Title

Evaluating the effects of a short-term feed restriction period on the behavior and welfare of Atlantic salmon, *Salmo salar,* parr using social network analysis and fin damage

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Abstract

Social network analysis was used to quantify the role of behavioral interactions on the frequency and severity of fin damage in Atlantic salmon, *Salmo salar*, parr subjected to a short feed restriction period of 10 days. Dorsal fin erosion was observed in both feed-restricted (FR) and control (C) groups of fish, but was significantly more frequent and severe in FR groups. FR fish had a significantly lower weight, length and poorer body condition in comparison to C groups. Social networks based on aggressive interactions showed significantly higher overall degree-centrality, clustering coefficients, out and in-degree centralities in FR groups. This led to the formation of clusters of fish into initiators and receivers of aggression. Only the receivers of aggression exhibited dorsal fin damage, while initiators did not. Initiators and receivers of aggression in FR groups retained their roles even after control conditions were restored, suggesting that short periods of feed restriction can lead to permanent modifications in aggressive behavior. The present study demonstrates the applied value of using social network analysis to investigate the longer term effects that aggressive behavioral interactions have on fin damage and welfare in Atlantic salmon.

Keywords

Network, salmon, fin damage, food, restriction, welfare.

Introduction

The potential factors that affect the welfare of farmed fish have been the subject of numerous scientific research and review papers in recent years (e.g. Cañon Jones *et al*. 2010, Ashley 2007, Huntingford *et al.* 2006). Numerous husbandry factors such as handling (Barthel *et al*. 2003) and water quality (Person-Le Ruyet *et al*. 2008) can be detrimental to fish welfare in addition to other factors such as feed availability and feed quality (Ashley 2007). A common operational welfare indicator in fish is fin damage (see Ellis *et al.* 2008 for review) as this represents direct injury to live tissue possessing nociceptors capable of perceiving pain locally that will be integrated centrally and therefore cause suffering (Becerra *et al.* 1983). Fin damage can be caused by direct aggression between fish as confirmed by a number of recent studies in Atlantic salmon, *Salmo salar*, (Cañon Jones *et al.* 2010, Cañon Jones *et al.* 2011a, MacLean *et al.* 2000a). In addition to being detrimental to fish welfare, fin damage may also lead to the colonization of pathogenic bacteria such as *Flavobacterium columnare* at the point of injury and predispose to the development of the clinically important disease of flavobacteriosis (Loch and Faisal 2015). Nutritional and feed management factors known to affect fin damage include: type of diet (Lellis and Barrows, 1997) long periods (30 or more days) of feed restriction (Cañon Jones *et al*. 2010, Damsgård *et al.* 2006, Hatlen *et al.* 2006) and the choice of feed delivery strategy/system, be it a fixed ration feeding or a responsive ration feeding strategy (Noble *et al*. 2008).

Short periods of feed restriction (where fish are not fed to satiation) can occur in farmed fish in a variety of circumstances including: i) when feeding is standardized according to feed tables which do not account for variability in group appetite levels within and between days (Noble *et al*. 2008), ii) when feed delivery systems fail, iii) when environmental conditions or extreme weather situations prevent fish from being fed to full satiation. Fish can also be exposed to short periods of feed withdrawal where they are completely starved of feed such as prior to grading, during transport and during transfer from freshwater to seawater in anadromous species (Lucas and Southgate 2003). Although there is little documented evidence available on how short-term feed restriction or withdrawal periods affect the behavior and welfare of farmed fish, a previous study on Atlantic salmon parr by Cañon Jones *et al*., (2010) documented a detrimental effect of long-term underfeeding (30 days) upon aggression levels and fish welfare.

The present study was designed to elucidate and quantify potential short-term effects of feed restriction on fish behavior and welfare utilizing social network analysis (SNA) to quantify direct and indirect relationships occurring within groups of individuals (Wasserman and Faust 1994) while identifying and quantifying the roles of key individuals (Lusseau and Newman 2004). Social network analysis is increasingly used in applied (Cañon Jones *et al*. 2010, 2011) and ecological (Croft 2005, Croft *et al.* 2004) behavioral studies in fish.

The aim of the present study was to quantify the impact of a short period of feed-restriction on the welfare of Atlantic salmon parr specifically related to aggression and fin damage in relation to changes in the frequency and type of behavioral interactions amongst fish.

Methods

Animals and experimental groups

The experiment was carried out during the summer of 2009 at the Aquaculture Research Station in Tromsø, Northern Norway (Norwegian Animal Research Authority registration number 124, Project Number 6039/09-006.1/H69/32/KNF). Procedures used adhered to current Norwegian Fish Welfare and Laboratory Animals legislation (Ministry of Agriculture and Food of Norway 2010) which follows the European Convention for the Protection of Vertebrates used for Experimentation and other Scientific Purposes (European Union 1998).

Eight groups of 10 clinically healthy year 1+ Atlantic salmon each (61.7±6.4 g of weight and 17.2±0.5 cm of length, mean ± SD) were used in the experiment. The fish were sourced commercially from Aqua Gen A/S, Tribe Standard, generation 2008. Fish were kept at stocking density of 10 kg m^{-3} which is the density used in the Aquaculture Research Station for holding fish at that stage and in accordance with recommended maximum commercial fish stocking densities (50 kg m^{-3}) (RSPCA 2010). This stocking density was chosen as previous studies using the same number of fish per tank had demonstrated that intermediate stocking densities had a greater impact on welfare of fish (Adams *et al.* 1998, Cañon Jones *et al.* 2011b, Turnbull *et al.* 2005). It is recognized that this stocking density may not reflect stocking densities used under commercial production and future studies should aim to reproduce this experiment under such conditions. Three experimental phases were used: 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).

Feed ('NutraParr 3mm', Skretting AS, Stokmarknes, Norway) was delivered at a rate of 1.5% of estimated fish body weight day^{-1} and adjusted weekly according to the expected weight gain and water temperature. Feed was delivered daily at 10:00 hrs for 30 minutes during the whole experiment from calibrated automatic feeders located 1 meter above each tank. After the 10-day pre-treatment period, four tanks were selected as feed restriction (FR) and four tanks as control (C) groups. During the 10-day treatment period, feed was restricted to 1/3 of the calculated daily allocation in FR groups. Feed restriction finished in FR groups at the beginning post-treatment period when feed was provided at 1.5% day^{-1}. Control groups received the full feed ration of 1.5% of estimated fish body weight day^{-1} during the whole experiment. It should be noted that a feeding regime of once daily may not represent a typical feeding regime for Atlantic salmon under commercial production. However, the daily feed amounts were in accordance with manufacturers recommendations for fish of this size and single daily meals at are not uncommon in applied laboratory studies. Whilst this single daily meal may have influenced behavior in the control groups in comparison to fish fed to the multiple meal feeding strategies that can be employed in commercial production, the objective of the study was to evaluate the effect of a comparative reduction in ration size on behavior of fish leading to fin damage in relation to controls and not to optimize feed conversion. Any differences in the behaviors between the treatment and control groups can only be attributed to the reduction in feeding. The single daily meal feeding regime was selected to make the study comparable with previous work that investigated the effect of longer feed reduction periods of 30 days in this species (Cañon Jones *et al*. 2010). No fish mortalities occurred during the study and all fish were euthanized using overdose of benzocaine chlorhydrate (> 250 mg l^{-1} freshwater, Benzoak Vet, A.C.D. Pharmaceuticals SA, Norway) at the end of the experimental period.

Containment and individual identification

Fish were individually tagged whilst anesthetised by immersion in a solution of benzocaine chlorhydrate (100 mg L^{-1} freshwater, Benzoak Vet, A.C.D. Pharmaceuticals SA, Norway) at the beginning of the experiment. All fish achieved full anesthesia within 3 minutes and tagging was carried out during the following minute. Tags were designed to allow individual identification of fish using a combination 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). 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 and no significant effect of tag type 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 after recovery from anesthesia. 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 assisted recovery or veterinary assistance was required.

Housing, water quality and environmental conditions

Fish were housed in 300 L plastic circular tanks (50 cm high and 78 cm diameter). Filtered ambient surface freshwater (300 microns, 9-10ºC) was provided throughout the experiment. Dissolved oxygen content (100.1±0.9 % of saturation) and water temperature (10.4±0.2°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 L minute^{-1} in an open flow system with water velocities of one fish body length second^{-1}. A 24 hour light photoperiod regime was used throughout the study.

Physical measures

Initial and final weights (g) and lengths (total tail-fork length in mm) were measured in each fish. Individual specific growth rates (SGR), feed conversion ratio (FCR) and Fulton's condition factor (K) were calculated for each fish. SGR was calculated as ln $w1$ – ln $w0$)/Δt, where $w1$ was the wet weight of fish (g) at sampling time 1, $w0$ was the wet weight of fish (g) at sampling time 0, and Δt was the number of days between sampling times. FCR was calculated as total feed given (Kg) / fish weight gain (g). K was calculated as $W/L3$, where W was the weight of the fish (g), $L3$ was the length of the fish to the power of 3.

Quantification of fin damage

Damage to 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. Fin damage was quantified using a categorical method for fin erosion. The intensity of fin erosion was based on an ordinal scale of 0 (0% of fin eroded), 1 (1% to 24% of fin eroded), 2 (25% to 49% of fin eroded) and 3 (> 50% of fin eroded) (Cañon Jones *et al*., 2010, 2011). Additionally, fin splits (separation of > 3 mm between fin rays) and other external lesions were quantified at the end of the experiment.

Behavioral observations and social interactions

Behavioral interactions were recorded using CCTV cameras system (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) located 1 m above each tank and connected to a DVD/HDD recorder (Pioneer© DVR-550H-S) located in an adjacent room. Ten-minute 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 feed delivery (11:30 to 11:40). 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.

Associative behavioral interactions

Associative behaviors between fish were recorded at 1-minute intervals for the entire video recording period. A fish was assessed 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.

Aggressive behavioral interactions

Aggressive behaviors were classified as attacks, displacements or fin-bites and quantified using the methods described in Cañon Jones et al., 2010. 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 A directed towards fish B, with fish B 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 A directed towards fish B, with fish B swimming away from fish A (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 A towards fish B accompanied by a rapid escape movement response (to more than one fish body length distant) in fish B in response to the biting. In practice therefore, fish were fully capable of evading aggressor(s) except in the case of biting. The information from the aggressive behavior analysis was used to calculate and compare data relating both to the total amount of aggressive interactions and the sub-classifications of aggressive behaviors (attack, displacement and fin-biting) between experimental groups. 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.

Social network analysis

Social network analysis of the associative and aggressive interaction matrices was carried out using UCINET 6© (Borgatti *et al.* 1999). At the group level, quantified network variables were degree-centrality, clustering coefficient, transitivity, distance and density. At the individual level, quantified network variables were degree-centrality, out and in-degree centralities, clustering coefficients and distances. Detailed explanations of these network variables have been described previously (Cañon Jones *et al*. 2010). Briefly, degree-centrality is a measure based on the number of interactions an individual has with others within the network and represents how central and influential the individual is within the network. In the case of associative interaction matrices, these interactions are always symmetrical and reciprocal and therefore only overall degree-centrality was measured. On the other hand, aggressive interactions could be reciprocal or non-reciprocal and usually non-symmetrical; therefore 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). We then classified fish as initiators or receivers of aggression based on the relative differences between in-degree and out-degree centralities. A fish was classified as an initiator (I) if its out-degree centrality was at least four times greater than its in-degree centrality. A fish was classified as a receiver (R) if its in-degree centrality was at least four times greater than its out-degree centrality. Otherwise, fish were classified as neither I or R (I/R). All centrality measures were calculated as normalized in relation to the total number of individuals in the network and expressed as percentages (Hanneman and Riddle 2005). Density quantifies the amount of interactions between individuals and indicates the cohesion of the network. Clustering coefficient quantifies the extent to which two neighbors of an individual are themselves neighbors. High clustering coefficients suggest that individual fish are surrounded by others that are well connected with each other forming subgroups, sub-populations or clusters within the network. Network distance represents the mean number of connections between the members of all possible pairs of individuals within a network. High distance values indicate fewer interactions between individuals within the network.

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

Structural and spatial position measures

The structure and position of each fish were quantified from the video recordings at 1-minute intervals. Fish were classified as being either schooling or shoaling (Cañon Jones *et al*. 2010, 2011). Schooling was defined as a coordinated behavior where two or more fish were within association length/width and orientated in the same direction. On the other hand, shoaling was defined as an uncoordinated behavior where fish were within association length/width but showed no coordinated orientation and direction (Parrish *et al.* 2002). Additionally, any schooling fish was recorded as being located at the front, middle or back of the school when more than 50% of the fish body length was located either in the first, second or last third of the school respectively.

Statistical analyses

The Shapiro-Wilkes test of normality, descriptive analyses and one-way analyses of variance were carried out on weight, length, fin damage (splits and bites), SGR and K (Zar 2009). A general linear model described by y = a + bx where a is the intercept and b is the slope (effect of treatment) was carried out to clarify the effect of short-term feed restriction on the weight and length of fish (Zar, 2009). The Kruskal-Wallis non-parametric test was used to analyze the effect of tagging system on weight, length and fin damage between experimental groups. Chi-square tests and the Chi-square tests for trends (Zar, 2009) were used to evaluate any statistical differences between treatments in dorsal fin erosion. Correlations between dorsal fin erosion and other variables were analyzed using the Pearson rank correlation (Zar, 2009) and network distance and density were analyzed by one-way analysis of variance (Zar, 2009). Kruskal-Wallis tests were utilized to quantify differences in aggressive behaviors (biting, displacements, attacks and total aggressive behavior) as well as centralities (overall, in-degree and out-degree), clustering coefficients and densities between experimental groups. Mantel tests were carried out for associative and aggressive interaction matrices between pre-treatment, treatment and post-treatment periods in order to evaluate whether any differences would be attributed to statistically significant changes in the behavior of fish rather than by chance (Zar, 2009). All statistical analyses were carried out using R statistical software (R Development Core Team, 2008).

Results

Fin erosion was only observed on the dorsal fin and frequencies were significantly higher in FR compared to C groups (12.5% vs 7.5% of fish affected, $P = 0.03$). Moreover, moderate and severe dorsal erosion was present only in FR groups and not in C groups ($X_{23} = 4.21$, $P = 0.03$) as shown in Table 1. Dorsal fin erosion was positively correlated with the observation of biting in FR groups ($r^2 = 0.70$, $P = 0.02$). No fin splitting was recorded at the end of the experiment irrespective of treatment.

FR groups showed a significantly higher frequency of all types of aggression in comparison to C groups (21.82 vs. 12.32 interactions hour^{-1}, $H_1 = 5.33$, $P = 0.02$). Detailed analysis of the type of aggressive interaction showed a significantly higher frequency of attacks (21.58 vs. 11.68 interactions hour^{-1}, $H_1 = 5.33$, $P = 0.02$) and a tendency for higher biting frequencies (0.31 vs 0.1 interactions hour^{-1}, $H_1 = 3$, $P = 0.08$) in FR compared to C groups, as shown in Figure 1. These results suggest that feed restriction conditions triggered an increase in the frequency of aggressive behavior and that aggression was mainly in the form of 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%, $H_1 = 5.33$, $P = 0.02$), clustering coefficient (0.16 vs. 0.07, $H_1 = 5.33$, $P = 0.02$), out-degree centrality (54.33% vs. 35.69%, $H_1 = 4.08$, $P = 0.04$) and in-degree centrality (15.94% vs. 6.19%, $H_1 = 5.33$, $P = 0.02$) than networks in C groups. Also, the networks in FR groups were significantly more dense (16.07 vs. 6.98, $H_1 = 5.39$, $P = 0.02$) than in C groups. Network distance was lower (1.06 vs. 1.07) in FR compared to C groups while transitivity was high (84.87% vs. 79.93%) in both FR and C groups but no statistical significant differences were observed ($P > 0.05$).

These group-level results suggest that short-term feed restriction induced a particular separation of roles where fish with a specific arrangement of clusters separate 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%, $H_1 = 5.48$, $P = 0.02$). The graphical representation of the separation of roles of fish and clusters of initiators and receivers in the networks is shown in Figures 2 and 3 for C and FR groups respectively. In the FR group, initiators had no dorsal fin erosion but all receivers did (0 vs. 5 fish) but there were no significant differences ($P > 0.05$) in final weight (61.9 g vs. 60.5 g) or length (17.1 cm vs. 17.6 cm) between initiators or receivers of aggression (Table 2).

In addition, linear regression modelling showed differences in degree centralities only in FR groups with clusters of fish with high out-degree ($F_{1.78} = 47.021$, $P < 0.01$) and clusters of fish with high in-degree centrality ($F_{1.78} = 3.85$, $P = 0.05$) allowing the confident differentiation of individuals fish as I or R of aggression as shown in Figure 4.

Fish in the FR groups had lower final weights (60.3 g vs. 64.9 g, $F_{1.78} = 6.6$, $P = 0.04$), lower final lengths (17.4 cm vs. 17.7 cm, $F_{1.78} = 4.02$, $P = 0.04$) and poorer body condition (3.45 vs. 3.65, $H_1 = 5.74$, $P = 0.04$) compared to C groups. In fact, FR groups did not gain but lost weight compared to Control groups (-0.1 g vs. 1.3 g), which was also reflected in the FCR of both groups (1.72 vs. 1.20).

Mantel tests for aggressive interaction matrices between the pre-treatment and treatment periods were significantly different ($P < 0.05$) suggesting fish become more aggressive due to feed restriction. Mantel tests between treatment and post-treatment periods showed no significant differences ($P > 0.05$) demonstrating that once established, fish retain their roles as initiators or receivers of aggression even when full feed rations are restored.

One important aspect in the study is that the time when fin damage occurred (during feed-restriction or during return to normal feeding) was not directly confirmed. Future studies should focus on elucidating when, who and where fin damage occurs. The present study provides the basis for such studies as the regression analysis and Mantel test results strongly suggest that fin damage occurred because of the feed restriction and did not decrease after the return to normal feeding.

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

Discussion

Fin erosion was only observed on the dorsal fin in both experimental groups and was significantly higher in FR groups. Furthermore, moderate and severe dorsal fin erosion was only present in FR groups. These results agree with previous findings of a higher frequency of fin damage in feed-restricted rainbow trout (St. Hilaire *et al*. 2006) and Atlantic salmon (Cañon Jones *et al*. 2010). It is recognized that results from fin damage are limited in number (Table 1 and 2) but they represent strong and novel evidence of fin damage under a short feed restriction period.

FR groups not only exhibited the most severe dorsal fin erosion but also exhibited a trend towards the highest biting frequency suggesting aggression was the most probable cause of dorsal fin erosion in this study. The exact timing of fin damage occurrence could not be determined but the results of the Mantel test strongly suggest that fin damage was the result of aggressive behavior induced by feeding restriction. These results extend previous findings on the effect of a longer 30 day period of feed restriction resulting in the development of fin damage in Atlantic salmon parr (Cañon Jones *et al*. 2010). Taken together with the results of the present study, this provides further support for the hypothesis that dorsal fin damage in salmonids is primarily the result of aggression between fish as has been previously suggested (MacLean *et al.* 2000b, Turnbull *et al.* 1998, Turnbull and Huntingford 2012, Ellis *et al.* 2008). Other factors such as nutritional status and water quality (biotic and abiotic) (Bosakowski and Wagner 1994a, Bosakowski and Wagner 1994b, Ellis *et al*. 2008, Latremouille 2003, Moutou *et al.* 1998) are likely to predispose or perpetuate fin damage that originated from active physical damage occurring between fish rather than by causing the damage *per se*.

The results of the current study suggest that feed restriction increases the total amount of aggressive interactions amongst fish manifested by significantly more attacks and a tendency for more biting events ($P = 0.08$). The lack of statistical differences in the frequency of biting events may be related to the relatively short-term period of feed restriction (10 days) as a prolonged period of feed restriction (30 days) in Atlantic salmon parr has previously been shown to result in significantly increased levels of biting (Cañon Jones *et al*. 2010).

Social network analysis of aggressive interactions revealed that FR groups had higher overall degree-centrality and clustering coefficients, suggesting the presence of key individuals and clusters of individuals initiating and receiving aggression within the network. Detailed social network analysis revealed marked differences in the out and in-degree centrality of individuals in FR groups resulting in fish being classified as either initiators or receivers of aggression. Initiators of aggression were fish that had higher out-degree centrality and lower in-degree centrality and were therefore responsible for most of the aggression but did not receive aggression. As previously reported for longer periods of feed restriction (Canon Jones *et al*. 2010), highly central individuals are more influential within the network and more likely to gain access to 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 and rarely initiated aggressive interactions or retaliated. No statistical differences were found in the network parameters of transitivity, weight or length between initiators and receivers of aggression in the FR groups. A possible explanation for this is that fish were only subjected to a short 10-day feed restriction period rather than a longer period of 30 days where significant differences in physical parameters between initiators and receivers were seen (Cañon Jones *et al*. 2010). This is also supported by previous studies where the effects of aggressive dominance on physical parameters such as weight or length have been shown to require at least 7 days to develop (Huntingford *et al*. 1990). Initiators of aggressive interactions did not have any dorsal fin erosion providing further evidence that initiators may have been dominating feed resources without receiving aggression.

Strikingly, the results from the Mantel test showed that fish did not change their behavior after restoration to control conditions (from Treatment phase to Post-treatment phase). Initiators and receivers maintained their roles within the network even after the restoration of the pre-treatment feeding regime suggesting that a period of feed restriction as little as 10 days can have a lasting impact on behavior and welfare of fish even after the fish resume feeding at full ration. Farmed fish can be subjected to repeated short feed restriction periods during the production cycle such as when feed tables are inaccurate, or when farmers fail to match their feeding practices to changes in daily appetite (Noble *et al*. 2008) and subject to feed withdrawal prior to vaccinations, transport or slaughter. It is possible that the effect of such short periods of feed restriction on behavior and welfare may be cumulative if repeated short periods of feed restriction occur. However, confirmation of this will require future studies.

The use of social network analysis enabled the clear identification of the existence of socially important key individuals in groups of fish but whether these key individuals are responsible for causing dorsal fin erosion requires further research. The current results support the findings of previous studies by Canon *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 feed restriction affects fish behavior and welfare but does not necessarily affect physical or other phenotypic characteristics of the fish.

Feed restriction did not affect the structural distribution of fish in the water column, or their association within the networks. Fish did not appear to prefer to school or shoal and did not show any preference to associate with specific fish within the network. These results are in contrast to previous findings of a distinctive structural (schooling) and association preference in groups of fish subjected to a long period of feed restriction (Cañon Jones *et al*. 2010).

In terms of production performance, fish subjected to reduced daily ration for 10 days were shorter, lighter and in poorer condition than their corresponding controls at the end of the experimental period even after a further 10-day recovery period where fish were fed full ration. This finding further highlights the potentially detrimental effects of short periods of feed restriction on production performance in farmed fish as further discussed by Noble *et al*. (2008).

Conclusions

The present study demonstrated the applicability and value of using social network analysis to understand and quantify the role of short-term feed restriction on the behavior and welfare of farmed fish. The study showed that a short period of 10 days feed restriction can have a profound impact on the behavior of fish, leading to a differentiation of roles within the group of fish resulting in high levels of dorsal fin erosion. This behavior persisted even after the restoration of full feeding conditions. Further studies are needed to elucidate whether the highly aggressive individuals are the ones causing fin damage and also distinguish the effect of previous and current feeding regimes on the occurrence of fin damage under commercial conditions in order to improve our knowledge of the welfare of farmed fish.

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