Growth and enrichment

The provision of enrichment did not appear to affect growth (Chapter 4). Growth was neither improved nor worsened by the presence of enrichment. Previous studies on the effect of environmental enrichment on salmonid growth parameters is varied and appears to be species and developmental stage specific. Other studies have shown no effect of environmental enrichment (Arndt et al., 2001; Näslund et al., 2013; Brockmark et al., 2010). However, there are reports of better growth in structurally enriched environments as well as detrimental effects of growth (Bosakowski & Wagner, 1995). An adequate spatial distribution of food is essential for good growth (Jorgensen et al., 1996) and perhaps the enrichment structure impeded the distribution of food or altered the water flow such that food distribution was not optimal. Further investigations are required on the effect of the enrichment on food distribution. Elevated cortisol levels are known to suppress appetite in fish and hence reduce growth (Pankhurst et al., 2008; Pickering, 1993) although at the time of sampling the plasma cortisol levels of fish in enriched and non-enriched tanks were consistent with unstressed fish. Post vaccination and grading fish in non-enriched tanks were in poorer body condition but no difference was detected prior to transport to sea. However, a period of poorer body condition could lead to a compromised immune function in sub-optimal conditions leading to poor welfare (Castro et al., 2011).

7.5 Behaviour and enrichment

The use of qualitative behaviour assessment (QBA) showed a clear indication that fish in enriched tanks were more likely to be assessed as calm/tranquil and non-enriched tanks as tense/stressed (Chapter 6). This is the first study to use QBA techniques to study the behavioural effects of structural enrichment on fish. QBA was further validated by quantitative assessment of behaviour which indicated that swimming behaviour in enriched tanks was more ‘settled’ than that in non-enriched tank (Chapter 5 & 6). Experienced farmers can recognise these states. However, it is encouraging that observers with varied fish-based experience could also consistently score animals as content or tense although there were some issues with poor inter-observer reliability for some observers, which may be addressed with better training. Both farmers and RSPCA agree that we should encourage fish to behave in a relaxed manner, as opposed to an ‘unsettled’ manner. Monitoring behaviour as a welfare indicator is ranked highly by farmers although they have expressed opinion that it is difficult to quantify and must be interpreted in the context of ‘normal’ behaviour (North et al., 2008). QBA potentially addresses these concerns. Quantifying behaviour is difficult and time consuming therefore unsuitable for day-day welfare assessment on-farm. Subtle differences in behaviour may be missed due to the time it takes to quantify individual behaviours and analyse the data. In comparison, QBA is relatively quick (a list of 20 descriptive items can be assessed in < 5 minutes). This means it is capable of capturing dynamic changes in body language which may be important for welfare assessment (Fleming et al., 2016). Terrestrial animals can be viewed in real-time or from videos. On Atlantic salmon fish farms underwater cameras would be required with a monitor and /or recording facility. Underwater cameras are routinely used in sea cages so could be adapted for use in QBA. Underwater cameras are generally not used in on-shore freshwater systems however cameras such as Go Pros® are relatively inexpensive. Once the monitoring facility is in place virtually no other equipment is required to run a QBA assessment. As QBA is looking at body language then QBA assessments could probably be run under less than optimal light conditions, although this would still need to be checked. QBA can potentially be used as an alert system which could then followed up with more in-depth screening if required. QBA could also has the potential to be used as an auditing tool by farmers, welfare auditors, welfare standards and food safety authorities. In terrestrial animals QBA has successfully distinguished between different transport conditions for sheep (Wickham et al., 2012) and cattle (Stockman et al., 2013). QBA was also able to differentiate between pigs treated with a neuroleptic drug and those not treated (Rutherford et al., 2012). The possibility of different QBA scores depending on treatment indicates a potential use in nutritional studies and vaccine trials. Validation is still in its infancy, but initial indications are encouraging.

7.6 Non-Invasive vs Invasive monitoring of fin damage

Chapter 4 showed that good results could be obtained monitoring fin damage using an underwater camera and laser system and obviating the need to kill any animals. This satisfies the principles of the 3Rs (Replacement, Reduction and Refinement) towards a more ethical use of animals in testing. Light levels may be an issue but where this can be overcome, instrumentation of this type can be automatically recording over extensive periods of time, in inhospitable places.

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7.7 Future work

We now know that food deprivation leads to increased fin damage. The ability to consistently instigate fin biting by fasting the fish will allow studies to be designed to investigate the motivation for fish to bite fins. It is becomingly increasingly important to understand the motivation, biological mechanisms and individual differences underlying fin biting behaviour in order to prevent it and may provide the opportunity for additional control strategies. Is it redirected foraging, hunger, boredom or aggression? Studies on feather pecking in laying hens highlight redirected foraging as a likely motivation and can be reduced using environmental enrichment. Recent advances in understanding fish cognition may be key to exploring the underlying characteristics of fin biting by providing mechanisms to test motivation and providing enrichment that is ecologically relevant to fish may reduce the prevalence of fin damage. Experimental designs to test motivation are common in many species of terrestrial animals and birds but have only been applied to a limited extent in fish (Galhardo et al., 2011) with no comprehensive methodological reports for Atlantic salmon. The QBA validation process should continue and there are other issues still to be addressed such as investigating the effect of structural enrichment on water flow and food distribution. Also, whether there is any benefit in terms of increased productivity or better ability for fish to adapt to a new environment when transported between systems (e.g. freshwater tanks – sea cages). In the on-farm study fish were only fed during daylight hours so it would be interesting to monitor behaviour during the night and in particular at the first feed in the morning for evidence of fin biting.

7.8 Conclusions

Food withdrawal was identified as a major risk factor in the development of fin damage and could be improved by minimising periods of food withdrawal and providing structural enrichment. The significance of the effect of food withdrawal on fin damage has not previously been reported and this is the first report of enrichment effects at the farm-level. There was some indication that growth was better in enriched environments in the early developmental stages. However, by the end of the freshwater phase, growth was comparable between enriched and standard production tanks. Fish appeared less stressed and were more spatially distributed in the enriched environment suggesting an overall improvement in general welfare. This was the first time that qualitative behaviour assessment was used to assess the effect of a treatment (enrichment) and its potential benefit as an on-farm assessment tool is encouraging

7.9 References

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