MANIPULATING AGGRESSION AMONG JUVENILE ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) IN CULTURE CONDITIONS

Kate Greaves

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Division of Environmental and Evolutionary Biology,
Institute of Biomedical and Life Sciences,
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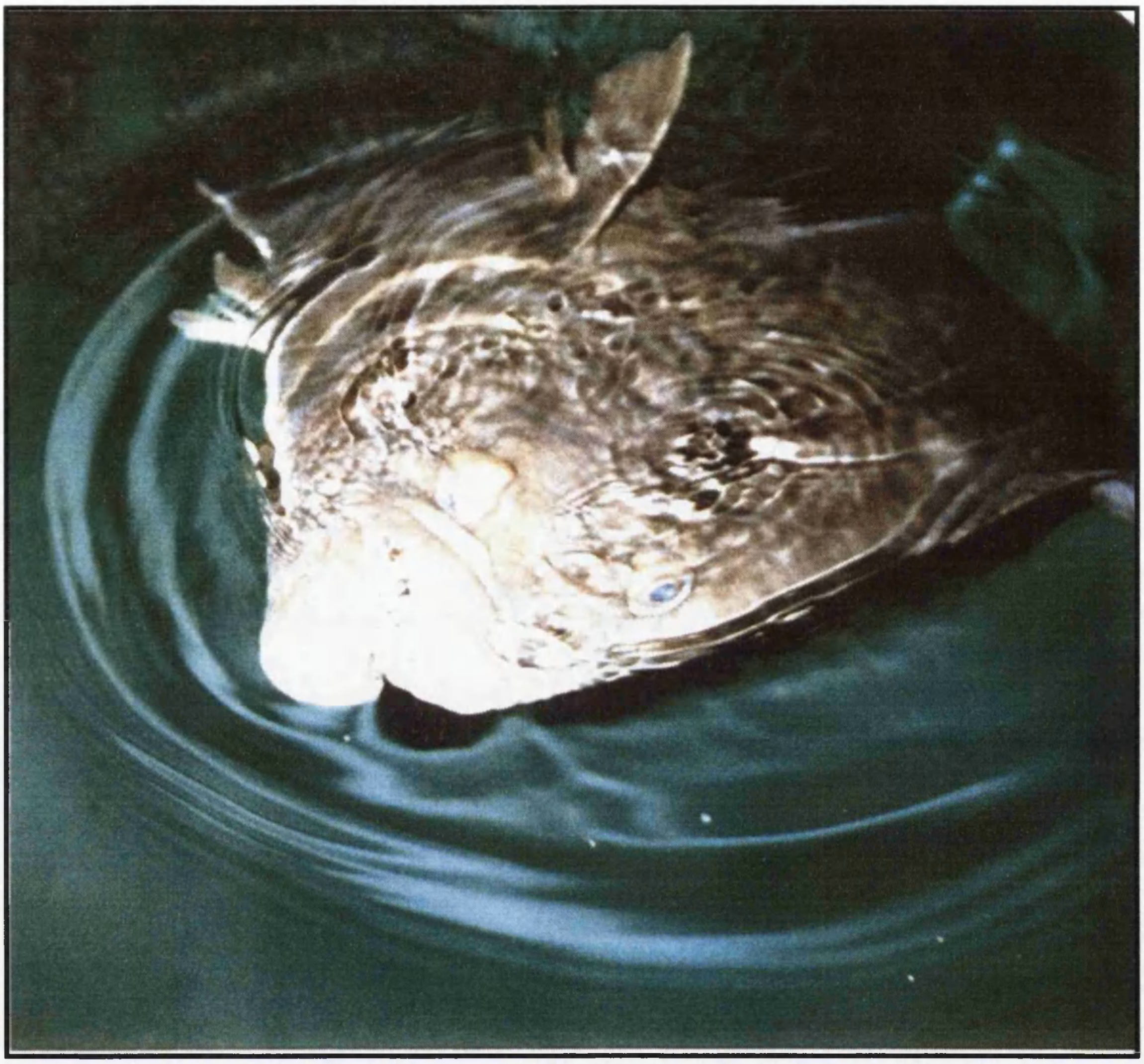

Hippoglossus hippoglossus

For Mum and Dad

Declaration

I declare that the work in this thesis has been carried out by myself unless otherwise cited or acknowledged. The data from halibut in Austevoll (Norway), presented in Chapter 2, was collected by Stig Tuene and jointly analysed for our paper. Otherwise, this thesis is entirely of my own composition and has not, in whole or in part, been submitted for any other degree.

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Chapter 1

General Introduction

1.1 The Atlantic halibut and halibut aquaculture

The Atlantic halibut (*Hippoglossus hippoglossus*) is the largest of all flatfish species, belonging to the family pleuronectidae (right eye flounders). This marine teleost can exceed 3 metres in length and weigh over 300 Kg, the maximum reported age being 50 years. Distribution is widespread, from the Arctic Ocean throughout the North Atlantic and occasionally as far south as Virginia in the USA. Atlantic halibut are typically found along the continental shelf of the sub-Arctic North Atlantic and are common off the Faroese, Icelandic, Norwegian and Canadian coasts (Haug, 1990; Trumble *et al.*, 1993). Adult halibut are associated with deep-water channels running between fishing banks (Bowering, 1986), are most abundant at depths between 200-500 metres but have been found as deep as 1000 metres.

Halibut exhibit sexual dimorphism, with males maturing between 4-5 years of age (from 1 Kg), while females mature later between 7-9 years (from 12 Kg, Bromage *et al.*, 2000). Adult halibut are largely solitary, but aggregate on spawning grounds between November and March to reproduce. Eggs and sperm are released into the water column and, once fertilised, the neutrally buoyant eggs drift until they hatch around 85 degree days (some 18 days later, at 5°C). Atlantic halibut eggs are 3mm diameter, considerably larger than most marine teleosts, and contain a large yolk reserve. Hatched yolk sac larvae are incapable of exogenous feeding, having no functional eyes or mouth, and larvae are unable to take prey until 220-290 degree days post-hatch when they begin to feed on marine zooplankton. Development is slow at such low temperatures and the pelagic phase is believed to last for 6-7 months (Trumble *et al.*, 1993).

Data on the early life history stages of this deep-water species in the wild are scarce, but larvae are thought to become demersal before completing pigmentation and metamorphosis (where the left eye migrates around the head to the right side). Larvae appear to settle in well-defined coastal nursery areas (20-60m depth) with sandy substrate (Haug and Sundby, 1987). Two such areas are off the Faroe islands and in Faxa Bay on the Icelandic west coast (Sigurdsson, 1956). Juvenile halibut (<30 cm) feed almost exclusively on invertebrate prey including hermit crabs, mysids and prawns (Haug, 1990). However, as fish grow the proportion of teleost fish in the diet increases and, beyond a length of 70cm, the halibut diet consists of mainly pelagic fish (redfish, pollock, haddock, sand-eels, herring and capelin), as well as some flatfish, including smaller halibut (McIntyre, 1952). The inclusion of conspecifics in the diet has also been seen in the closely related Pacific halibut (*H. stenolepis*). Best and St-Pierre (1986) analysed 250 Pacific halibut stomachs, of which 7% contained smaller conspecifics. Indeed, cannibalism has been directly associated with piscivory (Hunter and Kimbrell, 1980), and the majority of predatory fish are cannibalistic (Davis, 1985).

Halibut is a highly valued species, and can be seriously affected by over-fishing due to slow growth rate and the late onset of sexual maturity. Commercial catches have steadily declined from 10,000 tonnes world-wide in the mid-1980s to less than 3,000 tonnes in 2000. Research interest in the farming of this species began in Norway in 1985 when two weaned halibut were produced (Olsen *et al.*, 1999). A comprehensive scientific research programme followed in 1987 and over the past two decades has expanded to commercial production in Iceland, Norway, Scotland, Canada and now Chile.

Farmed juveniles can be weaned from live prey to an inert manufactured diet at a weight of 0.1-0.5 grams. Metamorphosis typically occurs between 50-80 days post-hatch at a total larval length of 34-47 mm (50-100 mg wet weight), giving a large size range within a sibling batch. Juveniles remain in nursery facilities for a further 6-9 months before transfer to larger on-growing tanks or sea cages (from 100 grams), and attain harvest weights between 2-6 Kg after another 24-36 months (Bromage *et al.*, 2000). Despite this lengthy production cycle, halibut is an attractive species for northern aquaculture. It commands a high market price (currently up to three times that of Atlantic salmon) and grows year-round in cool northern waters. Existing salmon tank facilities have needed little modification for halibut on-growing, whilst the addition of a tensioned net base has been required for cages. Because halibut is demersal, cage sites need to be very sheltered to minimise cage motion. Such sites have become less economical for salmon due to low biomass restrictions related to environmental carrying capacity. As halibut has a much higher value, these sites have become more economically attractive for this species, encouraging some salmon farmers to diversify.

To date, the reliable production of juveniles has been the principal constraining factor in the development of halibut aquaculture. Although this is now being overcome with increasingly successful rearing techniques, juveniles up to around 5g weight remain susceptible to two diseases. Nodavirus and infectious pancreatic necrosis (IPN) have both been identified as the cause of major mortalities in hatcheries in recent years. Fortunately, it appears that outbreaks can be prevented by the filtration and UV treatment of incoming water, and by keeping rearing temperatures below 10°C. With large numbers of juveniles now being produced for on-growing, new constraints have been emerging in this later phase.

Early experiences of on-growing on a commercial scale have resulted in excellent growth rates, but the mortality/loss figures particularly in the early stages (<300g) have been disappointing. Losses from 100g to 1.0 Kg have been recorded from 3-20% in individual cage populations (D. Mitchell, pers. comm.), whereas losses from 1.0 Kg to harvest size have been low, typically 1-3%. Given that the majority of recovered mortalities have suffered from eye loss, intraspecific aggression is suspected.

1.2 Aggression and aquaculture

Aggressive individuals frequently benefit from their behaviour by acquiring a disproportionate amount of food, thereby growing at a faster rate than social subordinates. In aquaculture systems, intra-specific competition of this kind exacerbates variability in growth rates and increases the size distribution of fish over time (*Salmo gairdneri*, Abbott & Dill, 1989; *Oncorhynchus mykiss*, McCarthy *et al.*, 1992; *Oncorhynchus keta*, Ryer & Olla, 1996). Furthermore, as dominant fish monopolise food, subordinates may be inhibited from feeding (Abbott & Dill, 1989; *Salmo salar*, Metcalfe *et al.*, 1989).

In many fish species, variation in growth rates is linked to social dominance hierarchies based on body size and/or territoriality (Noakes and Leatherland, 1977; Doyle and Talbot, 1986). In general terms, larger individuals tend to be dominant and aggressive, and smaller fish are often subordinate. Large body size is recognised as being an important factor in determining the outcome of conflicts. There is a continuing debate, however, whether large fish become dominant by virtue of size or aggressive fish become large by growing faster because of increased access to food. Huntingford *et al.* (1990) showed that in Atlantic salmon (*Salmo salar*) large body size is the consequence, rather than the cause, of social dominance, although studies in rainbow trout have suggested that the competitive advantage of large body size declines with increasing group size (Petterson *et al.*, 1996). These discrepancies may be due to environmental conditions.

Social hierarchies mediated by aggression have far-reaching consequences. Subordinates suffer chronic stress by having restricted access to food and being under constant threat of attack from behavioural dominants. Injured fish incur added energetic and metabolic costs in terms of tissue repair (Abbott & Dill, 1989), and injuries resulting from aggressive interactions also increase risk of disease (Turnbull *et al.*, 1996). Subordinate fish that cannot compete directly with dominants may choose to adopt alternative strategies. They may avoid aggression and reduce the risk of injuries by feeding opportunistically at different times of day or at different locations (Kadri *et al.*, 1997, post-smolts (*Salmo salar*), Adams *et al.*, 1998, salmon parr (*Salmo salar*)). Although these fish feed less often and more erratically as a result, there may be a trade-off between slower growth and avoiding competitive encounters with dominants. In this way they can continue to grow, albeit at a reduced rate.

An extensive literature on aggressive behaviour has shown considerable variability in levels of aggressiveness between individuals. This may be explained from a cost-benefit perspective, as being aggressive incurs possible risks of injury to oneself (Huntingford and Turner, 1987).

While it is generally accepted that subordinates suffer more from social stress than dominant fish, recent studies indicate that social dominance incurs subtle costs too. Creel *et al.*, (1996) found elevated levels of glucocorticoid stress hormones in two carnivorous species with complex social structures: dominant dwarf mongooses (*Helogale parvula*) and African wild dogs (*Lycaon pictus*). Agonistic interactions among fish are likewise energetically and metabolically costly (Li & Brocksen, 1977; Metcalfe, 1986). Noakes and Leatherland (1977) demonstrated an energetic cost to agonistic behaviour and activity in the form of elevated renal activity, indicative of stress. Their studies with rainbow trout (*Salmo gairdneri*) showed that mid-ranking fish in the hierarchy had the lowest inter-renal activity, suggesting that a mid-ranking social position was less costly. In their study of the social behaviour and growth of the carnivorous goby, *Odontobutis obscurus*, Yamagishi *et al.* (1974) found that dominant fish did not grow as well as second ranking fish. They surmised that the dominants expended more energy maintaining their social positions and territories, and suggested that aggression itself was stressful. Social dominance may incur additional costs such as missed feeding opportunities and increased energy expenditure due to swim acceleration when initiating attacks (Metcalfe, 1986; Nicieza and Metcalfe, 1999).

In the natural environment, aggressive behaviour serves to exclude conspecifics from preferential feeding areas and can limit their foraging success. Atlantic salmon parr are known to be highly aggressive, and compete intensely over food, shelter and territories (Cutts, Metcalfe and Taylor, 1998). Metcalfe *et al.*, (1989) used direct observations to show that aggressive dominant fish monopolised the food supply by occupying the best feeding stations, and also demonstrated higher competitive ability when contesting food. This can have important consequences for life history patterns. Faster growing individuals enter the upper modal group (UMG) and achieve earlier seaward migration than lower modal fish which remain another year or more in fresh water. Upper modal fish would appear to have the more successful life-history strategy, and feed aggressively to achieve the threshold size for migration. However, this strategy is not without cost. Nicieza and Metcalfe (1999) found that salmon in the upper modal group (UMG) were more aggressive than lower modal group fish (UMG), but were also more often attacked themselves. Therefore, faster-growing fish suffered significantly higher aggression rates from fellow UMG fish than slower growing LMG salmon. Understanding the natural ecology of the species has, therefore, shed light on the reasons that aggression prevails among cultured salmon parr. To date, however, there is no knowledge about aggressive behaviour in wild Atlantic halibut juveniles that could provide similar insights into the aggression observed in culture systems.

Given that Atlantic halibut is a deep-water species, the culture environment is highly artificial. Fish are kept in shallow tanks or cages, confined in small areas and farming economics dictate crowding at high densities. Moreover, because the majority of farmed juveniles are still the progeny of wild caught broodstock they are relatively undomesticated. Aggression appears to present a significant problem in the rearing of this species and is particularly acute during the nursery stage between 20 – 150 grams weight. In culture, the function or motivation for aggressive behaviour is unclear. Food is provided in excess, and is widely distributed to prevent monopolisation by a few dominant individuals. The environment is simple with no apparently preferential territories. However, competitive interactions between fish can affect variation in growth rates, even in well-managed environments when feed is available in abundance. Purdom (1974) found that the effect of the dominance hierarchy on heterogeneous growth persisted in plaice/flounder hybrids despite feeding them to excess.

Aggressive behaviour under culture conditions is likely to be a maladaptive response to stress of the artificial environment. This thesis concentrates on the behaviour of juveniles in culture where aggression-related problems in production have come to light. The objective of this research was to identify environmental variables that influence levels of aggression, with an aim to improve rearing conditions by manipulating these factors accordingly.

1.3 Outline and aims of Thesis

When are halibut aggressive and how is aggression manifest? There are few published accounts of the behaviour of juvenile flatfish in general and Atlantic halibut specifically. At the beginning of this project, there was no documented information on the form and context of aggressive behaviour in this species. Chapter Two describes aggression in Atlantic halibut across several age classes, providing information on when aggression occurs and the nature of agonistic interactions between halibut in culture conditions.

Many studies have ignored or belittled the influence of social behaviour in cultivated fish species. When fish behaviour has been considered, it has all too often been oversimplified at the population level, and differences in the behaviour of individuals have not been taken into account. How do aggression and social relationships affect individual feed intake and growth? How do subdominant fish cope? Data presented in Chapter Three comes from an experiment involving small groups of six halibut. This study examined the impact of aggression and social relationships between fish on feed intake and growth, and identified alternative feeding strategies adopted by social subordinates that could or would not directly compete with dominants.

A primary aim of finfish aquaculture is to maximise the biomass yield while incurring minimal costs to the producer (Purdom, 1974). Effective production entails the efficient use of facilities and food, and will determine rearing densities (Jobling *et al.*, 1995). However, several studies have demonstrated that stocking density can have a major impact on fish behaviour and performance (Wallace *et al.*, 1988; Brown *et al.*, 1992; Hecht and Uys, 1997). Alanärä and Brannäs (1996) found that several individual Arctic charr monopolise the food when held at low density (less than 20Kg/m^2). They attributed the resulting depressed growth to strong social hierarchy, corroborating other studies (Christiansen & Jobling, 1990; Brannäs & Alanärä, 1994; Wallace *et al.*, 1998). At high densities social hierarchies become less stable, presumably because repeated attacks on the same individuals and the defence of favourable territories cannot easily be maintained (Kalleberg, 1958; Fenderson & Carpenter, 1971). Does stocking density similarly affect levels of aggression among weaning halibut, and what are the consequences of aggression to recipients?

The experiment described in Chapter Four examined the effect of three stocking densities on aggressive behaviour in weaning Atlantic halibut. Behaviour throughout the weaning phase was studied in detail, and the frequency of aggressive interactions per minute of observed time was recorded at each density. In addition, each fish was examined at the end of the experiment, and scored for the location and severity of any injuries. Video footage of observed aggression at each density confirmed that the three body areas showing the most damage (ventral, dorsal and caudal fins) were also most frequently targeted.

Throughout the course of this study, halibut culture in Scotland has progressed from research to commercial scale, and the occurrence of fish with eye injuries has emerged as a genuine concern. Some on-growers have attributed mortality levels of up to 10% to eye injuries, which clearly represents a major hurdle to the successful on-growing of this species. Given that it had already been established that aggression was the primary cause of injury, the production-scale trial described in Chapter Five was designed to answer two specific questions. Can size-grading juvenile halibut control aggression levels and significantly reduce the incidence of eye injuries? How do eye injuries affect individual halibut growth rates? Three control populations were established, representing the normal size range in standard production tanks, and their growth and injury levels were compared with three more tightly graded populations over a four-month period. Population level data was complemented by data on individual fish, made possible by the use of two marking systems. This method made it possible to measure the effect of eye injuries and eye loss on the growth of individuals; provided insights into the development of eye injury over time, and the ability of fish to recover. The implications of eye injury-related losses to halibut producers in terms of economics, fish welfare and product quality are also discussed in this chapter.

Are farmed halibut cannibalistic? There is a growing body of evidence to suggest the occurrence of cannibalism in farmed halibut, supported by early research into the stomach contents of wild fish where smaller conspecifics have been recorded (McIntyre, 1952; Kohler, 1967). Although this was not studied specifically, cannibalism among farmed carnivorous fish species is widespread, and it was considered important to draw together data from cultured halibut that infers its occurrence in this species. Chapter Six relates strong anecdotal evidence from halibut production sites and experiments to documented cannibalism in many other farmed animals and fish.

The closing chapter of this thesis summarises the findings of the whole project and relates them to issues of commercial importance in the rearing of this species.

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Chapter 2

The form and context of aggressive behaviour in farmed Atlantic halibut (*Hippoglossus hippoglossus L.*)

2.1 Abstract

Physical damage among farmed Atlantic halibut, comprising injuries to eyes, pectoral fins and tails, first becomes apparent post-weaning. This study examined social interactions in farmed halibut to determine whether potentially serious aggression occurred in fish of different age classes in which injury had been reported. Specific aims were to understand the context in which such behaviour happens and to examine individual variation in aggressiveness.

Behavioural data were collected via direct observation and video footage from halibut of 6 size classes in Norway and the UK. Food was delivered to experimental tanks one pellet at a time to enable the identification of consumers and the recording of all social activity. In 5 size classes (45g - 3000g), potentially damaging contact among fish occurred solely during feeding and comprised targeted aggression (nips and chases) and collisions due to misdirected feeding attempts. Feed intake was positively correlated with levels of aggression, and fish were most aggressive early in a feeding session. A mean 85% of attacks were directed at fish that had won pellets from

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aggressors. The frequency of aggression declined markedly with fish size, and no aggression was observed in the largest size class (2.5 – 10 Kg).

Our studies indicate that the majority of aggression occurs early in hand feeding sessions, when fish are particularly hungry. To reduce the occurrence of this behaviour, we suggest that feed should be delivered by a combination of hand and automatic feeders. In this way, feed can be well-dispersed and provided throughout the day, thereby preventing a build-up of hunger levels and ensuring that fish are fed to satiation.

2.2 Introduction

The production of Atlantic salmon in both Scotland and Norway has grown steadily over the last twenty years, and aquaculture has become a major industry and source of employment for many outlying communities. However, salmon is no longer considered a luxury food item, and increased tonnages and intense competition among producers have reduced prices in recent years. As a consequence, there has been considerable interest in diversifying the range of species for cold water marine aquaculture. Atlantic halibut is a high-value fish, capable of commanding up to four times the market price of salmon. Native to the Arctic and Atlantic oceans, halibut grows well year-round in the ambient waters of Scotland, Norway and Canada. For these reasons, much concerted effort has recently been made in the intensive rearing of this species.

A consistent finding by researchers and producers is that there is a significant incidence of injury early on in the culture process, i.e. from weaning (0.3 grams weight). Weaning is defined as the transitional period when a live prey of enriched artemia and copepods is replaced by an inert manufactured crumb diet. Fish may lose weight at this time while they learn to accept a new diet, and nipping and physical damage to some individuals is first observed at this stage (M. Spreadborough pers, comm.; Greaves, unpublished data). Injuries are sustained to the eyes, tails, and pectoral fins. In some cases, cannibalism has been reported. Badly bitten fins and tails can invite secondary bacterial infection, and eye injuries in such young fish are cause for concern as fish become stressed, feeding is impaired and mortality may occur. It is now estimated that 3-5% of halibut juveniles (150 grams weight) have eye injuries, and up to 30% have tail and/or pectoral fin damage. The nature of these injuries suggests that the cause is unlikely to be handling or tank contact, but interactions between fish, possibly of an aggressive nature. Aggressive interaction, i.e. behaviour that inflicts non-accidental injury on other animals (Huntingford and Turner, 1987) is widespread in the animal kingdom and common among fish. This is well-documented and occurs in a variety of contexts, but mainly where fish are contesting limited resources, such as food (Magnuson, 1962; McCarthy *et al.*, 1993). A common finding of aggression studies in farmed fish is that individuals vary widely in the extent to which they use aggression to acquire resources (Shelbourne, 1964). Marked aggression among juveniles has been documented in halibut (Ottesen and Strand, 1996), and for other flatfish species: plaice (Shelbourne, 1964); greenback flounder (Carter *et al.*, 1996) and sole (Howell, pers. comm.).

The level and intensity of aggression in any given species depends on a balance between the advantageous and disadvantageous consequences of this behaviour for the individuals concerned (Krebs and Davies, 1987). Aggression can be minimised in farmed fish by increasing the costs or reducing the benefits associated with this behaviour (Christiansen and Jobling, 1990; Grand and Grant, 1994). However, before adopting this approach, one must understand what fish are fighting over and why aggression arises.

Few data are available on the activity or distribution of wild halibut, though it is known that individuals spend up to four years in coastal nursery grounds (Haug, 1990; Trumble *et al.*, 1993). They are essentially solitary fish, and conditions prevalent in hatcheries and on-growing facilities are in stark contrast to their natural environment. High stocking densities in tanks may increase stress and induce aggressive interactions. On the other hand, if fish are held at artificially high densities, injuries may be the result of accidental collisions during feeding rather than of targeted aggression. Given that these interactions are not mutually exclusive, the purpose of this study was to examine halibut behaviour in culture conditions and to identify any behavioural interactions that could cause the kinds of injuries seen in farmed fish. The specific aims were: to examine social interactions in farmed halibut to see whether potentially injurious aggression occurs in the different size classes in which injury has been reported; to understand the context in which such behaviour happens; and to investigate individual variation in the level of aggressive behaviour.

2.3 Materials and methods

2.3.1 Study sites and culture systems

The data described in this paper were collected from farmed halibut of different origins and various ages held in a range of husbandry systems in the UK and Norway. The UK site was a halibut on-growing farm (Marine Harvest McConnell, Scotland), and Norwegian data were collected at the Austevoll Aquaculture Research Station. The UK fish originated from wild-caught Icelandic and Faroese broodstock; Norwegian fish were hatchery-reared in Norway. They were hand-fed daily to apparent satiation (defined as the time when no more fish rose towards the surface when food was offered; McCarthy *et al.*, 1993). Experimental fish in Norway were held in 2-3 metre diameter tanks and hand fed once per day on either dry or moist pellets. Fish of 50-150 grams (size class 2) were maintained on heated water (13°C), larger fish were held in ambient conditions (6-9°C).

2.3.2 Collection of behavioural data

Size classes and experimental conditions are defined in Table 2.1. Data were collected using established methods of behavioural recording (direct observations and video footage, Bateson and Martin, 1995), and analysed to provide detailed descriptions of behaviour patterns in farmed halibut. Behavioural data were collected from halibut juveniles in Scotland (mean weight 45 grams, size class 1), and from halibut of 100-5250 grams (size classes 2-6) conducted in Norway.

In Scotland, 15 of the 35 fish were panjetted with Alcian Blue dye on the ocular side such that each fish could be identified by sight. Selected fish met certain criteria:

complete eye migration, no mouth or skeletal deformities and no physical damage at the outset. Food was delivered to a specific area of the tank, one pellet at a time, in order to register the identity of consumers and all social activity. Feeding continued until 10 uneaten pellets were visible on the tank base and fish showed no further interest in feeding. Following an acclimation time of one week, fish were filmed from two perspectives simultaneously: via a top view camera, and another recording through an observation window. Feeding sessions were recorded in full, and fish were filmed for 10 minutes at two hourly intervals throughout the rest of the day.

Aggressive interactions were also recorded in five experiments conducted in Norway using the registration methods of Tuene and Nortvedt (1995). Fish were fed once a day and individuals carried tags that could be identified from the tank side. Feed pellets were delivered one by one and feed intake and behavioural data were registered manually on a portable computer. Time was automatically recorded at each input. Although aggression was not registered every day in these experiments, fish were always fed to satiation, and the duration of the feeding session for each tank ranged from 10 to 45 minutes.

Table 2.1: Experimental conditions for behavioural studies with halibut.

Halibut size class	Mean weight / total length (g / cm)	No. of fish per tank	No. of tanks	Total days duration	Tank diameter (m)	Mean temperature (°C.)
1	45 / 16	35	1	14	1	13
2	101 / 21	18	8	28	2	12.4
3	391 / 33	14	12	32	2	8.6
4	1321 / 45	16	12	22	2	6.5
5	1690 / 50	14	9	14	2	7.5
6	5250 / 70	12	9	14	3	6.5

2.4 Results

2.4.1 The behaviour of Atlantic halibut in tanks

The behaviour of Atlantic halibut was observed in tanks and was categorised as: aggression (A), feeding (F), stress response (S) or neutral (N). An ethogram describing these behaviours is given in Table 2.2 (after Shelverton and Carter, 1998).

2.4.2 The relationship between aggression and feeding activity

The majority of physical interactions occurred during feeding sessions. At this time, fish came into potentially damaging proximity when several targeted the same pellet. Two types of behavioural interactions were distinguished: accidental collisions and targeted aggression. Non-targeted collisions associated with feeding activity occurred when two or more fish clashed while striking at a pellet (a lunge and suction action), the momentum of the feeding movement presumably making strikes difficult to abort. The force of impact was sometimes hard, and head to head clashes dramatic. Fish often reacted by fleeing the immediate area at high speed. At other times fish targeted a pellet, but sheered away on the approach swim if another fish contested it. This was interpreted as avoidance behaviour where the fish had time to change course to prevent physical contact at the cost of losing a pellet. Some behavioural patterns were clearly directed at other fish rather than at food pellets. Such targeted aggression included nipping, where the attacker bit the fins of other fish, and chasing where conspecifics were pursued around the tank. This nipping could clearly be distinguished from missed feeding attempts as there was often an associated delay between pellet consumption and subsequent biting.

Table 2.2: Ethogram of observed behavioural units in juvenile Atlantic halibut. Behaviour is categorised as to aggression (A), feeding (F), neutral (N) or stress response (S).

Number:	**Behaviour:**	**Description:**
1 (A)	Approach lunge	A forward movement of one fish towards another either while swimming or in contact with the tank base. The aggressor normally accelerates.
2 (A)	Nip	Brief contact with the mouth onto targeted fish. Fish normally accelerates as it executes the nip.
3 (A)	Bite	Contact of upper and lower jaw on to target fish – usually causing injury. Occasionally the bite is prolonged.
4 (A)	Chase	Aggressor pursues another fish around the tank. This is often followed by nipping or biting.
5 (A)	Posturing	A threat posture by the aggressor. The fish arches the head and mid-body high off the tank base and inclines the head forward, forming an 's' shape. Eyes are normally fixed on the target. Fish attempt to tower above the other fish, opercula often flared.
6 (A)	Flee	Response to aggression. Rapid swimming or darting away from an aggressor.
7 (F)	Ingestion	Feeding action. Fish moves forward for a pellet and simultaneously draws the material into the mouth. At speed, the fish lunges for the food.
8 (F)	Collision	Accidental impact of two or more fish targeting the same item of food.
9 (F)	'Miss'	Failed feeding attempt where fish fail to capture a targeted pellet.
10 (F)	Veer	Avoidance behaviour to prevent collision. Fish changes course suddenly.
11 (N)	Burying	Series of rapid tail and dorsal/anal fin undulations against the tank base, (resulting in burying in sand substrate).
12 (N)	Ascent	Fish swims vertically up through the water column, usually for food items.
13 (N)	Cruising	Unidirectional swimming throughout the water column, maintaining a slow speed. Pectoral fins are held perpendicular to the body and used as a steering aid.
14 (N)	Hover	Fish maintains a stationary position mid-water by gently undulating dorsal, anal and caudal fins.
15 (N)	Turn	Change of direction when swimming or hovering – can be rapid.
16 (S)	Surface spit	Fish hangs vertical at the surface, raising its head repeatedly and ejecting water from the mouth.
17 (S)	Burst swimming	Fish swims very rapidly around tank – sometimes hitting the side or colliding with another fish. Fish also often breaks water surface.
18 (S)	Surface circling	Halibut swim in very tight circles at the water surface, ocular side innermost, head raised out of the water.

Aggressive interactions occurred exclusively during feeding, and at other times there was little activity or social interaction. In size class 1, 390 minutes of video footage was recorded, of which 148 minutes (38%) comprised feeding sessions. The relative frequency of targeted versus non-targeted physical encounters in size class 1 was 23% and 77% respectively. During feeding eleven fish were involved in physical encounters (bodily contact) with conspecifics. These interactions were divided into overt aggression (bites, nips and chases) and contests for pellets (where 2 fish both targeted the same pellet and one only just out-competed the other). In these latter cases, the fish were so close that they were unable to avoid contact. There was a significant relationship between targeted aggression and contests over pellets (Pearson's correlation $Rs = 0.76$, $N = 11$, $p<0.01$), showing that the most overtly aggressive fish were also highly competitive.

In the remaining observed sessions, only targeted interactions were quantified. Within feeding periods, the majority of targeted aggression occurred at the beginning, and individuals were aggressive early on in their feed intake (Figure 2.1). Although aggression levels were variable between groups in size class 2, 55% of all aggressive attacks occurred in the initial five minutes of a feed session, and a further 20% during the next five minute period. This trend was evident among all size classes examined here. The number of targeted aggressive acts recorded during feeding is shown in Table 2.3. More than half of all the aggressive acts in size class 2 were initiated by individuals that at the time had not yet eaten a single pellet. In order to elucidate the relationship between aggression and feeding, the time of the aggressive act relative to competition over pellets was examined for all size classes (Table 2.4). A mean 85%

of attacks were made against fish that had won pellets from the aggressors and the percentage of attacks that occurred directly after the contested pellet ranged from 42% in size class 5 to 92% in size class 1.

2.4.3 Variation in levels of aggression

Within size class 1 (35 fish) it was clear that some fish were more aggressive than others. Of the 11 fish involved in physical contact (comprising both overt aggression and accidental collisions while competing for pellets), 4 halibut were responsible for 31% of all encounters and for 59% of elevated aggression during feeding. The levels of aggression recorded in our experiments decreased markedly with increasing fish size (Table 2.3). The number of attacks registered for size class 1 halibut (45g) was more than double that for size class 2 fish (100g), and diminished further in the remaining size classes.

2.4.4 Consequences of aggression for victims

In the above experiments, the consequences of the aggressive acts for the victims seemed minor. Only 2 of the 160 aggressive acts registered in size classes 2-5 resulted in the attacked individual letting go of the pellet it had taken (both in size class 2). In size class 1, only one bite was deemed severe, causing the fish to break the water surface as it fled the attacker. No eye injuries were recorded in any of the groups throughout the experimental periods.

Figure 2.1: Aggressive acts recorded during feeding periods over 5 days in size class 1 halibut (mean weight 45 grams). All aggression was targeted.

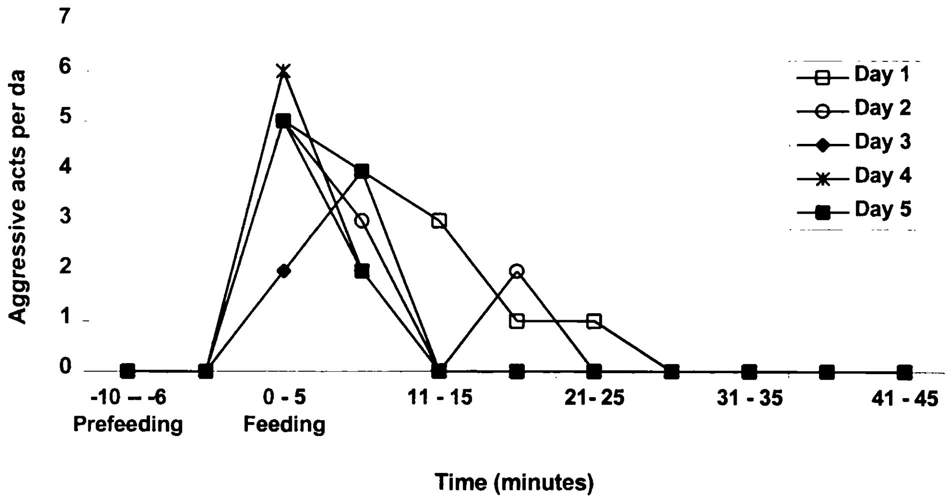

Table 2.3: Targeted aggressive acts recorded during feeding in halibut of six size classes. 80% of the aggressive acts in the above experiments occurred immediately after contest for a pellet. The recipients of aggression were targeted after they had won feed pellets from aggressors.

Halibut size class	Weight Range (g)	No. of fish per tank	No. of days observed	Total aggressive acts	Mean aggressive acts / fish / day
1	33 - 55	35	5	45	0.260
2	50 - 150	18	6	91	0.108
3	200 - 600	14	10	56	0.035
4	500 - 3000	16	5	5	0.005
5	1200 - 3000	14	10	12	0.009
6	2500 - 10000	12	10	0	0.000

Table 2.4: The relationship between contest over pellets, aggression and attack time in halibut. Attacks listed as 'directly after a pellet' were registered after that pellet had been consumed and before the next pellet was eaten. In cases where attacked recipients had not taken any of the 3 pellets preceding the aggressive act, attacks were classified as 'unrelated'. The total number of aggressive acts that were possible to classify in these ways is also shown (N).

Halibut size class	Attacks directly after pellet (%)	Attacks 1 pellet later (%)	Attacks 2 pellets later (%)	'Unrelated' fish attacked (%)	Total attacks (N)
1	92	3	0	5	38
2	74	7	1	18	88
3	83	4	0	13	47
4	60	20	0	20	5
5	42	25	16	17	12

2.5 Discussion

The primary aim of these preliminary behavioural studies was to describe social interactions of farmed halibut, and to determine the extent to which injurious aggression, as opposed to misplaced feeding actions, occurred. Atlantic halibut were found to have aggressive encounters that, although too brief to be labelled "fights", could cause injury. These were recorded in a range of age and size classes in different husbandry systems in Norway and the UK, suggesting that this behaviour is a consistent characteristic of the species and not due purely to environmental conditions or system design.

This work has shown that injurious aggression among halibut larger than 30g is prevalent when fish are actively feeding, and that this behaviour relates to resource competition. In our studies between 80 and 95% of all attacks were directed at fish that won pellets from attackers. Many victims were often aggressors themselves (i.e. those fish actively competing over food). Nipping and chasing were frequently observed immediately after one fish (the attacker) had been out-competed for food by another (the recipient of the subsequent aggression). During such interactions, both fish often missed out on further feeding opportunities. Attacks directed at fish that had not won pellets from aggressors may have been cases of mistaken identity as a consequence of crowding in the feeding area.

These findings are likely to have been influenced by temperature, age and metabolic rate. Gut transit time and the return of appetite would have been faster among small halibut held at high temperatures than for the larger fish held in cooler water. In addition, feeding fish just once a day would have generated different hunger levels among size classes, with small fish being most hungry at the start of daily feeds. The absence of aggression in size class 6 may be attributed to the fact that fish of this size do not all necessarily feed on a daily basis. Therefore, both hunger and competition would diminish. Taking account of these factors means that we cannot here prove that smaller, younger halibut are more aggressive than larger fish, though we believe this to be the case.

Although fish densities are low in the experiments presented here, experience from feeding halibut groups at high densities support some of our conclusions. Thus, aggression, collisions and activity are highest at the start of feeding and much higher during feeding sessions than at other times (Greaves, unpublished observations). However, aggression seems to be less frequent at higher densities and is also probably less precisely directed; the mode of feed delivery in our experiments (one pellet at a time) may well have exacerbated competition and aggression.

Data from our studies are consistent with the incidence of physical damage reported for all different halibut size classes on commercial farms. Halibut appear to be most aggressive up to around 100 - 150 grams weight, and particularly so in the nursery phase. An estimated 20 – 30 % of farmed stocks have bite damage to the upper pectoral fins and tails. Although there was no eye damage among our study fish, eye injuries are now a major concern for halibut on-growers as the production of juveniles increases. Given that eye injuries have such profound consequences in terms of growth, market value and fish welfare, on-growers are investigating the influences of stocking density, light levels, size grading and feed regimes in an effort to curtail this problem. Current estimates from Scottish on-growing sites of juveniles with at least one eye removed or damaged are in the range of 3-5%. Once fish exceed this size range, aggression is rarely cited as a major issue in culture conditions.

Putting these results in a cost benefit context, where food resource is limited, possible benefits of aggressive behaviour could be that competitors are driven off and immediate competition reduced. On several occasions fish exhibited avoidance behaviour, where they conceded pellets by veering off course to avert collisions. Such behaviour has previously been reported for Atlantic salmon parr by Metcalfe (1989), and in halibut (Davenport *et al.*, 1990). In a study of Atlantic salmon parr that involved the serial removal of dominants, Adams *et al.*, (1998) found a significant relationship between high feed intake and received aggressive attacks. This showed that there was a cost to feeding for these fish, as fish that were neither aggressive nor competed for food were attacked less often. In coho salmon high levels of aggression similarly correlated with high feed intake (Ejike and Schreck, 1980). In studies with charr, Adams *et al.* (1995) reported that all charr in the tank were recipients of aggression, irrespective of their social rank, but that only 5 of 10 fish actually obtained food and that the most aggressive individuals had definite feeding advantages.

Disproportional food acquisition has been held chiefly responsible for growth depensation in several farmed species (Koebele, 1985; Ryer and Olla, 1995). Given that aggressive interactions are prevalent during feeding they will be difficult to eradicate completely. However, understanding the underlying behavioural mechanisms responsible and ensuring widespread feed dispersal can reduce aggression and inadvertent physical contact from misdirected feeding attempts. The temporal concentration and spatial dispersal of feed renders it indefensible, and can prevent monopolisation by dominant or aggressive individuals (Grand and Grant, 1994; Ryer and Olla, 1996; Kadri *et al.*, 1997). In this context, information gleaned from fish behavioural studies has formed the basis of some valuable management strategies.

For juvenile halibut it appears important to provide feed over as long a time period as possible (via automatic feeders) thus preventing a build up of appetite, and to maintain at least two hand feeds per day. To reduce aggression in halibut tanks, we advocate a careful hand feeding strategy where food is spatially dispersed but concentrated in time. Our studies indicate that the majority of aggression occurs early in hand feeding sessions when fish are particularly hungry. A typical feeding session may last between ten and twenty minutes. Therefore, during the initial 5 minutes or so of the feeding session, feed should be delivered rapidly and in excess across the entire area of the tank. This will accommodate the sudden increase in fish activity and reduce the occurrence of competitive aggression and accidental collisions. For the remainder of the feed session, feeding should be less intense, and feed may be supplied at the rate at which the fish eat it. The remainder of the daily ration can then be dispensed via automatic feeders throughout the day, giving all fish the opportunity to feed to satiation and achieve maximum growth potential.

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Chapter 3

The effects of aggressive behaviour and hierarchies on feed intake variability in juvenile Atlantic halibut

3.1 Introduction

3.1.1 Performance and light levels in farmed halibut

Environmental factors such as water temperature and light levels are known to influence halibut behaviour and growth in aquaculture (Hallaråker *et al.*, 1995; Björnsson and Tryggvadóttir, 1996). In the majority of halibut culture systems, juveniles are transferred from indoor nursery facilities to covered outdoor tanks at an average weight circa 10-15 grams. This is chiefly due to spatial constraints and the diminished requirement for halibut to be maintained on filtered, UV-treated water once fish exceed 10 grams[1]. However, intra-specific aggression, physical injuries and abnormally high swimming activity noticeably increase with this environmental change. These adversely affect fish growth and may be indicative of stress linked to excessive light levels in the production facilities.

Behavioural interactions between fish, such as aggression, social hierarchy formation and competition have all evolved to enhance an individual's access to food, territories and mates. However, while adaptive under natural conditions, these can have profoundly negative impacts in the aquaculture environment. Persistent aggression among steelhead trout (*Salmo gairdneri*) manifests as fin and body

[1] Although there are no published results on the ontogeny of the immune system, preliminary studies would suggest that juvenile halibut have a fully established immune system between 5-10 grams weight.

damage, and subordinates show reduced growth rates presumably due to the energetic costs of chronic stress as well as restricted access to food (Abbott & Dill, 1989).

This study, conducted at the Norwegian Institute of Fisheries and Aquaculture in Tromsø, Norway, was originally designed to investigate the effects of light intensity on the behaviour of juvenile halibut in tanks. The hypothesis was that relatively high light intensities currently prevalent in juvenile rearing facilities may directly exacerbate management problems (fish stress, aggression and associated physical damage). It was predicted that lower light intensities would reduce stress, aggressive interactions and overall activity. To test this, nine experimental groups were set up, three at each of the following light intensities: 10 lux, 100 lux and 750 lux. In the event, there was no evident difference in the behaviour or feed intake of the fish on the three light regimes while the experiment was underway. Subsequent analysis on growth, feed intake and aggression confirmed this, showing no significant differences between groups. There are two plausible explanations for this: that fish had been exposed to high light levels prior to the experiment, and that the 750 lux intensity was not sufficiently high to induce a stress response (see Discussion below).

3.1.2 Behavioural examination of interactions between fish

Despite the lack of effect of light intensity on behaviour (the initial hypothesis), the results provided an opportunity to study the social relationships between individually marked fish in detail, and to explore ways of measuring dominance in small groups of halibut in relation to access to food. This chapter, therefore, will examine the social dynamics of each group, the effect of aggression and hierarchy on feed intake, and different feeding strategies adopted by individuals.

3.1.3 Studies of dominance relationships in research

There is an extensive literature on dominance interactions among species, from pair-wise encounters (Adams & Huntingford, 1996), and small groups (Adams *et al.*, 1998), to larger groups (Winberg *et al.*, 1993; Carter *et al.*, 1996). A common finding is that aggressive, dominant fish achieve superior growth because they have unrestricted access to food, and may actively deter their competitors from feeding. However, the relationship between feed intake and aggression rank is more complex then at first appears. In a study on competitive strategies and feed acquisition in juvenile Atlantic salmon (*Salmo salar*), Adams *et al.* (1998) found that although there was a significant association between aggression rank and food intake, this relationship was weak. Two categories of fish were identified as responsible for this surprisingly weak correlation: aggressive non-feeding fish and those that fed without engaging in aggressive bouts. The non-feeding aggressors compromised their feed intake by being too preoccupied with fighting, and their non-aggressive counterparts avoided confrontation but managed to acquire feed by being watchful and darting briefly into the feeding area as soon as food appeared. These latter individuals showed that alternative strategies to get food could still yield good growth rates. Aggression alone, therefore, is not always indicative of feeding success, and the relationship between dominance, aggression and feed intake needs to be clarified.

3.1.4 What do we mean by dominance and how can it be measured? Assessing dominance status and testing for a linear hierarchy

In 1922, Schjelderupp-Ebbe described the dynamics of hierarchical relationships among domestic fowl, which he termed the pecking order. Since then, the concept of dominance has been widely applied in ethology, and provides useful insights into social relationships between individuals in a group. However, there is a longstanding debate on the best way to measure dominance, and by what criteria dominance ranking should be ascribed. Drews (1993) comprehensively reviewed and discussed no less than thirteen different definitions of dominance used in behavioural studies. In spite of this array of definitions, it is generally accepted that dominance refers to agonistic interactions, and he offered the following structural description: 'Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterised by a consistent outcome in favour of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate.'

Occasionally, dominance hierarchies can be perfectly linear, and the dominance relation is transitive. Accordingly, the relationships between any three individuals may be defined as follows: if A is dominant over B, and B is dominant over C, then A will be dominant over C (de Vries, 1995). The Linearity Index, developed by Landau (1951), ranges between 0 and 1, where a value of 0 means that every individual dominates an equal number of other individuals. Conversely, a value of 1 indicates complete linearity. Although perfectly linear hierarchies do occasionally exist, non-linear relationships are far more commonplace (Manning, 1979; Appleby, 1983).

Conflict matrices provide a useful starting point in describing the aggressive relationships within a group. These illustrate the number of aggressive acts initiated by an individual towards the other group members, and also the number of times each individual was targeted, and by whom. However, determining the relationships between fish or their relative positions in a dominance order is not always straightforward. This is particularly true when there are tied ranks (i.e. two animals direct an equal number of aggressive actions towards each other), or when no aggressive interactions between two individuals are recorded. De Vries (1995) has developed a linearity test largely based on Landau's *h* Linearity Index, but which also takes into account tied and unknown relationships (zero dyads). Firstly a matrix of dominance relationships is constructed where dominants are given a value of 1, tied dominants a value of 0.5, and zero dyads a value of 0. There follows a two-step randomisation procedure which generates an unbiased estimate *h'* of Landau's Index *h* and the probability that the value *h'* will be attained or exceeded by chance.

Dominance relationships have also been expressed in terms of ordinal ranks. However, this system has proved problematic because it implies that the distance between adjacent ranks is the same, though this is rarely the case. Researchers have therefore devised their own more realistic indices (Boyd & Silk, 1983), resulting in a multitude of different classifications systems on offer. In short, although the concept of dominance is widely applied, ethologists have not always clearly defined the term in the context of their work, and these many definitions prove confusing.

3.1.5 Dominance in the context of small groups

It is widely accepted that dominance refers to agonistic behaviour. The adaptive significance of such behaviours is that more aggressive individuals often attain greater access to limited resources (Clutton-Brock *et al.*, 1979; Bond, 1989; Wagner & Gauthreaux, 1990). Aggression refers to a spectrum of behaviours, from threat displays and postures to overt physical attack. Many species communicate aggressive intent by use of aggressive displays that can obviate the need for overt physical attacks, thereby avoiding the risk of injury (Tinbergen, 1965; Dawkins & Krebs, 1978). Bond (1989) suggests the concept of 'behavioural efference' or positive feed back from the display of aggressive intent to the animal's internal motivational state. This controls the rate of intensification of aggressive interactions as, if an opponent concedes to a threat display, escalation to actual physical attack is unnecessary. The avoidance of escalated fights has been taken as evidence that a communication system has evolved (Sade, 1981).

In this way, the pattern of aggressive behaviour often changes over time within small groups. Initial levels of aggression can be relatively high when the social relationships between individuals are being established. However, as previously mentioned, maintaining the same level of overt aggression over time is profitable neither for the aggressor nor the subordinate recipient, because of the stress and energy expenditure associated with aggression and the potential risk of injury to both parties. Instead, the intensity of agonistic encounters frequently diminishes, and the same information is conveyed by threat displays and converse deference or avoidance behaviour. Therefore, the key to dominant-social relationships is the overall pattern of interactions rather than the total number of aggressive acts (Bernstein, 1976; Hinde, 1978; Drews, 1993).

Intriguingly, the number and complexity of elements in an aggressive repertoire varies from species to species, and there appears to be a relationship between the size of the repertoire and the severity of potential injuries. A fine example is Serpell's (1982) study of aggressive displays in 9 species of lorikeets.

Lorikeets are small, aggressive parrots that range from large heavy-billed species to smaller birds with a less powerful bite. The former can inflict serious injuries on one another, and Serpell described a repertoire of 20 ritualised display behaviours before a conflict would escalate to an attack. In contrast, smaller species had just 5 behaviours and overt attacks occurred more frequently.

3.1.6 Interindividual and intra-individual variability in food acquisition

Several researchers have suggested that repeated measurements of feed intake by individual fish can be used more easily than direct observation to indicate the social relationships within larger groups (Carter *et al.*, 1996; McCarthy *et al.*, 1992). This assessment presumes that dominant individuals gain preferential access to food and therefore have higher rates of consumption than subordinate fish. Many studies have substantiated this theory (Thorpe *et al.*, 1990; Grant and Kramer, 1992; Metcalfe and Thorpe, 1992). A second assumption is that dominant fish will feed to satiation on a daily basis because food is readily available to them. It follows then, that these fish will show little day-to-day variation in the amount of food consumed (i.e. dominants will have low coefficient of variation (CV_I). Conversely, subordinate fish, which may be actively prevented or inhibited from feeding, will display more uneven feeding patterns and greater variability (high CV_I values) (Jobling *et al.*, 1995). In a non-competitive environment, where all fish have access to food, one would expect stable feed intake and little day-to-day variation. The CV values can, therefore, be used to test for the presence of feeding hierarchies. Although this rationale holds true for the majority of fish, it is problematic when dealing with subordinate individuals. These fish that feed little or not at all will show highly consistent daily feed intake with low CV_I values but this obviously does not mean that they have fed to satiation.

Specific questions that were addressed with this data set were:

- How variable was feed intake during meals within small groups of juvenile halibut?
- How consistent were individuals in their feed intake over time?
- How did feed intake relate to growth rate?
- What was the nature of aggressive interactions within small groups of halibut?
- Was there a clear dominance structure?
- Was there a relationship between aggressive interactions and access to food?

3.2 Materials and Methods

3.2.1 Experimental conditions

Nine groups of six tagged individuals (initial weight 1 - 4 grams) were monitored at three broad light intensities of 10 lux, 100 lux and 750 lux in continuous light conditions. The nine experimental tanks used were adjacent to each other (in a segmented trough). This system had been specifically built for video recording the behaviour of small groups of fish. Each tank had three opaque sides and an opaque base so they were visually isolated from fish in neighbouring tanks. The front of the tank was transparent and filming was done from this perspective. Three bulbs linked to dimmer switches provided overhead lighting to tanks. Lux levels were set at the start of the experiment, and remained unaltered thereafter. To ensure an even distribution of light across the tank, an opaque cover was placed between the lights and water surface. The three replicates for each light treatment were adjacent to each other, and were isolated by black polythene screens to ensure set light intensities were preserved. Feed was delivered to each tank via a feed tube at the front. Panasonic WV-CP220 colour CCTV cameras were used for behavioural recording. These have built-in AGC (Automatic Gain Control) and aspherical high-speed lenses allowing a clear image under low light conditions. Cameras were mounted on a rail facing the front of the tanks and positioned so as to contain the whole tank in the field of view (Figure 3.1). The whole structure was contained within a light-proof polythene tunnel housed in a dark room.

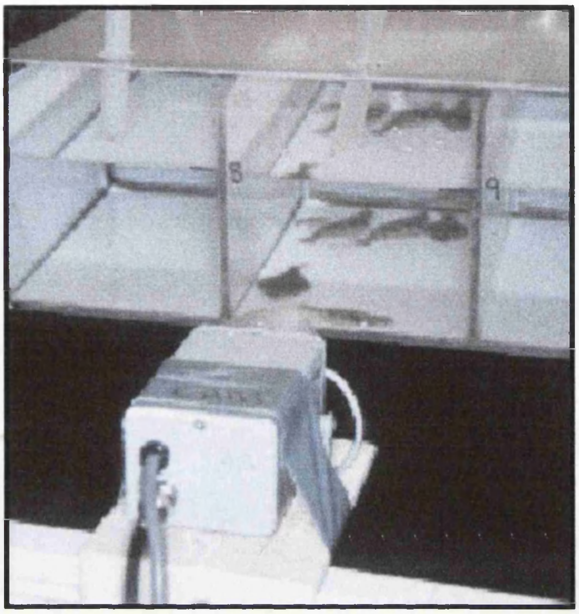
3.2.2 Individual fish marking

The ability to recognise individuals in this study was essential to enable the recording of the behaviour and feed intake of each fish. Therefore, each fish had to be marked in some way. The marking method had to satisfy several criteria:

- be visible at the lowest light condition (10 lux)
- be discerned from the video tapes
- be unobtrusive to the fish so that behaviour was not affected

Fish marking, especially for small individuals, continues to present a problem to researchers. There are a variety of methods currently available but none were considered entirely suitable for this experiment. Alcian blue dye and panjet marks are successfully used on salmonids (Herbinger *et al.*, 1990), where the contrast against silver scales is pronounced and there are many body sites to choose from. However, such marks on a pigmented halibut are difficult to see, especially indirectly from video tape. On such small fish, the choice of sites is also severely limited. Halibut tend to overlie one another which precludes siting marks on the fins as these

Figure 3.1: Lateral view of halibut observation tank showing video camera for recording fish behaviour. Tanks had three opaque sides and an opaque lid. The entire experimental set-up was enclosed in a black polytunnel to minimise disturbance and exclude any external light.



are commonly obscured from view. Some available marking methods, such as Floy Tags (Floy Tags Inc., U.S.A.), are anchored in the flesh and protrude. Though relatively large, clearly visible and available in different colours, these invasive tags have been associated with sores developing around the entry point. As fish behaviour was key to this study, the potential risk of wounding or distress was deemed too great and they too were discounted. Instead, a tagging method was devised which consisted of a coloured band around the caudal peduncle. This provided a relatively large mark that proved easy to see. Tags were made from the colour-coded sleeves for insulating wires within electrical cable. Cable was stripped down and the inner strands removed and cut. The wire within each strand was then pulled out, leaving a pliable material. This was cut into short sections, and the ends of each held together to form a loop. The two ends were then threaded through a wider sleeve, creating a lightweight, smooth coloured tail tag. This method was chosen because it avoided the use of knots, which could cause chafing, and the removal of protective mucous over time. Tags were tested prior to this experiment on several halibut held in the aquaria at the University of Glasgow. After three weeks there was no sign of abrasion damage caused to the fish, and no evident effect on fish behaviour.

Caudal tags were quick and easy to apply. Loose loops that could be slipped over the tail were pre-constructed in assorted colours. Fish were anaesthetised with Benzocaine (100ppm), and then each placed in a petri dish containing normal sea water, the tail resting on the side above the water surface. A loop was positioned around the tail and tweezers used to move the securing sleeve and tighten the loop. The protruding ends were then trimmed short. Loops were made tight enough to remain in place but loose enough to allow for growth (Figure 3.2). Although a wide choice of colours was available, under the lowest light conditions only yellow, red, green and blue were unmistakable. To obtain six tags per tank, variations of yellow and red tags were devised by alternating the colour of the securing sleeve and placing same colour tags on different sized fish.

3.2.3 Fish size and condition

At the beginning and end of the experiment, fish weight, total length and any physical damage was recorded according to the method in Table 3.1.

3.2.4 Daily husbandry and feeding

Access to tanks for feeding and daily husbandry was via a walkway along the back of the polytunnel. Fish were fed commercial fry feed (Ewos 2.0 crumb) at a rate of 1.5 % bodyweight/day. Feed was delivered via the feeding tube. Each tank received two 45-minute meals per day, one in the morning and one in the afternoon. On days when feeding behaviour was recorded, the entire morning session for all tanks was taped. Crumbs were non-uniform in size and shape, but the average weight of a crumb was 1.20 mg. Three tanks were fed at a time, one from each replicate. Food was added at a rate of 5-6 crumbs at a time. Some of these sank immediately while others floated on the surface before sinking in other areas of the tank. Given that feeding all nine tanks took two hours and fifteen minutes (3 x 45-minute meals), the order of tank feeding was randomised daily. Waste feed and faeces were removed each afternoon by siphoning and tanks were flushed.

Figure 3.2: Photograph of Tromso halibut showing the coloured tail tags for individual identification.

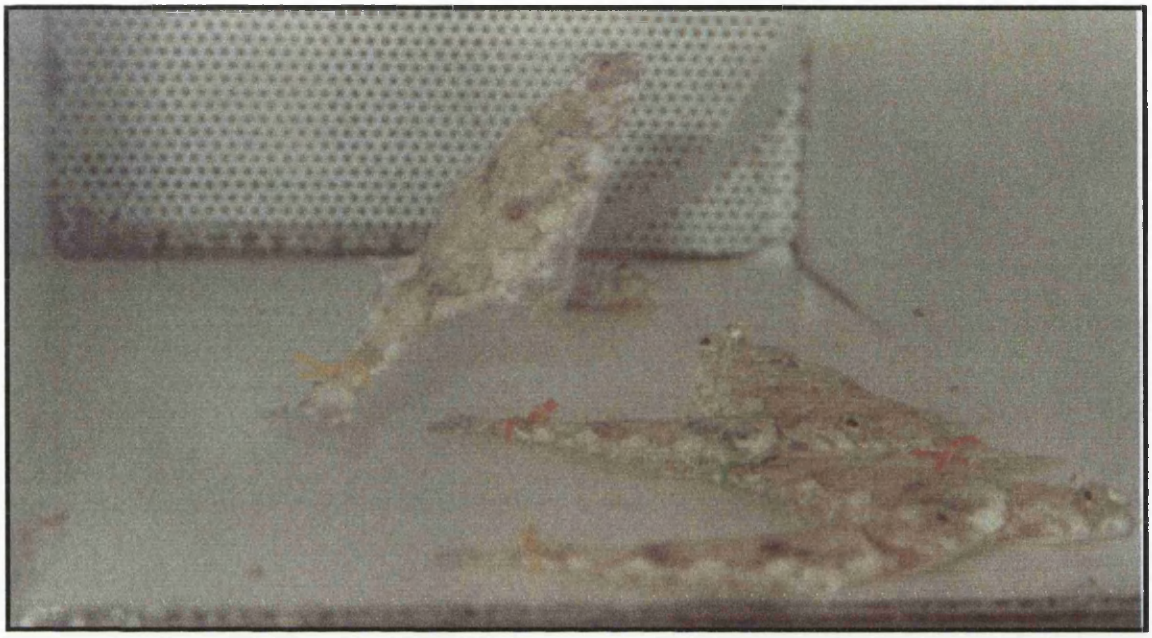


Table 3.1: The scale used to score dorsal, anal and caudal fin damage:

Score:	Damage Description:
0	Fins complete, no damage
1	< 30% fin damaged or missing
2	30 - 50% fin damaged or missing
3	> 50% fin missing (fin may be bitten down to base)

3.2.5 Behavioural analysis

Using *The Observer Video-Pro* (Noldus, Wagerningen, The Netherlands) behavioural analysis programme, videos were analysed for individual feed intake, the order of fish feeding, and aggressive behaviours (body posturing, displacement, nips, bites and chases). Conflict matrices were complied for daily aggressive interactions and also cumulatively across five days.

3.2.6 Cumulative feed intake plots and behavioural profiles

The daily feed intake of individual fish within groups is presented as cumulative feed intake plots. These illustrate the different feeding patterns of individuals, some feeding often and early in meals, others feeding sporadically or very little. Conflict matrices were compiled to clarify the dominance relationships in each group of six fish. In a matrix, the columns represented aggressors and rows represented the recipients of aggression. For each tank, both a daily matrix and a cumulative matrix were constructed. A behavioural profile for each fish was also compiled from the video footage, indicating when it chose to feed, how competitive it was and its position in the tank.

These behavioural profiles complemented the conflict matrices, providing information on fish-fish interactions, particularly those of an aggressive nature, and were helpful indicators of the social characteristics of the group. Registered information included:

- relative aggressiveness and the effect of received aggression on behaviour

- feeding motivation: feeding early or late in a meal, taking food from the surface or in the water column as it entered the tank, or consuming feed settled on the tank base, directly competing for food or feeding opportunistically
- the habitual positions occupied by fish in the tank: at the front beneath the feed tube, or on the side walls, rear screen or at the back of the tank; whether gregarious or solitary.

3.2.7 Statistical analyses

Concordance of feed intake and aggression across days was analysed non-parametrically by Kendall's Coefficient of Concordance (Siegel & Castellan, 1988). Fish were ranked according to the amount of feed ingested on each of the five days. This was adjusted to reflect the fish size by calculating the weight-specific feed intake. Specific growth rate was calculated according to the following formula:

$SGR = (LnW2-LnW1) / t_2-t_1 \times 100$

where Ln is natural log, W1 and W2 are the weights of fish recorded at times 1 and 2, and t_2-t_1 is the interval in days between weighing. Spearman's Rho correlations were carried out on feed intake, weight-specific feed intake, specific growth rate (SGR), initial fish weight, initial fish length, aggression given and aggression received (SPSS 9.0). Patterns of individual feed intake during meals are represented by meal profiles (cumulative feed intake plots). These were constructed from Observer data files for each tank on separate days. Feed intake was highly variable both between groups and between days. Generally a few fish dominated feeding in most tanks (1-3), some fed throughout the meal, some only sporadically, and some not at all. The majority of these plots are presented in Appendix I, though several are presented in the main text to illustrate specific points.

Inter-individual variability was calculated according to the formula: CV = [SD / Mean] x 100. Intra-individual variation in daily feed intake was similarly calculated according to the formula: CV (%) = (SD x 100) / Mean weight-specific consumption. The absolute amount of feed items ingested by an individual during a meal is one measure of feeding success. However, this becomes more meaningful if adjusted to be weight-specific, especially when fish in the tanks are of variable size.

Weight-specific feed intake (WSFI) = feed intake / average weight.

where feed intake = (weight per food item) x (number of items).

3.3 Results

3.3.1 The influence of light intensity on fish growth

There was no significant difference between SGR at the three different light intensities (Kruskal-Wallis test, $H = 0.10$, d.f. = 2, $p = 0.950$), although the coefficient of variation in weight was highest for low light and lowest for the high light treatments (CV 99.86 and 51.25 respectively).

3.3.2 Variability in Feed Intake

Kendall's coefficient of concordance was used to assess the agreement of feed intake ranks across five days for the six fish in each group. This showed that feed intake was remarkably concordant across days for all tanks (Table 3.2).

3.3.3 Patterns of individual feed intake during meals: cumulative feed intake plots

Feed intake was highly variable both between groups and between days. Generally a few fish dominated feeding in most tanks (1-3), some fed throughout the

Table 3.2: Kendall's coefficient of concordance for feed intake across days in 9 groups of 6 halibut:

Tank Number	X^2r Value (m = 5, n = 6)	Level of Significance
1	13.57	$P < 0.01$
2	13.65	$P < 0.01$
3	20.09	$P < 0.001$
4	20.16	$P < 0.001$
5	16.54	$P < 0.001$
6	21.8	$P < 0.001$
7	21.23	$P < 0.001$
8	15.83	$P < 0.001$
9	11.43	$P < 0.01$

[For m = 5, n = 6, significance at 0.05 level = 9.067, at 0.01 level = 11.87, and at 0.001 level = 15.20].

meal, some only sporadically, and some not at all. Meals are shown in the form of cumulative feed intake plots where each fish is represented by a different colour, and the pattern of daily feed intake may be followed (Appendix I).

3.3.4 The context of aggression and the nature of aggressive interactions

i) Aggressive displays

Aggression was characterised by either raised body display or overt physical contact. Display behaviour was unmistakable. The aggressor raised itself high off the tank base from the mid-body and curled the head forwards into an exaggerated s-bend (Figure 3.3). The gills were often flared at this time, and the fish appeared to try to tower above the receiver, eyes fixed upon it. Displays like these served to displace the other fish, which either swam off at speed, or flattened themselves against the tank base and backed away in a shuffling movement. Occasionally fish would turn away and retreat a short distance.

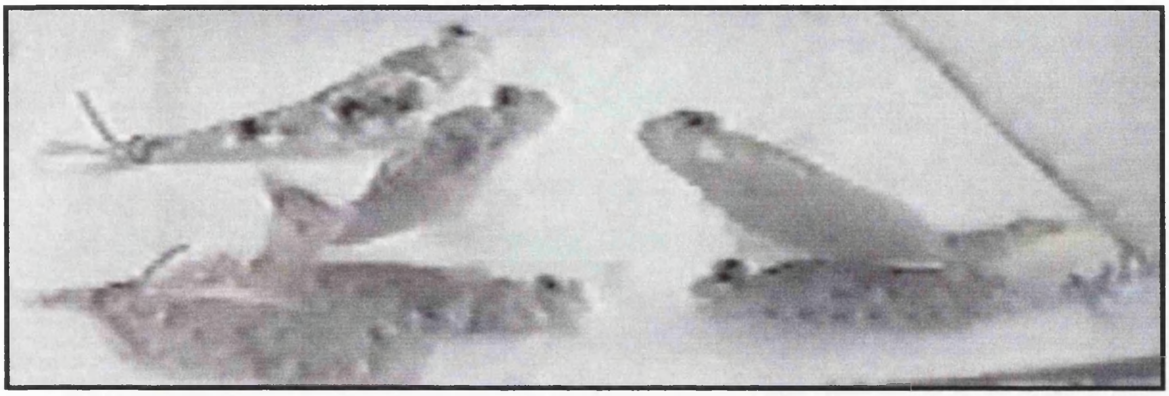
ii) Overt aggression

Overt aggression was brief and non-reciprocated, and no novel fin damage was recorded when fish were examined at the end of the study (Table 3.1). The majority of aggressive acts (mean 84%) were directed specifically at competitors, especially when the aggressor was out-competed for food items. Usually, two fish would target a food item and the successful feeder would then be chased, nipped or bitten by the loser. This was the provocation for all aggressive acts initiated by fish in tank 4 on 27/10 and 4/11. Figure 3.4 illustrates an aggressive attack against a potential competitor and the response of the victim.

Figure:3.3 The distinctive halibut threat posture.
The aggressor (on the left) raises its head and mid-body high off the tank base and inclines the head forward to form an 's' shape. Image taken from video footage.

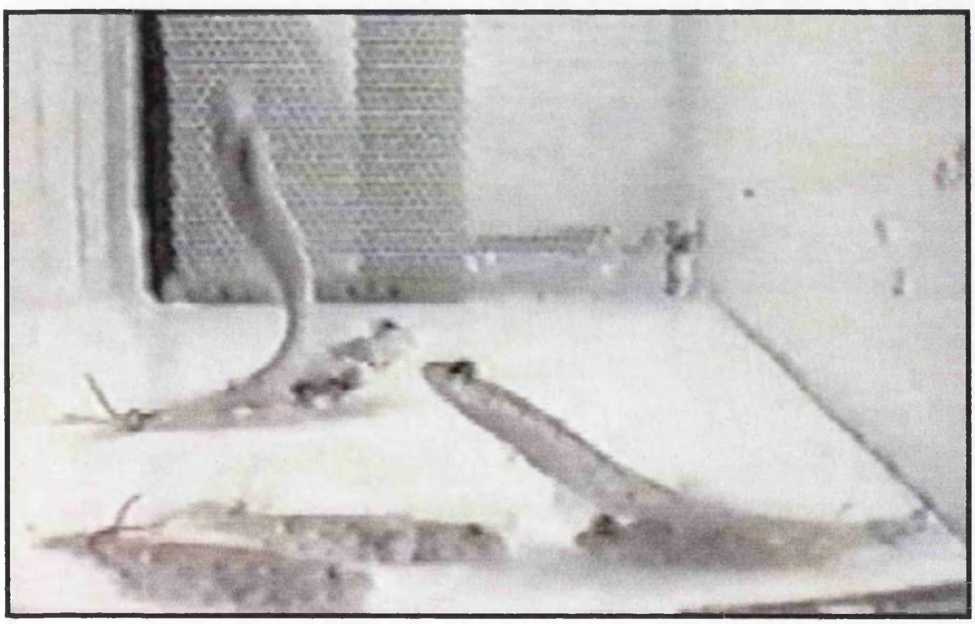
Figure 3.4: Sequence of images showing an attack by a halibut. Images are stills obtained from video footage.

a) the aggressor (on the right) approaches…



b) bites the victim on the mouth…

c) the victim recoils from impact and flees the scene.



3.3.5 Variability in Aggression

Aggression was highly variable, both between and within groups. These results present problems with interpretation, therefore, in the most part each tank shall be discussed separately. Aggression was concordant across the 5 days in only two groups, and Spearman rank correlation matrices for these tanks are presented below. Correlations for non-concordant tanks are in Appendix II. In this section, tanks of significant interest will be highlighted.

3.3.6 Testing for a linear hierarchy

De Vries' linearity test was performed on each group and the results are shown below (Table 3.3). The randomisation procedure was run 10,000 times but hierarchies were non-linear in all groups. Therefore, no clear dominance structure was evident within these groups. Indeed, results obtained from Kendall's coefficient of concordance for aggression across days showed that aggression was not consistent between fish from one day to the next in seven of the nine tanks (Table 3.4).

3.3.7 The relationship between aggression and access to feed in Aggression-concordant groups (tanks 2 & 3)

Absolute feed intake and body length were strongly positively correlated in both tanks (Tank 2: $R_s = .943$, $N = 6$, $p < 0.01$; Tank 3: $R_s = 1.00$, $N = 6$, $p < 0.01$). In Tank 2, all the fish showed reasonable growth throughout the experimental period, and their growth was the most homogeneous of all the groups. Aggression was also concordant across days in this group (Friedman X^2r $m = 5$, $n = 6$, $p < 0.05$), and aggression was significantly correlated with absolute feed intake ($R_s = .899$, $N = 6$, $p < 0.05$, Table 3.5). However, when this value was adjusted to weight-specific feed

Table 3.3: Results of De Vries' linearity test based on Landau's h statistic. The dominance relationship between each dyad was randomised 10,000 times.

Tank	Landau's h'	p linearity	p non-linearity
Tank 1	0.59816	0.338	0.735
Tank 2	0.774103	0.145	0.931
Tank 3	0.543086	0.420	0.724
Tank 4	0.541703	0.415	0.714
Tank 5	0.400474	0.566	0.525
Tank 6	0.484674	0.492	0.684
Tank 7	0.485783	0.401	0.599
Tank 8	0.719897	0.208	0.885
Tank 9	0.629109	0.299	0.805

Table 3.4: Kendall's coefficient of concordance for aggression across days in 9 groups of 6 halibut:

Tank Number	X^2r Value ($m = 5, n = 6$)	Level of Significance
1	4.23	N/S
2	9.23	$P < 0.05$
3	14.29	$P < 0.01$
4	4.50	N/S
5	7.89	N/S
6	8.66	N/S
7	2.49	N/S
8	7.17	N/S
9	9.74	$P < 0.05$

[Critical values of the Friedman X^2r Distribution for $m = 5$, $n = 6$: significance at 0.05 level = 9.067, at 0.01 level = 11.87, and at 0.001 level = 15.20].

intake (WSFI), the relationship was no longer significant. The most aggressive fish was the second largest individual, and initiated 80% of all aggressive interactions it was involved in. The three largest halibut overall exhibited the most aggression towards others, and showed a positive highly significant relationship between aggression and body length ($R_s = 1.00$, $N = 6$, $p < 0.01$).

In Tank 3, aggression and feed intake were perfectly positively correlated ($R_s = 1.00$, $N = 6$, $p < 0.01$), but when adjusted to WSFI, although still positive, the relationship was again no longer significant ($R_s = 0.657$, $N = 6$, $p = 156$), Table 3.6. All but the smallest individual fed and grew well. This fish took just one feed item throughout the observation days and had lost weight by the end of the experimental period (SGR = -0.71). This individual spent most of its time on the tank wall or base at the rear of the tank. It was never directly targeted aggressively by other fish, but was displaced on three occasions. This halibut was characteristically unresponsive and uninterested in feed, and interacted little with the other fish. It flattened itself right against the tank base, as if attempting to be as unobtrusive as possible. Given its relatively small size, it may have been inhibited by other fish from feeding.

3.3.8 Aggression and variability in feed intake:

Directly competitive fish fed well but were most often involved in aggressive interactions. They were typically highly motivated feeders, fed early in the meal and often took food from the surface as it entered the water. However, a cost associated with high feed intake was high levels of received aggression. Although this relationship was not significant at the group level, it applied to several individuals within groups. An example is fish #1 in tank 1 (Figure 3.5). This halibut had the

Table 3.5: Spearman's rank order correlations for aggression-concordant groups,

Tank 2:

		I Weight	I Length	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient		.829*	.771	.029	.667	-0.58	-.543
	significance		.042	.072	.957	.148	.913	.266
I Length	Correlation coefficient	.829*		.943**	.314	.841*	-.058	
	significance	.042		.005	.544	.036	.913	
FI	Correlation coefficient	.771	.943**		.371	.899*	.058	-.086
	significance	.072	.005		.468	.015	.913	.872
WSFI	Correlation coefficient	.029	.314	.371		.029	.638	.429
	significance	.957	.544	.468		.957	.173	.397
Agg.	Correlation coefficient	.667	.841*	.899*	.029		-.338	-.116
	significance	.148	.036	.015	.957		.512	.827
Agg. Rec.	Correlation coefficient	-0.58	-.058	.058	.638	-.338		.377
	significance	.913	.913	.913	.173	.512		.461
SGR	Correlation coefficient	-.543	-.371	-.086	.429	-.116	.377	
	significance	.266	.468	.872	.397	.827	.461	

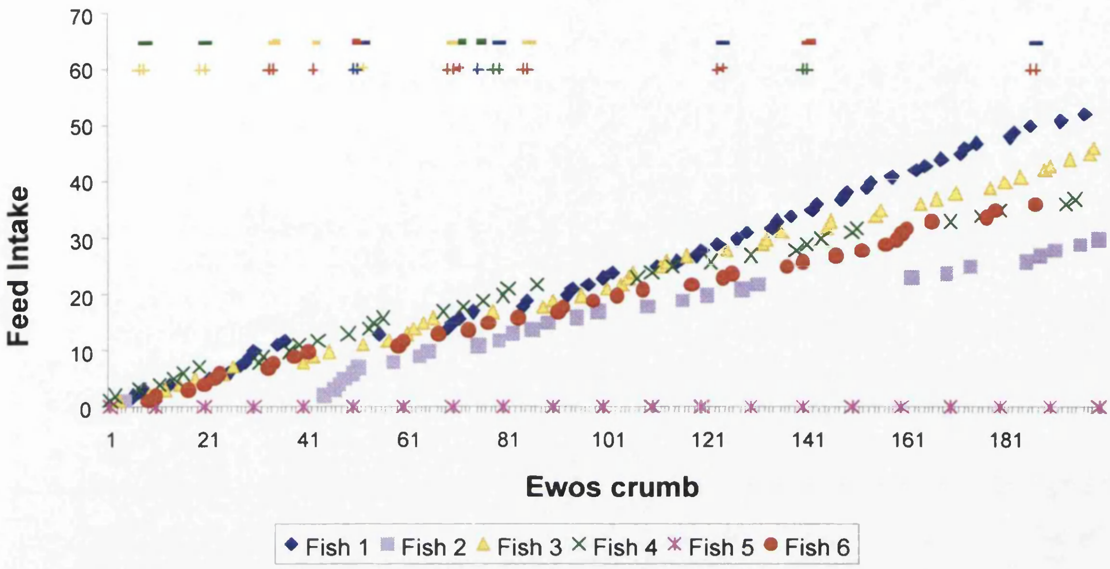
I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.
** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Table 3.6: Spearman's rank order correlations for aggression-concordant groups: Tank 3

		I Weight	I Length	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient		.943**	.943**	.600	.943**	.216	.657
	significance		.005	.005	.208	.005	.681	.156
I Length	Correlation coefficient	.943**		1.000**	.657	1.000**	.494	.829*
	significance	.005			.156		.320	.042
FI	Correlation coefficient	.943**	1.000**		.657	1.000**	.494	.829*
	significance	.005			.156		.320	.042
WSFI	Correlation coefficient	.600	.657	.657		.657	.525	.714
	significance	.208	.156	.156		.156	.285	.111
Agg.	Correlation coefficient	.943**	1.000**	1.000**	.657		.494	.829*
	significance	.005			.156		.320	.042
Agg. Rec.	Correlation coefficient	.216	.494	.494	.525	.494		.802
	significance	.681	.320	.320	.285	.320		.055
SGR	Correlation coefficient	.657	.829*	.829*	.714	.829*	.802	
	significance	.156	.042	.042	.111	.042	.055	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.
** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Figure 3.5: Tank 1 meal profile for 27/10. The cumulative intake of each fish is shown throughout the meal. Aggression is indicated by crosses (at y-axis value 60), the recipient by dashes at value 65. Both are colour-coded to denote the identity of the aggressor or recipient. Fish 1 (blue) had the highest feed intake but received most aggression. Fish 5 (pink) did not feed at all.



highest feed intake on this day and also received the most aggression from other individuals (attacked 7 times). In tank 3 (Figure 3.6), fish 1, 3 and 6 all directly competed for food. The feed intake of all three fish was very close but fish #3, the marginally more successful, was the recipient of 9 aggressive attacks from the other fish.

Other fish adopted non-aggressive strategies to attain food. These individuals were less competitive, and some managed to attain reasonably good feed intake though not as consistently as fish in the first category. They tended to distance themselves from the others in the tank and avoided direct competition for food. For the most part, they evaded aggression. Some waited late in meals before starting to feed, often taking food items lying on the tank base. Some adopted positions on the tank walls, which they left only briefly to snatch food. Fish on walls oriented themselves downwards and leaned out to take food off the tank base (keeping their tail end in contact with the wall), or maintained a position just below the surface facing upwards and spied feed items moving along the water surface. They would then take the item and return to their position. Fish #3 in tank 2 was one such opportunistic fish (Figure 3.7).

A third category of fish was apparent. These were viewed as subordinate individuals, had little or no feed intake and generally lost weight over the trial period. Such fish were very unresponsive to incoming food and to other fish in the groups. Somewhat surprisingly, there was an individual of this kind in 6 of the 9 experimental tanks. Fish would lie at the rear of the tank or in the corners, and often remained unmoving in the same position throughout an entire recording session. Occasionally,

Figure 3.6: Tank 3 meal profile for 27/10. The cumulative intake of each fish is shown throughout the meal. Aggression is indicated by crosses (at y-axis value 60), the recipient by dashes at value 65. Both are colour-coded to denote identity of aggressor or recipient. Fish 1 (blue), 3 (yellow) & 6 (red) all directly competed for food. Fish 3 had marginally better intake and was the recipient of 9 aggressive attacks.

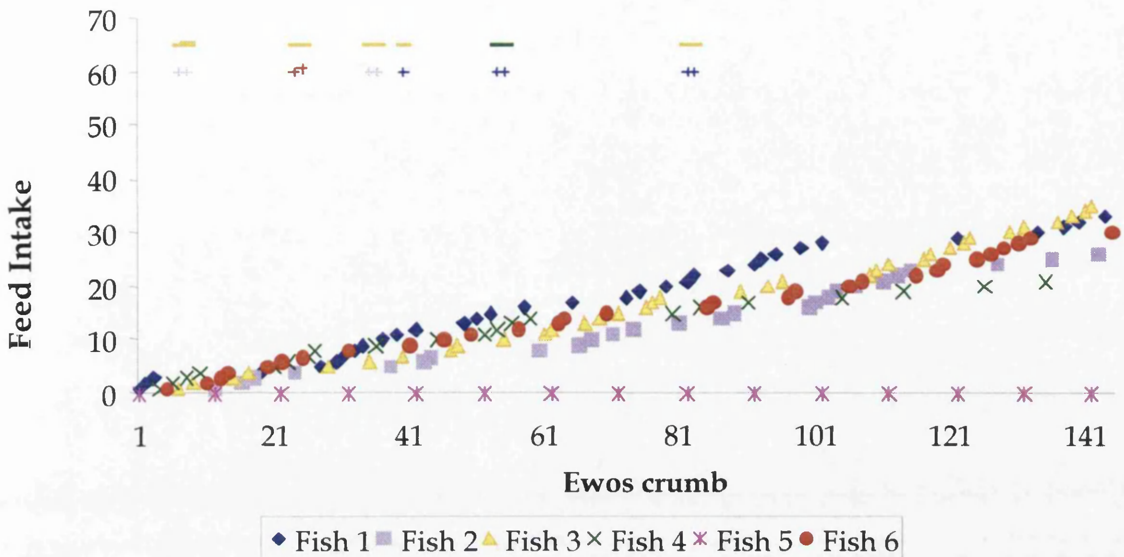
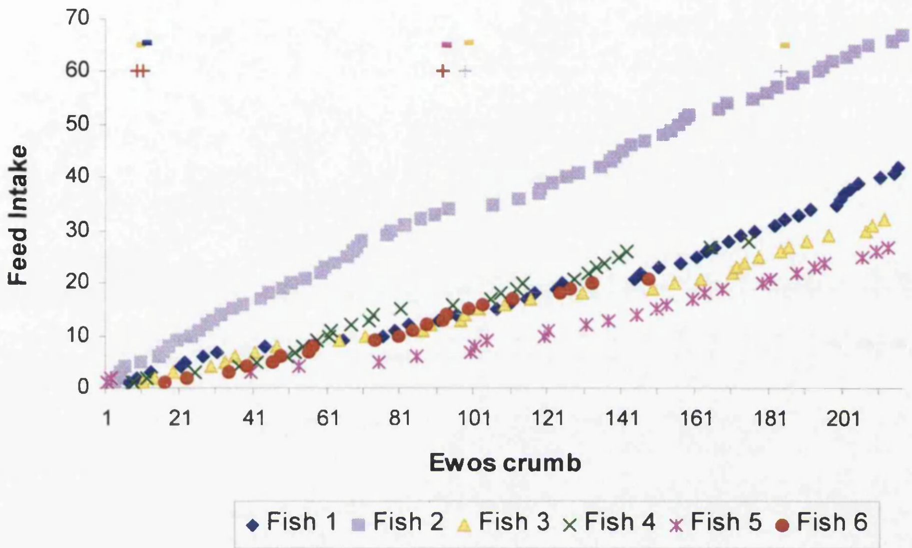


Figure 3.7: Tank 2 meal profile for 30/10. The cumulative intake of each fish is shown throughout the meal. Aggression is indicated by crosses (at y-axis value 60), the recipient by dashes at value 65. Both are colour-coded to denote identity of aggressor or recipient. Fish 3 (yellow) fed at the back of the tank or from the side wall and avoided direct competition, yet still managed reasonable intake.



other fish arched over their bodies to take a food item from the tank base but they did not react at all. These fish looked listless and lay very flat against the tank base. These fish received no aggression from the rest of the group (e.g. fish #5 in tank 3, fish # 5 in tank 5, and fish # 2 in tank 5 (Appendix I). Although in 4 of these tanks it was the smallest individuals that behaved in this way, in Tank 5, fish #2 was the second largest fish yet still did not feed on three of the five days. A couple of fish in this category demonstrated highly disturbed behaviour. On 4/11/99, one such individual in tank 3 (fish #6) that had been lying motionless suddenly dashed around the tank surface mid-meal and then again towards the end, before resuming its position in a corner. This action was entirely unprovoked by other fish and remains unexplained. One possible explanation is that the caudal tag proved irritating, though all fish were examined when tags were removed at the trial end and none had incurred any visible abrasion damage. Apart from this incident, there was no evidence that the presence of tags hindered the normal movement and swimming behaviour of the fish, and tags were ignored by other fish.

3.3.9 Direct effect of aggression on feed intake – the cessation of feeding

Aggression and high food intake are often associated with large relative body size. However this is not always the case. In tank 5, the second largest fish at the trial start (fish # 2) was a poor feeder and was the only fish in the tank to have a negative SGR at the end of the experiment. On the first day feeding behaviour was recorded, this individual was the recipient of the most aggression. This fish took the third feed item but was then nipped by fish #1 (the largest) and did not feed again. This fish did not feed on 3 of the subsequent 4 days yet received no aggression.

Similarly, in tank 3 (02/11), fish #4 took three items at the beginning of the meal but was then out-competed on three occasions by bigger fish and attacked 3 times by fish #1. There followed a prolonged gap in feeding from fish #4, which did not feed again until item 88. Fish #4 was the second smallest fish in the group and its relative body size may have been a contributing factor. Likewise, in tank 6 (30/10), fish #4 was the first to feed and took 4 items early in the meal. This halibut was then bitten on the head and there followed a long gap of 17 feed items before it fed again. In the same tank again, on 2/11 fish # 6 had a steady intake until it out-competed fish #2 for item 60 and was immediately bitten. There followed a gap of 27 items before it fed again.

3.4 Discussion

As mentioned in the Introduction, the original intent of the experiment had been to monitor the effect of different light intensities on halibut behaviour and growth. However, no significant differences were found, and two plausible explanations for this lack of effect are proposed. Firstly, these halibut had been used in a previous trial under high light conditions (c750 lux). Secondly, and in view of this, the highest lux reading used in this experiment of 750 lux at the water surface, was probably too conservative to elicit an effect. Therefore, the presumed 'stress' of the high light treatment was not realised, and the transition from indoor nursery to outdoor tanks as experienced by fish on the farms was not reproduced.

The focus of the study turned instead to the social relationships between individuals in each of the nine groups, and the following questions were posed:

- How variable was feed intake during meals within small groups of juvenile halibut?
- How consistent were individuals in their feed intake across days / over time?
- How did feed intake relate to growth rate?
- What was the nature of aggressive interactions within small groups of halibut?
- Was there a clear dominance structure?

Although the behaviour of each group of halibut in this study was distinct from the next, individual feed intake within groups was highly consistent. Despite the lack of effect of the differing light levels, this experiment with small groups of marked fish allowed the feed intake and aggressive behaviour of individuals to be explored over days and to examine the social relationship between individuals in the group and how this related to feed intake. Aggression was undisputedly linked to competition during feeding, and arose after failed feeding attempts. In the majority of cases, recipients of aggressive attacks (nips, bites) had just ingested food items targeted by the aggressor. In the remaining incidents, potential competitors were chased from the area or displaced by threat displays.

Although no clear dominance structures (in terms of linear hierarchies) were apparent in any of the nine groups, fish with high feed intake did expose themselves to high levels of received aggression. This can be viewed as a cost to successful feeding. However, aggression was minor in terms of consequences for the receiver, as no physical injuries were scored at the end of the experiment.

Fish could be divided into three categories on the basis of their behaviour:

- Directly competitive fish
- Fish adopting non-aggressive strategies to attain food
- Subordinate fish that had little or no feed intake and generally lost weight over the trial period.

The success or performance of an individual is frequently determined by its social status within a group. This being the case, ranking fish on the basis of dominance is common practise in behavioural research. In most cases this is justified as dominant individuals gain preferential access to food and other resources (by occupying the best territories) and maintain their status through aggression. In the main, dominant individuals can exert more influence over small groups than larger ones. However, aggression and feeding success are not always correlated, and ranking fish this way does not help to tease apart sometimes complex relationships between individuals. In this study, each group had its own characteristics, making groups distinct and difficult to relate to each other.

Although Tank 5 was non-concordant for aggression, fish #1 (the largest at the outset) was most aggressive on 4 of 5 days. Cumulative intake plots here are very interesting. On the first 2 days that feeding behaviour was recorded, this individual was highly aggressive (initiating 9 and 13 aggressive acts respectively) yet fed relatively poorly (ingesting just 1 food item on the first day). At first examination, aggression at this stage appeared to be highly counter-productive behaviour. However, it may have served to establish the aggressive fish as dominant to the others on subsequent days. Close examination of the video footage showed that other fish seemed to be actively deferring to fish #1 and conceding pellets from the third day that feeding was recorded. On latter days, therefore, aggression from this fish lessened while its feed intake became significantly higher.

Fish in this experiment were compared in terms of feeding behaviour, strategies adopted to gain access to food, and relative feed intake. Fish that fed well were, unsurprisingly, the most active individuals, often taking food from the surface as soon as it entered the tank. Some would even hover expectantly high in the water column beneath the feed tube. These fish were clearly highly motivated feeders. Conversely, fish that fed little or not at all throughout the experiment behaved very differently. These halibut were largely immobile and unresponsive, lay flattened in the corners or high on the tank walls away from other fish. Similar behaviours were observed in subordinate arctic charr by Winberg *et al.*, (1992) in their study of the social relationships between groups of four individual charr. They suggested that subordinates were attempting to hide from the other fish in the group. The behaviour of subordinate halibut in the present study could reasonably be interpreted in the same way. Although true that in groups of considerable size range larger fish generally dominate, social rank in Atlantic salmon juveniles did not always correlate with fish size (Huntingford *et al.*, 1990). The authors of this study concluded that size was a consequence of dominance, and not the cause of it.

Two variables have been shown to influence monopolisation of food resources by dominant individuals: increased access to the food supply and larger group size (Li & Brocksen, 1977; Jobling & Baardvik, 1994; Alanärä & Brännäs, 1996).

Subordinates and subdominant fish face the risk of aggressive attacks from dominant individuals. Therefore, these fish may make a decision not to compete directly and to adopt alternative feeding strategies. This is a trade-off between expending time and energy competing (largely unsuccessfully) or feeding opportunistically but less consistently on uncontested feed items that either drift along the surface or have previously fallen to the tank base.

Kadri *et al.*, (1996) showed that one-sea-winter Atlantic salmon adopt these so-called "sit-and-wait" strategies. Fish with the highest feed intake fed at the water surface and contested many pellets. Conversely, subordinates fed at different times seemingly to avoid aggression (Kadri *et al.*, 1997). When food is available to excess in culture conditions, subordinates can still attain adequate food using these alternative feeding strategies (Metcalfe *et al.*, 1999).

Within populations, high variability between individuals in metabolic rate and growth rates cause even well size-matched fish to diverge over time. In addition, aggression mediated social hierarchies contribute to this growth depensation (where small initial size differences become more pronounced over time) (Jobling, 1985; Jobling & Wandsvik, 1983).

The majority of studies of aggressive interactions in fish have focussed on small groups (less than 20 individuals, triads or pair-wise encounters). Several researchers have shown that in such small groups pronounced social hierarchies develop, where 1 or 2 individuals dominate the rest by monopolising the food supply, directly reducing the feed intake and subsequent growth of subordinates (Jobling & Wandsvik, 1983; Koebele, 1985; Huntingford *et al.*, 1993; Adams *et al.*, 1998).

Past studies have demonstrated that dominant fish are generally more aggressive than subordinates, and subordinates thereby usually receive more aggression and physical injury (Abbott & Dill, 1989; Fenderson & Carpenter, 1971; Moutou *et al.*, 1998). However, results obtained from studies of small groups do not necessarily apply to larger groups. MacLean *et al.*, (2000) make the point that findings from small-scale studies cannot be assumed to hold true for larger groups in culture conditions. Social interactions may vary significantly with group size. This was neatly demonstrated by Adams & Huntingford (1996), in their study of Arctic charr juveniles. Fish were first subjected to pair-wise encounters, in which the most aggressive fish attained the most food. However, when these same individuals were placed in large groups in culture conditions they lost their growth advantage and growth rate was no different from that of previously subordinate charr. This evidence supports the belief that social hierarchies are less stable in larger groups (Fenderson & Carpenter, 1971). In this study of halibut, video footage confirms that aggressive fish compete directly and inflict aggression upon each other.

There is little information on the social and territorial behaviour of halibut in the wild. Published studies have concentrated on the diet, distribution, spawning and migration of halibut rather than its behaviour, presumably because of the difficulties of observing this fish in its natural state (McIntyre, 1952, Haug, 1990, Trumble *et al.*, 1993,). No information is available on the frequency or intensity of aggressive interactions, or on what circumstances provoke aggression. Knowledge of these basic aspects of the natural history of this species would be valuable for interpreting findings described in this experiment and the thesis as a whole. In the absence of such data, small-scale studies like the one described above can increase our understanding of the behavioural interactions between individuals and the context in which aggression occurs. While the conditions in this experiment were highly artificial, this study has generated some useful data. It has, at the very least, confirmed the findings of previous studies showing that aggression was associated with feeding behaviour, and enabled the behaviour of halibut to be scrutinised at close range.

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Chapter 4

The influence of stocking density on the behaviour and growth of Atlantic halibut throughout the weaning period

4.1 Abstract

In this study the behaviour and growth of juvenile halibut held at different stocking densities was monitored over five weeks; all of these being within the range used by commercial hatcheries to date (2000 fish/2m^2, 4000 fish/2m^2 and 8000 fish/2m^2 respectively). The behaviour of fish in each tank was filmed from above on three separate days, and videos were analysed for the frequency of aggressive interactions. Aggression was characterised by bites, nips and chases, and recipients of aggression were without exception sedentary individuals on the tank base. Agonistic behaviour was not correlated with feeding activity, suggesting that it is not directly competition-induced. The frequency of aggressive acts was significantly greater at the lowest density, as was the incidence and severity of physical injury to fish in these tanks. The results of the current study indicate that stocking density within the range currently used has a strong influence on halibut behaviour and growth at this developmental stage, and that fish reared at the highest density attained superior growth rates.

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4.2 Introduction

The growth and behaviour of farmed fish can be influenced by biotic factors such as food availability and fish density (Sakakura and Tsukamoto, 1997; Papoutsoglou *et al.*, 1998). Aggression is one mechanism by which stocking density influences production, and the potential economic losses in aquaculture from cannibalism and aggressive behaviour have been the impetus for numerous experimental studies of these factors (Smith and Reay, 1991; Ruzzante, 1994; Sakakura and Tsukamoto, 1997). Excessive growth variation due to social behaviour (termed growth depensation) has similarly been documented in several fish species (Koebele, 1985; Ryer and Olla, 1996). Husbandry techniques can however be modified in order to reduce aggressive interactions and growth depensation. For example, Arctic charr (*Salvelinus alpinus*) reared at higher densities initiated significantly fewer aggressive interactions and attained higher mean weights than did charr at lower densities (Brown *et al.*, 1992). Hecht and Uys (1997) and Papoutsoglou *et al.* (1998), working with African catfish (*Clarias gariepinus*) and European sea bass (*Dicentrarchus labrax*) respectively, also found that the growth rate of juveniles increased with increasing density. The present study was carried out to examine the influence of stocking density on agonistic behaviour and growth of Atlantic halibut at the weaning stage.

Levels of aggression and consequent physical damage prevalent in farmed Atlantic halibut appear far higher than for other species of flatfish, but there is very little published information at this time. Carter *et al.* (1996) cited fin biting as a rare occurrence in greenback flounder, and Purdom *et al.* (1972) observed only one case of fin biting in 383 turbot held for 20 months. Farmed halibut are normally transferred from larval rearing facilities at an age of 650 – 700 degree days into weaning tanks. It typically takes two to three weeks for all the fish in a batch to be fully weaned from exogenous live prey items (copepods and enriched artemia) onto an inert, manufactured crumb diet. Fish weight and growth may be depressed while fish learn to accept a new diet, and nipping and physical damage to some individuals is first observed at this stage (Greaves, unpublished data). There is considerable variation in fish size and development at weaning. Some individuals are already settled out on the tank base, metamorphosed and recognisable as halibut in miniature; at the opposite extreme are pellucid individuals that are still pelagic and resemble larvae (Klokseth and Øiestad, 1999). Mortalities throughout the weaning period generally occur in fish that either fail to wean and starve, or are attacked by conspecifics.

Aggression among juvenile and older halibut has been shown to be feed-related (Chapter 2). Observations of weaning halibut show that, although aggression at weaning might be exacerbated by the browsing behaviour of individuals on the tank base, it does not appear to be entirely feed-related. Evidence to date suggests that attacks are generally unprovoked and given by fish that cruise along the tank base and randomly target sedentary individuals. Thorough hand feeding, supplemented by auto-feeders and widespread feed dispersal throughout the day can discourage this browsing by supplying fresh feed throughout the water column. However, aggression has been recorded in the presence of fresh feed, so the motivation for these attacks remains unclear (Greaves, unpublished data).

As commercial production increases, weaning tank space and chilled water supply become limiting. Therefore, information on the optimum and/or maximum density for good growth, condition and survival would be valuable. The chief aim of this study was to monitor the behaviour of weaning halibut at different stocking densities as a step to identifying an optimum rearing density. Three stocking densities were chosen from the range already used in commercial rearing tanks. Higher densities were not tested in this experiment because of the potential risk of compromising fish health.

4.3 Materials and methods

4.3.1 Establishing the experimental populations

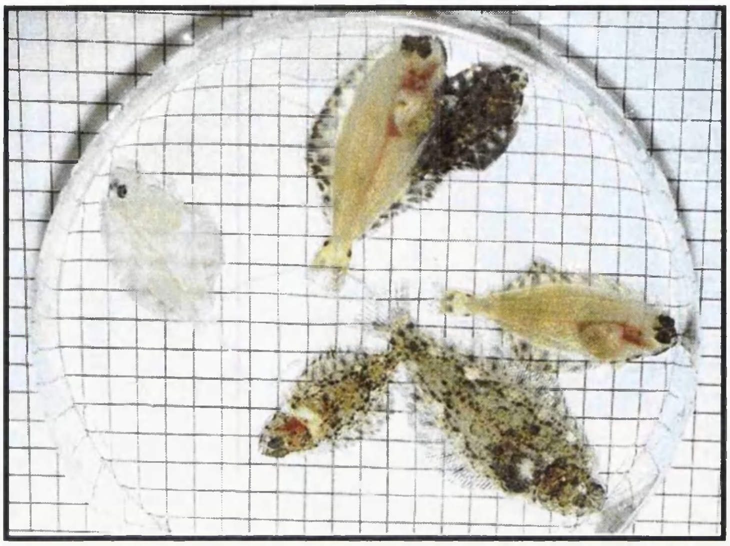
The trial was carried out at a halibut hatchery in Argyll, Scotland (Otter Ferry Seafish Ltd., Latitude 56^0 North). All trial fish originated from the same larval tank to avoid batch to batch variation. Cohort survival between start feeding and the end of larval rearing was 23.6%, (exceeding the normal mean of 20%), when fish were transferred into a standard $2m^2$ weaning tank (1257L) at 681 degree days. From this tank, fish were netted and randomly distributed among 9 50L volume (surface area 0.14 m^2) tanks (Table 4.1) to facilitate observations of fish behaviour during the experimental period. Three observation tanks were used per treatment. In order that the experimental populations were representative of a normal weaning batch, containing fish of variable size and developmental stage (Figure 4.1), only very poor fish were rejected (weak, non-feeders deemed unlikely to survive the next few days).

Each experimental tank population was bulk-weighed using standard husbandry procedures. All fish in a tank were contained in a single net and excess water was gently shaken and dabbed off. Fish were then transferred to a tared vessel and the total fish weight recorded. The mean weight of fish was then calculated.

Table 4.1: Number of fish per observation tank and stocking density, together with corresponding number of fish in the associated standard production tanks.

Density	Number of fish:	Number of fish /litre	Equivalent No. fish in standard $2m^2$ production tank:
Low	88	1.8	2000
Medium	175	3.5	4000
High	350	7	8000

Figure 4.1: Photograph showing the size and developmental variation within a halibut batch on transfer to weaning (681 degree days). Fish are shown in a standard petri dish (represented are fully pigmented, metamorphosed demersal halibut and a pelagic, slow-developer).



4.3.2 Husbandry

All environmental factors were standardised, and the routine husbandry regimes and conditions employed in the hatchery were followed as closely as possible (Table 4.2). Fish were fed to excess, such that uneaten feed was present on the tank base but water quality was maintained. Halibut are visual feeders (Klokseth and Øiestad, 1999) and, in order to encourage weaning onto the inert diet, feed was delivered at regular intervals throughout the day from automatic belt feeders, supplemented by hand feeds. Inert diet sank slowly through the water column or remained on the water surface for a time. Spread from the feeders was very good given the small size of the tanks good (covering the whole area), and feeding response to hand feeds was poor as a result, hence surplus feed was present throughout the day. As with production tanks, the observation tanks were flushed each morning to remove waste feed, and the base and walls gently brushed to reduce the build up of bacterial film. In order to avoid undue stress and disturbance to trial fish, weekly sample weights were obtained from the remainder of the cohort in the production tank for mean growth data. Mortality for each tank was very low and dead fish were not replaced. Any dead fish were removed, examined for physical damage and their total length and weight recorded.

4.3.3 Estimating growth and condition

At the end of the 5 week trial, experimental tank populations were bulk weighed in a tared vessel for biomass and mean weight data. Individual fish were then placed into a beaker of water (direct 'dry' handling was avoided to minimise stress), and scored for physical damage. A four-point scale based on the percentage

Table 4.2: Environmental and husbandry regimes in the observation tanks:

Tanks:	Circular 50 L volume
Water depth:	35cm
Water flow:	2L/minute
Water temperature	9 – 10 °C
Light regime:	24 hours, 80-180 lux at tank surface
Feed regime:	auto-feeders (20 hours), hand fed twice a day
Feed (% body weight / day):	weaning @ 15%, post-weaned @ 7%.
Mean initial fish weight	0.3 grams.

Table 4.3: The scale used to score dorsal, anal and caudal fin damage:

Score:	Damage Description:
0	Fins complete, no damage
1	< 30% fin damaged or missing
2	30 - 50% fin damaged or missing
3	> 50% fin missing (fin may be bitten down to base)

of damage was applied to dorsal, anal and caudal fins (Table 4.3). In addition to recording the number of fish with physical damage in each tank, an overall damage score was calculated for each individual by adding scores for all fins. Given that there was a maximum score of 3 for each body area and 3 affected areas (caudal, dorsal and anal fins), there was a maximum attainable score of 9.

Specific growth rate (SGR) was calculated according to the formula:

$$SGR = 100 \times (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are the weights of the fish at times t_1 and t_2 and $(t_2 - t_1)$ is the time in days between weighing.

4.3.4 Mortality

Total mortality in the observation tanks was very low (2.9%) and well within the normal expected value for production tanks of <10%. All fish that died were considerably under the mean weight of the production cohort (by as much as 0.65 grams by week 4, when mortality levels peaked at 30/week), suggesting they had failed to wean onto the crumb diet and had starved.

4.3.5 Behavioural observations and video filming

Throughout the course of the trial, each tank was filmed on three days. Qualitative data was collected to identify the context in which aggression occurred. An overhead camera (*iN-Former* colour camera, In-Depth Systems, Basingstoke, UK) was mounted on a rail, and moved from tank to tank on the days each was filmed. One tank was filmed per day during 15 x 4 minute intervals over 24 hours. Each tank was thus filmed every nine days. Due to the small size and large number of fish per tank, no individual identification and monitoring was undertaken. Videos were analysed for levels of aggression (aggressive acts/fish/minute), and the contact site of an attack on the body of the recipient fish. Feeding behaviour and any indication of territoriality or defence of specific tank base areas by individuals were also recorded.

4.3.6 Statistical analysis

All statistical analyses were performed with MINITAB Version 11 (MINITAB Inc., USA). Analysis of variance (ANOVA) was used to identify statistical differences between means for growth rates and frequency of aggression, and observation tanks at the same density were treated as replicates. Significant ANOVAs were followed by a Tukey multiple comparisons test to locate differences in behaviour and SGR between treatments. A significance level of 0.05 was used in all cases. Chi-square tests of association were used to determine the effect of stocking density on the number of fish with physical damage, and the targeted body areas where physical damage occurred. A Kruskal-Wallis was used to ascertain differences in damage severity between densities, and post-hoc testing on the average ranks was carried out to determine where the differences lay.

4.4 Results

4.4.1 Qualitative description of aggressive interactions

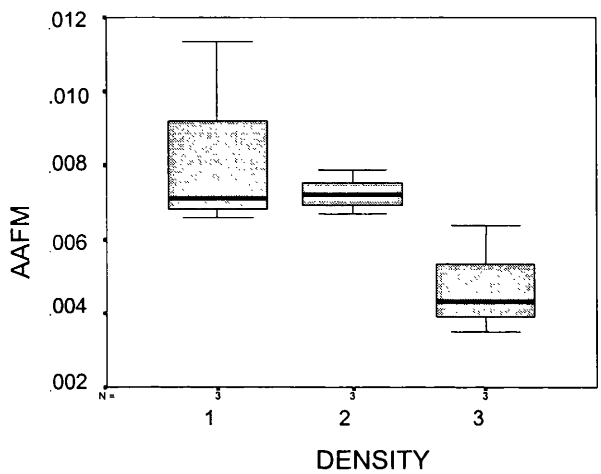
Aggressive interactions (mainly nips and chases) were not directly feed related. All attacks occurred on the tank base and none were seen mid-water. Demersal fish were not uniformly distributed in any of the tanks, and halibut tended to lie over each other and cluster in certain areas. Interestingly, aggressors rarely targeted fish that were part of a big group, but instead harassed those lying alone or in groups of 2-3 elsewhere in the tank. An aggressor typically targeted a sedentary individual from several inches distance, advanced slowly to within striking range, then lunged forward to bite. There appeared to be no territorial defence, as the aggressor usually moved away from the area almost immediately and pursued a different fish. Some fish were persistently aggressive and, at times, one halibut was the perpetrator of all aggression during an observation period. Such individuals could be seen cruising along the tank base biting one fish after another in a seemingly random manner.

Sedentary fish were often displaced by aggressive individuals, and moved away quickly as the other fish approached. Many fish left the tank base and settled instead on the walls, or started swimming high in the water column. On several occasions, pelagic individuals that swam down and settled on the tank base were targeted. Soon after settling, their tails were nipped at and, in most cases, the fish resumed swimming. Aggressors that targeted several fish in close succession caused an increase in the number of fish that swam off the tank base.

4.4.2 The frequency and target of aggressive interactions

Levels of aggression were broadly similar across the day. There were striking differences in the frequency of aggressive acts between the three densities, and good agreement between all replicate tanks (Figure 4.2). The frequency of aggression was comparable in the low and medium density tanks, but significantly lower in the high density replicates (ANOVA, $F_{2, 6} = 32.28$, $P<0.001$). The following body areas received nips and bites: caudal (38% of attacks) dorsal and anal fins (36%); head (23%); and eye (3%).

Figure 4.2: Box plot of aggressive acts / fish / minute (AAFM) for low, medium and high density tanks. Median AAFM are represented by the black lines in the boxes. Densities are 1 (low), 2 (medium) and 3 (high) respectively. The frequency of aggression was comparable in the low and medium density tanks, but significantly lower in the high density replicates (ANOVA, $F_{2,6} = 32.28$, $P<0.001$).



4.4.3 Physical damage

All physical injury was to the dorsal, anal and caudal fins and surviving fish had neither pectoral fin nor eye damage. Figure 4.3 shows the percentage of damaged fish in each treatment. The mean number of fish with physical injuries was significantly higher in the low density groups (36%) compared to either the medium density (9%) or the high density replicates (10%; $\chi^2 = 129.69$, d.f. = 2, $P<0.001$). The distribution of fin damage on injured fish is shown in Table 4.4. Most affected fish had either caudal or dorsal/anal fin damage, but some had both, and a significantly higher number of these fish were in low density tanks ($\chi^2 = 28.67$, d.f. = 4, $P<0.001$). Three fish with severe bite damage to their dorsal and/or anal fins and lesser caudal fin damage are shown in Figure 4.4.

The severity of injury was also calculated for each affected fish. Individuals scored between 0 and 7 on a severity scale with a maximum value of 9. The three groups differed significantly in the intensity of injuries, and fish held at low density had greater physical damage than fish in either of the remaining groups (Kruskal-Wallis test, H = 139.23, d.f. = 2, $P<0.001$). Post-hoc testing on the average ranks (Siegel and Castellan, 1988), showed significant differences in damage severity between low and medium and low and high density groups. The most severe fin damage (dorsal or anal fin, score 3), where the fin was bitten right down to the base, was only recorded in the low density tanks.

Figure 4.3: Box plot of percentage physical damage for low, medium and high density tanks. Median values are represented by the black lines in the boxes. Densities are 1 (low); 2 (medium) and 3 (high) respectively. The low density mean was significantly higher than those of the medium and high density tanks ($\chi^2 = 129.69$, d.f. = 2, P<0.001).

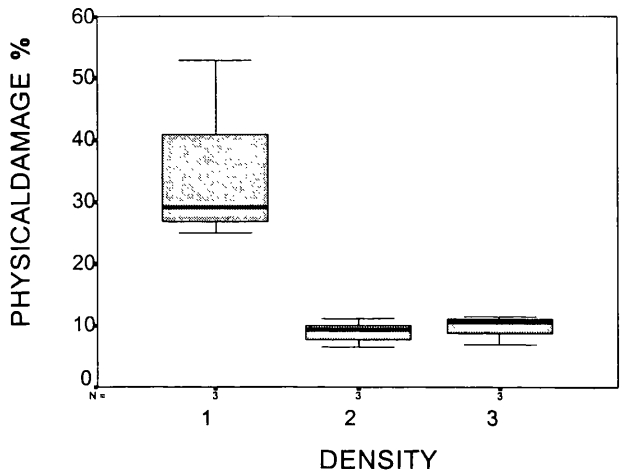


Table 4.4: The number of fish with physical damage to the caudal, dorsal / anal fins or to both areas is shown with the corresponding percentage value for each density group.

Damage Site:	Low density:		Medium density		High density:	
	# fish	% fish	# fish	% fish	# fish	% fish
Caudal fin only	32	13	23	5	71	7
Dorsal / anal fins	35	14	16	3	21	2
Caudal, dorsal & anal fins	24	9	5	1	8	1

Figure 4.4: Halibut from a low density tank showing severe physical damage to dorsal, anal and caudal fins as a result of intra-specific aggression. These were the most severely affected individuals in the experiment.

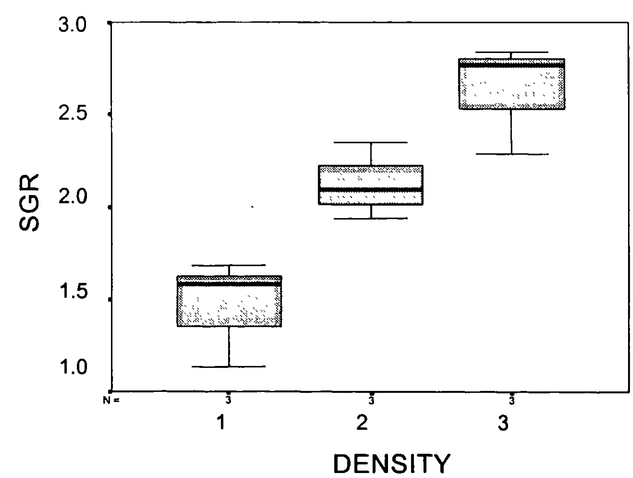
4.4.4 Growth

After five weeks, the high density replicates had consistently better SGR (ranging from 2.29 – 2.84), than did the low density replicates (1.13 to 1.68; Figure 4.5). The difference between mean SGR values for the three densities was statistically significant (ANOVA, $F_{2,6} = 14.21$, $P<0.005$). A Tukey test showed that the high density tanks had significantly better SGR values than all of the low density tanks, though there was no significant difference between low and medium or medium and high density treatments.

4.5 Discussion

The growth performance of halibut in the high density replicates was comparable to that of the production cohort (median SGR 2.77 and 2.72 respectively). At the remaining densities, fish growth and condition was poorer, and there was a strong suggestion that keeping fish at the lowest density was detrimental to both production and fish welfare. Although the level of aggression observed in this study may not appear very high (0.0043 acts/fish/minute in the low density tanks), when aggressive acts per day are calculated there is potential for 545 acts between 88 fish. Halibut farming in the UK is still in relative infancy, and techniques are continually being modified and refined. The acquisition of new broodstock and the growing expertise of staff has led to increased egg availability and improved survivals throughout larval rearing during the past two years. As a consequence, weaning fish are being held at higher densities and the lowest stocking density used in this study is no longer consistently applied.

Figure 4.5: Box plot of Specific Growth Rate (SGR) for low, medium and high density tanks. Median values are represented by the black lines in the boxes. Densities are 1 (low), 2 (medium) and 3 (high) respectively. The difference between mean SGR values for the three densities was statistically significant (ANOVA, $F_{2,6}$ = 14.21, P<0.005).



Turnbull *et al.*, (1998) suggested that the social environment may be as important as the physical environment to the health of farmed fish. Aggression not only causes physical injury, but also has the potential to inhibit the function of the immune system thereby increasing susceptibility to disease pathogens (Wedemeyer, 1997). Abbott and Dill (1989) also pointed out that tissue repair by injured fish incurs an added metabolic cost. Jobling (1985), and Brown *et al.* (1992), have suggested that improved growth rates observed in Arctic charr held at higher densities may be behaviourally mediated. A decrease in the frequency of aggressive interactions, coupled with net energy savings (through a reduction in swimming activity and aggression) could serve as the possible mechanism for differences in growth rates.

The type of physical damage sustained by weaning halibut differed from that seen in older farmed fish (Greaves and Tuene, 2001). Although caudal fin damage was common, there was no pectoral fin or eye damage recorded. Conversely, damage to the dorsal and anal fins (which is rare in older halibut) was prevalent in the present study. Some individuals were badly damaged, with both dorsal and anal fins ragged and partially removed. Several fish developed ragged caudal fins as a result of biting, and some of these individuals elevated these above the tank base and into the water column. Given that attacks by aggressors were only seen to occur on the tank base itself, this may have been an attempt to deter further injury. It is likely that the eye removal noted on some mortalities occurred either when moribunds were dying or when picked at after death. On two occasions observations of production tanks showed moribund fish being attacked and eyes were targeted during these encounters. Pectoral fin damage is relatively common among halibut from 5 grams weight (Chapter 2, and Greaves and Tuene, 2001). This most likely occurs in the water column when fish are feeding, as the fish employ the fin as a steering aid, holding it perpendicular to the body. In this position, it would be an easy fin for aggressors to target. The lack of damage to this fin at the weaning stage may be explained by the fact that all recorded attacks were directed at fish residing on the tank base, when this fin would be mainly flat against the body and not present an obvious target.

In their study of aggression in Atlantic salmon parr, Turnbull *et al.*, (1998) showed that specific body areas were attacked at a rate different from that expected based on their relative size. However, in our study of weaning Atlantic halibut, there is a strong relationship between the relative size of the body area and the number of attacks received. The dorsal, anal and caudal fins were most commonly targeted. It seems likely that the preferential targeting of these fins by aggressors occurred either because they constitute a large proportion of the fish area, or because they were easier to grasp and bite than the body itself. Data from the videos relating to targeted body areas are consistent with the actual damage scored at the end of the trial, and the higher incidence of fin damage in the low density tanks is concordant with the significantly greater frequency of aggression. Levels of aggression varied little across the day, perhaps unsurprising given that fish were maintained on 24 hours light.

The decision to perform our study in 50L tanks allowed us to obtain good quality behavioural data but may have amplified the effects of stocking density. However, daily observations of production tanks confirmed that overall behavioural profiles were the same in both systems (i.e. there were no behaviours seen in one and not the other). However, the relative probability of repeated attacks on the same individual would have increased in the smaller tanks. The limited tank volume gave little opportunity for fish to evade aggressors. If harassed fish left the tank base and resorted to swimming, they would have expended valuable energy, possibly reflected by the poorer specific growth rates. Those that remained on the tank base risked further attacks.

The higher levels of activity observed in the lower density tanks are indicative of stress, and may partially explain the poor growth attained. Jobling and Wandsvik (1983) suggested that poor growth in subordinate fish may have been caused by an "undefined psychological stress". Fish at the highest density not only grew better throughout the trial period but also suffered less damage. Therefore, we believe that growth was a function of density whereby the influence of aggressors at higher density was reduced, and individuals achieved better food conversion ratios.

4.6 Conclusions

This study has quantified the aggressive behaviour and growth of juvenile halibut held at different production densities over the critical weaning period. Fish in the lower density tanks attained significantly poorer growth and suffered the highest incidence of physical damage. Recipients of aggression did not respond aggressively to attacks but simply fled the scene. The nature of physical damage resembles that seen in production tanks, although in larger facilities damage may be less noticeable or severe because targeted fish are able to evade aggressors or flee to other areas of the tank. This study indicates that an optimal rearing density for weaning halibut may lie between the medium density treatment equivalent to 4000 fish/2m^2 production tank and the high density treatment equivalent to 8000 fish/2m^2. In this density range, the frequency of intraspecific aggression is reduced and the growth performance of the fish is enhanced. Additional work in larger systems is evidently required to determine whether increasing densities still further would be beneficial. These results have positive implications for halibut hatchery operators where tank space and chilled water supply may be constraints, and their goal is to produce healthy fast-growing fish.

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Chapter 5

The effect of size grading on the prevalence of eye damage in juvenile Atlantic halibut

5.1 Introduction

Atlantic halibut is a relatively new species for aquaculture. The majority of farmed halibut are the progeny of broodstock captured from the wild and are, therefore, relatively undomesticated. Unsurprisingly, they exhibit behaviours that, while adaptive for survival under natural conditions, prove problematic in an intensive rearing environment.

Behavioural interactions between fish such as intraspecific competition, aggression and dominance hierarchies have evolved to enhance an individual's success by increasing access to resources. In nature, where resources may be limiting, fish that compete aggressively can access more food, grow more quickly and maximise their fitness (Huntingford and Thorpe, 1992).

Intraspecific competition of this kind is a pervasive problem in the aquaculture of carnivorous fish (Magnuson, 1962; Abbott, 1991; Olla, Davis and Ryer, 1992). Dominance hierarchies are generally based on fish size and can form rapidly, particularly at low rearing densities (Brown *et al.*, 1992). Subordinate fish may suffer reduced growth rates (from interference competition or intimidation and chronic stress). This further reinforces the size hierarchy and generates positively skewed size distributions within populations, comprising a few larger individuals but many smaller fish (Noakes & Grant, 1992). This increase in variance of a size distribution with time, due to differential growth rates, is termed growth depensation (Magnuson 1962).

Aggressive behaviours including fin-nipping, biting and chasing are known to cause serious injuries to many farmed species including halibut (Wall, 1999; Greaves and Tuene, 2001) and salmonids (Turnbull *et al.*, 1998; MacLean *et al.*, 2000). Health problems as an indirect consequence of aggression between fish occur relatively frequently in culture systems.

The challenge for aquaculture is to identify the behavioural repertoire of a farmed species and to understand the underlying mechanisms. Farmers can then manipulate the culture environment accordingly and design husbandry systems that promote more efficient production while accommodating the behaviour of the fish.

One such strategy is periodic size grading, which has been adopted as routine management practice on many fish farms. It has proved advantageous in promoting more rapid and homogeneous growth among individuals in a population, as it serves to fragment established social hierarchies and reduce disparate growth rates between dominant and subordinate fish (Brown *et al.*, 1992; Seppa *et al.*, 1999). In addition it is believed that growth rates of smaller individuals improve in the absence of larger fish. Grading is used as a management tool to reduce aggression, to produce fish of more uniform size throughout the production cycle, particularly at harvest, and to simplify feed administration and the allocation of correct feed pellet and net mesh sizes.

Female halibut are batch spawners, producing batches of eggs at 2-3 day intervals. Spawning order hierarchies that occur between females further accentuate the linear production of juveniles. Mortality between egg and weaned juvenile is considerable, so for these reasons efficient production involves amalgamating several batches in order to make up sufficient numbers to stock on-growing tanks and cages. Juveniles of varying ages coming through hatcheries, coupled with a large range in individual growth rates means that size variation is difficult to avoid.

Eye injuries among juvenile halibut in Scottish on-growing facilities have become increasingly apparent over the last 12-18 months as production has intensified. Intraspecific aggression is the suspected cause, as there is a higher prevalence of damage to the more prominent non-migratory eye than to the outermost migratory eye. In support of this theory, many fish have concomitant bite damage to their upper pectoral fins, an easy target when fish swim in the water column. Such physical injuries to fins resulting from aggression appear to be especially prevalent in fish between 20 –150 grams weight, novel cases thereafter being rare (Greaves & Tuene, 2001).

Size-grading halibut is routine management practice from post-weaning, and is believed to enhance growth and reduce aggressive interactions between fish. A 10 - 15% prevalence of total eye removal in a Scottish stock of mixed size fish (within the first 4 months after stocking in a cage) has recently highlighted the issue of tight size grading during on-growing. However, there is still a lack of information on grading frequency and tightness of grade required to circumvent these problems, and no absolute proof that grading is the only influencing factor. In addition to the economic implications of this eye damage for producers, the fish welfare issue and market image of farmed halibut cannot be ignored. Anecdotal observations from farm staff indicated that eye removal severely debilitated fish. Injured halibut tended to exhibit abnormal swimming behaviour at or near the water surface and were clearly separated from the remainder of the group. Such fish may well be more likely to suffer further damage, either as a result of aggression or because of accidental collisions with the holding facility whilst the fish is distressed and unable to swim properly. There is a consensus that high levels of eye damage in fish from as small as 20 grams weight is one of the most significant problems facing halibut on-growers, and that more information on the effect of size grading on halibut behaviour is required.

This chapter describes a production-scale experiment designed to examine the effect of size grading on the prevalence of eye damage in juvenile halibut held in 5m diameter production tanks. The aims of the trial were to:

a) determine whether tighter size grading could significantly reduce the prevalence of eye damage in on-growing populations;
b) ascertain whether tighter size grading could benefit fish growth
c) determine whether eye injury leads to reduced growth
d) characterise the ontogeny of eye damage in groups of halibut over time

e) investigate the predictability of developing eye damage from relative fish weight or length.

5.2 Materials and methods

5.2.1 Establishing the experimental populations

In mid-February 2000, six halibut populations, comprising over 12,500 juveniles, were established at Otter Ferry Seafish Ltd, Loch Fyne, Argyll. Given the considerable number of fish involved in this experiment, and the time-consuming sorting of fish, grading was determined on the basis of fish total length. In order to ensure that we incorporated the entire range of fish in the on-growing population, setting up the tanks entailed several steps. The fish were first transferred out of nursery facilities into two 5m on-growing tanks, each containing some 6,500 halibut, mean weight approximately 20 grams. 1,000 fish were then length-measured and evenly distributed into the three tanks that would represent standard production populations with halibut of varying size (termed 'ungraded'). Length data from these 1000 fish was plotted to give a length frequency distribution.

It was apparent from this distribution that there were insufficient fish to stock 3 tightly graded tanks of identical size range without either omitting fish of certain length from these graded stocks, or seriously under-representing that size range of fish in the ungraded groups. Therefore, three populations of tightly graded fish, designated small, medium and large were established, and the remaining fish distributed among the other three tanks (Figure 5.1). Each tank contained approximately 2,100 halibut juveniles and length distributions are given in Table 5.1.

Figure 5.1: Length frequency distribution of 1000 halibut in February. Grade divisions (decided on the basis of fish length) are shown. Fish of all sizes were represented in ungraded populations.

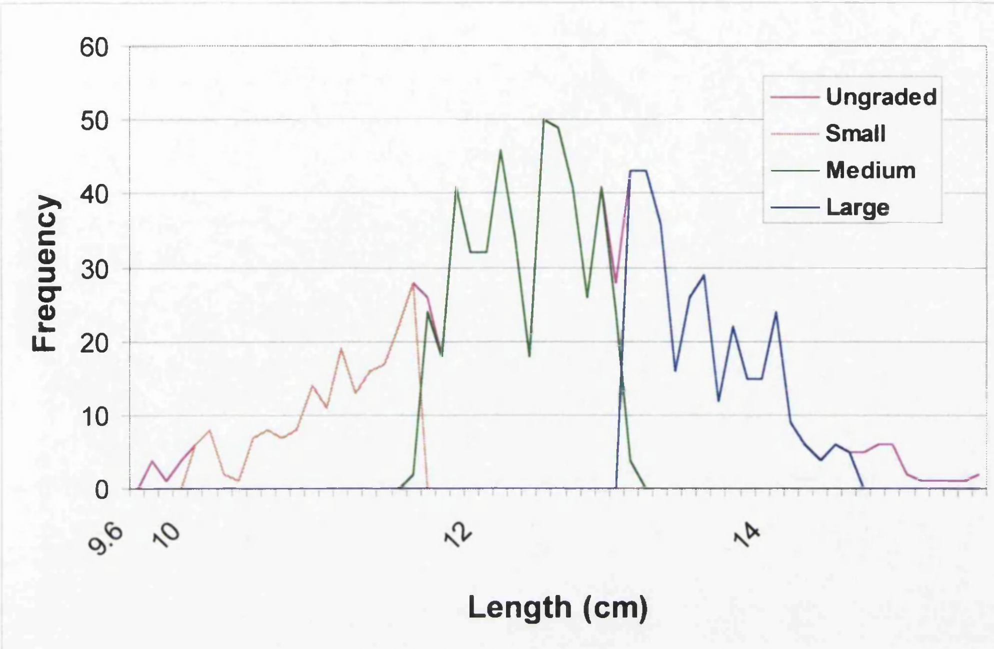


Table 5.1: Total fish length range for graded and ungraded populations at the trial start. Mean fish length, CV for length, and the number of fish in each tank is also stated.

Tank	Min. length (cm)	Max. length (cm)	Mean length (cm)	CV length [stdev/mean]*100	No. of Fish
Small Graded	10.0	11.5	10.95	4.11	2056
Medium Graded	11.5	13.0	12.26	3.23	2100
Large Graded	13.0	14.5	13.54	3.24	2100
Ungraded 1	9.7	16.2	12.58	8.58	2138
Ungraded 2	9.8	16.4	12.59	9.26	2149
Ungraded 3	9.7	16.2	12.52	7.86	2138

5.2.2 Experimental conditions

The trial commenced in mid-February 2000 and was terminated at the beginning of July. Halibut were maintained in sea water, salinity 34 ppt., and water temperature throughout the 21 week trial period ranged from a low of 7.6^{0}C in February to a high of 11.9 ^{0}C in July, mean temperature 9.1^{0}C. Flow rates maintained oxygen levels above 80% saturation, and tanks were aerated for added security. Tanks were fitted with protective green polythene covers that reduced ambient light intensity. An entry hatch was generally closed to exclude both high light levels (several thousand lux on bright days) and harmful UV rays. Throughout the course of the trial, day length ranged from a minimum 8.44 hours light in February to 17.23 hours in July. Estimated tank biomass at the start of the experiment, assuming a mean fish weight of 20 grams, ranged from 41.12 Kg (2.62 Kg/m^2) to 42.98 Kg (2.74 Kg/m^2). This was well below the recommended stocking density for this size of halibut of 10 Kg/m^2. In this study the number of fish per tank was held constant (except for mortalities), so biomass density increased as the fish grew.

Fish were fed a commercial diet (Trouw Aquaculture) of two pellet sizes: Marine Halibut 35 (3.5mm) with 56% Protein:16% Lipid, and Marine Halibut 50 (5mm) 52% Protein : 22% Lipid. Feed rate was maintained @ 2% biomass/day, and the rations were dispensed by 20-hour auto-feeders supplemented by three hand-feeds per day.

The halibut available for the trial were of wide size range, and there were insufficient numbers to set up three tightly graded groups of equivalent size.

Therefore, since grading was the factor in question, small, medium and large graded populations were established. It was important to determine whether these three groups could be regarded as true replicates. February – April SGR weight (because the grades were deemed most effective at that time) of Elastomer-marked halibut in the three graded populations was analysed using one-way ANOVA to see if fish were growing at a similar rate (Table 5.2). The result was highly significant (ANOVA, $F_{2, 381} = 32.58$, $P< 0.001$), and a *post-hoc* Tukey test showed differences in SGR weight between all groups, with the biggest grade performing best. CV_{SGR} revealed that there was considerable inter-individual variation in growth rates in the small graded tank.

5.2.3 Tracking individual halibut

One of the aims of this experiment was to monitor the time-scale over which eye damage occurred and to see whether minor eye damage could stabilise and recover, or if it invariably led to eye loss. It also allowed us to determine whether individual fish with eye damage become more susceptible to further injury (especially the loss of the other eye). To realise these aims, fish were individually marked so that they could be tracked throughout the trial.

A normally pigmented halibut has a white blind side providing a large area ideal for marking. Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc.) were used to mark the fish. Elastomer is a bio-compatible two part fluorescent material. Once mixed, it forms a liquid that can be injected into translucent tissue using a hypodermic syringe. Within 24 hours this liquid cures into a pliable solid, forming a well-defined permanent mark.

Table 5.2: The rate of weight gain (SGR) (% d^{-1}) and variation in growth (CV_{SGR}) in a subset of elastomer-marked fish in three graded populations of Atlantic halibut juveniles of different sizes between February and April.

Tank number and fish size	Number of fish	SGR W	CV_{SGR}
1 Small graded	119	0.84	46.74
4 Medium graded	131	1.07	27.36
5 Large graded	134	1.17	26.84

These tags were developed to provide externally visible internal marks for fish too small for other methods. Given that the experimental fish were production stock, these small but distinct marks were highly suitable. A marking scheme was devised whereby a mark to the blind side of the fish in a specific area corresponded to each sampling point (Figure 5.2a).

In order to obtain growth data from individuals, 150 normal (undamaged) fish in each tank were Elastomer-marked at the start of the trial. Using six distinct marking sites on the margins of the unpigmented blind side (Figure 5.2b) and four colours, we devised a total of 150 unique combinations per tank. Each fish was identified by two colour-coded marks (e.g. Fish #1: site 1 Green, site 3 Red). These fish were tracked throughout the trial, providing data on individual growth rates (weight and length measurements at each sample point), and the development of physical damage over time.

To verify that marked fish were selected as a representative sample of the group populations, a Wilcoxon Signed Rank test was used to compare the initial lengths of Elastomer-marked and unmarked fish in each tank at the trial start (Wilcoxon Statistic = 16.0, $N = 6$, $P = 0.295$). The median of the differences was not significantly different from zero (Table 5.3), therefore, in terms of initial total fish length, Elastomer-marked fish were a representative sample of the experimental populations.

Figure 5.2a: Halibut Eye Damage Marking Scheme: (View of halibut blind side)

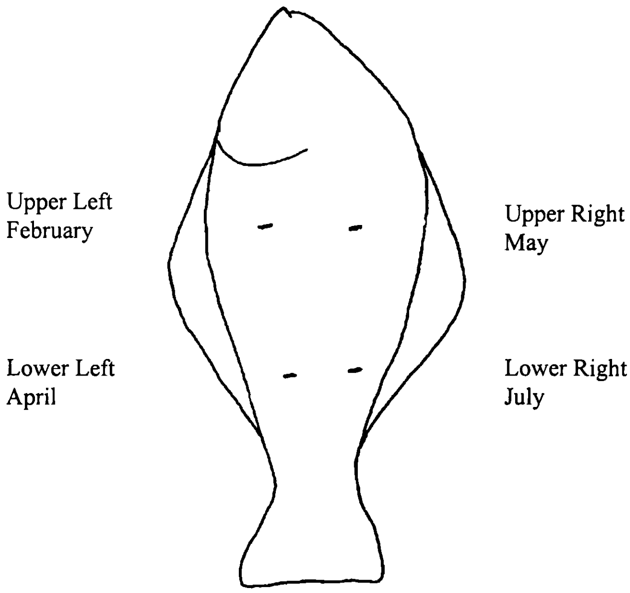


Figure 5.2b: Halibut Individual Marking Scheme: (View of halibut blind side)

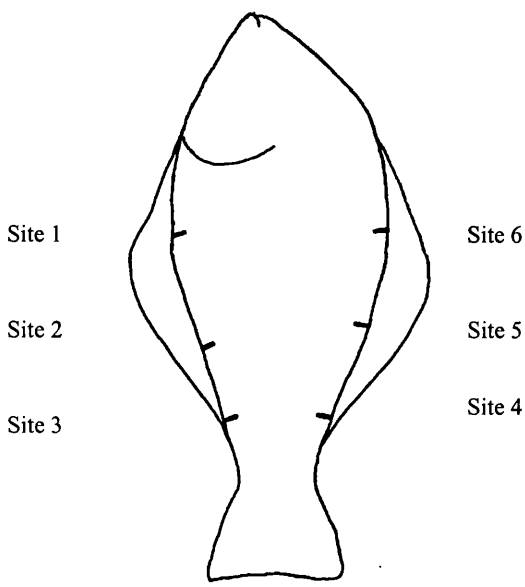


Table 5.3: Mean total fish length (cm) & CV length at the trial start for Elastomer-marked and unmarked fish in all tanks

Tank	Mean total fish length (cm) & CV length			
	Elastomer-marked	CV_L	Unmarked	CV_L
1 Small graded	11.07	4.95	10.95	4.2
4 Medium graded	12.41	3.7	12.26	3.2
2 Large graded	13.45	3.84	13.54	3.2
1 Ungraded	12.70	8.63	12.58	8.6
3 Ungraded	12.51	8.37	12.65	2.0
6 Ungraded	13.54	7.12	12.52	7.9

Table 5.4: Eye damage severity scores and a description of the Respective level of injury:

Eye Score:	Description of eye damage:
1	Bruising, swelling to the outer eye, or opacity on cornea (fish still sighted)
2	Haemorrhaging visible within eye, but eye still present (fish still sighted)
3	Eye completely missing, wound healed.
4	Eye completely missing. Fish has a fresh and bloody open wound.

5.2.4 Classifying and tracking eye damage

We identified four categories of eye damage, two deemed minor, where the eye was still present and functional, and the others severe where the eye had been removed (Table 5.4). These categories were necessarily broad to facilitate sampling, but encompassed all levels of injury observed (Figures 5.3 & 5.4).

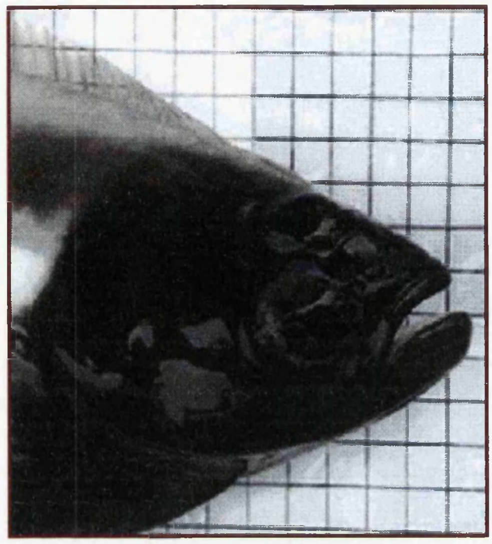
An Elastomer-marking scheme was used to track eye damage throughout the experiment: the position of a mark related to when injury was first noted, and the colour of the Elastomer indicated the severity of eye damage as shown in Table 5.5. For example, a mark in the upper left site denoted that eye damage to that fish was first evident in February. For a severity score of 2, a green Elastomer mark would be placed at that site. If, at the following sampling point in April, the affected eye was missing leaving a fresh wound, the fish would receive a second Elastomer mark in red to the lower left site. In this way, the development of eye damage over time for individual fish could be traced. For each eye-damaged fish we also recorded whether injury was to the migratory or non-migratory eye.

5.2.5 Sampling whole tank populations

Halibut were sampled at intervals of approximately seven weeks. Given the high number of fish in the trial, it was only feasible to record weights for marked individuals. However, production staff conducted monthly batch sample weights to adjust feed rates (100 fish bulk weighed and weight averaged).

Figure 5.3: Photographs depicting minor eye damage (scores 1 and 2):

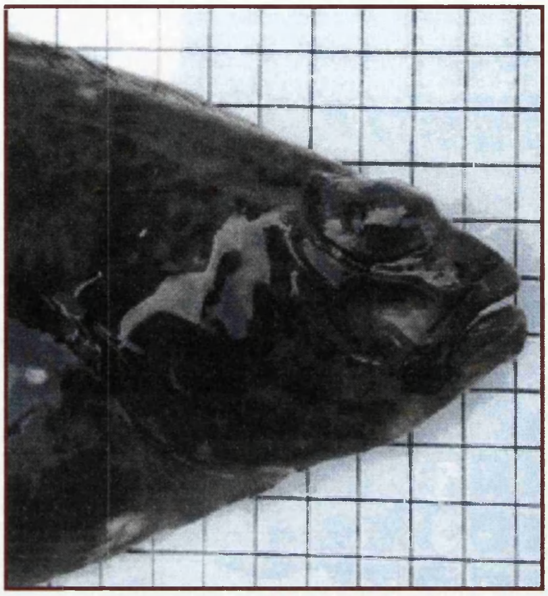
i) Eye damage score 1: tissue damage but eye still present.



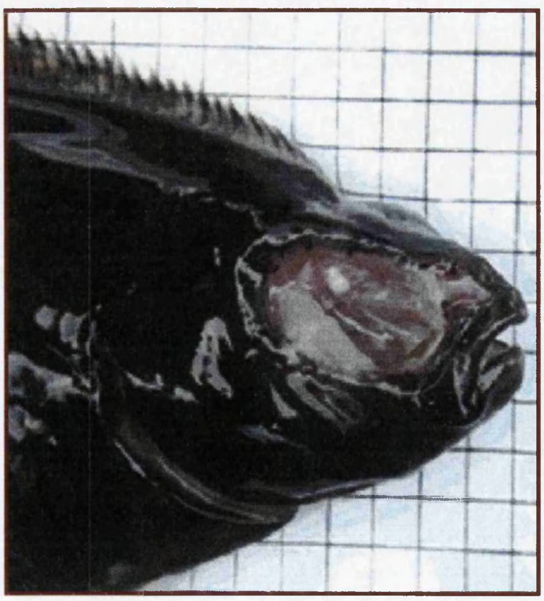
ii) Eye damage score 2: haemorrhaging visible within the eye, but eye still present

Figure 5.4: Photographs depicting eye loss (scores 3 and 4):

iii) Eye damage score 3: eye missing but wound completely healed.



iv) Eye damage score 4: eye missing, fresh open wound



At each sample point, all halibut were examined for eye damage. A crowder was used to confine the fish into one half of the tank and a grading trough placed on a table on the empty side. Fish were netted onto the trough and sorted into 3 categories: individually marked, eye-damaged or normal fish. Efforts were made to reduce handling and sampling stress. The sorting trough contained sufficient water for fish to swim up and down prior to examination, and normal fish were returned immediately to the tank. Although tank water levels were lowered, flows were maintained throughout to minimise stress to the majority of the fish. Damaged and marked fish were placed into buckets and removed for more detailed examination and required marking. These were anaesthetised with benzocaine and then weighed, measured or marked as appropriate. Previous marks were also registered. Following a recovery period in freshly oxygenated water, the fish were returned to their tank. A small number of fish in very poor condition with double eye loss were removed from the trial populations. These were humanely killed by overdose anaesthesia with benzocaine.

At each sample point the coefficient of variation for growth of Elastomer-marked fish in each tank was determined, and comparisons made of growth rates and total eye damage prevalence between populations. At the final sampling in July, we recorded the total length of every fish in the trial, and both length and weight data for any eye-damaged halibut and individually marked fish in all tanks, giving a comprehensive data set for the beginning and end of the experiment.

5.2.6 The impact of physical handling on the prevalence of eye damage

Chi-square (χ^2) tests were used to ascertain whether physical handling during trial set-up and sampling could predispose fish to developing eye damage. Given that Elastomer-marked individuals were handled on all sampling days throughout the trial, the number of marked and unmarked fish with eye damage was compared. Using July data (end sample) for these individual fish, we investigated whether handling increased the probability of these fish developing eye injuries. A Chi-Square (χ^2) test was used to compare the numbers of Elastomer-marked fish and unmarked halibut with no damage, minor eye damage and severe eye damage (eye loss) for each tank population. The results are given in Table 5.6.

There were no significant differences between means for tanks 1 – 5. Tank 6 was significant ($P < 0.05$), but had fewer severely damaged fish than expected in the Elastomer-marked group. Repetitive handling, as experienced by Elastomer-marked fish in this experiment, did not appear to predispose fish to developing eye damage.

5.2.7 Statistical Analyses

All statistical analyses were performed with MINITAB Version 11 (MINITAB Inc). Normality of data was checked using the Anderson-Darling test, and non-parametric tests were used for data of non-normal distribution. A probability level of $P < 0.05$ was considered significant for all tests. Chi-Square tests were used to compare the number of Elastomer-marked fish with no damage, minor eye damage and eye loss for each tank population, and the number of injured and uninjured fish at

Table 5.5: Eye damage severity scoring, a description of the injury and corresponding Elastomer marks.

Score:	Description of eye damage:	Mark Colour:
1	bruising/swelling/opacity to outer surface	Blue
2	eye haemorrhaged but still present	Green
3	eye missing, wound fully healed	Yellow
4	eye missing, fresh wound	Red

Table 5.6: Chi-Square (χ^2) comparing the numbers of Elastomer-marked fish and unmarked fish with no damage, minor eye damage and severe eye damage (eye loss) for each tank population. Handling per se did not increase the probability that marked fish develop eye injury.

Tank		No Damage		Minor Damage		Eye Loss		χ^2	P Value
		# Fish	%	# Fish	%	# Fish	%		
1 SG	Elastomer	78	80.4	8	8.2	11	11.3		
	Unmarked	1609	82.5	146	7.5	196	10.0	0.27	NS
4 MG	Elastomer	81	78.6	14	13.6	8	7.8		
	Unmarked	1372	71.3	324	16.8	228	11.9	2.70	NS
5 LG	Elastomer	66	67.0	20	20.0	13	13.0		
	Unmarked	1354	71.6	311	16.5	225	11.9	1.23	NS
2 UG	Elastomer	63	64.3	21	21.4	14	14.3		
	Unmarked	1391	66.7	357	17.1	337	16.2	1.29	NS
3 UG	Elastomer	73	78.5	12	12.9	8	8.6		
	Unmarked	1358	74.7	233	12.8	228	12.5	1.28	NS
6 UG	Elastomer	86	72.3	23	19.3	10	8.4		
	Unmarked	1336	68.2	294	15.0	329	16.8	6.49	$P < 0.05$

Chi-Square χ^2 significance $p < 0.05$, 2 d.f. = 5.99

particular sample points with their subsequent condition. Comparisons were also made of injury levels in graded and ungraded populations. Kruskal-Wallis tests were used to examine the effect of eye damage on growth rate. Specific growth rate (SGR) (% d^{-1}) was calculated according to the formula:

$[SGR = \underline{(LnW_2 - LnW_1)} / t_2 - t_1 \times 100]$

where Ln is natural log, W_1 and W_2 are the weights of fish recorded at times 1 and 2, and $t_2 - t_1$ is the interval in days between weighing respectively. The same formula was used to calculate the rate of increase in fish total length. Variability in growth (weight and length) was expressed as the coefficient of variation:

(CV = [SD/mean] x 100).

5.3 Results

Data in this section are presented in the same sequence as the experimental aims.

5.3.1 General growth of the production cohort

Figures 5.5 and 5.6 show the length frequency distribution for all experimental fish in February and July. In addition to a general increase in size, there was a marked increase in variance for fish weight and length over the experimental period. In February, mean and median total length was 13.4cm, standard deviation 2.15 and CV 16.11. By July mean and median total length was 20.4 cm, standard deviation and CV increased to 6.23 and 30.57 respectively. Halibut ranged in size from 9.7cm – 16.4cm at the trial start, and 9.7cm to 31.1cm in July. Figure 5.7 gives the average weight increase over time of all Elastomer-marked individuals, and Figure 5.8 depicts the growth performances.

Figure 5.5: Juvenile halibut length frequency distribution at the trial start, February 2000

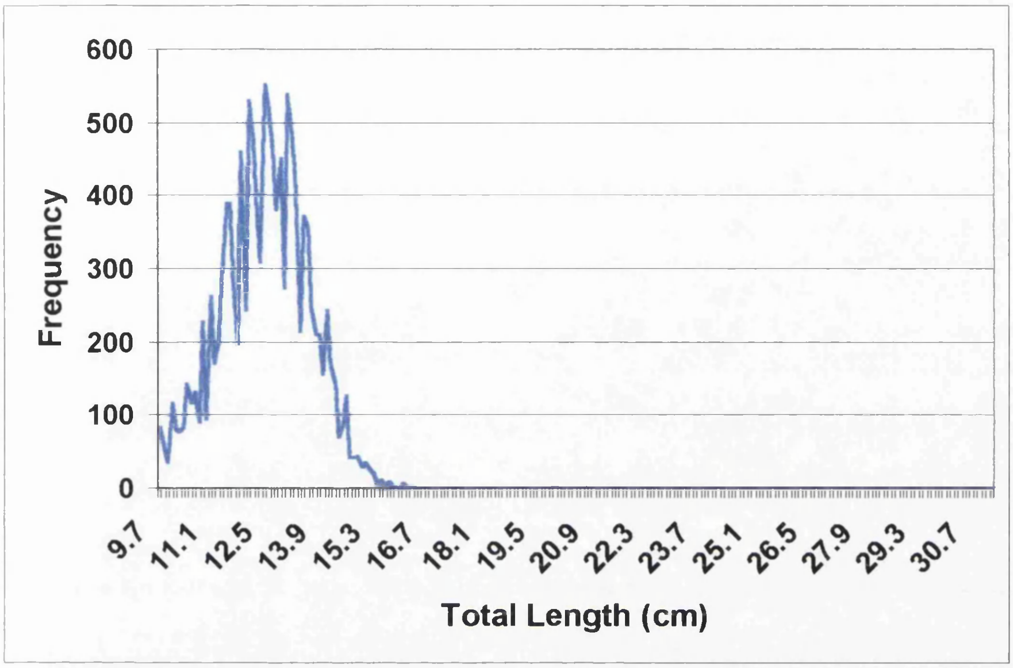


Figure 5.6: Juvenile halibut length frequency distribution at the trial end, July 2000

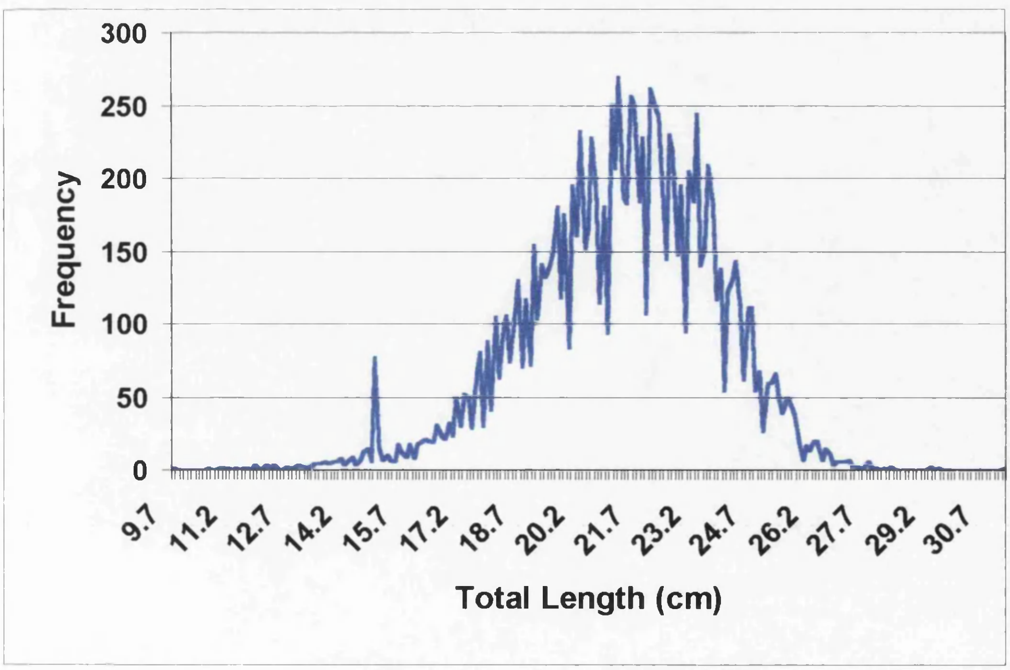


Figure 5.7: Mean weight increase over time of all Elastomer marked fish, February-July 2000. Error bars represent standard deviation of the mean.

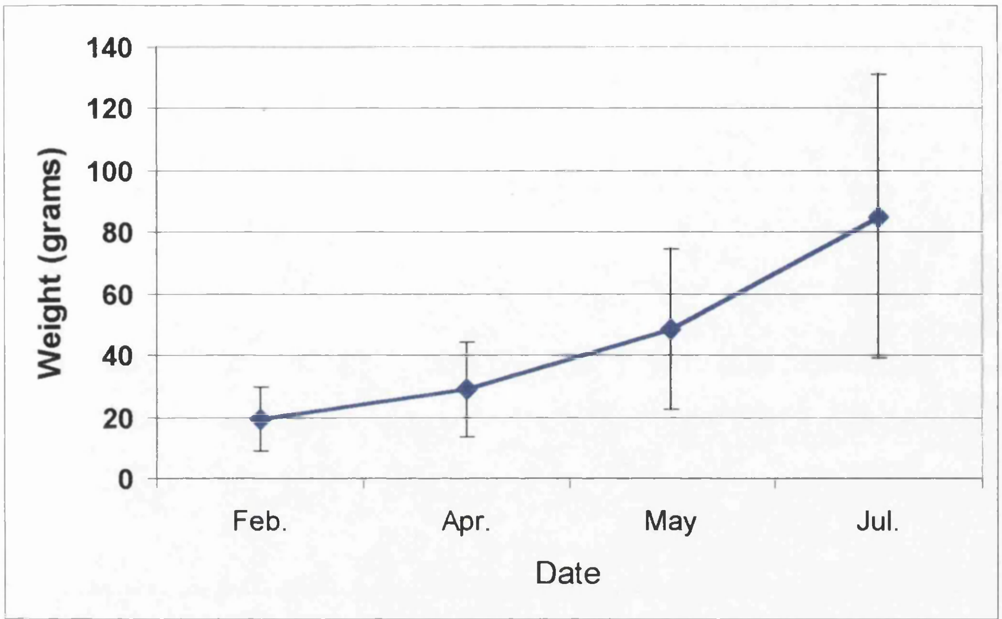


Figure 5.8: Mean specific growth rate over time of all Elastomer marked halibut. Error bars represent standard deviation of the mean.

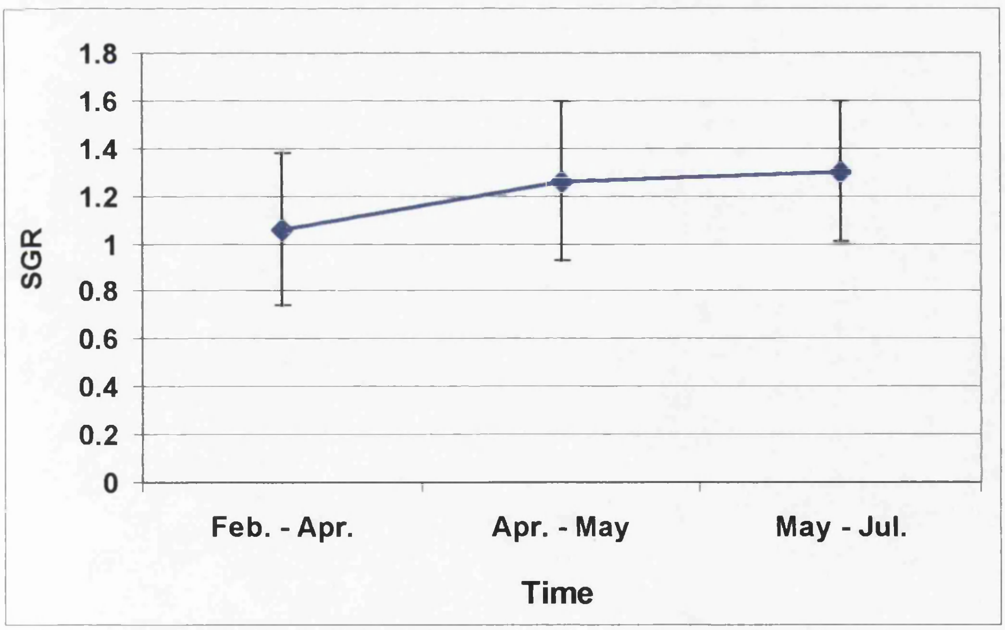


Figure 5.9: Cumulative eye injuries (minor and severe) for all populations over time February – July 2000

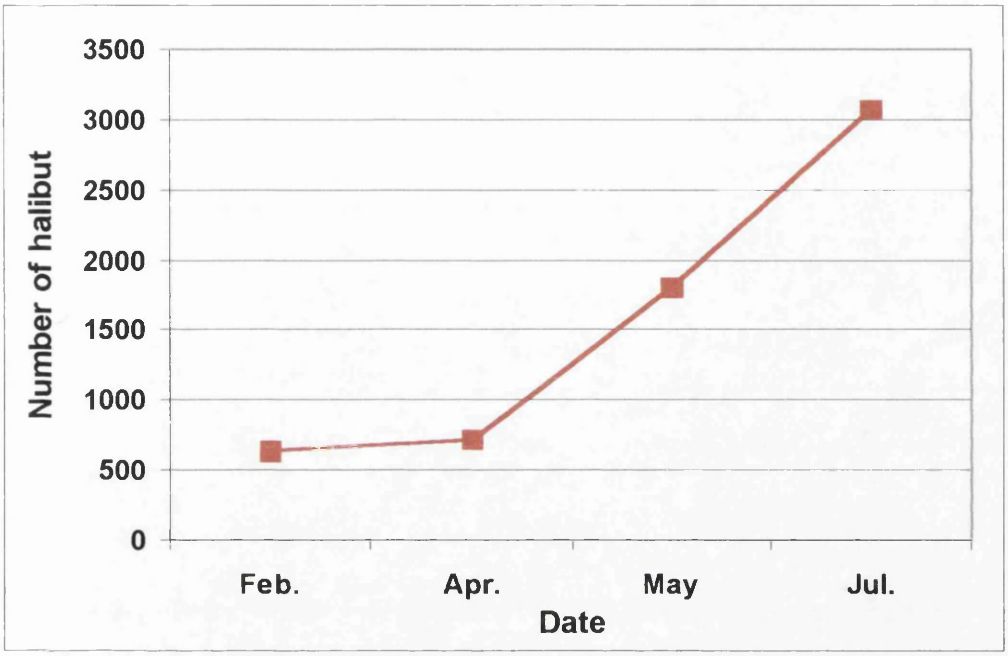
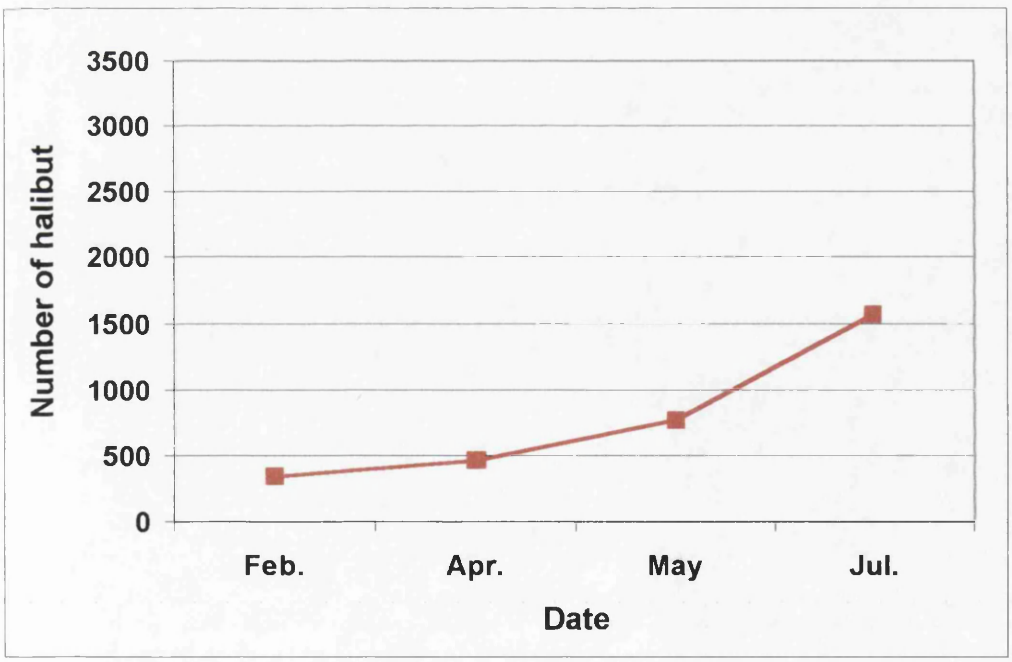


Figure 5.10: Cumulative eye losses for all populations over time February – July 2000



5.3.2 The prevalence of eye damage

When halibut populations were set up in February, 5% of the fish had already incurred eye damage of some description while in the nursery facilities. By the trial end in July, this percentage total had risen to 26%, 13% of which had suffered eye losses. Figure 5.9 shows cumulative eye injuries (minor and severe) over the course of the trial. A rapid increase in eye damage prevalence was evident following the April sampling point (seven weeks into the trial), and injuries increased in a linear manner thereafter. Actual eye losses are shown in Figure 5.10. Here, again the rise is dramatic between April and May.

From a total of 336 halibut with eyes missing in February, a further 125 fish had lost eyes by the April sampling date. This represents a 37 % increase in fish with eye losses in just seven weeks. As the experiment continued, novel occurrences became more frequent, escalating to a 67% increase between April and May, and a 105% increase between May and July respectively. There was an overwhelming bias of damage to the non-migratory eye over the migratory eye, 2744 (87%) to 248 (8%), with only 171 (5%) halibut incurring damage to both eyes. This 10:1 ratio is clearly highly significant.

The prevalence of worsening eye injury was determined by comparing the condition of previously injured fish with uninjured fish using a Chi-Square test (Table 5.7). To determine whether halibut with eye damage were predisposed to develop more severe eye injuries than fish uninjured in the previous period, we recorded the number of injured and normal (Elastomer-marked and unmarked) fish in February.

Table 5.7: Chi-Square χ^2 test comparing the numbers of injured and normal fish in each tank population at a particular sample point, and their subsequent condition.

5.7 a) February to April

Tank		April: Not Worse		April: Worse		χ^2	P value
		# Fish	%	# Fish	%		
1 SG	Normal in Feb.	1926	98.9	2	1.1		
	Injured in Feb.	98	90.7	10	9.3	44.17	$P < 0.01$
4 MG	Normal in Feb.	2018	99.1	18	0.9		
	Injured in Feb.	50	78.0	14	22.0	207.90	$P < 0.01$
5 LG	Normal in Feb.	1992	98.3	35	1.7		
	Injured in Feb.	61	83.6	12	16.4	69.90	$P < 0.01$
2 UG	Normal in Feb.	1983	97.3	56	2.7		
	Injured in Feb.	75	75.8	24	24.2	121.31	$P < 0.01$
3 UG	Normal in Feb.	2044	96.8	67	3.2		
	Injured in Feb.	25	65.8	13	34.2	100.71	$P < 0.01$
6 UG	Normal in Feb.	1956	97.4	52	2.6		
	Injured in Feb.	112	86.2	18	13.8	48.79	$P < 0.01$

Table 5.7: Chi-Square χ^2 test comparing the numbers of injured and normal fish in each tank population at a particular sample point, and their subsequent condition.

5.7 b) May to July

Tank		July: Not Worse		July: Worse		χ^2	P value
		# Fish	%	# Fish	%		
1 SG	Normal in May	1609	88.7	204	11.3		
	Injured in May	122	74.9	41	25.2	27.37	P < 0.01
4 MG	Normal in May	1378	78.0	390	22.0		
	Injured in May	146	78.9	39	21.1	0.14	NS
5 LG	Normal in May	1341	79.7	342	20.3		
	Injured in May	165	62.3	100	37.7	39.89	P < 0.01
2 UG	Normal in May	1363	77.6	393	22.4		
	Injured in May	198	67.6	95	32.4	13.69	P < 0.01
3 UG	Normal in May	1355	83.4	269	16.6		
	Injured in May	138	68.0	65	32.0	29.14	P < 0.01
6 UG	Normal in May	1329	79.1	351	20.9		
	Injured in May	194	79.2	51	20.8	0.00	NS

These fish were again registered in April as either unchanged or in worse condition (i.e. with novel eye damage or more severe injury). The same criteria was used to compare fish in May and July.

Over the course of the experimental sampling, there were 47 (0.4%) halibut with severe damage to both eyes that were humanely culled by overdose anaesthesia. Several mortalities also suffered double eye loss but these were omitted from analysis as eye losses likely occurred after fish had died.

5.3.3 Consequences of eye injury for growth

To determine whether eye injury had an effect on individual growth rates, Kruskal-Wallis tests were used to compare median growth rates of Elastomer-marked halibut with no eye injury, those with minor injury (corneal abrasion or haemorrhage), and those that had lost an eye over the course of the experiment. SGR ($\%d^{-1}$) for fish weight and total length were examined for each category.

Tables 5.8 and 5.9 show that in all populations, undamaged fish grew at a significantly faster rate than halibut with eye injuries. Eye injury, particularly eye loss, had a consistently negative effect on growth in terms of both fish weight and length. Therefore, it appears that absolute (overall) growth is depressed. A weight deficit alone would imply poor condition factor, but here fish which have suffered eye injuries are growing at a slower rate than their uninjured conspecifics.

Table 5.8: Results of Kruskal-Wallis test giving median weight gain (SGR) (%d^{-1}) in relation to eye injury severity score for all experimental populations

5.8 a): Median weight gain (SGR) (%d^{-1}) in relation to eye injury severity score for all experimental populations, February – July 2000

Tank	Median SGR (# fish) for each eye score						H Test-statistic	Degrees of freedom	Probability value & significance (* ** or ***)	
	0		1		2					
	SGR #	Fish	SGR #	Fish	SGR #	Fish				
1 SG	1.23	78	0.98	7	0.96	12	16.01	2	P = 0.000	***
4 MG	1.27	101	1.11	12	1.07	8	21.70	2	P = 0.000	***
5 LG	1.2	64	1.01	19	1.02	14	28.51	2	P = 0.000	***
2 UG	1.18	60	1.01	20	0.90	15	31.87	2	P = 0.000	***
3 UG	1.24	68	1.09	12	0.99	7	19.83	2	P = 0.000	***
6 UG	1.24	84	1.04	23	0.92	10	31.02	2	P = 0.000	***

5.8 b): Median weight gain (SGR) (%d^{-1}) in relation to eye injury severity score for all experimental populations, April – July 2000

Tank	Median SGR (# fish) for each eye score						H Test-statistic	Degrees of freedom	Probability value & significance (* ** or ***)	
	0		1		2					
	SGR #	Fish	SGR #	Fish	SGR #	Fish				
1 SG	1.34	69	1.12	6	1.10	9	12.82	2	P = 0.002	**
4 MG	1.37	73	1.12	12	1.01	8	36.35	2	P = 0.000	***
5 LG	1.21	54	0.90	6	1.04	8	22.23	2	P = 0.000	***
2 UG	1.23	45	1.09	18	0.98	8	17.09	2	P = 0.000	***
3 UG	1.31	57	1.09	10	1.05	3	15.10	2	P = 0.001	***
6 UG	1.26 77		1.07 19		0.82 9		25.50	2	P = 0.000 ***	

Table 5.9: Results of Kruskal-Wallis test giving median length gain (SGR) ($\%d^{-1}$) in relation to eye injury severity score for all experimental populations,

5.9 a) Median length gain (SGR) ($\%d^{-1}$) in relation to eye injury severity score for all experimental populations, February – July 2000

Tank	Median SGR & # fish for each eye score						H Test-statistic	Degrees of freedom	Probability value & significance (* ** or * **)
	0		1		2				
	SGR L	# Fish	SGR L	# Fish	SGR L	# Fish			
1 SG	0.41	78	0.36	7	0.32	12	13.91	2	P = 0.001 ***
4 MG	0.42	101	0.39	12	0.38	8	16.93	2	P = 0.000 ***
5 LG	0.39	64	0.33	19	0.34	14	25.20	2	P = 0.000 ***
2 UG	0.39	60	0.35	20	0.29	15	30.72	2	P = 0.000 ***
3 UG	0.41	68	0.37	12	0.34	7	22.89	2	P = 0.000 ***
6 UG	0.41	84	0.38	23	0.32	10	21.30	2	P = 0.000 ***

5.9 b): Median length gain (SGR) ($\%d^{-1}$) in relation to eye injury severity score for all experimental populations, April – July 2000

Tank	Median SGR & # fish for each eye score						H Test-statistic	Degrees of freedom	Probability value & significance (* ** or ***)
	0		1		2				
	SGR L	# Fish	SGR L	# Fish	SGR L	# Fish			
1 SG	0.45	69	0.36	6	0.34	9	10.48	2	P = 0.005 **
4 MG	0.47	73	0.41	12	0.37	8	33.61	2	P = 0.000 ***
5 LG	0.40	54	0.33	6	0.32	8	22.73	2	P = 0.000 ***
2 UG	0.42	45	0.39	18	0.34	8	14.07	2	P = 0.001 ***
3 UG	0.46	57	0.37	10	0.37	3	14.81	2	P = 0.001 ***
6 UG	0.44	77	0.39	19	0.32	9	17.85	2	P = 0.000 ***

Figure 5.11 depicts SGR over time of individuals with eye injuries first apparent in May. The figure is colour-coded to indicate the severity of injury. There were 9 fish with eye injuries in May and a mean SGR value is shown for fish with the same injury scores to simplify the figure. Eight of the nine halibut showed markedly reduced growth rates post-injury, presumably due to trauma and less efficient feeding capacity. By July, seven of the nine fish showed improved growth rates, and four fish were growing at a faster rate. Of particular interest are two fish that, in May, had eyes missing and fresh wounds that healed by July (M4, J3). Growth rates for these fish were reduced for a time following eye loss but, once the wound had healed, they recovered and their SGRs for period 3 exceeded those attained in period 1. Halibut, therefore, have the capacity to recover and adapt to disability in a relatively short time.

Figure 5.12 depicts SGR over time of 13 fish with novel eye damage in July, and mean SGR for 57 undamaged fish. Again, mean values are shown for fish with the same Eye injury scores. All fish grew at a steady rate between period 1 (February and April) and period 2 (April and May). Eye damage after this period reduced growth rates as expected. This was especially dramatic for a fish that had recently lost an eye, whose SGR plummeted from 1.14 to 0.32.

In July there was a 60:40 % ratio of fish with both eyes damaged (double) in ungraded and graded tanks respectively. The February - July SGR of double eye injury fish and normal uninjured fish were compared for this period. As there were

Figure 5.11: Comparison of SGR over time for individual Elastomer-marked halibut that suffered eye injuries between April and May (N = 9). The dotted line represents SGR for uninjured fish. The level of injury is indicated by the key (according to the classification given in Table 5.5) and the colour-coded lines i.e. M4J3 (red between April and May, and yellow between May and July) indicates that the fish had lost an eye (was freshly wounded when examined in May) and had a healed scar by July. (M = May sample point, J = July sample point; and associated numbers and colours refer to the level of damage).

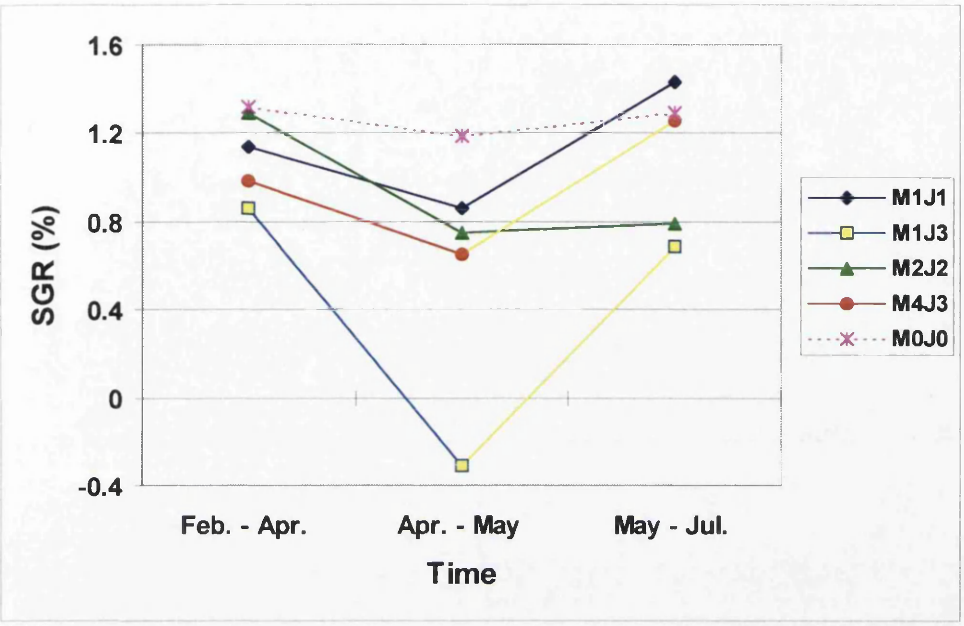
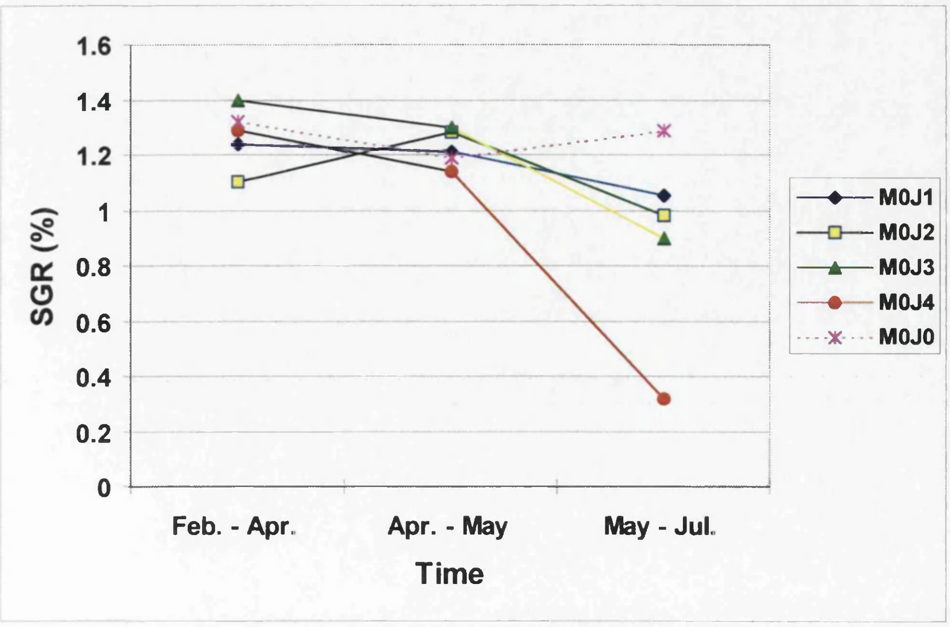


Figure 5.12: Comparison of SGR over time for individual Elastomer-marked halibut that suffered eye injuries between May and July (N = 13). The dotted line represents SGR for uninjured fish. The level of injury is indicated by the key (according to the classification given in Table 5.5) and the colour-coded lines i.e. M0J4 (a blue line between April and May, and a red line between May and July) indicates that the fish was uninjured in May, but had an eye missing and a fresh wound in July.



just eight Elastomer-marked fish with double eye damage, all populations were combined for analysis. A Mann-Whitney U test on these samples revealed highly significant differences between median values of uninjured fish SGR 1.24 and fish with both eyes damaged SGR 0.85 ($N = 455, 8$, $W = 107031.0$, $P < 0.001$).

5.3.4 The effect of grading on the prevalence of eye damage

Cumulative eye injuries (minor and severe) and cumulative eye losses in graded and ungraded tanks throughout the experiment are shown in Figures 5.13 and 5.14 respectively. The distribution of fish with eye losses by July was 57% in ungraded tanks and 43% in graded tanks. While the established size grades remained effective, the rate of eye damage increase in graded tanks was much slower than for ungraded tanks. However, there was a sharp increase in the number of fish with eye losses (and all levels of eye injury) between May and July, by which time these grades had broken down, as evidenced by the increase in CV.

Results of a Chi-Square test comparing the numbers of halibut in graded and ungraded tanks with no eye damage, minor or severe injury at each sample point are shown in Table 5.10. There was no significant difference between the numbers of injured fish in graded and ungraded populations at the trial start, but highly significant differences had developed by April. The graded tanks had significantly fewer damaged fish than expected after just seven weeks. Conversely, there were higher than expected injured fish among the ungraded populations.

Figure 5.13: Cumulative eye damage in graded and ungraded halibut populations, February – July 2000

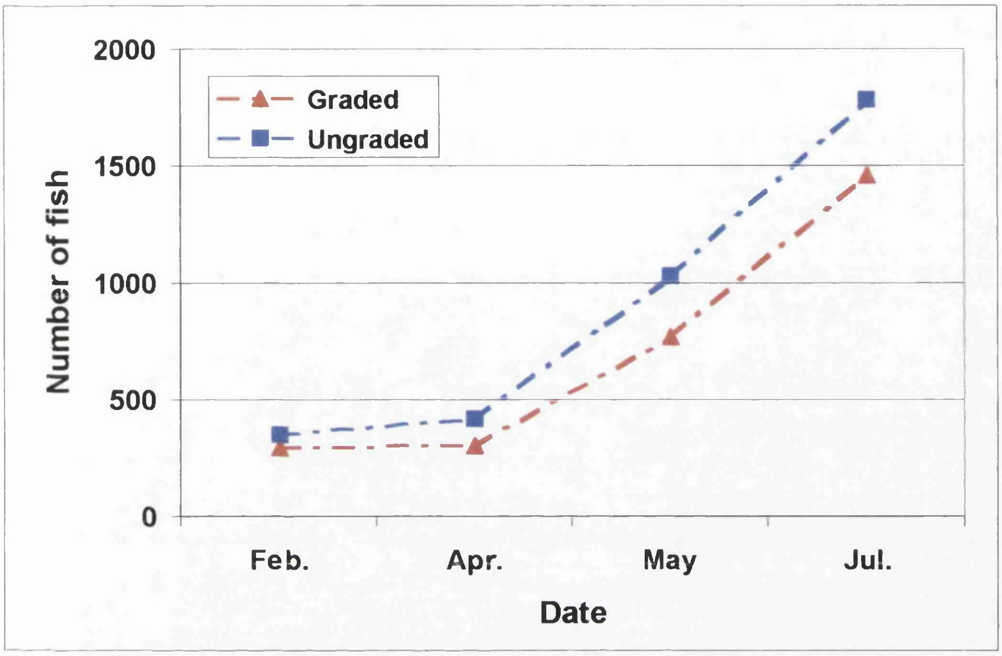


Figure 5.14: Cumulative eye losses in graded and ungraded halibut populations, February – July 2000

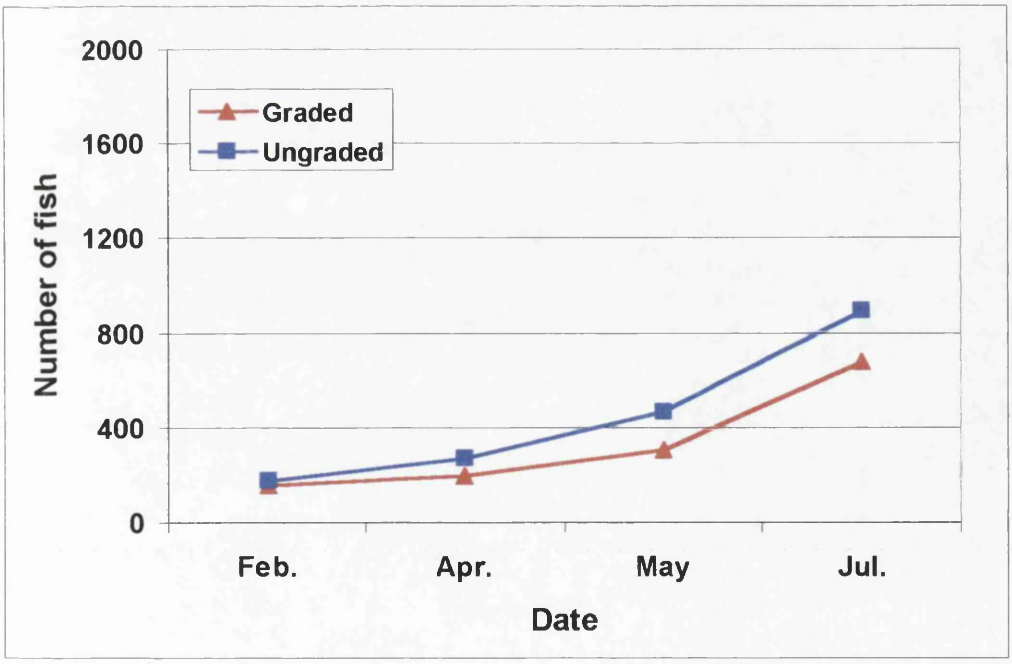


Table 5.10: Chi-Square (χ^2) comparing the numbers of graded and ungraded fish with no eye damage, minor eye damage and severe eye damage (eye loss) at each sample point.

Date		No Damage		Minor Damage		Eye Loss		χ^2	P Value
		# Fish	%	# Fish	%	# Fish	%		
Feb.	Graded	5963	95.3	136	2.2	157	2.5		
	Ungraded	6074	94.5	172	2.7	179	2.8	4.50	NS
April	Graded	5956	95.2	106	1.7	194	3.1		
	Ungraded	6009	93.5	149	2.3	267	4.2	16.67	P < 0.001
May	Graded	5299	87.4	641	7.6	304	7.5		
	Ungraded	5123	83.0	586	9.5	464	7.5	50.63	P < 0.001
July	Graded	4604	75.9	781	12.9	679	11.2		
	Ungraded	4395	71.2	884	14.3	894	14.5	39.43	P < 0.001

Chi-Square χ^2 significance $p < 0.05$, 2 d.f. = 5.99; $p < 0.001$, 2 d.f. = 13.82

The overall percentage increase in eye-damaged halibut throughout the course of the experiment is shown in Figure 5.15. Figure 5.16 represents the relative distribution of eye damage severity (1 – 4), colour-coded in accordance with the marking scheme used in the trial. In February, 48% of injured fish had minor damage (score 1-2) and 52% had lost eyes. Data from April showed that eye loss had increased to 60% of all injured fish. However, May data shows a sharp increase in novel minor eye damage as the prevalence of injury escalated. By July, there were equal numbers of fish in the minor and severe eye damage categories.

Five percent of all eye-damaged fish had injuries to both eyes by July. The relative distribution of these fish is shown in Figure 5.17. It is interesting to note that the prevalence increases with fish size among the graded tanks, being especially high for the large graded fish. Ungraded Tank 3 had fewer eye-damaged fish than the other ungraded populations and also fewer fish with double eye injury. Reasons for this are not yet clear, although it may be linked to mortality data.

5.3.5 Mortalities

Mortality levels throughout the experiment, ranged from 2.4% to 4.6% in five of the six tanks (mean 4.8%). Tank 3 (ungraded), however, had the much higher figure of 11.2% of fish by the trial end, many of which had not been removed from the tank and recorded as mortalities. This may be explained by a problem with the tank centre grid, discovered just after the start of the trial. The grid had dislodged from its position over the tank drain, leaving a small gap. It is likely that fish were

Figure 5.15: The percentage of eye-damaged halibut over time, February – July 2000

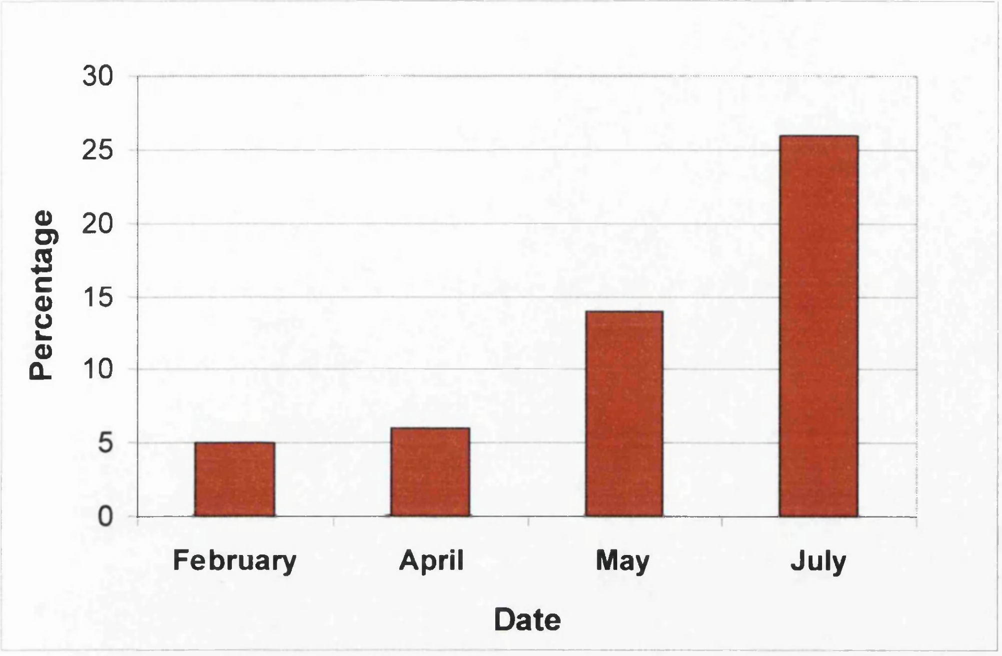


Figure 5.16: The distribution of eye damage severity, February – July 2000

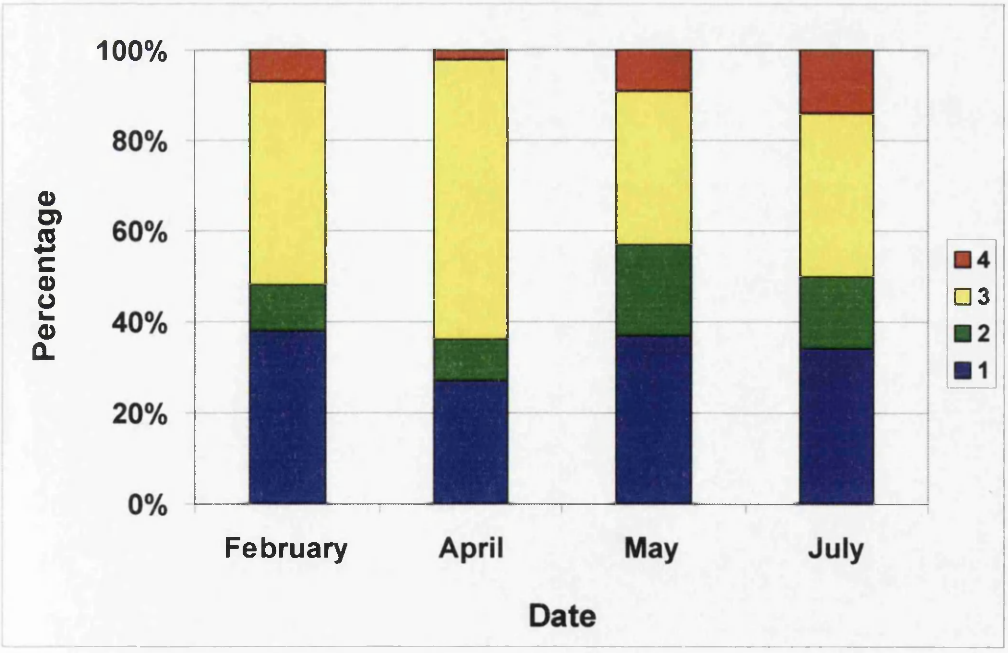
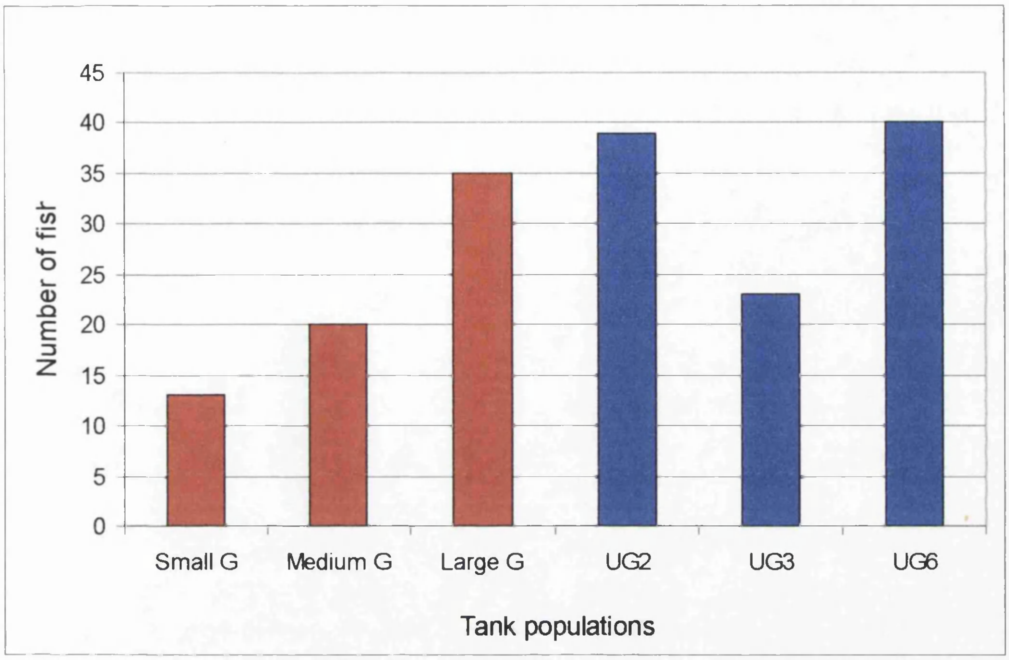


Figure 5.17: The number of fish with both eyes damaged in graded and ungraded populations, July 2000



lost from the tank before this was discovered which would account for the discrepancy.

5.3.6 Grading and growth

Experimental populations were established in February on the basis of halibut total length. Figure 5.18 a) and 5.18 b) represent CV (%) for length and weight over time. There is a clear difference between graded and ungraded populations at the outset, and less variation between fish in graded tanks as intended. Throughout the entire experimental period, all graded tanks maintained lower CV values than their ungraded counterparts. However, as the grades disintegrated, variation between fish in the populations increased. Only the large graded fish (LG5) had consistently low CV values.

Growth trends in graded and ungraded tanks are represented in Figures 5.19: a, b, and 5.20: a, b, respectively. At the population level, grading did not significantly benefit growth, and fish in all tanks appear to have grown at similar rate. However, given the higher prevalence of eye damage in the ungraded tanks, and the negative impact of eye injury on growth, grading indirectly affected the growth of individuals.

5.3.7 The relationship between eye injury and body size

At the end of the trial, the length distribution of halibut in each tank was plotted against the number and percentage of fish with eye damage in each length category. This was done separately for each tank but all showed the same strong relationship. The prevalence of eye damage was consistently higher in the smaller

Figure 5.18a: Coefficient of variation for Elastomer-marked fish weight over time [CV = (Standard deviation/mean x 100)]

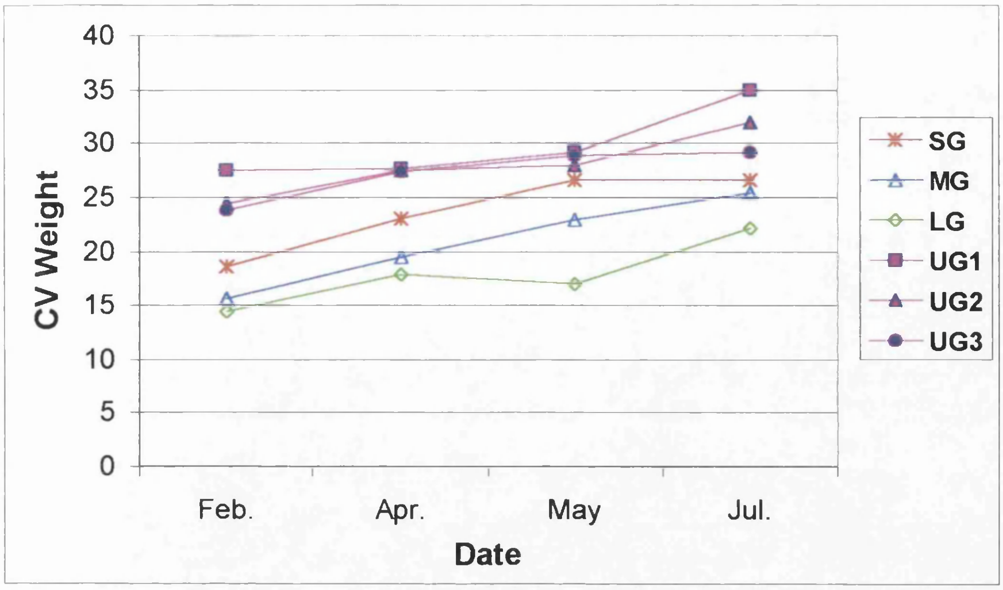


Figure 5.18b: Coefficient of variation for Elastomer-marked fish length over time [CV = (Standard deviation/mean x 100)]

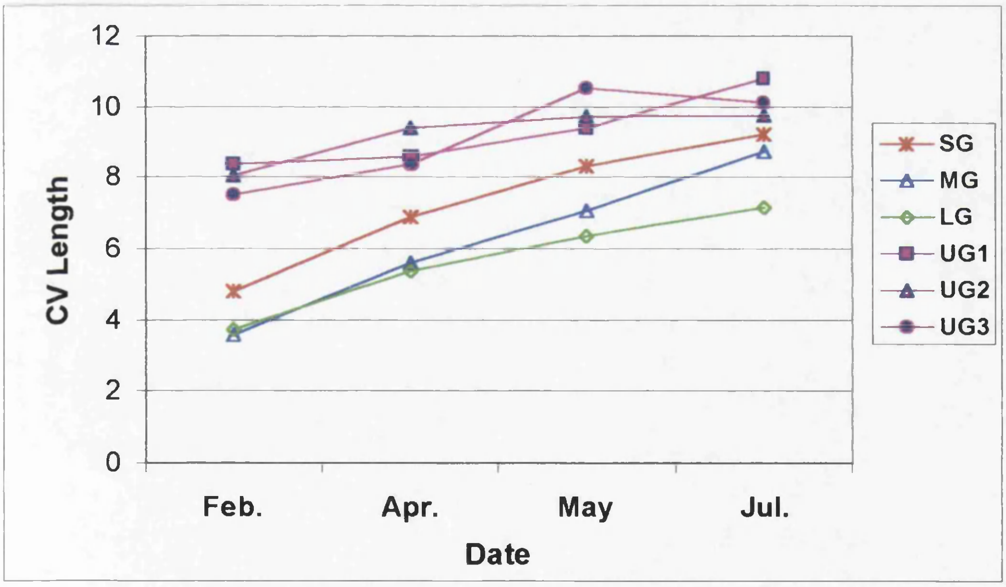


Figure 5.19a: Average length increase over time in graded populations (small, medium and large respectively). Error bars represent standard deviation from the mean.

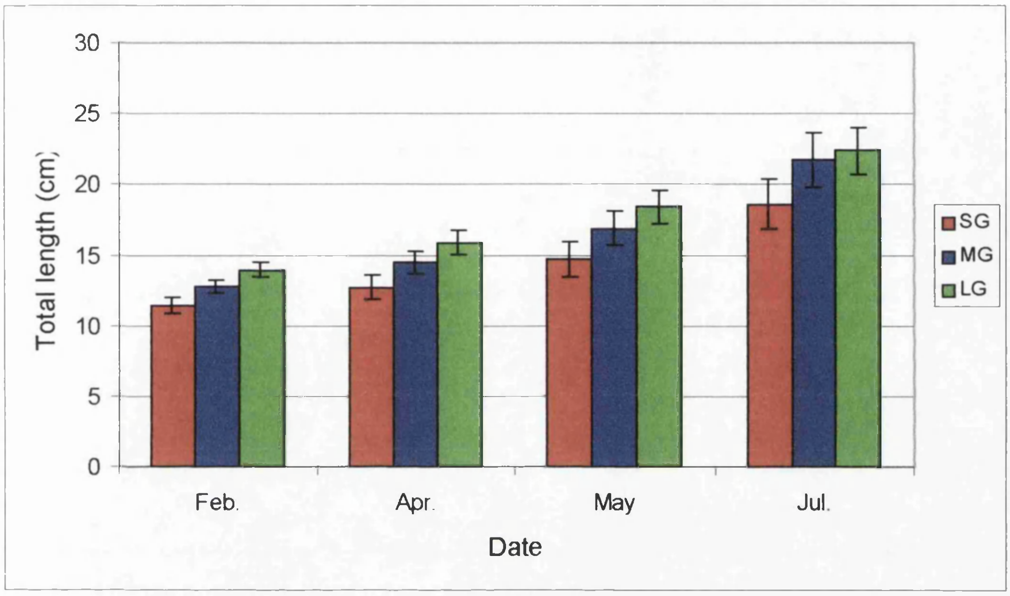


Figure 5.19b: Average length increase over time in the three ungraded populations (1,2 and 3). Error bars represent standard deviation from the mean.

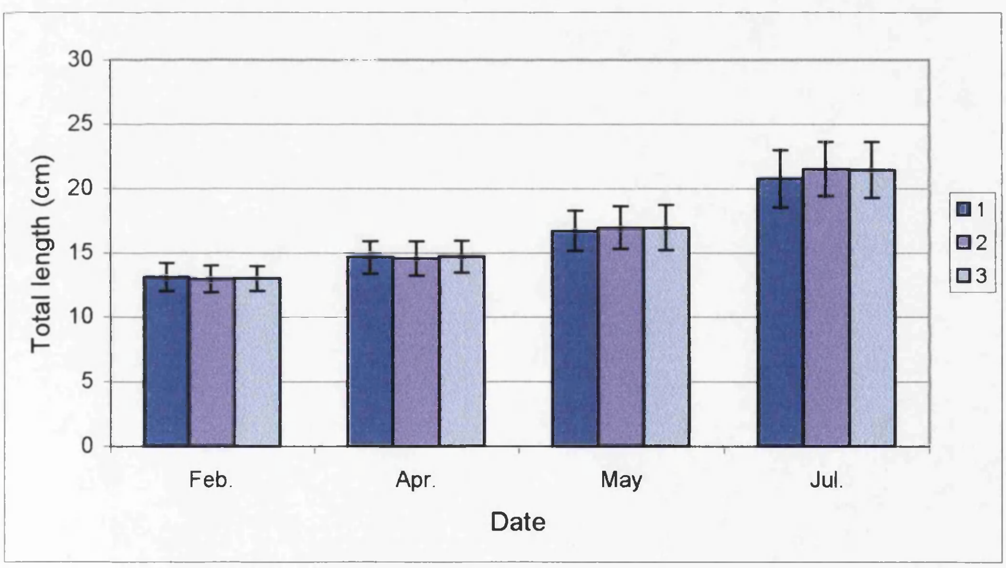


Figure 5.20a: Average weight increase over time in graded populations (small, medium and large respectively). Error bars represent standard deviation from the mean.

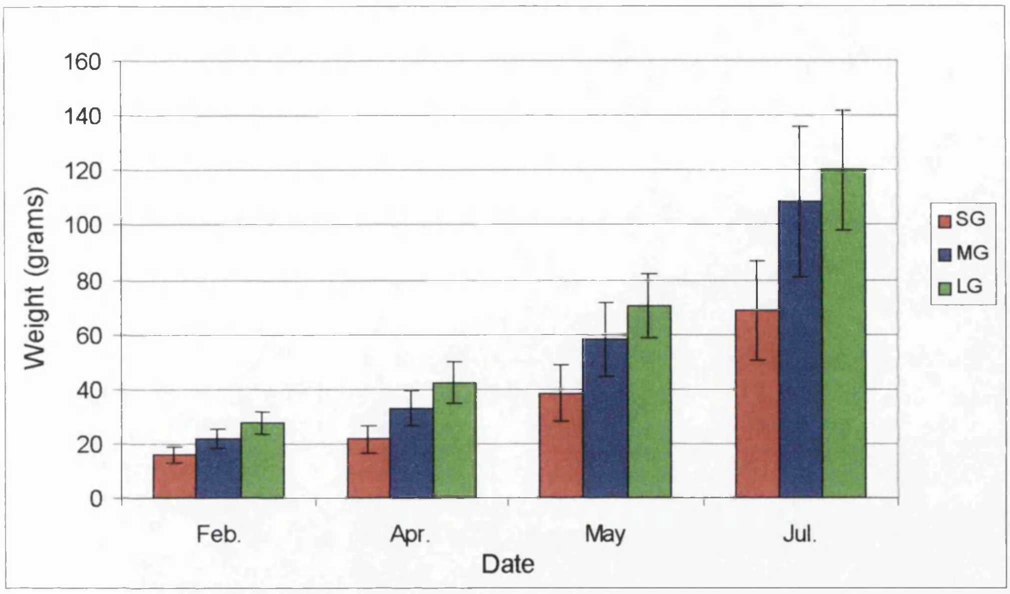
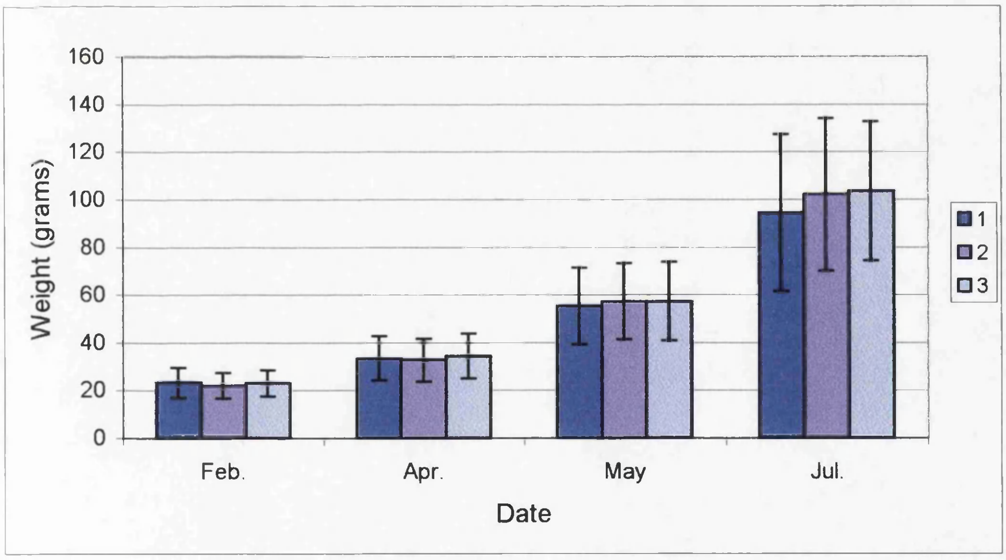


Figure 5.20b: Average weight increase over time in the three ungraded populations (1,2, and 3). Error bars represent standard deviation from the mean.



size fraction of each population. A composite of all tanks is shown in Figure 5.21, and the figures relating to individual tanks are given in Appendix III. Although small fish were more susceptible to eye damage, some of the largest fish also incurred eye injuries during the trial. Most of these individuals otherwise had good body condition.

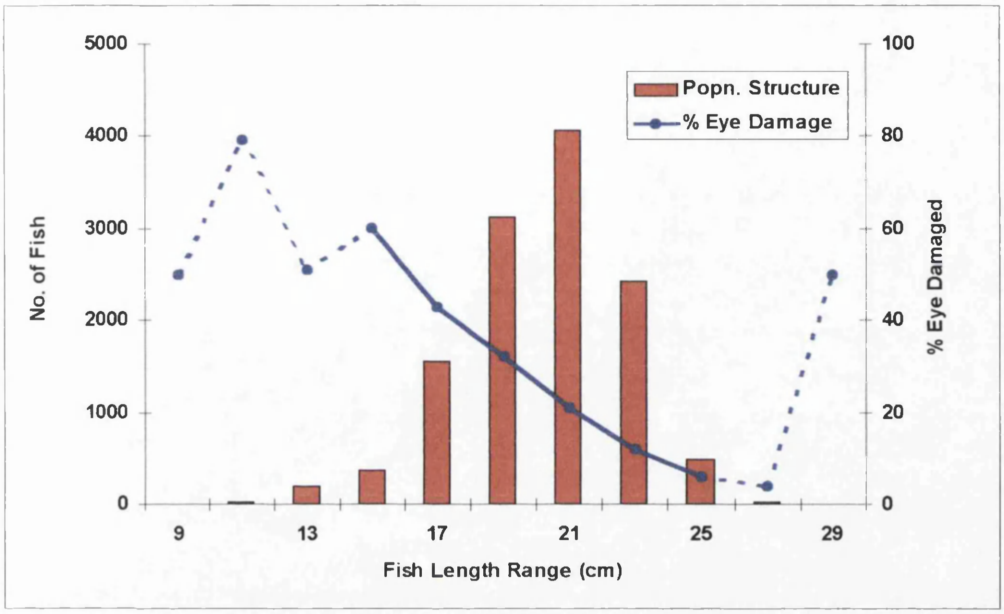
5.4 Discussion

The study of the aggressive behaviour of farmed juvenile halibut is a relatively novel area and there is little published data available. This being the case, there is a need to draw on information provided by husbandry staff. Observations and personal communications of this kind have proved invaluable in interpreting experimental data and putting it into context. I believe, therefore, that it is appropriate to include some of these insights here.

The average growth rates of halibut in the experimental tanks were on a par with or better than those expected by commercial stocks at these temperatures (Marine Harvest Halibut Growth Tables). This was interesting because, although initial stocking was below the recommended density, it appeared to have had no adverse affect on fish growth.

Throughout the experimental period the prevalence of eye injuries increased from 5% to 26% of the population, 13% of which were eye losses. This study was the first to examine the ontogeny of eye damage over time in individual halibut. The

Figure 5.21: Composite of all tanks length distribution in July. The dotted line indicates a very small sample size. The general trend is very clear, that smaller fish suffer more eye damage.



results indicate that, although some injuries can heal, the majority of eye damage becomes worse over time and leads to eventual eye loss.

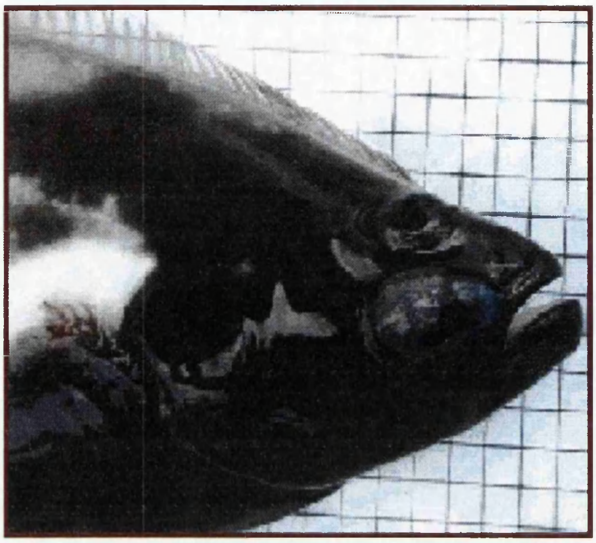
It is suggested that eye damage is caused by intraspecific aggression rather than physical damage from the tank environment. Of all eye damage, 87% was to the more prominent non-migratory eye. This eye is located higher up on the head than the other eye, which may explain its preferential targeting by aggressors. It is suggested that if eye injuries were caused by physical contact (with the tank sides, aeration devices or netting), then the migratory eye, located on the side of the head, would be more vulnerable. This view is corroborated by Norwegian researchers, Nortvedt and Tuene, (1995). However, severe eye injuries may be induced by apparently superficial lesions on the cornea surface. These can rapidly degenerate due to osmotic tissue damage, and the ensuing dessication and rupture of the cornea result in the eye being lost. Although little is known about the corneal physiology of teleost eyes (Wilcock and Dukes, 1989), such lesions may induce more serious eye injuries in halibut juveniles and should be properly investigated.

One-eyed blindness has proved to be a common problem in Norwegian commercial halibut farming too. In their 3-month study involving 12 tanks each of 60 fish (mean weight 72 grams), between 8 and 28% of halibut in the groups lost an eye. 7 fish (1%) lost both eyes. Nortvedt and Tuene observed that 76% of eye damage was to the non-migratory eye, and attributed blindness to biting from other halibut or by accidental collisions during feeding. Reduced feeding success by one-eyed fish also led to poorer growth, and the majority of one-eyed fish were from the smallest size fraction of the tank populations. They concluded that being small at the start increased the risk of becoming one-eyed over the course of the experiment.

In further support of the aggression theory, many eye-damaged fish in this trial also had damage to the upper pectoral fins (Figure 5.22). While this was not measured directly, it was estimated that more than 50% of all eye-damaged halibut in the tank populations were so affected. In a concurrent study comparing the prevalence of eye damage in a recirculation system and flow through tanks, eye damage has proved a serious concern, affecting up to 11% of fish in some tank populations. The recirculation system halibut were more prone to eye damage, running at a higher temperature of 14^0C as opposed to ambient 8^0C. The smaller individuals again suffered the majority of damage, and the non-migratory eye was consistently more frequently damaged. Researchers have estimated 40–60% of eye damaged halibut also have pectoral fin damage (D.Hunter, Marine Harvest Ltd., pers. comm.). When swimming, halibut use the pectoral fin as a steering aid and hold it out perpendicular to the body. We are all agreed that this provides an easy target for aggressive fish to grasp.

In a related study of farmed halibut eye pathology conducted by Tony Wall (Fish Vet. Group, Inverness), halibut from two Scottish on-growing farms were surveyed between March and September 1999. The mean fish weight at the start was in excess of 25 grams and fish over 400 grams were recorded at the end. Farms were visited monthly and 50 fish were netted at random, anaesthetised and their eyes examined under a slit lamp. More detailed ophthalmic examination and histology was

Figure 5.22: Halibut with eye and pectoral fin damage. The fin has been bitten off completely, leaving just the fin base.



then carried out on a sub-sample of 10 fish (5 with damaged eyes and five normal halibut).

In Wall's study a total of 25% of fish sampled from both farms had some form of eye damage, which concurs well with our findings. The extent of eye damage was frequently severe, including haemorrhaging, scarring, dislocated lenses and changes to the cornea. The earliest and least affected halibut that were screened had corneal lesions consistent with bite marks, which appeared as linear scars and lesions. There was a 4:1 ratio of non-migratory to migratory eye injury among the fish sampled, showing a similar trend to my results and those of the Marine Harvest study.

In this experiment individual fish were successfully tracked over time, and the effect of eye damage severity on fish growth and condition was measured. Poor growth as a result of eye injury was widespread. This is likely due to impaired feeding ability as well as appetite loss and trauma associated with injury. However, the recovery of growth rates demonstrated by some fish once wounds had healed was encouraging (Figure 5.11). Fish with one good eye remaining can clearly adapt to disability, resume feeding and grow well. However, loss of the remaining eye would likely lead to poorer performance and could ultimately prove fatal.

The use of Elastomer marks made it possible to follow the development of eye damage in individual fish, and to monitor the rate of healing. Atlantic halibut have been shown to have a highly efficient immune system in comparison with salmonids (Bricknell, 1999). When the experimental populations were established in

February, there were already 45 fish that had recently lost eyes, leaving bloody, open wounds. By the April sampling point, all of these had healed, leaving scarring and a depression where the eye had been. Somewhat more noteworthy was the fact that fish with novel scarring were recorded at this time. These latter individuals had, therefore, lost eyes and healed the wounds perfectly during the seven-week period between sampling, at mean water temperature 7.6^{0}C. Totally blind halibut, of several kilos weight, have also been found in sea cages in good condition (Greaves, pers. obs., D.Thomas, Marine Harvest, pers. comm.). Presumably, they survived by browse feeding along the tarpaulin base of the cage. The inherent resilience of halibut and their ability to recover from injury in a relatively short time period should be encouraging to producers.

Fish with eye damage, particularly eye loss, displayed two extremes of behaviour. Many could be seen swimming at or near the water surface of production tanks, the body held vertical or at a 45^{0} angle, with the head uppermost. Some individuals spun in slow circles, swimming with a rocking motion. Fish behaving in such a disturbed manner were frequently emaciated, showed no interest in food, and reacted slowly to environmental stimulus. In contrast, other injured halibut tended to rest on the tank base, moving little. When sampling the tank populations, we noticed that the final nets of fish contained a higher proportion of eye-damaged fish than average. Many injured fish were found lying adjacent to the crowding device, up against the tank walls or beneath other fish. Some eye-damaged fish thereby conceal themselves or at least lie quietly, perhaps in an effort to heal damage and avoid further conflict or interactions. This behaviour could partially explain why the extent of eye damage among farmed stocks has been under-estimated in the past.

Grading effectively slowed the increase of eye damage prevalence over time. There was no significant difference in levels of eye damage at the trial start between the graded and ungraded populations but, after just seven weeks, halibut in the graded tanks were incurring significantly less cumulative damage. Evidently, maintaining fish in narrow size distributions can effectively mitigate intraspecific aggression. However, grading alone will not eradicate the problem, and other factors must be addressed.

In Wall's study, the prevalence of eye damage was not equal at the two farms (Farm B had almost 50% more eye damaged fish than Farm A). He compared the husbandry conditions (tank environment) on the farms and identified several important differences. Farm A had smaller dimly lit tanks with higher current flow, and maintained fish at higher stocking densities than Farm B. Wall concluded that all ocular pathology seen was consistent with bite marks as a result of aggression from other fish, and that by manipulating environmental factors farms could significantly reduce the problem.

A case in point is a Canadian halibut facility where the rearing environment differs from UK farms, and the prevalence of eye damage is just 2-3% (N. Brown, pers. comm). The farm is a small recirculation unit, all tanks are indoors and maintained at 13^{0}C. Light levels are extremely low (10-20 lux at the water surface), conditions are kept constant, and there is minimal disturbance. This is in stark contrast to fish kept in outdoor tanks, subjected to daily fluctuations in outside noise, light (several thousand lux on bright days) and changing weather conditions. The Canadian tanks are square with rounded corners, 1-1.5m depth and, to maximise the use of tank space, there are wire mesh shelving units on each side which provide 2 extra layers of resting space. These shelves occupy most of the tank area leaving a limited area of open space around the central standpipe. Stocking densities are high, 20-25Kg/m^2 (c15,000 20g fish / tank initial density), and tanks have high flow rates. Halibut use the shelves well, evenly distributing themselves even on the top shelf which provides no cover. This would perhaps be unexpected in systems with higher light levels.

Shelves may confer several benefits. The increased surface area available to fish, at varying depth, optimises the use of the water column, allowing the farmer to stock tanks at higher density while circumventing some of the associated risks (hypoxic conditions due to crowding an area). Shelving may also alter halibut behaviour. In conventional rearing tanks, flatfish can often be seen 'burying' i.e. undulating the dorsal and anal fins that would conceal them in substrate. In bare tanks, fish instead bury /burrow beneath other fish, presumably seeking cover. The burying behaviour, therefore, increases potential interactions between fish. By contrast, shelving transforms the tank into a more complex environment and reduces encounter rates and activity. Halibut may also prefer lying on shelves for the texture they provide and water circulation around and beneath them.

Indications that high stocking densities, low light and shelf systems are favourable for halibut rearing are supported by information from an Icelandic facility (Fiskeldi Eyfaljardar Ltd, D.Mitchell, pers. comm.). Here, tanks are again shelved and indoors, densities of 30Kg/m^2 are used (inclusive of the shelf area), but water temperature is considerably lower at 7-8°C and fish are generally less active.

The initial stocking densities in the current trial were below the recommended optimum for this size of halibut. Although this had no impact on growth rates, it may well have influenced the prevalence of eye damage. To date stocking density trials on flatfish have given disparate results. Juvenile halibut at the weaning stage (from 0.3g) showed improved growth rates at the highest stocking density (7 fish/L, 0.14m^2 surface area) correlated with a decrease in the frequency of aggressive interactions (Chapter 4: Greaves *et al.*, submitted 2000). Conversely, in a study comparing the growth rates of turbot juveniles held at four densities, the growth of some individuals was suppressed as density increased (Irwin *et al.*, 1999). However, higher rearing densities have successfully curbed agonistic behaviour in many species and improved growth rates (Wallace & Kolbeinshavn, 1988; Christiansen & Jobling, 1990, Brown *et al.*, 1992).

In spite of size grading, differences in individual growth rates can increase the coefficient of variation for fish size in a matter of weeks, and larger fish often still monopolise feed (Olla *et al.*, 1992). The mode of feed delivery, feeding frequency and ration size can all be successfully manipulated to prevent this occurrence.

Fish culture tanks are simple environments where feed is normally well distributed, the need for resource competition is reduced, and there is no predation risk. The high level of eye damage in our experiment raises the question of adequate daily feed rate. Feed rations were dispensed from automatic feeders mounted on the tank sides, supplemented by hand feeds. Flow rates were sufficient to ensure widespread feed distribution across the tank, seemingly making it difficult to defend. Why then, were damage levels so high? It is suggested that the feed rate of 2% bodyweight/day may not have been high enough to satiate fast-growing fish, especially when halibut metabolic rate increased with water temperature. In support of this hypothesis, the prevalence of eye damage among the graded populations was highest in the large graded fish. By definition, these fish represented the largest size fraction of the cohort at the start, being fast-growing successfully competitive fish.

The rate at which feed is presented to the fish is also important. Dominant fish can still monopolise feed if it is delivered too slowly and continuously (Gillis & Kramer, 1997). Pulsing feed into several meals promotes scramble feeding, and also regulates fluctuating oxygen demand associated with increased activity (Noakes & Grant, 1992).

In circular production tanks, the water inlets at the tank sides are angled to generate a current round the tank that disperses slow-sinking feed pellets over a wide area. However, there is still the potential for localised "hotspots" where feed concentrates. Such areas exacerbate intraspecific competition and facilitate resource monopolisation by dominant individuals and the concurrent intimidation of subordinate fish. Extending the period of time that feed is made available throughout the day and providing feed in excess allows subordinate fish to feed at different times than dominants. Fish farmers must compromise between providing high feed rations so that competition for food is less intense and incurring costs associated with feed wastage and environmental pollution.

To date, there has been no work on the natural feeding rhythms of halibut. In salmonids, behavioural research in this area has prompted the design of demand-feeding systems which give fish control over when they feed via a feed back loop (Jobling & Kostela, 1996; Kadri *et al.*, 1997). Similar studies on halibut would provide much-needed information on daily and seasonal appetite fluctuations, and auxilliary information for the management of aggressive behaviour. In addition, adjusting feed regimes to accommodate the demands of the fish should optimise feed conversion efficiency.

Results of this study indicate that farmed halibut juveniles with eye injuries incur a cost to on-growers in terms of biomass losses due to reduced growth rates. To illustrate this, I compared the growth rates of uninjured Elastomer-marked halibut with those with minor eye damage and eye loss respectively in the 3 replicate ungraded populations (Table 5.11).

Using mean SGR values for each injury level, the biomass increase (if all fish grew at these rates) was calculated according to the following formula (Laird & Needham, 1991):

$w_t = w_0\, e^{((Gt)/100)}$

where w_t is the weight of halibut after t days, w_0 is the initial weight of the fish in February, G is the daily SGR as percent per day, t is the number of days of growth, and e is the exponential constant = 2.718282 (Table 5.12). The average start weight of fish in the experiment was 20 grams, and the experiment spanned 127 days between February and July. Therefore, the initial biomass of 299 fish @ 20 grams was 5.98 Kg.

This model assumes that uninjured fish have an SGR of 1.22%/day. Therefore, if none of the 299 halibut were injured their projected biomass would be 25.18 Kg after 127 days. This represents a biomass gain of 19.2 Kg. In our small sample alone, the combined cost of the reduced growth rates of 87 halibut with minor and severe eye injury in terms of biomass was 1.27 Kg for just 127 days. Given the substantial number of fish currently affected by eye damage in production tanks, this evidently has serious implications for farming efficiency. Physically damaged halibut may be classed as an inferior product at the processing level which adversely affects product price. In addition, the issues of fish welfare and the market image of farmed fish are important considerations.

Given that grading successfully reduced the prevalence of eye damage in this study, recommendations given here are based on the graded tanks alone. Coefficient of variation (%) and eye damage/loss increased sharply between the April and May sampling dates (fourteen weeks after grading). On the basis of my data, I would advocate a size-grading interval of six to eight weeks for 20-40 gram halibut held at

Table 5.11: Median SGR for the period February – July (127 days) among Elastomer-marked halibut in three ungraded populations (299 fish):

Eye Injury	T2 Median SGR	T3 Median SGR	T6 Median SGR	Mean SGR	Number of Fish
0 Injury	1.18	1.24	1.24	1.22	212
1 Minor	1.01	1.09	1.04	1.05	55
2 Eye loss	0.90	0.99	0.92	0.94	32

Table 5.12: The projected biomass losses on account of eye-damaged fish after 127 days according to the model (Laird and Needham, 1991)

Eye Score	SGR	Number of fish	Initial biomass (Kg)	Fish weight after 127 days (grams)	End biomass (Kg)	Biomass gain (Kg)	Relative biomass deficit (Kg)
0 Injury	1.22	212	4.24	84.2	17.85	13.61	0
1 Minor	1.05	55	1.1	72.4	3.98	2.88	0.65
2 Eye loss	0.94	32	0.64	64.6	2.07	1.43	0.62

this density. Alternatively, the farmer could monitor the increase in the coefficient of variation over time. The ranges in the coefficient of variation for graded Elastomer fish weight in April and May were17.81 – 23.01 and 16.97 – 26.56 respectively. The most homogeneous were the large graded fish, largest variation being in the small graded group. By July, when damage prevalence was high due to breaking down of the grades, the range of CV for weight had increased to 22.09 – 26.60. According to these figures, it would appear advisable to maintain CV below 20 %.

However, this is one preliminary study and further work is required before precise grading intervals can be determined. Given that stocking density has been identified as one of the most important influencing factors, complementary studies are required to establish the relationship between grading frequency and higher density. Keeping halibut at higher production densities can, in itself, suppress overt aggressive behaviour. Therefore, it is possible that size grading may not need to be as stringent as it was in this trial, and the grading interval could likely be extended to two to three months.

The decision to grade is not a straightforward one, and farmers have to weigh up the pros and cons. Grading is labour-intensive and time-consuming, and can be economically expensive too. The combined effect of a 1-day starvation period prior to grading, and appetite loss post-grading as a direct result of handling stress, can translate to the loss of several days' growth. In addition, there is potential for physical damage to fish from the grader or nets. Grading may also have a negative effect on the behaviour of the fish. Baardvik and Jobling (1990) provide evidence that altering group structure induces high levels of social interactions and adversely affects growth rates in Arctic charr (*Salvelinus alpinus*). It is generally accepted that the formation of a social hierarchy is characterised by a period of fierce competition, but, once established, aggressive interactions become less frequent and intense (Wedemeyer, 1997). Grading, therefore, disrupts the social structure and may induce a further period of intense aggression when new hierarchies are being established. However, this phenomenon has yet to be examined in halibut, and the consequences of not maintaining halibut of this size range in tightly graded populations appear to outweigh these potential problems.

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Chapter 6

Cannibalism among farmed Atlantic halibut

6.1 Cannibalism and welfare in farmed animals

The welfare of any intensively farmed species, from poultry to fish, is an increasingly important consideration to consumers and producers alike. Basic welfare requirements include adequate food and space, suitable temperature, and protection from disease and unnecessary pain. Cannibalism is an undisputed welfare concern because of the pain caused to victims, and presents a serious problem to the profitable culture of many fish species. Similar difficulties have arisen in the poultry industry, where outbreaks of cannibalism among flocks can have major economic consequences (Yngvesson, 1997). In halibut culture, significant mortality coupled with the number of surviving but injured juveniles is obviously an economic and welfare consideration to producers. Eye injuries impair vision and open wounds cause physiological and behavioural distress. Blind halibut or those with eye injuries do not feed as efficiently and therefore have reduced growth rates (Nordvedt & Tuene, 1995; Greaves, unpublished data). In addition, fin damage and skin lesions caused by aggressive biting behaviour render fish susceptible to secondary infections and many subsequently die.

In this chapter evidence will be presented that suggests the occurrence of cannibalism among farmed Atlantic halibut. Although no experimental data will be presented, the circumstantial evidence gained from production sites and as a by-product of formal experiments is compelling, and is considered to be significant enough to warrant a separate chapter in this thesis. This information will be related to experimental data from other farmed species, and the relationship between aggression and predation, and their association with cannibalism will be discussed. Firstly, these three classes of behaviour will be defined.

6.2 Definition of terms

Aggression refers to three basic types of behaviour: aggressive display (threat), attack, and fighting. Behaving aggressively usually confers advantages such as securing favourable territories, protecting young and gaining access to food and other resources. Examples include stags fighting over females during rutting (Clutton-Brock *et al.*, 1992), and salmonid parr defending profitable feeding areas in streams from conspecifics (Abbott and Dill, 1989). However, there are costs associated with aggression and, in most cases, fighting is a last resort.

Predation may be defined as "the pursuit, capture and killing of animals for food" (Encyclopedia Britannica 2000). The victim here is usually killed immediately and there is no preliminary threat involved. Predators are by definition carnivorous, like the owl hunting the mouse, and kill for survival.

6.3 The natural history of cannibalism:

Cannibalism, the practice of ingesting one's own kind (either partially or whole) is a relatively common phenomenon in the animal kingdom occurring in mammals, insects, amphibians and fish. However, the function and motivation for this behaviour differs from species to species. In order to understand the mechanisms underlying cannibalism and aggression in a captive species, hypotheses with biological bases are necessary. Polis (1981) suggested three explanations of cannibalism:

1. It is adaptive for the cannibalistic individual when the population is too large or the density too high.
2. It is an extension of normal feeding behaviour
3. It is an abnormal response to stress caused by the captive environment. The animal fails to adapt or to cope and performs behaviours it would not in its natural environment.

In addition, cannibalism may be an extreme form of aggressive behaviour. In practice, these explanations are not necessarily mutually exclusive.

One function of cannibalism is to promote the growth and survival of the cannibalistic individual. Tiger salamander larvae (*Ambystoma tigrinum*) normally exist as 'small-headed' morphs and feed chiefly on aquatic invertebrates. However, under crowded conditions, 'cannibal' morphs with larger, specialised heads enabling them to cannibalise siblings, are induced facultatively. Hoffman & Pfennig (1999) demonstrated that the development of the cannibalistic morph was elicited by tactile cues from other salamanders.

In their studies on African catfish, *Clarias gariepinus*, Hecht and Appelbaum (1988) identified distinct types of cannibalism which have subsequently been recognised in other fish species. Type I cannibalism, where prey is only partially ingested, can occur among similar-sized fish, whereas complete type II cannibalism (where prey is swallowed whole) is constrained by the mouth gape size of the cannibal and can only be practised by considerably larger predators (Hecht and

Appelbaum, 1988; Baras, 1999). This is true of pike, *Esox lucius*, where sibling cannibalism is characterised by prey being swallowed whole and head first. Therefore, the success of attempted cannibalistic attacks is dependent on the size ratio of the cannibal to its prey, and mouth gape size is an important constraint determining the maximum size of the victim (Giles, Wright and Nord, 1986; Bry *et al.*, 1992).

Size variation, therefore, largely controls the rate of cannibalism in many species (Polis, 1981). However, the relationship between size variation, cannibalism and population dynamics is evidently more complex because, as smaller individuals are removed, size variation in the population alters. As many as 30% of tiger salamanders in a larval population may be cannibalistic, and exhibit dramatically increased growth rates. Such larvae metamorphose earlier and at a larger body size than those consuming other prey, and their stomachs may contain several smaller larvae at a given time (Ziemba & Collins, 1999). Competitive interactions can increase size variation still further, and Ziemba & Collins (1999) hypothesise that the predation threat posed by larger tiger salamanders may indirectly interfere with the feeding behaviour of smaller conspecifics. Similar observations were made by Giles *et al.,* (1986), where small pike fry remained still for much of the time because sudden movements often initiated cannibalistic attacks. In this way they were deterred from feeding, and mortalities were frequently emaciated despite an abundance of suitable natural prey.

Cannibalism in some contexts may be regarded as an adaptive behaviour that increases an individual's fitness or propensity to survive. In general terms cannibalism is characterised by differences in age, developmental stage, size and strength (Yngvesson, 1997). In a landlocked population of Arctic *charr (Salvelinus alpinus)* in Spitzbergen, cannibalism of small individuals is believed to be the chief cause of juvenile mortality and the major determinant of population structure (Svenning & Borgstrom, 1995). Adult-adult cannibalism is rare because of the high cost associated with attacking an individual of similar size and weight (Elgar & Crespi, 1992).

The most intense intracohort sibling cannibalism yet documented in fish is practised by dorada, *Brycon moorei*, a migratory species with potential for aquaculture in Latin America. The emergence of type I cannibalistic individuals is exceptionally early, starting as soon as the oral teeth are fully developed 21 hours post-hatch (Baras *et al.*, 2000). At this stage, the head cannot be ingested and a novel type of incomplete cannibalism (type III) is also occasionally observed. Here, a smaller individual is attacked and progressively broken up into smaller pieces by several cannibalistic siblings taking bites out of it. Complete type II cannibalism, which is limited by the prey to cannibal size ratio, is established 48 hours after hatching. Lateral and tail attacks on victims are commonly observed. Dorada normally become fully developed juveniles 14 days post-hatch. However, cannibalistic fish have a highly significant growth advantage, reaching this stage within just 8 days. There are high risks associated with this strategy. Cannibalistic larvae with prey in their mouths exhibit erratic swimming behaviour making them easy targets for other predatory siblings. Chains of up to seven individuals have been observed partially ingesting other fish (Baras *et al.*, 2000). The fact that dorada preferentially ingest smaller conspecifics is presumably because easier and more rapid prey handling reduces their own risk. In the late larval stage (0.2 grams weight), cannibals can grow remarkably fast (52 percent increase per day, consuming up to 130 percent of their bodyweight per day). For most species cannibalism is most intense at the larval and early juvenile stages than in older fish, which achieve lower specific growth rates and require less energy (Hecht and Pienaar, 1993). Both aggression and cannibalism in dorada cease when fish are 15-30 grams weight, some 60 days post-hatch.

6.4 Cannibalism among farmed fish

The rearing environment is known to influence intraspecific aggression and cannibalism in many fish species (Smith & Reay, 1991). Cannibalism among cultured species has been associated with differences in body size, limited food supply, lack of shelter or refuge and high light levels (Hecht & Appelbaum, 1988; Katavic *et al.*, 1989; Qin & Fast, 1996). Several studies indicate that size variation and limited food availability are the primary causes of cannibalism (Katavic *et al.*, 1989 (Sea bass, *Dicentrarchus labrax*); Folkvord & Ottera, 1993 (Cod, *Gadus morhua*); Dou *et al.*, 2000 (Japanese flounder, *Paralichthys olivaceus* T.)). Larger body size often confers social dominance and early experience appears critical. Insufficient food has been shown to elicit cannibalistic responses in Striped bass (*Morone saxatilis*) larvae and, once accustomed to this, many larvae never learned to accept artificial food (Paller & Lewis, 1987). For many species, relative rather than absolute size is the key criterion determining vulnerability to cannibalism. Cannibalism is well-documented among juvenile cod, and Folkvord & Ottera (1993) found that large size differences between fish elicited cannibalism, and that 50% of mortalities removed from tanks also had visible injuries. Strict size grading thereafter successfully reduced this occurrence. Similarly, 19% of larval walleye (*Stizostedium* *vitreum*) that were attacked but not ingested died within 24 hours from injuries inflicted by other fish (Loadman *et al.*, 1986). In another study Katavic *et al.* (1989) recorded eye losses and damage to the caudal fins and abdomens of Gilthead sea bream (*Sparus aurata*) and interpreted their findings as evidence of cannibalistic behaviour.

Like pike, striped Snakeheads (*Channa striatus*) swallow their prey whole, and fish size variation determines cannibalism rate. This ranged from 40% cannibalisation after 5 days when prey-predator length ratio was 0.64: 1, to 100% of smaller fish cannibalised when prey-predator ratio was 0.35:1 (Qin & Fast, 1996). Average prey size was 32% that of predators when cannibalism rates were high. To reduce intraspecific cannibalism the authors recommend that prey : predator size ratio should not exceed 0.33:1. Feeding frequency is another critical factor, and the rate of cannibalism among Sea bass (*Dicentrarchus labrax*) fingerlings increased if fish were not fed in the morning (37% of fish in the experiments ate their siblings). Katavic *et al.*, (1989) demonstrated that the extent of cannibalism could be markedly reduced in the weaning period if fish were fed *ad libitum.*

Wild Arctic charr (*Salvelinus alpinus*) are opportunistic feeders with a wide-ranging diet, and larger fish may be both piscivorous and cannibalistic (Amundsen *et al.*, 1995). At the end of a series of 24 hour trials investigating prey selection in this species, Amundsen *et al.* (1995) often found dead fish in the tanks, many of which had open wounds on their lateral sides. They also observed visible bite marks on the flanks of some surviving fish. Subsequent direct observations of charr behaviour in the tanks confirmed that charr attacked from the side. Cannibalistic Arctic charr preferentially consumed prey averaging 22% of their body length and Amundsen *et al.* (1995) concluded that this was probably due to the difficulty of handling larger prey.

In another study on charr prey specialisation, Amundsen *et al.* (1995) tested individual preferences for pellets or smaller live conspecifics. Three groups of 10 larger predators were kept for a seven week period, and fed pellets only, pellets supplemented by smaller live charr added 3 times a week, or small charr alone. Each diet was fed for one week only. X-radiography was used to ascertain individual diet choice. The smaller charr were anaesthetised and force-fed lead shot (X-ray dense particles) prior to their introduction to the tanks, and pellets contained Ballotini beads. In this way, stomach contents were determined by the size of particles from the X-rays. Individuals exhibited remarkably consistent feeding preferences throughout the experiment, and were either non-feeders, pellet-eaters or cannibalistic. Pellet-eaters did not become cannibalistic when only live prey was offered, neither did cannibalistic charr consume pellets in the absence of smaller charr. Interestingly, there were no significant differences between initial length or weight of fish in these categories. Between 2-4 fish per tank of 10 became cannibalistic, and were not necessarily the largest individuals. Overall, 37% of formerly naïve hatchery-reared charr became cannibalistic, suggesting a strong latent response in this species.

Another species apparently predisposed to cannibalism is Japanese flounder (*Paralichthys olivaceus* T.). This species is hatchery-reared in large numbers and released as part of a marine stock enhancement programme in Japan (Dou *et al.*, 2000). Chasing, attacking and biting among conspecifics are frequently observed in

Japanese flounder rearing tanks, and considerable losses of newly settled flounder are attributed to predation from older conspecifics post-release (Dou *et al.*, 2000; Miyazaki *et al.*, 2000). In their experiments, Dou *et al.* (2000) tested the effects of size variation, starvation, light, density and sand substrate on cannibalism when food was available and throughout a starvation period. They determined that size variation and starvation were the principal factors affecting cannibalism in juvenile flounder. The rate of cannibalism was low when food was plentiful, but became more prevalent with time over the starvation period, presumably due to rising hunger levels. The presence of sand substrate enabled flounder to bury themselves, and effectively reduced predation of smaller fish in the heterogeneous size groups. However, significant differences were found in the incidence of cannibalism between homogeneous and heterogeneous size groups, more cannibalism occurring in the latter even when sufficient food was available. Evidently, uneven growth rates between fish that allow siblings to feed on smaller individuals in their population facilitated cannibalism (Fitzgerald & Whoriskey, 1992; Dou *et al.*, 2000).

6.5 Cannibalism in wild Atlantic halibut

The Atlantic halibut is the largest of the flatfish and is known to be an efficient predator. Stomach content analysis of wild halibut shows that diet composition alters with fish age. The juvenile diet has a large proportion of crustacea and molluscs, but that of mature fish is chiefly composed of cephalopods and pelagic fish, such as gadoids and capelin. However, a smaller percentage of demersal fish are also consumed, including smaller conspecifics and other flatfish (McIntyre, 1952). Halibut produce millions of eggs during spawning that develop while drifting in the sea and, like many other species of marine fish, there is no parental care. It seems reasonable to suppose that smaller halibut, therefore, are not recognised as conspecifics and may just be viewed as other prey items.

6.6 Evidence of cannibalistic behaviour from farmed halibut

There are various factors indicating the occurrence of cannibalism among farmed Atlantic halibut:

6.6.1 Missing fish

In halibut tanks and cages, from nursery facilities to on-growing at sea, regular discrepancies between the number of fish stocked and subsequent counts have come to light. Intraspecific aggression in farmed halibut is prevalent between weaning (>1 gram) and 150-200 grams weight. Thereafter, evidence of this behaviour and physical damage to conspecifics is rare (Greaves & Tuene, 2001). However, two main incidences of suspected cannibalism have been reported in halibut of several kilos weight in sea cages (Thomas, Marine Harvest, pers. comm.). In 1997 the British Halibut Association long-lined wild halibut from Icelandic waters and stocked them in a cage in the Western Isles site as potential broodfish. From their arrival in Scotland, they were fed only commercial pellets due to the risk of disease infection from trash fish. The number of retrieved mortalities in this pen was low but the size range among fish was considerable (0.5 – 6 Kg). When fish were weighed and pit-tagged after four months, the majority of smaller fish had 'disappeared' and many survivors had suffered eye losses. Greaves and Mitchell (unpublished observations) suggest that these undomesticated fish did not readily accept the pelleted diet and had, therefore, resorted to cannibalising smaller fish. Anecdotal evidence of differences in behaviour between hatchery-reared and these wild-caught fish corroborates this view. Juvenile saithe resident in cages of hatchery-reared halibut swam amongst the fish and were largely ignored. However, any live saithe netted out and placed into the wild halibut cage were consumed almost immediately, halibut lunging at them with great speed and swallowing them whole. In a separate incident in 1999, a deficit of 1000 fish in a production cage of hatchery-reared halibut was discovered (Thomas, Marine Harvest, pers. comm.). The remaining fish were all large individuals and, as predator netting and security systems were in place, cannibalism of smaller individuals again looked likely.

In a recent trial examining the effect of size grading in halibut on aggression and eye injuries (Chapter 5), count discrepancies became apparent at the trial end after recorded mortalities had been taken into account. On average, almost 50% of missing fish were unaccounted for on the mortality record for all six experimental tanks.

6.6.2 The condition of dead fish removed from systems

Many dead fish removed from systems show bite damage to the eyes, gut and marginal fins. Eyes are frequently missing, though tank observations indicate that this may occur after death as fish are known to pick at dead individuals, and the heads tend be eaten away rapidly. The presence of mortalities also elicits intraspecific aggression between individuals (George and Spreadborough, Otter Ferry Seafish Ltd, pers. comm.; Thomas, Marine Harvest, pers. comm). Attacks have been observed on smaller fish, moribunds and injured halibut in both tanks and cages, particularly in heterogeneous size groups.

6.6.3 Direct observations of behaviour

Specific evidence from halibut farm stock includes observations of moribund fish in halibut populations swimming erratically and being attacked. One example captured on film showed a moribund fish exhibiting brief bursts of swimming up into the water column followed by a slow 'floating back' down to the tank base (Greaves, unpublished data). This behaviour attracted the attention of several individuals that gathered from across the tank, surrounded the moribund fish and started nipping at its fins. One fish then seized the moribund firmly by the gut and shook it vigorously from side to side.

General observations indicate that halibut with open wounds (following recent eye loss) are show signs of stress, swimming slowly at or near the water surface, sometimes circling, or raising their heads out of the water and exhibiting a 'flapping' movement. They show no interest in feed and may bump into the tank sides, presumably unable to see properly with the remaining eye. These fish may be more susceptible to further attacks because of the visual stimulus of blood and trailing tissue, and/or because of their uncharacteristic behaviour. It is suggested that cannibalism among farmed Atlantic halibut may be viewed as an extreme form of aggressive behaviour linked to a latent predatory response.

Another example from video footage of caged juveniles in Norway (Tuene, pers. comm.) shows a smaller fish being pursued up through the water column by larger conspecifics, several more joining the chase. This was interpreted as predatory behaviour because of the small size of the victim and the nature of attack. The above examples provide a strong argument that cannibalism occurs among farmed halibut. In production tanks, similar sized fish are reared together and size grading is practised. Only occasionally is size variation disparate enough for halibut to ingest conspecifics whole, so Type II cannibalism seems unlikely in the main. However, weaning fish (where size variation is considerable) have occasionally been seen with other fish in their mouths (head or tail protruding), and some die in the process of ingestion (Greaves, Spreadborough, Otter Ferry Seafish Ltd., pers. comm). Figures 6.1 and 6.2 show a dead halibut removed from a weaning tank that had choked on a smaller conspecific (6.4 : 2.8 cm total body length respectively). Tails and marginal fins (dorsal and anal) are also targeted. Throughout the weaning period, where aggression and cannibalism first becomes apparent in halibut, the reduced availability of live artemia prey could induce this shift in behaviour. The regular unexplained count losses from nursery tanks and on-growing cages over time ('missing' fish) indicate that whole fish are consumed, but evidence from direct observations indicates this probably occurs in stages (i.e. a fish dies or is killed first) and by several fish (Figure 6.3). It is suggested that latent predatory and cannibalistic behaviour may be stimulated by the appearance of small, weaker fish or fish behaving erratically.

6.7 Similarities between halibut farming and intensive poultry production

Cannibalism in poultry, where hens inflict damage to the skin and tissue of other birds, is a longstanding problem, documented as early as 1938. It may be opportunistic, and elicited by artificially high densities in production systems. Individual hens learn by observing the behaviour of other hens and, if the behaviour is of value, will adopt it (Nicol, 1995). Yngvesson (1997) postulated that cannibalism among hens could indeed be a learned behaviour where the first bite may be investigative, but positive feedback gained from this action (taste) could induce the development of a cannibalism habit.

Researchers have drawn a distinction between feather pecking, aggression and cannibalism. Feather pecking can be gentle and is part of the normal behavioural repertoire of the bird where pecks are directed at particles in the plumage of other birds. No threat behaviour precedes this action (Hoffemeyer, 1969; Hughes & Duncan, 1972; Keeling, 1994), and bleeding only occurs if a feather is pulled out accidentally. The majority of feather pecks are directed from behind the bird being pecked. Conversely, aggressive pecking and threats from one bird to another usually occur from above, and the head of the receiving bird is targeted. However, severe feather pecking is associated with body cannibalism, when the blood drawn attracts other birds and may lead to the rapid death of the pecked individual. A similar scenario seems plausible for halibut suffering recent eye loss or damage where blood triggers attacks. Feather pecking in loose-housing poultry systems can affect up to 99% of birds (Gunnarsson *et al.*, 1999). In severe cases, the feather pecking bird vigorously pulls out feathers from the victim and can leave it almost totally denuded. The denuded bird presents both economic and welfare problems to the farmer. It must eat more to maintain its body temperature (incurring higher egg production costs), and suffers pain as a result of feathers being pulled out (McAdie & Keeling, 2000). In poultry, there is evidence for a genetic component to abnormal feather pecking behaviour, as it differs between strains of hens (Hughes & Duncan, 1972; Craig & Muir, 1996) and can, therefore, be selected against (Keeling & Wilhelmson, 1997).

Figure 6.1: A post-weaned halibut found dead in the tank with a fish protruding from its mouth. This fish is presumed to have choked while trying to swallow the smaller halibut.

Figure 6.2: Photograph showing the relative size of the cannibalistic halibut to its victim.

The larger fish measured 6.2 cm total length, the smaller fish 2.8 cm.

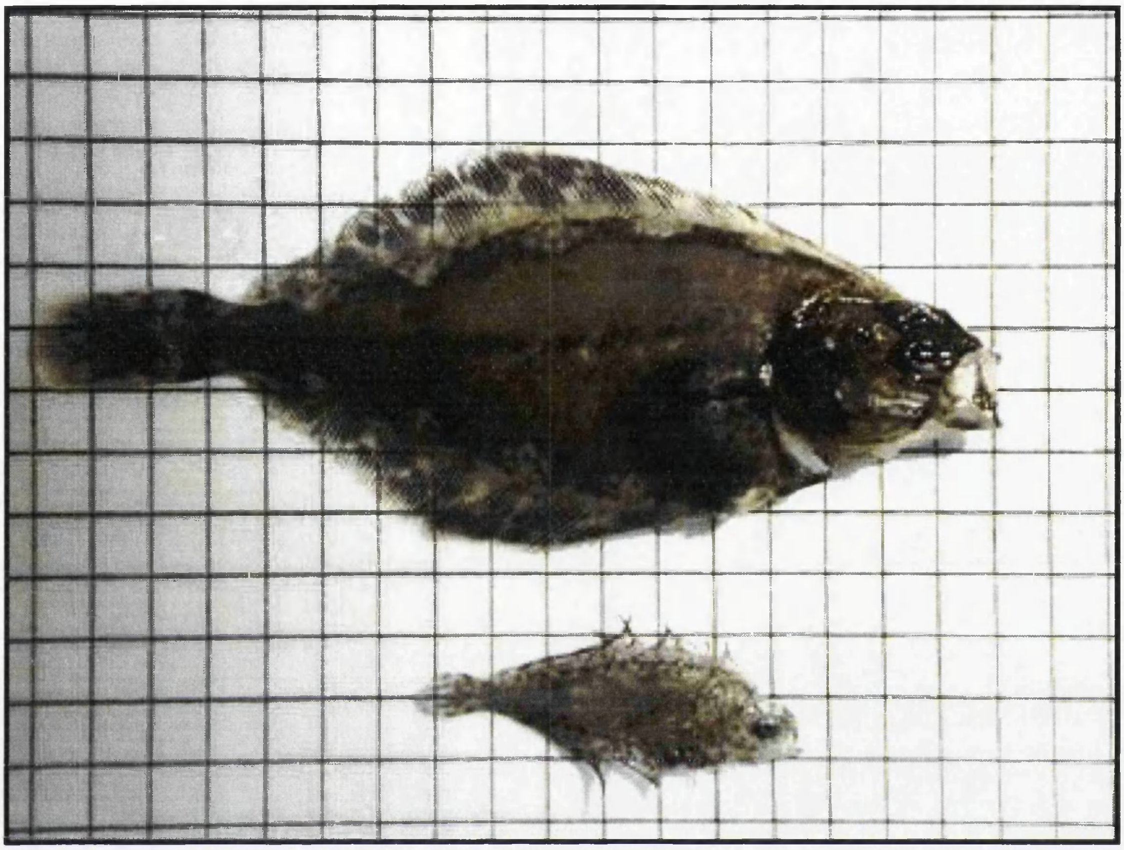
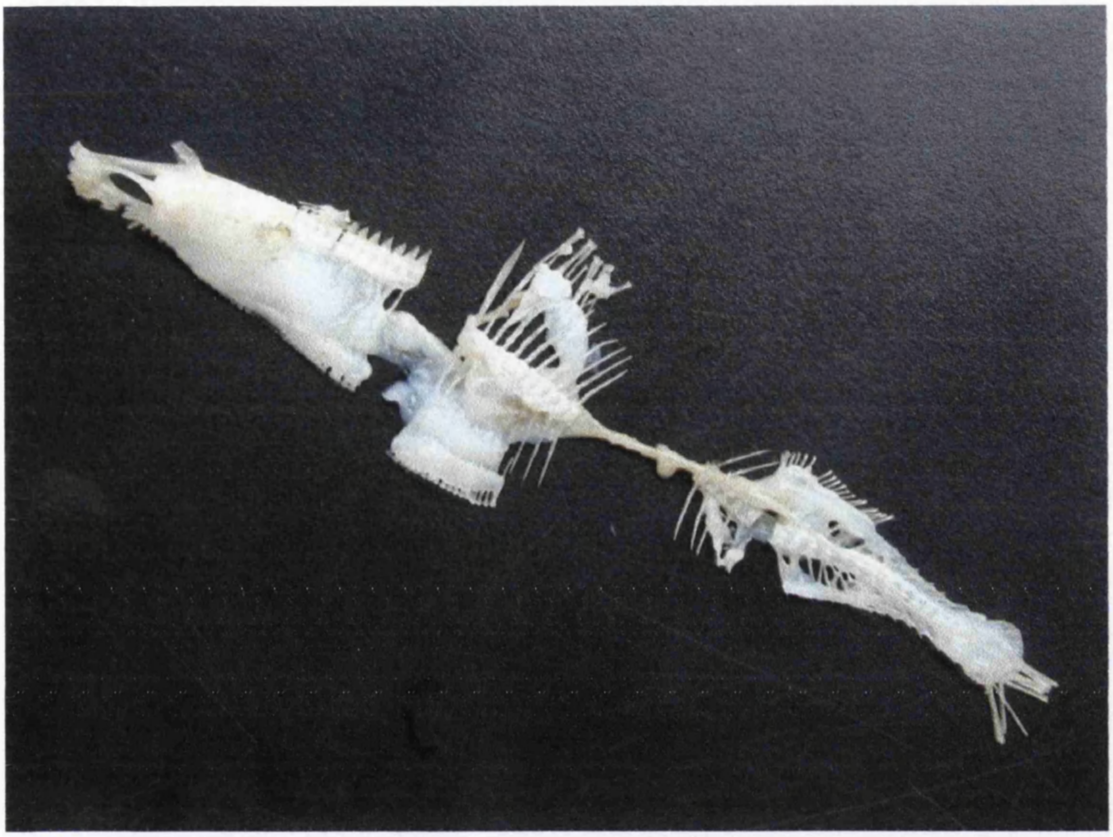


Figure 6.3: Possible evidence of cannibalism: the remains of a mortality removed from an on-growing tank (mean population fish weight 50 grams).



In a study designed to test the attractiveness of ruffled or damaged feathers as targets for feather pecking, McAdie & Keeling (2000) manipulated the feathers on 8 out of 11 birds in 16 pens by cutting tail feathers short, ruffling or removing rump feathers. Only one manipulation was performed on each individual. Over a two week period, observations were made of feather peckers, their victims and the body areas damaged. Damaged feathers received more severe feather pecks than undamaged ones. This supports the assumption that damaged feathers prove attractive targets to feather peckers, thereby eliciting the spread of feather pecking throughout flocks. In this study there was an unexpected outbreak of cannibalism in 8 of the 16 experimental groups. When the researchers deliberately manipulated feathers at the start, no damage to the skin was caused. However, 13 of 16 attacked hens (from a total 176 in the study) were wounded in the area of the manipulated feathers.

As only a small proportion of hens cannibalise other birds, it may seem reasonable to remove them from the group. However, in loose housing systems finding these birds among the flock is usually impractical (Keeling, 1994). In many countries feather pecking and cannibalism are regulated by confining birds to small cages (battery) or by beak trimming. However, beak trimming has been shown to cause long-term pain in hens (Gentle *et al.*, 1991), and is illegal in some countries. Sweden outlaws both these methods on welfare grounds and other alternatives must be found.

6.8 Overview

The circumstantial evidence presented above provides a strong argument that cannibalism occurs among farmed halibut stocks. There are several possible explanations for the occurrence of cannibalism in this farmed species:

1. It confers a growth advantage to the individual.
2. It may be an extension of normal feeding behaviour
3. It may be a form of extreme aggression
4. It may be a learned behaviour

6.8.1 Cannibalism confers a growth advantage to the individual

In other documented cannibalistic species (e.g. African catfish, cod & tiger salamanders), cannibalistic behaviour is evident from an early stage: larvae ingest other larvae, and the biological basis for this behaviour appears to lie in definite growth advantages. Catfish and salamanders have huge growth spurts and can contain several siblings in the gut at one time. Conversely, the growth rates of cannibalistic halibut are far less pronounced so this seems an improbable basis for this behaviour. However, this is not known for certain, and specific research in this area is needed. There are three more likely explanations for cannibalism in this species which are not necessarily mutually exclusive.

6.8.2 Cannibalism may be an extension of normal feeding behaviour

Wild halibut are very efficient predators of pelagic fish and also of demersal species, including flatfish and smaller conspecifics. Many constituents of aggressive behaviour (e.g. chasing, biting) are also used for the catching and killing of prey. Therefore, the impetus for cannibalism in farmed halibut may be an extension of normal feeding behaviour, exacerbated by a response to crowding and the artificial rearing environment. In addition, current stocks are relatively undomesticated as the majority of juveniles come from wild-caught broodstock. Therefore, such behaviours are likely to persist for several generations.

6.8.3 Cannibalism may be a form of extreme aggression

Extreme aggression involves wounding another fish to the point of death, but this may or may not involve cannibalism. Similarities exist between halibut and Arctic charr, which also inflict severe wounds to conspecifics (termed cannibalism by Amundsen *et al.*, 1995). However, charr that die from wounding in the tank are not eaten, whereas halibut will pick at mortalities and partially consume the carcasses. It seems reasonable, therefore, to distinguish between the two species and to classify charr as extremely aggressive fish and halibut as having more cannibalistic tendencies.

Intraspecific aggression and subsequent eye injuries among halibut are known to increase markedly under starvation conditions (i.e. prior to fish transport), hunger being the most likely motivating factor. Experiments have shown that when feed is poorly distributed or delivered in limited quantities, halibut will compete aggressively for it (Greaves & Tuene, 2001). However, it is still unclear why fish are aggressive towards one another when food is well distributed and abundantly available. Production tanks and cages are relatively simple & uniform environments, and there is no evidence to date that halibut defend territories or areas in these systems. Previous work has also shown that aggression is less prevalent at higher stocking densities (Chapter 4, Greaves *et al.*, submitted to Aquaculture, 2000), and behavioural evidence shows that halibut do not engage in fights. Rather, aggressive interactions between farmed halibut are brief episodes, one individual pursuing and biting or nipping another. The recipient of aggression generally responds by fleeing the scene.

6.8.4 Cannibalism may be a learned behaviour

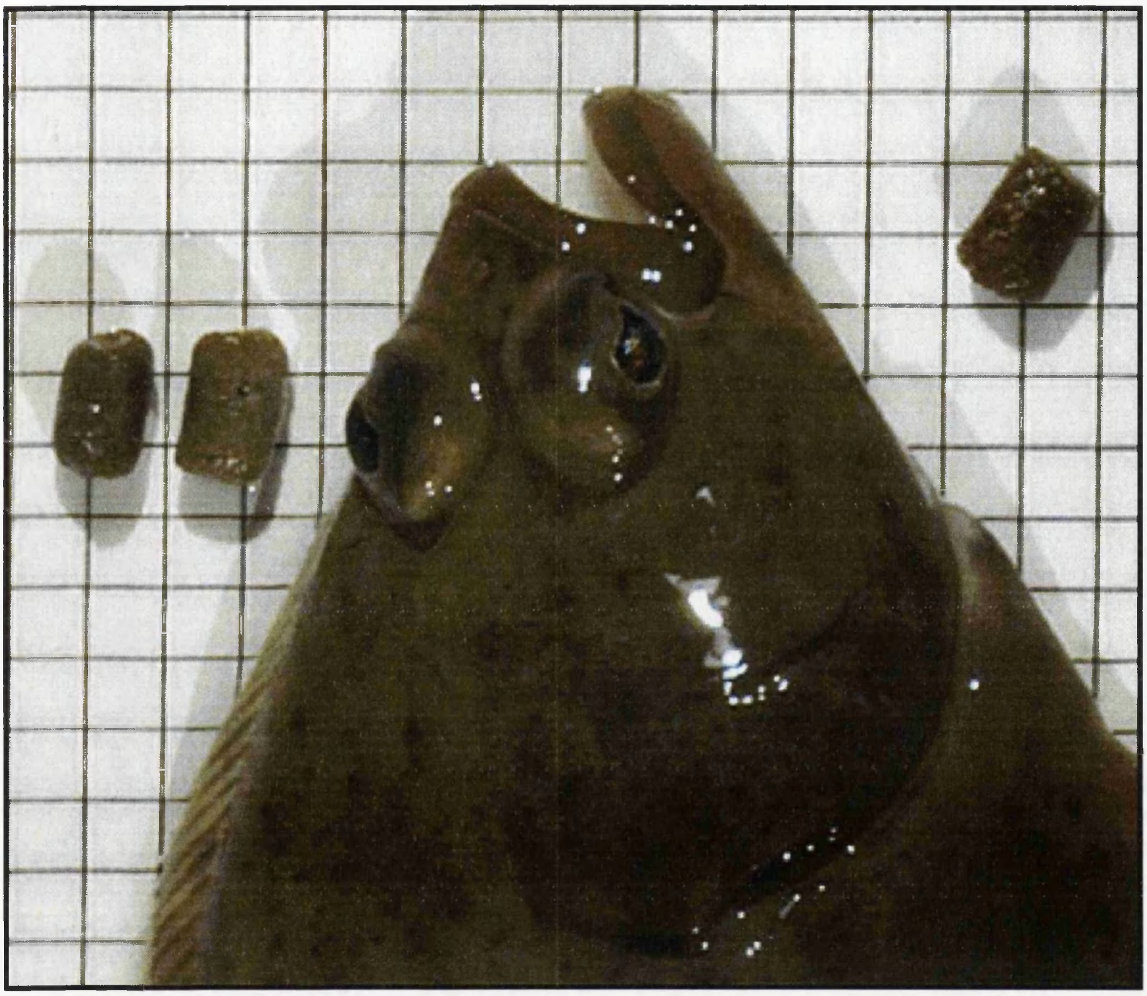
A striking similarity has been noted between the size, shape and colour of halibut eyes and feed pellets fed to juveniles throughout the on-growing phase (Figure 6.4). Appropriate pellet size is chiefly determined by the mouth gape of halibut, and larger pellets are preferred because feeding effort and energy expenditure is reduced. The possibility exists that at least some of the eye damage observed in culture systems may be caused by fish inadvertently striking at the eyes of fish resting on the bottom, believing them to be fallen pellets. Video footage confirms that this is especially plausible if the 'attacker' approaches from behind because the eye itself is not clearly visible from this direction. The halibut non-migratory eye is positioned prominently on the top of the head and is, therefore, vulnerable to this mode of attack. Although initial attacks may be accidental, an element of 'learned' behaviour could follow where a fish develops a taste for eyes, and a cannibalistic habit forms, as has been suggested in poultry.

6.9 Reducing the incidence of cannibalism among halibut

In order to elucidate the behaviour of halibut, the above hypotheses should be tested by conducting further experiments. One could be based on that of Amundsen *et al.* (1995) on prey selectivity. This would involve a heterogeneous size group with halibut prey ranging in size from 30% or less that of the larger fish. Stocking densities and feed rations would be manipulated and observations made of the nature of attacks on other fish. One would hope to determine the way halibut kill and consume conspecifics, and the preferred size of prey. A second experiment would test the hypothesis that eyes are being mistaken for fallen pellets due to similarities between eye and pellet size, shape and colour. Feed colour and shape could be altered for an experimental population and the amount of eye damage/loss compared with that of a control population on the current feed. A surplus of feed on the tank base has until now been viewed as positive because fish can continue to browse feed after the main meal. While this may benefit smaller or subordinate fish by prolonging food availability, it may have negative implications if this hypothesis proves correct. Further research into appropriate feed regimes for this species is of paramount importance, and it may be best to ensure that fish feed predominantly in the water column.

If one accepts that eye losses are due at least in part to cannibalism, then this partial type I cannibalism has greater impact and is more widespread than type II cannibalism among farmed halibut. Therefore, as Hecht and Appelbaum (1988) suggested for African catfish, *Clarias gariepinus*, efforts to mitigate this behaviour should focus on type I cannibalism. Evidence to date suggests that cannibalism in halibut is opportunistic and that conspecifics are not the primary food resource. In the culture environment, key factors in controlling this behaviour appear to be strict size grading (despite the fact that the predator : prey ratio is not critical in this case), good availability and widespread distribution of feed, and relatively high stocking density.

Figure 6.4: The striking similarity between eye and pellet size, shape and colour. It is suggested that some eye damage may be caused by eyes being mistaken for pellets lying among fish on the tank base. This seems especially plausible if the actor approaches from behind the resting fish as the pupil of the eye will not be visible.



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Chapter 7

General Discussion

7.1 Summary of main findings

Aggression among halibut takes the form of brief, non-reciprocated interactions rather than fights (Chapter 2). Fish engage in threat displays (e.g. posturing, Chapter 3), and overt aggression comprising nips, bites and chases. This research has shown that aggressive behaviour is strongly associated with feeding (Chapters 2, 3), and is specifically directed at competitors, especially fish that out-compete aggressors over food items. Earlier studies demonstrated that aggression diminishes with fish size/age, and production experience indicates that it is especially prevalent in juveniles between 20-150 grams weight.

In small groups of fish, dominance structures were apparent and three types of fish were classified on the basis of their behaviour (Chapter 3). Aggressive dominants competed actively for food whereas less competitive individuals chose alternative strategies whereby they fed opportunistically and/or in different areas of the tank from dominants. Highly subordinate halibut inter-acted little and many lost weight over the course of the experiment, indicating considerable levels of stress. In the Tromso experiment involving groups of 6 fish, feed intake by individuals was surprisingly consistent across days (Chapter 3).

Rearing densities were also shown to have a profound impact on aggressive behaviour. Agonistic interactions were more frequent among fish at low densities, and physical damage was not only more prevalent but also more severe than at higher densities (Chapter 4). Direct observations of behaviour showed a strong positive relationship between the targeted attack site on the body of the fish and the nature of physical injuries recorded at the end. This study confirmed that aggression was the primary cause of injuries in halibut of this size class, and that aggression could be reduced and controlled if fish were stocked at higher densities.

Likewise, size grading halibut juveniles significantly diminished the incidence of eye injuries (Chapter 5). The progression of ocular pathology was also detailed here and, while most eye injuries became worse over time, minor damage did sometimes recover completely, even where haemorrhaging within the eye had been noted. Unsurprisingly, eye injuries had a profoundly negative effect on individual growth rates, but fish showed a remarkable ability to heal quickly even at low water temperatures ($7^{o}C$). Encouragingly for farmers, halibut had the capacity to adapt to disabilities and recover growth over a relatively short time period.

There is mounting evidence that cannibalism occurs among farmed halibut populations of varying size (Chapter 6), and cannibalism has been suspected in Atlantic halibut populations from weaning up to 5 Kg weight. Although no formal experiments were carried out to investigate this phenomenon I, (with others), have personally observed it in post-weaned halibut and it may persist in older populations. I suggest that the eye damage investigated in Chapter 5 may or may not constitute partial cannibalism (Type I). However, further studies are needed to demonstrate a direct causal relationship between eye pathology and cannibalism if this hypothesis is to be proved correct.

Behavioural studies have already made valuable contributions to improving rearing conditions and reducing agonistic interactions among farmed fish. For example, Christiansen and Jobling (1990) reduced the aggressiveness of Arctic charr by increasing flow velocity and forcing them to actively swim against the current; and African catfish were less aggressive and showed enhanced growth when reared at densities above which shoaling behaviour was initiated (Hecht and Uys, 1997).

7.2 Dominance hierarchies and the context of aggression

In small groups of halibut, where individual recognition and frequent encounters between the same individuals occur, dominance hierarchies founded on aggressive interactions and intimidation appear to form (Chapter 3). In the experiments described here, fish were classified as competitive-aggressive, opportunistic sneaky feeders or highly subordinate non-feeding fish. However, in large populations (of several hundred or thousand individuals) the presence of such dominance structures has yet to be conclusively proven. I have shown that aggression among halibut is chiefly feed-related, and predominantly follows failed feeding attempts by aggressors. A mean 85% attacks in experiments outlined in Chapter 2, and a mean of 83% in the study of groups of 6 individuals described in Chapter 3, were expressly directed at fish that had won contested pellets. In this context, aggression relates to competition rather than dominance.

Aggressive interactions between halibut are brief and non-random, successful competitors being deliberately sought out by out-competed individuals. While aggressive behaviour is clearly associated with competition over food, this response may be prompted by frustration. Pigeons and rats conditioned to expect a food reward when they press a key will attack their companions if anticipated food does not arrive (Huntingford and Turner, 1987). The frustration-aggression hypothesis contends that frustration alone is sufficient motivation for attack. While this may apply to halibut, they do not simply attack the nearest fish but actively pursue the very individual that out-competed them.

7.3 The effect of density

Weaning and post-weaned fish behave rather differently from older halibut, and aggression does not relate to feed intake (Chapter 4). Instead, aggressors cruise along the tank base and target fish lying alone, or in small groups of 2-3 individuals. On occasions, aggressors attack fish bigger than themselves, so body size is not always associated with aggressiveness. Aggression is also far more prevalent in lower density groups than at either medium or high densities. In the low density trial tanks, the distribution of halibut was patchy across the tank base, and the observation that fish lying in groups of more than three or four individuals were not targeted by aggressors may be significant. Is there safety in numbers? Perhaps aggressors are deterred from attacking fish lying in groups where the whole fish may not always present a clear target if partially concealed by other halibut. This may explain in part why the frequency of aggressive attacks diminishes as stocking density increases. Alternatively, the reduced frequency of aggression at higher stocking densities could be because aggressors cannot maintain control over large groups of fish.

There appears to be a striking contrast in the seemingly random attacks perpetrated by weaning halibut and the highly specific attacks made by older fish. The combined use of video observations and physical damage scoring at the trial end showed that there was good correspondence between the observed frequency of attacks, the level of physical damage scored, and the number of physically damaged fish at the three different densities.

7.3.1 Scale

A continuing problem in applied research is the reconciliation of results obtained from small-scale studies to production scenarios. While small-scale studies enable researchers to obtain detailed information in a controlled environment, there is some doubt as to the validity of their results when applied to larger systems. This PhD research was carried out in a variety of systems, small and production-scale. In the stocking density experiment (Chapter 4), I suggest that the impact of aggression was amplified in the trial tanks by virtue of confinement in a small area and the inability of fish to evade aggressors. However, the results obtained do reflect production tank experience and, as commercial rearing densities have increased, the incidence of aggression and injuries in this size class of halibut has declined.

7.4 Size grading

The importance of size grading (Chapter 5) has been realised, and it is being practised more frequently throughout the halibut production cycle. Current economic losses are due not only to outright mortalities, but also to increased production costs as a result of reduced growth rates associated with eye injury or blindness. The size grading trial (Chapter 5) involved the close examination and classification of degrees of eye damage (simplified into four categories). The marking system, whereby damage severity was colour-coded and mark placement indicated the time that injury occurred, provided quality data on the development of eye damage over time. This trial not only illustrated the progression of eye damage, but also the remarkable ability of halibut to recover physically and in terms of growth rate. Although aggression was certainly the primary cause of initial eye injuries, this trial did not conclusively prove that further deterioration was the result of additional agonistic attacks. Injuries could also worsen by secondary bacterial infection or the progressive degeneration of damaged eye tissue. A detailed study of halibut ophthalmic physiology would, therefore, be valuable to more fully understand the development of eye damage.

The incidence of physical damage is a reliable indicator of aggression levels in aquaculture tanks, and has been used in several large-scale studies where direct behavioural observations have not been feasible (Turnbull *et al.*, 1998; MacLean *et al.*, 2000). The examination of all trial fish individually showed a strong and consistently positive relationship between pectoral fin damage and eye injuries. The ratio of 10:1 non-migratory: migratory eye damage further supports the assertion that aggression is responsible, as this eye is positioned higher on the head, making it a more obvious target. Contrary to expectations, small fish were not the only ones to incur physical injuries during the size grading experiment. Indeed, some of the largest fish suffered eye injuries or loss, suggesting that in large populations of halibut, dominant fish with large body size compete aggressively against one another for food. This outcome supports that of MacLean *et al.*, (2000) who found that large successfully feeding salmon parr *(Salmo salar)* in culture conditions were more prone to attack by a factor of six than smaller parr with lower feed intake. Therefore, for both species, the risk of aggressive attacks represents a cost to high feed intake.

Despite being time-consuming, labour-intensive and stressful to the fish, size grading can significantly reduce aggression and related injuries among farmed halibut juveniles (Chapter 5). On balance then, it appears to be a valuable management tool. There was no evidence that grading improved fish growth, concurring with the study by Sunde *et al.*, (1998) with turbot. Whilst this experiment demonstrated that size grading halibut was worthwhile, the required grading frequency was not fully investigated. One of the shortcomings of my experiment was the failure to grade fish once the trial was underway. This was not feasible because the chosen marking system involved duplicating marking sites and colour-codes between tanks. More sophisticated individual identification (e.g. Passive Integrated Transponder (PIT) tags or Visible Implant (VI) tags, Northwest Marine Technology, U.S.A.) were beyond the scope of available funds for this project, but would enable fish to be mixed at sampling points and tightly graded populations to be maintained throughout. Even so, despite only an initial size grade, the results obtained were conclusive. It is suggested that, had a tight grade been preserved across the four months, the level of damage in the graded populations would be far less than in the ungraded ones. These findings indicate that grading is advisable at 6-8 week intervals for fish weighting between 20-150 grams. However, the initial densities in the trial tanks were lower than normal and stocking at higher densities may help to control aggression levels. Future work should endeavour to more precisely determine the optimum grading interval.

Although there are no known reports of wild-caught halibut with eye damage or loss, one cannot conclude that intraspecific aggression of this kind does not occur. Affected individuals may perish as a result of wounds or impaired feeding ability. In aquaculture tanks, where food is plentiful across a small area and there are no predators, fish that would have died in natural conditions may be able to survive. However, in my opinion, the considerable amount of eye pathology is indicative of environmental stressors. Interestingly, two indoor halibut systems in Canada and Iceland have fewer problems of this kind, and possible reasons for this are broached later in this chapter.

7.5 Cannibalism

Various reports from research and production sources (and personal observations) confirm that cannibalism occurs among farmed Atlantic halibut. Stomach content analyses of wild Atlantic and Pacific halibut suggest that cannibalism is a natural phenomenon, rather than an abnormal behaviour produced solely at high fish densities in intensive culture conditions. However, environmental stressors prevalent in intensive systems may exacerbate this behaviour. Sakakura and Tsukamoto (1997) found that even well fed Yellowtail (*Seriola quinqueradiata*) were cannibalistic. Various types of cannibalism have been described, from whole body ingestion to partial consumption of body parts. In Chapter 6, it was suggested that the eye damage problem may relate to cannibalism rather than being exclusively aggression-induced. If so, it is possible that cannibals established throughout the weaning phase go on to develop this habit as they grow. In populations of post-weaned halibut there are occasionally a few very large individuals, some seen with smaller fish protruding from their mouths (M. Spreadborough; D.Patterson, pers. comm.). Here, cannibalism is likely to confer a growth advantage. However, such fish are thought to represent a negligible percentage of the population, and subsequent grading would reduce the opportunity. Cannibalism in halibut has not yet been properly investigated, but it appears that the weaning phase is the most critical for whole body ingestion of conspecifics because of the large size and developmental range in a cohort. The stress of handling delicate fish prior to 2 grams weight, and the perceived risk of losses as a result, outweigh the benefits of grading at this time. Although some losses are known to occur, cannibalistic halibut are not thought to be as voracious or common as some other documented species (African catfish, yellowtail). In any event, constrained by mouth gape size, cannibals target the smaller, weaker fish that may die anyway.

7.6 Other important factors that influence aggression

7.6.1 Feeding activity and natural feeding rhythms

Feeding is perhaps the most important area for further research studies on aggression in halibut. If fish are being fed to excess (or at least to satiation), and there is no need to compete over food, then why is aggression still so prevalent in farmed halibut tanks? Hungry fish exhibit a strong feed response, and many fish will compete for the same pellets. In this situation, there is the potential for heightened intra-specific aggression and also accidental collisions that may inadvertently cause injury (Chapter 2). It appears important with this species to satisfy fish appetite and prevent the build-up of hunger levels by providing feed throughout the day. However, further studies are required to determine the optimal way of delivering feed to tanks/cages, and also the minimum amount of food entering the tank at any time to curb competitive aggression. The total daily ration may be ample, but if food is added too slowly (too few pellets at any one time from an automatic feeder) then a competitive situation is created and aggression is stimulated. I surmise that this occurs in halibut tanks and it could partially explain the continuing aggression. In addition, fish that browse for fallen pellets on the tank base among resting halibut may also cause some eye damage (Chapter 5). Ideally, a feeding regime should be developed that encourages fish to feed in the water column, and pellets must be sufficiently numerous and dispersed to circumvent competition. The time of day when feed is distributed can affect both physiological and behavioural processes, such as growth rate and nutrient partitioning. Matching the temporal pattern of feeding to the natural feeding rhythm of the fish should, in turn, improve production efficiency because fish can feed when appetite is highest (Kadri *et al.*, 1991).

7.6.2 Light levels

Maintaining halibut at constant low light levels can substantially reduce both swimming activity and aggressive interactions between juveniles. This is most probably due to a reduction in environmental stress. The indoor rearing systems employed in Canada and Iceland appear to be successfully managing these problems. However, it will be difficult to implement similar systems in Scottish ongrowing facilities because existing systems comprise outdoor tanks. Although tanks do have shade covering, and access hatches are kept closed throughout the day to minimise light penetration as far as possible, the fish are exposed to daily fluctuations in light levels. In addition, outdoor systems are prone to more disturbances from changing weather (storms, rain, wind) and also general noise on a production site. It is interesting to note that in Scottish systems aggression becomes problematic on transfer from indoor nursery tanks to outdoor facilities where light levels increase considerably. Further research in this area is obviously important.

7.6.3 The addition of shelves in tanks

Juvenile Atlantic halibut may be kept at densities where fish layer 2-3 deep on the tank base. Behavioural observations show that fish are constantly shuffling position, those on the upper layer burrowing beneath other fish. These dorsal/anal fin undulations would normally cause them to bury in sand substrate and may be indicative of fish attempting to find cover. The recent addition of shelves in halibut on-growing tanks and cages looks to be a promising innovation. Shelves not only provide the fish with increased surface area for resting, but may also reduce aggressive interactions because fish are spatially dispersed, and have fewer encounters. When feeding, fish either leave the shelves to feed in the open area of the tank or feed *in situ* (Nick Brown, pers. comm.). Shelves along the tank sides also provide a degree of shading and shelter, and reduced activity has been noted in these systems. An interesting observation is that smaller fish, or those with injuries, seek refuge on the uppermost shelf. This may allow them recovery time without being under constant threat of attack or harassment (S.Wilde, pers. comm.). Shelving is a relatively new initiative but has the potential to markedly improve the tank environment for fish and research is currently ongoing.

7.6.4 Stress in aquaculture

Intensive aquaculture imposes many unavoidable stressors on the fish being farmed, for example: handling, grading and transport. Stressors may be acute (handling, transport) or prolonged and chronic (social hierarchy effect, overcrowding, poor water quality). Responses to stress can be broadly categorised as physiological, neuroendocrinological and behavioural. The physiological stress response is initially adaptive, adjusting metabolic processes to enhance the fish's ability to cope better with the situation. However, frequent activation of the stress response by repeated exposure to acutely stressful stimuli and adverse conditions is detrimental to the fish and has repercussions in terms of reduced growth, increased susceptibility to disease because of immunosuppression and poor body condition (Pickering, 1998). Plasma cortisol, the principal corticosteroid in teleosts, is regularly used to measure how stressful particular stimuli are to fish. Both the magnitude and the duration of the response convey the degree of stress. Given the detrimental effects of stress on the general growth and health of the fish, stress should be minimised wherever possible throughout the production cycle. This even extends to larval fish during inherently stressful developmental periods, for example, metamorphosis in flatfish. I have studied the behaviour of halibut juveniles in culture and provided some useful information that can be (and has been) directly applied in the culture of this species.

My work with post-weaned halibut has shown that aggressive interactions and consequent physical injuries were significantly higher at the low stocking density than at two higher densities. Social interactions among halibut can, therefore, impose a chronic stress on subordinate fish. This work has shown that it is possible to manipulate aggressive behaviour in halibut by adjusting rearing densities, and that social stress can be alleviated by keeping fish at higher densities. However, while behavioural explanations can be insightful, physiological and neural factors also affect aggressive behaviour. A more comprehensive understanding would be obtained by examining all three of these components, and co-ordinated research between physiologists and behavioural scientists would be fruitful.

7.6.5 Aggression and Domestication

Several interesting studies have highlighted the influence of domestication on the behaviour of cultivated species. Vincent (1960) demonstrated a significant difference in the performance and behaviour of domesticated brook trout (*Salvelinus fontinalis*) and those only one generation from wild stock. Domesticated stock, after 90 years of cultivation, were substantially tamer, less afraid and also showed superior growth rates than fish derived from wild parents. Perhaps the best example of domestication in fish is the common carp, (*Cyprinus carpio*), which is the longest established domestic species having been cultivated for over 2000 years. Weatherley and Gill (1981) infer that it is no accident that this species is known for its docile nature and tolerance of crowding. In contrast, the majority of Atlantic halibut juveniles in Scottish hatcheries are still the progeny of captured broodstock and, therefore, only one generation removed from the wild. Although the broodfish themselves have a placid nature and appear to have adapted reasonably well to captivity, their progeny are likely to have inherited genetic traits that exacerbate behavioural problems in culture conditions.

7.7 Final remarks

Commercial aquaculture is, by definition, intensive and fish are kept in highly artificial conditions. The principal factor of interest to any fish farmer is growth, and the challenge for commercial production is to manipulate fish social behaviour to promote equal access to feed by the whole population and achieve more uniform growth performance. The primary challenge in aquaculture is to understand the behavioural mechanisms that generate differential access to resources and adjust the environmental conditions so that this advantage is reduced.

Many environmental factors influence fish growth and production efficiency, among them water temperature, fish size and age, environmental stressors and stocking density. Throughout my research I have identified and examined several environmental factors that impinge on halibut behaviour, and specifically aggression. However, while each variable studied is undoubtedly important, I believe that successfully managing the problem behaviours of this species requires attention to a combination of factors. Fortunately, the significance of behaviour in relation to the profitable production of halibut has been realised, and improvements are steadily being implemented as we learn how to handle this species.

Atlantic halibut is known to be an aggressive predatory fish, and the highly artificial and simplistic culture environment may exacerbate latent aggressive tendencies. Therefore, aggression is unlikely to be completely eradicated, but it can be better managed and controlled. The magnitude of physical damage caused primarily by intraspecific aggression is likely due, at least in part, to imposed environmental stressors. My work has shown the plasticity of halibut behaviour and the scope for manipulating this in culture systems. As the scale of production increases new challenges emerge, and there is much work to be done on further mitigating and ameliorating the culture environment. Alterations to farming practice can markedly reduce aggression levels and alleviate the effects of chronic stress in cultured fish.

Addendum

Commercial producers and behavioural scientists have different objectives and there is often a degree of compromise on both sides in the design and running of an experiment. The goal of the fish farmer is to get the maximum number of quality fish through the production cycle and to generate a profit. Therefore, persuading farmers to grant access to large numbers of fish for trial purposes can be difficult as it imposes a risk

of sub-optimal growth performance and even losses. Given the high value of halibut juveniles, I am extremely grateful to the BMFA and particularly Otter Ferry Seafish Ltd. for their generosity and trust in allowing me the opportunity to conduct my experiments.

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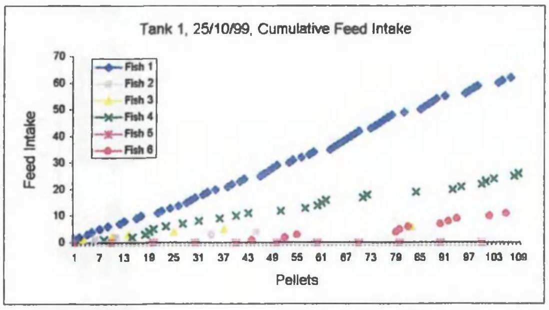
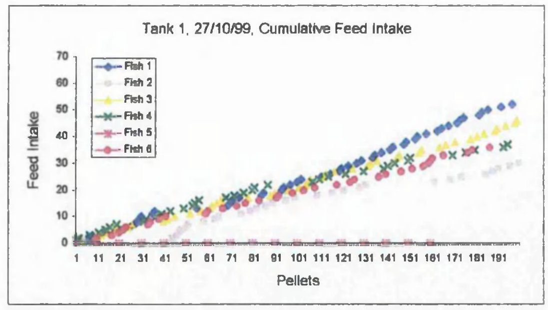
APPENDIX I

CUMULATIVE FEED INTAKE PLOTS OVER FIVE DAYS FOR NINE GROUPS OF SIX HALIBUT (CHAPTER 3)


Tank 1, 25/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6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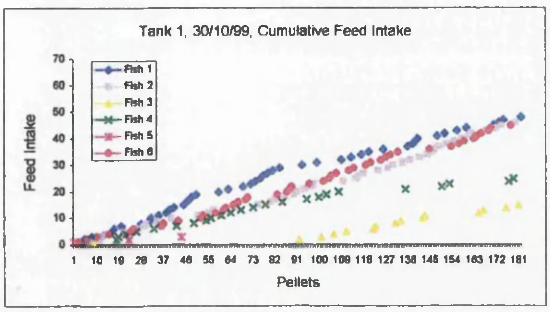
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6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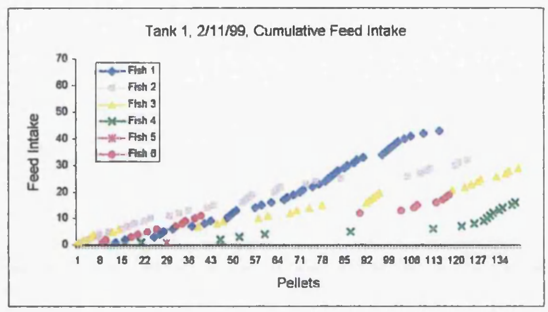
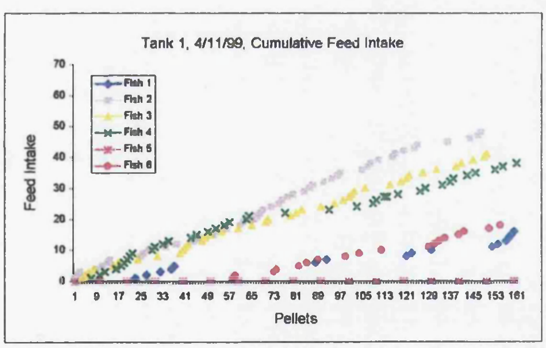
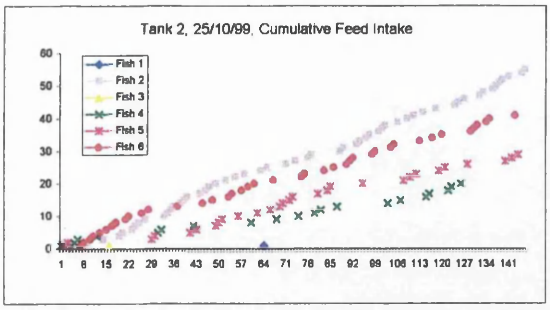
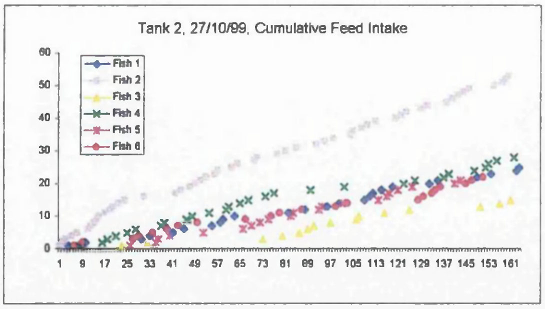
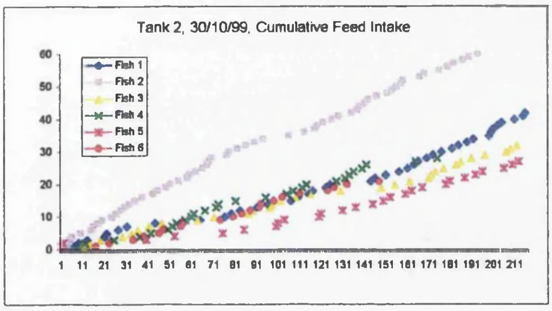
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

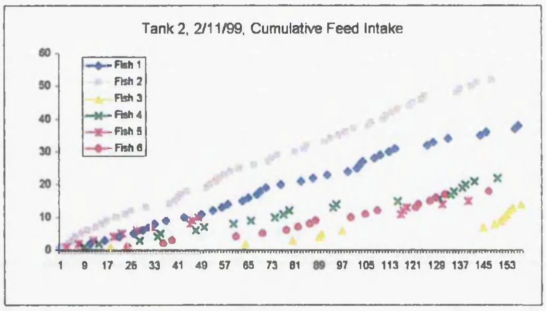

Tank 1, 2/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

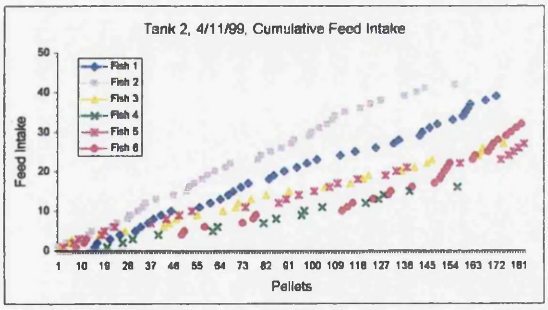

Tank 1, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

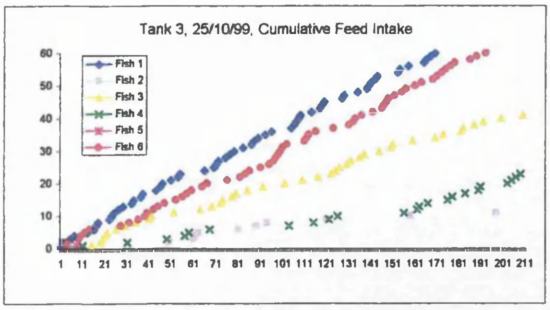

Tank 2, 25/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

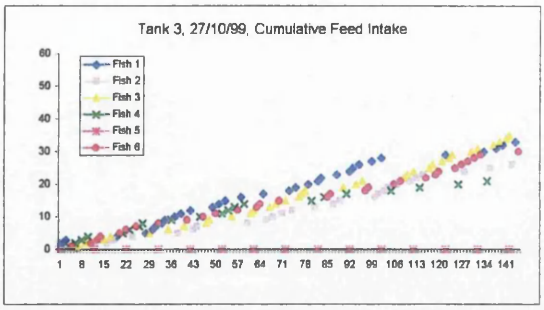

Tank 2, 27/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

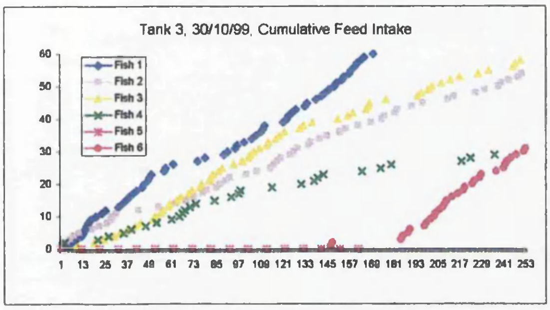

Tank 2, 30/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

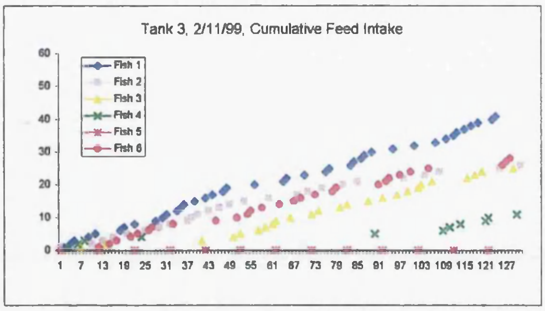

Tank 2, 2/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

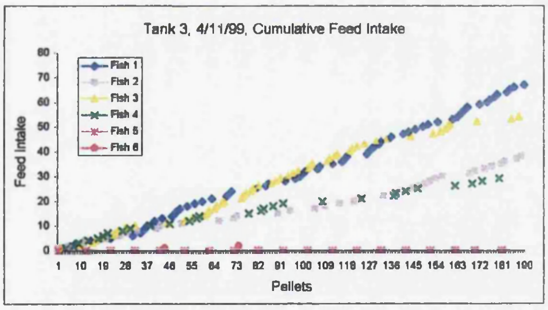

Tank 2, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

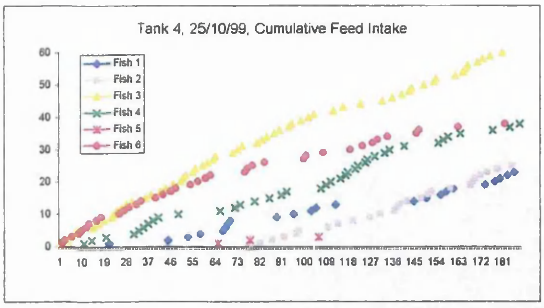

Tank 3, 25/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

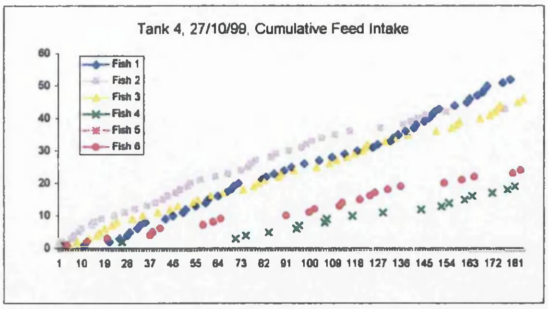

Tank 3, 27/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

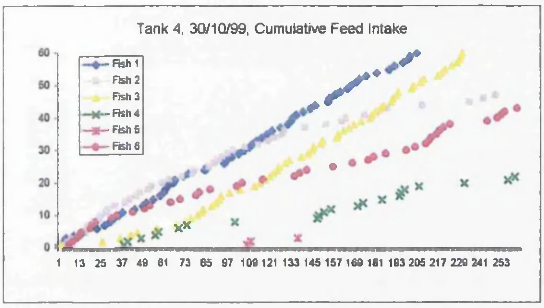

Tank 3, 30/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

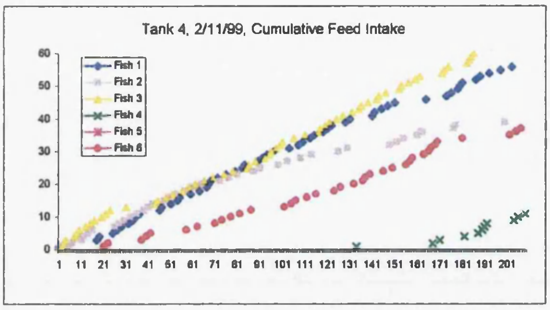

Tank 3, 2/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

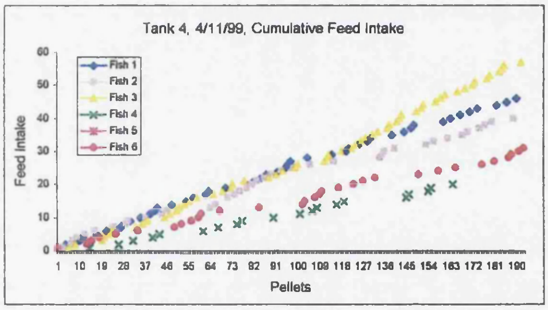

Tank 3, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

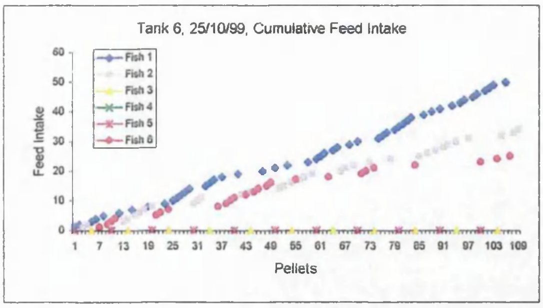

Tank 4, 25/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6


Tank 4, 27/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

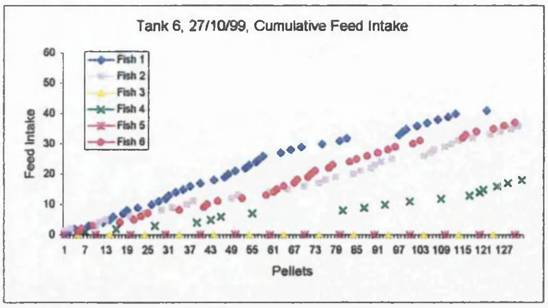

Tank 4, 30/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6


Tank 4, 2/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

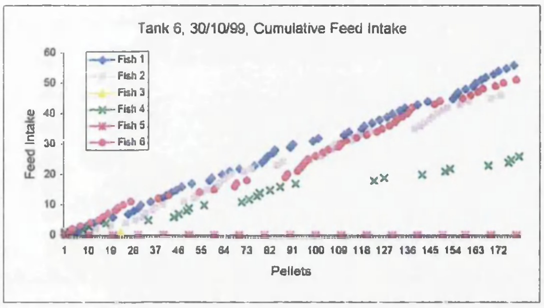

Tank 4, 4/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets



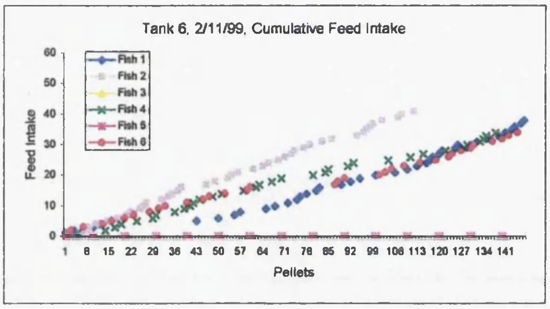
Tank 6, 25/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



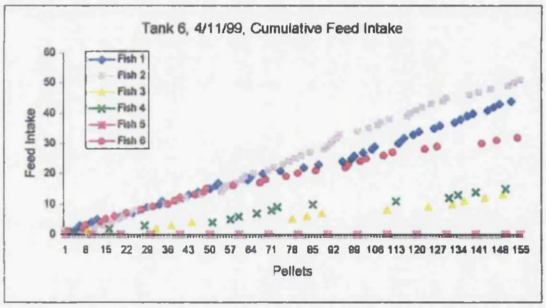
Tank 6, 27/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



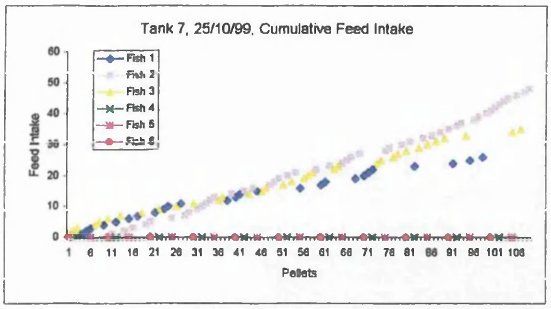
Tank 6, 30/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

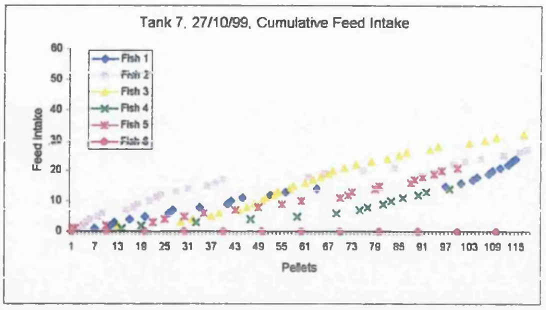


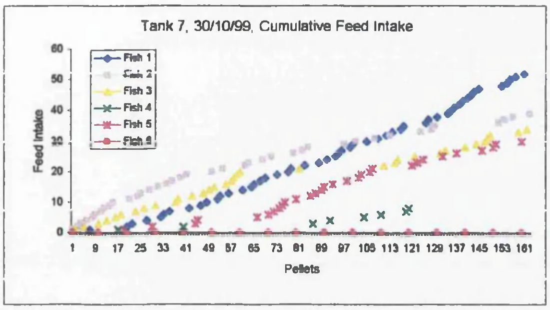
Tank 6, 2/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

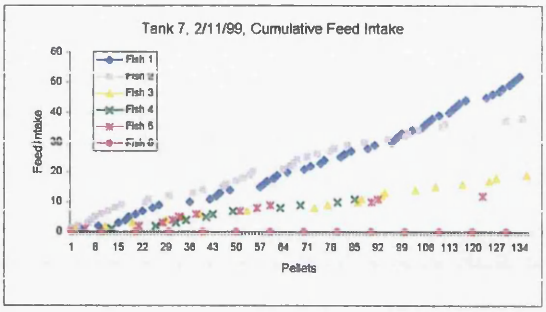


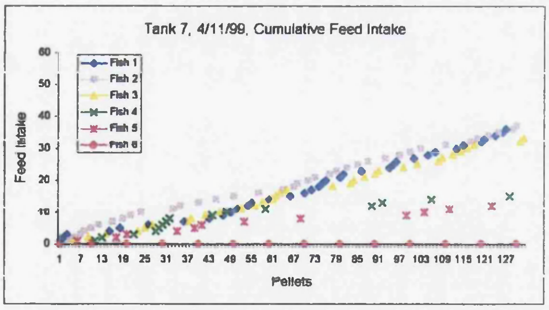
Tank 6, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

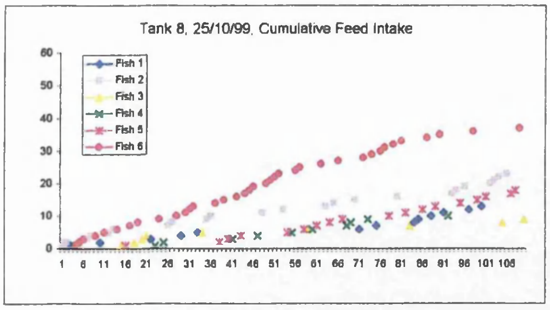

Tank 7, 25/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6


Tank 7, 27/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

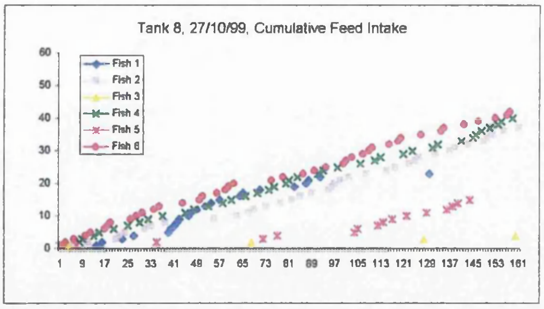

Tank 7, 30/10/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6


Tank 7, 2/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6

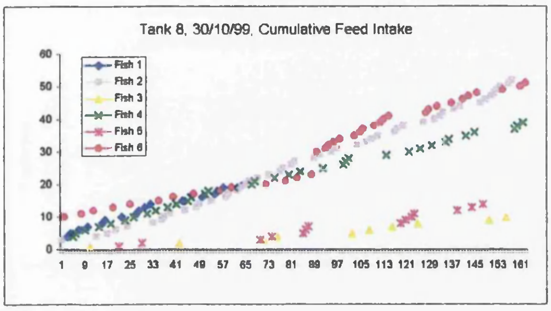

Tank 7, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



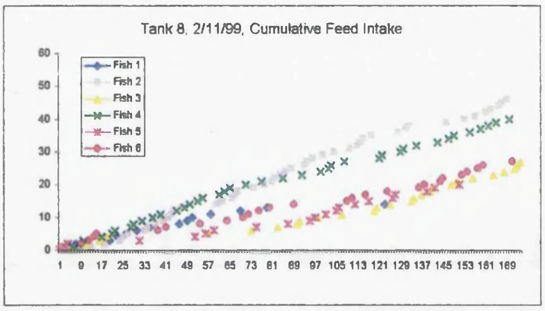
Tank 8, 25/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



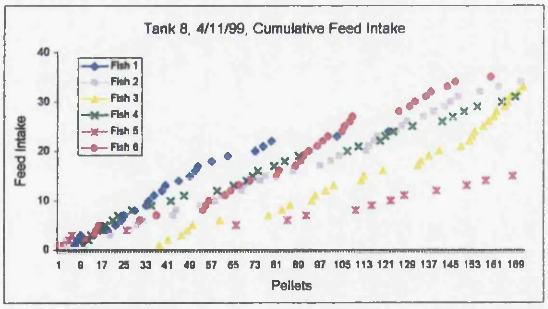
Tank 8, 27/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



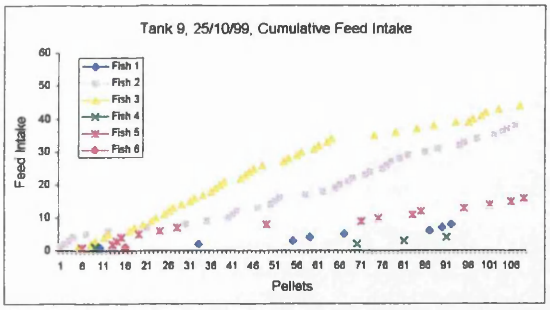
Tank 8, 30/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



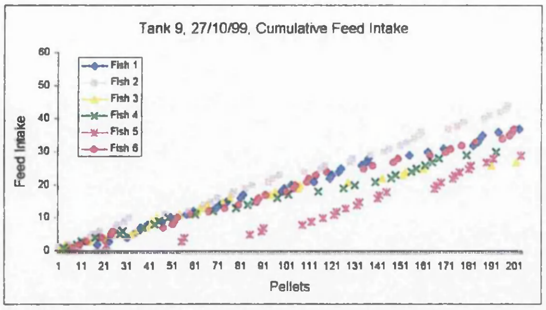
Tank 8, 2/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



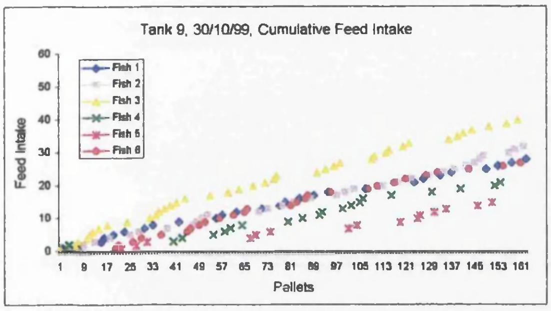
Tank 8, 4/11/99, Cumulative Feed Intake
Feed Intake
Pellets
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6



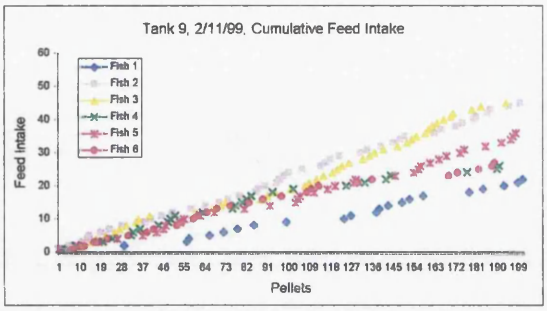
Tank 9, 25/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets



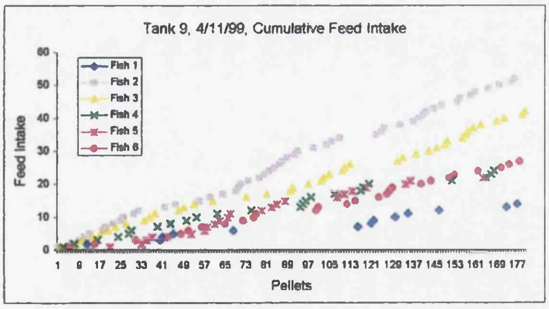
Tank 9, 27/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets



Tank 9, 30/10/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets



Tank 9, 2/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets



Tank 9, 4/11/99, Cumulative Feed Intake
Fish 1
Fish 2
Fish 3
Fish 4
Fish 5
Fish 6
Feed Intake
Pellets

APPENDIX II

SPEARMAN'S RHO CORRELATION MATRICES FOR GROUPS OF TROMSO HALIBUT WHERE AGGRESSION WAS NOT CONCORDANT (CHAPTER 3)

Tank 1

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		0.314 0.544	-.232 .658	0.029 .957	-.290 .577	-0.314 .544
FI	Correlation coefficient significance	0.314 0.544		.783 0.066	-.058 .913	.609 .200	.714 .111
WSFI	Correlation coefficient significance	-.232 .658	.783 0.066		.250 .633	0.882* .020	.986** .000
Agg.	Correlation coefficient significance	0.029 .957	-.058 .913	.250 .633		.515 .296	.319 .538
Agg. Rec.	Correlation coefficient significance	-.290 .577	.609 .200	0.882* .020	.515 .296		.928* .008
SGR	Correlation coefficient significance	-0.314 .544	.714 .111	.986** .000	.538 .319	.928* .008	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Tank 4

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		.771 0.072	-0.029 .957	0.029 .957	0.406 .425	0.029 .957
FI	Correlation coefficient significance	.771 0.072		.522 .288	.429 .397	.493 .321	.600 .208
WSFI	Correlation coefficient significance	-0.029 .957	.522 .288		.174 .742	.603 .205	.899 .015
Agg.	Correlation coefficient significance	0.029 .957	.429 .397	.174 .742		.577 -.290	.486 .329
Agg. Rec.	Correlation coefficient significance	0.406 .425	.493 .321	.603 .205	.577 -.290		.461 .377
SGR	Correlation coefficient significance	0.029 .957	.600 .208	.899 .015	.486 .329	.461 .377	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Tank 5

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		.257 .623	-.543 .266	.174 .742	-.657 .156	-.314 .544
FI	Correlation coefficient significance	.257 .623		.486 .329	.464 .354	.029 .957	.771 .072
WSFI	Correlation coefficient significance	-.543 .266	.486 .329		.700 .203	.771 .072	.829* .042
Agg.	Correlation coefficient significance	.174 .742	.464 .354	.700 .203.		-.377 .461	.913 .058
Agg. Rec.	Correlation coefficient significance	-.657 .156	.029 .957	.771 .072	-.377 .461		.600 .208
SGR	Correlation coefficient significance	-.314 .544	.771 .072	.829* .042	.913 .058	.600 .208	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level

Tank 6

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		.886* .019	.771 .072	.714 .111	.829* .042	.771 .072
FI	Correlation coefficient significance	.886* .019		.943** .005	.829* .042	.714 .111	.771 .072
WSFI	Correlation coefficient significance	.771 .072	.943** .005		.943** .005	.600 .208	.829* .042
Agg.	Correlation coefficient significance	.714 .111	.829* .042	.943** .005		.657 .156	.943** .005
Agg. Rec.	Correlation coefficient significance	.829* .042	.714 .111	.600 .208	.657 .156		.829* .042
SGR	Correlation coefficient significance	.771 .072	.771 .072	.829* .042	.943** .005	.829* .042	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level

Tank 7

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		.943** .005	.657 .156	.812* .050	.771 .072	.886* .019
FI	Correlation coefficient significance	.943** .005		.829* .042	.754 .084	.657 .156	.943** .005
WSFI	Correlation coefficient significance	.657 .156	.829* .042		.348 .499	.429 .397	.771 .072
Agg.	Correlation coefficient significance	.812* .050	.754 .084	.348 .499		.406 .425	.580 .228
Agg. Rec.	Correlation coefficient significance	.771 .072	.657 .156	.429 .397	.406 .425		.771 .072
SGR	Correlation coefficient significance	.886* .019	.943** .005	.771 .072	.580 .228	.771 .072	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level

Tank 8

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient significance		.314 .544	.872* .086	.290 .577	.319 .538	-.928** .008
FI	Correlation coefficient significance	.314 .544		.771 .072	.725 .103	.812* .050	.784 -.145
WSFI	Correlation coefficient significance	.872* .086	.771 .072		.928** .008	.841* .036	.913** .058
Agg.	Correlation coefficient significance	.290 .577	.725 .103	.928** .008		.691 .128	912** -.059
Agg. Rec.	Correlation coefficient significance	.319 .538	.812* .050	.841* .036	.691 .128		.592 -.279
SGR	Correlation coefficient significance	-.928** .008	.784 -.145	.913** .058	.912** -.059	.592 -.279	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level

Tank 9

Spearman's rank order correlations:

		I Weight	FI	WSFI	Agg.	Agg. Rec.	SGR
I Weight	Correlation coefficient		.543	.290	.600	.371	-.600
	significance		.266	.577	.208	.468	.208
FI	Correlation coefficient	.543		.928**	.714	.714	.314
	significance	.266		.008	.111	.111	.544
WSFI	Correlation coefficient	.290	.928**		.696	.580	.551
	significance	.577	.008		.125	.228	.257
Agg.	Correlation coefficient	.600	.714	.696		.787	.787
	significance	.208	.111	.125		.143	.143
Agg. Rec.	Correlation coefficient	.371	.714	.580	.787		.872*
	significance	.468	.111	.228	.143		.086
SGR	Correlation coefficient	-.600	.314	.551	.787	.872*	
	significance	.208	.544	.257	.143	.086	

I Weight = initial weight; FI = feed intake WSFI = weight specific feed intake; Agg. = aggression; Agg. Rec. = aggression received.

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level

APPENDIX III

JULY LENGTH FREQUENCY DISTRIBUTIONS AND THE RELATIVE PERCENTAGE OF FISH AFFECTED BY EYE DAMAGE

Figure AIII.1: Tank 1 (Small, graded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. Small sample sizes are indicated by a dotted line.

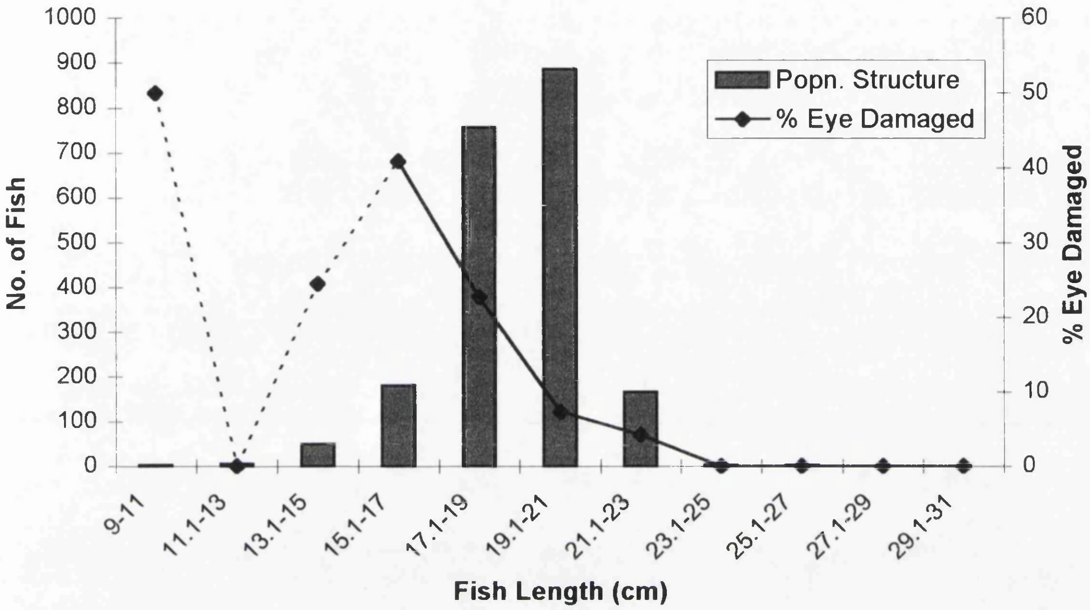


Figure AIII.2: Tank 2 (Ungraded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. sample sizes are indicated by a dotted line.

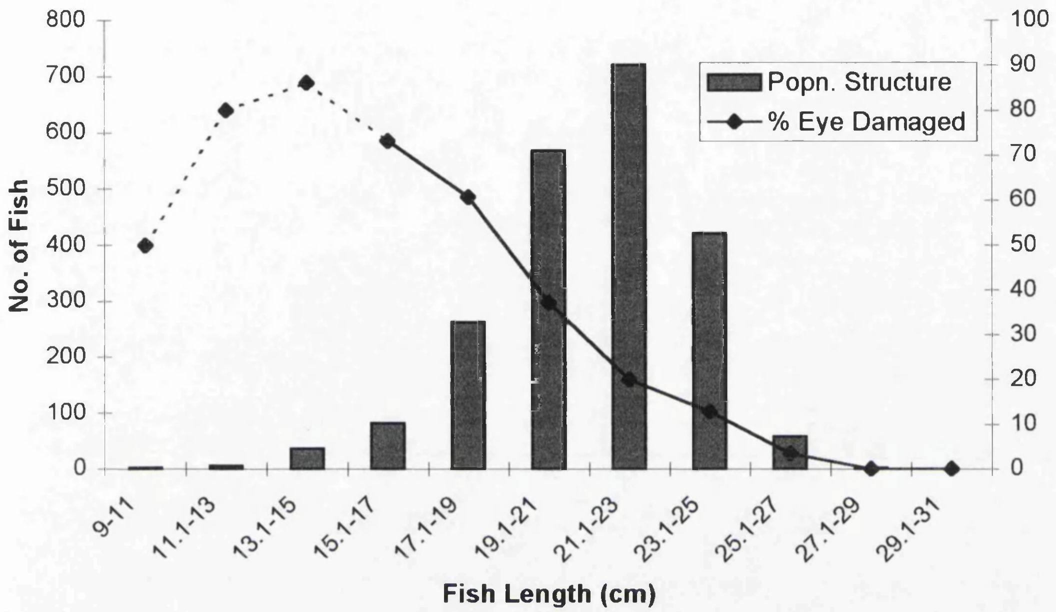


Figure AIII.3: Tank 3 (Ungraded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. Small sample sizes are indicated by a dotted line.

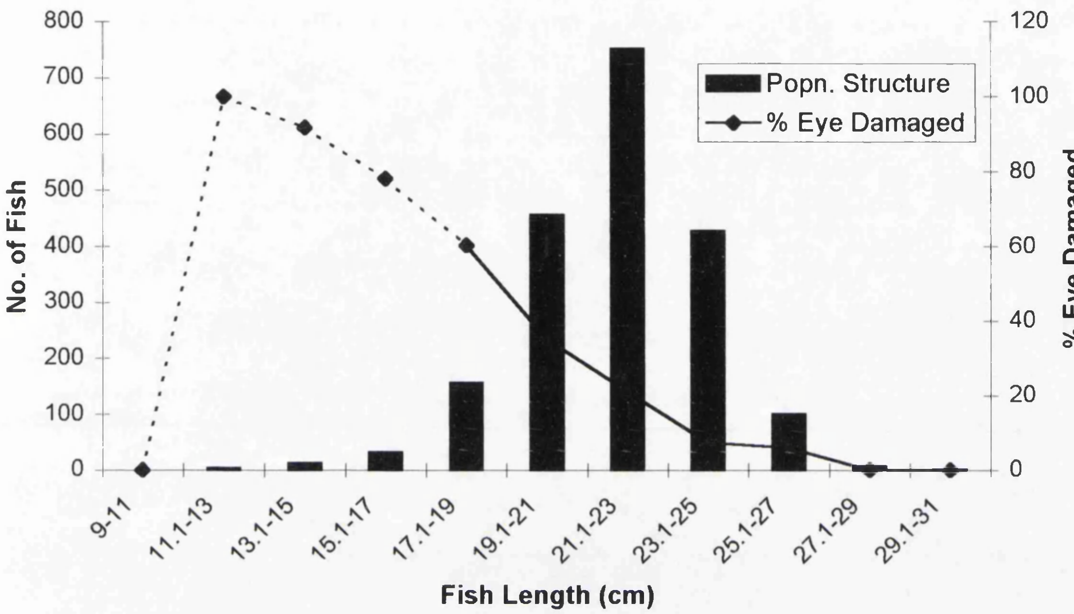


Figure AIII.4: Tank 4 (Medium, graded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. Small sample sizes are indicated by a dotted line.

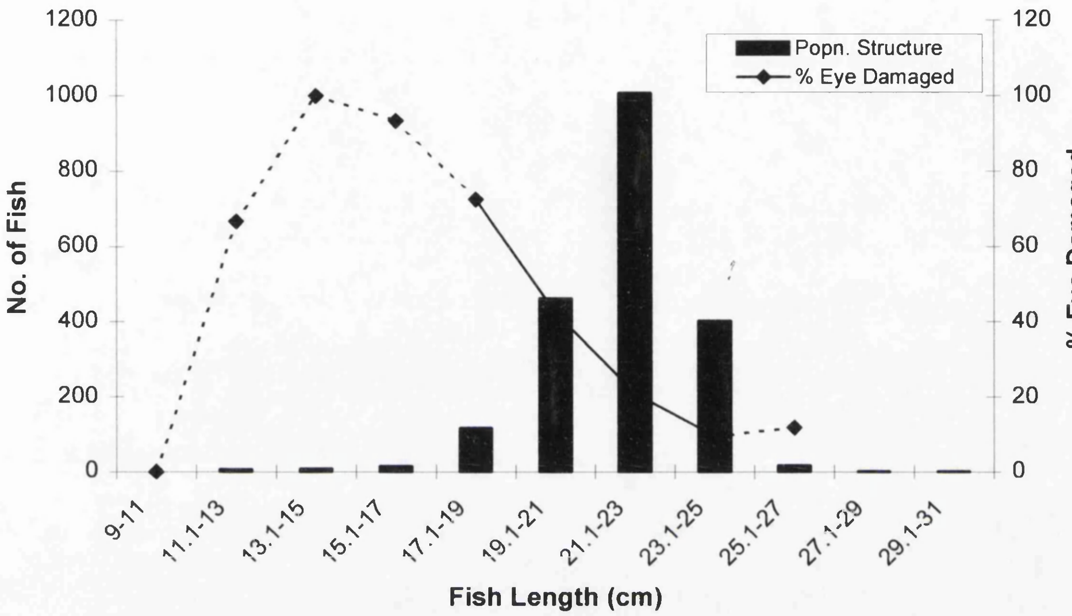


Figure AIII.5: Tank 5 (Large, graded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. Small sample sizes are indicated by a dotted line.

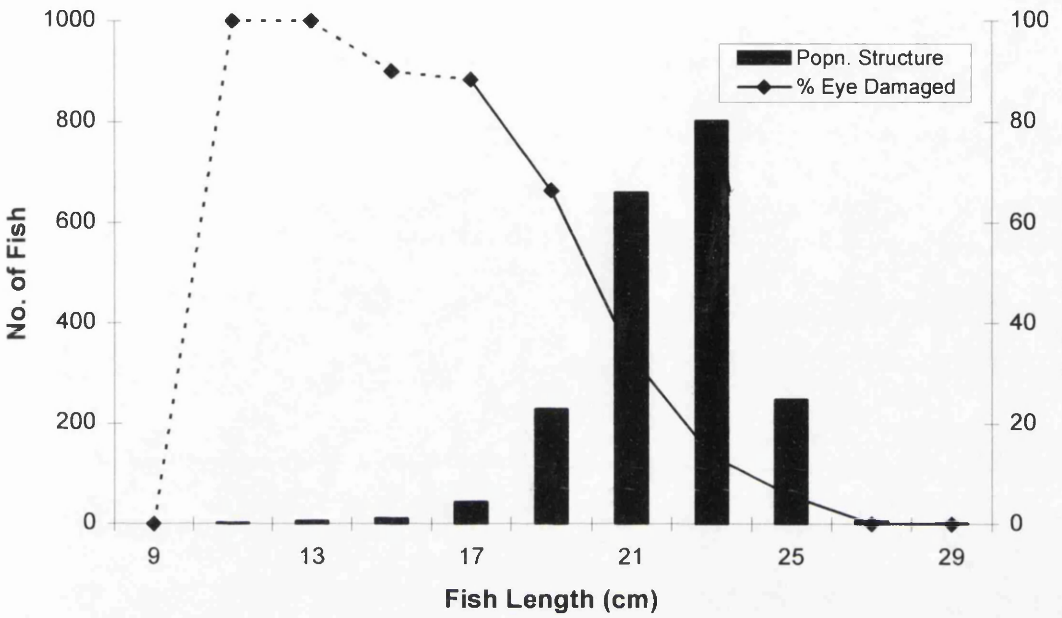


Figure AIII.6: Tank 6 (Ungraded) July length frequency distribution and the relative percentage of fish affected by eye damage. Smaller fish suffered more eye damage. Small sample sizes are indicated by a dotted line.