
Social network analysis of behavioural interactions
influencing the development of fin damage in
Atlantic salmon (*Salmo salar*)

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Preface

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. The text does not exceed 60,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

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Summary

The development of fin damage in Atlantic salmon parr was investigated using social network analysis of behavioural interactions occurring under different feeding and stocking conditions. Four separate experiments were carried out in which groups of fish were subjected to a long food restriction period (30 days) described in Chapter 2, high (30 kg/m³) or low (8 kg/m³) fish stocking densities (Chapter 3), predictable or unpredictable food delivery regime (Chapter 4) or a short food restriction period (10 days) described in Chapter 5. Dorsal fin damage (erosion, splits and fin index) was significantly higher in groups of fish subjected to food restriction periods (short and long), held at high stocking density (30 kg/m³) or with an unpredictable food delivery regime. No other fins were found to be affected by fin damage irrespective of the treatment. The social networks based on aggressive interactions showed higher centrality, clustering coefficients, in-degree centrality, out-degree centrality and less dense networks in groups subjected to food restriction (short and long), low stocking densities 8 kg/m³, and unpredictable food delivery. The high centralities and clustering coefficients indicated separation of fish within the groups into initiators of aggression and receivers of aggression. This separation of roles was seen only in the food restricted group, high density groups and unpredictable food delivery groups. Initiators had higher out-degree centrality while receivers showed high in-degree centrality. Also, initiators of aggressive interactions had less fin erosion, higher final weights and higher body lengths than receivers of aggression. The severity of fin damage was significantly higher when this role differentiation occurred, and it was highly correlated with fin biting

events. Additionally, overall aggression was higher in food restricted groups, low density groups and unpredictable groups. The dynamic analyses of networks over time (Chapter 6) showed that fish classified as initiators of aggression did not change this behaviour after normal/control environmental conditions were restored (i.e. *ad libitum* food delivery, low stocking density or predictable food delivery). The latter result indicates that individual fish maintained their behaviour irrespective of their social context, which is consistent with the definition of behavioural syndromes, personalities or temperament defined for other non-human animals. Overall these studies demonstrated the importance of using social network analysis to clearly identify and quantify roles that individual fish assume within their network group and through time based on their behavioural interactions leading to dorsal fin damage and differential physical characteristics. The results are potentially applicable within the commercial aquaculture industry as a valuable technique to evaluate and improve the welfare of farmed fish.

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Table of contents

Preface	ii
Summary	iii
Acknowledgments	v
Table of contents	viii
1. Chapter 1. General introduction	1
1.1. Aquaculture: production and relevance	1
1.2. Welfare of fish: concepts and general introduction	3
1.3. Welfare in fish and the issue of pain, consciousness and awareness	5
1.4. Welfare of farmed fish related to harvest and slaughter	6
1.5. Welfare of fish related to feed restriction	9
1.6. Welfare of fish related to stocking densities	10
1.7. Fin damage, aggression and welfare of farmed fish	14
1.8. Social behaviour aspects related to welfare in farmed fish	19
1.9. Social network analysis as tool to evaluate the effect of social behaviours in the welfare of fish	22
1.10. The use of social network analysis in fish	28

2. Chapter 2. Social network analysis of behavioural interactions on fin damage development in Atlantic salmon (<i>Salmo salar</i>) during a long (30 days) food restriction period.	30
2.1. Introduction	30
2.2. Materials and methods	32
2.2.1. Animals and experimental groups	32
2.2.2. Containment and individual identification	33
2.2.3. Water quality and environmental conditions	35
2.2.4. Physical measures	35
2.2.5. Quantification of fin damage	36
2.2.6. Behavioural observations and social interactions	37
2.2.6.1. Association interaction measurements	37
2.2.6.2. Aggressive interactions measurements	38
2.2.7. Social network analysis	40
2.2.8. Schooling/shoaling and spatial position measurements	41
2.2.9. Statistical analyses	42
2.3. Results	43
2.4. Tables and figures	47
2.5. Discussion	58
2.6. Conclusion	66

3. Chapter 3. Social network analysis of the behavioural interaction	67
that influence the development of fin damage in Atlantic salmon	
parr (<i>Salmo salar</i>) held at different stocking densities.	
3.1. Introduction	67
3.2. Materials and methods	70
3.2.1. Animals and experimental groups	70
3.2.2. Containment and individual identification	72
3.2.3. Housing, water quality and environmental conditions	73
3.2.4. Physical measures	73
3.2.5. Quantification of fin damage	74
3.2.6. Behavioural observations and social interactions	74
3.2.6.1. Association interactions measurements	75
3.2.6.2 Aggressive interactions measurements	75
3.2.7. Social network analysis	76
3.2.8. Structural and spatial position measures	76
3.2.9. Statistical analyses	77
3.3. Results	78
3.4. Tables and figures	80
3.5. Discussion	86
3.6. Conclusions	90

4. Chapter 4. Social network analysis of behavioural interactions	92
influencing the development of fin damage in Atlantic salmon parr (<i>Salmo salar</i>) subjected to predictable and unpredictable food delivery schedules.	
4.1. Introduction	92
4.2. Materials and methods	94
4.2.1. Animals and experimental groups	94
4.2.2. Containment and individual identification	95
4.2.3. Housing, water quality and environmental conditions	96
4.2.4. Physical measures	97
4.2.5. Quantification of fin damage	97
4.2.6. Behavioural observations and social interactions	98
4.2.6.1. Associative behavioural interactions	98
4.2.6.2. Aggressive behavioural interactions	99
4.2.7. Social network analysis	99
4.2.8. Structural and spatial position measures	100
4.2.9. Statistical analyses	100
4.3. Results	101
4.4. Tables and figures	103
4.4. Discussion	108
4.5. Conclusion	112

5. Chapter 5. Social network analysis of behavioural interactions	113
influencing the development of fin damage in Atlantic salmon parr	
(<i>Salmo salar</i>) subjected to short period (10 days) of food restriction.	
5.1. Introduction	113
5.2. Materials and methods	114
5.2.1. Animals and experimental groups	114
5.2.2. Containment and individual identification	115
5.2.3. Housing, water quality and environmental conditions	116
5.2.4. Physical measures	117
5.2.5. Fin damage quantification	117
5.2.6. Behavioural observations and social interactions	117
5.2.6.1. Aggressive behavioural interactions	118
5.2.6.2. Associative behavioural interactions	118
5.2.7. Social network analysis	119
5.2.8. Structural and spatial position measures	119
5.2.9. Statistical analyses	120
5.3. Results	121
5.4. Tables and figures	123
5.5. Discussion	128
5.6. Conclusions	131

6. Chapter 6. Dynamic social network analysis of aggressive behavioural interactions in Atlantic salmon parr (<i>Salmo salar</i>) leading to fin damage: an insight into personality traits.	133
6.1. Introduction	133
6.2. Materials and methods	137
6.2.1. Data collection	137
6.2.2. Description of models for dynamic network analysis	139
6.2.3. Model specification	140
6.2.4. Model estimation	142
6.3. Results	143
6.4. Tables and figure	145
6.5. Discussion	151
6.6. Conclusion	154
7. General discussion and conclusions	156
8. References	162
Appendix	200

1. Chapter 1. General introduction.

1.1. Aquaculture: production and relevance.

The farming of aquatic species for human consumption, or aquaculture, has become one of the most important animal production sectors in the world, and it is projected to expand more in future years (F. A. O., 2009). In 2004, world aquaculture production was estimated at 59.4 million tons, representing 32.4% of the total production and extraction of fish, crustaceans, molluscs and other aquatic animals (F. A. O., 2009). Compared with other types of animal production, aquaculture is an expanding activity with an estimated annual production growth rate of 8.8% each year compared with terrestrial animal production rates of 2.8% (F. A. O., 2009). Altogether, this has been translated in an increase of food availability from aquaculture from 0.7 kg per person in 1970 to 7.8 kg per person in 2004 (F. A. O., 2009). Furthermore, if extraction and processing from fisheries industries maintain this current fish extraction and processing level, it is estimated that all fish consumed by humans by 2050 will come from aquaculture (F. A. O., 2009).

Aquaculture, as such, comprises over 240 animal and plant species and has a major economic and productivity impact in developing countries (F. A. O., 2009). For example, freshwater aquaculture production (comprising fish, molluscs and crustaceans) represents 56% of the total production by quantity and 50% of the total by value (F. A. O., 2009). On the other hand, marine aquaculture contributes to 36% of the

total production and 33% of the total value produced (F. A. O., 2009). The main species of fish used in aquaculture are carp and other cyprinids, salmonids, tilapia (*Tilapia rendalli*) and other cichlids (F. A. O., 2009).

As previously mentioned, aquaculture has an important local economic and social impact, especially in developing countries. Globally, 11 million of people are working in the aquaculture productive sector with 96% of them belonging to southeast Asiatic countries (F. A. O., 2009). Furthermore, it is estimated that women actively participate in aquaculture in these countries (F. A. O., 2009).

Current aquaculture production systems based on optimal intensification of animal production aims to produce high quality animal food and other products to an increasing human population, while minimizing production costs and expenses (Lucas and Southgate, 2003). Although a positive image of the fish industry and its outputs amongst consumers has been previously reported (Hanson et al., 1994), consumers have become increasingly interested in issues such as the sustainability of fish farming and the welfare of farmed fish (Altintzoglou et al., 2010). For example, a recent study (Olesen et al., 2010) showed that consumers are willing to pay an additional 15% for salmon raised using higher welfare standards (RSPCA, 2007) or under conditions that have less environmental impact (Debio, 2010).

1.2. Welfare of fish: concepts and general introduction.

The concern for animal welfare in the United Kingdom dates back to the early 1800s with the germinal ideas and concepts of animal suffering and rights by Jeremy Bentham (Bentham, 1823). The concept of a definition of animal welfare is still a matter of discussion in the biological sciences as it touches upon different disciplines such as behaviour, psychology, physiology and health (Fraser, 2008). Therefore, animal welfare definitions range from the coping ability of individuals, taking into account their subjective experiences, to a more nature-based one where the animal needs in the wild are taken into account (Broom, 1991a).

The most accepted and meaningful definition of animal welfare is the ability of animal to cope with its environment (Broom, 1986, Broom, 1991a). Subsequently, this definition has been broadened to include new concepts such as the recognition of subjective experience as an essential part of the welfare of the animal (Mendl et al., 2009, Mendl and Paul, 2004).

Aquatic animal welfare has been the subject of a more rigorous scientific approach over the last decade (Huntingford, 2008). As well as in other animal species, the assessment of animal welfare in fish includes physiological, immunological, productive and behavioural measurements (Broom, 1986, Broom, 1988, Broom, 1991b, Broom, 1991a, Broom, 1991c, Broom and Fraser, 2007). For example, physical welfare parameters such as mortality, growth, food conversion, size variation, condition factor, weight, and physiological parameters such as haematocrit and plasma cortisol

concentrations, as well as indicators of trauma, such as fin condition, have been used in salmonids (Adams et al., 2007, Branson, 2008, Ellis et al., 2002).

Behavioural measurements are of particular importance in animal welfare studies because they provide broader answers to complex questions compared to single physiological parameters associated with welfare (Broom, 1986, Broom, 1988). Moreover, measurement of behaviours and behavioural changes in animals can be used in field conditions to directly evaluate the welfare of animals (Broom, 1991c).

There is scientific evidence that fish can perceive noxious stimuli associated with pain and have the physiological and cognitive capabilities of demonstrated behaviours associated with suffering (Braithwaite et al., 2008, Chervova, 1997, Sneddon, 2003a). Therefore, it has been suggested that the welfare of farmed fish should be protected and enhanced as in other animal species (Broom, 2007). Additionally, several organisations have produced legislation and regulations on the welfare of farmed fish in later years in the United Kingdom specially those related to the Freedom Food scheme produced by the Royal Society for the Protection of Cruelty to Animal for Atlantic salmon (FSBI, 2002, RSPCA, 2007) and Rainbow trout (Quality Trout UK, 2007). Regionally, the European Union (European Union, 1998) and worldwide organisations such as the World Organisation for Animal Health (World Organisation for Animal Health, 2010) have produced similar pieces of legislation. This legislation and regulatory framework is based on scientific evidence in order to achieve a threshold of good welfare standards for fish such as rainbow trout (*Onchorynchus mykiss*) (European Food Safety Authority, 2009a), Atlantic salmon (*Salmo salar*) (European Food Safety Authority, 2009c,

European Food Safety Authority, 2008) and turbot (*Scophthalmus maximus*) (European Food Safety Authority, 2009b) .

1.3. Welfare in fish and the issue of pain, consciousness and awareness.

Pain has been defined as an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1979) and it has been described as a fundamental element for welfare evaluation and assessment (Broom, 1991b, Broom, 1991c).

In non human animals, including fish, the main approaches to evaluate pain have been anatomical, physiological and behavioural (Bateson, 1991, Weary et al., 2006). Pain receptors have been found in a variety of fish, peripherally and internally, showing the same physiological properties as in mammals and other animals (Lynn, 1994, Sneddon, 2003a, Sneddon, 2003b). However, central nervous system structures differ from those of mammals in spatial distribution and degree of development (Sloman et al., 2005, Sneddon et al., 2003). Behavioural changes occur when chemical and physical noxious stimuli are applied to fish such as rapid swimming, mouth rubbing and a decrease in feeding/foraging, demonstrating pain perception capabilities (Sneddon, 2003a, Sneddon, 2003b). Moreover, pain related behaviour decreases when analgesic drugs are given to fish (Sneddon, 2003b). This empirical evidence strongly suggests that fish do not need the same anatomical structures as in mammals in order to be capable of feeling pain.

It is important to point out that there has been much controversy in the awareness and cognition capabilities of fish (Rose, 2002, Rose, 2007). However, several studies have shown cognitive and awareness capabilities in fish such as assortive social preferences (Brown and Laland, 2001, Brown and Laland, 2003, Laland, 2003), spatial learning (Braithwaite et al., 1996, Sovrano et al., 2007), eavesdropping abilities (Oliveira et al., 1998, Valone, 2007), recognition of co-specifics and predators (Metcalf and Thomson, 1995, Griffiths, 2003, Hawkins et al., 2004, Korzan et al., 2007, Scheurer et al., 2007), olfactory awareness (Sommerville and Broom, 1998, Leduc et al., 2007) and decision making abilities (Johnsson and Akerman, 1998).

1.4. Welfare of farmed fish related to harvest and slaughter.

Several handling procedures are associated with a decrease in overall welfare of fish (Branson, 2008) but the most investigated management procedures in terms of pain and stress have been the welfare of fish during harvest (Robb, 2008) and transport (Erikson et al., 1997, Portz et al., 2006).

Harvest in aquaculture refers to several industrial processes beginning from taking the fish from production confinement areas such as sea cages, runways, ponds, tanks to the final processing for human food consumption (Lucas and Southgate, 2003). Fish are commonly starved or food restricted for a few days in order to allow gastrointestinal tract to empty, reduce microbial content in the gut and minimise food

contamination during slaughter and processing (Robb, 2008). However, harvesting fish is time consuming, and may take many days, with the consequence that some fish are starved for many days (Robb, 2008). There is little known about the maximum starving times but the normal range varies between 5 to a maximum of 14 days without causing a pathological starvation and decrease in final product quality (Einen et al., 1998a, Einen et al., 1998b, Einen et al., 1999). Crowding follows the starving of the fish (when fish receive no food), and consists of reducing the space in which fish swim and thus increasing fish stocking densities in order to facilitate extraction from the cages (Lucas and Southgate, 2003). Scientific evidence indicates that crowding is a highly stressful event leading to increases in plasma corticosteroids concentrations (Erikson et al., 1999) with a concomitant decrease in the quality of meat of Atlantic salmon (Berg et al., 1997, Erikson et al., 1997, Sigholt et al., 1997). Transfer of fish from cages is performed using water pumps, which collect and carry fish from the cage to wellboats designed to transport several hundreds or thousands of fish to on-shore facilities where fish are stunned, killed and processed (Branson, 2008).

Rendering the fish insensate by stunning, or other methods, has been regarded as a highly stressful event for the fish (European Food Safety Authority, 2009a, European Food Safety Authority, 2009c, Robb and Roth, 2003). Different stunning methods have been evaluated using behavioural responses, such as swimming, reactivity to touch, ventilation and eye control (Kestin et al., 2002) or direct brain activity (Robb et al., 2000, Robb and Roth, 2003). For example, carbon dioxide narcosis by the immersion of fish in water with saturated carbon dioxide has been investigated with unfavourable results as fish behave aversively before complete narcosis and

immobilization, and normal eye movement is present, suggesting a complete conscious state while bleeding out (Roth et al., 2006, Robb and Kestin, 2002, Robb et al., 2000). Live chilling has been utilised as a method of stunning in fish which has the additional benefit of reducing bacterial contamination (Skjervold et al., 2001b, Skjervold et al., 2002). The main effect of live chilling is a decrease in locomotor activity of fish. Fish are immersed in large holding tanks with recirculating cold water (2 to 4°C) for 30 to 40 minutes (Skjervold et al., 1999, Rorvik et al., 2001). However, there is no evidence of any anaesthetic effect or reduction of consciousness when using live chilling alone and another stunning method has to be used in conjunction (Skjervold et al., 2001a). It is important to point out that carbon dioxide and live chilling are not commonly used and suggested methods of stunning in the United Kingdom (RSPCA, 2007; Quality Trout UK, 2007) but may still be in use in some other countries.

Other methods such as electrical stunning of rainbow trout and Atlantic salmon have been recently investigated and suggest that low current as well as low frequency are necessary for several minutes to achieve a humane stunning without any flesh and meat alterations (Lines et al., 2003, Robb et al., 2002, Robb and Roth, 2003).

Undoubtedly, the most common and successful method of stunning farmed fish is cranial percussion which achieves a total, immediate and permanent lack of consciousness and sensibility (Kestin et al., 2002, Morzel et al., 2003, Robb and Kestin, 2002, Roth et al., 2007, van de Vis et al., 2003). Slaughter finishes with the total bleeding of fish which is carried out by severing at least 3 to 4 gill arches (Robb, 2008). It is important that slaughter be performed on fully stunned fish, as studies have demonstrated that severe aversive muscle movements occur when fish are conscious

during this process, which may cause internal or external lesions leading to poor quality derived products (Robb and Kestin, 2002).

1.5. Welfare of fish related to feed restriction.

Farmed fish may be subjected to several episodes of feed restriction throughout the production cycle. As previously mentioned, fish are subjected to a short period of feed restriction or starvation of less than a week before slaughter (Robb, 2008). Fish are also subjected to short periods of feed restriction of two or three days before vaccination in order to reduce the likelihood of contamination while injecting the vaccine intraperitoneally (Berg et al., 2006, Lucas and Southgate, 2003). Short feed restriction periods are also used before fish are transported and graded to reduce the possibility of faecal cross contamination in small spaces and accumulation of toxic faecal and urinary chemicals, for example ammonia (Erikson et al., 1997, Lucas and Southgate, 2003). It is important to point out that the oxygen requirements of fish decreases whenever food is not fully available (Brett and Groves, 1979) contributing to the confounding effect of feed restriction on welfare of fish.

Fish may be subjected to long periods of feed restriction, weeks or months, while they are wait to be harvested and they are required to grow less, thus food delivery is reduced to levels that are sufficient for their the basal metabolic needs (Lucas and Southgate, 2003, Stefansson et al., 2009). Fish may be subjected to long periods of feed restriction of at least two to three weeks whenever they have been medicated with

antimicrobials or antiparasitic drugs and withdrawal times are needed (Lucas and Southgate, 2003).

There have been no previous studies investigating the effect of short or long periods of feed restriction on the behaviour of fish and fin damage as measures of fish welfare.

1.6. Welfare of fish related to stocking densities.

A currently relevant and controversial topic in the welfare of farmed fish is the consequences of high rearing stocking densities (Adams et al., 2007, Ellis et al., 2002, Ellis et al., 2008, North et al., 2006, Turnbull et al., 2005). In terrestrial farmed animals, stocking densities may be measured in terms of kilograms of animals per square meter (kg/m^2), while in aquatic animals it may be expressed as weight of animals in each cubic meter (kg/m^3). The addition of this third dimension when considering stocking densities in fish implies special mathematical and physical considerations (Norris and Schilt, 1988, Viscido et al., 2007). It should also be noted that identical fish stocking densities can be achieved with different numbers of fish with different body weights.

The association between welfare of fish and stocking densities has been addressed by various studies, but results have been contradictory and the effect of stocking densities on the welfare in salmonid fish is still unclear (Huntingford et al., 2006, Adams et al., 2007). The difficulty in reaching a consensus is related to the

several confounding factors that must be taken into account such as species of fish, life stage, size, group size, feeding schedules, and environmental factors such as dissolved oxygen and ammonia (Ellis et al., 2002). Recent studies using multivariate statistical models and principal component analysis used the analysis of different welfare measures in order to obtain a welfare score (Turnbull et al., 2005). The major problem using these types of models is that they are usually context dependent and specific to the life stage, size or weight of fish (Turnbull et al., 2005).

It has been suggested that the life stage may play an important role in the effect of stocking densities on the welfare of fish (Adams et al., 2007, Huntingford et al., 2006). There is evidence of a strong behavioural component at different life stages, with fry and parr being mainly territorial, solitary and more aggressive (Huntingford et al., 1993, Huntingford et al., 1990, Pitcher and Magurran, 1993), while smolts and adult fish are less aggressive, more social and acquiring a schooling behaviour preference (Kjartansson et al., 1988, Pitcher, 1993). However, salmonid parr can school when increasing water flow (Shirvell, 1994) or stocking densities (Hosfeld, 2009).

Recent studies have quantified the effect of high and low stocking densities on physiological and physical welfare indicators in Atlantic salmon under commercial farm conditions (Adams et al., 2007, Turnbull et al., 2005). Both studies assessed similar welfare parameters such as condition factor, fin condition, plasma glucose concentration and plasma cortisol concentration using stocking densities of 15, 25 and 35 kg/m³. The number of animals in each experimental group was varied while the space was kept constant. For example, Turnbull et al. (2005) used approximately 720, 1200 and 1680 fish of 2.5 kg to establish the experimental stocking densities of 15, 25

and 35 kg/m³, respectively. Likewise, Adams et al., 2007 used approximately 47, 80 and 112 fish weighing 0.98 kg +/- 0.24 kg to achieve similar experimental stocking levels as Turnbull et al. (2005). Both studies concluded that stocking densities of 22-25 kg/m³ or higher had an adverse effect on welfare parameters, as did very low densities. Importantly, both studies suggested that the impact of densities is related to social interactions between fish, although no direct visual observations of social behaviours at the group or individual level of behaviour were performed.

Studies have also been conducted with similar results in rainbow trout (Ellis et al., 2002, North et al., 2006) and Arctic charr (Jørgensen et al., 1993). Jørgensen et al., 1993, using 25, 100 and 200 fish achieved stocking densities of 15, 60 and 120 fish found a decreased in growth only the lowest stocking densities. Also, North et al., 2006, used freshwater rainbow trout weighing 180 grams at group sizes of 100, 370 and 710 individuals to achieve densities of 10, 40 and 80 kg/m³, respectively. Although the authors found that densities of 80 kg/m³ did not decrease physiological welfare parameters, fin erosion was increased at this density, as well as, at the lowest density, indicating that factors other than those measured in this study, such as plasma cortisol glycaemia, haematocrit, dissolved oxygen oxygen, among other, must be responsible for the decreased welfare scores seen in fish at highest and lowest stocking densities such as social interactions.

A meta-analysis conducted by Ellis et al., 2002 reviewed 43 experimental studies investigating the effects of stocking densities on rainbow trout. Almost 70% of the studies demonstrated that high stocking densities (>45 kg/m³) had a negative effect on growth and 20% of the studies showed a direct effect on increasing mortality of fish.

Ellis et al., (2002), concluded that in order to fully understand the effect of stocking densities, it is imperative to integrate information from different fish welfare measurements both biotic, such as social interactions and behaviour, and abiotic, such as water quality, water temperature and feeding practices.

Various physiological welfare parameters have been linked to the effect of stocking densities on welfare of fish. For example, high stocking densities have been associated with the release of physiological stress responses such as increasing plasma cortisol concentration and hematocrit while decreasing plasma glucose concentration (Iwama et al., 1997, Pickering, 1981, Portz et al., 2006, Schreck, 2000). These physiological measures are accurate physiological indicators of fish welfare in response to stress (Conte, 2004), but they are impractical to carry out in commercial aquaculture.

Physical injuries have been related to high stocking densities in fish, mainly fin damage (Ellis et al., 2002, Ellis et al., 2008, Rasmussen et al., 2007, St.Hilaire et al., 2006), and it has been suggested that the main cause of this is the increase of aggressive behavioural interaction between fish leading to the biting of fins (Turnbull et al., 1998).

The determination of optimal stocking densities in aquaculture will help increase output efficiency, expressed as the relationship between the number of fish in a cage, pond or tank at the end of the production cycle over the number of fish initially stocked. The most efficient management will involve adjusting the amount of fish inside the

cages according to, size and life stage of the fish while increasing the welfare of fish through minimizing fish damage.

1.7. Fin damage, aggression and welfare of farmed fish.

Fin damage in farmed fish is widely used as a welfare indicator as it gives evidence of injury related to physical, chemical, bacteriological, nutritional and behavioural quality of the environment in which fish are reared (Blanchet et al., 2006, Charif et al., 2005, Damsgård et al., 2006, Hoyle et al., 2007, Rasmussen et al., 2007, St.Hilaire et al., 2006). Fin damage may be a predisposing factor for infectious diseases in fish such as furunculosis produced by *Aeromonas salmonicida* (Scheinder and Nicholson, 1980, Turnbull et al., 1996, Wedemeyer, 1997).

There are many and varied factors that contribute to fin damage and improving welfare by decreasing fin damage remains a major unresolved problem (Ashley, 2007, Branson et al., 2008, Huntingford et al., 2006).

Fin damage is useful as a welfare indicator firstly because they are easily available for evaluation and, secondly they contain the anatomical structures capable of perceiving noxious and painful external stimuli. Becerra et al. (1983) found abundant nerves bundles alongside blood vessels underneath the epidermal and dermal layer of fins. Two years later, Geraudie and Singer, 1985, while studying the regeneration process of amputated fins in mummichog fish (*Fundulus* sp) concluded that

regeneration was only viable if the damaged tissue had an adequate nervous supply. The nociceptive capability of fins in rainbow trout and Atlantic cod was confirmed by Chervova (1997); the most sensitive fins were the caudal, dorsal and pectoral fins. Chervova (1997) also demonstrated an increase in the pain threshold, measured by a decrease in behavioural responses associated with pain, when an analgesic was administered.

Several other studies have evaluated fin damage in farmed fish (Kindschi et al., 1991; Adams et al., 1993; Bosakowski and Wagner, 1994; Moutou et al., 1998; Latremouille, 2003; Hoyle et al., 2007; Person-Le Ruyeta et al., 2007). Some of them utilised categorical methods recording fin erosion, i.e. fin splitting, fin thickening and fin tissue loss (Hoyle et al., 2007). Others have used the relative fin index which is the relation between the longest ray fin of the fin and the length of the fish (Bosakowski and Wagner, 1994, Ellis et al., 2009, Kindschi, 1987). However, there is no evidence to which method fits better for the purpose as an operational welfare indicator.

The earliest reports of fin damage in salmonids comes from studies conducted in rainbow trout (Newman, 1956), juvenile Coho salmon (Chapman, 1962), Atlantic salmon (Keenleyside and Yamamoto, 1962), all of which described the aggressive behaviour as a direct biting/nipping of fins, mainly to the dorsal and pectoral fins.

More recently, experimental and field studies have suggested that the dorsal and pectoral fins are the main sites of attack in Atlantic salmon and rainbow trout (Abbott and Dill, 1985, Abbott and Dill, 1989, Cole and Noakes, 1980, Maclean and Metcalfe, 2001, Moutou et al., 1998, Noble et al., 2007a, Noble et al., 2008, St.Hilaire et al.,

2006). Turnbull et al. 1996 found that the appearance of fin lesions were consistent with fish bite profiles. They also noted that the absence of bacteria or swelling, epithelial hyperplasia and cellular inflammatory response that indicated active healing was occurring in the damaged tissue, and that nervous structures are required to give the necessary central and peripheral sensorial input to the fish.

Environmental factors such as high ammonia levels, alkalinity, exposure to ultraviolet light and low nutritional quality of the diet may be involved in the maintenance, aggravation and even the generation of fin damage (Bosakowski and Wagner, 1994b, Kindschi et al., 1991, Latremouille, 2003, St.Hilaire et al., 2006). There have been inconsistent reports that irregular surface of tanks or nets are associated with fin damage (Branson et al., 2008, St.Hilaire et al., 2006).

Although environmental factors clearly have an influence on the extent of fin damage in farmed fish there may be situations when the control of these environmental factors is not practical or not economically viable. Therefore, it seems apparent that the only way to control the occurrence of fin damage in farmed fish is through an understanding of the factors that govern aggressive behavioural interactions amongst fish.

Conceptually, aggression in human and non-human animals has been defined as the delivery of noxious or potential harmful stimuli to other co-specifics (Archer, 1988, Huntingford and Turner, 1987, Johnstone, 2001, Lorenz, 1996). Conflict may be an inevitable side-effect of living in groups, as well as playing a pivotal role in how group

members relate to one another (Huntingford and Turner, 1987, Krause and Ruxton, 2002, Levine and Moreland, 2006).

Aggressive behavioural interactions may arise due to incompatibility of common goals between members of the same group, such as foraging, competition for resources (e.g. as food, space or reproductive success), and is usually self-limiting (Krause and Ruxton, 2002, Levine and Moreland, 2006, Morrell and James, 2008, Smith and Price, 1973).

Fin biting or nipping in fish can be interpreted as an interaction between two individuals in an aggressive response related as part of the social interactions resulting from group living (Huntingford and Adams, 2005, Huntingford and Turner, 1987, Krause and Ruxton, 2002). Although aggression usually occurs in a dyadic relationship, it must be analysed in a wider social context and in isolation (Malloy et al., 2005). The latter seems especially important in non-human animals where different aggregations, group formations and social activities are common in the wild (Arnold, 1985, Gueron et al., 1996, Krause and Ruxton, 2002) and is imposed upon domesticated and farmed animals (Estevez et al., 2007, Syme and Syme, 1979).

Much of the work done in humans and non-humans has stated that the existence of a social context is necessary for the expression of behaviours, and variations in behaviour (Broom and Fraser, 2007, Fraser, 1985, Gosling, 2008, Malloy et al., 2005). It has been suggested that excessive aggressive behaviour expressed by some animals must be carefully assessed in order to differentiate extreme aggressive behaviour from true pathological aggressive behaviour in animals (Sih et al., 2004, Goslin, 2008, Sih

and Bell 2008). In humans and non-human animals, pathological aggressive behaviour may originate from an internal dysfunction, somatic or non-somatic, and separate from personality traits, copying styles or behavioural syndromes (Sih et al., 2004). In fact, from an evolutionary point of view, personality traits have evolved to increase individual and social fitness (Smith and Blumstein, 2008) and might be regarded as a way of coping with a stressful situation (Koolhaas, 2008, Koolhaas et al., 2007, Øverli et al., 2007). Further, a study conducted by Sørensen et al. (2007) found an increase in neurogenesis in subordinate fish which may be a consequence of aggressive social interaction between fish.

In natural environments, aggressive animal behaviour is typically triggered by competition for resources such as space, food and reproduction (Huntingford and Turner, 1987, Krebs and Davis, 1978, Smith and Price, 1973). One of the main causes of aggressive behaviour is dispute over boundaries by wild animals where the concepts of 'individual space' and 'social space' has been widely recognised (Brereton, 1971, McBride, 1971, Myers et al., 1971, Wynne-Edwards, 1971, Zayan, 1985). It is accepted that individual and social spaces are context dependent and the individual space required by any animal, including fish, ultimately depends on intrinsic factors such as size, age, social interaction strategies and the relationship with other external resources such as food, and environmental quality (Couzin et al., 2002, Godin and Godin, 1997, Syme and Syme, 1979).

However, farming systems such as aquaculture impose artificial groups and environments on individuals who may be unable to escape the contact of aggressive individuals, choose companions or manipulate group size by their own means. As

mentioned before, although group sizes and stocking densities have been studied in farmed fish, no substantial understanding of the social mechanisms and social effects have been fully investigated.

In fish, particularly in farmed salmon, aggression related to food has been widely studied, as it is accepted that food and feeding represents approximately 70% of all the production costs of farmed fish (Personal communication, Biomar Chile SA). Food competition associated with social behaviours, structures and dynamics have been mostly studied as in classical dominance or hierarchical process (Adams et al., 1998, MacLean et al., 2000). Additionally, in field studies at a commercial level, the effect of food restriction on the occurrence of fin damage in farmed salmon has been studied at the population level (Adams et al., 2007, Noble et al., 2008, Noble et al., 2007b, Rasmussen et al., 2007).

Therefore, it has been recognised that the current main welfare topics in farmed fish are associated with food availability and there is a need to fully understand the underlying social mechanisms governing aggressive behavioural interactions in farmed fish (Volpato et al., 2007, Ashley, 2007, Huntingford et al., 2006).

1.8. Social behaviour aspects related to welfare in farmed fish.

Most animals, including humans, have a strong tendency to live in groups, during part or their entire life (Krause and Ruxton, 2002, Levine and Moreland, 2006). From an evolutionary perspective, the main benefits of living in groups have been associated to a

reduction of predation risk and an increase in predator detection (Arnold and Fraser, 1985, Krause and Ruxton, 2002, Krebs and Davis, 1978, Morrell and James, 2008, Pulliam and Caraco, 1978). Also, living in groups leads to the synchronization of several behaviours such as feeding, foraging, resting and reproduction (Krause and Ruxton, 2002).

Social interactions such as grooming, social learning and playing, and reduction on the energetic cost of movement have been associated positively to living in groups in animals, including fish (Krause and Ruxton, 2002, Slater, 1999, Sloman et al., 2005). However, and as mentioned before, living in groups is also associated with costs related to competition for valuable resources, such as food, water and shelter (Krause and Ruxton, 2002).

Group formation is considered to be a self-regulated process and it is considered a by-product of environmental conditions in non captive animals, whereas in farming conditions this process can be manipulated by controlling the resources (food and water) available to all animals (Lucas and Southgate, 2003). Domestication of animals, including fish, has focused on maximization of productivity traits and heavily selecting for parameters such as increased growth rate and increased reproduction rates with a lack of consideration of behavioural traits leading to the development of several behavioural syndromes and increased disease susceptibility in terrestrial (Abeni and Bertoni, 2009, Broom, 2001, Koolhaas et al., 1999) and aquatic animals (Huntingford and Adams, 2005, Huntingford, 2004, Ruzzante, 1994, Koolhaas et al., 1999, Broom, 2001, Andersen et al., 2006).

Behavioural syndromes (also named as copying styles, personalities or temperaments), defined as consistent individual behavioural variation maintained across different social contexts, have been identified in non-human animals (Gosling, 2008, Sih et al., 2004) including fish (Conrad et al., 2011). It has been suggested that this consistency may originate from the individual as it recognises others within a group (Hoare et al., 2004, Levine and Moreland, 2006, Malloy and Kenny, 1986). Empirical evidence in fish has shown distinct behavioural syndromes, mainly related to the shyness-boldness personality axis (Conrad et al., 2011, Huntingford, 2004). Also, it has been shown that certain coping styles can be selected and genetically transmitted to future generations in Rainbow trout (Øverli et al., 2004). The evaluation and quantification of behavioural syndromes in farmed fish is of particular importance since repetitive undesirable behaviours of some individual fish during stressful situations such as feed restriction or high stocking densities might lead to poor welfare due to an increase in aggressive interactions that finally leads to fin damage or low growth in others (Huntingford and Adams, 2005).

Fish can decide to pack together, for example by schooling, to avoid or distract potential predators, forage or reproduce (Griffiths and Magurran, 1997, Partridge et al., 1980, Pitcher and Partridge, 1979, Soria et al., 2007). In farmed salmonids, group decision has been suggested to be determined by feeding demand and predator avoidance, where circular schooling movements are seen especially in the growing phase (Adams et al., 1998, Dill, 1977, Noble et al., 2008, Puckett and Dill, 1985, Wankowski, 1979). The establishment of a hierarchical ladder has been investigated and found to be strongly associated with aggressive behaviour of individuals due to

territoriality and food availability (Adams et al., 1998, Dill, 1977, Estevez et al., 2007, Noble et al., 2008, Puckett and Dill, 1985, Wankowski, 1979).

Several theories and models have been implemented to investigate the social aspect of living in groups in human and non-human animals, such as the social relations model (Kenny and La Voie, 1984, Malloy and Kenny, 1986, Chase et al., 2002), association indexes (Whitehead, 1997, Cairns and Schwager, 1987) and more recently social network analysis (Bode et al., 2011, Croft, 2005, Drewe, 2010, Drewe et al., 2009, McCowan, 2007). On the basis that collective individual social actions and behaviours affect group dynamics, structure and behaviour, Bryson et al. (2007), Hemelrijk and Kunz (2005), Snijders (2001), Snijders et al. (2007), Snijders et al. (2010), have implemented and used actor or agent-based models for the evaluation social networks and groups. No work has been carried out to study the persistence of individual differences in salmonids. It may be useful to identify and quantify if the aggressive behaviour of certain members of a group is maintained in longitudinally and cross-situational studies in salmonids fish associated with welfare related consequences such as fin damage.

1.9. Social network analysis as a tool to evaluate the effect of social behaviours involved in the welfare of fish.

In recent years, social network theory and analysis have been introduced and applied to animal behaviour and health studies (Bell et al., 1999, Croft, 2005, Croft et

al., 2008, Croft et al., 2004). The use of social network analysis addresses complex biological questions as it investigates the direct and indirect relationships occurring among individuals of a group better than other measures such as association indices, and it can identify roles of individuals animals within the group and its social interactions influencing the structure and dynamics of a given group (Krause et al., 2007, Newman, 2003, Newman and Park, 2003, Wasserman and Faust, 1994, Wey et al., 2008). Adequate comparison among networks can be carried out, and group differences can be explained using standard mathematical formulas for measuring individual and group social characteristics (Boccaletti et al., 2006, Wasserman and Faust, 1994, Watts, 2004, Watts and Strogatz, 1998, Wey et al., 2008).

There are several ways to analytically study social networks ranging from complex networks theories (Boccaletti et al., 2006, Rosvall and Bergstrom, 2007) to 'small world' theories (Watts and Strogatz, 1998). All social network analysis theories are based on the following main principles (Wasserman and Faust, 1994, Wellman and Berkowitz, 1988):

a) Ties between individuals are usually asymmetrically reciprocal, differing in content and intensity, thus asymmetries arise in relations, intentionally or unintentionally.

b) Ties link members indirectly and directly and are defined in terms of longer network structures. Also, indirect ties and relations are abundant and quantifiable using network analysis.

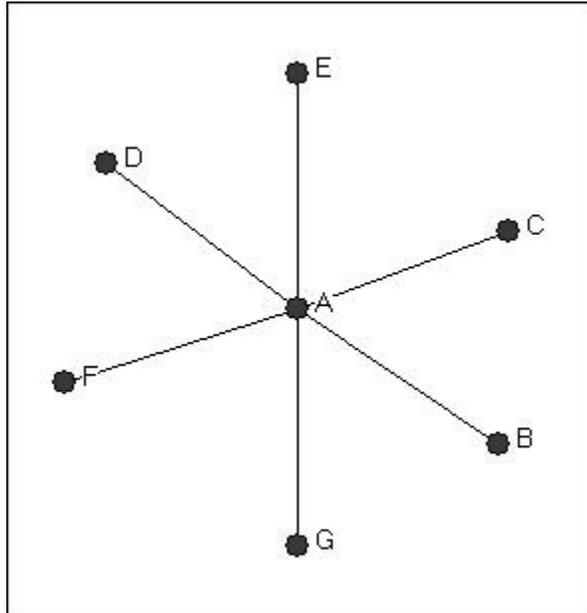
c) The structure of social ties creates non random networks, forming clusters and subgroups, as individuals have a certain number of ties available and have to decide with whom to tie to.

d) Behavioural interactions do not flow evenly or randomly in a network structure, because of formed asymmetric ties between individuals. It is important to note that positions in the group can become resources in the structure, with the formation of specific roles such as gatekeepers and brokers. Gatekeepers control access to the organization leader and therefore, have more influence in the network. Brokers can link two networks while having no affiliation to either of them.

Briefly, the following social network variables have been studied in animal groups and further details are reviewed in Wasserman and Faust (1994):

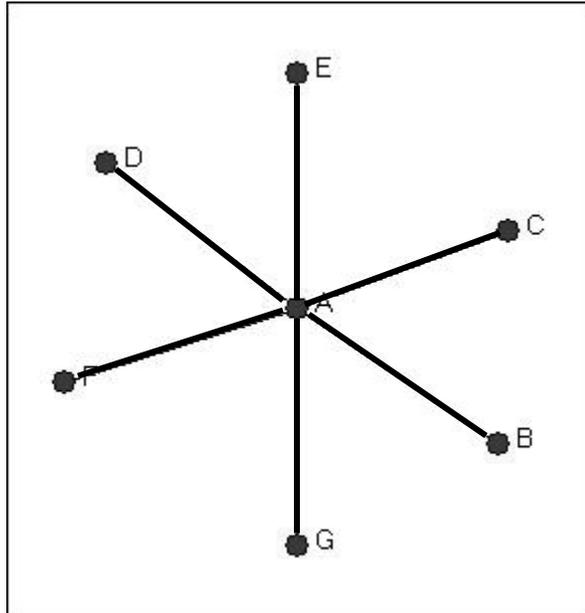
a) Centrality: measures the quantity of direct connections an individual has with others within the network (Wasserman and Faust, 1994). Centrality has been demonstrated to be one of the best network measures for quantifying transmission of infection in humans (Bell et al., 1999) and social association behaviours in mammals (Manno, 2008). It can be used to classify individuals according to their behaviours. As shown in Diagram 1.1., highly central individuals are connected to most or all members of the network (A), and non-central individuals (B,C,D,E,F,G) are connected to only a few, in this case just one (A).

Diagram 1.1. Graphical representation of centrality.



- b) Density: quantifies the amount of potential connections between individuals that are actually present. A high density indicates network saturation, meaning that almost all potential interactions are present while low densities indicate sparse networks meaning fewer potential interactions between individuals are present. Diagram 1.2. shows the representation of density where the thickness of the line represent how dense are connections between individuals within the network and it is clearly denser than network in Diagram 1.

Diagram 1.2. Graphical representation of density.



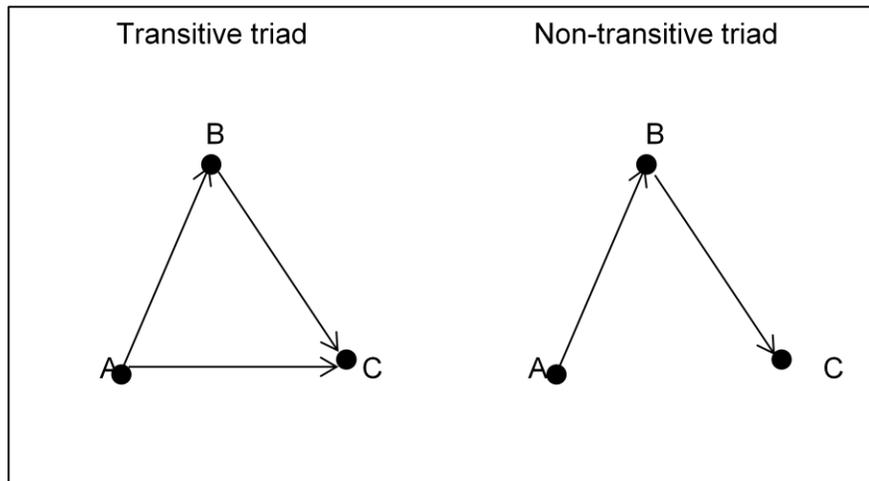
- c) Clustering coefficient: measures the extent to which two neighbours of an individual are themselves neighbours. High clustering coefficients suggest that individuals are surrounded by individuals that are well connected with each other forming subgroups or populations within the network.

- d) Distance: measures the mean number of connections between the members of all possible pairs of individuals within a network. High distance values mean fewer interactions.

- e) Transitivity: quantifies the degree of connection of three connected individuals or triads. Triads are considered important social structures as they represent the minimal connection every individual can have, such as ego, alter and

other (Newman and Park, 2003). Low transitivity indicates abnormal social systems and therefore social disturbances may occur due to non stable relationships among individuals. High transitivity has been described as a basis of stable social systems (Wasserman and Faust, 1994). Diagram 1.3. shows the graphical representation of transitivity. A triad is said to be transitive whenever A is connected to B and B is connected then A has to be connected to C.

Diagram 1.3. Graphical representation of transitivity.



1.10. The use of social network analysis in fish.

In fish, only a few studies in wild fish have addressed the use of social network analysis. Earlier studies of Croft et al., 2003, investigating the social mechanism in shoal composition of guppies, found that there was a structure in shoal composition due to mainly to social factors. Further studies using social network analysis of wild guppies suggested the existence of strong, stable social structure with altruism characteristics such as cooperative behaviour for localising predators (Croft et al., 2004).

However, no studies have been conducted using social network analysis in order to give a deeper understanding of social dynamics within groups of fish, as well as to determine the individual roles of each member in the group which could give answers to the underlying mechanisms in fin damage and aggression.

As described in the previous subsections, the causes of fin damage are multifactorial and the need for further research has been emphasised in food related issues and stocking densities. In that respect, the effect of long and short food restriction periods, unpredictable food delivery and different stocking densities on the behavioural changes in aggression of Atlantic salmon leading to fin damage have not clearly elucidated. The following chapter described the research carried out using social networks and networks dynamics in order to clarify the occurrence and the dynamic changes in development of fin damage as a welfare indicator. The methodology is similar but full description of the experiment is included in each.

The hypotheses of the present dissertation were that:

- a) Feed restriction periods (long or short), high stocking densities and unpredictable food delivery schedules would not have an effect on the behavioural interactions of Atlantic salmon parr leading to fin damage.
- b) Short feed restriction, high stocking densities and unpredictable food delivery schedule would not cause a persistence of aggressive behaviours in Atlantic salmon fish over time after restoration to control or normal conditions.
- c) The quantification and identification of socially important fish within a group can be assessed using social network analysis, dynamic social network and other physical measurements.

2. Chapter 2. Social network analysis of behavioural interactions on fin damage development in Atlantic salmon (*Salmo salar*) during a long (30 days) food restriction period.

(The following chapter has been published in Applied Animal Behaviour Sciences and attached as an Appendix)

2.1. Introduction.

Fin damage is increasingly being used as a potential indicator of the welfare of farmed fish (Ashley, 2007, Broom, 2007, Broom and Fraser, 2007). It has been associated to competition as a result of inadequate housing and husbandry conditions, both of which are known to influence fish welfare. Fin damage can be caused by increased aggression in both rainbow trout and Atlantic salmon (Abbott and Dill, 1985, Turnbull et al., 1998) and the tissue damage can be maintained and aggravated by other risk factors such as poor water quality resulting mainly from high ammonia and low dissolved oxygen levels (Person-Le Ruyet et al., 2008). However, the relative importance of the factors has not been fully elucidated and have often been confounded in previous studies (Adams et al., 2007).

Also, fish species are social and form aggregations known as schools or shoals (Ruzzante, 1994, Viscido et al., 2007). In this context, fin damage has been investigated

in terms of dyadic interactions related to aspects of group-living such as competition and aggression over food resources, space and territory (Huntingford and Adams, 2005, Huntingford and Turner, 1987). Intraspecific competition, associated with access to food has been described in fish (Ward et al., 2006) both in the wild (Dunbrack et al., 1996) and under farming conditions (Noble et al., 2007a). It can also lead to the formation of dominance hierarchies and territories (Metcalfe et al., 2003).

As mentioned previously in Chapter 1, the use of novel tools such as social network analysis to quantify relationships between aggression and fin damage in farmed fish will help to identify the precise social role of potentially key individuals and their social position within groups causing the damage. It can also quantify the social effects of feed-restriction, a common procedure carried out in aquaculture during sampling periods, grading and the transport of farmed fish (Lucas and Southgate, 2003).

The aims of this study were to quantify the impact of feed-restriction on the development of fin damage in juvenile Atlantic salmon and to relate this to changes in social dynamics, structure and organisation in order to quantify the underlying role of behavioural interactions in the development of fin damage.

2.2. Materials and methods.

2.2.1. Animals and experimental groups.

The experiment was conducted at the Aquaculture Research Station in Tromsø, Northern Norway during the summer of 2008 (Project Number H08/26). The study was conducted in accordance with current Norwegian Fish Welfare legislation (Ministry of Agriculture of Norway, 1996, Ministry of Agriculture and Food of Norway, 2010, 2002). These regulations also adhere to the European Convention for the Protection of Vertebrates used for Experimentation and other Scientific Purposes (European Union, 1998b).

Eight experimental groups were used each consisting of six clinically healthy year 1+ Atlantic salmon weighing 137.5 ± 20.1 g (mean \pm SD). The fish were sourced commercially from Haukvik Kraft-Smolt A/S, Tribe Batnfjord, generation 2007. Fish were kept for 420 days prior to the experiment in a 1 m³ holding tank according to the Aquaculture Research Station Standard Procedure. There were two experimental periods: (1) Pre-treatment (from day 0 to day 13) and (2) Treatment period (from day 14 to day 44). During the pre-treatment period fish were left to acclimatise and a maintenance level of commercial pelleted feed ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway, containing 21–22.8% fat, 50–52% protein and 22.2–22.8 MJ/kg) was delivered to all groups automatically from overhead feeders once a day at 10:00 hrs for 30 minutes at a rate of 1.5% of fish body weight/day. The amount of food delivered was adjusted weekly according to the expected weight gain and water

temperature following manufacturer feeding tables. After the 14 days of acclimatisation, four groups were selected randomly as Control groups (C) and four groups as Feed-restriction (FR) groups. In order to minimise the effect of management disturbances, two C groups and two FR groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the door. During the treatment period, C groups were given the same feeding regime as used during the acclimatisation period (1.5% body weight/day), whereas the FR groups were given a restriction ration representing expected food consumptions of 0.3% body weight/day. The feeding regime was maintained for 30 days. All fish were killed humanely using an overdose bath of benzocaine chlorhydrate (> 250mg/l freshwater) at the end of the experiment. The layout of the experimental room is given in Figure 2.1.

2.2.2. Containment and individual identification.

Fish were held in 300 litre plastic tanks with initial stocking densities of 4.1 ± 0.1 kg/m³ (mean \pm SD). All fish were individually identified at the beginning of the experiment under anaesthesia using a bath of benzocaine chlorhydrate (100 mg/l freshwater). Anaesthesia of fish was carried out using the recommended dosage of benzocaine chlorhydrate (100 mg/l freshwater). All fish entered into deep surgical anaesthesia within 3 minutes inside the anaesthetic bath. Surgical anaesthesia was determined when there was total loss of equilibrium and muscle tone, decreased respiratory rate and no response to stimuli when the base of the tail was firmly squeezed, as described in

several previous studies (Ferreira et al., 1979, Gilderhus, 1989, Gilderhus, 1990). Tagging was carried out during the phase of deep surgical anaesthesia within a period of less than 1 minute for each fish. After tagging, fish were transferred to designated experimental tanks and observed for 30 minutes after fully recovered from anaesthesia. Good recovery of anaesthesia was achieved in all fish and it was assessed as a progression of behaviours starting with evidence of opercular movements, then presence of regular and increased opercular movements accompanied by gross body movements and finally re-establishment of equilibrium with presence of preanesthetic appearance as described in previous studies (Ferreira et al., 1979, Gilderhus, 1989, Gilderhus, 1990).

An emergency recovery tank with highly oxygenated freshwater (>99% dissolved oxygen injected through diffusers connected to oxygen gas tanks) was available adjacent to the tanks in case of any fish did not recovered from anaesthesia within 5 minutes of transfer to the designated tank. However, it was not necessary to use the emergency recovery tank as all fish recovered from anaesthesia within 5 minutes in their corresponding designated tank. Nevertheless, as stated previously, fish were observed for a further 30 minutes after recovery from anaesthesia in order to detect the possible need of veterinary assistance.

Tags were designed using markings (2.5 by 2.5 cm) made of plastic printing paper (Xerox® Special Advanced Media Digital Colour, Premium Never Tear 95µ Polyester paper, PN: 003R98056) using combinations of black/white geometric designs and attached behind the dorsal fin of each fish using strong silk thread and standard

commercial Floy Tags as shown in Figure 2.2 (Hallprint®, Polyepalticthylene streamer tags, series PST).

2.2.3. Water quality and environmental conditions.

Filtered freshwater was provided throughout the experiment. Dissolved oxygen content ($98\pm 2\%$) and water temperature ($10\pm 2^\circ\text{C}$) were maintained, measured and recorded daily. Water flow was controlled at an exchange rate of 5 litres/minute and velocities of 0.75 fish body lengths/second. A 6:18 light-dark photoperiod regime was used during the study.

2.2.4. Physical measures.

The weight (g) and length (total tail-fork length in mm) of each fish were measured at the beginning and end of the experimental period. Specific growth rate (SGR) was calculated as: $\text{SGR} = (\ln w_1 - \ln w_0)/\Delta t$, where w_1 is the wet weight of fish (g) at sampling time 1, w_0 is the wet weight of fish (g) at sampling time 0, and Δt is the number of days between sampling times. Fulton's condition factor (K) was calculated from tail-fork length and mass of individual fish as: $K = W/L^3 \times w_1 \text{ or } 2$, where W is the weight of the fish (g), L^3 is the length of the fish to the power of 3, s is the total tail-fork length in mm and $w_1 \text{ or } 2$ is the weight of fish (g) at sampling time 1 or 2.

2.2.5. Quantification of fin damage.

Digital photographs were taken at the beginning and end of the experimental period and fin damage was evaluated in every fish using the Relative Fin Index (RFI) as described in Bosakowski and Wagner, 1994b. RFI has been suggested to allow reliable and objective measurement of the degree of fin damage (Person-Le Ruyet et al., 2007). Briefly, RFI was obtained by dividing the maximum total fin length (longest fin-ray length from body) by the fork length in each individual fish. All pectoral, ventral, anal, caudal (upper and lower) and dorsal fins were measured and quantified using this index. In addition, the total number of fin splits (separation of fin rays greater than 3 mm) was recorded in each fish.

Fin erosion was measured using a modified method utilising an ordinal scale of 0, 1, 2 and 3, corresponding to no erosion (0% of fin eroded), mild erosion (1% to 24% of fin eroded), moderate (25% to 49% of fin eroded) and severe erosion (>50% of fin eroded), respectively (after Hoyle et al., 2007). An example of fin erosion is shown in Figure 2.3.

Additionally, fish were visually examined for other external lesions in their bodies as evidence of biting during sampling periods and at the end of the experiment.

2.2.6. Behavioural observations and social interactions.

The light inside the experimental were automatically turned on at 08:00 hrs and turned off at 14:00 hrs. Ten minute video recordings of the fish in each tank were carried out at 09:00 to 09:10, 10:00 to 10:10 and 11:30 to 11:40 on each day of the experimental period. This allowed recordings 1 hour before feeding, during the first ten minutes of feeding and 1 hour after last food delivery from the automatic feeders. Video recordings were made using CCTV colour cameras (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) located 1 m above each tank. Recordings were captured and digitalised using a DVD/HDD recorder (Pioneer© DVR-550H-S).

2.2.6.1. Association interaction measurements.

Association interaction matrices were constructed using data collected from the video recordings at 1 minute intervals. One fish was recorded as being associated with another fish when they were within two fish body lengths or widths when parallel or perpendicular to their body axis respectively.

2.2.6.2. Aggressive interactions measurements.

Attacks, displacement and fin-biting were quantified using all occurrences recording (Lehner, 1996) from video recordings to obtain the total number of events for each fish. Attacks were defined as a rapid swimming movement(s) of fish X directed to fish Y with fish Y swimming away rapidly (to more than one fish body length distant) and with no physical contact occurring between fish during the attack. Displacements were defined as a slow swimming movement of fish X directed to fish Y with fish Y swimming away from fish X (to more than one fish body length distant) and with no physical contact between fish during the displacement. Biting was defined as a direct physical contact between fish X towards fish Y with a rapid escape movement response (to more than one fish body length distant) in fish Y after the biting. Therefore, fish were in practice fully capable of evading aggressor(s) except in the case of biting. Identification of initiator(s) and receiver(s) of aggression were recorded and weighted matrices for social network analysis were constructed for each video sampling period.

The information from the aggressive behaviour was used to calculate and compare the basic data relating both to the total amount of aggressive interactions and the sub-classifications of aggressive behaviours (attack, displacement and fin-biting) between experimental groups. The detailed information from the aggressive interactions (initiator and receiver of every aggressive interaction) was used to calculate social network analysis parameters within each experimental group. This approach was used to enable comparison of differences in aggressive interactions between groups using

total amount of aggressive interactions and also differences in aggressive behavioural interaction within each group by social network analysis.

An anticipated termination of the experiment was considered if there were cases of 'severe aggressive interactions'. A 'severe aggressive interaction' was defined as whenever a biting interaction produced observable bleeding at the biting point. However, it is important to note that no such severe aggressive interactions occurred at any time during the experiment.

Additionally, an early termination of the experiment was considered if fish were observed not to eat any feed pellets for three consecutive days. This threshold was decided on the basis of current recommendations suggesting that 72 hours (3 days) is required for the complete emptying of the fish gut before slaughtering whilst minimising adverse welfare effects (Humane Slaughter Society, 2005, RSPCA, 2007). This finding is supported by further evidence from studies quantifying the effects of food restriction in fish (Einen et al., 1999). Therefore, this previous scientific evidence was considered to support the adoption of these thresholds to indicate in what circumstances it would be necessary on ethical grounds to terminate the experiment. However, the need to terminate the experiment for any of these reasons did not occur.

2.2.7. Social network analysis.

Social network analysis was carried out using associative and aggressive interaction matrices using UCINET© software (Borgatti et al., 1999). Centrality, density, clustering coefficient, distance and transit were measured. A short description of these network variables quantified are presented as follows specifically for this experiment (refer to Chapter 1 for a more detailed explanation). Centrality measures the quantity of direct connections an individual has with others within the network (Wasserman and Faust, 1994). In the case of association interaction matrices, only the overall degree of centrality was measured, as interactions were symmetrical and reciprocal. Because aggressive interactions could be reciprocal or non-reciprocal and usually non-symmetrical, in-degree centrality (amount of aggression received) and out-degree centrality (amount of aggression generated) measures were calculated for each fish. Using this analysis, fish could be classified as initiators or receivers of aggression. Initiators were classified as fish whose out-degree centrality was four times greater or more than their in-degree centrality. Conversely, receivers were classified as individual fish whose in-degree centrality was four times greater or more than out-degree centrality. Otherwise, fish were classified as both initiator and receiver. In order to adequately compare networks, Degree Centralities were calculated as normalised to the total numbers of individuals in the network and thus expressed as percentages (Hanneman and Riddle, 2005).

All network analyses were carried out both for the acclimatisation (days 0 to 14), treatment (days 14 to 44) and entire experimental periods (days 0 to 44).

2.2.8. Schooling/shoaling and spatial position measurements.

Fish were recorded as schooling or shoaling at 1 minute intervals during the video recordings. Schooling was defined as a coordinated grouping behaviour where two or more fish were within association length/width and in the same orientation and direction, as described previously in the Association interaction measurements section.

Shoaling was defined as an uncoordinated grouping behaviour where fish were not within association length/width, as indicated in Chapter 1, section 2.2.6.1. Association interaction measurements, and showed no coordinated orientation and direction (Parrish et al., 2002).

Spatial positioning analysis for each schooling fish was recorded and defined arbitrarily and classified categorically as in the front, middle or back whenever more than 50% of the fish body length was located either in the first third, second third or last third of the school, respectively, measured from the nostril of the fish positioned in front of the school to the tail of the fish positioned in the rear of the school.

2.2.9. Statistical analyses.

Descriptive statistical analyses, the Shapiro-Wilkes test of normality and one-way analyses of variance were carried out on physical (weight and length), RFI, fin damage (splits and bites), SGR and K (Zar, 2009). In order to clarify the effect of treatment a general linear model described by $y = a + bx$, where a is the intercept (C group) and b the slope (effect of treatment), was carried out for weight and length variation (Zar, 2009). Differences in degree of dorsal fin erosion amongst treatments were analysed using the Chi-square test and the Chi-square test for trends (Zar, 2009). Correlations between dorsal fin erosion and other variables were analysed using the Pearson rank correlation (Zar, 2009). Kruskal Wallis tests were used to analyse the effect of tag type on SGR, final weight, dorsal fin erosion and weight variation, differences in total number of aggressive behaviours (bites, attacks and displacements), differences in centrality network parameters (overall, in-degree and out-degree), clustering coefficient, transitivity and reciprocity, and schooling and shoaling positions between C and FR groups, in addition to the effect of tag type on the type of individual (initiator, initiator/receiver and receiver of aggression) and schooling/shoaling positioning (Zar, 2009). Network distance and density were analysed by analysis of variance (Zar, 2009). Mantel tests (1000 permutations) were carried out for association and aggression matrices between acclimatisation and treatment periods in order to show that any differences were attributed to statistically significant changes in the behaviour of fish and not by chance (Zar, 2009). All statistical analyses were performed using R statistical software (R Development Core, 2008).

2.3. Results.

Fish under feed-restriction had significantly lower final weight ($F_{1, 46} = 4.39$, $P = 0.04$), SGR ($F_{1, 46} = 13.60$, $P < 0.01$), condition factor ($F_{1, 46} = 5.76$, $P = 0.02$) and weight gain ($F_{1, 46} = 14.24$, $P < 0.01$) compared with unrestricted fed fish. However, there were no treatment differences in final length of fish ($F_{1, 46} = 0.60$, $P = 0.44$). In addition, there was a significantly higher variation in weight gain ($F_{1, 46} = 14.24$, $P < 0.01$) within each FR group (CV: 95.09) but not in C groups where the variation was smaller (CV: 15.08). Length variation and weight gain of each fish in the experimental groups are shown in Table 2.1 indicating that some fish lost weight. This further supported by the results from the linear regression model showing a significant effect on weight variation while belonging to FR compared to C group ($y = 23.783 - 17.221x$, $P < 0.001$). The same situation did not happen for length variation ($y = 1.279 - 0.437x$, $P = 0.29$).

Total values for fin damage evaluated in dorsal fin in Table 2.2. Only 31.25% of all fish had any dorsal fin erosion (degrees 1, 2 and 3). However, fish under feed-restriction showed more moderate (16.6% of fish) or severe (7.2% of fish) amounts of fin erosion compared with control fish ($\chi^2_3 = 6.88$, $P = 0.07$) in dorsal but not in other fins ($P > 0.050$). There was a linear relationship between the total number of fish with erosion and the degree of erosion (χ^2 trend for proportions = 6.54, $P > 0.05$). Dorsal fin erosion was positively correlated with the occurrence of dorsal fin bites in FR groups ($r^2 = 0.54$, $P = 0.03$). Moreover, there was a strong correlation between the number of dorsal fin bites and dorsal fin erosion ($r^2 = 0.84$, $P < 0.01$). There were no significant

differences between FR and C groups in initial and final RFI in all fins evaluated ($P > 0.05$).

Aggressive behavioural interactions for each type of aggression in each group are presented in Table 2.3. Total aggression was significantly higher in FR groups (232.5 mean events/group/30 hours observation period) than in C groups (135.35 mean events/group/30 hours observation period) ($H_1 = 4.08$, $P = 0.04$). The number of attacks between fish was also significantly higher in feed-restricted fish compared to control fish ($H_1 = 5.39$, $P = 0.02$). Although biting was observed only to fins during sampling periods, there was no visual evidence of lesions elsewhere in the fish at the end of the experiment.

Differences in social network variables between groups were found only during the treatment period and not during the acclimatisation period ($P > 0.05$) using 390 matrices for each type of interaction. Therefore, comparisons between groups were carried out during the treatment period (day 14 to day 44). In addition, the increase in the number of aggressive interactions in feed-restricted fish was not due to chance ($Z = 18486$, $P = 0.01$) confirming that the increased aggression between fish was due to a real change in the behaviour of fish subjected to feed-restriction. The same situation did not occur in fish fed to satiation ($Z = 701$, $P > 0.05$). The results showing the values obtained for social network variables are presented in Table 2.4.

FR groups showed significantly lower transitivity compared to C groups ($H_1 = 7.04$ $P < 0.01$). Social distance between fish was significantly lower in the FR groups and centrality tended to be higher ($F_{1, 22} = 3.34$, $P = 0.05$ and $H_1 = 2.09$, $P = 0.11$, respectively). Figures 2.4 and 2.5 present the networks based on degree of centrality in

individual fish and the association tie strength in C and FR groups, respectively. These findings suggest that fish in feed-restricted groups tended to associate uniformly and the associations were stronger with all other member of the groups compared to fish in C groups.

Networks were significantly denser and less distant in FR groups ($F_{1, 22} = 9.72$, $P < 0.01$ and $F_{1, 22} = 3.64$, $P = 0.05$, respectively) compared with control fish. FR groups showed higher out-degree ($H_1 = 7.49$, $P < 0.01$), and in-degree centrality ($H_1 = 17.91$, $P < 0.01$) compared to C groups. Feed-restricted fish networks had higher clustering coefficients values ($H_1 = 8.33$, $P < 0.01$). Figures 2.6 and 2.7 show the out-degree centrality for C and FR group networks respectively.

These findings indicate that there were distinct differences in out and in degree centrality leading to particular differentiation of roles within the group of feed-restricted fish. Typically in each group, two fish were found to be highly aggressive and were classified as initiators of aggression while two fish were usually the receivers of the aggression. This differentiation led to the formation of clusters of initiators and receivers in each group as can be seen in Figure 2.7.

Additionally, initiators of aggression were fish that gained more weight while receivers of aggression were fish maintaining or losing weight, as in Table 2.5.

Tag type was not significantly associated with whether fish were initiators or receivers of aggression ($H_5 = 3.04$, $P > 0.05$).

As shown in Table 2.5, fish in FR and C groups did not differ in their schooling preference ($H_1 = 0.88$, $P > 0.05$) or shoaling preference ($H_1 = 0.89$, $P > 0.05$). However, it can be observed that initiators of aggression tended to maintain central schooling

positions whenever feed-restricted fish schooled ($H_2 = 5.49$, $P = 0.06$). Receivers, on the other hand, did not have any consistent position while schooling ($P > 0.05$).

There were no significant effects of tag type on condition factor ($H_{5,42} = 1.45$, $P > 0.05$), SGR ($H_{5,42} = 4.83$, $P > 0.05$), final weight ($H_{5,42} = 1.82$, $P > 0.05$), dorsal fin erosion ($H_{5,42} = 3.75$, $P > 0.05$) or weight gain ($H_{5,42} = 3.97$, $P > 0.05$). Thus, tag type had no effect on any of the welfare-related variable assessed in this study.

2.4. Tables and figures.

Table 2.1. Length variation (L, in mm), weight variation (W, in g) in fish and Coefficient of Variation (CV) according to experimental group.

Fish	Feed-restriction								Control							
	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7		Group 8	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
A	1.3	12.5	0.7	2.3	0.1	-0.3	0.4	0.8	0.5	1.6	0.5	1.7	0.4	1.2	0.0	-0.3
B	1.4	21.5	0.1	-0.2	0.4	0.9	0.4	0.8	0.1	0.0	0.4	1.3	0.1	-0.2	0.3	0.8
C	1.6	22.5	0.6	1.9	0.3	0.4	0.3	0.2	0.7	2.2	1.2	4.3	0.4	1.0	0.4	0.9
D	0.7	6.5	0.1	0.0	0.1	0.2	0.2	0.5	0.1	0.2	0.2	0.6	-0.1	-0.8	0.4	1.5
E	0.8	4.5	0.2	0.2	0.0	-0.4	0.0	-0.5	0.2	0.4	0.2	0.6	0.3	0.8	0.2	0.3
F	0.6	1.5	-0.2	-1.4	0.3	0.8	0.2	0.4	0.1	0.2	0.3	1.1	-0.1	-0.6	0.2	0.7
CV	39.2	77.4	135.6	297.4	77.5	203.6	60.7	132.1	90.4	118.3	80.9	86.8	140.3	83.3	60.7	93.2

Table 2.2. Number of splits, dorsal fin bites and degree of erosion of dorsal fin of fish (N=24 for each treatment).

Group	Dorsal fin damage							
	Degree of Erosion (number and %)					Bites Splits		
	0 [#]	1 [#]	2 [#]	3 [#]	Sum 1,2,3			
Control (N=24)	20(83.4%)	4 (16.6%)	0	0	4 (16.6%)	4	8	
Feed-restriction (N=24)	13 (51.2%)	6 (25%)	4	1 (7.2%)	11 (45.8%)	8	12	
			(16.6%)					
Total (N=48)	33 (68.5%)	10 (20.8%)	4 (8.3%)	1 (2.4%)	15 (31.25%)	12	20	

[#]: 0= No erosion (0% of erosion); 1=Mild erosion (1 to 25% erosion); 2=Moderate erosion (26 to 50%); 3= Severe erosion (> 50% of erosion).

Table 2.3. Mean aggressive behaviours according to type of aggression and experimental group.

	Biting	Displacement	Attack	Total
Control	12.5	88.75	34	135.35
Feed-restriction	23.25	153.5	55.75*	232.5*

* = $P < 0.05$

Table 2.4. Values of social network analysis variables (mean \pm SD) according to aggressive and associative interactions network in control and feed-restriction groups.

<i>Associative interaction networks</i>						
	Degree Centrality (%)		Density	Clustering coefficient	Distance	Transitivity (%)
Control	15.58+/-1.69		1.04+/-0.08	0.010+/-0.009	1.18+/-0.04	92.55+/-3.72
Feed-restriction	16.29+/-0.85		0.86+/-0.05	0.087+/-0.006	1.09+/-0.02*	71.96+/-2.53*

<i>Aggressive interactions networks</i>						
	Centrality (%)		Density	Clustering coefficient	Distance	Transitivity (%)
	Out-degree	In-degree				
Control	31.18+/-4.18	14.25+/-1.24	1.44+/-0.15	0.014+/-0.015	1.41+/-0.01	64.44+/-1.92
Feed-restriction	38.63+/-1.58*	38.63+/-2.38*	5.91+/-0.59*	0.055+/-0.068*	1.20+/-0.04*	78.08+/-4.63

* : $P < 0.05$

Table 2.5. Weight gain, length variation, schooling/shoaling preference and spatial position when schooling of fish (mean \pm SD) according to behavioural classification of fish (I= Initiator of aggression, I/R= Initiators and receivers of aggression, R= Receivers of aggression) in treatment groups.

Group	Type of individual	Weight gain (g)	Length variation (g)	Schooling (% of time)	Shoaling (% of time)	Position when schooling (% of time)		
						Front	Middle	Back
Control (N=24)	I/R (N=24)	23.8 \pm 19.8	1.3 \pm 0.5	53.3 \pm 18.0	46.7 \pm 18.0	20.2 \pm 14.3	53.9 \pm 16.5	25.9 \pm 15.5
Feed Restriction (N=24)	I (N=6)	12.2 \pm 8.4	1.0 \pm 0.3	45.7 \pm 15.6	54.3 \pm 15.6	13.9 \pm 5.2	62.1 \pm 19.6	24 \pm 17.8
	I/R (N=12)	6.5 \pm 8.4	0.8 \pm 0.4	52.5 \pm 21.0	47.5 \pm 21.1	25.5 \pm 19.6	54.4 \pm 21.1	20.1 \pm 17.6
	R (N=6)	1.2 \pm 10.5	0.8 \pm 0.5	43.8 \pm 26.8	56.2 \pm 26.6	31.8 \pm 25.7	34.9 \pm 12.2	33.3 \pm 30.9

Figure 2.1. Experimental room layout. Tanks, automatic feeder and CCTV are depicted in the photograph.

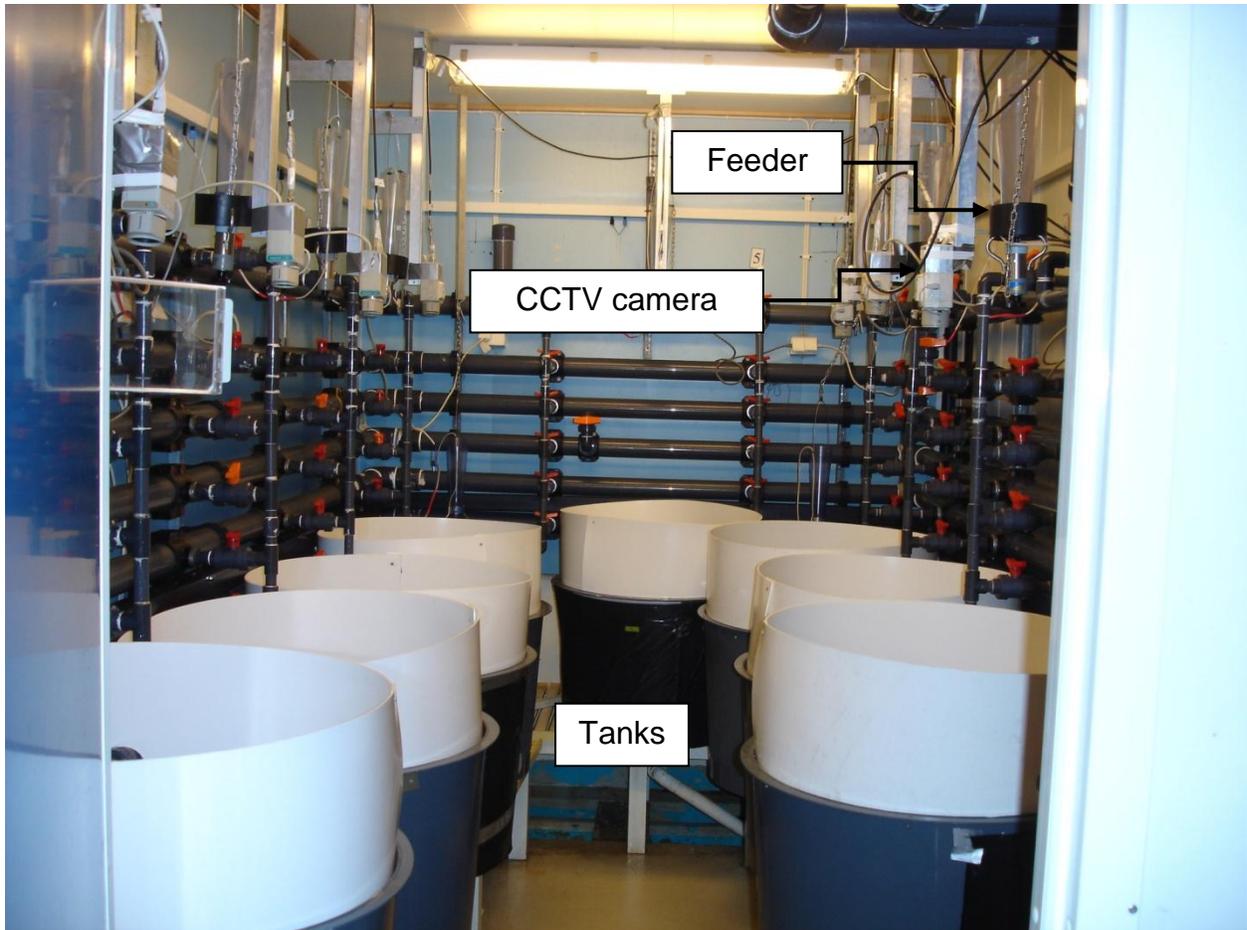


Figure 2.2. Photograph of fish after tagging showing the type of tag used in the experiments.



Figure 2.3. Photograph of a fish with moderate dorsal fin erosion indicated by the arrow.

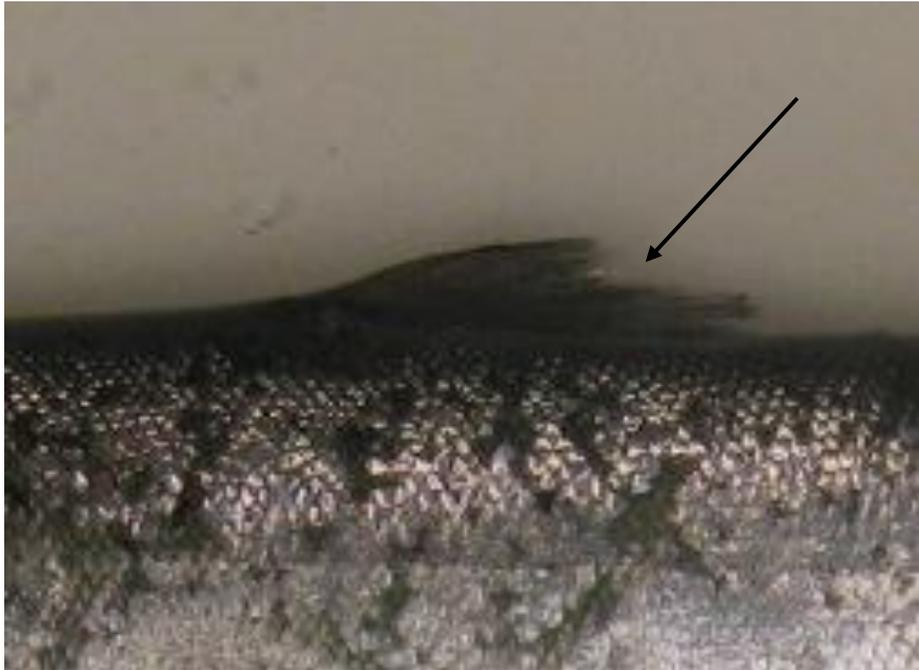


Figure 2.4. Social networks for associative interactions in C groups. Individual fish are depicted by circles in which the diameter represents the magnitude of the centrality degree and line thickness represents association strength.

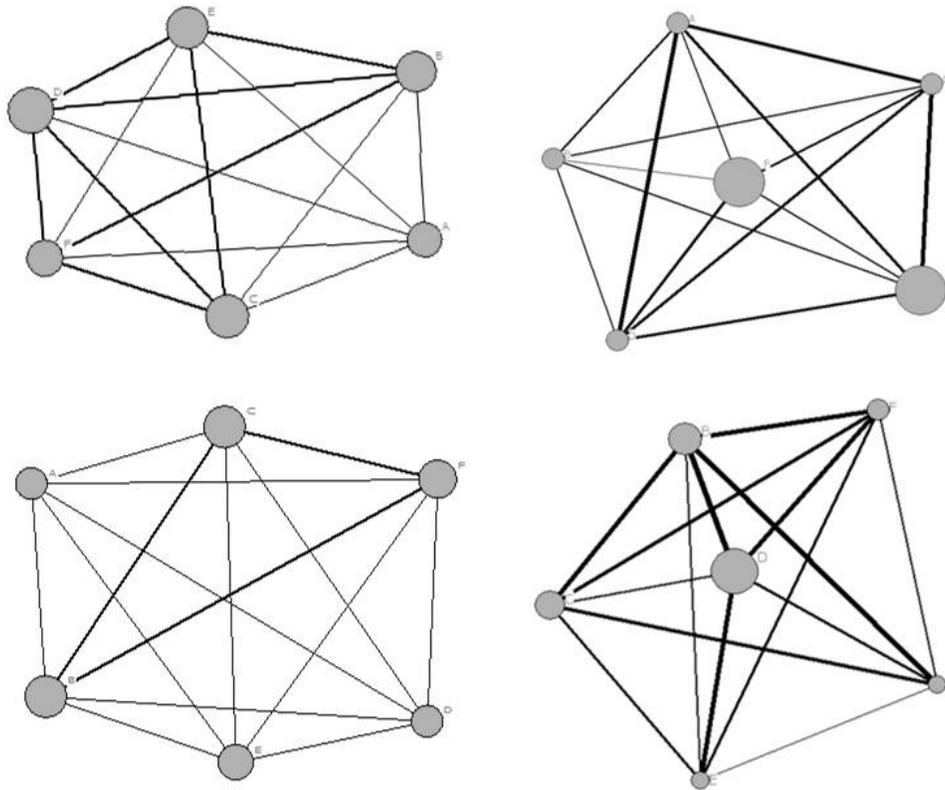


Figure 2.5. Social networks for associative interactions in FR groups. Individual fish are depicted by circles in which the diameter represents the magnitude of the centrality degree and line thickness represents association strength

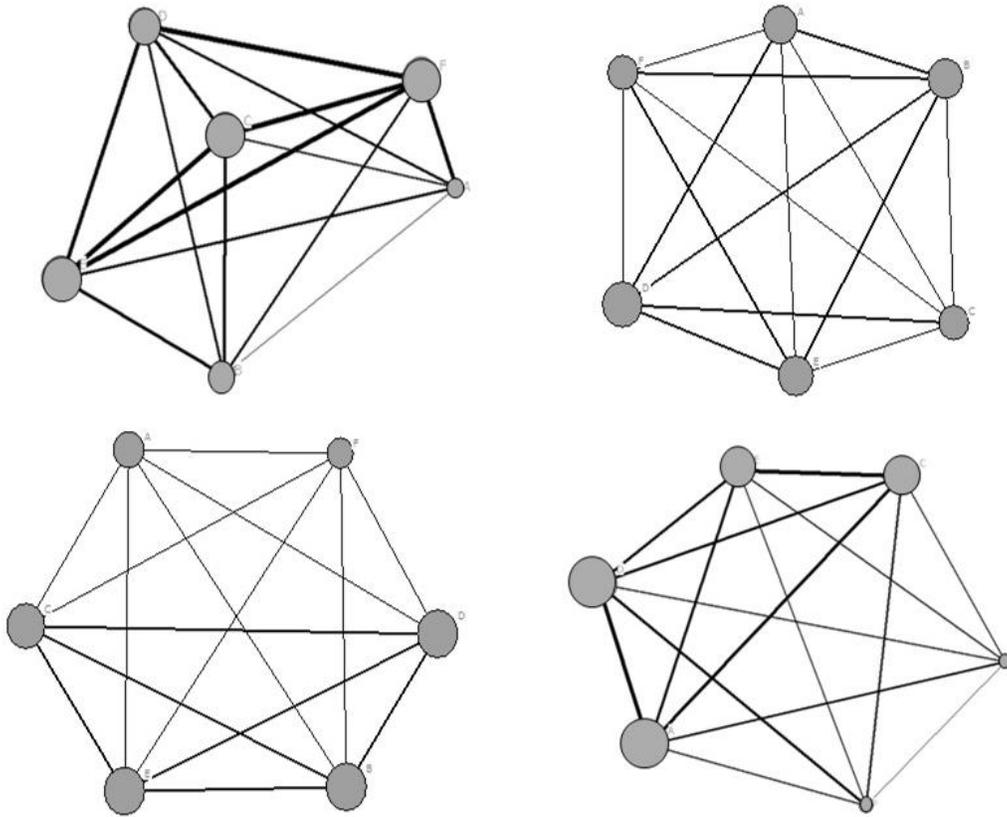


Figure 2.6. Social networks for aggressive interactions in C groups. Individual fish are depicted by circles in which the diameter represents the magnitude of the out-degree centrality degree (aggression expressed by fish) and line thickness represents aggression strength.

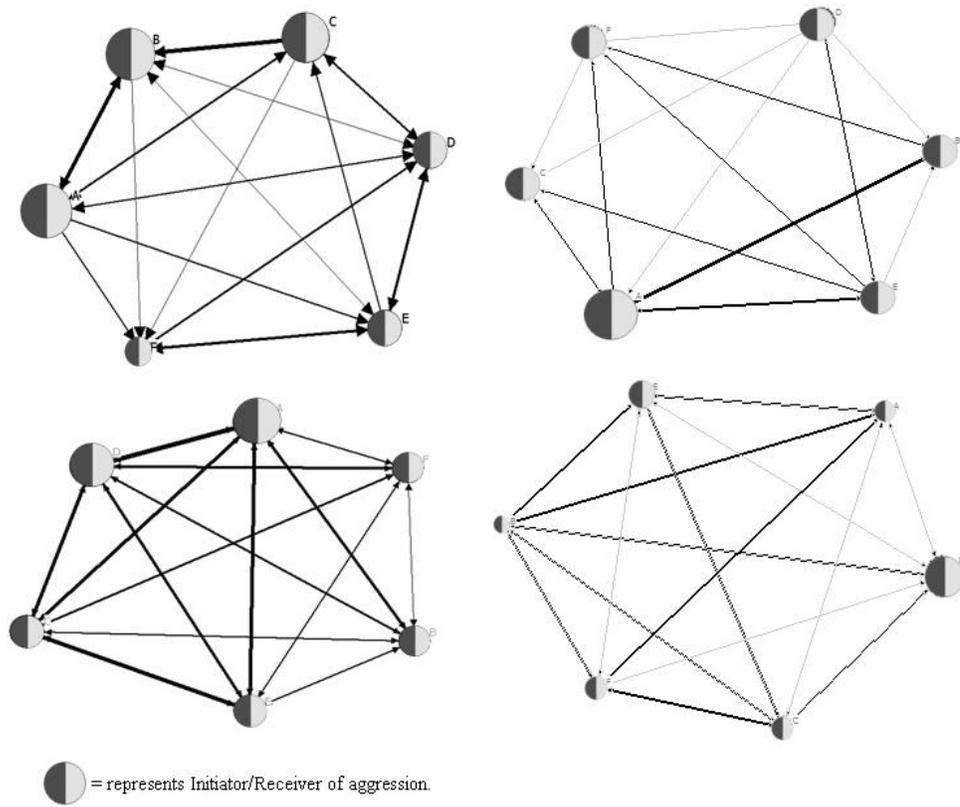
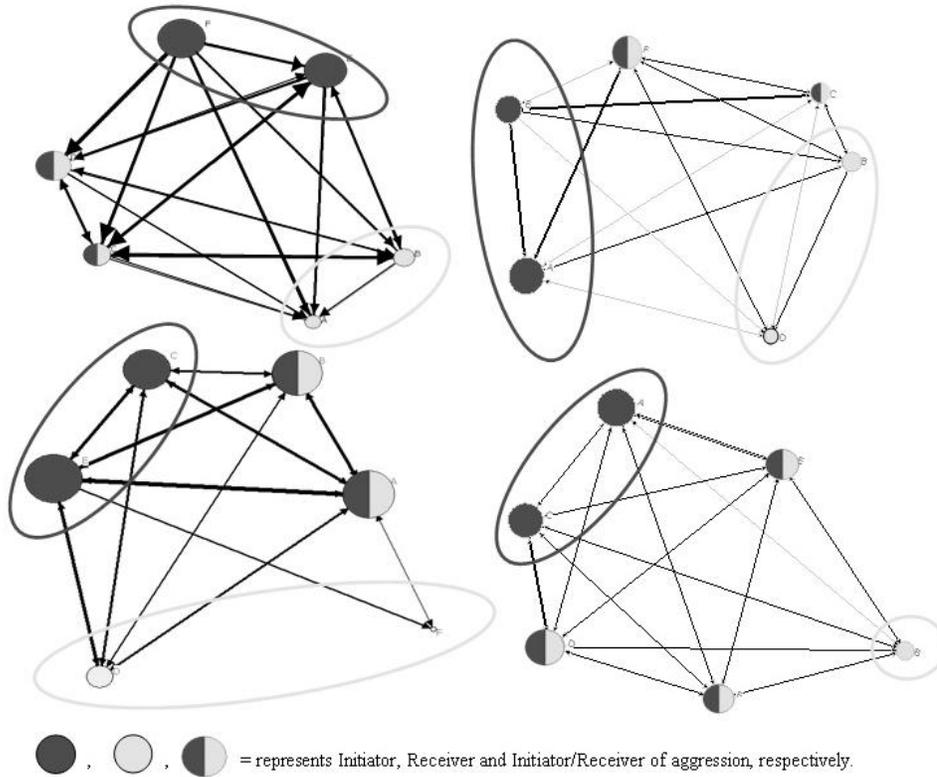


Figure 2.7. Social networks for aggressive interactions in FR groups. Individual fish are depicted by circles in which the diameter represents the magnitude of the out-degree centrality degree (aggression expressed by fish) and line thickness represents aggression strength. Cluster of Initiators or Receivers of aggression are encircled.



2.5. Discussion.

Dorsal fin damage was observed in all groups but was significantly higher in FR groups, especially dorsal fin erosion, splits and bites. This finding is supported by other studies using feed-restriction in salmonids and confirmed the potential value of using dorsal fin damage evaluation as a good on-farm indicator of welfare related to increased levels of aggressive social interactions in rainbow trout (St.Hilaire et al., 2006) and in Atlantic salmon (Noble et al., 2007a, Noble et al., 2008). Other studies have suggested that tanks or net surfaces play a role in fin abrasion or erosion (for a review see Latremouille, 2003). However, in the present study detailed inspection of tanks before introduction of fish indicated no physical abrasive material that could cause fin abrasion, erosion or damage. Moreover, there was a positive correlation between the occurrence of dorsal fin bites, behavioural biting events and dorsal fin erosion, which strongly indicated that the cause of fin damage was aggressive behaviour between fish leading to biting and fin injury. This leads to the conclusion that the only cause of the fin damage observed in the present study was direct social aggressive interaction among fish which increased when fish were feed-restricted. This evidence supports the hypothesis that fin damage in salmon aquaculture is largely the result of aggressive behaviour as suggested in previous studies in salmonid fish (Abbott and Dill, 1985; Turnbull et al., 1996; Turnbull et al., 1998; Ellis et al., 2008).

There were no significant differences in fin erosion between treatments, when measured using the RFI method. However, there were significant treatment differences

in dorsal fin erosion when erosion was measured using categories. This difference may highlight the limitations of using the RFI method to evaluate fin damage, as it only utilises data from the longest single fin ray of each fin. Researchers and other end users should be cautious when using this technique to quantify fin erosion (for an in depth review see Ellis et al., 2008).

Aggressive interactions have been previously suggested as a factor causing fin damage in numerous farmed and wild trout species such as cut-throat trout (*Oncorhynchus clarki*), brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (Bosakowski and Wagner, 1994), in addition to other species such as Atlantic salmon (MacLean et al., 2000), cod (*Gadus morhua*) (Brawn, 1961) and Arctic charr (*Salvelinus alpinus*) (Jobling and Wandsvik, 1983). The present study supports these findings and showed that aggressive interactions lead to fin damage and potentially the establishment and maintenance of social hierarchies that could affect social structures in Atlantic salmon.

Social network analysis of aggressive interactions revealed that FR groups presented denser and less distant networks indicating that aggression was a social interaction rapidly transmitted within the members of the network with fish identifying rapidly the initiators of aggression. Most importantly, the out and in-degree centrality differences revealed that feed-restriction disaggregated members according to their levels of aggression resulting in fish becoming either initiators or receivers of aggression. Initiators of aggression had higher out-degree centrality and were therefore extensively involved in interactions within the network due to having more ties with other fish within the group. This is particularly important as economic and sociological theory

indicates these fish are more influential and are more likely to gain resources (Wasserman and Faust, 1994). On the other hand, receivers of aggression had fewer interactions and their spatial positions within the group were dependent on initiators. These high in-degree and low out-degree values of receivers indicated that these individuals seldom initiated aggressive interactions and did not retaliate or counterattack their aggressor(s).

The higher clustering coefficient observed in the networks of feed-restricted fish indicated the formation of highly connected groups that coincided with the differentiation in aggressive roles. Importantly, initiators of aggressive interactions were fish that gained more weight in their respective experimental groups, whilst receivers were fish maintaining or decreasing weight gain.

The present experiment also demonstrated that during periods of feed-restriction initiators of aggressive interactions exhibited less fin damage, suggesting that initiators of aggressive behaviour and fin-biting tended to dominate the food resource without receiving aggression. Additionally, using social network analysis it was possible to clearly identify the existence of important key individuals (possessing high aggressive out-degree centrality and lower distance within a network) that were likely to be responsible for causing most of the fin damage.

The identification of these individuals could have important implications in the control of fin damage in aquaculture whenever reduced or non-feeding periods occurs such as those that occur during fish grading, transport, slaughter and other farm management practices such as vaccination of fish. In that respect, it has been

demonstrated that removing dominant fish increases aggression on the remaining fish in small groups as a compensatory effect of removal (Adams et al., 2000). However, this effect has yet to be investigated in commercial farm situations. This finding is also important in the light of new research revealing the importance of the detection of key individual behaviours in the spread of infectious diseases in humans and non-humans (Bansal et al., 2007). For example in humans, Bansal et al., 2007 while investigating six contact-network in humans found that contact patterns within individuals in the network were heterogeneous instead of homogeneous as is usually assumed within classical epidemiological models demonstrating the importance of quantifying interactions at the individual level in disease spread. Also, Read et al., 2008, demonstrated that individuals differ in the type and quantity of contacts revealing the importance of understanding mixing patterns and behavioural differences in the spread of diseases. Similar findings have been recently demonstrated in non-human studies such as that of Perkins et al., 2009, in the yellow-necked mice (*Apodemus flavicollis*), which confirmed the importance of identification of socially important individuals in understanding disease transmission in wild populations. Additionally, Madden et al. (2009, 2011) and Drewe (2010), using social network analysis in meerkats found that receivers of aggression and animals that groomed, instead of being groomed, were more susceptible to become infected and develop tuberculosis (*Mycobacterium bovis*), demonstrating the importance of social network analysis to elucidate the importance of specific behavioural interactions between individuals and potentially control disease spread within animal populations.

Social network analysis of associative behaviour showed lower transitivity in FR group networks indicating less social equilibrium due to unbalanced relationships

between members of the group. This social instability is supported by the fact that in the present study there was a distinct differentiation of roles and aggressive behaviour potentially leading to formation of hierarchies in the feed-restricted groups as it has been described in previous studies on fish, including salmonids (Huntingford et al., 1993, MacLean et al., 2000). Similar findings have been described in socio-ecological models of instability in other animals such as elephants (*Loxodonta africana*) (Wittemyer and Getz, 2007) and non-human primates (Isbell and Young, 2002) when competing for resources such as food and shelter. In addition, there were lower mean distances between fish. Degree of centrality tended to be higher in FR networks, indicating that fish were often associated closely and strongly within the network under feed-restriction conditions. The results of the present experiment showed a direct negative effect on the structural stability of associations in feed-restricted fish as compared with control fish.

Previous ecological studies using social network analysis in fish showing assertive characteristics in groups have demonstrated that some fish actively try to maintain specific inter-individual associations and interactions (Croft, 2005, Croft et al., 2004). This suggest that fish can distribute themselves in subpopulations and attain specific roles within their network according to their relationships. However, these previous studies focused on exploring temporal association networks in large populations of fish rather than specific behavioural interactions that occurs between individual fish within smaller groups. The present experiment is the first to explore and quantify social network variables in small groups of fish and relate potential differences in network interactions and spatial positions to welfare.

The spatial and structural analysis of schooling behaviour showed that, within a group, aggressive individuals tended to school more often and attained more central schooling positions, whereas receivers of aggression tended to shoal or be around the periphery of the school. This finding is in agreement with theoretical background of the distribution of fish within schools according to aggression or dominance (Viscido et al., 2007). It is clear from the present study that positions within schools are important during feed-restriction periods when central positions are adopted by more aggressive individuals who probably benefit from better overall surveillance of other more peripherally placed fish that in turn are more exposed to fin damage and lower weight gain.

Aggressive interactions within groups of farmed animals such as cows (*Bos taurus*) (Phillips and Rind, 2001) and pigs (*Sus scrofa*) (Sherritt et al., 1974) have been associated with detrimental production effects on growth, weight, condition and length of animals and welfare parameters. In the present study, analysis of production welfare related measures indicated that fish tail-fork length did not significantly differ between FR and C groups. This suggests that, although some fish decreased or lost weight, they continued to grow in terms of length, becoming thinner rather than having an overall restriction in growth. This finding has not been directly reported before and indicates a direct physical compensatory response that allows individual fish to stay within a competitive length. This is supported by empirical evidence on social competitive abilities in Atlantic salmon parr (Huntingford et al., 1990) showing a positive correlation between length and dominance during the parr phase. A longer period of food restriction might have had different effects on growth, especially considering that previous studies

have demonstrated that length growth is less sensitive to short term food supply fluctuations and dependant more on endogenous factors (Dutta, 1994, Stefansson et al., 2009).

There was a wide variation in weight gain in all groups suggesting that aggressive behaviour was associated with the potential of establishing classical models of dominance hierarchies, where dominant fish take most of the food resources. This has been previously described in studies when food resources are scarce or restricted in Atlantic salmon (Maclean and Metcalfe, 2001), brown trout (Petersson and Järvi, 2003), Arctic charr (Damsgård et al., 1997), Atlantic cod (Hatlen et al., 2006 and tilapia (McCarthy et al., 1999). Growth variation in FR groups was greater than that of C groups and some fish lost weight. This was likely to be caused by competition for food resources leading to a higher frequency and intensity of aggressive interactions. This is in accordance to other studies showing that reduced access to food does lead to differences in feed intake amongst fish such as Atlantic salmon (Adams et al., 1998; Ward et al., 2006 for a review).

In addition, SGR in the FR groups were significantly lower than in C groups indicating an overall growth restriction in feed-restricted fish. This is in accordance to several previous studies using food restriction in Atlantic salmon (Jurss et al., 1987, Huntingford et al., 2000, Ward et al. ,2006) and it has important applications in aquaculture as the existence of wide weight variation within groups imposes management costs and increases final product price due to the need for frequent grading of fish to obtain fish of similar weight and length. This finding may also indicate that maintained aggressive conditions in FR groups could have resulted in the

intermittent and repeated release of cortisol inducing catabolic physiological states in subordinate fish, as has been described previously in rainbow trout (Fernandes-de-Castilho et al., 2008, Øverli et al., 2004, Yue et al., 2006). It is important to point out that in this study six individuals per tank were used which is likely to have induced a despotic situation where one or two fish monopolized the food resource by interference competition. In a more commercial situation with more fish and higher stocking densities, the social situation could be different likely favouring a scramble competition where, according to the resource defence theory, no contact or aggression should occur among individuals (Grant and Godin, 1997, Milinski et al., 1997). Paradoxically, resource defence theory does not sufficiently explain the empirical evidence of high levels of aggression and fin damage incidence in high stocking densities in Atlantic salmon (Brockmark et al., 2007, Ellis et al., 2008, Hosfeld et al., 2009, Kjartansson et al., 1988) and rainbow trout (Ellis et al., 2008, Hosfeld et al., 2009, North et al., 2006). Also, empirical evidence suggests that physical contact occurs when fish compete with restricted food leading to physical damage but not necessarily mediated through aggression (Ashley, 2006).

Also, the nutritional quality and quantity of the feed delivered have been linked to fin erosion through the putative influence of deficiencies of specific lipids (linoleic acid), amino acids (lysine, arginine, histidine, isoleucine, threonine, valine and tryptophane), minerals (copper) and vitamins (Vitamin C) (see Latremouille, 2003 for a full review). A decrease in the availability of nutrients could also be a consequence of diseases affecting the digestive system of fish. For example, Ferguson et al., 1986a, Ferguson et al., 1986b, found high fin erosion and ulcerations of affected fish during an outbreak of

Pancreas Disease. However, nutritional deficiencies of any origin are not likely to be a primary aetiological factor as previous studies have demonstrated that isolated fish had no fin damage compared to fish held communally and fed under the same feeding regime (Kindschi et al., 1991; Turnbull et al., 1998). In addition, levels of stress hormones such as cortisol have been shown to increase during acute infections in Atlantic salmon (Ackerman et al., 2000) which in turn have been demonstrated to impair skin structure and reparation of dermis in salmonids (Iger et al., 1995, Roubal and Bullock, 1988).

2.6. Conclusion.

The present study demonstrated the applicability and value of social network analysis in understanding the development of fin damage in fish by the quantification and identification of highly aggressive fish within the network. The use of social network analysis offers considerable potential in contributing to the improvement of farmed fish welfare through the correct identification of socially important aggressive individuals. Further detailed studies of the effects of feed-restriction on the occurrence of fin damage in other social contexts at different life stages and different stocking densities will be necessary in order to fully understand the underlying social causes of fin damage in relation to food resources and hence indicate ways to improve the welfare and productivity of farmed fish.

3. Chapter 3. Social network analysis of the behavioural interactions that influence the development of fin damage in Atlantic salmon parr (*Salmo salar*) held at different stocking densities.

(The following chapter has been published in Applied Animal Behaviour Sciences and attached as an Appendix)

3.1. Introduction.

As previously mentioned in Chapter 1, the association between high or low stocking densities and fin damage has been previously investigated in Atlantic salmon in both laboratory and commercial situations but this work has produced contradictory results. For example, a higher incidence of fin damage has been associated with both high fish stocking densities (Turnbull et al., 2005; Adams et al., 2007; Brockmark et al., 2007) and low fish stocking densities (Turnbull et al., 2005; Adams et al., 2007). However, as densities are generally measured in kilograms of fish per cubic meter, identical stocking densities can be achieved with different numbers of fish with different body weights. In fact, most studies investigating the effect of stocking densities in Atlantic salmon achieved the desired stocking densities by increasing/decreasing the number of fish or group size, without taking into account the possible social and behavioural consequences of such changes. In that respect, for example, Kjartansson

et al. (1988) investigated the effects of three stocking densities on the physiological parameters of Atlantic salmon by manipulating stocking densities by increasing the number of fish in each tank, but removing fish on four occasions, reducing the total number of fish to ca. 50% of the original number. Also, Soderberg and Meade (1987), removed fish (ca. 15% of the original number) during their experiment in order to maintain the defined stocking densities. Similar situations occurred while investigating the effect of stocking densities in rainbow trout where fish had to be removed during the experiment to ca. 50% (North et al., 2006) or 20% (Person-Le Ruyet et al., 2008) of the original number of fish. Also, other studies using fixed group sizes at two stocking densities showed no significant differences in fin damage (Rasmussen et al., 2007). However, stocking densities increased more than two-fold from the beginning to the end of the study, irrespective of treatment. The causal relationships between density and fish welfare are complex (see review by Ellis et al., 2002 and North et al., 2006). Many density experiments have not compensated for changes in water flow or oxygen levels, and it is thus difficult to evaluate if the documented effects are caused by fish density or by changes in water quality (e.g. Hosfeld et al., 2009, North et al., 2006, Soderberg and Meade, 1987).

It is widely accepted that the number of individual animals in a group have a direct impact on the affiliative behaviour of each individual within the group (Krause and Ruxton, 2002, Massen et al., 2010). In addition, it has been demonstrated that fish are capable of recognising other conspecifics in groups up to 15 to 20 individuals and tend to create stable relationships among members of the group when housed in these group sizes (Griffiths, 2003, Ward and Hart, 2003). Therefore, there is a need to clarify and

quantify the effect of different stocking densities in farmed fish held at constant group sizes on production, behaviour and welfare parameters.

Social network analysis has recently been used to quantify the behaviour of fish in ecological studies (Croft, 2005, Croft et al., 2004) and more recently in studies investigating fin damage during a feed restriction period in Atlantic salmon (Cañon Jones et al., 2010). Social network analysis describes and quantifies direct and often hidden indirect relationships, social ties and influences among individuals (Wasserman and Faust, 1994). It can be used to identify the specific roles of individuals within the group who have a higher and specifically variable number of contacts and interactions and, therefore, may be more socially important and influential (Lusseau and Newman, 2004, Wasserman and Faust, 1994).

The main advantage of using social network analysis is that it enables the correct identification and quantification of social position, social influence and role of key individuals within groups and their involvement in the development of fin damage under different stocking densities. Subsequent control measures based on this identification of roles could then be developed and implemented in order to improve the welfare of farmed fish. Recently, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) has recommended fish stocking density limits of 17 and 30 kg/m³ for Atlantic salmon in freshwater production tanks and seawater enclosures, respectively (RSPCA, 2007). However, they have acknowledged that robust scientific knowledge is still lacking in this area.

In this Chapter, social network analysis was used to quantify the behavioural interactions that influence the occurrence of fin damage in Atlantic salmon parr held in constant group sizes at stocking densities that represent the low and high end of the spectrum currently used in the salmon industry.

3.2. Materials and methods.

3.2.1. Animals and experimental groups.

The experiment was conducted in the summer of 2009 at the Aquaculture Research Station in Tromsø, Northern Norway. Eight experimental groups, consisting of ten clinically healthy year 1+ Atlantic salmon weighing 113.24 ± 10.7 g (mean \pm SD) and with mean body lengths of 20.3 ± 0.6 cm were used in the study. The fish were sourced commercially from Haukvik Kraft-Smolt AS, Tribe Batnfjord, generation 2008. There were three experimental phases: Phase 1 or Pre-treatment (from day 1 to day 10), Phase 2 or Treatment period (from day 11 to day 20) and Phase 3 or Post-treatment (from day 21 to day 30).

Feed was delivered automatically at 10:00 hrs. for 30 minutes at a rate of 1.5% of estimated fish body weight/day from feeders located above the experimental tanks. A commercial pelleted feed ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway) was used throughout the study. The amount of food delivered was adjusted weekly

according to the expected weight gain and water temperature according to guidelines from the feed manufacturer.

After the pre-treatment phase, four tanks of fish were randomly selected as High Density (HD, 30 kg/m³) and four tanks as Low Density (LD, 8 kg/m³) groups. For the HD groups, a tubular shaped flexible plastic mesh ring net (Biltema®, 1 mm thick thread with 13 mm spacing) was inserted inside the HD tanks at the start of Phase 2, as shown in Figure 3.1. Fish were then housed in this ring to increase density to 30 kg/m³. Therefore, only space was reduced while environmental qualities such as water column height, volume, current, velocities, fish tank, number and initial volume of fish were similar in each group. At the start of Phase 3 (post-treatment phase) the plastic ring net was removed from the HD groups in order to observe the effect on the aggressive behaviour of fish after changing to low density.

In order to minimise any effect of management disturbances, two LD groups and two HD groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the entrance.

No mortalities occurred during the experiment and all fish were euthanised at the end of the study using immersion in an overdose bath of benzocaine chlorhydrate (> 250 mg l/1 freshwater).

3.2.2. Containment and individual identification.

Fish were individually identified under anaesthesia induced by submersion in a bath of benzocaine chlorhydrate (100 mg/l freshwater) at the beginning of the experiment. Tags were designed using unique combinations of black or white geometric designs (circles, triangles, squares, rectangles and crosses of 2.5 by 2.5 cm) made from plastic printing paper (Xerox® Special Advanced Media Digital Colour, Premium Never Tear 95µ Polyester paper) and inserted under the skin behind the dorsal fin of each fish using strong silk thread and standard commercial Floy Tags (Hallprint®, Polyepaltichylene streamer tags, series PST). Damage to the skin was minimal and no significant effect of tagging system or type of tag on weight, length or fin damage was observed between experimental groups. All fish achieved full anaesthesia within 3 minutes and tagging was carried out during the following minute. After tagging, fish were transferred to the experimental tank with initial stocking densities of $7.9 \pm 0.1 \text{ kg/m}^3$ (mean \pm SD) and observed for 30 minutes following recovery from anaesthesia. An emergency recovery tank with highly oxygenated freshwater (> 99% dissolved oxygen injected through diffusers connected to oxygen gas tanks) was available during tagging in case fish needed assisted recovery or veterinary assistance.

3.2.3. Housing, water quality and environmental conditions.

Fish were housed in 300 litres high density plastic circular tanks measuring 78 cm of diameter and 50 cm of height. Naturally aerated and filtered ambient freshwater from a nearby river was provided throughout the experiment. Dissolved oxygen content ($93.3\pm 3.0\%$) and water temperature ($12.4\pm 1.7^\circ\text{C}$) were measured and recorded twice daily. Water flow was controlled at an exchange rate of 10 l/minute in an open flow system and velocities of one fish body length/second. A 24 hour light photo-period regime was used during the study.

3.2.4. Physical measures.

The weight (g) and length (total tail-fork length in mm) of each fish were measured at the beginning and the end of the experimental period. Specific growth rate (SGR) was calculated as: $\text{SGR} = (\ln w_1 - \ln w_0)/\Delta t$, where w_1 is the wet weight of fish (g) at sampling time 1, w_0 is the wet weight of fish (g) at sampling time 0, and Δt is the number of days between sampling times. Fulton's condition factor (K) was calculated from the tail-fork length and mass of individual fish as: $K = W/L^3 \times w_1 \text{ or } 2$, where W is the weight of the fish (g), L^3 is the length of the fish to the power of 3, s is the total tail-fork length in mm and $w_1 \text{ or } 2$ is the weight of fish (g) at sampling time 1 or 2.

3.2.5. Quantification of fin damage.

Digital photographs of both sides of every fish were taken at the beginning and end of the experimental period. Fin damage was evaluated from these pictures using the Relative Fin Index (RFI) as described in Bosakowski and Wagner (1994). An additional categorical method was also used to quantify fin erosion utilising an ordinal scale of 0, 1, 2 and 3, corresponding to no erosion (0% of fin eroded), mild erosion (1% to 24% of fin eroded), moderate (25% to 49% of fin eroded) and severe erosion (> 50% of fin eroded), respectively (see Chapter 2 and Cañon Jones et al., 2010).

Every left and right pectoral, ventral, anal, caudal (upper and lower) and dorsal fin was measured and quantified using RFI and the categorical erosion index. In addition, the total number of fin splits (separation of fin rays greater than 3 mm) was recorded for each fish.

Additionally, fish were visually examined for other external lesions on their bodies as evidence of the occurrence of biting during sampling periods and at the end of the experiment.

3.2.6. Behavioural observations and social interactions.

Ten minute video recordings of each tank were carried out at 09:00 to 09:10, 10:00 to 10:10 and 11:30 to 11:40 on each day of the experimental period. This

recording regime quantified fish behaviour 1 hour before feeding, during the first ten minutes of feeding and 1 hour after the last food delivery from the automatic feeders. CCTV colour cameras (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) located 1 m above each tank were used to record the behaviour of fish. Each tank had a perforated water inlet pipe submerged to the water level and a double central perforated standpipe to prevent rippling on the water surface. Digital recordings were made using a DVD/HDD recorder (Pioneer© DVR-550H-S).

3.2.6.1. Association interactions measurements.

Data extracted collected from the video recordings at 1 minute intervals were used to construct association matrices. A fish was considered as associated with another fish when they were within two fish body lengths if parallel to each other, or within two body widths if perpendicular to each other.

3.2.6.2 Aggressive interactions measurements.

Aggressive behaviour was classified as an attack, a displacement or a fin-bite and quantified using the methods described in Chapter 2 and Cañon Jones et al. (2010). Initiator(s) and receiver(s) of any aggressive interaction were recorded and weighted matrices for social network analysis for each video sampling period were

constructed. Aggressive interactions were also used to calculate and compare data relating the total amount of aggressive interactions and attacks, displacements and fin bites between experimental groups.

3.2.7. Social network analysis.

Social network analysis was carried out with associative and aggressive interaction matrices using UCINET© (Borgatti et al., 1999). The quantified network variables were the degree of centrality, clustering coefficient, transitivity, distance and density. Detailed explanations of these network variables based on Wasserman and Faust (1994) and Hanneman and Riddle (2005) are described in Chapter 2 and Cañon Jones et al., 2010.

Network analyses were carried out for the pre-treatment (days 1 to 10), treatment (days 11 to 20), and post-treatment (days 21 to 30) periods and for the entire experimental period (days 0 to 44).

3.2.8. Structural and spatial position measures.

Each individual fish was classified as being schooling or shoaling at 1 minute intervals from the video recordings using methodology described in Chapter 2 and Cañon Jones et al., 2010.

3.2.9. Statistical analyses.

Descriptive statistical analyses, the Shapiro-Wilkes test of normality and one-way analyses of variance were carried out on weight, length, RFI, fin damage (splits and bites), SGR and K (Zar, 2009). In order to clarify the effect of treatment, a general linear model described by $y = a + bx$, where a is the intercept (LD group) and b the slope (effect of high density), was carried out for weight and length variation (Zar, 2009). Kruskal Wallis tests were used to measure the effect of tagging system on weight, length and fin damage between experimental groups. Differences in degree of dorsal fin erosion amongst treatments were analysed using the Chi-square test and the Chi-square test for trends (Zar, 2009). Correlations between dorsal fin erosion and other variables were analysed using the Pearson rank correlation (Zar, 2009) and network distance and density were analysed by analysis of variance (Zar, 2009). Kruskal Wallis tests were used to analyse the differences in aggressive behaviours (biting, displacements, attacks and total aggressive behaviour) as well as for centralities

(overall, in-degree and out-degree), clustering coefficients and densities between experimental groups. Mantel tests were carried out for association and aggression matrices between acclimatisation and treatment periods in order to ascertain whether any differences were attributed to statistically significant changes in the behaviour of fish rather than by chance (Zar, 2009) . All statistical analyses were performed using R statistical software (R Development Core Team, 2008).

3.3. Results.

Fin erosion was only present on the dorsal fin and it was significantly higher in HD groups (6 vs. 1 fish, $\chi^2 = 3.91$, $P = 0.03$) which also showed significantly lower RFI (11.5 vs. 12.5, $F_{1,68} = 10.9$, $P < 0.01$) as shown in Table 3.1. In addition, moderate and severe dorsal erosion was present only in HD groups and not in LD groups ($\chi^2_3 = 8.1$, $P = 0.02$) as well as dorsal splits and bites ($P < 0.05$). Dorsal fin erosion was positively correlated with the occurrence of biting in HD groups ($r^2 = 0.68$, $P = 0.04$). There were no statistical differences in the RFI and erosion for all other fins evaluated ($P > 0.05$).

LD groups had significantly more total aggression than HD groups (55.43 vs. 41.46 interactions/hour, $H_1 = 5.33$ $P = 0.03$) but HD groups had a significantly higher frequency of fin biting (0.35 vs. 0.13 interactions/hour, $H_1 = 5.39$, $P = 0.03$), as shown in Figure 3.2. This suggests that LD conditions increased the frequency of aggressive behaviour in comparison to HD groups, but this aggression was significantly less severe than at higher densities.

Social networks based on aggressive interactions in HD groups showed higher centrality (32.9% vs. 27.7%, $H_1 = 3.1$, $P = 0.04$), clustering coefficient (0.41 vs. 0.36, $H_1 = 4.9$, $P < 0.01$), in-degree centrality (35.2% vs. 20.8%, $H_1 = 13.6$, $P < 0.01$), out-degree centrality (46.1% vs. 36.9%, $H_1 = 15.2$, $P < 0.01$) and the network were less dense (29.4 vs. 60.8, $H_1 = 5.33$, $P = 0.02$) than networks in LD groups.

These findings indicated a distinctive separation of roles of the fish according to aggression in HD groups accompanied by formation of separate clusters of initiators and receivers of aggression in these groups. Initiators had high out-degree centrality (59.3% vs. 5.5%, $H_1 = 7.0$, $P < 0.01$) while receivers showed high in-degree centrality (21.7% vs. 11.1%, $H_1 = 6.1$, $P < 0.01$). Figures 3.3 and 3.4 show the graphical representation of these network formations in each HD and LD groups, respectively. Within the HD groups, initiators of aggression had less dorsal fin erosion (1 fish vs. 5 fish), higher final weight (121.6 g vs. 105.0 g, $F_{1,9} = 4.9$, $P = 0.04$) and length (21.7 cm vs. 20.7 cm, $F_{1,9} = 5.9$, $P = 0.04$) compared to receivers of aggression (Table 3.2).

Furthermore, linear regression modelling showed differences in degree centralities only in HD groups with clusters of fish with high in-degree ($F_{1,78} = 106.9$, $P < 0.01$) and clusters of fish with high out-degree centrality ($F_{1,78} = 23.5$, $P < 0.01$). These results allowed the classification of individuals as I or R of aggression as is shown in Figure 3.5. Fish in the LD groups had lower final weights (106.0 g vs. 111.8 g, $F_{1,78} = 4.3$, $P = 0.04$), lower final lengths (20.7 cm vs. 21.1 cm, $F_{1,78} = 6.2$, $P < 0.01$) and lower body condition (5.1 vs. 5.2, $H_1 = 3.6$, $P < 0.03$) compared to fish held at high stocking density. Additionally, a significantly higher variation in weight gain was seen in LD groups compared to HD groups (7.3 vs. 6.3 g, $F_{1,78} = 5.1$, $P = 0.02$).

There were no statistical differences ($P > 0.05$) in social network parameters for associative behaviour between experimental groups. Similarly, fish did not show detectable structural (schooling or shoaling) or positional preferences in any of the experimental groups ($P > 0.05$).

3.4. Tables and figures.

Table 3.1. Number and percentage of fish with fin erosion, bites, splits and relative fin index (RFI, mean \pm SD) in the dorsal fin measured in the experimental groups (HD= high stocking density; LD= low stocking density).

	Fin erosion (%)				Bites	Splits	RFI
	No erosion	Mild	Moderate	Severe			
LD groups	39 (97.5%)	1 (2.5%) ^a	0 (0%) ^a	0 (0%) ^a	1	1	11.55 \pm 1.51 ^a
HD groups	34 (85.0%)	4 (10.0%) ^b	1 (2.5%) ^b	1 (2.5%) ^b	3	4	12.53 \pm 1.09 ^b

^a and ^b = statistical differences at $P < 0.05$ between experimental groups.

Table 3.2. Fish length and weight (mean \pm SD) and number of fish showing fin erosion according to type of individual (initiators or receivers) in high stocking density groups.

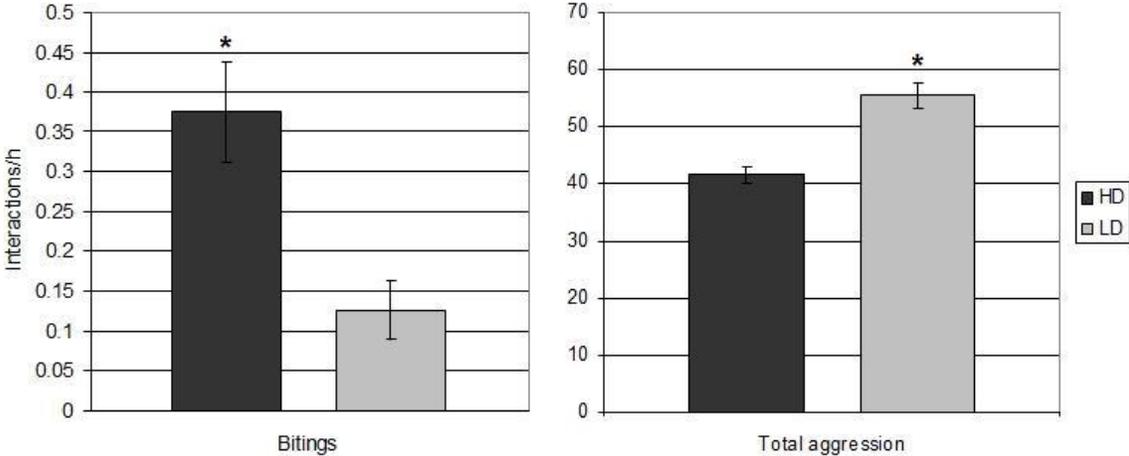
	Length (cm)	Weight (g)	Fish showing fin erosion
Initiators	21.77 \pm 0.47 ^a	121.60 \pm 12.89 ^a	1
Receivers	20.70 \pm 0.46 ^b	105.00 \pm 9.87 ^b	5

^a and ^b = statistical differences at $P < 0.05$ between experimental groups.

Figure 3.1. Photograph of circular plastic mesh inserted in the treatment tanks during Phase 2 of the experiment for the high stocking density groups.



Figure 3.2. Mean occurrences of biting and total aggressive interactions in experimental groups (HD= high stocking density; LD= low stocking density).



* = statistical differences at $P < 0.05$ between experimental groups.

Figure 3.3. Social network graphs of aggressive interactions in each high stocking density (HD) group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) and the thickness of the connecting lines represents the magnitude of the interaction. White, grey and black represent the type of individual classified based on their out and in-degree centralities as initiators, receivers and initiators/receivers of aggression, respectively. Encircled are clusters of initiators and receivers. + symbol represents fish with dorsal erosion.

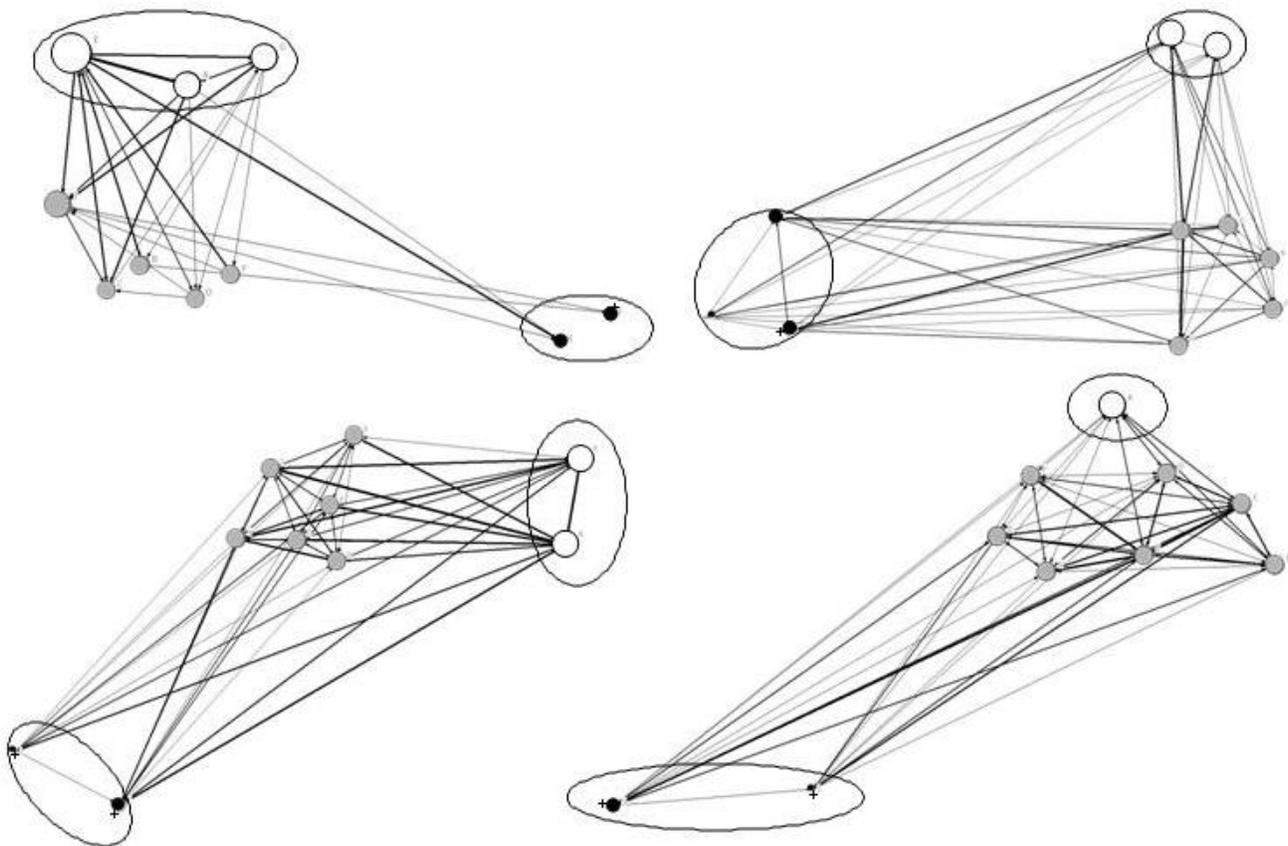
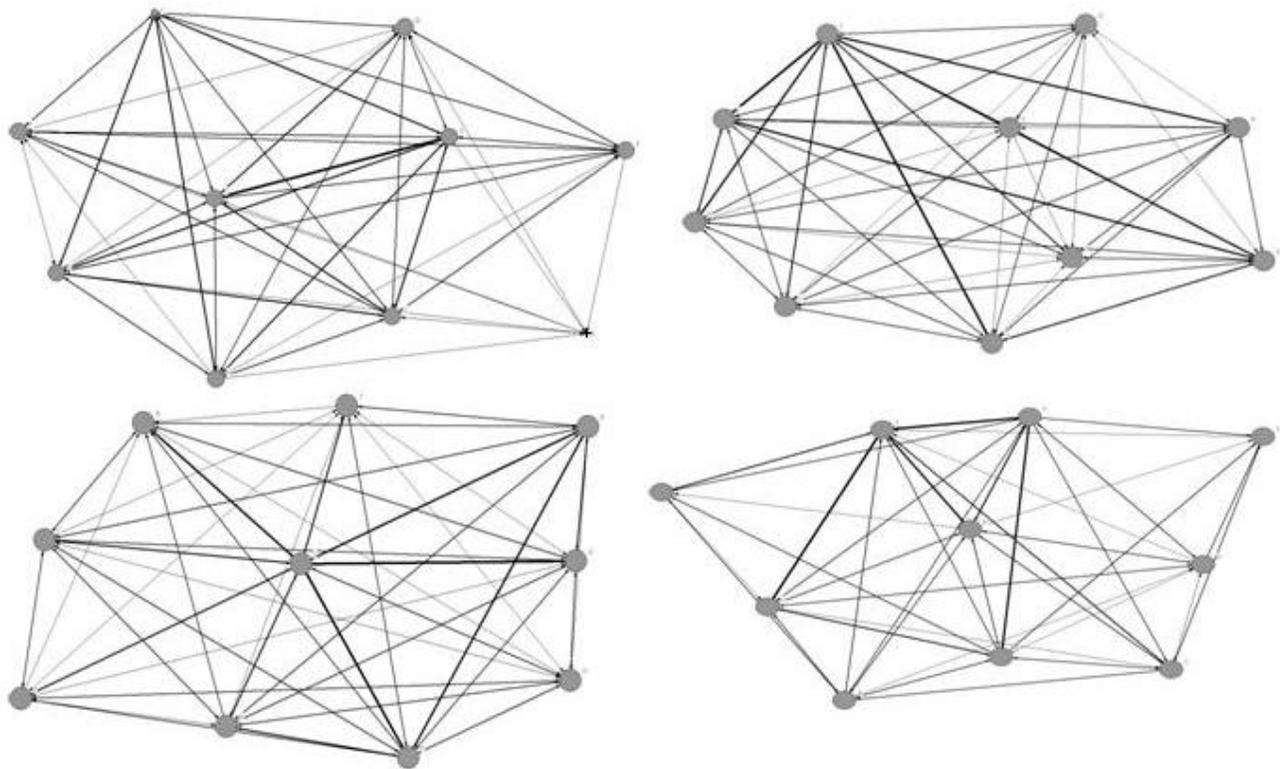


Figure 3.4. Social network graphs of aggressive interactions in each low stocking density (LD) group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) and the thickness of the connecting lines represents the magnitude of the interaction. Only initiators/receivers based on the out and in-degree centralities of aggression were found in LD groups.



3.5. Discussion.

Dorsal fin damage was significantly higher in HD groups in terms of fin erosion and a lower dorsal RFI. These findings are in agreement with other studies that correlate high stocking density with increased levels of fin damage in rainbow trout (Ellis et al., 2002, North et al., 2006, Person-Le Ruyet et al., 2008) and Atlantic salmon (Turnbull et al., 2005, Adams et al., 2007, Brockmark et al., 2007). However, in Chapter 2 and Cañon Jones et al. (2010) showed that RFI was not associated with fin damage and therefore not a very robust welfare measure, suggesting the value of using direct examination of fins in this study. It is in contrast with a small number of other studies (e.g. Rasmussen et al., 2007) where lower levels of fin damage were reported in fish stocked in high densities. Interestingly, no other fins were significantly affected by fin damage in relation to stocking density. The present experiment suggests increased dorsal fin biting leads to higher dorsal fin damage in Atlantic salmon confirming that dorsal fin damage can be used as a morphological Operational Welfare Indicator (OWI) for aggression (Ellis et al., 2002, Ellis et al., 2008, Noble et al., 2008).

The results of the present study strongly suggest that fin-biting was the most likely cause of fin damage in the HD groups and this is supported by the findings of previous studies in both farmed and wild fish (Bosakowski and Wagner, 1994, MacLean et al., 2000, Hatlen et al., 2006, Jobling and Wandsvik, 1983).

Detailed examination of aggressive interactions in the current study showed that the total number of aggressive interactions (attacks, displacements and fin bites) was

higher in LD groups than in HD groups. However, HD groups had a significantly higher frequency of biting than LD groups. Therefore, although the overall frequency of aggression was higher in LD groups, the form of aggression (fin biting) was significantly more intense at higher stocking densities. This evidence strongly supports the conclusion that the most likely cause of fin damage was overt aggression in the form of fin biting at high stocking densities. The low frequencies of fin damage in our study (8.75%) are in general agreement with levels reported in previous studies in Atlantic salmon kept under commercial situations (c. 6% in Noble et al., 2008) and Rainbow trout kept experimentally (c. 9% in Rasmussen et al., 2007). These results show the relevance of relatively low frequencies of highly intense aggressive behaviour such as fin biting on the development of fin damage and welfare of fish under different stocking densities. This finding also underlines the importance of separating and discriminating the types of behavioural interactions which occur between fish in studies where fin damage is used as an indicator of welfare.

Social network analysis of aggressive interactions showed that high stocking densities had a significant effect on increasing the centrality, clustering and density in the social networks of each group. Importantly, in and out-degree centrality in HD groups showed a distinct separation of individual members of the groups according to their roles within the network into initiators and receivers of aggression. Interestingly, although LD groups were more aggressive, the separation of roles occurred only at high stocking densities and not at low stocking densities. Initiators of aggression had higher out-degree centrality and showed a higher number of interactions among members within the group, suggesting that initiators were more influential and more likely to gain

access to resources (Wasserman and Faust, 1994), such as space/position and food and assert more influence on the behaviour of other fish in this experiment. In fact, receivers of aggression had higher in-degree centrality and lower out-degree centrality, indicating that they seldom initiated aggressive interactions with no retaliation or counter-attacks. Additionally, detailed analysis of physical measures showed that initiators of aggressive interactions were fish that gained more weight, achieved longer body lengths and exhibited less fin damage compared to receivers of aggression. The correct identification and possible removal of these individuals as those who are bigger and longer may have potential implications for attempts to control fin damage and increase the welfare of farmed salmon whenever high densities are maintained. However, the removal of dominants may be beneficial only in the short term, as some laboratory studies have observed that the removal of the dominant fish allows the next ranked fish to take the dominant place and results in a transient increase in aggression (Adams et al., 1998; Adams, et al., 2000). The separation of roles within groups of fish seen in this study is in agreement with previous studies using social network analysis in Atlantic salmon under feed restriction (Chapter 2 and Cañon Jones et al., 2010) where it was possible to identify highly aggressive individuals using social network analysis.

Social network analysis has been used studies of guppies (*Poecilia reticulata*) to show that individuals can maintain specific and differential associations and interactions between individuals within a group (Croft et al., 2004, Croft, 2005). However, to the author's knowledge, the current study is the first to quantify the effect of behavioural interactions at different stocking densities in relation to fin damage using social network analysis in aquaculture species.

Although the primary objective of this study was to quantify the effects of stocking density upon fin damage, it is important to recognise the relative detrimental effect of low stocking densities on fish welfare in the current study. Fish held at LD had a lower weight, length and body condition, higher total aggressive interactions but less fin damage compared to fish in HD groups. Fish in LD were not losing weight and were subjected to less intense aggressive behaviour (fin-biting) compared to HD groups. However, it is suggested that the lower growth of fish in LD groups, probably because of an increased physical activity due to higher aggressive behaviour of fish, represents reduced welfare based on the well accepted definition of poor welfare being any difficulty in coping or reduction in fitness by an animal (Broom, 1988, 1991). However, it is not necessary for fish to lose weight or become anorexic to have a poor welfare status. These findings are in agreement with previous studies demonstrating negative physical effects of fish held at low stocking densities (Turnbull et al., 2005, Person-Le Ruyet et al., 2008). Therefore, results of the current study indicated a negative welfare effect of low rearing densities, highlighting the differential detrimental welfare effects which can occur at both low and high stocking densities in farmed fish. However, the numbers of fish used in the current study were low compared to farming conditions, but were not low compared to other experimental studies investigating stocking density (Alanara and Brannas, 1996, Turnbull et al., 1998).

The spatial and structural analysis of schooling behaviour showed that stocking densities did not affect the spatial distribution of fish according to their aggressive behaviour. These results are in contrast to the theoretical evidence that aggressive or dominant individuals should attain central positions when schooling (Viscido et al.,

2007) and the only empirical evidence of this occurring was in Atlantic salmon subjected to feed restriction (Chapter 2 and Cañon Jones et al., 2010). It is possible that this phenomenon was not observed due to the fact that both high and low stocking densities used in this study had a negative impact on the behaviour and welfare of fish, resulting in the fish not being able to achieve preferential schooling or shoaling behaviour. The lack of these behaviours could also be explained by the fact that salmon parr tend to be highly aggressive (Bardonnet and Baglinière, 2000).

3.6. Conclusions.

This study demonstrated the applicability and value of social network analysis in understanding behavioural interactions underlying the development of fin damage in fish held at low and high stocking densities. Both high and low stocking densities had a differential detrimental welfare effect on fish; high stocking densities resulted in a differentiation of roles of fish within their network into initiators and receivers of aggression. This led to an increase in the frequency of biting and resulted in increased dorsal fin erosion. The factors that determine which fish becomes an initiator or a receiver of aggression are still unclear and future studies are needed to investigate this. Low stocking densities did not result in this differentiation of roles but aggression amongst fish within the LD groups was higher and fish grew at a slower rate. The use of social network analysis in behavioural studies is likely to contribute to the understanding and improvement of the welfare of farmed fish by the correct identification of socially

important aggressive individuals. However, more studies are needed to adequately quantify the effect of other social contexts such as group size, different life stages, intermediate stocking densities or feed delivery systems on the development of fin damage and hence indicate appropriate and practical ways to improve the welfare and productivity of farmed fish.

4. Chapter 4. Social network analysis of behavioural interactions influencing the development of fin damage in Atlantic salmon parr (*Salmo salar*) subjected to predictable and unpredictable food delivery schedules.

4.1. Introduction.

Time and regime of feeding has been identified as one of the most important factors influencing the welfare of farmed fish (Huntingford et al., 2006). In fact, variations in the predictability of husbandry practices such as feeding in captive animals can have a negative effect on physiological variables such as plasma cortisol concentrations, reflected into behavioural changes such as increase activity, due to the fact that animals lose control over their environment (Bassett and Buchanan-Smith, 2007). Also, unpredictability of feeding times has been related to poor welfare in terrestrial farmed animals such as pigs, showing a decrease in growth (Carlstead, 1986), and stereotypical behaviour in horses (Ninomiya et al., 2004).

In fish, feeding times have been demonstrated to be a strong entrainment factor of the circadian rhythms (Blanco-Vives and Sánchez-Vázquez, 2009, Boujard and Leatherland, 1992, Del Pozo et al., 2011). Unpredictable feeding times in sea bream (*Sparus aurata*) increased locomotor activity, plasma cortisol and glucose compared to fixed feeding times (Sánchez et al., 2009). Likewise, sea bass (*Dicentrarchus labrax*) gained more weight when feeding time matched the natural feeding rhythms associated to dawn and dusk (Azzaydi et al., 1999). Also, groups of goldfish (*Carassius auratus*)

subjected to predictable feeding times showed an entrainment pattern consisting of higher food anticipatory activity, high amylase and low plasma cortisol levels at feeding times (Vera et al., 2007). These results suggested that predictable feeding times are less stressful events than unpredictable feeding time and with less negative impact on the welfare of fish. Currently, farmed Atlantic salmon are generally fed once or twice daily at fixed times during the day (Kaushik, 2000).

However, no studies have been reported that investigate the influence of food delivery on the behaviour of Atlantic salmon leading to fin damage as welfare indicator. Unpredictability of food delivery may occur whenever there is a malfunction of automatic feeders, electrical failures, self-feeders or hand feeding are used.

The use of social network analysis enables the potential precise identification and quantification of social positions, social influences and the role of key individuals within groups and their involvement in the development of fin damage under unpredictable and predictable feeding times.

This study therefore used social network analysis to quantify the behavioural interactions that influence the occurrence of fin damage in Atlantic salmon parr held under predictable or unpredictable food delivery.

4.2. Materials and methods.

4.2.1. Animals and experimental groups.

The experiment was carried out in the spring of 2010 at the Aquaculture Research Station in Tromsø, Northern Norway (Project Number 7006/10-006.1/H10/20/KNF) and followed the current Norwegian Fish Welfare and Laboratory Animals legislation (Ministry of Agriculture and Food of Norway, 2010; Ministry of Agriculture of Norway, 2010) adhering to the European Convention for the Protection of Vertebrates used for Experimentation and other Scientific Purposes (European Union, 1998).

Eight experimental groups, consisting of ten clinically healthy year 1+ Atlantic salmon weighing 94.64 ± 7.52 g (mean \pm SD) and with mean body lengths of 19.87 ± 0.55 cm were used in the study. The fish were sourced commercially from Aqua Gen A/S, Tribe Standard, generation 2009. There were three experimental phases: Pre-treatment period (from day 1 to day 14), Treatment period (from day 15 to day 28) and Post-treatment period (from day 29 to day 42).

During the pre-treatment period, food was delivered every day at 10:00 hrs over a 30 minutes period from calibrated automatic feeders located 1 meter above each experimental tank. After the pre-treatment period and during the treatment period, four tanks were randomly selected as Predictable food delivery schedule (PD) and four

tanks as Unpredictable food delivery schedule (UD) groups. PD groups received food each day at 10:00 hrs for 30 minutes. UD groups received food at a random time each day for 30 minutes. In order to improve the food unpredictability it was decided that the feeding time on each day was delivered 2 or more hours apart from the previous day and during 8:30 and 16:30. At the start of post-treatment period, UD and PD groups were fed each day at 10:00 hrs. for 30 minutes. Food was delivered using commercial pelleted feed ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway) at a rate of 1.5% of estimated fish body weight/day and adjusted weekly according to the expected weight gain and water temperature following the guidelines of the feed manufacturer. It is interesting to point out that a predictable food delivery schedule was used in Chapters 2, 3 and 5.

Additionally, and in order to minimise any effect of management disturbances, two PD groups and two UD groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the entrance.

No mortalities occurred during the experiment and all fish were euthanised by an overdose bath of benzocaine chlorhydrate (> 250 mg/l freshwater, Benzoak Vet, A.C.D. Pharmaceuticals SA, Norway) at the end of the experiment.

4.2.2. Containment and individual identification.

Fish were individually tagged whilst anaesthetised by submersion in a bath of benzocaine chlorhydrate (100 mg/l freshwater, Benzoak Vet, A.C.D. Pharmaceuticals

SA, Norway) at the beginning of the experiment. All fish achieved full anaesthesia within 3 minutes and tagging was carried out within the following minute. Tags were designed to allow individual identification using combinations of black or white geometric designs (circles, triangles, squares, rectangles and crosses of 2.5 by 2.5 cm) made from plastic printing paper (Xerox® Special Advanced Media Digital Colour, Premium Never Tear 95µ Polyester paper) and inserted under the skin behind the dorsal fin of each fish using strong silk thread and a standard commercial Floy Tags (Hallprint®, Polyepaltichylene streamer tags, series PST). Macroscopic tissue damage of the skin was minimal and no significant effect of the tagging procedure or the type of tag on weight, length or fin damage was observed between experimental groups. After tagging, fish were transferred back to the designated experimental tank and observed for 30 minutes during recovery from anaesthesia. An emergency recovery tank containing highly oxygenated freshwater (> 99% dissolved oxygen injected through block diffusers connected to oxygen gas tanks) was permanently available during tagging procedure in case fish needed assisted recovery or veterinary assistance.

4.2.3. Housing, water quality and environmental conditions.

Fish were housed in 300 litres high density plastic circular tanks (50 cm high and 78 cm diameter). Filtered ambient freshwater was provided throughout the experiment. Dissolved oxygen content ($100.15 \pm 0.98\%$) and water temperature ($10.47 \pm 0.21^\circ\text{C}$) were measured and recorded twice daily using a calibrated sensor (OxyGuard© Handy

Alpha, OxyGuard International A/S). Water flow was controlled at an exchange rate of 10 litres/minute in an open flow system with water velocities of 1 fish body length/second. A 24 hours light:0 hour darkness photoperiod regime was used during the study.

4.2.4. Physical measures.

Initial and final weight (g) and length (total tail-fork length in mm) were measured in each fish. Specific growth rate and Fulton's condition factor were calculated for each fish of the experimental groups as described in previous chapters.

4.2.5. Quantification of fin damage.

The fin damage of the dorsal, pectoral, ventral, anal, upper and lower caudal fins was evaluated from digital photographs of every fish taken at the beginning and end of the experiment using the Relative Fin Index (RFI) and a categorical method for fin erosion (CM) as described in previous chapters. Additionally, fin splits (separation of > 3 mm between fin rays) and other external lesions were quantified at the end of the experiment.

4.2.6. Behavioural observations and social interactions.

Social behavioural interactions were recorded using an automated CCTV cameras system (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) located 1 m above each tank. Ten minutes video recordings were carried out 1 hour before feeding, during the first ten minutes of feeding and 1 hour after the last food delivery from the automatic feeders. PD groups were recorded at 09:00, 10:00 and 11:30 while UD groups were recorded at times relative to their feeding times periods. Rippling on the water surface was prevented using a perforated water inlet pipe submerged to water level and a double central perforated standpipe in each tank. Digital video recordings were made using a DVD/HDD recorder (Pioneer© DVR-550H-S).

4.2.6.1. Associative behavioural interactions.

Associative behaviour between fish were recorded at 1 minute interval during all video recordings. A fish was recorded as associated with any other fish when it was observed within two fish body lengths (if parallel to each other), or within two body widths (if perpendicular to each other). Association matrices were constructed for each sampling period and quantified using social network analysis.

4.2.6.2. Aggressive behavioural interactions.

Aggressive behaviours were classified as attacks, displacements or fin-bites and quantified using the methods described in Chapter 2 and Cañon Jones et al. (2010). The initiator(s) and the receiver(s) of any aggressive interaction were recorded and weighted matrices for social network analysis were constructed. Aggressive interactions were also used to calculate and compare the total amount of aggressive interactions and attacks, displacements and fin bites within and between experimental groups.

4.2.7. Social network analysis.

The social network analysis of the associative and aggressive interaction matrices was carried out using UCINET© (Borgatti et al., 1999). At the group level, the quantified network variables were degree-centrality, clustering coefficient, transitivity, distance and density. At the individual level the quantified network variables were degree-centrality, out and in-degree centralities, clustering coefficients and distances. Detailed explanations of these network variables have been described in Chapter 2, and in Cañon Jones et al. (2010), Cañon Jones et al. (2011).

Network analyses were carried out for the pre-treatment, treatment, and post-treatment periods and for the entire experimental period.

4.2.8. Structural and spatial position measures.

The structure and position of each fish was quantified from the video recording at 1 minute intervals. Fish were classified as being schooling or shoaling (as in Chapter 2 and Cañon Jones et al., 2010, 2011a).

4.2.9. Statistical analyses.

The Shapiro-Wilkes test of normality, descriptive analyses and one-way analyses of variance were carried out on weight, length, RFI, fin damage (splits and bites), SGR and K (Zar, 2009). A general linear model described by $y = a + bx$, where a is the intercept (PD group) and b the slope (effect of treatment), was carried out to clarify the effect of predictability of food delivery on weight and length of fish (Zar, 2009). The Kruskal Wallis non-parametric test was used to analyse the effect of tagging system on weight, length and fin damage between experimental groups. Chi-square test and the Chi-square test for trends (Zar, 2009) was used to evaluate any statistical differences between treatment on dorsal fin erosion. Correlations between dorsal fin erosion and other variables were analysed using the Pearson rank correlation (Zar, 2009) and network distance and density were analysed by one-way analysis of variance (Zar, 2009). Kruskal Wallis tests were utilised to quantify differences in aggressive behaviours (biting, displacements, attacks and total aggressive behaviour) as well as for

centralities (overall, in-degree and out-degree), clustering coefficients and densities between experimental groups. Mantel tests were carried out for associative and aggressive interactions matrices between acclimatisation and treatment periods in order to evaluate whether any differences would be attributed to statistically significant changes in the behaviour of fish rather than by chance (Zar, 2009). All statistical analyses were carried out using R statistical software (R Development Core Team, 2008).

4.3. Results.

Dorsal fin erosion was present in both experimental groups but affected a significantly higher number of fish in UD compared with PD groups (6 vs. 3 fish, $X^2 = 3.52$, $P = 0.04$). Additionally, moderate and severe dorsal erosion was only present in UD groups ($X^2_3 = 3.76$, $P < 0.01$) as can be seen in Table 4.1. However, neither the RFI indexes for all the fins nor number of fin splits differed between experimental groups ($P > 0.05$).

UD groups showed significantly less total aggression than PD groups (45.08 vs. 60.61 interactions/hour, $H_1 = 5.33$ $P = 0.03$). Also, attacks were less frequent in UD groups than in PD (40.66 vs. 56.04 interactions/hour, $H_1 = 5.33$, $P = 0.03$), as shown in Figure 4.1. These results suggested that predictable food delivery regimes increased aggressive behaviour in fish compared with unpredictable food delivery.

Social networks analysis based on aggressive interactions showed a significantly higher degree centrality (43.51% vs. 31.72%, $H_1 = 5.39$, $P = 0.02$), out-degree centrality (46.57% vs. 36.88%, $H_1 = 5.33$, $P = 0.02$) and clustering coefficient (14.12% vs. 10.60%, $H_1 = 5.33$, $P < 0.05$), but less dense networks (10.60 vs. 16.17, $H_1 = 5.33$, $P = 0.02$) in UD groups compared with PD groups.

The high clustering coefficient found in UD groups suggested a disaggregation of the network in groups and indicated a marked separation of roles of the fish in their social network produced by aggressive interactions in UD groups into initiators and receivers of aggression. Initiators had higher out-degree centrality compared with receivers (60.97% vs. 8.33%, $H_1 = 6.81$, $P < 0.01$) while receivers showed higher in-degree centrality compared with initiators (21.87% vs. 10.43%, $H_1 = 3.93$, $P = 0.03$). The networks in UD and PD groups can be seen in Figures 4.2 and 4.3, respectively. Additionally, initiators of aggression had less dorsal fin erosion (1 fish vs. 5 fish), higher final weight (108.8 g vs. 89.6 g, $F_{1,8} = 31.24$, $P < 0.01$) and length (21.2 cm vs. 20.2 cm, $F_{1,8} = 8.92$, $P = 0.02$) compared with receivers of aggression (Table 4.2).

Differences in degree centralities were only seen in UD groups with clusters of fish with high in-degree ($F_{1,78} = 4.7e^{31}$, $P < 0.01$) and also clusters of fish with high out-degree centrality ($F_{1,78} = 1.39e^{32}$, $P < 0.01$). The results allowed to confidently differentiate individuals fish as I (I) or R (R) of aggression as is shown in Figure 4.4.

There were no significant differences in the final weights (102.1 g vs. 102.5 g, $P > 0.05$), lengths (20.81 cm vs. 20.89, $P > 0.05$) and body condition (4.88 vs. 4.92, $P > 0.05$) between PD and UD groups.

There were no statistical differences in social network parameters for associative behaviour between experimental groups ($P > 0.05$). Similarly, fish did not show detectable structural (schooling or shoaling) or positional preferences ($P > 0.05$) in any of the experimental groups.

4.4. Tables and figures.

Table 4.1. Number and percentage of fish with fin erosion, bites and relative fin index (RFI, mean \pm SD) in the dorsal fin measured in the experimental groups.

	Fin erosion (%)				Bites	Splits	RFI
	No erosion	Mild	Moderate	Severe			
Predictable	37 (92.5%) ^a	3 (7.5%)	0 ^a	0 ^a	1	1	8.44 \pm 1.60
Unpredictable	34 (85%) ^b	3 (7.5%)	2 (5%) ^b	1 (2.5%) ^b	5	2	8.28 \pm 1.59

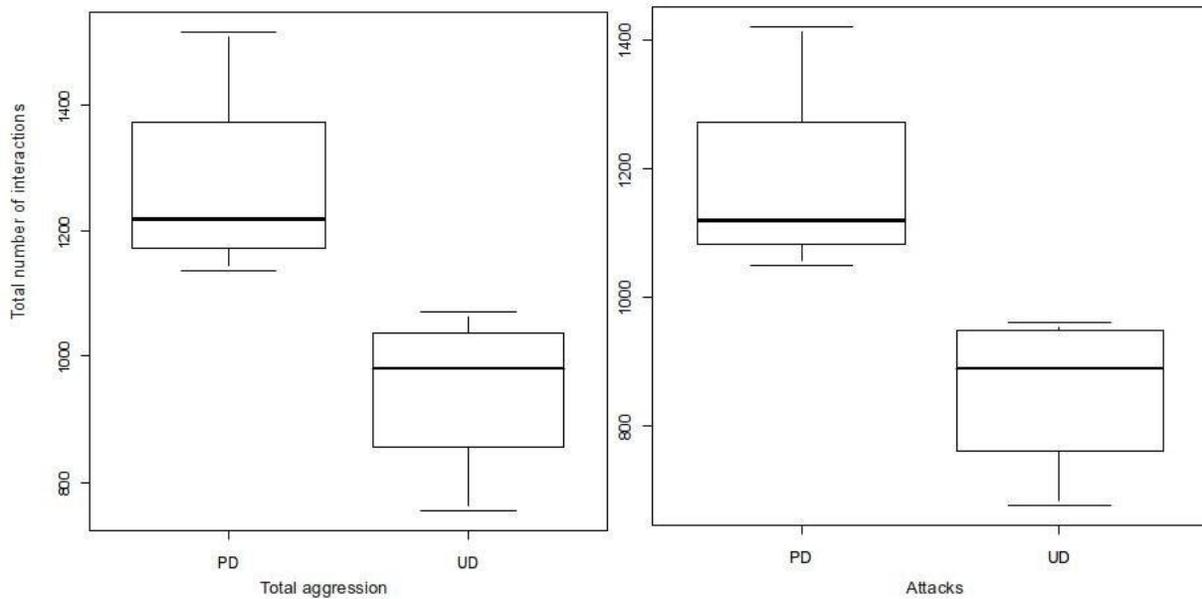
^a and ^b = statistical differences at $P < 0.05$ between experimental groups.

Table 4.2. Fish length and weight (mean±SD) and number of fish showing fin erosion according to type of individual (initiators or receivers) in unpredictable food delivery (UD) groups.

	Length (cm)	Weight (g)	Number of fish with fin erosion
Initiators	21.2±0.50 ^a	108.8±4.86 ^a	1
Receivers	20.2±0.54 ^b	89.6±5.9 ^b	5

^a and ^b = statistical differences at $P < 0.05$ between experimental groups.

Figure 4.1. Mean occurrences of total aggressive interactions and attacks according to experimental groups (PD= predictable food delivery; UD=unpredictable food delivery).



* = statistical differences at $P < 0.05$ between experimental groups.

Figure 4.2. Social network graphs of aggressive interactions in each unpredictable food delivery (UD) group. Each fish is represented by a circle. The diameter represents the out-degree centrality (amount of aggression initiated by the fish) while the thickness of lines represents the magnitude of the interaction. White, grey and black colour represent the type of individual based on their out and in-degree centralities and classified as initiators, initiators/receivers and receivers of aggression, respectively. Encircled are clusters of initiators and receivers.

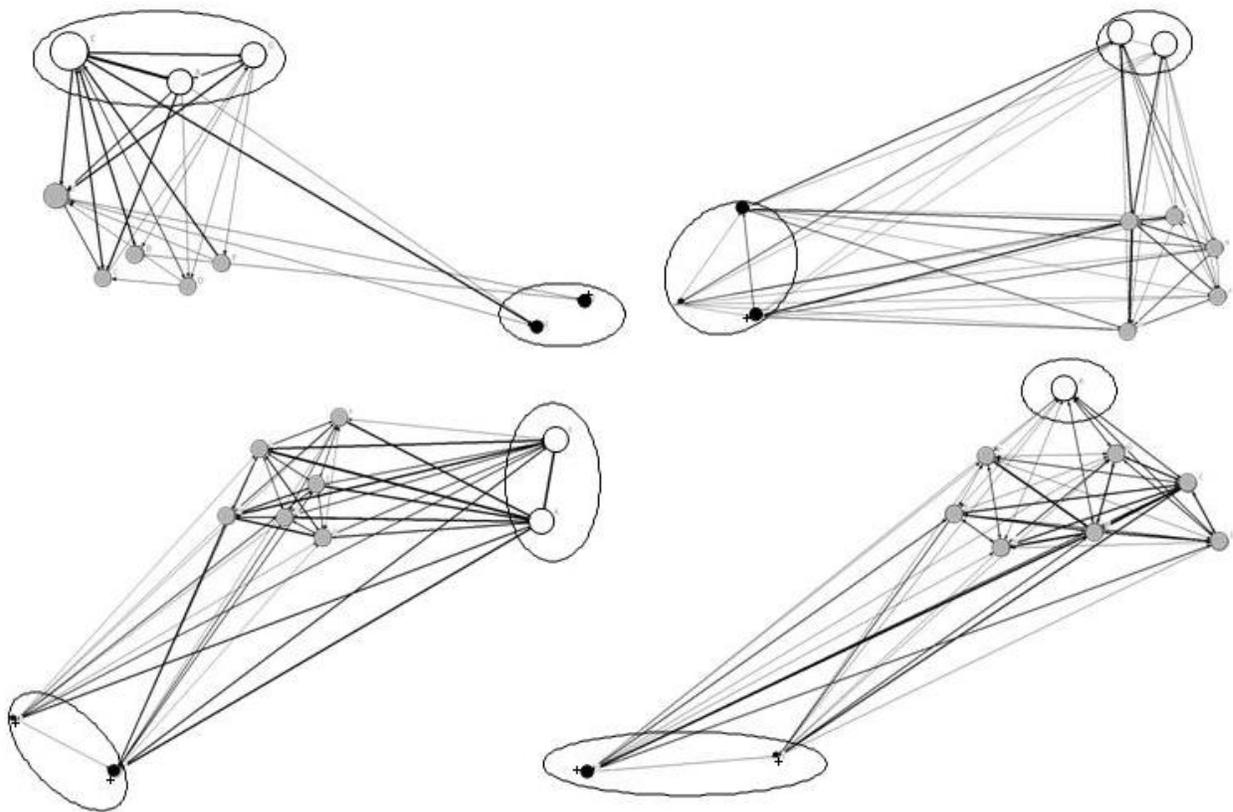


Figure 4.3. Social network graphs of aggressive interactions in each predictable food delivery (PD) group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) while the thickness of the lines represents the magnitude of the interaction. Only initiators/receivers defined on the basis of the out and in-degree centralities of aggression were found in PD groups.

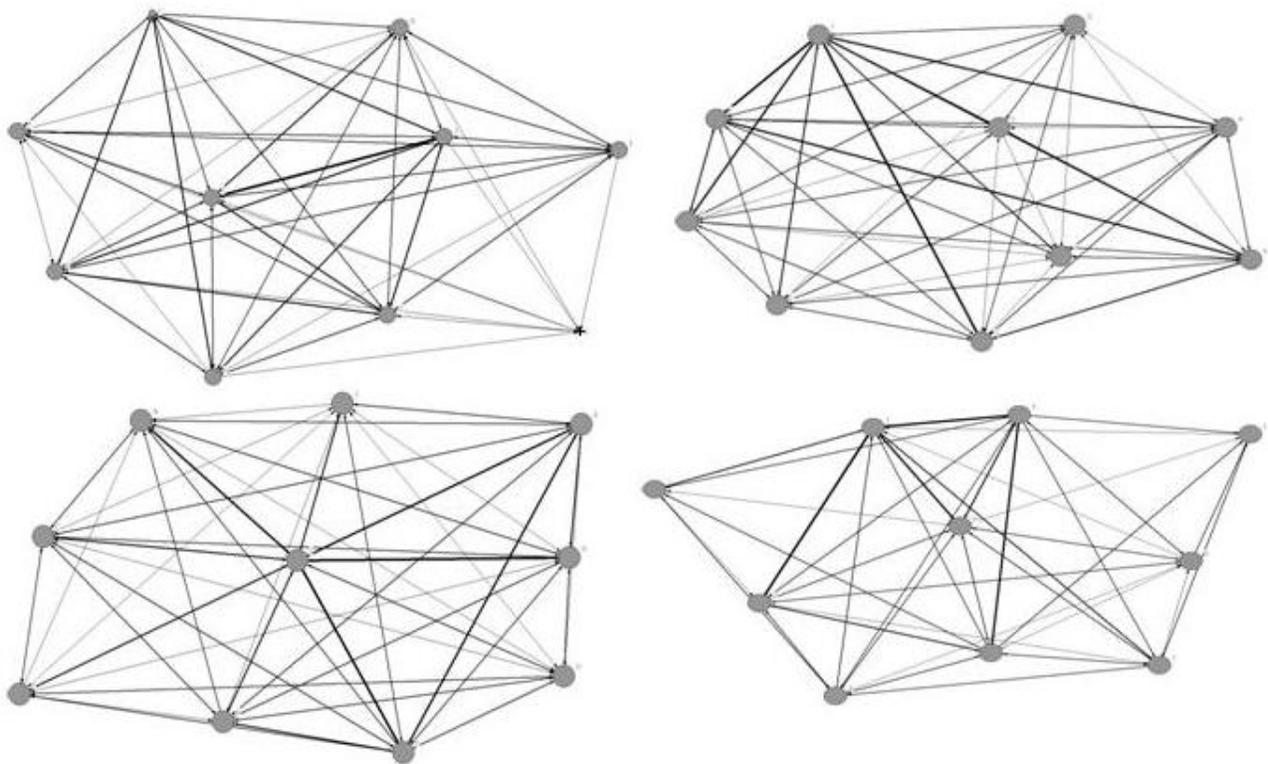
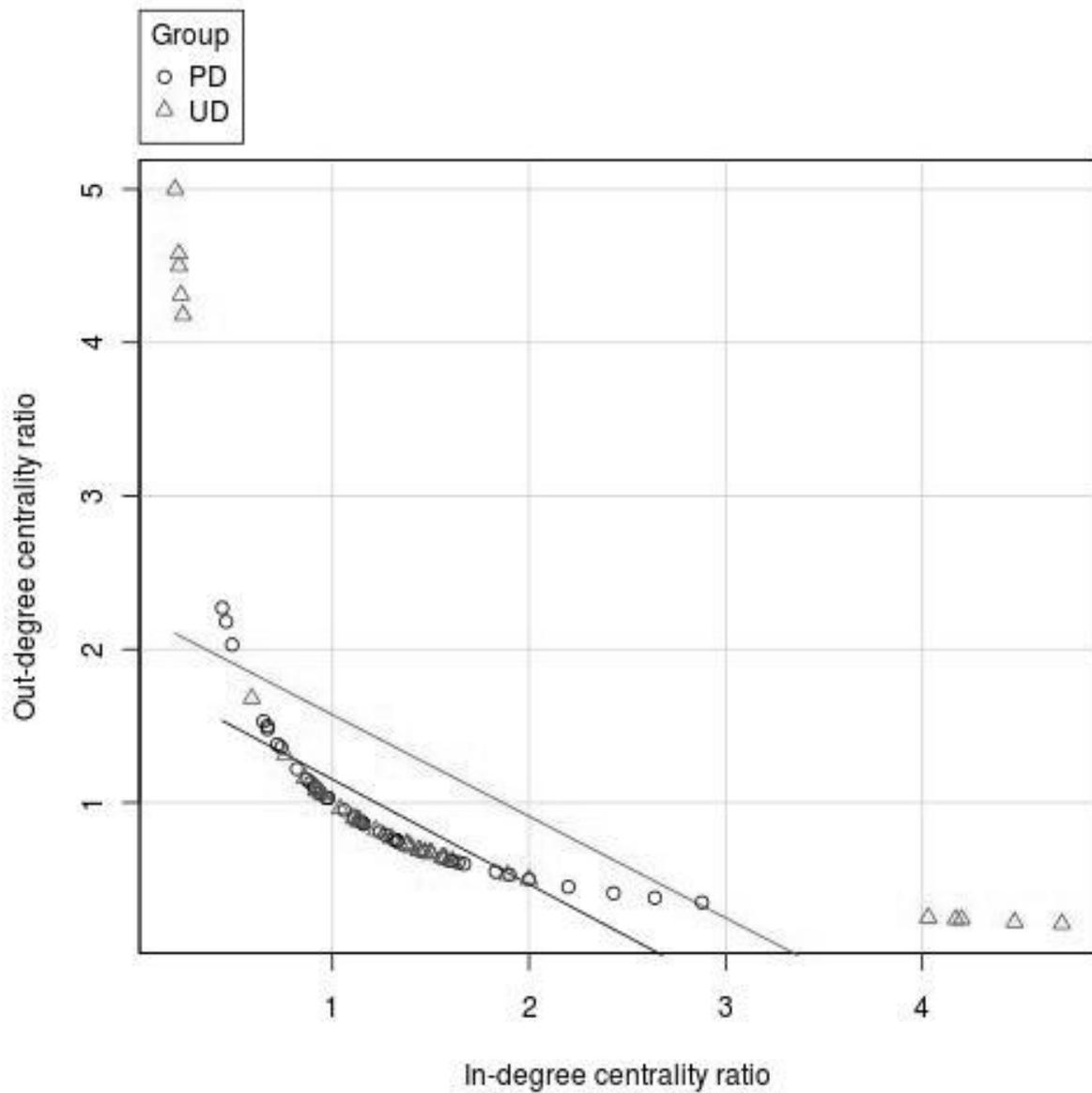


Figure 4.4. Out-degree and in-degree centrality ratios of individual fish in predictable food delivery (PD) and unpredictable food delivery (UD) groups. Initiators and receivers of aggression were only present in UD groups. Lines represent the centrality cut-offs used for the classification of individuals as initiators or receivers of aggression. Dotted lines represent the regression analysis at statistical difference of $P < 0.05$.



4.4. Discussion.

Fish subjected to unpredictable food delivery had higher number and more severe dorsal fin damage compared to predictable food delivery group. The negative effect of unpredictable food delivery on dorsal fin damage in Atlantic salmon has not been reported before and confirms the value of direct visual assessment of fin condition as a good operational welfare indicator as suggested by previous studies in Atlantic salmon (Noble et al., 2007, 2008), rainbow trout (St.Hilaire et al., 2006) and cod (Hatlen et al., 2006). However, no differences were found between groups using the RFI indicating the limitations of using this method to evaluate fin damage, as it only utilises the data from the longest single fin ray of each fin. Similar results have been demonstrated in Atlantic salmon in Chapter 2 and Cañon Jones et al. (2010), suggesting that RFI should be used with caution when quantifying dorsal fin erosion. The levels of fin damage in the present experiment (7.5% and 15%, for PD and UD, respectively) are in general agreement with levels reported in commercially reared Atlantic salmon (c. 6% in Noble et al., 2008) and experimentally housed rainbow trout (c. 9% in Rasmussen et al., 2007).

Fish under predictable food delivery showed higher total aggressive interactions, specifically attack events, compared with unpredictable food delivery subjected fish. Aggressive interactions have been demonstrated to be a good indicator of food anticipatory activity in rainbow trout (Heydarnejad and Purser, 2009). However, this is the first experimental evidence of differences in aggressive behaviour in Atlantic salmon

subjected to predictable and unpredictable food delivery. These findings are in contrast to high levels of food anticipatory activity, most probably involving aggressive interactions, previously reported in other fish such as goldfish (Vera et al., 2007) and sea bream (Sánchez et al., 2009). Thus, our results may suggest a species-specific difference in food anticipatory activity behaviour perhaps coupled to the fact that behavioural interactions were not measured during the whole day.

The social networks based on aggressive behavioural interactions of fish subjected to unpredictable food delivery had increased centrality, clustering coefficient and were less dense compared to those under predictable food delivery. These results suggest a disaggregation of the networks into clusters according to the amount of aggressive behaviour shown. Additionally, the higher in and out-degree centralities quantified in UD groups compared to PD demonstrated a distinct separation of roles of individual members within the network. It is important to note that the separation of the network into clusters and individuals according to their roles did not occur in PD groups even though there was significantly more aggression in PD compared to UD. At the individual network level, initiators of aggression had higher out-degree centrality and more of interactions within the network suggesting a more influential role over the behaviour of other fish and likely that these individuals were able to gain access to resources (Wasserman and Faust, 1994) such as food. Receivers of aggression, on the other hand, had higher in-degree centrality and lower out-degree centrality which indicated that they did not initiate aggression and counter-attacked less often. Importantly, initiators of aggressive interactions were heavier, longer and had less fin damage compared to receivers of aggression (Table 4.2). These results are in

agreement with previous chapter and published studies in Atlantic salmon under long (1 month) food restriction (Chapter 2 and Cañon Jones et al., 2010) and high stocking densities (Chapter 3 and Cañon Jones et al., 2011) and confirm the usefulness of social network analysis for the identification of role of individual fish within their networks. This suggest that in a commercial setting, social network analysis could be used to identify and possibly remove highly aggressive individuals who are heavier and longer in order to reduce fin damage and increase the welfare of farmed salmon if unpredictable food delivery occurs as previously described. Nevertheless, laboratory studies have shown that removing highly dominant fish only allows other fish to take the dominant place with an accompanying short period of increased aggression (Adams et al., 1998, 2000). Social network analysis has been used in ecological studies in other fish specie such as guppies (*Poecilia reticulata*) showing that fish can maintain specific and preferential associations and interactions (Croft et al., 2004, Croft, 2005) and, more recently, social network analysis has been suggested to be a powerful tool in quantification and classification of personalities, behavioural syndromes or temperament in non-human animal (Krause et al., 2010). This study is the first to quantify the effect of predictability of food delivery on the behavioural interactions in relation to fin damage using social network analysis in commercially important farmed species.

There were no differences on the final weight, length and condition of fish between PD and UD groups. One possible reason for this result may be the short period (15 days) during which fish were subjected to unpredictable delivery in this study. This is supported by the evidence from Sanchez and Sanchez-Vazquez, 2009, who found a transient decrease in weight only after 30 days of random food delivery in sea bream.

However, the present experiment identified higher fin damage in fish subjected to unpredictable delivery indicating a poorer welfare on these fish

It is also important to point out that the numbers of fish used in the current study were low compared with commercial aquaculture conditions and caution should be applied when extrapolating these findings to commercial settings. However, the group sizes were higher or similar than others experimental studies investigating predictability in fish (Vera et al., 2007, Sanchez and Sanchez-Vazquez, 2009, Feliciano et al., 2011).

Finally, fish did not differ in their preference to school or shoal behaviour in PD or UD groups. The lack of any preference could be due to the tendency of Atlantic salmon to be highly aggressive and solitary at the parr stage (Bardonnnet and Baglinière, 2000). However, our results are in contrast to theories stipulating that aggressive or dominant individuals should attain central positions in a school of fish (Viscido et al., 2007) and the previous empirical evidence of this occurring in Atlantic salmon (Chapter 2 and Cañon Jones et al., 2010).

4.5. Conclusion.

This study demonstrated the value of using social network analysis in understanding the underlying behavioural causes of fin damage in fish under predictable and unpredictable food delivery schedules. Unpredictable feeding practices resulted in a separation and differentiation of roles of fish within their network into initiators and receivers of aggression and culminating in higher frequencies of dorsal fin damage to receivers. However, more studies are necessary to elucidate the determinants of which fish become an initiator or a receiver of aggression within such a network. Fish subjected to a predictable food delivery schedule did not separate into these roles but overall aggression was higher. Also, physical characteristics and production parameters did not differ at the inter-group level, but did differ between individual fish within the unpredictable delivery groups. The use of social network analysis in applied animal behaviour studies at a more commercial scale is likely to contribute to the understanding of the effect of predictability of food delivery in Atlantic salmon and the improvement of the welfare by the correct identification of socially important individual fish. However, more studies are needed to adequately quantify additional factors influencing the behaviour of fish leading to fin damage such as water temperature, water quality, or different life stages both experimentally and at a larger scale, thus enabling us to suggest the development of practical but scientifically based methods to improve the welfare and productivity of farmed fish.

5. Chapter 5. Social network analysis of behavioural interactions influencing the development of fin damage in Atlantic salmon parr (*Salmo salar*) subjected to short period (10 days) of food restriction.

5.1. Introduction.

Short food restriction periods are commonly applied to fish before practices such as prior to vaccination to empty gastrointestinal tract, sampling of fish to estimate production and correct feeding practices, grading of fish, and transport and transfer from freshwater to seawater (Lucas and Southgate, 2003). However, little is known regarding the effect of a short food restriction period on the behaviour of fish that ultimately may lead to fin damage. The only evidence of the latter occurring is described as in Chapter 1 and section 2.3 Results of Chapter 2 and by Cañon Jones et al. (2010) where a long food restriction period of 30 days was used. Thus, there was a need to investigate and quantify the social effects of short food restriction period.

The aim of the present study was to quantify the impact of a short feed-restriction period in the development of fin damage in Atlantic salmon parr due to changes in behavioural and social structure and organisation.

5.2. Materials and methods.

5.2.1. Animals and experimental groups.

The experiment was carried out in the summer of 2009 at the Aquaculture Research Station in Tromsø, Northern Norway under the Project Number 6039/09-006.1/H69/32/KNF and it adhered to the current Norwegian Fish Welfare and Laboratory Animals legislation (Ministry of Agriculture and Food of Norway, 2010, Ministry of Agriculture of Norway, 2010) .

Eight groups of ten clinically healthy year 1+ Atlantic salmon each (61.73 ± 6.45 g of weight and 17.22 ± 0.52 cm of length, mean \pm SD) were used in the experiment. The fish were sourced commercially from Aqua Gen A/S, Tribe Standard, generation 2008. Three experimental phases were planned: Pre-treatment period (from day 1 to day 10), Treatment period (from day 11 to day 20) and Post-treatment period (from day 21 to day 30).

Food ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway) was delivered at a rate of 1.5% of estimated fish body weight/day and adjusted weekly according to the expected weight gain and water temperature. Food was given every day at 10:00 hrs for 30 minutes during the whole experiment from calibrated automatic feeders located 1 meter above each tank. After the pre-treatment period four tanks were selected as food restriction (FR) and four tanks as control (C) groups. During the treatment period, food

was restricted to 1/3 of the calculated daily in FR groups. Control groups received the full food ration during the whole experiment. At the beginning of the post-treatment period, food was fully re-established in the FR.

Two FR groups and two C groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the entrance in order to even the effect of daily management disturbances.

There were no fish mortalities during the experiment and all fish were euthanised by immersing them in an overdose bath of benzocaine chlorhydrate (> 250 mg/l freshwater, Benzoak Vet, A.C.D. Pharmaceuticals SA, Norway) at the end of the experiment.

5.2.2. Containment and individual identification.

As previously described, fish were individually tagged under anaesthesia by an immersion bath of benzocaine chlorhydrate (100 mg/l freshwater, Benzoak Vet, A.C.D. Pharmaceuticals SA, Norway) at the beginning of the experiment. Fish achieved full anaesthesia within 3 minutes and tagging was carried out within the following minute. Especially suited tags were created with black or white geometric designs (circles, triangles, squares, rectangles and crosses of 2.5 by 2.5 cm) using plastic printing paper (Xerox® Special Advanced Media Digital Colour, Premium Never Tear 95µ Polyester paper). The tags were inserted under the skin behind the dorsal fin of each fish using

strong silk thread and a standard commercial Floy Tag (Hallprint®, Polyepalticthylene streamer tags, series PST). Macroscopic tissue damage of the skin was minimal with no significant effect of the tagging procedure or the type of tag on weight, length or fin damage was observed between experimental groups. Fish were moved back to the designated experimental tank and observed for 30 minutes after recovery from anaesthesia. An emergency recovery tank with highly oxygenated freshwater (> 99% dissolved oxygen injected through block diffusers connected to oxygen gas tanks) was available permanently during tagging of fish in case of assisted recovery or veterinary aid.

5.2.3. Housing, water quality and environmental conditions.

Fish were held in 300 litres high density plastic circular tanks (50 cm high and 78 cm diameter). Ambient temperature freshwater was obtained from a local uncontaminated river and filtered before used. Dissolved oxygen content ($96.87 \pm 2.80\%$) and water temperature ($11.40 \pm 1.70^\circ\text{C}$) were measured and recorded twice daily using a calibrated sensor (OxyGuard© Handy Alpha, OxyGuard International A/S). An open flow system with water exchange rates of 10 litres/minute and velocities of 1 fish body length/second was set-up in every tank. A 24 hours light: 0 hours darkness photoperiod was maintained throughout the experiment.

5.2.4. Physical measures.

Body weight (g) and length (total tail-fork length in mm) were measured in each fish at the beginning and end of the experiment. The specific growth rate and the Fulton's condition factor were subsequently calculated for each fish of the experimental groups as described in Chapter 2 and Cañon Jones et al. (2010).

5.2.5. Fin damage quantification.

Digital photographs of every fish were taken at the beginning and end of the experiment and used for the evaluation of damage of the dorsal, pectoral, ventral, anal, upper and lower caudal fins using the Relative Fin Index (RFI) and a categorical method for fin erosion (CM) as previously described in Chapter 2 and Cañon Jones et al. (2010).

5.2.6. Behavioural observations and social interactions.

Fish behaviours were captured and recorded using CCTV cameras (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) placed 1 m above each tank and connected to a DVD/HDD recorder (Pioneer© DVR-550H-S) located in an adjacent

room. Ten minutes video recordings were obtained each experimental day at 1 hour before feeding time (09:00 to 09:10), during the first ten minutes of feeding (10:00 to 10:10) and 1 hour after the last food delivery (11:30 to 11:40) from the previously described automatic feeders. Surface water rippling was prevented using a perforated water inlet pipe, allowing the water to come into the tank under the water level, and a double central perforated standpipe.

5.2.6.1. Aggressive behavioural interactions.

Aggressive behaviour were classified as attacks, displacements or fin-bites, and recorded as described in Chapter 2 and Cañon Jones et al. (2010).

5.2.6.2. Associative behavioural interactions.

Associative behaviour between fish was recorded every 1 minute intervals in the video recordings. Association was defined as whenever two fish were within a distance of two fish body lengths (if parallel to each other), or two body widths (if perpendicular to each other). Association matrices were constructed and quantified using social network analysis.

5.2.7. Social network analysis.

Social network analyses of the aggressive and associative interaction matrices were carried out using UCINET© (Borgatti et al., 1999). The quantified group-level network variables were degree-centrality, clustering coefficient, transitivity, distance and density. At the individual level, the quantified network variables were degree-centrality, out and in-degree centralities, clustering coefficients and distances. In depth descriptions of these network variables have been described previously in Chapter 2 and Cañon Jones et al. (2010).

Network analyses were carried out for the pre-treatment, treatment, and post-treatment periods and for the entire experimental period.

5.2.8. Structural and spatial position measures.

The structure of the group of fish and the position of each fish in the tank was recorded from the video recording at 1 minute intervals. Structurally, fish were classified as being schooling or shoaling as described in Chapter 2 and Cañon Jones et al. (2010).

5.2.9. Statistical analyses.

The Shapiro-Wilkes test of normality, descriptive analyses and one-way analyses of variance were carried out on weight, length, RFI, fin damage (splits and bites), SGR and K (Zar, 2009). A general linear model described by $y = a + bx$, where a is the intercept (C group) and b the slope (effect of food restriction), was carried out to clarify the effect of predictability of food delivery on weight and length of fish (Zar, 2009). The Kruskal Wallis non-parametric test was used to evaluate the effect of tagging system on weight, length and fin damage between experimental groups. Chi-square test and the Chi-square test for trends was used to evaluate any statistical differences between treatment on dorsal fin erosion. Kruskal Wallis tests were utilised to quantify differences in aggressive behaviours (biting, displacements, attacks and total aggressive behaviour) as well as for centralities (overall, in-degree and out-degree), clustering coefficients, distances and densities between experimental groups. Mantel tests were carried out for associative and aggressive interactions matrices between acclimatisation and treatment periods in order to evaluate whether any differences would be attributed to statistically significant changes in the behaviour of fish rather than by chance (Zar, 2009). All statistical analyses were carried out using R statistical software (R Development Core Team, 2008).

5.3. Results.

Fin damage was observed to occur only in the dorsal fin and frequencies were significantly higher in FR compared to C groups (12.5% vs 7.5% of fish affected, respectively). Moreover, moderate and severe dorsal erosion was present only in FR groups and not in C groups ($\chi^2 = 4.21$, $P = 0.04$) as shown in Table 5.1. Dorsal fin erosion was positively correlated with the observation of biting in FR groups ($r^2 = 0.70$, $P = 0.03$). Interestingly, there were no differences in dorsal fin RFI or any other fin evaluated ($P > 0.05$).

FR groups showed significantly more total aggression than C groups (21.82 vs. 12.32 interactions/hour, $H_1 = 5.33$, $P = 0.03$). Detailed analysis of the type of aggressive interaction showed a significantly higher amount of attacks (21.58 vs. 11.68 interactions/hour, $H_1 = 5.33$, $P < 0.03$) and a tendency of higher biting (0.31 vs. 0.1 interactions/hour, $H_1 = 3$, $P > 0.05$) in FR compared to C groups (0.35 vs. 0.13 interactions/hour, $H_1 = 5.39$, $P < 0.03$), as shown in Figure 5.1. These results suggest that food restriction conditions trigger an increase in the frequency of aggressive behaviour, and that the aggression is mainly through attacks.

At the group level, social networks analyses based on aggressive interactions showed that FR groups had higher overall degree-centrality (47.94% vs. 35.93%, $H_1 = 5.33$, $P = 0.03$), clustering coefficient (0.16 vs. 0.07, $H_1 = 5.33$, $P = 0.03$), out-degree centrality (54.33% vs. 35.69%, $H_1 = 4.08$, $P = 0.02$) and in-degree centrality (15.94% vs. 6.19%, $H_1 = 5.33$, $P = 0.03$) than networks C groups. Also, the networks in FR groups were significantly more dense (16.07 vs. 6.98, $H_1 = 5.39$, $P = 0.03$) than in C groups.

Network distance was lower (1.06 vs. 1.07) while transitivity was high (84.87% vs. 79.93%) in both FR and C groups, but no statistical difference was observed ($P > 0.05$).

These group-level results suggested that food restriction induced a particular separation of roles of fish with a specific arrangement of clusters into groups of initiators and receivers of aggression. Network analysis at the individual level showed that initiators had high out-degree centrality (64.76 vs. 3.24%, $H_1 = 11.38$, $P < 0.01$) while receivers showed high in-degree centrality (22.64% vs. 14.41%, $H_1 = 5.48$, $P = 0.03$). The graphical representation of the separation of roles of fish and clusters of initiators and receivers in the networks can be seen in figures 5.2 and 5.3 for C and FR groups, respectively. In FR group, initiators had no dorsal fin erosion (0 vs. 5 fish), but there were no significant differences ($P > 0.05$) in final weight (61.91 g vs. 60.54 g) or length (17.13 cm vs. 17.65 cm) compared with receivers of aggression (Table 5.2).

Additionally, the linear regression modeling showed differences in degree centralities only in HD groups with clusters of fish with high out-degree ($F_{1,78} = 47.02$, $P < 0.01$) and clusters of fish with high in-degree centrality ($F_{1,78} = 3.85$, $P = 0.03$) allowing to confidently differentiate individuals fish as I or R of aggression as shown in Figure 5.4.

Fish in the FR groups had lower final weights (60.33 g vs. 64.92 g, $F_{1,78} = 6.6$, $P = 0.02$), lower final lengths (17.44 cm vs. 17.72 cm, $F_{1,78} = 4.02$, $P = 0.03$) and lower body condition (3.45 vs. 3.65, $H_1 = 5.74$, $P = 0.04$) compared with C groups. In fact, FR groups did not gain and lost weight compared with Control groups (-0.16 g vs. 1.35 g), reflected also in the food conversion ratio (FCR) of both groups (0.20 vs. 1.72).

Statistical differences were not found in social network parameters based on associative behaviour between experimental groups ($P > 0.05$). Likewise, fish did not show any detectable structural (schooling or shoaling) or positional preference in the experimental groups ($P > 0.05$).

5.4. Tables and figures.

Table 5.1. Number and percentage of fish with fin erosion, bites and relative fin index (RFI, mean \pm SD) in the dorsal fin measured in the experimental groups.

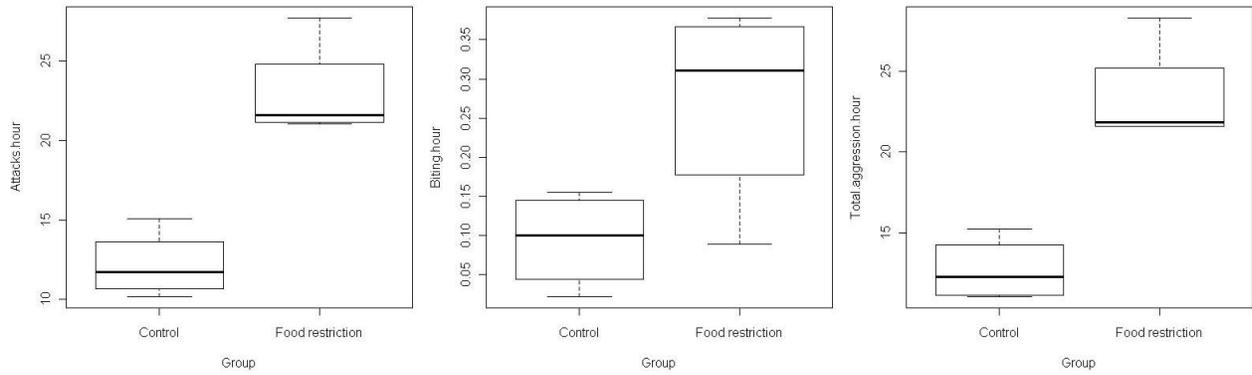
	Fin erosion (%)				Bites	RFI
	No erosion	Mild	Moderate	Severe		
Control	37 (92.5%) ^a	3 (7.5%) ^b	0 ^a	0 ^a	0	11.66 \pm 2.03
Food restriction	35 (87.5%) ^b	3 (7.5%) ^b	1 (2.5%) ^b	1 (2.5%) ^b	1	11.22 \pm 1.67

^a and ^b = statistical differences at $P < 0.05$ between experimental groups.

Table 5.2. Fish length and weight (mean \pm SD) and number of fish showing fin erosion according to type of individual (initiators or receivers) in food restriction (FR) groups.

	Length (cm)	Weight (g)	Number of fish with fin erosion
Initiators	17.65 \pm 0.59	61.91 \pm 7.24	0
Receivers	17.13 \pm 0.68	60.54 \pm 8.45	5

Figure 5.1. Mean occurrences of total aggressive interactions and attacks according to experimental groups.



* = statistical differences at $P < 0.05$ between experimental groups.

Figure 5.2. Social network graphs of aggressive interactions in food restriction (FR) groups. Fish are represented by circles. The diameter represents the out-degree centrality (amount of aggression initiated by the fish) while the thickness of connecting lines represents the magnitude of the interaction. Black, grey and white colour indicate the type of individual classified as initiators, initiators/receivers and receivers of aggression, respectively. Clusters of initiators and receivers are encircled.

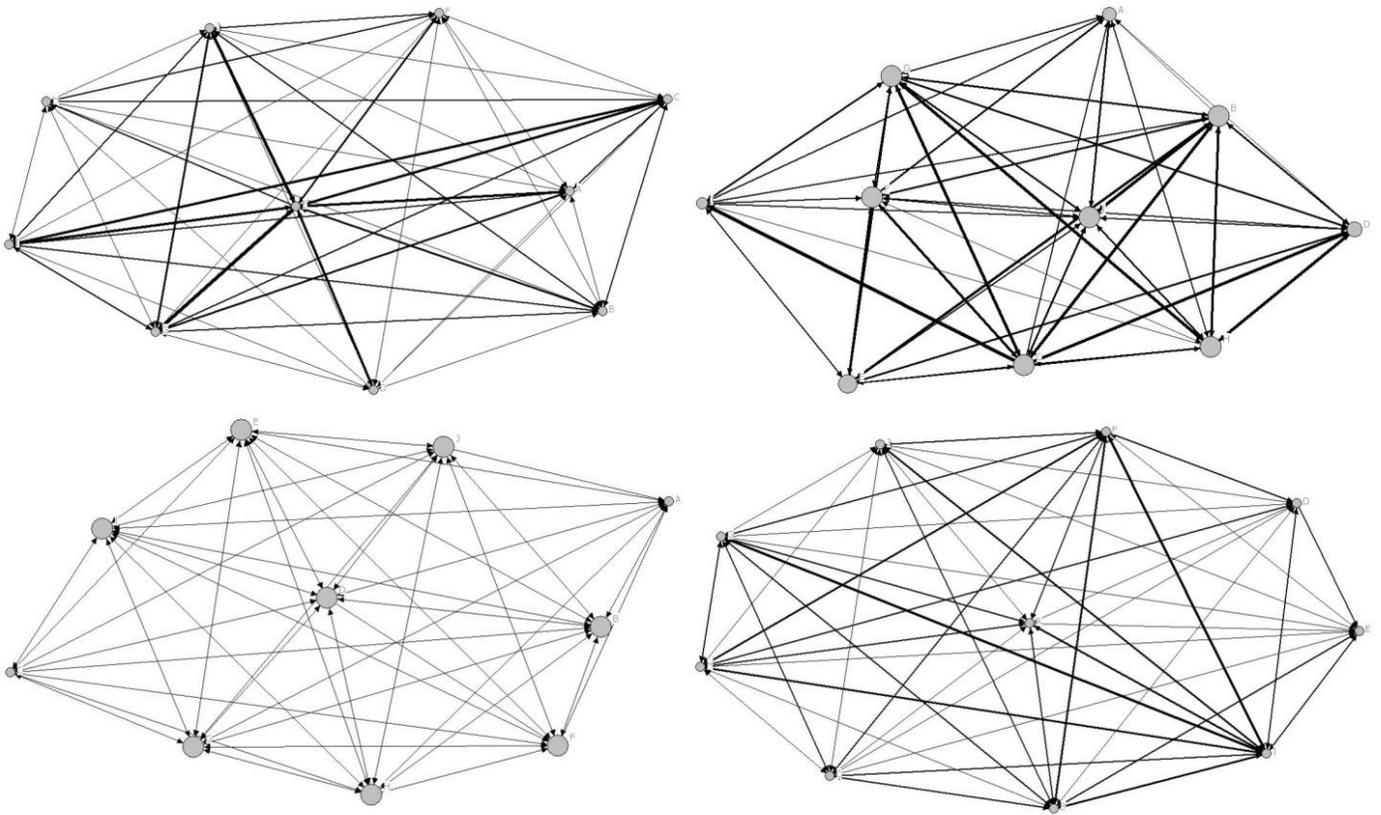


Figure 5.3. Social network graphs of aggressive interactions in each control (C) group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) and the thickness of the lines represents the magnitude of the interaction. Only initiators/receivers were found in C groups.

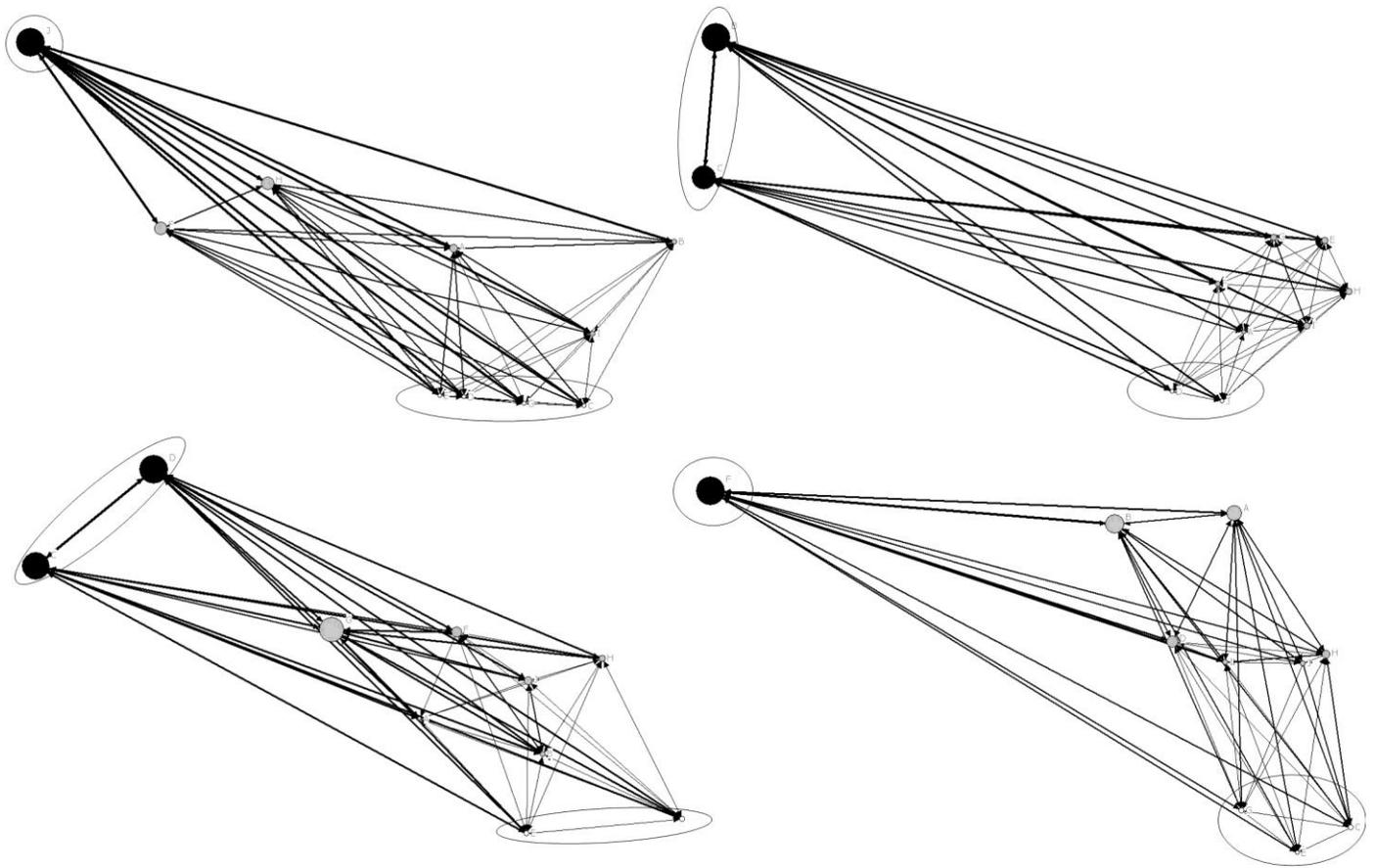
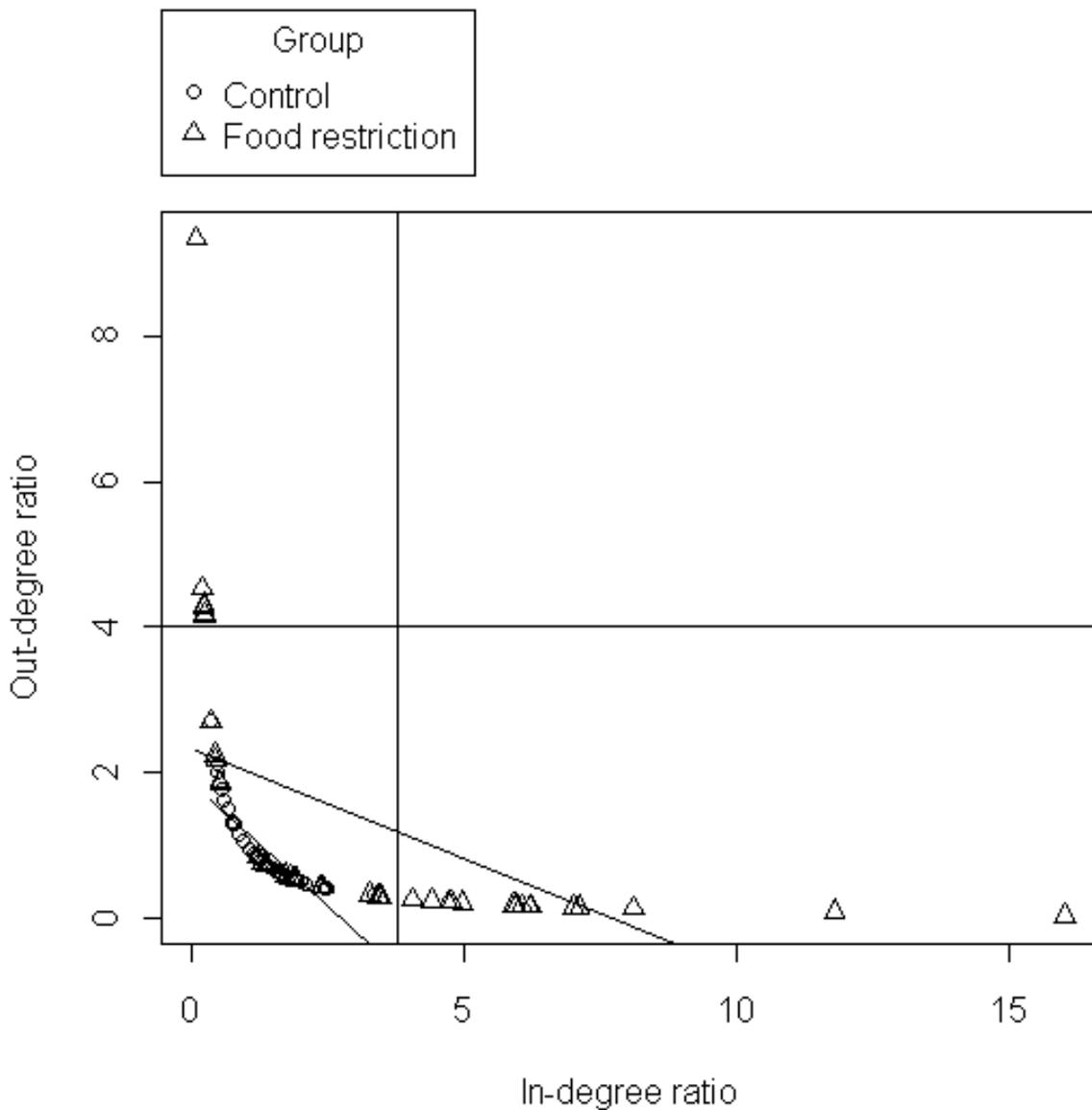


Figure 5.4. Out-degree and in-degree centrality ratios of individual fish in control (C) and food restriction (FR) groups. Lines represent the centrality cut-offs used for the classification of individuals as initiators or receivers of aggression which were only present in FR groups. Dotted lines represent the regression analysis at statistical difference of $P < 0.05$.



5.5. Discussion.

Fin damage was only observed in the dorsal fin in both experimental groups, but it was significantly higher in FR groups. Furthermore, moderate and severe dorsal fin erosion was only present in FR groups. These results are in accordance with previous findings of higher fin damage in food-restricted rainbow trout (St.Hilaire et al., 2006) and Atlantic salmon (Noble et al., 2007, Noble et al., 2008). However, no differences were found using RFI as an indicator of fin damage. A possible explanation for this is that RFI takes the longest fin-ray (Bosakowski and Wagner, 1994) which may not be the section of the fin actually eroded or damaged. Nevertheless, our results confirm the utility of direct observational of the condition of the fin as an indicator of the both the occurrence of damage and the degree of the erosion and highlight the limitations of using RFI.

Fin damage was observed in the same groups where high biting events occurred, suggesting that the most probable cause of fin damage was direct aggressive interactions. It has been suggested that rough tanks or net surfaces may excoriate fins producing (Latremouille, 2003). However, there is no empirical evidence demonstrating a direct cause-effect relationship of this in farmed situations and in this study, each tank was inspected before the experiment for the presence of any abrasive material which could cause fin abrasion, erosion or damage. These results are in agreement with the previous findings on the effect of a long period of 30 days of food restriction on the development of fin damage in Atlantic salmon as described in Chapter 2 and Cañon Jones et al. (2010). The results support the hypothesis that fin damage in salmonids

may be the result of aggression between fish (Abbott and Dill, 1985, Turnbull et al., 1996, 1998, Ellis et al., 2008).

The results showed that food restriction increased the amount of total aggressive interactions amongst fish, manifested mainly by attacks with a tendency of more biting events ($P > 0.05$). The fact that no statistical differences were found in biting events may be because fish were not exposed to a prolonged food restriction period where aggression could be higher in intensity, as has been described in Chapter 2 and Cañon Jones et al. (2010).

The social network analysis based on aggressive interactions revealed that FR groups had higher overall degree-centrality, clustering coefficients suggesting the presence of key individuals and clusters of individuals initiating and receiving aggression within the network. Detailed social network analysis unveiled marked differences in the out and in-degree centrality of individuals in FR groups resulting in fish being able to be classified as either initiators or receivers of aggression. Initiators of aggression were fish having higher out-degree centrality and with lower in-degree centrality and, therefore produced most of the aggression without retaliation. As previously found in long period of food restriction in Chapter 2 and Canon Jones et al. (2010), highly central individuals are more influential within the network and more likely to gain resources (Wasserman and Faust, 1994). Receivers of aggression were fish with high in-degree and low out-degree centralities reflecting that they were mostly recipients of aggression, almost never initiated aggressive interactions and did not strike back. However, no statistical differences were found in transitivity, weight or length, between initiators and receivers of aggression in the FR groups. A possible explanation

for this is that fish were subjected only to 10 days food restriction period rather than a long period of 30 days of food restriction which showed significant differences in physical parameters between initiators and receivers (Chapter 2, Cañon Jones et al., 2010). This is also supported by the fact that the effect of aggressive dominance on physical parameters such as weight or length requires at least a week to build up (Huntingford et al., 1990). Also, it is important to point out at this moment that these results are only for FR groups and that statistical differences in weight and length were found between FR (lighter and shorter) and C groups. Furthermore, initiators of aggressive interactions did not have any fin damage, corroborating that initiators may have been dominating food resources without receiving aggression.

Thus, the use of social network analysis enabled to clearly identify the existence of socially important key individuals which were responsible for causing most of the fin damage. These results are in accordance to previous studies by Cañon Jones et al. 2010 where initiators and receivers were identified and their effects quantified using social network analysis. More importantly, the results showed that a short period of food restriction affects the behaviour of fish increasing aggression that leads to fin damage, but not necessarily affecting physical or other phenotypical characteristic of the fish.

The correct identification of these highly aggressive individual fish will have important practical implications in the control of fin damage in aquaculture whenever reduced or non-feeding periods occur such as the repeated routines before of fish grading, vaccinations, transport or slaughter. Nevertheless, future studies are needed to test the current experimental findings under more commercial farmed situations.

Food restriction did not affect the structural distribution of fish in the water column or their association in the networks ($P > 0.05$). Fish did not appear to prefer to school or shoal, and did not show any preference to associate with specific fish in the network. These results are in contrast to previous finding of a distinctive structural (schooling) and association preference in groups of fish subjected to a long period of food restriction (Chapter 2 and Cañon Jones et al., 2010). These may be due to the fact that Atlantic salmon parr tends to be highly aggressive solitary animals (Bardonnnet and Baglinière, 2000) or due to the short period of food restriction to which the fish were subjected.

5.6. Conclusions.

The present study established the applicability and the value of social network analysis in understanding the development of fin damage in Atlantic salmon under short food restriction on the social and behavioural characteristic of fish. The study showed that a short period of 10 days had a profound impact on the behaviour fish, triggering internal cognitive processes pointing to a differentiation of roles within a group of fish leading to high fin damage. The utilisation of social network analysis brings a potential for the improvement of farmed fish welfare through the precise and correct identification and quantification of these socially important aggressive individuals. More studies are needed to elucidate the effect of different food restriction regimes on the occurrence of

fin damage as well as in other social contexts and thus continuing to improve the welfare of farmed fish.

6. Chapter 6. Dynamic social network analysis of aggressive behavioural interactions in Atlantic salmon parr (*Salmo salar*) leading to fin damage: an insight into personality traits.

6.1. Introduction.

As mentioned previously in Chapter 1, humans and non-humans animals tend to aggregate and form social groups (Krause et al., 2002). Previous research has defined these social groups as social networks where individual animals (nodes) are connected with each other through repeated behavioural interactions (edges) such as aggression, grooming, reproduction and proximity (Croft et al., 2008, Coleing, 2009, Bode et al., 2011). Social network analysis has been the subject of increasing scientific interest in the behavioural sciences recently (Krause et al., 2007, Wey et al., 2008). The advantages of a social network approach include the ability to quantify the direct and indirect relationships that occur between individuals, their social ties and influences and allowing the identification of key individuals within the network and their roles that they play within the group (Wasserman and Faust, 1994).

Animal social network analysis has been utilised in non-captive animals such as meerkats (*Suricatta suricatta*) (Drewe et al., 2009), elephants (*Loxodonta africana*) (Wittemyer and Getz, 2007) and guppies (*Poecilia reticulata*) (Croft et al., 2004, Croft, 2005), in captive animal such as rhesus macaques (*Macaca mulatta*) (McCowan, 2007) and in farmed animals such as cows (*Bos taurus*) (Gygax et al., 2010) and Atlantic

salmon (*Salmo salar*) (Chapters 2 and 3, Cañon Jones et al., 2010, Cañon Jones et al., 2011). In most of these studies observations of behavioural occurrences were recorded, combined and quantified using social network analysis in order to give a static representation of the network in time. However, social networks are seldom static but dynamic entities, where relationships between individuals can be initiated and either maintained or do not persist in time (Snijders et al., 2010).

Dynamic network analysis is a technique that takes into account the evolution and changes that occur within a network over time and several models have been proposed recently for the quantification of these changes in human behavioural studies (Snijders, 2001, Boccaletti et al., 2006, Koskinen and Snijders, 2007, Hock et al., 2010). All dynamic social network analysis models are based on comparing repeated observed social networks against theoretical generated networks, usually using a stochastic approach employing Markov chains processes and Monte Carlo simulations (Snijders, 2001). In this way, dynamic network analyses allows the testing of whether changes in the network are the result of chance alone or, if they are the product of intentional and maintained types of relationships (for example, friendship or dominance between individuals) within the network.

Stochastic actor-based models have been proven useful to investigate the dynamic changes in relationships between social networks of students (Steglich et al., 2010) and the tendency of behaviours such as smoking in adolescents (Mercken et al., 2010) but no studies have been conducted in non-human animals using these type of models. Actor-based models quantify changes in the network caused by simultaneous mechanisms such as changes in centrality, transitivity or reciprocity of the relations of

individuals within the network (Snijders et al., 2007). The main advantage in using actor-based models for dynamic network analysis is that they quantify behavioural tendencies of individuals in the network (Snijders et al., 2010).

Animals have also been suggested to possess personality traits also known as behavioural syndromes, copying styles or temperaments, and defined as a suite of behavioural traits that consistently co-vary across different social contexts and situations (Sih et al., 2004, Gosling, 2008). The concept of personality is supported by the evolutionary view that certain personalities increases fitness and survival of individuals in a particular environment, for example bold animals securing enhanced access to scarce food resources (Dingemanse and Reale, 2005, Reale et al., 2007, Réale et al., 2010). Empirical evidence in fish has shown distinct personality traits related to the shyness-boldness, exploration-avoidance, activity, sociability and aggressiveness personalities axes (Huntingford, 2007, Conrad et al., 2011). Moreover, the evaluation and quantification of personalities in farmed fish is of particular importance (Huntingford and Adams, 2005) since repetitive undesirable behaviours of some individual fish leads to poor welfare such as in the case of aggressive interactions in Atlantic salmon leading to fin damage (Chapters 2 and 3, Cañon Jones et al., 2010, Cañon Jones et al., 2011) and reduced growth (Noble et al., 2007b). Furthermore, it has been shown that selection for certain coping styles is feasible in rainbow trout (Øverli et al., 2007) opening the possibility of selection based on behavioural traits leading to improved welfare of farmed fish.

Recently, several authors have suggested the use of social network analysis and dynamic network analysis to investigate personalities in animals because it can quantify

consistent behavioural tendencies of the individuals within the network (Krause et al., 2007, Krause et al., 2010, Sih and Bell, 2008). However, to the author's knowledge, no studies have yet investigated the changes in animal social network through time using dynamic social network analysis yet.

The effect of long (one month) and short (ten days) periods of food restriction, high stocking densities and unpredictable food delivery on the social networks based in aggressive behaviour in Atlantic salmon leading to fin damage has been described in previous Chapters. In these experiments using traditional social network analysis it was possible to identify and quantify the roles of particular fish within their network as initiators and receivers of aggression. Initiators had less fin damage while the receivers had more fin damage. However, dynamics changes in the networks during these studies were not quantified and it is unknown if the effect of treatments involved produced only a short term or a sustainable effects on network behaviour over time.

The present Chapter investigated temporal changes in social networks using dynamic social network analysis of aggressive interactions leading to fin damage in Atlantic salmon involving the corresponding quantification of personalities in individual fish.

6.2. Materials and methods.

6.2.1. Data collection.

The data used in the present study were collected from the three previous Chapters using social network analysis in farmed Atlantic salmon. In depth experimental design of each study is explained in detail in Chapters 3, 4 and 5. Briefly, experiment 1 (Stocknet) investigated the effect of high (30 kg/m^3) and low (8 kg/m^3) stocking densities the aggressive behavioural interactions leading to fin damage as described in Chapter 3 and Cañon Jones et al. (2011). Experiment 2 (Prednet) investigated the effect of unpredictable food delivery on aggressive behaviour influencing the development of fin damage and described as in Chapter 4. Experiment 3 (Shortnet) quantified the effect of a short food restriction (30% of *ad libitum* daily ration) period (10 days) in the occurrence of aggressive behaviour leading to fin damage in Atlantic salmon as described in Chapter 5.

All experiments were carried out in three experimental phases: a) Pre-treatment b) Treatment, and, c) Post-treatment phase, consisting of ten days each for the Stocknet and Shortnet experiments and 14 days each for the Prednet experiment. As shown in Table 6.1, fish were left to acclimatise to the new environmental conditions during the pre-treatment phase; during the treatment phase, half of the experimental groups were subjected to either high stocking density, unpredictable food delivery schedule or food restriction in Stocknet, Prednet and Shortnet, respectively; in the post-

treatment phase environmental conditions were restored to pre-treatment (control) conditions. All environmental conditions such as water flow, dissolved oxygen, holdings tanks, automatic feeders, video recording equipments were identical in each experiment except for the particular treatments.

Aggressive behavioural interactions were quantified each day and cumulative aggressive behaviour matrices were constructed for the pre-treatment, treatment and post-treatment periods for each experimental group in each study. Each matrix represented 15 hours of behavioural observations with a total of 45 hours for each experimental tank in every study, producing three waves of social networks for each experimental tank in each study for dynamic network analysis.

It is important to point out that using traditional social network analysis it was possible to identify and quantify individuals forming subgroups or clusters of initiators of aggression (fish that had high out-degree centrality and low in-degree centrality) and clusters of receivers of aggression (fish with high in-degree centrality and low out-degree centrality) only in the treatment groups. Initiators were fish gaining more weight and with less or no fin damage whereas receivers were fish with more and severe fin damage which usually grew less.

6.2.2. Description of models for dynamic network analysis.

Dynamic network analysis was carried out using RSiena package in the R statistical program (R Development Core, 2008). RSiena carries out dynamic social network analysis using statistical models of repeated social networks measures based on stochastic actor-based models. Briefly, actor-based models aim to represent the dynamics of observed longitudinal network data which are driven by different network parameters such as centrality, density and reciprocity (Snijders et al., 2010). The models assume that time is continuous and that the change in the network is the product of a Markov process where all current states of the network contain all previous information and thus determine its future evolution in a stochastic manner with no predetermined future behaviour (Snijders, 2001, Steglich et al., 2010). It also assumes that individuals in the network control the ties with others in the network but the changes in the network are triggered only by those initiating connections and that the rate of this change depends on the position (for example, centrality) of each individual (Snijders, 2001). Thus, the stochastic nature of the actor-based models enabled to test hypothesis about significant persistent tendencies in variables under study such as in and out-degree centrality of individuals within the network.

The use of these types of models provides insight into if (and how) initiators and/or receivers of aggression continue to behave in these ways over time after control situations have been restored. More specifically the model enables to test whether fish are behaviourally capable or flexible enough to return to previous roles following

restoration of normal or control situations, or whether fish acquired persistent behavioural roles independently of current context. Persistence of role differentiation would be in accordance with the concept of personalities in humans (Digman, 1990, Clifton et al., 2009) and other non humans animals (Sih et al., 2004, Sih and Bell, 2008, Gosling, 2008).

6.2.3. Model specification.

The dynamic network depends on the change of choices of interactions individuals make with others in the network, and it is described by an *evaluation function*:

$$f^{net}(x) = \sum_k \beta_k^{net} s_{ik}^{net}(x)$$

where $f^{net}(x)$ is the value of the function for any individual i at time x of a network parameter k , β_k^{net} are rate parameters and s_{ik}^{net} are the effects parameters of network variables (Snijders et al., 2008, Snijders et al., 2010). The rate parameters describe the rate at which the network changes and it has no real interpretation as the rate of change between the observed networks must be always positive. The effects parameters represent the effects of network variables such as degree centrality on the dynamics of individuals (tendencies to behave) within the network. It follows then that the evaluation function is the weighted sum of effects s_{ik}^{net} .

The selection of the effect parameters is based on empirical or theoretical backgrounds (Snijders et al., 2010). In the present model specification, based on the previous empirical evidence of the separation of roles of individual fish within networks into initiators and receivers of aggression, we selected the following effect parameters for the evaluation function:

- a) *Density effect* which tests the tendency of the network and its individuals to be highly dense (more connections) or not, and it is described as,

$s_{i1}^{net}(x) = \sum_j x_{ij}$, where x_{ij} indicates the presence or absence of an interaction between individual i to individual j ;

- b) *Reciprocity effect* which tests the tendency of reciprocated interactions among individuals. In this study the reciprocity effect evaluates the tendency of retaliation or counterattacks of the receiver of the aggression towards their aggressor (initiator)

This effect is defined as: $s_{i2}^{net}(x) = \sum_j x_{ij}x_{ji}$, where x_{ij} indicates the presence or absence of an interaction between individual i to individual j , and x_{ji} indicates the presence or absence of an interaction between individual j to individual i

- c) *In-degree related popularity effect* which reflects the tendency of individuals with high in-degree centralities to attract more incoming interactions because they have in-degree centralities. In the present study it evaluates if receivers of interactions receive more aggression because they are receivers. It is mathematically represented as the sum of the in-degree centralities of the others individuals to whom is interacting (x_{+j}), and defined as:

$$s_{i3}^{net}(x) = \sum_j x_{ij} x_{+j}, \text{ and};$$

d) *Out-degree activity effect* reflects the tendency for individuals with high out-degree centralities to generate more outgoing interactions because of their high out-degree centralities. In the present study, this effect evaluates the tendency of initiators to keep being aggressive and it is represented as the squared out-degree centrality of the individual defined as:

$$s_{i4}^{net}(x) = x_{i+}^2$$

Thus, the evaluation function of the model is represented as,

$$f^{net}(x) = s_{i1}^{net}(x) + s_{i2}^{net}(x) + s_{i3}^{net}(x) + s_{i4}^{net}(x), \text{ or}$$

$$f^{net}(x) = \text{density} + \text{reciprocity} + \text{indegree popularity} + \text{outdegree activity}$$

6.2.4. Model estimation.

The model was evaluated with the above specifications and estimated using a stochastic approximation algorithm based on repeated simulations of the evolution of the network. The estimation process was based on the Method of Moments (Snijders, 2001, Snijders et al., 2007) which compares the observed networks with hypothetical networks generated during the simulation allowing for non-constant rate parameters with 3000 iterations or simulations.

Convergence of the model is a measure of how good the model fits the observed data and it was tested using the t-ratios for convergence obtained during the estimation process. The t-ratio for convergence considers the deviation between the simulated values with the observed values. Therefore, if the deviations are small, convergence of the model is good. Convergence is excellent when the t-ratios are less than 0.1, reasonable between 0.1 and 0.2 and moderate between 0.2 and 0.3 (Snijders et al., 2007).

The t-statistic, defined as the estimated value of the parameter divided by its standard error, was utilised to statistically quantify the effect parameters on the network. As such, absolute t-statistic values of 2 represent statistical differences at the 0.05 significant level.

6.3. Results.

The t-ratios for convergence of the model were ≤ 0.10 , as it can be seen in Tables 6.2, 6.3 and 6.4, for the Stocknet, Shortnet and Predent experiments, respectively. The results indicate an excellent fit of the model with the empirical data and that the results from the estimation are significantly representative of the social dynamics within each network.

In the Stocknet experiment, the effect of high stocking density treatment was significant for the dynamics of network density, in-degree popularity and out-degree

activity in all groups. In contrast, low stocking density (Control groups) had a significant effect on the dynamics of reciprocity and in-degree popularity only, as shown in Table 6.5.

Similar results were obtained in the Shortnet experiment, where the treatment had significant effect for the dynamics of network density, in-degree popularity and out-degree activity while two out of four control networks were also significant for density and out-degree activity, as shown in Table 6.6.

The results for the evolution of the Prednet model are shown in Table 6.7. Predictable food delivery (Control groups) had a significant effect only in the dynamics of the in-degree popularity and one group showing a significant in network density. In contrast, unpredictable food delivery (Treatment groups) had a significant effect on the dynamics of network density, reciprocity, in-degree popularity and out-degree activity.

The statistical significance (P values) of the evaluated function parameters are shown in Table 6.8 demonstrating the highly significant values for out-degree activity and density ($P < 0.01$).

The evolution of the networks in the Stocknet, Shortnet and Prednet can be observed in Figure 6.1 showing the persistence of the effects over time even after restoration to control conditions (post-treatment period) .

6.4. Tables and figure.

Table 6.1. Experimental conditions of each study, group (4 tanks with 10 fish each) and experimental phases utilised for dynamic network analysis.

Study	Stocknet		Prednet		Shortnet	
	Control	Treatment	Control	Treatment	Control	Treatment
Pre-treatment	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .
Treatment	FF at 10:00 fish held at 8 kg/m ³ (Low stocking density)	FF at 10:00 fish held at 30 kg/m ³ (High stocking density)	FF at a predictable time (10:00) ; fish held at 8 kg/m ³ .	FF at unpredictable time between 8:30 to 16:30 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	Food restricted (1/3 of FF), delivered at 10:00; fish held at 8 kg/m ³ .
Post-treatment	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .	FF at 10:00 fish held at 8 kg/m ³ .

FF = Full food ration.

Table 6.2. T-ratios for convergence between the actual observed amount of aggression and amount of aggression estimated by the model for groups in the Stocknet experiment.

Effect parameter	Control groups (Low stocking density)				Treatment group (High stocking density)			
	1	2	3	4	1	2	3	4
Density	0.090	0.042	0.008	0.090	0.017	0.105	0.104	0.100
Reciprocity	0.019	0.087	0.079	0.010	0.035	0.001	0.026	0.018
Indegree popularity	0.031	0.073	0.107	0.083	0.031	0.024	0.048	0.028
Outdegree activity	0.011	0.045	0.098	0.031	0.044	0.032	0.026	0.053

Values ≤ 0.1 are considered to represent an excellent convergence of the model.

Table 6.3. T-ratios for convergence between the actual observed amount of aggression and amount of aggression estimated by the model for groups in the Shornet experiment.

Effect parameter	Control groups (Ad libitum)				Treatment group (Feed restricted)			
	1	2	3	4	1	2	3	4
Density	0.083	0.020	0.048	0.084	0.101	0.026	0.073	0.062
Reciprocity	0.037	0.040	0.047	0.047	0.018	0.070	0.099	0.005
Indegree popularity	0.004	0.045	0.044	0.012	0.019	0.025	0.046	0.034
Outdegree activity	0.107	0.028	0.024	0.099	0.054	0.013	0.080	0.001

Values ≤ 0.1 are considered to represent an excellent convergence of the model.

Table 6.4. T-ratios for convergence between the actual observed amount of aggression and amount of aggression estimated by the model for groups in the Prednet experiment.

Effect parameter	Control groups (Predictable)				Treatment group (Unpredictable)			
	1	2	3	4	1	2	3	4
Density	0.016	0.055	0.023	0.036	0.017	0.030	0.079	0.055
Reciprocity	0.040	0.021	0.041	0.021	0.051	0.011	0.005	0.101
Indegree popularity	0.012	0.096	0.086	0.103	0.064	0.103	0.023	0.105
Outdegree activity	0.087	0.039	0.044	0.007	0.072	0.107	0.010	0.075

Values ≤ 0.1 are considered to represent an excellent convergence of the model.

Table 6.5. Evaluation of effects parameters (mean \pm standard error) according to experimental groups in Stocknet experiment.

Effect parameter	Control groups (Low stocking density)				Treatment group (High stocking density)			
	1	2	3	4	1	2	3	4
Density	-1.14 (0.37)	-1.01 (0.62)	-2.27 (1.74)	-5.73 (3.01)	-2.28 (0.43)*	-2.52 (0.44)*	-3.06 (0.62)*	-7.68 (1.55)*
Reciprocity	1.28 (0.47)*	0.79 (0.36)*	1.07 (0.40)*	2.35 (1.13)*	0.35 (0.99)	0.24 (0.41)	0.68 (2.17)	0.98 (2.60)
Indegree popularity	-0.04 (0.14)	-0.20 (0.14)	0.01 (0.17)	0.40 (0.41)	0.17 (0.10)*	0.29 (0.10)*	0.19 (0.02)*	0.65 (0.27)*
Outdegree activity	0.13 (0.05)*	0.18 (0.03)*	0.27 (0.06)*	0.37 (0.10)*	0.14 (0.03)*	0.2 (0.05)*	0.37 (0.11)*	0.86 (0.31)*

* = significance at $P < 0.05$ level

Table 6.6. Evaluation of effects parameters (mean±standard error) according to experimental groups in Shortnet experiment.

Effect parameter	Control groups (Non-food restriction)				Treatment group (Food restriction)			
	1	2	3	4	1	2	3	4
Density	-0.94±0.82	-1.45±0.65*	-1.18±1.04	-1.61±0.56*	-1.57±0.39*	-2.45±0.83*	-1.42±0.59*	-2.64±0.73*
Reciprocity	0.44±0.03*	1.21±0.45*	0.38±0.03*	0.80±0.20*	1.20±0.82	2.96±1.73	1.04±0.52	0.80±0.42
Indegree popularity	-0.13±0.14*	-0.21±0.12*	-0.12±0.2	-0.06±0.09	-0.09±0.04*	-0.46±0.15*	-0.23±0.06*	-0.74±0.12*
Outdegree activity	0.16±0.02*	0.21±0.02*	0.20±0.03*	0.17±0.02*	0.13±0.02*	0.31±0.04*	0.19±0.02*	0.27±0.07*

* = significance at P < 0.05 level

Table 6.7. Evaluation of effects parameters (mean±standard error) according to experimental groups in Prednet experiment.

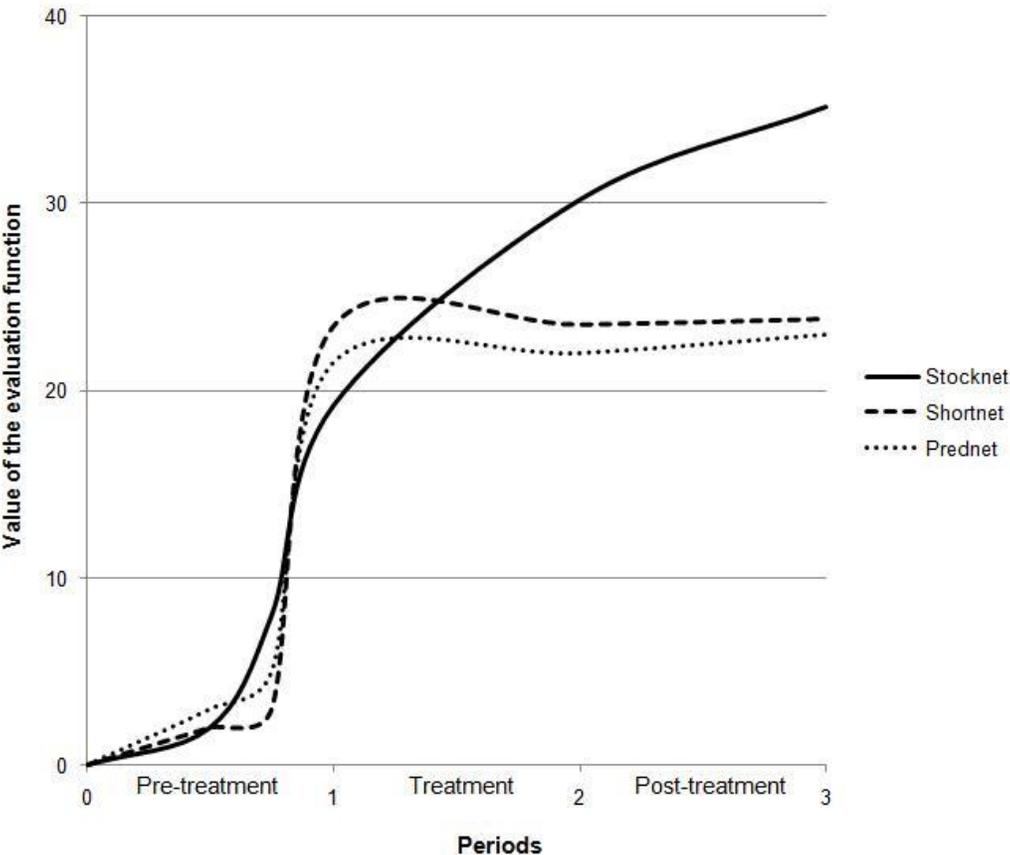
Effect parameter	Control groups (Predictable delivery)				Treatment group (Unpredictable delivery)			
	1	2	3	4	1	2	3	4
Density	-1.22±0.60*	-0.68±1.07	-1.54±0.9	-1.57±0.62	-1.49±0.30*	-1.91±0.97	-1.42±0.65*	-2.76±0.53*
Reciprocity	0.40±0.30	0.43±1.48	0.41±0.32	0.35±1.06	1.19±0.34*	2.89±0.79*	1.17±0.40*	0.79±0.43*
Indegree popularity	-0.06±0.09	-0.40±0.22	-0.06±0.17	-0.12±0.11	-0.15±0.07*	-0.57±0.21*	-0.26±0.10*	-0.36±0.15*
Outdegree activity	0.16±0.02*	0.22±0.02*	0.20±0.03*	0.19±0.02*	0.12±0.02*	0.29±0.05*	0.20±0.02*	0.28±0.06*

* = significance at P < 0.05 level

Table 6.8. Significance level, P , of the effect parameters in treatments groups in treatment groups in Stocknet, Shornet and Prednet experiments.

Effect parameter	Stocknet	Shortnet	Prednet
Density	0.0000001	0.0011938	0.0003585
Reciprocity	0.6873571	0.08201282	0.0029410
Indegree popularity	0.0000385	0.00013478	0.0138809
Outdegree activity	0.0002236	<0.0000001	<0.0000001

Figure 6.1. Graphical representation of the evaluation functions in Stocknet, Shortnet and Prednet experiment.



6.5. Discussion.

Convergence was excellent in all dynamic network analysis estimations for each experiment. Thus, the model showed a good representation of the dynamic of the networks in the study which are in agreement with other studies showing the use of actor-based models for the evaluation of the evolution of behaviours in humans (Mercken et al., 2010, Steglich et al., 2010). However, this the first study to use actor-based models to represent the evolution of behaviours in non-human animals.

In the Stocknet experiment, fish held at high stocking densities significantly maintained a higher out-degree activity, in-degree popularity but no reciprocated interactions and the network was denser. These results support the previous evidence that high stocking density change the behaviours of some fish disaggregating the networks into initiators and receivers of aggression (Chapter 3, Cañon Jones et al., 2011). Moreover, these results indicate that initiators (defined as individuals with high out-degree centrality and low in-degree centrality), maintained their aggressive behaviours even when the conditions were restored to low stocking density (Figure 6.1). Likewise, receivers did not prefer to change their behaviour as receivers of aggression when low density was restored and kept their roles in the network. The latter is further supported by the evidence that there was a preference of non-reciprocating the aggression. Fish in the control groups, which were not exposed to high stocking densities, had high out-degree activity but their interactions were not reciprocated and there was no significant in-degree popularity tendency. These results showed that all

fish tended to initiate aggressive interactions at some point during the experiment with a tendency to keep generating aggression themselves. However, the results showed that fish receiving the aggression did not receive more aggression during the experiment (in-degree popularity) and that the aggression was reciprocal and therefore, counterattacked. Overall, the results suggest that holding fish at high stocking densities, even for as little as ten days, had a significant impact on the behaviour of fish which continued to even after low stocking density was imposed. High stocking density triggered a change in the behaviour of fish, separating their roles in the network into initiators and receivers of aggression and these behavioural changes were not recovered resulting in fish maintaining their roles within the network even after restoration of normal conditions. The evidence in the present study is in accordance to the definition of personalities in fish and studies in rainbow trout where persistence of individual differences in aggressive behaviour existed for at least one week have been reported (Øverli et al., 2004). Importantly, these results suggest that initiators of aggression will continue to initiate more aggression leading to fin damage to receivers and consequently poorer welfare which is especially important in aquaculture whenever high stocking densities are maintained such as at the end of the growing phase or during transport.

Detailed analysis of the Shortnet experiment indicated similar results as those found in the Stocknet. Food restriction, as short as 10 days, triggered a significant change in the network and behaviour of fish separating fish into initiators, who maintained their high aggressive behaviour during the whole duration of experiment even after restoring to full daily ration, and receivers who continued to receive

aggression without retaliation (Figure 6.1). Once again, the persistence of roles of individuals in aggressive behaviour, independently of the context, indicates that fish have different personalities. Previous experiments in Atlantic salmon (Huntingford et al., 1993) and rainbow trout (Jobling and Koskela, 1996) had shown that food restriction triggers the formation of dominance hierarchies but this is the first experiment demonstrating the effect of food restriction on behaviour classification using dynamic social network analysis. As a consequence, there is a significant impact beyond the food restriction period on the networks and behaviour of fish, where some fish will continue to be highly aggressive and producing fin damage and other receiving the aggression and with more fin damage and poorer welfare. It is likely that repeated periods of short food restriction, such as those used in commercial aquaculture prior to vaccinations, transport or grading, could have a similar and cumulative effect on fish social behaviour. However, further research is needed to confirm these findings under commercial conditions.

The results from the evolution of the Prednet study showed that unpredictable food delivery triggered a persistence of highly aggressive behaviours in initiators while receivers kept receiving aggression after restoration of a predictable food delivery schedule (Figure 6.1). It is important to point out that there was a tendency for aggression to be reciprocated by all fish, including the receivers under predictable food delivery conditions. These results have not been reported before and a tendency of reciprocation of aggression during unpredictable food even after restoring predictable food delivery might be explained by the fact that fish were alert to delivery of food all the time after unpredictable food delivery periods. Once again, these results may be

important in commercial aquaculture situation whenever unpredictable food delivery occur, such as the malfunction of food delivery equipments. However, the results indicate that unpredictable food delivery had a significant persistent effect both on those fish initiating aggression inducing them to maintain their roles as initiators of aggression as well as an effect on fish receiving aggression inducing them to continue to receive aggression leading ultimately to more fin damage and poorer fish welfare under these conditions.

6.6. Conclusion.

The present study demonstrated the utility of stochastic actor-based models for dynamic social network analysis of behavioural interactions in fish under various environmental conditions. It was possible to describe the dynamics of the networks of fish under high stocking density, short food restriction and unpredictable food delivery. It was demonstrated using dynamic network analysis that fish separate into roles of initiators and receivers of aggression and that these roles were maintained after restoration of control conditions suggesting that the effect of treatments continued beyond the actual treatment period. Furthermore, the results indicated that individuals differ in a persistence and constant way indicating the existence of different personalities in fish.

These findings have practical significance as initiators are fish that are responsible for causing fin damage to other individuals in their network (Chapters 2 and

3, Cañon Jones et al., 2010, Cañon Jones et al., 2011), are unlikely to stop their aggressive behaviour even after the triggering factor (high stocking density, food restriction or unpredictable food delivery) is removed. Conversely, fin damage is mainly found in receivers of aggression, which are fish that do not retaliate and keep on receiving aggression from initiators, leading to sustained poor welfare in these fish. Future research should therefore investigate other triggering factors such as intermediate stocking densities, short periods of increased stocking densities as well as longer observational periods in order to increase further elucidate the importance of social factors leading to fin damage in farmed Atlantic salmon which can ultimately be used to improve the welfare of farmed fish.

7. General discussion and conclusions.

The results of the experiment in Chapters 2, 3, 4, 5 and 6 provided scientific evidence that food restriction periods (long and short), high stocking densities and unpredictable food delivery have an effect on the behaviour of Atlantic salmon leading to fin damage analysed and quantified using social network analysis. Additionally, new knowledge was obtained on the quantification and identification of socially important aggressive individuals within the network causing the fin damage as well as receivers of that aggression. The latter findings were only possible to be obtained using social network analysis and represent the first evidence of this occurring in farmed fish. More importantly, new understanding on the profound effects of episodic and short events of feed restriction, high stocking densities and unpredictable food delivery on behavioural interactions of fish which triggered a persistent aggressive behaviour even when conditions are restored to normal was found. These episodic events are common in the aquaculture industry and to the author's knowledge this is the first evidence of this occurring in Atlantic salmon. Thus, this new knowledge may be of the potential use for fish farmers as these possible unrecorded and possible repeated events may have long term consequences on the welfare of fish mediated through aggressive behaviour leading to fin damage.

More specifically, dorsal fin damage was consistently a good welfare indicator and was found to be significantly higher in groups of fish subjected to food restriction periods (short and long), held at high stocking density or with an unpredictable food

delivery regime. The severity of fin damage was significantly higher when this role differentiation occurred, and it was highly correlated with fin biting events. These results indicate that fin damage evaluation using visual and direct measurements is a good operational welfare indicator in field conditions.

Additionally, the results suggest that aggressive behaviour can be reliably used in social network analysis as a good indicator of the development of fin damage in Atlantic salmon. Social networks based on aggressive interactions showed higher centrality, clustering coefficients, in-degree centrality, out-degree centrality and lower dense networks in groups subjected to food restriction (short and long), high stocking densities, and unpredictable food delivery. The high centralities and clustering coefficients found in these groups indicated separation of fish within the groups into initiators of aggression and receivers of aggression. This separation of roles was seen only in the food restricted group, high density groups and unpredictable food delivery groups. Initiators had higher out-degree centrality while receivers showed high in-degree centrality.

It is important to point out that in order to transform the new knowledge generated by this research into practical and useful strategies to control fin damage at a farm level, additional processes need to be completed, mainly referring to the scale of the experiment. Futures studies should be conducted to a more commercial level and explore if the results are replicable in this new scenario.

One acknowledged issue in the preceding Chapters was the number of fish utilized in each study. The number of fish in the first experiment was 6 and the

subsequent experiment 10 fish were used. The decision of choosing this number of fish was based on several factors including that previous studies under laboratory conditions have used similar group sizes, it enables the correct detection and identification of every single fish within each tank and also on the basis of the evidence that fish are capable to remember around 10 to 15 fish (Griffiths, 2003). Nevertheless, the main disadvantage of choosing a small group size is that extrapolation of the result to a more commercial aquaculture setting where hundreds or thousands of fish co-exist should be made with great care. Although replicates (4 replicates for each experimental group) were considered in the experimental design, each experiment as whole unit was only conducted once. It is likely that similar results may be obtained if more repetitions of the whole experiments were to be conducted due to the advantages of using social networks analysis. Social network theory indicates that networks should behave similar whenever a critical group size is reached (Newman, 2003, Newman and Park, 2003), in this case is 10 to 15 fish. One can imagine a sea-cage with thousand of fish inside consisting of several subnetworks joined together to form a big network and behaving similarly (Newman, 2003) and future studies using similar experimental framework should be conducted to confirm this hypothesis. However, one known limitation of using social networks analysis is that the results are dependent on the behaviour that is measured.

The retrieval of information from the video recordings was carried out by direct observation. The use of direct human observation imposes long periods of observation but it enables to identify clearly every single fish within the tank in the tank even during period of obstruction with other fish. At the moment there is no automated artificial

technology (computer software) capable to track fish in this manner. Potential new technologies probably will involve the use of ultrasound system to track aquatic animal.

Another issue that should be addressed in futures studies is the effect of tanks and tags used. It was acknowledged from the beginning that the use of tags inserted through the skin of the fish and the tanks design imposed a non-natural environment for the fish. It was not possible to carry additional experiments consider these issues. Nevertheless, the experimental design chosen did consider that all experimental fish were subjected to the same conditions. However, future studies should be conducted to understand the effect of tagging and tank design alone.

The ultimate cause of fish becoming an initiator or receiver of aggression is still an unsolved question. Future studies may explore the molecular genetics basis of this occurring in fish, given the fact that the whole Atlantic salmon genome will be fully codified by 2012 (Davidson et al., 2010) and that recent studies have identified the expression of certain genes related to copying styles in mammals and rainbow trout (Graff and Mansuy, 2008, MacKenzie et al., 2009). These studies could reinforce Darwinian theories suggesting that behavior, and behavioural changes, evolved under the same evolutionary principles and pressures as other physical characteristics in animals (Darwin, 1859, Darwin, 1872) which increases the fitness and reproductive success of the species, in this case Atlantic salmon. Additionally, specific genes related with behavior could be identified and potentially incorporated into aquaculture genetic selection programs or as a animal model for human behavioural studies.

Using dynamic network analysis, it was possible to identify persistent aggressive and submissive behaviours in initiators and receivers, respectively, which was suggested to be consistent with the existence of personalities defined for human and other non-human animals. Futures studies should investigate whether these persistent changes in behaviour are maintained for longer time than those used here and if these results are also found in a more commercial setting. However, the discovery that fish may be able to possess similar behavioural tendencies as in other vertebrates including humans (Sih and Bell, 2008), opens new research areas in comparative psychology where fish can be used as animal models.

As mentioned at the beginning of this Chapter, the results are potentially applicable within the commercial aquaculture industry as a valuable technique to evaluate and improve the welfare of farmed fish. It important to point out that in order to transform the new knowledge generated by this research into practical and useful strategies to control fin damage at a farm level, additional processes need to be completed, mainly referring to the scale of the experiment. Again, futures studies should be conducted to a more commercial level and explore if the results are replicable in this new scenario.

The results of the thesis provided new understanding for the long term effect on the behaviour of fish of short term husbandry practices commonly used in commercial setting. These findings also open the possibility for future studies using of social network analysis for explaining disease transmission in farmed fish. Studies using social network analysis may identify specific individuals in a group who have more socially active (higher out-degree centrality) with an important role in mediating disease

transmission. Likewise, it may be possible to identify those fish that may be most at risk of becoming infected (higher in-degree centrality) which in turn may be the one transmitting the disease further in the network. Additionally, it may be possible to evidence if the disease mediates persistent changes in behaviour that could lead to a higher disease transmission.

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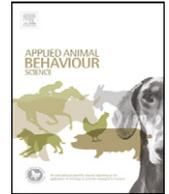
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Appendix

Published manuscripts



Social network analysis of behavioural interactions influencing fin damage development in Atlantic salmon (*Salmo salar*) during feed-restriction

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ABSTRACT

The role of behavioural interactions in the development of fin damage amongst Atlantic salmon (*Salmo salar*) undergoing feed-restriction was investigated using social network analysis. Dorsal fin damage, particularly erosion, was seen only in groups subjected to feed-restriction. The amount of dorsal fin damage was positively correlated with aggression and fin-biting. Feed-restricted fish exhibited significantly lower weight gain, reduced growth-rate and body condition but no differences in total length. Social networks based on aggressive interactions in feed-restriction groups showed significantly lower distance, and higher density, higher clustering coefficient and higher in and out degree centrality. These findings indicated higher and more intense aggressive interactions in feed-restricted fish. A distinctive separation of roles according to aggression was found in feed-restriction groups where initiators had high out-degree centrality and receivers had high in-degree centrality. Fish initiating aggressive interactions had less fin damage, gained more weight and attained more central positions within the school. Fish receiving aggression had more fin damage and gained less weight. Association networks in the feed-restricted groups had significantly lower values for transitivity and distance with a tendency for higher centrality. These findings indicate higher levels of interaction and an imbalance in their relationships. We demonstrated the value of social network analysis in investigating behavioural interactions associated with aggression and the development of fin damage in Atlantic salmon.

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1. Introduction

Fin damage is increasingly being used as a potential indicator of the welfare of farmed fish (Ashley, 2007; Broom, 2007; Broom and Fraser, 2007). It has been asso-

ciated with inter-fish competition as a result of inadequate housing and husbandry conditions, both of which are known to influence fish welfare. Fin damage can be caused by increased aggression in both steelhead trout (*Salmo gairdneri*) and Atlantic salmon (*Salmo salar*) (Abbott and Dill, 1985; Turnbull et al., 1998) and the tissue damage can be maintained and aggravated by other risk factors such as poor water quality resulting mainly from high ammonia and low dissolved oxygen levels (Person-Le Ruyet et al., 2008). Although the effect has not been fully elucidated, fish stocking densities have been also implicated

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in the occurrence of fin damage in Atlantic salmon. For example, higher fin damage has been described at high fish stocking densities (Turnbull et al., 2005; Adams et al., 2007; Brockmark et al., 2007) as well as in low fish stocking densities (Adams et al., 2007; Turnbull and Kadri, 2007) in Atlantic salmon in hatcheries and farm conditions. Aggression amongst fish can be exacerbated by periods of feed-restriction (Damsgård et al., 1997; Hatlen et al., 2006), or inappropriate feeding regimes such as under-feeding (McCarthy et al., 1999; Noble et al., 2007a,b).

Other potential risk factors for fin damage associated with aquaculture systems include abrasion from tank surfaces and raceways (St. Hilaire et al., 2006; Branson, 2008) and poor feed management (Noble et al., 2007a,b; Rasmussen et al., 2007). Fin damage has also been suggested to be a predisposing factor for the colonisation of pathogens in the damaged tissue and consequently the development of economically important infectious diseases such as furunculosis (Turnbull et al., 1996). In addition, a poor welfare status related to aggressive social interactions in farmed fish has been associated with reduced immuno-competence linked with an increase in bacterial disease susceptibility (Peters et al., 1988). Fin damage may also be associated with pain, as fish possess nociceptors needed for the perception of painful stimuli in their fins (Becerra et al., 1983). The relative importance of all these factors and interactions has not been fully elucidated and has often been confounded in previous studies (Adams et al., 2007).

Many fish species are social and form aggregations known as schools or shoals (Ruzzante, 1994; Viscido et al., 2007). In this context, fin damage has been investigated in terms of dyadic interactions related to aspects of group-living such as competition and aggression over food resources, space and territory (Huntingford and Turner, 1987; Krause and Ruxton, 2002; Huntingford and Adams, 2005). Intra-specific competition, associated with access to food has been described in fish (Ward et al., 2006) both in the wild (Dunbrack et al., 1996) and under farmed conditions (Noble et al., 2007a,b). It can also lead to the formation of dominance hierarchies and territories (Metcalf et al., 2003).

It has been suggested from theoretical work using simulation models that the more aggressive fish become dominant, occupy more central positions within the school, benefit from increased protection from predators and have reduced energetic costs of locomotion and consequently show an increased growth rate (Parrish, 1989; Parrish et al., 2002). In addition, subordinate fish have been suggested to occupy peripheral positions during schooling and are required to be more vigilant due to increased exposure to potential predators and consequently show a reduced growth rate compared to aggressive fish in simulation models (Morrell and Romey, 2008). Field studies of dominance hierarchies in fish suggest that the advantages of being dominant are less evident and that laboratory conditions measuring dominance using dyad interactions may bias these effects (Sloman and Armstrong, 2002). These findings have exposed the lack of understanding of the effects of dominance in fish under laboratory and commercial conditions with more complex interactions to

be expected than those seen using only dyadic encounters. Therefore, empirical quantification of the behavioural interactions, structure and dynamics involved in the development and occurrence of aggressive behaviour among farmed fish and its relationship with schooling behaviour is needed. Social network analysis may provide useful information about the behavioural interactions underlying the occurrence of fin damage and poor welfare in farmed fish.

Social network theory and analysis has been introduced and applied to fish behaviour recently and mainly only within ecological contexts (Croft et al., 2004, 2005). It has been recently used in health studies in both humans (Bell et al., 1999) and animals (Bohm et al., 2008; Drewe et al., 2009). Social network analysis addresses complex biological questions as it describes the direct and indirect relationships occurring amongst individuals within a group (Wasserman and Faust, 1994) and quantifies the social ties and influences among connected individuals. The information obtained from social network analysis gives a better understanding of interactions and can be used to identify the roles of key individuals within a group (Lusseau and Newman, 2004) with higher numbers of contacts and interactions and thus the power to influence social relationships within and between groups (Wasserman and Faust, 1994). The use of novel tools such as social network analysis to quantify relationships between aggression and fin damage in farmed fish will help to identify the precise social role of key individual fish and their social position within groups associated with the development of fin damage. This methodology can also be used to quantify the social effects of the feed-restriction commonly carried out in commercial aquaculture during sampling periods, grading and the transport of farmed fish (Lucas and Southgate, 2003).

It has been shown that feed restriction in farmed fish leads to the formation of hierarchies and aggressive competition for food under both laboratory and commercial situations and has been associated with heterogeneous and uneven growth within the fish population (Jobling and Koskela, 1996; Damsgård et al., 1997; Hatlen et al., 2006; Noble et al., 2008).

The aim of the present study was to quantify the impact of feed-restriction on the development of fin damage in juvenile Atlantic salmon and to relate this to changes in social dynamics, structure and organisation within groups of fish in order to quantify the underlying role of behavioural interactions in the development of fin damage.

2. Methods

2.1. Animals and experimental groups

The experiment was conducted at the Aquaculture Research Station in Tromsø, Northern Norway (Project Number H08/26) in accordance with current Norwegian Fish Welfare legislation: 'Regulations on experiments with animals' produced by the Norwegian Ministry of Agriculture and Food (Ministry of Agriculture, 1996) and the 'Animal Welfare Act' from the Norwegian Ministry of Agriculture and Food (Ministry of Agriculture and Food, 2009). These regulations adhere to the European Convention for

the Protection of Vertebrates used for Experimentation and other Scientific Purposes (Commission of the European Union, 1999). Guidelines for the treatment of animals in applied animal behavioural research were also adhered to (Sherwin et al., 2003).

Eight experimental groups were used each consisting of six clinically healthy year 1+ Atlantic salmon weighing 137.5 ± 20.1 g (mean \pm SD). The fish were sourced commercially from Haukvik Kraft-Smolt A/S, Tribe Batnfjord, generation 2007. There were two experimental periods: (1) acclimatisation period (from day 0 to day 13) and (2) treatment period (from day 14 to day 44). During the 14 day acclimatisation period a maintenance level of commercial pelleted feed ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway, containing 21–22.8% fat, 50–52% protein and $22.2\text{--}22.8$ MJ kg⁻¹) was delivered to all groups automatically from overhead feeders once a day at 10:00 h for 30 min at a rate of 1.5% of fish body weight day⁻¹. The amount of food delivered was adjusted weekly according to the expected weight gain and water temperature following manufacturer feeding tables. After the 14 days of acclimatisation, four groups were randomly selected as Control groups (C) and four groups as feed-restriction (FR) groups. In order to minimise the effect of management disturbances, two C groups and two FR groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the entrance. During the treatment period, C groups were given the same feeding regime as used during the acclimatisation period (1.5% body weight day⁻¹), whereas the FR groups were given a restricted ration representing expected food consumptions of 0.3% body weight day⁻¹. The feeding regime was maintained for 30 days. This level of food restriction represented the level of food provided during routine practices used in commercial aquaculture such as food deprivation before slaughter and food withdrawal periods used following medication (Einen et al., 1998; Houlihan et al., 2001). This level of food restriction also reflects periods of natural food deprivation in wild fish determined by seasonal food availability (Weatherley and Gill, 1987). All fish were killed humanely at the end of the experiment using an overdose bath of benzocaine chlorhydrate (>250 mg l⁻¹ freshwater).

We anticipated an early termination of the experiment if either, cases of severe aggressive interactions occurred, biting interactions produced observable bleeding at the biting point or if fish were observed not to eat any feed pellets for 3 consecutive days. These thresholds were designed to minimise adverse welfare effects based on current recommendations that 72 h (3 days) are required for the complete emptying of the fishes' gut before slaughter (Humane Slaughter Association, 2005; RSPCA, 2007). This is further supported by evidence from studies quantifying the effects of food restriction in fish (Einen et al., 1999).

2.2. Containment and individual identification

Fish were kept for 420 days prior to the experiment in a 1 m³ holding tank according to the Aquaculture Research Station Standard Procedure. Table 1 shows the corresponding light regime, water quality and feeding procedures that

were applied during this period. On the first day of the experiment (day 0) fish were individually identified under anaesthesia induced by submersion in a bath of benzocaine chlorhydrate (100 mg l⁻¹ freshwater). All fish were observed to enter into deep surgical anaesthesia within 3 min of being placed inside the anaesthetic bath and tagging was carried out within a period of less than 1 min after the onset of full anaesthesia for each fish. After tagging, fish were transferred to designated 300 l plastic experimental tanks with initial stocking densities of 4.1 ± 0.1 kg (m³)⁻¹ (mean \pm SD) and observed for 30 min after fully recovering from anaesthesia. Good recovery from anaesthesia was achieved in all fish as assessed by the progression of behaviours from onset of opercular movement then accompanied by gross body movements and finally re-establishment of full equilibrium with the resumption of pre-anaesthetic appearance as described in previous studies (Ferreira et al., 1979; Yesaki, 1988; Gilderhus, 1989, 1990; Gilderhus et al., 1991). An emergency recovery tank with highly oxygenated freshwater (>99% dissolved oxygen injected through diffusers connected to oxygen gas tanks) was available at all times during procedures in case fish needed assisted recovery or veterinary assistance.

Tags were designed using markings (2.5 cm \times 2.5 cm) made of plastic printing paper (Xerox® Special Advanced Media Digital Colour, Premium Never Tear 95 μ m Polyester paper, PN: 003R98056) using unique combinations of black/white geometric designs and attached behind the dorsal fin of each fish using strong silk thread and standard commercial Floy Tags (Hallprint®, Polyepaltichylene streamer tags, series PST).

2.3. Water quality and environmental conditions

Filtered freshwater was provided throughout the experiment. Dissolved oxygen content ($98 \pm 2\%$) and water temperature (10 ± 2 °C) were maintained, measured and recorded daily. Water flow was controlled at an exchange rate of 5 l min⁻¹ and velocities of 0.75 fish body lengths s⁻¹. These conditions have been linked to increased aggressive interactions in Atlantic salmon (Jørgensen and Jobling, 1993) and Arctic charr (*Salvelinus alpinus*) (Adams et al., 1995). A 6:18 light–dark photoperiod regime was used during the study.

2.4. Physical measures

The weight (g) and length (total tail-fork length in mm) of each fish were measured at the beginning (day 0) and end (day 44) of the experimental period. Specific growth rate (SGR) was calculated as: $\frac{w_1 - w_0}{w_0 \times \Delta t}$, where w_1 is the wet weight of fish (g) at sampling time 1, w_0 is the wet weight of fish (g) at sampling time 0, and Δt is the number of days between sampling times. Fulton's condition factor (K) was calculated from tail-fork length and mass of individual fish as: $K = W/L^3 \times s$, where s is the total tail-fork length in mm and $w_{1 \text{ or } 2}$ is the wet weight of fish (g) at sampling time 1 or 2.

Table 1
Light regime, water quality and feeding procedures of fish prior to experiment.

Year	2007						2008		
Month	April–May	June	July	August	September	October–December	January–May	June	July
Light	24:0	24:0	24:0/6:18	6:18	6:18	6:18	6:18	6:18	6:18
Temperature	Nat	Nat	Nat	Nat	Nat/4	Nat/4	Nat/4	Nat/4	Nat
Water	FW/CF	FW/CF	FW/CF	FW/CF	FW/CF	FW/CF	FW/CF	FW/CF	FW/CF
Feeding	Ad lib	Ad lib	Ad lib	Ad lib	Ad lib	Ad lib	Ad lib	Ad lib	Ad lib

Light: 24:0 = continuous light; 6:18 = 6 h light and 18 h of darkness.

Temperature: Nat, ambient water temperature ranging from 8 to 10 °C; Nat/4: ambient water temperature supplemented with heated water to maintain at least 4 °C, if necessary.

Feeding: Ad lib, *ad libitum* feeding using type of food according to fish life stage. Source of food = Skretting.

Water: FW/CF, fresh water with continuous flow of water.

2.5. Quantification of fin damage

Digital photographs were taken at the beginning and end of the experimental period and fin damage was evaluated in every fish using the Relative Fin Index (RFI) as described by Bosakowski and Wagner (1994). RFI has been suggested to allow reliable and objective measurement of the degree of fin damage (Person-Le Ruyet et al., 2007) and was obtained by dividing the maximum total fin length (longest fin-ray length from body) by the fork length in each individual fish. All pectoral, ventral, anal, caudal (upper and lower) and dorsal fins were measured and quantified using this index. In addition, the total number of fin splits (separation of fin rays greater than 3 mm) was recorded in each fish.

Fin erosion was measured using a modified method utilising an ordinal scale of 0, 1, 2 and 3, corresponding to no erosion (0% of fin eroded), mild erosion (1–24% of fin eroded), moderate (25–49% of fin eroded) and severe erosion (>50% of fin eroded), respectively (after Hoyle et al., 2007).

Additionally, fish were visually examined for other external lesions on their bodies as evidence of the occurrence of biting during sampling periods and at the end of the experiment.

2.6. Behavioural observations and social interactions

Ten minute video recordings of the fish in each tank were carried out at 09:00–09:10, 10:00–10:10 and 11:30–11:40 h on each day of the experimental period. This allowed recordings 1 h before feeding, during the first 10 min of feeding and 1 h after last food delivery from the automatic feeders. Video recordings were made using CCTV colour cameras (Panasonic® VWR42 with Panasonic® WV-LA4R5C3B lenses) located 1 m above each tank. Each tank had a perforated water inlet pipe submerged to the water level and a double central perforated standpipe to prevent rippling in the water surface. Recordings were made using a DVD/HDD recorder (Pioneer® DVR-550H-S).

2.7. Association interaction measurements

Association interaction matrices were constructed using data collected from the video recordings at 1 min intervals. One fish was recorded as being associated with another fish when they were within two fish body lengths

or widths when parallel or perpendicular to their body axis, respectively.

2.8. Aggressive interactions measurements

Attacks, displacement and fin-biting were quantified using all occurrences recording (Lehner, 1996) from video recordings to obtain the total number of events for each fish. Attacks were defined as a rapid swimming movement(s) of fish X directed towards fish Y with fish Y swimming away rapidly (to more than one fish body length distant) but with no physical contact occurring between the two fish during the attack. Displacements were defined as a slow swimming movement of fish X directed towards fish Y with fish Y swimming away from fish X (to more than one fish body length distant) but with no physical contact between fish during the displacement. Biting was defined as a direct physical contact between fish X towards fish Y accompanied by a rapid escape movement response (to more than one fish body length distant) in fish Y in response to the biting. In practice therefore, fish were fully capable of evading aggressor(s) except in the case of biting. Identification of initiator(s) and receiver(s) of aggression was recorded and weighted matrices for social network analysis were constructed for each video sampling period.

We used the information from the aggressive behaviour analysis to calculate and compare data relating both to the total amount of aggressive interactions and the sub-classifications of aggressive behaviours (attack, displacement and fin-biting) between experimental groups. We then used the detailed information from each aggressive interaction (initiator and receiver of every aggressive interaction) in order to calculate social network analysis parameters within each experimental group as detailed in Section 2.9 below. We used this approach to enable comparison of differences in aggressive interactions between groups based on total amount of aggressive interactions and also differences in aggressive behavioural interactions within each group using social network analysis.

2.9. Social network analysis

Social network analysis was carried out with associative and aggressive interaction matrices using UCINET® software (Borgatti et al., 1999). A short description of the network variables quantified are presented as follows and further detailed explanations of the network variables used

in the analysis being available elsewhere (Wasserman and Faust, 1994; Hanneman and Riddle, 2005). *Centrality* measures the quantity of direct connections an individual has with others within the network (Wasserman and Faust, 1994). Centrality has been demonstrated to be one of the best network measures for quantifying transmission of infection in humans (Bell et al., 1999) and social association behaviours in mammals (Manno, 2008). In the case of association interaction matrices, only the overall degree of centrality was measured, as interactions were symmetrical and reciprocal. Because aggressive interactions could be reciprocal or non-reciprocal and usually non-symmetrical, in-degree centrality (amount of aggression received) and out-degree centrality (amount of aggression generated) measures were calculated for each fish. Using this analysis, fish could be classified as initiators or receivers of aggression. Initiators were classified as fish whose out-degree centrality was four times or more greater than their in-degree centrality. Conversely, receivers were classified as individual fish whose in-degree centrality was four times or more greater than out-degree centrality. Otherwise, fish were classified as both initiator and receiver. This four times greater cut-off was selected as it represents more than 50% of all possible individual contacts of any one fish and has been used previously to classify individuals according to their degree of centrality in human studies (Clifton et al., 2009). In order to adequately compare networks, degree centralities were calculated as normalised to the total numbers of individuals in the network and thus expressed as percentages (Hanneman and Riddle, 2005). *Density* quantifies the amount of potential connections between individuals that are actually present. A high density indicates network saturation, meaning that almost all potential interactions are present while low densities indicate sparse networks meaning few potential interactions between individuals are present. *Clustering coefficient* measures the extent to which two neighbours of an individual are themselves neighbours. High clustering coefficients suggest that individuals are surrounded by individuals that are well connected with each other forming subgroups or populations within the network. *Distance* measures the mean number of connections between the members of all possible pairs of individuals within a network. High distance values mean fewer interactions. *Transitivity* quantifies the degree of connection of three connected individuals or triads. Triads are considered important social structures as they represent the minimal connection every individual can have, such as *ego*, *alter* and *other* (Newman, 2003). For aggressive interactions, transitivity quantifies the number of interactions when fish A attacks fish B, fish B attacks fish C and fish A attacks fish C. Low transitivity indicates abnormal social systems that can be described in this experiment as 'the aggressor of my direct aggressor does not behave aggressively to me' and therefore social disturbances may occur due to non stable relationships among individuals. High transitivity has been described as a basis of stable social systems (Wasserman and Faust, 1994). Transitivity was calculated as percentage of transitive triads that could actually complete the triad (Hanneman and Riddle, 2005). We considered it important to calculate this parameter because it would be expected

to give a better understanding of the stability of the social system in the network based on previous evidence suggesting a classical hierarchical process where aggression should be higher in individuals closest in hierarchical rank and lower or non-existent in individuals lower in the hierarchy (Bradbury and Vehrencamp, 1998). These types of simple quantifications of hierarchies have been described previously in salmonids (Huntingford et al., 1990; Bailey et al., 2000), but to the authors' knowledge, no experiment has explored the use of other social measurements such as transitivity.

All network analyses were carried out for the acclimatisation (days 0–14), treatment (days 14–44) and entire experimental periods (days 0–44).

2.10. Schooling/shoaling and spatial position measurements

Fish were classified as schooling or shoaling at 1 min intervals from the video recordings. Schooling was defined as a coordinated grouping behaviour where two or more fish were within association length/width and positioned in the same orientation and direction, as described previously in Section 2.7.

Shoaling was defined as an uncoordinated grouping behaviour where fish were within association length/width, as indicated in Section 2.7, but showed no coordinated orientation and direction (Parrish et al., 2002).

Spatial positioning analysis for each schooling fish was recorded and classified categorically as in the front, middle or back, defined by direction of movement, whenever more than 50% of the fish body length was located either in the first third, second third or last third of the school, respectively, measured from the nostril of the fish positioned in front of the school to the tail of the fish positioned in the rear of the school.

2.11. Statistical analyses

Descriptive statistical analyses, the Shapiro–Wilkes test of normality and one-way analyses of variance were carried out on physical (weight and length), RFI, fin damage (splits and bites), SGR and *K* (Zar, 2009). In order to clarify the effect of treatment, a general linear model described by $y = a + bx$, where *a* is the intercept (C group) and *b* the slope (effect of treatment), was carried out for weight and length variation (Zar, 2009). Differences in degree of dorsal fin erosion amongst treatments were analysed using the Chi-square test and the Chi-square test for trends (Zar, 2009). Correlations between dorsal fin erosion and other variables were analysed using the Pearson rank correlation (Zar, 2009) and network distance and density were analysed by analysis of variance (Zar, 2009). Mantel tests (1000 permutations) were carried out for association and aggression matrices between acclimatisation and treatment periods in order to ascertain whether any differences were attributed to statistically significant changes in the behaviour of fish rather than chance (Zar, 2009). All statistical analyses were performed using R statistical software (R Development Core Team, 2008).

Table 2Length variation (*L*, in mm), weight variation (*W*, in g) in fish and Coefficient of Variation (CV) according to treatment.

Fish	Feed-restriction								Control							
	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7		Group 8	
	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>
A	1.3	12.5	0.7	2.3	0.1	-0.3	0.4	0.8	0.5	1.6	0.5	1.7	0.4	1.2	0.0	-0.3
B	1.4	21.5	0.1	-0.2	0.4	0.9	0.4	0.8	0.1	0.0	0.4	1.3	0.1	-0.2	0.3	0.8
C	1.6	22.5	0.6	1.9	0.3	0.4	0.3	0.2	0.7	2.2	1.2	4.3	0.4	1.0	0.4	0.9
D	0.7	6.5	0.1	0.0	0.1	0.2	0.2	0.5	0.1	0.2	0.2	0.6	-0.1	-0.8	0.4	1.5
E	0.8	4.5	0.2	0.2	0.0	-0.4	0.0	-0.5	0.2	0.4	0.2	0.6	0.3	0.8	0.2	0.3
F	0.6	1.5	-0.2	-1.4	0.3	0.8	0.2	0.4	0.1	0.2	0.3	1.1	-0.1	-0.6	0.2	0.7
CV	39.2	77.4	135.6	297.4	77.5	203.6	60.7	132.1	90.4	118.3	80.9	86.8	140.3	83.3	60.7	93.2

Table 3Number of splits, dorsal fin bites and degree of erosion of dorsal fin of fish (*N* = 24 for each treatment).

Group	Dorsal fin damage					Bites	Splits
	Degree of erosion		Total number (% of fish affected)				
	0 ^a	1 ^a	2 ^a	3 ^a	Sum 1, 2, 3		
Control (<i>N</i> = 24)	20 (83.4%)	4 (16.6%)	0	0	4 (16.6%)	4	8
Feed-restriction (<i>N</i> = 24)	13 (51.2%)	6 (25%)	4 (16.6%)	1 (7.2%)	11 (45.8%)	8	12
Total (<i>N</i> = 48)	33 (68.5%)	10 (20.8%)	4 (8.3%)	1 (2.4%)	15 (31.25%)	12	20

^a 0 = no erosion (0% of erosion); 1 = mild erosion (1–25% erosion); 2 = moderate erosion (26–50%); 3 = severe erosion (>50% of erosion).

3. Results

3.1. Physical measures

Fish under feed-restriction had significantly lower final weight ($F_{1,46} = 4.39$, $P = 0.04$), SGR ($F_{1,46} = 13.60$, $P < 0.01$), condition factor ($F_{1,46} = 5.76$, $P = 0.02$) and weight gain ($F_{1,46} = 14.24$, $P < 0.01$) compared with unrestricted control fish. However, there were no treatment differences in final length of fish ($F_{1,46} = 0.60$, $P = 0.44$). In addition, there was a significantly higher variation in weight gain within each FR group (CV: 95.09) compared to C groups (CV: 15.08) ($F_{1,46} = 14.24$, $P < 0.01$). Length variation and weight gain of each fish in the feed-restricted groups are shown in Table 2 indicating that some fish lost weight. This is further supported by the results from the linear regression model showing a significant effect of weight variation in FR fish compared to C fish ($y = 23.783 - 17.221x$, $P < 0.001$) but no effect of length variation ($y = 1.279 - 0.437x$, $P = 0.29$).

3.2. Quantification of fin damage

Total values for dorsal fin damage are shown in Table 3. Only 31.25% of all fish had any dorsal fin erosion (degrees 1, 2 and 3). However, fish under feed-restriction showed more moderate (16.6% of fish) or severe (7.2% of fish) amounts of fin erosion compared with control fish ($\chi^2_3 = 6.88$, $P = 0.07$) in dorsal but not in other fins ($P > 0.10$). There was a linear relationship between the total number of fish with erosion and the degree of erosion (χ^2 trend for proportions = 6.54, $P = 0.01$). Dorsal fin erosion was positively correlated with the occurrence of dorsal fin bites in FR groups ($r^2 = 0.54$, $P = 0.03$). Moreover, there was a strong correlation between the number of dorsal fin bites and dorsal fin erosion ($r^2 = 0.84$, $P < 0.01$). There were no differences between FR and

C groups in initial and final RFI in all fins evaluated ($P > 0.10$).

3.3. Quantification of aggression

Aggressive behavioural interactions for each type of aggression in each group are presented in Table 4. Total aggression was significantly higher in FR groups (232.5 mean events/group/30 h observation period) than in C groups (135.35 mean events/group/30 h observation period) ($H_1 = 4.08$, $P = 0.04$). The number of attacks was also significantly higher in FR groups compared to C groups ($H_1 = 5.39$, $P = 0.02$). Biting to fins was observed only during sampling periods and there was no visual evidence of lesions elsewhere in the fish at the end of the experiment.

3.4. Social network analysis

Differences in social network variables between groups were found only during the treatment period and not during the acclimatisation period ($P > 0.10$) using 390 matrices for each type of interaction. Therefore, comparisons between groups were carried out during the treatment period (day 14–44). In addition, the increase in the number of aggressive interactions in feed-restricted fish was not due to chance ($Z = 18486$, $P = 0.01$) confirming that the increased aggression between fish was due to a real change in the behaviour of fish subjected to feed-

Table 4

Mean aggressive behaviours according to type of aggression and experimental group.

	Biting	Displacement	Attack	Total
Control	12.5	88.75	34	135.35
Feed-restriction	23.25	153.5	55.75*	232.5*

* $P < 0.05$.

Table 5Values of social network analysis variables (mean \pm SD) according to aggressive and associative interactions network in control and feed-restriction groups.

Associative interaction networks						
	Degree Centrality (%)		Density	Clustering coefficient	Distance	Transitivity (%)
Control	15.58 \pm 1.69		1.04 \pm 0.08	0.010 \pm 0.009	1.18 \pm 0.04	92.55 \pm 3.72
Feed-restriction	16.29 \pm 0.85		0.86 \pm 0.05	0.087 \pm 0.006	1.09 \pm 0.02*	71.96 \pm 2.53*
Aggressive interactions networks						
	Centrality (%)		Density	Clustering coefficient	Distance	Transitivity (%)
	Out-degree	In-degree				
Control	31.18 \pm 4.18	14.25 \pm 1.24	1.44 \pm 0.15	0.014 \pm 0.015	1.41 \pm 0.01	64.44 \pm 1.92
Feed-restriction	38.63 \pm 1.58*	38.63 \pm 2.38*	5.91 \pm 0.59*	0.055 \pm 0.068*	1.20 \pm 0.04*	78.08 \pm 4.63

* $P < 0.05$.

restriction. The same situation did not occur in fish fed to satiation ($Z = 701$, $P = 0.286$). The results showing values obtained for social network variables are presented in Table 5.

3.5. Social network analysis of associative behaviour

FR groups showed significantly lower transitivity compared to C groups ($H_1 = 7.04$, $P < 0.01$). Social distance between fish was significantly lower in the FR groups and centrality tended to be higher ($F_{1,22} = 3.34$, $P = 0.05$ and $H_1 = 2.09$, $P = 0.11$, respectively). Figs. 1 and 2 present the networks based on degree of centrality in individual fish and the association tie strength in C and FR groups, respectively. These findings suggest that fish in feed-restricted groups tended to associate uniformly with stronger associations with all other member of the groups compared to fish in C groups.

3.6. Social network analysis of aggressive behaviours

Networks were significantly denser and less distant in FR groups ($F_{1,22} = 9.72$, $P < 0.01$ and $F_{1,22} = 3.64$, $P = 0.05$, respectively) compared with control fish. FR groups showed higher out-degree ($H_1 = 7.49$, $P < 0.01$), and in-degree centrality ($H_1 = 17.91$, $P < 0.01$) compared to C groups. Feed-restricted fish networks had higher clustering coefficients ($H_1 = 8.33$, $P < 0.01$). Figs. 3 and 4 show the out-degree centrality for C and FR group networks, respectively.

These findings indicate that there were distinct differences in out and in-degree centrality leading to particular

differentiation of roles within the group of feed-restricted fish. Typically in each group, two fish were found to be highly aggressive and were classified as initiators of aggression while two fish were usually the receivers of the aggression. This differentiation led to the formation of clusters of initiators and receivers in each group as can be seen in Fig. 4.

Additionally, initiators of aggression were fish that gained more weight while receivers of aggression were fish maintaining or losing weight as shown in Table 6.

3.7. Schooling/shoaling preference and spatial position measurements

As shown in Table 6, fish in FR and C groups did not differ in their schooling preference ($H_1 = 0.88$, $P > 0.10$) or shoaling preference ($H_1 = 0.89$, $P > 0.10$). However, it can be observed that initiators of aggression tended to maintain central schooling positions whenever feed-restricted fish schooled ($H_2 = 5.49$, $P = 0.06$). Receivers, on the other hand, did not show any consistent position while schooling ($P > 0.10$).

4. Discussion

Dorsal fin damage was observed in all groups but the amount was significantly higher in FR groups, particularly that recorded as dorsal fin erosion, splits and bites. This finding agrees with other studies using feed-restriction in salmonids and confirms the potential value of using dorsal fin damage evaluation as a good on-farm indicator of welfare related to increased levels of aggressive

Table 6Weight gain, length variation, schooling/shoaling preference and spatial position when schooling of fish (mean \pm SD) according to behavioural classification of fish (I, initiator of aggression; I/R, initiators and receivers of aggression; R, receivers of aggression) in treatment groups.

Group	Type of individual	Weight gain (g)	Length variation (g)	Schooling (% of time)	Shoaling (% of time)	Position when schooling (% of time)		
						Front	Middle	Back
Control (N=24)	I/R (N=24)	23.8 \pm 19.8	1.3 \pm 0.5	53.3 \pm 18.0	46.7 \pm 18.0	20.2 \pm 14.3	53.9 \pm 16.5	25.9 \pm 15.5
Feed Restriction (N=24)	I (N=6)	12.2 \pm 8.4	1.0 \pm 0.3	45.7 \pm 15.6	54.3 \pm 15.6	13.9 \pm 5.2	62.1 \pm 19.6	24.0 \pm 17.8
	I/R (N=12)	6.5 \pm 8.4	0.8 \pm 0.4	52.5 \pm 21.0	47.5 \pm 21.1	25.5 \pm 19.6	54.4 \pm 21.1	20.1 \pm 17.6
	R (N=6)	1.2 \pm 10.5	0.8 \pm 0.5	43.8 \pm 26.8	56.2 \pm 26.6	31.8 \pm 25.7	34.9 \pm 12.2	33.3 \pm 30.9

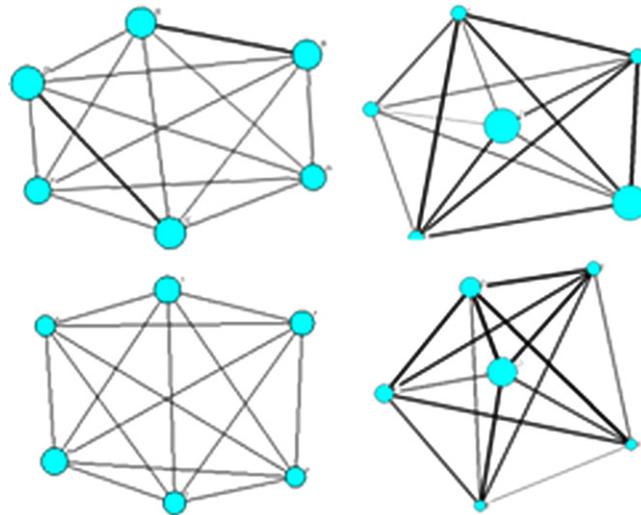


Fig. 1. Social networks for associative interactions in Control groups. Fish are represented by circles in which the diameter represents the magnitude of centrality, and line thickness represents association strength.

social interactions in rainbow trout (*Onchorynchus mykiss*) (St. Hilaire et al., 2006) and in Atlantic salmon (Noble et al., 2007a,b, 2008). Previous studies have suggested that tanks or net surfaces play a role in fin abrasion or erosion (for a review see Latremouille, 2003). However, in the present study, detailed inspection of tanks before introduction of fish indicated no physical abrasive material that could cause fin abrasion, erosion or damage. Moreover, the present study clearly demonstrated a positive correlation between the occurrence of dorsal fin bites, behavioural biting events and dorsal fin erosion, indicating that the cause of fin damage was aggressive behaviour between fish leading to fin injury from biting. Other studies have linked fin erosion to both the nutritional quality and quantity of feed delivered (see Latremouille, 2003 for a full review). How-

ever, nutritional deficiencies were unlikely to be a primary aetiological factor in the present experiment – a conclusion supported by studies demonstrating that isolated fish had no fin damage compared to fish held communally and fed under the same feeding regime (Kindschi et al., 1991; Turnbull et al., 1998). This leads to the conclusion that the only cause of the fin damage observed in the present study was direct social aggressive interactions amongst fish which increased when fish were feed-restricted. This evidence supports the hypothesis that fin damage in salmon aquaculture is largely the result of aggressive behaviour as suggested in previous studies (Abbott and Dill, 1985; Turnbull et al., 1996, 1998; Ellis et al., 2008). In addition, aggressive interactions have been previously suggested as a factor causing fin damage in numerous farmed and

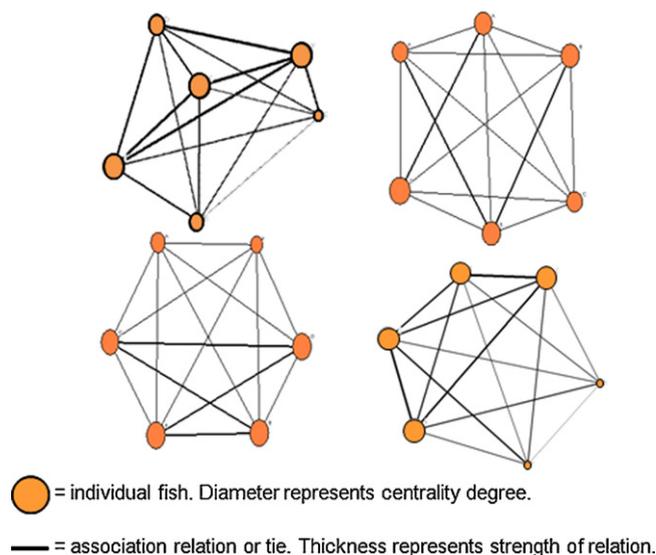


Fig. 2. Social networks for associative interactions in feed-restriction groups. Fish are represented by circles in which the diameter represents the magnitude of centrality, and line thickness represents association strength.

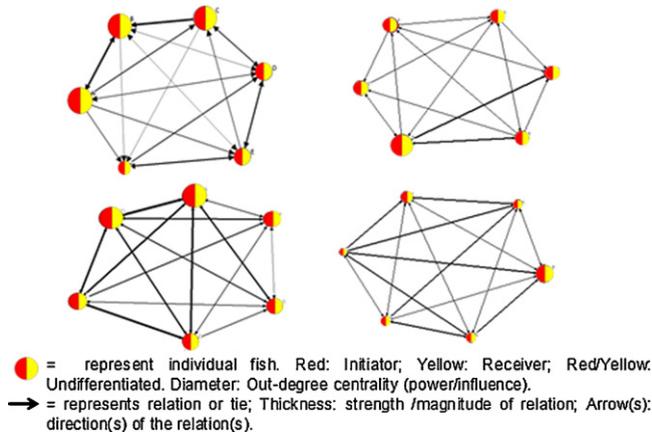


Fig. 3. Social networks for aggressive interactions in Control groups. Fish are represented by circles in which the diameter represents the magnitude of out-centrality (aggression expressed by fish), and line thickness represents association strength. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

wild trout species (Bosakowski and Wagner, 1994), Atlantic salmon (MacLean et al., 2000), Baltic cod (*Gadus callarias*) (Brawn, 1961) and Arctic charr (Jobling and Wandsvik, 1983). The present study supports these findings and further demonstrates that aggressive interactions lead to fin damage and the establishment of social hierarchies that influence the social structure of groups of Atlantic salmon.

Interestingly, there were no significant differences in fin erosion between treatments, when measured using the RFI method. However, there were significant treatment differences in dorsal fin erosion when erosion was measured using categories. This difference may highlight the limitations of using the RFI method to evaluate fin damage, as it only utilises data from the longest single fin ray of each fin. Researchers and other end users should therefore be cautious when using this technique to quantify fin erosion as reviewed by Ellis et al. (2008).

Social network analysis of aggressive interactions revealed that FR groups presented denser and less distant networks indicating that aggression was a social interaction rapidly transmitted within the members of

the network with fish rapidly identifying the initiators of aggression. Most importantly, the out and in-degree centrality differences revealed that feed-restriction disaggregated members according to their levels of aggression resulting in fish becoming either initiators or receivers of aggression. Initiators of aggression had higher out-degree centrality and were therefore extensively involved in interactions within the network due to having more ties with other fish within the group. This is particularly important as economic and sociological theory indicates that these fish are highly influential and are more likely to gain resources (Wasserman and Faust, 1994). On the other hand, receivers of aggression had fewer interactions and their spatial positions within the group were dependent on initiators. These high in-degree and low out-degree values of receivers indicated that these individuals seldom initiated aggressive interactions and did not retaliate or counterattack their aggressor(s).

Interestingly, the higher clustering coefficient observed in the networks of feed-restricted fish indicated the formation of highly connected groups that coincided with the

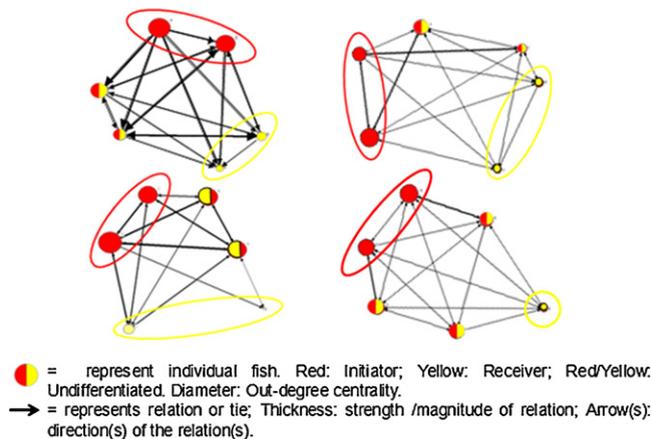


Fig. 4. Social networks for aggressive interactions in feed-restriction groups. Fish are represented by circles in which the diameter represents the magnitude of out-centrality (aggression expressed by fish), and line thickness represents association strength. Encircled are initiators of aggression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

differentiation seen in aggressive roles. Importantly, initiators of aggressive interactions were fish that gained more weight in their respective experimental groups, whilst receivers were fish maintaining or decreasing weight.

The present experiment also demonstrated that during periods of feed-restriction, initiators of aggressive interactions exhibited less fin damage suggesting that initiators of aggressive behaviour and fin-biting tended to dominate the food resource without receiving aggression from others. Additionally, using social network analysis we were able to clearly identify the existence of important key individuals (possessing high aggressive out-degree centrality and lower distance within a network) that were likely to be responsible for causing most of the fin damage within the group.

The identification of these individuals could have important implications in the control of fin damage in aquaculture whenever reduced or non-feeding periods occur such as during fish grading, transport, slaughter and other farm management practices such as vaccination. Although some studies have demonstrated that removing dominant fish increases aggression in the remaining fish in small groups as a compensatory effect of removal (Adams et al., 2000; Sneddon, 2006), this effect has yet to be investigated in commercial farm situations.

The importance of the detection of key individuals in this experiment also agrees with studies of infectious disease transmission in humans and animals (Bansal et al., 2007). For example, Bansal et al. (2007) found that contact patterns between individual people were heterogeneous rather than homogeneous thus demonstrating the importance of quantifying interactions at the individual level to investigate mechanisms of disease spread. Also, Read et al. (2008), demonstrated that individuals differ in the type and quantity of contacts indicating the importance of understanding mixing patterns and behavioural differences in the spread of infectious disease. Similar findings have been recently demonstrated in animals by Drewe (2010) in wild meerkats (*Suricata suricatta*) and Perkins et al. (2009), in the yellow-necked mice (*Apodemus flavicollis*).

Social network analysis of associative behaviour showed lower transitivity in FR group networks indicating less social equilibrium due to unbalanced relationships between members of the group. The presence of unstable relationships between individuals of the group is supported by the finding that there was a distinct differentiation of roles and aggressive behaviour potentially leading to formation of hierarchies in the feed-restricted groups as it has been described in previous studies on fish, including salmonids. Similar findings have been described in socio-ecological models of instability in other animals such as elephants (*Loxodonta africana*) (Wittemyer and Getz, 2007) and non-human primates (Isbell and Young, 2002) when competing for resources such as food and shelter. In addition, there were lower mean distances between fish and degree of centrality tended to be higher in FR networks, indicating that fish were often associated closely and strongly within the network under feed-restriction conditions. Our findings show a direct negative effect on the structural stability of associations in feed-restricted fish as compared with control fish.

Previous ecological studies using social network analysis in fish showing assertive characteristics in groups have demonstrated that some fish actively try to maintain specific inter-individual associations and interactions (Croft et al., 2004, 2005). This suggests that fish can distribute themselves in subpopulations and attain specific roles within their network according to their relationships. However, these previous studies focused on exploring temporal association networks in large populations of fish rather than specific behavioural interactions that occur between individual fish within smaller groups. The present experiment is the first to explore and quantify social network variables in small groups of fish and relates potential differences in network interactions and spatial positions to animal welfare.

The spatial and structural analysis of schooling behaviour showed that within a group, aggressive individuals tended to school more often and attain more central schooling positions, whereas receivers of aggression tended to shoal or be positioned around the periphery of the school. This experimental finding supports previous predictions of spatial distribution of fish within schools according to aggression or dominance (Viscido et al., 2007). It is clear from the present study that positions within schools are important during feed-restriction periods when central positions are adopted by more aggressive individuals who probably benefit from better overall surveillance of other more peripherally placed fish that in turn are more exposed to fin damage and lower weight gain.

Aggressive interactions within groups of farmed animals such as cows (*Bos taurus*) (Phillips and Rind, 2001) and pigs (*Sus scrofa*) (Sherritt et al., 1974) have been associated with detrimental production effects on growth, weight, condition and length of animals as well as various measures of animal welfare. In the present study, analysis of production welfare related measures indicated that fish tail-fork length did not significantly differ between FR and C groups. This suggests that, although some fish lost weight, they continued to grow in terms of length becoming thinner rather than having an overall restriction in growth. This finding has not been directly reported before but indicates a direct physical compensatory response that allows individual fish to stay within a competitive length which is in accordance to studies of allometric and compensatory growth in fish (Ali et al., 2003). This is supported by empirical evidence on social competitive abilities in Atlantic juvenile salmon (parr) (Huntingford et al., 1990) showing a positive correlation between length and dominance during the parr phase. A longer period of food restriction may have had different effects on growth, especially considering that previous studies have demonstrated that length growth and condition are less sensitive to short term food supply fluctuations and dependant more on endogenous factors (Dutta, 1994; Stefansson et al., 2009).

There was a wide variation in weight gain in all groups suggesting that aggressive behaviour was associated with the potential of establishing classical models of dominance hierarchies where dominant fish take most of the food resources. This has been previously described in studies when food resources were scarce or restricted in Atlantic salmon (Maclean and Metcalfe, 2001), brown

trout (Pettersson and Järvi, 2003), Arctic charr (Damsgård et al., 1997), Atlantic cod (*Gadus morhua*) (Hatlen et al., 2006) and tilapia (*Tilapia rendalli*) (McCarthy et al., 1999). In the present study, growth variation in FR groups was greater than that of C groups and some fish lost weight. This was likely to have been caused by competition for food resources leading to a higher frequency and intensity of aggressive interactions possibly associated with intermittent and repeated release of cortisol inducing catabolic physiological states in subordinate fish as has been described previously in rainbow trout (Øverli et al., 2004; Yue et al., 2006; Fernandes-de-Castilho et al., 2008). The existence of wide weight variation within groups in a commercial situation would be expected to impose significant management costs and increase final product price due to the need for frequent grading of fish to obtain marketable fish of similar weight and length. However, our study investigated relationships between only six individuals per tank – circumstances which are likely to have induced a despotic situation where one or two fish monopolized the food resource resulting in interference competition. In a more commercial situation with more fish and higher stocking densities, no or very little contact or aggression may occur between individuals where scramble competition occurs (Grant, 1997; Milinski et al., 1997). However, empirical evidence suggests that this is not the case in Atlantic salmon (Kjartansson et al., 1988; Brockmark et al., 2007; Ellis et al., 2008; Hosfeld et al., 2009) and trout (North et al., 2006; Ellis et al., 2008; Hosfeld et al., 2009) where high levels of aggression and fin damage incidence have been repeatedly reported to occur at high stocking densities.

5. Conclusion

The present study has demonstrated the applicability and value of social network analysis in understanding the development of fin damage in fish. The use of social network analysis offers considerable potential in contributing to the improvement of farmed fish welfare through the correct identification of socially important aggressive individuals. Further detailed studies of the effects of feed-restriction on the occurrence of fin damage in other social contexts at different life stages and different stocking densities will be necessary in order to fully understand the underlying social causes of fin damage in relation to food resources and hence indicate ways to improve the welfare and productivity of farmed fish.

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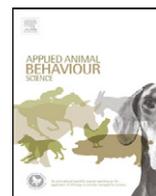
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Social network analysis of the behavioural interactions that influence the development of fin damage in Atlantic salmon parr (*Salmo salar*) held at different stocking densities

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ABSTRACT

Social network analysis of behavioural interactions was used to quantify the effect of high (HD, 30 kg m⁻³) and low (LD, 8 kg m⁻³) stocking densities on the frequency and severity of fin damage in Atlantic salmon. Dorsal fin damage (erosion, splits, fin index) was significantly higher in HD compared to LD groups with higher amounts of dorsal fin erosion. The prevalence of dorsal fin splitting was also significantly higher in HD groups. No other fins were affected by fin damage irrespective of density. Social networks based on aggressive interactions showed that HD groups exhibited higher centrality, clustering coefficient, in-degree centrality, out-degree centrality and were less dense than LD groups. High centralities and clustering coefficients indicated a distinctive separation of fish within HD groups into initiators of aggression (out-degree four times higher than in-degree) and receivers of aggression (in-degree four times higher than out-degree). This separation of roles was seen only in HD groups where initiators had higher out-degree centrality while receivers showed high in-degree centrality. Initiators of aggressive interactions had less fin erosion, higher final weights and higher body lengths than receivers of aggression. Fish in HD groups were significantly less aggressive than fish in LD groups in terms of the total number of aggressive behaviours observed but they exhibited significantly more overt aggression in terms of biting frequency. This can explain the occurrence of higher levels of fin damage in HD groups. Fish in LD groups had lower final weights, final body lengths and body condition than those in HD groups. This study shows that fish grew better and were in better condition when held at higher densities, but has significantly more overt aggression and fin damage than fish at lower densities. Density therefore has a differential detrimental effect upon performance and welfare depending upon the choice of welfare indicator (e.g. growth and condition vs. aggression and fin damage).

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1. Introduction

Aquaculture is one of the fastest growing global animal producing sectors and contributes to approximately 50% of all fish consumed (Food and Agriculture Organization of the United Nations, 2010). Although a positive image of the fish industry and derived products amongst consumers has been previously reported (Hanson et al., 1994), consumers have become increasingly interested in issues such as the

sustainability of fish farming and the welfare of farmed fish (Altintzoglou et al., 2010). For example, a recent study (Olesen et al., 2010) showed that consumers are willing to pay an additional 15% for salmon raised using higher welfare standards (Royal Society for the Prevention of Cruelty in Animals, 2010) or under conditions that have less environmental impact (DEBIO, 2009). The welfare of farmed fish has been the focus of much scientific research leading to several recommendations, guidelines, regulations and pieces of legislation (Huntingford, 2008; European Food Safety Authority, 2008, 2009a, 2009b, 2009c; World Organisation for Animal Health, 2010).

Fin damage has been frequently used as a welfare indicator (Noble et al., 2007, 2008) as fins have the nociceptors (Chervova, 1997) that have been implicated in pain perception in fish (Sneddon, 2003; Sneddon et al., 2003). Fin damage has been associated with aquaculture environmental variables such as abrasion from tank and cage surfaces (St. Hilaire et al., 2006), poor water quality, such as low dissolved oxygen and high ammonia content (Person-LeRuyet et al., 2008) and also with industry practices such as on-demand feeding delivery systems (Noble et al., 2007, 2008), high and low stocking densities (Turnbull et al., 2005; Brockmark et al., 2007) and increased aggression between fish associated to territoriality and food (Abbott and Dill, 1985; Turnbull et al., 2005). However, the relative importance of these various factors has not been fully investigated and has often been confounded (Adams et al., 2007).

The association between high or low stocking densities and fin damage has been investigated in Atlantic salmon (*Salmo salar*) in both laboratory and commercial situations but this work has produced contradictory results. For example, a higher incidence of fin damage has been associated with both high fish stocking densities (Turnbull et al., 2005; Adams et al., 2007; Brockmark et al., 2007) and low fish stocking densities (Turnbull et al., 2005; Adams et al., 2007). However, as densities are generally measured in kilograms of fish per cubic meter, identical stocking densities can be achieved with different numbers of fish with different body weights. In fact, most studies investigating the effect of stocking densities in Atlantic salmon achieved the desired stocking densities by increasing/decreasing the number of fish or group size, without taking into account the possible social and behavioural consequences of such changes. For example, Kjartansson et al. (1988) investigated the effects of three stocking densities on the physiological parameters of Atlantic salmon by manipulating stocking densities by increasing the number of fish in each tank, but removing fish on four occasions, reducing the total number of fish to ca. 50% of the original number. Also, Soderberg and Meade (1987) removed fish (ca. 15% of the original number) during their experiment in order to maintain the defined stocking densities. Similar situations occurred while investigating the effect of stocking densities in rainbow trout (*Oncorhynchus mykiss*) where fish had to be removed during the experiment to ca. 50% (North et al., 2006) or 20% (Person-LeRuyet et al., 2008) of the original number of fish. Also, other studies using fixed group sizes at two stocking densities showed no significant differences in fin damage (Rasmussen et al., 2007). However, stocking densities increased more than two-fold from

the beginning to the end of the study, irrespective of treatment. The causal relationships between density and fish welfare are complex (see review by Ellis et al., 2002). Many density experiments have not compensated for changes in water flow or oxygen levels, and it is thus difficult to evaluate if the documented effects are caused by fish density or by changes in water quality (e.g. Soderberg and Meade, 1987; North et al., 2006; Hosfeld et al., 2009).

It is widely accepted that the number of individual animals in a group has a direct impact on the affiliative behaviour of each individual within the group (Krause et al., 2002; Massen et al., 2010). In addition, it has been demonstrated that fish are capable of recognising other conspecifics in groups up to 15–20 individuals and tend to create stable relationships among members of the group when housed in these group sizes (Griffiths, 2003; Ward and Hart, 2003). Therefore, there is a need to clarify and quantify the effect of different stocking densities in farmed fish held at constant group sizes on production, behaviour and welfare parameters.

Social network analysis has recently been used to quantify the behaviour of fish in ecological studies (Croft et al., 2004; Croft, 2005) and more recently in studies investigating fin damage during a feed restriction period in Atlantic salmon (Cañon Jones et al., 2010). Social network analysis describes and quantifies direct and often hidden indirect relationships, social ties and influences among individuals (Wasserman and Faust, 1994). It can be used to identify the specific roles of individuals within the group who have a higher and specifically variable number of contacts and interactions and, therefore, may be more socially important and influential (Lusseau and Newman, 2004; Wasserman and Faust, 1994).

The main advantage of using social network analysis is that enables the correct identification and quantification of social position, social influence and role of key individuals within groups and their involvement in the development of fin damage under different stocking densities. Subsequent control measures based on this identification of roles could then be developed and implemented in order to control the welfare of farmed fish. Recently, the Royal Society for the Prevention of Cruelty to Animals has recommended fish stocking density limits of 17 and 30 kg m⁻³ for Atlantic salmon in freshwater production tanks and seawater enclosures, respectively (Royal Society for the Prevention of Cruelty in Animals, 2010). However, they have acknowledged that robust scientific knowledge is still lacking in this area.

This current study used social network analysis to quantify the behavioural interactions that influence the occurrence of fin damage in Atlantic salmon parr held in constant group sizes at stocking densities that represent the low and high end of the spectrum currently used in the salmon industry.

2. Materials and methods

2.1. Animals and experimental groups

The experiment was conducted in the summer 2009 at the Aquaculture Research Station in Tromsø, Northern

Norway (Norwegian Animal Research Authority (NARA) registration number 124). The study (Project Number 6040/09-006.1/H09/32) followed the current Norwegian Fish Welfare and Laboratory Animals legislation (Ministry of Agriculture of Norway, 1996; Ministry of Agriculture and Food of Norway, 2010), which adheres to the European Convention for the Protection of Vertebrates used for Experimentation and other Scientific Purposes (Council of the European Union, 1998). Additionally, the guidelines for the treatment of animals in applied animal behavioural research were adhered to during the experiment (Sherwin et al., 2003).

Eight experimental groups, consisting of ten clinically healthy year 1+ Atlantic salmon weighing 113.24 ± 10.7 g (mean \pm SD) and with mean body lengths of 20.3 ± 0.6 cm were used in the study. The fish were sourced commercially from Haukvik Kraft-Smolt AS, Tribe Batnfjord, generation 2008. There were three experimental phases: Phase 1 pre-treatment (from day 1 to day 10), Phase 2 treatment period (from day 11 to day 20) and Phase 3 or post-treatment (from day 21 to day 30).

Feed was delivered automatically at 10:00 h for 30 min at a rate of 1.5% of estimated fish body weight day^{-1} from feeders located above the experimental tanks. A commercial pelleted feed ('NutraParr 3 mm', Skretting AS, Stokmarknes, Norway) was used throughout the study. The amount of food delivered was adjusted weekly according to the expected weight gain and water temperature according to guidelines from the feed manufacturer.

After the pre-treatment phase, four tanks of fish were randomly selected as high density (HD, 30 kg m^{-3}) and four tanks as low density (LD, 8 kg m^{-3}) groups. A tubular shaped flexible plastic mesh ring net (Biltema®, 1 mm thick thread with 13 mm spacing) was inserted inside the HD tanks at the start of Phase 2. Fish were then housed in this ring to increase density to 30 kg m^{-3} . Therefore, only space was reduced while environmental qualities such as water column height, volume, current, velocities, fish tank, number and volume of fish were similar in each group. At the start of Phase 3 (post-treatment phase) the plastic ring net was removed from the HD groups in order to observe the effect on the aggressive behaviour of fish after changing to low density.

In order to minimise any effect of management disturbances, two LD groups and two HD groups were allocated to tanks near the entrance to the experimental room while the remaining groups were allocated away from the entrance.

No mortalities occurred during the experiment and all fish were euthanised at the end using immersion in an overdose bath of benzocaine chlorhydrate ($>250 \text{ mg l}^{-1}$ freshwater).

2.2. Containment and individual identification

Fish were individually identified under anaesthesia induced by submersion in a bath of benzocaine chlorhydrate (100 mg l^{-1} freshwater) at the beginning of the experiment. Tags were designed using unique combinations of black or white geometric designs (circles, triangles, squares, rectangles and crosses of $2.5 \text{ cm} \times 2.5 \text{ cm}$) made from plastic printing paper (Xerox® Special Advanced

Media Digital Colour, Premium Never Tear 95μ Polyester paper) and inserted under the skin behind the dorsal fin of each fish using strong silk thread and standard commercial Floy Tags (Hallprint®, Polyepaltichylene streamer tags, series PST). Damage to the skin was minimal and no significant effect of tagging system or type of tag on weight, length or fin damage was observed between experimental. All fish achieved full anaesthesia within 3 min and tagging was carried out during the following minute. After tagging, fish were transferred to the experimental tank with initial stocking densities of $7.9 \pm 0.1 \text{ kg m}^{-3}$ (mean \pm SD) and observed for 30 min following recovery from anaesthesia. An emergency recovery tank with highly oxygenated freshwater ($>99\%$ dissolved oxygen injected through diffusers connected to oxygen gas tanks) was available during tagging in case fish needed assisted recovery or veterinary assistance.

2.3. Housing, water quality and environmental conditions

Fish were housed in 300l high density plastic circular tanks measuring 78 cm of diameter and 50 cm of height. Naturally aerated and filtered ambient freshwater from a nearby river was provided throughout the experiment. Dissolved oxygen content ($93.3 \pm 3.0\%$) and water temperature ($12.4 \pm 1.7^\circ\text{C}$) were measured and recorded twice daily. Water flow was controlled at an exchange rate of 10 l min^{-1} in an open flow system and velocities of $1 \text{ fish body length s}^{-1}$. A 24 h light photo-period regime was used during the study.

2.4. Physical measures

The weight (g) and length (total tail-fork length in mm) of each fish were measured at the beginning and the end of the experimental period. Specific growth rate (SGR) was calculated as: $\text{SGR} = (\ln w_1 - \ln w_0) / \Delta t$, where w_1 is the wet weight of fish (g) at sampling time 1, w_0 is the wet weight of fish (g) at sampling time 0, and Δt is the number of days between sampling times. Fulton's condition factor (K) was calculated from the tail-fork length and mass of individual fish as: $K = W / L^3 \times w_{1 \text{ or } 2}$, where W is the weight of the fish (g), L^3 is the length of the fish to the power of 3, s is the total tail-fork length in mm and $w_{1 \text{ or } 2}$ is the weight of fish (g) at sampling time 1 or 2.

2.5. Quantification of fin damage

Digital photographs of both sides of every fish were taken at the beginning and end of the experimental period. Fin damage was evaluated from these pictures using the relative fin index (RFI) as described in Bosakowski and Wagner (1994a). Briefly, RFI relates the length of the fin over the total length of the fish and it has been suggested to allow reliable and objective measurement of the degree of fin erosion (Person-LeRuyet et al., 2007). An additional categorical method was also used to quantify fin erosion utilising an ordinal scale of 0, 1, 2 and 3, corresponding to no erosion (0% of fin eroded), mild erosion (1–24% of fin eroded), moderate (25–49% of fin eroded) and severe ero-

sion (>50% of fin eroded), respectively (Cañon Jones et al., 2010).

Every left and right pectoral, ventral, anal, caudal (upper and lower) and dorsal fin was measured and quantified using RFI and the categorical erosion index. In addition, the total number of fin splits (separation of fin rays greater than 3 mm) was recorded for each fish.

Additionally, fish were visually examined for other external lesions on their bodies as evidence of the occurrence of biting during sampling periods and at the end of the experiment.

2.6. Behavioural observations and social interactions

Ten minute video recordings of each tank were carried out at 09:00 to 09:10, 10:00 to 10:10 and 11:30 to 11:40 on each day of the experimental period. This recording regime quantified fish behaviour 1 h before feeding, during the first 10 min of feeding and 1 h after the last food delivery from the automatic feeders. CCTV colour cameras (Panasonic® VWR42 with Panasonic® WV-LA4R5C3B lenses) located 1 m above each tank were used to record the behaviour of fish. Each tank had a perforated water inlet pipe submerged to the water level and a double central perforated standpipe to prevent rippling on the water surface. Digital recordings were made using a DVD/HDD recorder (Pioneer® DVR-550H-S).

2.7. Association interactions measurements

Data extracted collected from the video recordings at 1 min intervals were used to construct association matrices. A fish was considered as associated with another fish when they were within two fish body lengths if parallel to each other, or within two body widths if perpendicular to each other.

2.8. Aggressive interactions measurements

Aggressive behaviour was classified as an attack, a displacement or a fin-bite and quantified using the methods described in Cañon Jones et al. (2010). Initiator(s) and receiver(s) of any aggressive interaction were recorded and weighted matrices for social network analysis for each video sampling period were constructed. Aggressive interactions were also used to calculate and compare data relating the total amount of aggressive interactions and attacks, displacements and fin bites between experimental groups.

2.9. Social network analysis

Social network analysis was carried out with associative and aggressive interaction matrices using UCINET® (Borgatti et al., 1999). The quantified network variables were the degree of centrality, clustering coefficient, transitivity, distance and density. Detailed explanations of these network variables based on Wasserman and Faust (1994) and Hanneman and Riddle (2005) are described in Cañon Jones et al. (2010).

Briefly, centrality measures the quantity of direct connections or behavioural interactions an individual has with others within the network. In the case of association interaction matrices, only the overall degree of centrality was measured, as interactions were symmetrical and reciprocal. On the other hand, the aggression in the aggressive interactions matrices could be reciprocal or non-reciprocal and usually non-symmetrical, thus we calculated the in-degree centrality (amount of aggression received by each individual or group) and the out-degree centrality (amount of aggression generated by each individual or group). Furthermore, fish could then be classified as initiators or receivers of aggression. Initiators (I) were classified as fish whose out-degree centrality was four times greater or more than their in-degree centrality. Receivers (R) were classified as individual fish whose in-degree centrality was four times greater or more than their out-degree centrality. Otherwise, fish were classified as neither I nor R. Differences between an individual's in-degree and out-degree centralities have been used previously to classify humans with different personality disorders (Clifton et al., 2009). The in-degree/out-degree ratio of 4 was used to classify fish as Receivers or Initiators, as shown in Fig. 4. All centrality measures were calculated as normalised to the total numbers of individuals in the network and expressed as percentages (Hanneman and Riddle, 2005).

Density quantifies the amount of potential connections between individuals that are actually present. A high density indicates saturation of the network, where almost all potential interactions are present. Low densities indicate sparse networks where fewer potential interactions between individuals are present.

Clustering coefficient quantifies the extent to which two neighbours of an individual are themselves neighbours. High clustering coefficients suggest that individuals that are well connected with each other forming subgroups, subpopulations or clusters within the network surround individuals.

Network distance measures the mean number of connections between the members of all possible pairs of individuals within a network. High distance values mean fewer interactions between individuals within the network.

Network analyses were carried out for the pre-treatment (days 1–10), treatment (days 11–20), and post-treatment (days 21–30) periods and for the entire experimental period (days 0–44).

2.10. Structural and spatial position measures

Each individual fish was classified as being schooling or shoaling at 1 min intervals from the video recordings using methodology described in Cañon Jones et al. (2010). Briefly, schooling was defined as a coordinated grouping behaviour where two or more fish were within association length/width and orientated in the same direction. Shoaling was defined as an uncoordinated grouping behaviour where fish were within association length/width and showed no coordinated orientation and direction (Parrish et al., 2002). Additionally, any schooling fish were classified as being located in the front, middle or back of the

Table 1Number and percentage of fish with fin erosion, bites, splits and relative fin index (RFI, mean \pm SD) in the dorsal fin measured in the experimental groups.

	Fin erosion (%)				Bites	Splits	RFI
	No erosion	Mild	Moderate	Severe			
LD groups	39 (97.5%)	1 (2.5%) ^a	0 (0%) ^a	0 (0%) ^a	1	1	11.55 \pm 1.51 ^a
HD groups	34 (85.0%)	4 (10.0%) ^b	1 (2.5%) ^b	1 (2.5%) ^b	3	4	12.53 \pm 1.09 ^b

^(a,b)Statistical differences at $P < 0.05$ between experimental groups.

school whenever more than 50% of the fish body length was located either in the first third, second third or last third of the school, respectively.

2.11. Statistical analyses

Descriptive statistical analyses, the Shapiro–Wilkes test of normality and one-way analyses of variance were carried out on weight, length, RFI, fin damage (splits and bites), SGR and K (Zar, 2009). In order to clarify the effect of treatment, a general linear model described by $y = a + bx$, where a is the intercept (LD group) and b the slope (effect of high density), was carried out for weight and length variation (Zar, 2009). Kruskal Wallis tests were used to measure the effect of tagging system on weight, length and fin damage between experimental groups. Differences in degree of dorsal fin erosion amongst treatments were analysed using the Chi-square test and the Chi-square test for trends (Zar, 2009). Correlations between dorsal fin erosion and other variables were analysed using the Pearson rank correlation (Zar, 2009) and network distance and density were analysed by analysis of variance (Zar, 2009). Kruskal Wallis tests were used to analyse the differences in aggressive behaviours (biting, displacements, attacks and total aggressive behaviour) as well as for centralities (overall, in-degree and out-degree), clustering coefficients and densities between experimental groups. Mantel tests were carried out for association and aggression matrices between acclimatisation and treatment periods in order to ascertain whether any differences were attributed to statistically significant changes in the behaviour of fish rather than by chance (Zar, 2009). All statistical analyses were per-

formed using R statistical software (R Development Core Team, 2008).

3. Results

Fin erosion was only present on the dorsal fin and it was significantly higher in HD groups (6 vs. 1 fish, $\chi^2 = 3.91$, $P < 0.05$) which also showed significantly lower RFI (11.5 vs. 12.5, $F_{1,68} = 10.9$, $P < 0.01$) as shown in Table 1. In addition, moderate and severe dorsal erosion was present only in HD groups and not in LD groups ($\chi^2_3 = 8.1$, $P < 0.05$) as well as dorsal splits and bites ($P < 0.05$). Dorsal fin erosion was positively correlated with the occurrence of biting in HD groups ($r^2 = 0.68$, $P = 0.04$). There were no statistical differences in the RFI and erosion for all other fins evaluated ($P > 0.1$).

LD groups had significantly more total aggression than HD groups (55.43 interactions/h vs. 41.46 interactions/h, $H_1 = 5.33$, $P < 0.05$) but HD groups had a significantly higher frequency of fin biting (0.35 interactions/h vs. 0.13 interactions/h, $H_1 = 5.39$, $P < 0.05$), as shown in Fig. 1. This suggests that LD conditions increased the frequency of aggressive behaviour in comparison to HD groups, but this aggression was significantly less severe than at higher densities.

Social networks based on aggressive interactions in HD groups showed higher centrality (32.9% vs. 27.7%, $H_1 = 3.1$, $P < 0.05$), clustering coefficient (0.41 vs. 0.36, $H_1 = 4.9$, $P < 0.01$), in-degree centrality (35.2% vs. 20.8%, $H_1 = 13.6$, $P < 0.01$), out-degree centrality (46.1% vs. 36.9%, $H_1 = 15.2$, $P < 0.01$) and the network were less dense (29.4 vs. 60.8, $H_1 = 5.33$, $P < 0.05$) than networks in LD groups.

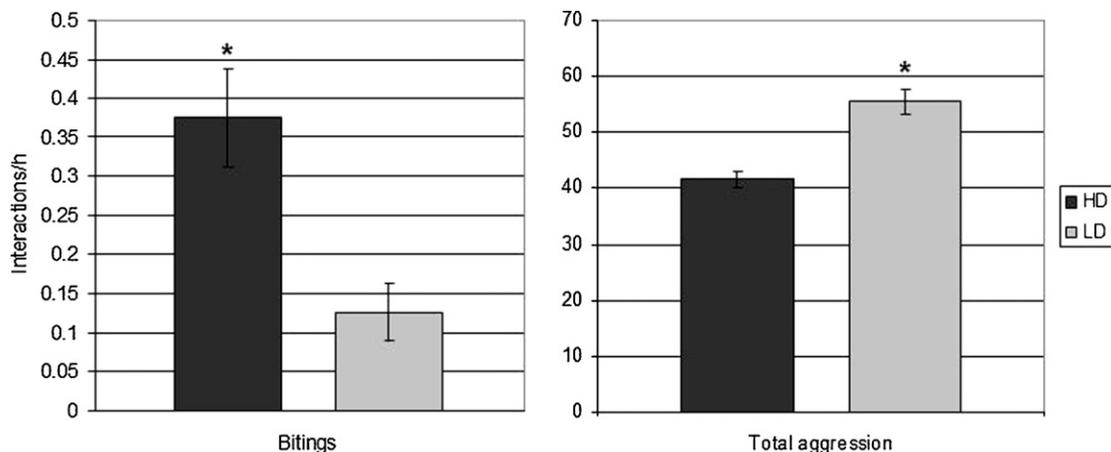


Fig. 1. Mean occurrences of biting and total aggressive interactions in experimental groups. *Statistical differences at $P < 0.05$ between experimental groups.

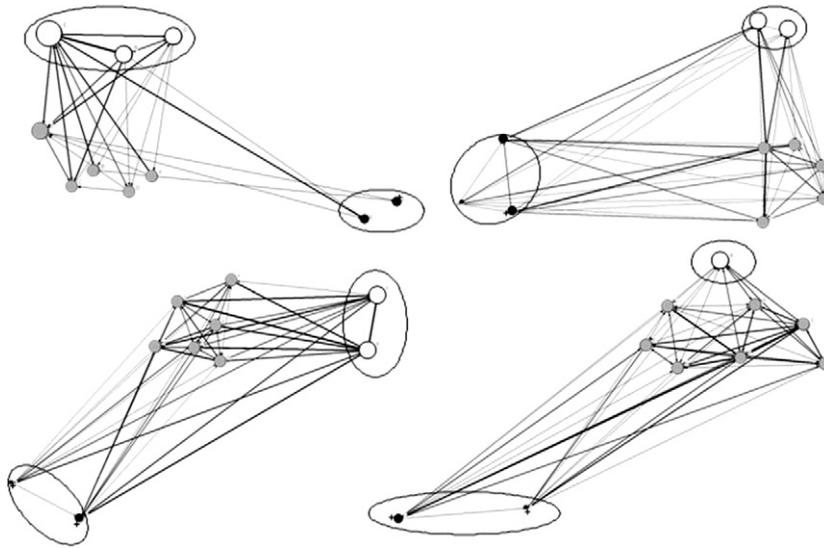


Fig. 2. Social network graphs of aggressive interactions in each HD group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) and the thickness of the connecting lines represents the magnitude of the interaction. White, black and grey represent the type of individual classified based on their out and in-degree centralities as initiators, receivers and initiators/receivers of aggression, respectively. Encircled are clusters of initiators and receivers. (+) Symbol represents fish with dorsal erosion.

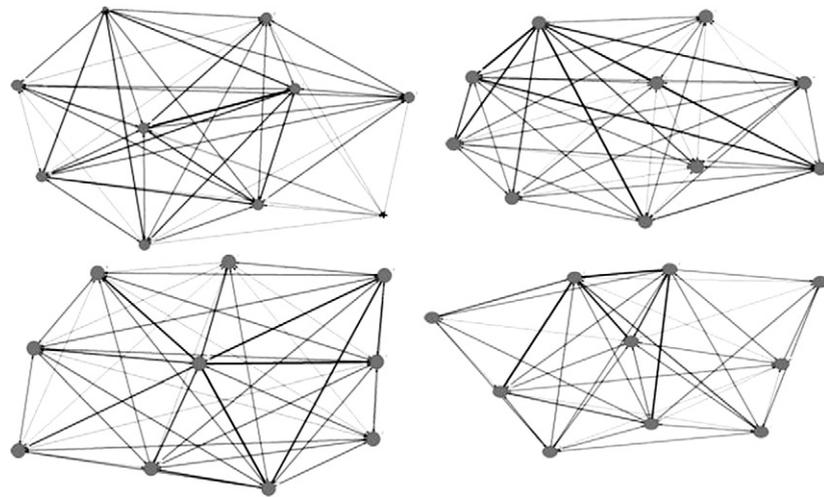


Fig. 3. Social network graphs of aggressive interactions in each LD group. Fish are represented by circles where their diameters represent out-degree centrality (amount of aggression initiated by the fish) and the thickness of the connecting lines represents the magnitude of the interaction. Only initiators/receivers based on the out and in-degree centralities of aggression were found in LD groups.

These findings indicate a distinctive separation of roles of the fish according to aggression in HD groups accompanied by formation of separate clusters of initiators and receivers of aggression in these groups. Initiators had high out-degree centrality (59.3% vs. 5.5%, $H_1 = 7.0$, $P < 0.01$) while receivers showed high in-degree centrality (21.7% vs. 11.1%, $H_1 = 3.1$, $P < 0.01$). Figs. 2 and 3 show the graphical representation of these network formations in each HD and LD groups, respectively. Within the HD groups, initiators of aggression had less dorsal fin erosion (1 fish vs. 5 fish), higher final weight (121.6 g vs. 105.0 g, $F_{1,9} = 4.9$, $P < 0.05$) and length (21.7 cm vs. 20.7 cm, $F_{1,9} = 5.9$, $P < 0.05$) compared to receivers of aggression (Table 2).

Furthermore, linear regression modelling showed differences in degree centralities only in HD groups with clusters of fish with high in-degree ($F_{1,78} = 106.9$, $P < 0.01$) and clusters of fish with high out-degree centrality

Table 2

Fish length and weight (mean \pm SD) and number of fish showing fin erosion according to type of individual (initiators or receivers) in HD groups.

	Length (cm)	Weight (g)	Fish showing fin erosion
Initiators	21.77 \pm 0.47 ^a	121.60 \pm 12.89 ^a	1
Receivers	20.70 \pm 0.46 ^b	105.00 \pm 9.87 ^b	5

(a,b) Statistical differences at $P < 0.05$ between experimental groups.

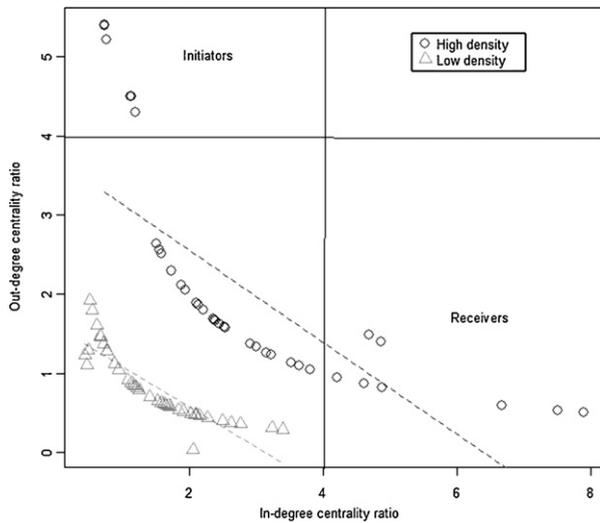


Fig. 4. Out-degree and in-degree centrality ratios of individual fish in HD and LD groups. Initiators and receivers of aggression are only present in HD groups. Lines represent the centrality cut-offs used for definition of individuals as initiators and receivers of aggression. Dotted lines represent the regression analysis with statistical difference at $P < 0.05$.

($F_{1,78} = 23.5$, $P < 0.001$). These results allowed us to differentiate individuals as I or R of aggression as is shown in Fig. 4. Fish in the LD groups had lower final weights (106.0 g vs. 111.8 g, $F_{1,78} = 4.3$, $P < 0.05$), lower final lengths (20.7 cm vs. 21.1 cm, $F_{1,78} = 6.2$, $P < 0.01$) and lower body condition (5.1 vs. 5.2, $H_1 = 3.6$, $P < 0.05$) compared to fish held at high stocking density. Additionally, a significantly higher variation in weight gain was seen in LD groups compared to HD groups (7.3 vs. 6.3 g, $F_{1,78} = 5.1$, $P < 0.05$).

There were no statistical differences in social network parameters for associative behaviour between experimental groups. Similarly, fish did not show detectable structural (schooling or shoaling) or positional preferences in any of the experimental groups.

4. Discussion

Dorsal fin damage was significantly higher in HD groups in terms of fin erosion and a lower dorsal RFI. These findings are in agreement with other studies that correlate high stocking density with increased levels of fin damage in rainbow trout (Ellis et al., 2002; North et al., 2006; Person-LeRuyet et al., 2008) and Atlantic salmon (Turnbull et al., 2005; Adams et al., 2007; Brockmark et al., 2007). However, previous studies by Cañon Jones et al. (2010) showed that RFI was not associated with fin damage and therefore not a very robust welfare measure, suggesting the value of using direct examination of fins in this study. It is in contrast with a small number of others studies (e.g. Rasmussen et al., 2007) where lower levels of fin damage were reported in fish stocked in high densities. Interestingly, no other fins were significantly affected by fin damage in relation to stocking density. The present experiment suggests increased dorsal fin biting leads to higher dorsal fin damage in Atlantic salmon confirming that dor-

sal fin damage can be used as a morphological operational welfare indicator (OWI) for aggression (Ellis et al., 2002, 2008; Noble et al., 2008).

The results of the present study strongly suggest that fin-biting was the most likely cause of fin damage in the HD groups and this is supported by the findings of previous studies in both farmed and wild fish such as cut-throat trout (*Oncorhynchus clarki*), brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (Bosakowski and Wagner, 1994b), Atlantic salmon (MacLean et al., 2000), Atlantic cod (*Gadus morhua*) (Hatlen et al., 2006) and Arctic charr (Jobling and Wandsvik, 1983).

Detailed examination of aggressive interactions in the current study showed that the total number of aggressive interactions (attacks, displacements and fin bites) was higher in LD groups than in HD groups. However, HD groups had a significantly higher frequency of biting than LD groups. Therefore, although the overall frequency of aggression was higher in LD groups, the form of aggression (fin biting) was significantly more intense at higher stocking densities. This evidence strongly supports the conclusion that the most likely cause of fin damage was overt aggression in the form of fin biting at high stocking densities. The low frequencies of fin damage in our study (8.75%) are in general agreement with levels reported in previous studies in Atlantic salmon kept under commercial situations ($\pm 6\%$ in Noble et al., 2008) and Rainbow trout kept experimentally ($\pm 9\%$ in Rasmussen et al., 2007). These results show the relevance of relatively low frequencies of highly intense aggressive behaviour such as fin biting on the development of fin damage and welfare of fish under different stocking densities. This finding also underlines the importance of separating and discriminating the types of behavioural interactions, which occur between fish in studies where fin damage is used as an indicator of welfare.

Social network analysis of aggressive interactions showed that high stocking densities had a significant effect on increasing the centrality, clustering and density in the social networks of each group. Importantly, in and out-degree centrality in HD groups showed a distinct separation of individual members of the groups according to their roles within the network into initiators and receivers of aggression. Interestingly, although LD groups were more aggressive, the separation of roles occurred only at high stocking densities and not at low stocking densities. Initiators of aggression had higher out-degree centrality and showed a higher number of interactions among members within the group, suggesting that initiators were more influential and more likely to gain access to resources (Wasserman and Faust, 1994), such as space/position and food and assert more influence on the behaviour of other fish in this experiment. In fact, receivers of aggression had higher in-degree centrality and lower out-degree centrality, indicating that they seldom initiated aggressive interactions with no retaliation or counter-attacks. Additionally, detailed analysis of physical measures showed that initiators of aggressive interactions were fish that gained more weight, achieved longer body lengths and exhibited less fin damage compared to receivers of aggression. The correct identification and possible removal of these individuals as those who are bigger and longer may

have potential implications for attempts to control fin damage and increase the welfare of farmed salmon whenever high densities are maintained. However, the removal of dominants may be beneficial only in the short term, as some laboratory studies have observed that the removal of the dominant fish allows the next ranked fish to take the dominant place and results in a transient increase in aggression (Adams et al., 1998, 2000). The separation of roles within groups of fish seen in this study is in agreement to previous studies using social network analysis in Atlantic salmon under feed restriction (Cañon Jones et al., 2010).

Social network analysis has been used in guppies (*Poecilia reticulata*) to show that individuals can maintain specific and differential associations and interactions between individuals within a group (Croft et al., 2004; Croft, 2005). However, to the authors' knowledge, the current study is the first to quantify the effect of behavioural interactions at different stocking densities in relation to fin damage using social network analysis in aquacultural species.

Although the primary objective of this study was to quantify the effects of stocking density upon fin damage, it is important to recognise the relative detrimental effect of low stocking densities on fish welfare in the current study. Fish held at LD had a lower weight, length and body condition, higher total aggressive interactions but less fin damage compared to fish in HD groups. It is important to point out that fish in LD were not losing weight and were subjected to less intense aggressive behaviour (fin-biting) compared to HD groups. However, we suggest that the lower growth of fish in LD groups, probably because of an increased physical activity due to higher aggressive behaviour of fish, represents reduced welfare based on the well accepted definition of poor welfare being any difficulty in coping or reduction in fitness by an animal (Broom, 1988, 1991). We believe that it is not necessary for fish to lose weight or become anorexic to have a poor welfare status. These findings are in agreement with previous studies demonstrating negative physical effects of fish held at low stocking densities (Turnbull et al., 2005; Person-LeRuyet et al., 2008). Therefore, results of the current study indicated a negative welfare effect of low rearing densities, highlighting the differential detrimental welfare effects which can occur at both low and high stocking densities in farmed fish. However, the numbers of fish used in the current study were low compared to farming conditions, but were not low compared to others experimental studies investigating stocking density (Alanärä and Brännäs, 1996; Turnbull et al., 1998).

The spatial and structural analysis of schooling behaviour showed that stocking densities did not affect the spatial distribution of fish according to their aggressive behaviour. Our results are in contrast to the theoretical evidence that aggressive or dominant individuals should attain central positions when schooling (Viscido et al., 2007) and the only empirical evidence of this occurring was in Atlantic salmon subjected to feed restriction (Cañon Jones et al., 2010). It is possible that we did not observe this phenomenon due to the fact that both high and low stocking densities used in this study had a negative impact on the behaviour and welfare of fish, resulting in the fish

not being able to achieve preferential schooling or shoaling behaviour. The lack of these behaviours could also be explained by the fact that salmon parr tend to be highly aggressive (Bardonnet and Bagliniere, 2000).

5. Conclusion

The present study demonstrated the applicability and value of social network analysis in understanding behavioural interactions underlying the development of fin damage in fish held at low and high stocking densities. Both high and low stocking densities had a differential detrimental welfare effect on fish: high stocking densities resulted in a differentiation of roles of fish within their network into initiators and receivers of aggression. This leads to an increase in the frequency of biting and resulted in increased dorsal fin erosion. The factors that determine which fish becomes an initiator or a receiver of aggression are still unclear and future studies are needed to investigate this. Low stocking densities did not result in this differentiation of roles but aggression amongst fish within the LD groups was higher and fish grew at a slower rate. The use of social network analysis in behavioural studies is likely to contribute to the understanding and improvement of the welfare of farmed fish by the correct identification of socially important aggressive individuals. However, more studies are needed to adequately quantify the effect of other social contexts such as group size, different life stages, intermediate stocking densities or feed delivery systems on the development of fin damage and hence indicate appropriate and practical ways to improve the welfare and productivity of farmed fish.

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