



Sarf043
Species Specific Requirements Of Marine Finfish



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SARF043**Species Specific Requirements of Marine Finfish
("Failed cod syndrome")**

Descriptor of project:

Social, stocking density and dietary effects on the failure of farmed cod *Gadus morhua*

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PROJECT SUMMARY

A large proportion of cod juveniles fail following transfer from the hatchery to sea cages. Failed cod take and then eject feed and are thin and emaciated fish of low weight compared with “normal” siblings. Failed fish comprised 70% of total mortalities and 10% of fish stocked in the first eight months of production on a cod farm in Scotland. Failed cod comprised from 0 to 8% of fish reared in twelve hatchery tanks over 3 months. These failed cod were graded from larger fish in five consecutive grades at three weekly intervals and, after being reared separately, over 90% of these fish “recovered”. No significant differences were found in food acceptance of six alternative trial diets and a standard marine commercial diet by failed cod. Smaller pellets or feed moistened in seawater were offered but this did not significantly increase the rate of feed acceptance. A novel demand feeder system was developed during the project and was used to assess whether alternative diets with high moisture and high attractant would be preferred to a standard diet by ungraded cod but there were no significant differences. The effects of stocking density on the development of failed cod were assessed by comparing performance at densities of 0.2, 2 and 20 kg m⁻³. Aggressive interaction was lowest at an intermediate stocking density of 2 kg m⁻³ and highest at a density of 20 kg m⁻³. Fin nipping was concentrated on the first dorsal fin and was not significantly different between stocking densities, but the other dorsal fins, pectoral fins and tail were also attacked more frequently at the highest stocking density. Growth was consistently highest at 2 kg m⁻³, although this was not significantly different from the other stocking densities. It is concluded that failed cod can be recovered by grading and feeding these cod separately from the main population. The demand feeding system developed in the project has been further applied to determining an optimum and acceptable diet for cleaner fish, wrasse, used in salmon farming.

Key findings:

- poor-doer cod are common and cause mortality in both Scotland and Norway
- it can occur in hatcheries but is less common than in sea cages

- poor-doer cod can recover following grading
- intake of food into the mouth and rejection of food after a feeding response are common and the latter seems to be the root of the problem, rather than a lack of feeding response
- morphological abnormalities do not appear to be the cause of rejection of food
- behavioural interactions are unproven as a cause of poor-doers
- manipulating diets to improve ingestion rates in failing cod is very difficult
- injuries occur to the fins, that may be associated with aggression (although this is not proven)
- stocking density may affect levels of injury

Keywords: failed cod, feeding, grading, aggression, stocking density, demand feeding system

1. Introduction

Farming of Atlantic cod *Gadus morhua* has been a major development in Norway, Iceland, Scotland and Canada over the last 10 years (Kjesbu et al., 2006). Although the economics of cod farming have been difficult recently and there are still some technical issues to resolve to achieve sustainable production, the farming of cod is likely to remain of commercial interest. Technical developments include vaccines for diseases such as *Vibrio anguillarum* and atypical *Aeromonas salmonicida* (Bricknell et al., 2006) and control of early maturation which adversely affects growth rate (Taranger et al., 2006). A major issue for the industry in Scotland (Adoff, 2011; Hanche-Olsen and Nilsen, 2009), Norway (K. Sørebo, pers. comm.) and Iceland (Kristmundsson et al., 2011) is a condition involving the failure of a high proportion

of farmed cod following transfer from the hatchery to sea cages. Although there is little published information on this condition there are several unpublished industry reports (from former NoCatch Ltd Shetland, A. Bourhill, pers. comm.; and A. Young, Biomar Ltd, pers. comm.). They reported thin and underweight cod compared with “normal” fish and these failed fish represented a high percentage of fish stocked. Failed cod syndrome is also a widespread problem in Norway (Fig. 1) and the farmers often lose 20-30% of a group of cod within the first 6-8 weeks after transfer to sea cages (L. Olav Sparboe, pers. comm.).

The failed cod condition has not been formally defined. Histopathology samples were taken of failed cod at Vidlin Voe, Shetland (NoCatch Cod) but these did not reveal a clinical condition, with only anorexia being reported (Prof. H. Ferguson, pers. comm.). However, the main feature of the condition is that soon after transfer from the hatchery to sea cages, the fish will take, then reject (‘spit out’), formulated feed, even if known to be hungry (A. Bourhill, pers. comm.). The fish thereafter become poor growers, lose condition, and probably become more susceptible to disease. Attempts to recover such fish have had limited success, and fish that do survive do not perform as well as the rest of the stock.

The practice in the UK was to transfer cod juveniles to ongrowing sites by lorry to Shetland from the mainland hatcheries at a mean weight around 10 g. This journey took ca. 24 hours and losses were variable but, with experience, survival increased and stress was reduced during transfer by more experienced net handling, adjustment of stocking densities to a maximum of 25 kg m³ and, in some cases, the addition of buffers to reduce changes in pH. The fish were stocked directly in to a sheltered nursery site in 10 m square cages. They were grown to a size of ca. 60 g before being vaccinated intraperitoneally and transferred to large polar circle cages in the main area of the voe (sea loch). The feed used in all hatcheries prior to and also after transfer was Biomar Pearl as it was the only organically approved feed. The fish were fed by hand to satiation in the morning and continuously fed throughout daylight hours with a Betten automatic feeder. The fish were graded 2 to 3 times while in the nursery cages. Where failed cod were present these fish were also fed by feed distributed by hand to the corners of the cages where these fish congregated (M. Johnson, pers. comm.).

A range of variables may be implicated in this failure, including feed size, formulation and texture, the social hierarchy in the cage, change in water quality between the hatchery and the sea site, stress on transfer to the cage site, population size distribution, a sudden change in stocking density, and change in environmental variables such as photoperiod, light intensity and current speed. The present work focuses on the influence of food preferences and behaviour of cod. Failed cod syndrome may be related to social structure and aggression within the cage population. However, there are widely conflicting accounts of aggressive behaviour in cod. Hoglund et al. (2005) showed that juvenile cod are highly aggressive, whereas Salvanes and Hart (2000) did not find indicators of aggression nor territorial defence among juvenile cod competing for feed. Other experimental studies have shown that cod do not defend a feeding territory (van Duren and Glass, 1992), whereas wild juvenile cod have been observed to show territorial behaviour (Tupper and Boutilier, 1995). In contrast, there have been several other reports of feed deprivation and restricted feeding without evidence of typical aggression related fin damage (Hawkins et al., 1985; Foster et al., 1993). Other studies have shown that juvenile cod compete for food without physical aggression (Hart and Salvanes, 2000; Salvanes and Hart, 2000).

The purpose of the SARF project was to establish the validity and extent of the failed cod condition given this mixed review of competition for feed of cod and the role of aggression. Social and behavioural factors were examined to determine if they influenced the development of failed cod through aggressive behaviour and to assess if stocking density affects the degree of this interaction. The value of segregating failed fish from the rest of the cod was investigated further by grading failed cod and feeding them separately. The role of dietary factors and food palatability in aiding the recovery of failed cod were also examined by offering alternative diets with high moisture content and attractants as a means of recovering fish.

2. Methods and results

2.1 *The proportion of failed cod in total mortalities of juveniles in Scotland and Norway*

The numbers of failed cod in Scotland in the first year of stocking in sea cages were obtained from stock records of the former company NoCatch Ltd, Shetland (A. Bourhill, pers. comm.). The company classified losses under six categories: Failed, deformities, predation, transport, *Vibrio anguillarum* and unclassified. The cumulative mortality of cod juveniles under each of these categories was calculated as a percentage of total mortalities and as a percentage of the total stock. Data from Norway were sourced from an unpublished study by fish veterinarians of mortalities on five cod farms in Nordland County (Hanche-Olsen and Nilsen, 2009). Weekly mortality figures in the first year of the production cycle were classified under nine headings: Failed fish, cadevorosis, ulcers, mechanical trauma, predation, egg bound females, intestinal lesions, Atypical furunculosis, unclassified mortalities (unidentified).

The cumulative mortality of cod juveniles at Vidlin Voe, Shetland assigned as “failed” fish in the first 8 weeks after stocking was 160,000 fish (Fig. 2), amounting to 70% of the total mortalities and 10% of the total stock of 1,686,417 fish. Failed cod was the main cause of losses in five cod farms in Nordland county in Norway (Fig. 3) and comprised 46% of mortalities.

2.2 *Frequency of failed cod in hatchery tanks*

An assessment was made of failed fish in hatchery tanks to determine if this mirrored the reported “failure” of cod in sea cages. The fish were stocked in 12 tanks of 1.5 diameter and 1 m depth, that held 900 l water. The flow rate was 5 l min⁻¹, temperature in the range 8 to 11°C, aeration was provided and lighting was continuous. The fish were feed twice each day to satiation with 3 mm Biomarine

Pearl diet. 100 fish of 12 g mean weight were stocked in each tank. After 12 weeks all fish were removed from each tank, anaesthetised in phenoxyethanol, and measured to fork length (mm) and weight (g). The numbers of “failed cod” were recorded and these were classified as thin fish, of low weight, and with a relatively large head compared with the body (Fig. 4). There was a differentiation in the smaller cod with some fish (see bottom two fish in Fig. 4) showing a deeper body. This would indicate that there had been some recovery in condition in these fish to some extent, and that there is a gradation in the condition. The condition factor of the fish was calculated as $K = (\text{weight (g)} * 100000 / \text{length (mm)}^3)$.

The details of the fish and environmental conditions in each of the trials in this project are shown in Table 1.

The numbers of failed cod varied from 2 to 8 in each tank per 100 fish stocked with no failed cod present in two of the tanks (0 to 8.8% in the 12 tanks) (Fig. 5). The total number of failed fish was 42 of 1058 fish stocked representing 3.9% of all cod. The failed fish were thin and in poor condition (Fig. 4). Failed cod were slightly darker in appearance and weighed a mean of 11 g compared with 48 g in “normal” cod and the length was 109 mm compared with 166 mm (Fig. 6). The weights of the failed fish were similar to the mean of 12 g at the start of the feeding trial. There was a clear differentiation between fish in good condition and weight frequency compared with failed cod (Fig. 6). The condition factor of normal growers was significantly higher (ANOVA, $P < 0.05$) at 1.06 compared with 0.70 in failed fish. There was a large variation in weight in the cod stock due to the presence of failed fish. This was reflected in the coefficient of variation (SD/mean) which was 0.35. In contrast, the CV was more restricted when calculated for the two modes in weight representing normal and failed fish, with a value of 0.2306 in normal growers compared with 0.2258 in failed cod.

2.3. Recovery of failed fish by grading

Attempts were made to “recover” failed cod in isolation from larger fish and to determine whether they would accept feed. 39 failed fish were stocked in a 1.5 m diameter tank of 1300 l volume with a flow rate of 5 l min⁻¹. The temperature was in the range 8 to 12°C, salinity varied from 32-34 ppt, and the fish were maintained in continuous light. The fish were fed 4 to 5 times each day with Biomar Pearl Marine of 2 mm pellet size. The feeding response of the fish was observed, a note made of the numbers of fish participating in feeding, and the degree of interaction and aggression between fish were assessed. The trial continued for 6 weeks and then the fish were removed, anaesthetised in phenoxyethanol, the length and weight were measured, and the numbers of failed cod were recorded. This experiment was repeated on three further occasions by removing the recovered cod from the tank and repeating the trial with the remaining failed fish, while the recovered fish were transferred to stock tanks. Occasional fish were removed for internal examination but were replaced with failed fish of similar weight.

Failed cod were graded five times at 4 to 6 weekly intervals to determine whether failed cod could be “recovered” by separation from the larger feeding fish (Fig. 7). During the first grade 42 failed fish of 11 g mean weight were obtained by grading 1058 fish distributed in 12 tanks. Three of these fish were killed and dissected to see if there were anatomical abnormalities of the mouth, the oesophagus and of the digestive tract, and none was observed. The remaining fish were measured after 6 weeks of feeding in a separate tank. 21 of 39 fish (53.9%) had recovered and were feeding well and were in good condition. Seven failed fish were autopsied and, after the failed cod were separated again, 7 of 14 fish recovered (50%) in the third grade, 7 of 13 fish in a fourth grade (61%), and 3 of 5 fish in the last grade (60%). The total recovery of failed fish in all of these grades was 37 of an initial number of 39 fish, representing a recovery rate of 95%.

2.4. Assessment of acceptance of different recovery diets by failed cod

A batch of 18 failed cod of mean weight 15 g was used to test the feeding response to a range of six potentially more acceptable diets of 2 mm pellet size produced by

Biomar Ltd. This was compared with the response to standard Biomar marine diet in the trial tanks and conditions are described in section 2.3. The feed was offered by hand 4 to 5 times each day with 20 observations made of fish feeding to assess acceptance or “spitting out” of the feed pellet. Typically in these cases fish would ingest the pellet and would spit the pellet out after 1 to 2 secs. The percentage of successful feeding attempts was calculated from 10 separate feeding assessments of each diet (10 assessments x 20 fish, typically over 2 days=200 individual observations), then another diet was tested on the same fish, until all 7 diets had been assessed. This series of trials was carried out in replicate with all trial diets. The trial diets (Table 2) tested against the standard marine diet were:

Diet A: Control Diet. A standard commercial cod recipe (Biomar).

Diet B: Standard Cod recipe with 10% addition of Krill Meal.

Diet C: Attractant diet. Liquid compound that was top-coated on to pellets, 0.5% of total recipe inclusion. A crustacean extract was used.

Diet D: a Salt Diet.

Diets E and F: High Moisture diets. The moisture content was 16% in the diets compared to the other experimental diets which had 7.5–8.0%.

Diet F also had “Mycocurb”, an organic salt to prevent mould formation.

Diet G: Smaller pellets

The average rejection rate of the 7 diets in the first trial was 15 pellets of 20 offered (n=10 feeding events x 20 fish in the case of each diet) (Fig. 8). The rejection rate of all diets was not statistically different from the rejection rate of the standard marine diet (A) (ANOVA $P>0.05$), although there was a lower level of rejection of control, standard commercial diet A. The cod had been accustomed to this diet A over the previous 12 weeks and this may explain the initial preference. The average rejection rate of the seven diets in the second trial (Fig. 9) was higher with 18 pellets rejected out of 20 offered and in this case diet A was also rejected to a high degree. There were no significant differences (ANOVA $P>0.05$) in rejection rate between any of the test diets and the control diet A.

Two further experiments were carried out with failed cod. The first examined the effect of pellet size and compared the acceptance of 2 mm with 3 mm pellets. The second trial examined the effects of first soaking pellets in seawater for 30 mins before feeding to the fish. This latter procedure was to determine whether “softening” of the diet by immersion would make the feed more acceptable to the fish.

The number of rejected diet A 3 mm pellets was 10.9 per 20 offered compared with 11.1 for the smaller 2 mm pellet size (ANOVA $P>0.05$) (Fig. 10), indicating that offering a smaller size did not improve the pellet acceptance rate.

Pellets of diet C were soaked for 60 mins and the rejection rate was lower at 14.1 per 20 offered (10 feeding events with 20 pellets) compared with 15.2 dry pellets rejected (Fig. 11), but this was not significant (ANOVA, $P>0.05$).

2.5. Stocking density as a possible facilitator of failed cod

Cod reared in commercial hatcheries are held at a relatively high density, e.g. in organic production up to 15 kg m^{-3} or up to $30\text{-}35 \text{ kg m}^{-3}$ in standard systems. Under such conditions, the opportunity for individual fish to create territories is limited as the fish swim as a shoal and there is no space available to defend a distinct area. However, when cod are transferred to sea cages of much larger volume, stocking densities can suddenly be reduced to around 2 kg m^{-3} . This may permit cod to establish territories and potentially a dominance hierarchy, where the dominant fish might prevent or inhibit the smaller fish from feeding, leading to runting or “failed cod syndrome”. The effects of different stocking densities on social hierarchy and interaction of juvenile cod were assessed as a possible initiator of the failed cod syndrome.

The effects of stocking density on failure of cod juveniles were examined in four experiments each of 6 weeks’ duration. Cod of initial weight $24.6 \text{ g} \pm 3.0 \text{ SD}$ were stocked in 6 tanks of 1.5 m diameter and 900 l volume. The tanks were covered with netting to prevent escapement. The flow rate was 5 l min^{-1} , lighting was a 24 h

photoperiod regime, salinity was in the range 32 to 34 ppt, temperature varied from 8 to 11°C and oxygen was in the range 8.1 to 9.8 mg l⁻¹. Cod were stocked at three densities in duplicate tanks:

Low 0.2 kg m⁻³ = 0.18 kg total weight, or 8 fish as a minimum; Medium 2 kg m⁻³ = 1.8 kg ; High 20 kg m⁻³ = 18 kg.

Fish were fed to satiation 3x each day with the commercial diet Biomar Pearl of 2 mm pellet size at a rate of ca. 2% body weight. The weight of feed offered to each tank of fish was recorded daily, as were water temperature and oxygen, and also mortalities. The differences in the feeding response were monitored and any interaction between the fish and exclusion of fish from feeding were recorded. The behaviour of the fish was monitored during feeding and recorded as: no interaction between cod, "Chasing" visible as one cod following quickly behind another to move it from the area with feed pellets, or "nipping" which was an apparent aggressive touching movement, the rejection of pellets, no response to pellets which was different from rejection in that there was no movement or interest in pellets, and high activity which was an increased movement generated by a feeding response. Each behaviour was quantified as a percentage of that behaviour noted in particular fish that were being monitored. The population size varied with fewer fish being available to observe at the lowest stocking density. However, the duration of the observation period remained the same for any fish at all stocking densities.

Aggression and interaction between fish was assessed on sampling when anaesthetised from a score of erosion of fins (there was no splitting) on three levels of severity and damage, all on the scale 0=none, 1=low/reddening of fin margins but no erosion, 2=moderate/open bleeding no erosion, 3= high/open wound and some erosion on distal edge of fins. The jaw and skin of the fish was also assessed on a scale; 0=no damage; 1=damaged.

Fish were anaesthetised in phenoxyethanol and individually measured to length (FL mm) and weight (nearest g) on commencement of the trial (day zero) and at the end of each experiment. All fish in the low and medium density tanks were measured and 50 fish from tanks with high density. The mean temperatures were used to calculate the thermal growth coefficient (TGC; $[(M_n^{0.33} - M_{n-1}^{0.33}) / (d \cdot \text{temp})] \cdot 1000$,

where M_n = mass at a measured point (g) and d = duration (days)) for the first and second growth periods. Food conversion ratio (FCR) was calculated for each growth period following the formula: $F/(BM_n - BM_{n-1} + BM_{lost})$, where F = food mass ingested (g) over a measured interval, BM_n = tank biomass (g) at a measured point (n) and BM_{lost} = mass of losses due to mortality (g). The period between both BM measuring points corresponds to the period over which F was fed.

The length and mean fish mass at the start of the trial were 374 mm \pm 32 SD and 24.6 g \pm 3 respectively. The weight of fish at the beginning of the trial was in a bell shape curve (Fig. 12), of restricted range, and there were no outliers (CV=0.23). Mean water temperature during the first, second, third and fourth growth periods (ca. six weeks' duration) were 11.3°C, 7.4°C, 6.7°C and 7.8°C respectively. Mean oxygen concentration was 8.6 mg l⁻¹. There was no indication of failed cod at any stocking density (Fig. 12). There were no mortalities related to stocking density with only occasional fish jumping from the tanks due to loose net screening.

The thermal growth coefficient (TGC) of fish at the 0.2 kg m⁻³ density was significantly lower in the first growth period than for the medium and high densities ($t=4.45$, $P=0.01$) (Table 3). Although the TGC was subsequently higher in the cod stocked at medium density, these differences were not significantly different from low or high densities in the second (ANOVA=0.22, $P=0.84$), third (ANOVA=0.32, $P=0.84$) or fourth growth periods (ANOVA=0.34, $P=0.90$) (Fig. 13).

There was no interaction between cod in over 80% of the feeding events (Fig. 14). Chasing and nipping events combined were significantly higher ($P<0.05$) in the tanks stocked at the medium density of 2 kg m⁻³. Cod stocked at the lowest density showed no feeding response on 5% of occasions and also showed a significantly lower level of activity compared with the highest stocked fish (20 kg m⁻³) that displayed the highest activity.

The lesions on the first dorsal fin of cod at medium and high densities were pronounced and noticeable from the surface of the tanks and were present in up to 19% of fish. In many other fish the first dorsal fin showed erosion although this had healed and damage was not current. No fin damage was present in cod stocked at the lowest density of 0.2 kg m⁻³ (Fig. 15). Fin damage was most frequent on the first dorsal fin of the cod at the medium stocking density while it was higher in the

highest stocked cod on the second and third dorsal fins and also the pectoral, pelvic and tail fins. However, there were no significant differences ($P>0.05$) in fin damage between cod at the 2 kg and 20 kg m⁻³ densities. Attacks appeared to be the likely cause of the damage and this was considered after seeing the fish interacting and showing some apparent “nipping” behaviour. There may also have been some bacterial infection but that would have been a response to the initial injury.

2.6. Ascertaining feed preferences of juvenile cod utilizing a demand feeding system

Two demand feeding units with triggers were fitted to each of three 1300 l capacity tanks (Fig. 16) which were stocked with 75 “normal” cod and weights and lengths were recorded. These same fish were used for assessing the 7 trial diets in sequence against the control diet. The feeder-trigger units were placed in identical positions relative to the water inflow and each other on each of the three tanks to minimise the risk of feeder selection being based on position rather than the feed it contained. Each trigger was connected via the electronic control system of the feeder to an analogue-digital converter fitted to a remote PC. Software for recording the time of activation of each of the six feeders was written using Visual Basic TM. This software automatically excluded the recording of any trigger actuations which took place whilst the feeder was delivering feed from a previous activation, and so each recorded trigger activation represented one delivery of feed. The amount of feed delivered during each trigger activation could be set by varying the duration of feeder operation after activation, so that the feed reward per trigger pull could be equalised for each feeder, and so minimised any feeder preference being due to a factor other than the feed.

Seawater of ambient temperature (trial average temperature 7.8°C, range 6.5-8.5°C) was supplied tangentially to each tank at a rate of 15 l min⁻¹. The outlet consisted of a sleeved standpipe with a 1.5 mm mesh screen to prevent uneaten food from being flushed to waste. Each tank was illuminated by a 38W Par floodlight situated 1.2 m above the water surface, with a dimmer controlling the light intensity to 150 lux at the water surface. The three tanks, being situated on their own in a

separate room, were effectively shielded from any outside disturbance other than through the 4x daily routine of checking the system and refilling the feeders. The trial commenced after an initial two week period of training in the use of the triggers. The two feeders on each tank were filled with each of the two test diets which were kept in previously weighed stock feed tubs for each feeder. The feeders were refilled from this stock tub as required during each two week trial. The amount of feed remaining in the feeder was returned to the stock tub at the end of each run and the tub re-weighed to give the amount of feed consumed in that period. At the end of the first two weeks, the two different feeds were exchanged in the feeders and the trial as resumed for a further two weeks. In this way any impact on the pattern of feed consumption that may have been due to effects such as the trigger or feeder position, rather than the feed itself, could be negated. The feeds listed in Table 2 were tested in alphabetical order.

Test diets were compared individually with the commercial control diet A in the demand feeding trials. The data are presented as the percentage of total food consumption in each diet in a tank combined for the two feeders and shown separately for the two trial periods, that is weeks 1 and 2 were compared with weeks 3 and 4 when the diets were changed between feeders. Note that statistical analyses, see below, were based on the combined data for the two periods.

There was no significant difference in the weight of food released between the two feeders ($P > 0.05$) indicating that there was no effect of feeder on the feeding response ($t = 0.0426$, $df = 35$, $P = 0.9663$). There was a high degree of variability in the proportion of each diet taken (Fig. 17) reflecting the wide variation between the three replicate tanks. There was no distinct preference for a given trial diet. No significant differences were found in the proportion of food fed per tank between any of the six test diets and the control diet. The only diet for which there was almost a significant effect was diet E compared with the control diet (ANOVA, $F = 4.5239$, $P = 0.0593$).

2.7. Statistical analyses

The lengths and weights of fish in different tanks were tested for homogeneity of variances by Bartlett's test and then by nested analysis of variance (ANOVA) using the statistical program Minitab 15. This was used to test different treatments and a P-value <0.05 was accepted as significant. The data from the demand feeding system were first arc sine transformed and a paired "t" test applied to compare the feeding response between the first and second feeders in weeks 1 and 2 with the feeders utilised in weeks 3 and 4 when the feed type was switched between feeders. After transformation of the data to normalise variances the acceptance of each pair of diets was compared by ANOVA in each of the three replicates. The damage scores at each stocking density for each of the fin areas were compared by ANOVA after testing for homogeneity of the data. To gain sufficient statistical power the data in the demand feeding trial were analysed together for the two periods: ANOVA was therefore applied to 2 periods x 3 replicates= 6 data points for each feed.

3. Discussion

3.1. Failed fish syndrome

The identification of mortalities of juvenile cod in Shetland and Norway indicates that failed cod is a recognised loss category that has been identified by veterinarians from symptoms such as anorexia and wasting and such losses comprise the foremost cause of mortality of cod in the first year in sea cages. These failed cod were also evident in a limited way at an early stage of development in cod held in tanks. Other species of fish also show failure of a proportion of the stock. The failure of salmon *Salmo salar* smolts on transfer to sea cages is common (Stradmeyer, 1994). This was characterised by an increase in mortalities and was attributed to the failure of some smolts to feed after transfer. Ostensibly this appears to be a similar phenomenon to the failed cod syndrome but, for salmonids, there is the complicating factor of smoltification. The failure of salmon "smolts" on transfer may be due to incomplete smoltification in some fish, possibly genetically related, and not to any other factor. Rather than being similar to "failed salmon smolt" which is due to inability to

osmoregulate the failed cod condition appears to be a different phenomenon. Feeding hierarchies in salmonids are well established to reduce feeding activity in subordinates. In cod there continues to be feeding activity but then subsequent rejection of food.

Runt white sturgeon *Acipenser transmontanus* have been reported (Georgiadis et al., 2000a) as poor growers, having underdeveloped muscle mass, swimming slowly and were found to be frequently in the upper water column. The runts were separated from the dominant fish and the percentage of runts recovered over a period of 46 to 89 days was high, ranging from 16 to 58%. The growth of white sturgeon was also higher in runts when they were separated from the dominant fish (Georgiadis et al., 2000b) and the growth rate of recovered fish was the same as in the rest of the population. It is concluded that social hierarchies play a key role in “runting” compared to some (unidentified) illness or ontogenetic abnormality. In laboratory trials here behavioural and social factors were studied. Small numbers of failed juvenile cod were repeatedly graded from the larger cod. However, it is less clear whether this repeat-grading approach can be implemented cost effectively on a commercial basis. In the former arrangement in Shetland, cod were first stocked in Nursery pens near the main farm. In that scenario it would be possible to grade the failed fish at the time when fish were vaccinated intra-peritoneally and before the fish are transferred to large on-growing pens.

Cod have been reported to have quality problems with swim bladder development (Kellet et al., 2005), inflation and in some cases overinflation. This is a potential cause of poor-doers when moving between systems, and especially of different depths and pressures. Latterly it has been suggested that swimbladder over-inflation is a stress response, particularly at the end of the larval rearing period. Failed cod were dissected and the swimbladder was present and appeared to have a normal appearance.

3.2. Social and aggression effects on feeding in cod

When failed cod in the hatchery were graded repeatedly and separated from normal juveniles a proportion of fish accepted dry feed and “recovered”. Some of the graded fish ingested and then rejected pellets in the presence of other fish that did ingest feed. There was, however, no evidence for feeding hierarchies in the graded failed cod, nor aggression affecting food retention. It therefore appears that food rejection due to dominance hierarchies has not been proven. Indeed, there was no indication of physical aggression by these dominant fish. Over 95% of initially “failed” cod were recovered following repeated grading. No physical reason for failure to swallow feed pellets was identified in the mouth, oesophagus or intestinal tract. As feeding hierarchies were set up in these graded and separated failed cod this would suggest that there are social and aggression influences on the failure of cod.

A large size variation within populations of farmed fish is common and, because of the risk of cannibalism, juvenile cod are graded robustly from weaning to around 5 g. However, there are still size discrepancies that are accentuated with time. Larger cod have been shown to have dominance hierarchies (Brawn, 1961) and display aggressive behaviour. Aggression in fish is frequently shown as fin damage (Turnbull et al., 1998) and this parameter has thus been used as a direct measure of aggression in salmonids (MacLean et al., 2000). There was a correlation between growth rank and fin damage in cod (Hatlen et al., 2006) as in rainbow trout *Oncorhynchus mykiss* (Moutou et al., 1998). However, in salmonids, faster growing fish have been reported to have more bite marks and this could be explained by subordinate fish withdrawing from confrontations for feed (Kadri et al., 1996).

Dominance and fin nipping have been reported as typical of juvenile cod (Hatlen et al., 2006). That study suggested that excess feeding may be important in reducing aggression, and the growth of the smallest fish was not affected by the largest fish in the trial. A restriction, for whatever reason, in feed availability may lead to increased aggression (Moutou et al. 1998; Gregory and Wood, 1999) and also increased variation in feed intake and growth (McCarthy et al. 1992; Jobling and Baardvik, 1994). Cod are cannibalistic and this behaviour is exacerbated when food is limited (Folkvord, 1991). Cannibalism is most pronounced in cod from weaning up to 1 g weight (Ottera and Folkvord, 1993) and can be an issue when there is a large range in size within the population (Blom and Folkvord, 1997). In contrast, there have been

several other reports of feed deprivation and restricted feeding in juvenile cod without evidence of typical aggression related fin damage (Hawkins et al., 1985; Foster et al., 1993). In some studies juvenile cod competed for food without physical aggression (Hart and Salvanes, 2000; Salvanes and Hart, 2000).

It should be noted that hierarchies in dominance (aggression) and feeding are not interchangeable. The term scramble competition implies a mass scramble for food in the absence of stylised aggression and in the latter food intake is determined by competitive ability (usually linked to body size). Contest competition implies a strong dominance (aggression) hierarchy that then results in an orderly feeding hierarchy. Size variation may therefore be a cause or effect of a feeding hierarchy.

Fin damage was apparent in cod fed at graded levels of food restriction but it varied between size groups (Hatlen et al., 2006). Smaller cod of 55 g mean weight displayed most damage on the dorsal fin, whereas pectoral fins were most damaged in fish of 250 g. Damage was high in these fish and it increased when feed was limited. In the largest fish, of 450 g, there was little fin damage and incidence did not depend on the amount of feed provided. As the fast growing fish displayed less incidence of fin nipping than the small fish it was concluded that the fish that received the most aggression were prevented from feeding. The variation in individual growth rates increased when feeding was restricted and the weight distribution was skewed according to feeding level. Hatlen et al. (2006) concluded that the growth of individual cod held in groups in culture can be limited by competition. However, in contrast, other studies on juvenile cod held in groups and deprived of feed (Foster et al., 1993; Peck et al., 2003) or fed a restricted ration (Hawkins et al, 1985; Peck et al., 2003) have not reported fin damage. In the study by Hatlen et al. (2006) skewness in weights was also high when feed was in excess suggesting that a small proportion of fish ate little or nothing. It was suggested that this could be due to lack of feed motivation or social exclusion, and the high incidence of fin damage in full-fed groups suggested that there might be social reasons.

3.3. Stocking density as a possible influence on failed cod

When cod were stocked at densities ranging from 0.2 to 20 kg m⁻³ the growth was consistently highest at the medium density of 2 kg m⁻³, although not significantly different from the other densities. However, aggression measured as fin nipping and damage was highest and affected a range of fins at the highest stocking density. High stocking densities in fish have been considered as a stressor (Wedemeyer, 1997), but may give improved survival, better growth and less size variation in some species such as Arctic charr *Salvelinus alpinus*, (Jorgensen et al, 1993) and catfish *Heterobranchus longifilis* (Baras et al., 1998). Increasing the stocking density beyond a threshold may also reduce agonistic interactions (Kaiser et al., 1995) and there may be reduced aggression where the fish are cultured in reduced light (Jorgensen and Jobling, 1993) and where there are moderate water currents (Jobling et al., 1993).

Social structure may be influenced not only by stocking density but by size heterogeneity and sex structure (Alanara and Brannas, 1996). In some cases aggression is not aimed at all fish but just those that are slightly smaller (Baras et al., 2000). In the context of cod culture juveniles are typically held at high density in hatchery tanks prior to transfer to sea cages but densities are reduced dramatically when fish are transported to sea cages, from ca. 30 to 1 kg m⁻³ and, where there is a larger available living area, this may permit cod to develop territories.

4. Conclusions

In practical terms, this final report does not relate directly back to all of the original six objectives. These were:

1. To assess in tanks the feed preference of juvenile cod in terms of moisture content of the diet, pellet size and colour and shape, and palatability. From this recommend best practice.
2. To field test the optimum diet, first in 5 metre cages at commercial stocking densities, and to compare this with current feeds for juvenile cod. Fish would be transferred at 10 grams weight.
3. To assess the proportion of fish that become poor doers.
4. To examine size distribution and social hierarchy on the effect of successful feed adaptation in sea pens.

5. To utilise demand feeding systems in sea pens to ensure the even distribution of feed and to determine optimum feeding practices. To modify the settings of this equipment to meet the needs of cod culture.
6. To assess the degree of efficiency of feed use and the likely environmental impact of each feeding regime.

It was reported early in the project that failed cod would continue to reject pellets based on alternative raw materials. Pellets of smaller sizes and moist pellets were also rejected, so the initial hypothesis that there was a dietary problem was invalid. Therefore it was agreed that the project would focus on laboratory work on the effects of grading, density effects and behaviour. These subjects could not be examined closely in sea cages so objectives 2, 4, 5 and 6 were curtailed. In the event, the cod farms in Shetland closed and further work in Scotland was not possible. However, recommendations were passed to cod farms in Norway through Biomar Ltd to enable cod farmers to take further.

The acceptance rate of a range of six alternative diets offered to failed cod by hand was not significantly different from the commercial marine diet. Other strategies of offering smaller pellets or soaking pellets for 60 minutes before presenting to fish did not improve acceptance rate. This study has examined the preference of fish for different diets utilizing a demand feeding system permitting the fish to activate feed triggers. This allowed comparison of uptake of a commercial diet with feeds incorporating high attractant or high moisture content in replicated tanks. No preference was shown for any of these alternative diets over the commercial feed. However, cod are normally insatiable feeders and they may be less inclined to discriminate between different flavours and might therefore take any feed that is presented regardless of composition.

The recovery of failed cod following grading from feeding fish suggests a social influence on the feeding success of juvenile cod and on the initiation of the failed cod condition. This aggressive interaction appears to be initiated on transfer of cod from high to low densities in sea cages. Recommendations for managing the problem include the recovery of failed cod by grading from the main population and rearing these fish separately.

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Tables

Table 1. Summary of environmental and biological parameters in the various trials. The reference numbers refer to the paragraph headings of the Methods section in the report.

Trial no.	Trial	Initial WT (g) (range)	Stocking density kg m ⁻³	Temp °C	Tank volume	Flow rate L min ⁻¹	Lighting	Photopd hrs light	Diet	Ration	Feeding regime x day ⁻¹
2.2.	Failed Frequency in hatchery Tanks	45 (9-70)	1.1	8-11	900	5	Strip light	24 h	Biomarine Pearl 3 mm	to satiation	twice
2.3.	Grading Trials (9-14)	11	0.22	8-12	1300	5	Strip light	24 h	Biomar Pearl 2mm	satiation	five
2.4.	Recovery Diets	15	0.14	10-12	1300	5	Strip light	24 h	Biomarine test diets	satiation	five
2.4.	Pellet size	15	0.14	10-12	1300	5	Strip light	24 h	2 vs 3 mm	satiation	five
2.4.	Soak pellet	15	0.14	10-12	1300	5	Strip light	24 h	Biomarine	satiation	five
2.5.	Stocking Density (18-30)	24	0.2, 2 and 20	8-11	900	5	Strip light	24 h	Biomarine Pearl 2mm	satiation	three
2.6.	demand Feeding (21-69)	48	2.5	8-11	1300	15	Par floodlight 150 Lux	24 h	Biomarine 8 trial diets	demand fed	continuous

Table 2. Composition of a standard marine diet (control) and recipes for trial diets manufactured by Biomar Ltd.

HM=high moisture; small=small pellet size, 1 mm.

LT FM=fish meal. Further details of diets in text.

Diet:	Control Diet	Standard plus krill	Crustacean attractant	2% NaCl	HM	HM + acid	Small pellet
LT FM	39.59	35.34	39.21	38.90	35.40	35.40	39.59
SA SUPER prime FM	38.67	34.00	39.65	38.00	35.40	35.40	38.67
Wheat	13.5	13.5	13.5	13	13	13	13.5
Lecithin	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Fish oil	7.04	5.99	6.99	7.18	6.37	6.37	7.04
Premix A	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Premix B	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix C	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Krill meal		10					
Attractant			0.5				
Mould stabiliser						0.3	
NaCl				2			
Moisture change	0.16	0.13	-0.38	-0.12	8.80	8.50	0.16
volume (%)	100	100	100	100	100	100	100
WATER	8.0	8.0	7.5	7.6	16.0	16.0	8.0
OIL %	15.0	14.8	15.0	15.0	13.6	13.6	15.0
PROTEIN %	56.0	56.0	56.4	55.0	50.7	50.7	56.0
ASH %	11.3	11.1	11.4	11.1	10.2	10.2	11.3
Pellet size (mm)	2	2	2	2	2	2	1

Table 3. Mean thermal growth coefficients (TGC) per tank and stocking density for the first (e.g. TGC 1), second, third and fourth sampling periods. Error bars denote standard deviation.

Density	TGC 1	Mean TGC 1	TGC 2	Mean TGC 2	TGC 3	Mean TGC 3
Low 0.2 kg m ⁻³	0.68	0.59±0.0	1.44	1.56±0.1	1.76	1.48±0.00
	0.5		1.68		1.20	
Medium 2.0 kg m ⁻³	1.76	1.84±0.11	1.88	1.83±0.1	1.76	1.61±0.04
	1.92		1.77		1.46	
High 20 kg m ⁻³	1.89	1.64±0.11	1.43	1.40±0.2	1.17	1.42±0.11
	1.38		1.36		1.66	

TGC 4	Mean TGC 4
1.59	1.44±0.90
1.30	
2.1	1.87±0.32
1.64	
1.55	

1.07	1.31±0.34
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Figures



Fig. 1. Failed cod from a veterinary survey in Nordland County, Norway. (Hanche-Olsen and Nilsen, 2009).

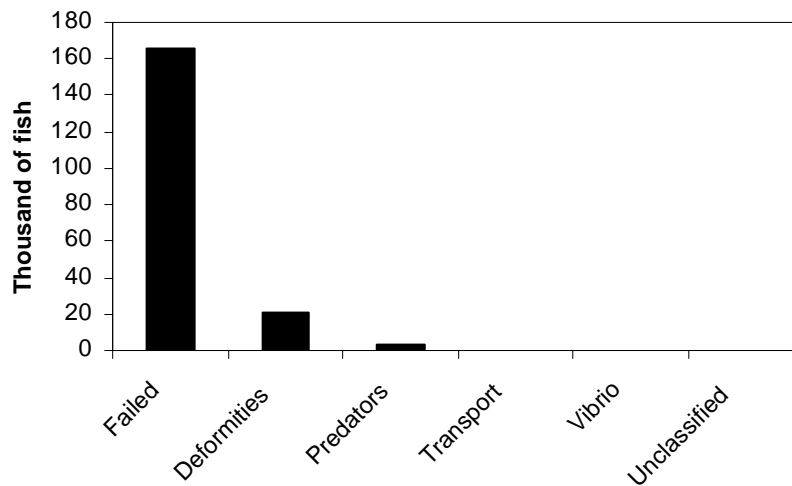


Fig. 2. Cumulative mortality of cod juveniles in the first 8 months in sea cages in Scotland (n= 1,686,417).

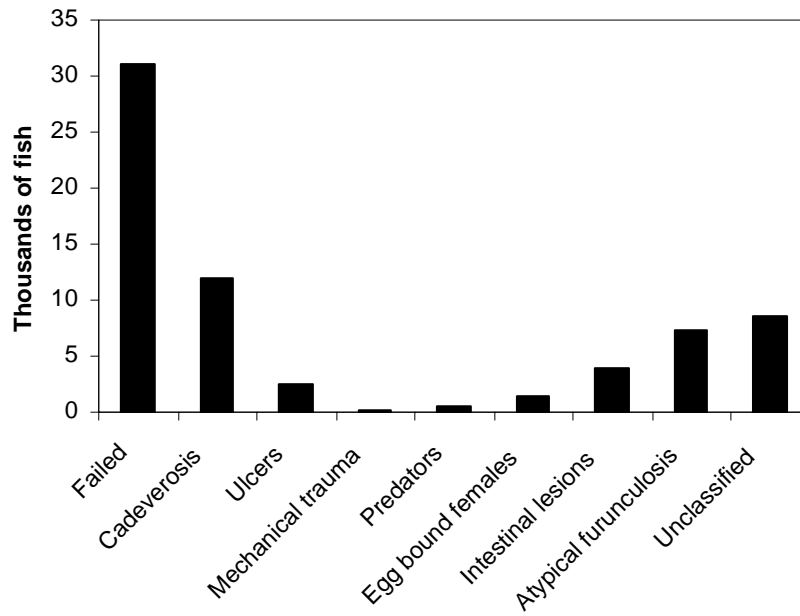


Fig. 3. Cumulative mortality of cod in four cage farms in Norway through the first year of the production cycle (data from Hanche-Olsen and Nilsen, 2009).



Fig. 4. Failed (mean weight 11 g) and normal cod (48 g) of the same age (250 days post hatch) from tank rearing. Note that two of the small cod (bottom) have a deeper body and show some signs of “recovery” in condition.

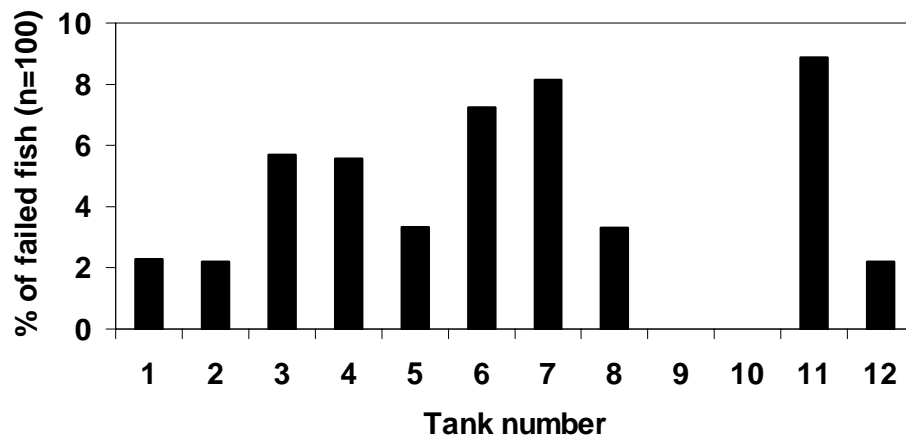


Fig. 5. The percentage of failed cod in 12 nursery tanks of 900 l volume after a 12 week study period. N= 100 fish stocked in each tank.

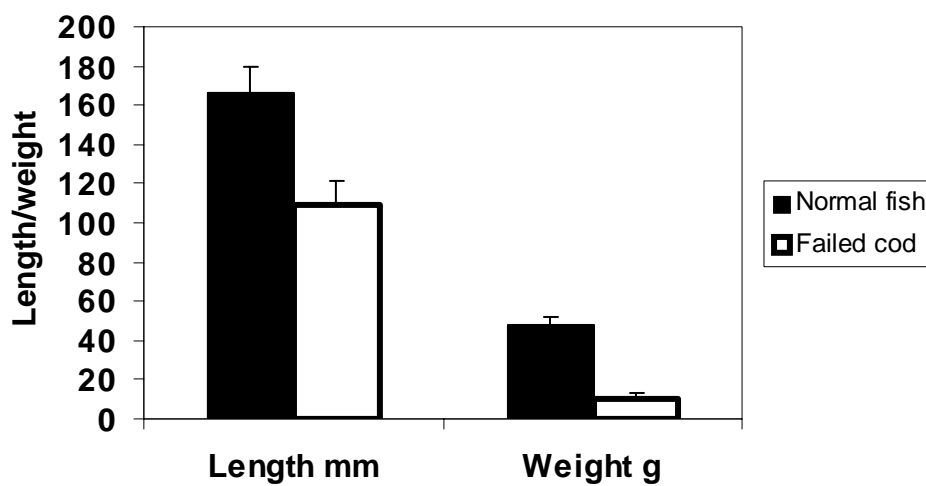


Fig. 6. Mean length mm and weight g of normal (=feeding) (166 mm, 48 g) and failed (109 mm, 11 g) cod, with SD as error bars.

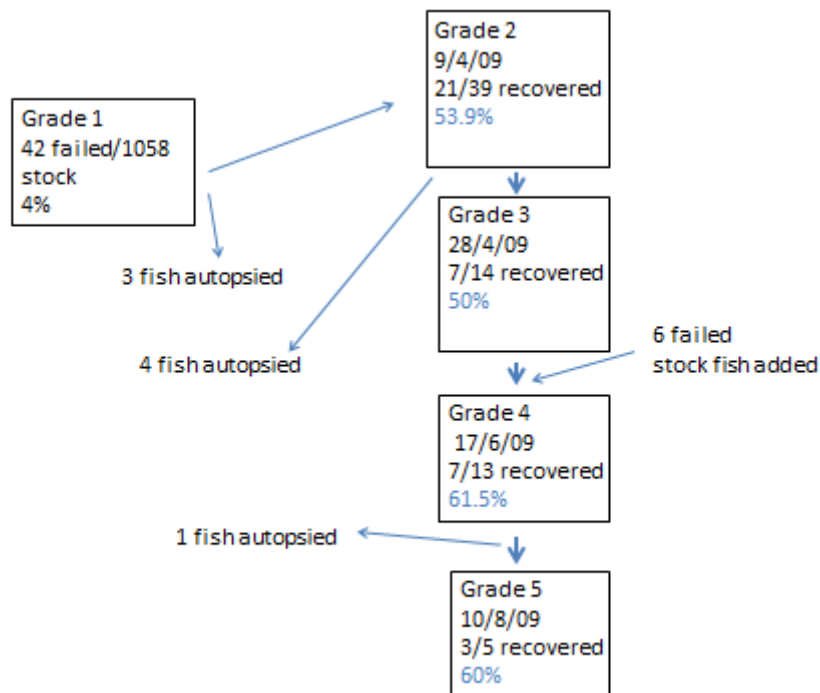


Fig. 7. Recovery rate of failed cod graded every 4 to 6 weeks. Recovered fish were removed at the end of each trial and transferred to a stock tank of “normal” fish. The second trial started with 39 fish as 3 fish were sampled for histology and further fish were also sampled for histological examination of the jaw, mouth, and intestine to search for obstructive abnormalities. Four further failed fish were autopsied prior to the grade giving 14 trial fish, and 6 failed cod were added to the 7 failed cod from grade 3 for the 4th trial. Five failed fish were stocked in the 5th trial after one fish was culled for autopsy.

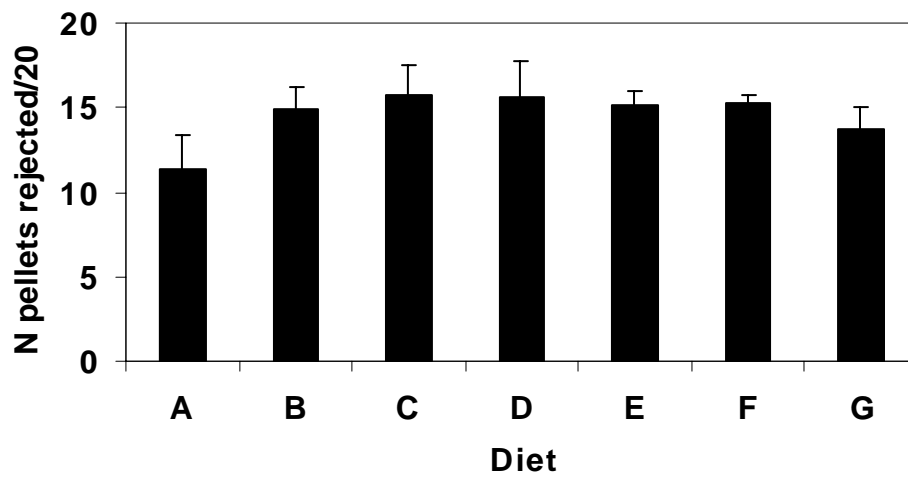


Fig. 8. Rejection rate of pellets (N rejected/20 offered per event) of trial diets (B-G) compared with the control diet A in trial 1. Mean rejection rate shown of 10 feeding events with SD as bar.

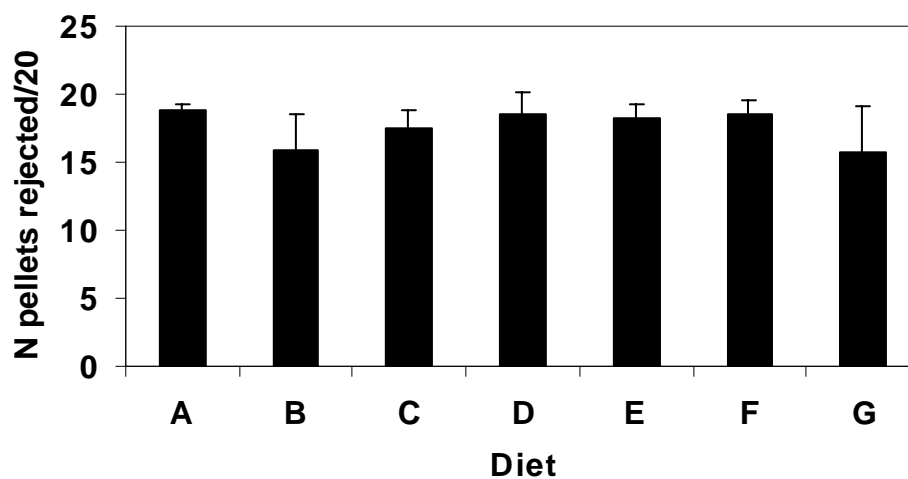


Fig. 9. Rejection rate of pellets (N/20) of trial diets (B-G) compared with the control diet A in trial 2. Mean rejection rate shown of 10 feeding events with SD as bar.



Fig. 10. Rejection rate of pellets (N/20) of control diet A of 2 mm compared with 3 mm size. Mean rejection rate shown of 10 feeding events with SD as bar.

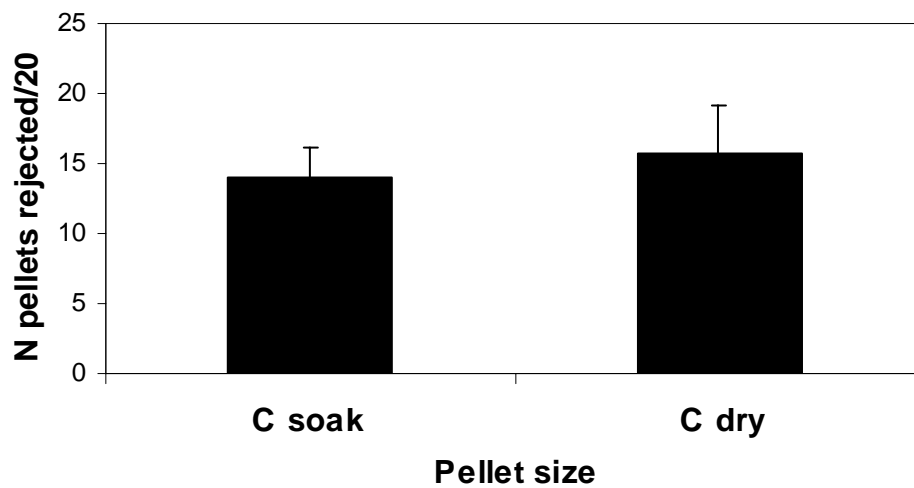


Fig. 11. Rejection rate of pellets (N/20) of dry or soaked for 60 mins prior to feeding of control diet A. Mean rejection rate is shown of 10 feeding events with SD as bar.

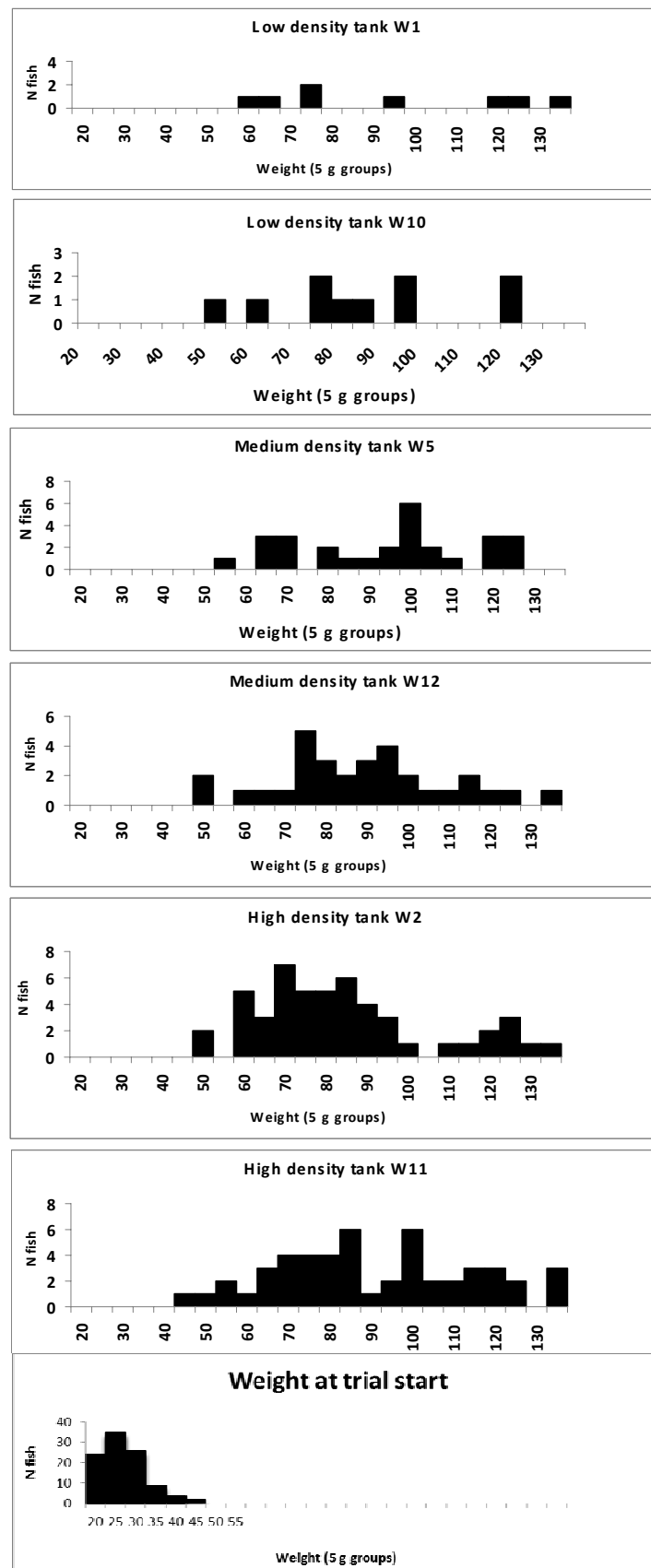


Fig. 12. Weight frequency distribution (in 5 g groups) of cod held at three stocking densities (0.2 , 2 and 20 kg m^{-3}) in duplicate tanks after 24 weeks of the trial. The weight on stocking was $24.6 \pm 3 \text{ g}$ ($\text{CV} = 0.23$). Sample size $n = 50$ fish at the highest stocking density and all fish were measured (ca. 30 and 8 respectively) at medium and low densities.

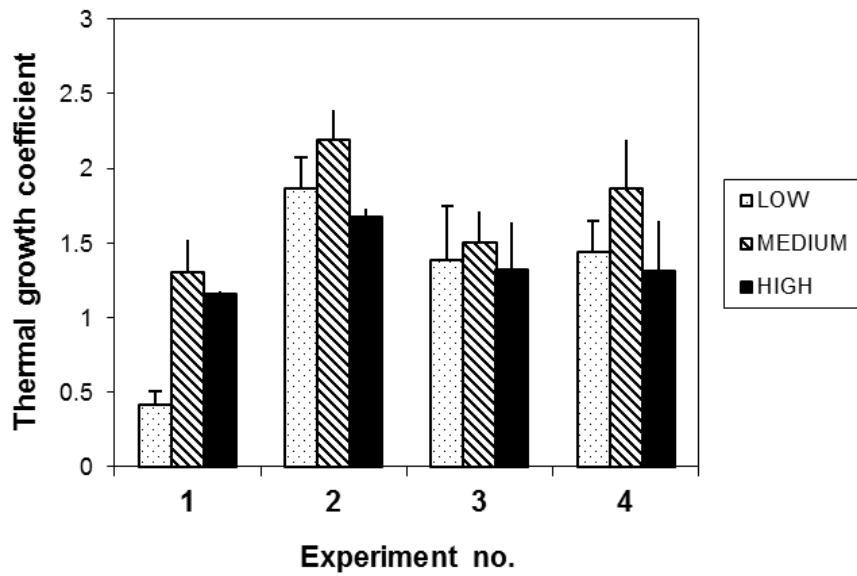


Fig. 13. Growth of cod as TGC at three stocking densities 0.2, 2 and 20 kg m⁻³, in four consecutive 6 week trials. Means of TGC and SD shown for duplicate tanks at each stocking density.

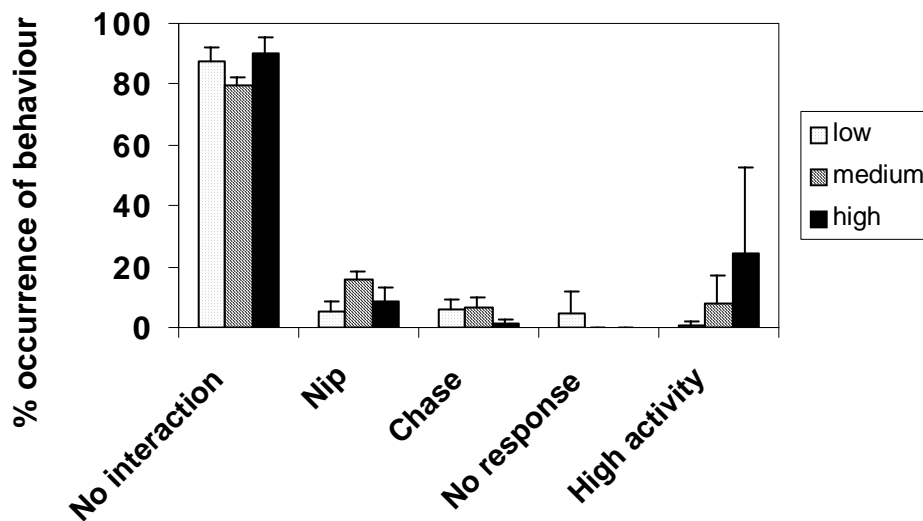


Fig. 14. The frequency of different feeding behaviours in three densities of cod recorded during feeding. The degree of response to feed being offered and the extent of interaction between fish is shown.

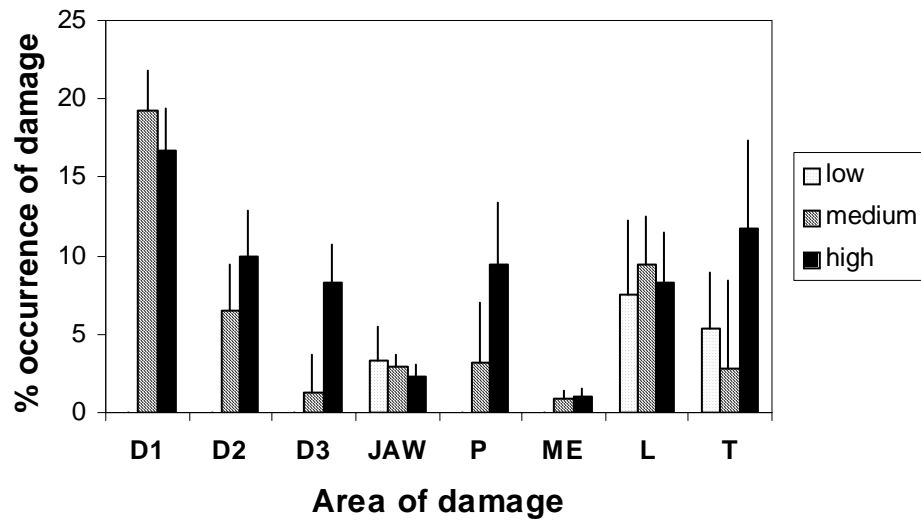


Fig. 15. The frequency of occurrence of damage in cod at low, medium and high stocking densities as: D=erosion to the dorsal fin at 1=low, 2=medium and 3=high levels of damage, P=the pectoral and pelvic fins, ME=missing eye, T=tail and L=lesions. Mean data are shown for 4 trials of 6 weeks' duration with SD indicated as error bars.

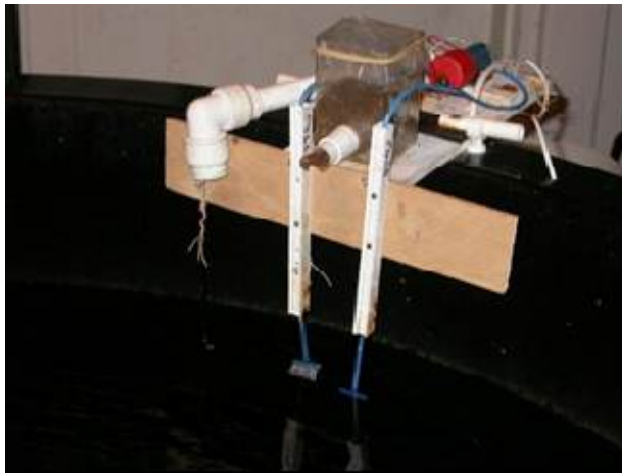


Fig. 16. Experimental demand feeding apparatus permitting cod to select one of two diets with one diet being the standard control marine diet. The cod pulled a ball trigger to activate the feeding mechanism and each calibrated feeding event was recorded on a computer. The experiment was conducted in triplicate tanks. Below, the cod demand feeding system modified to determine the best trigger mechanism from three options for later work on ballan wrasse.

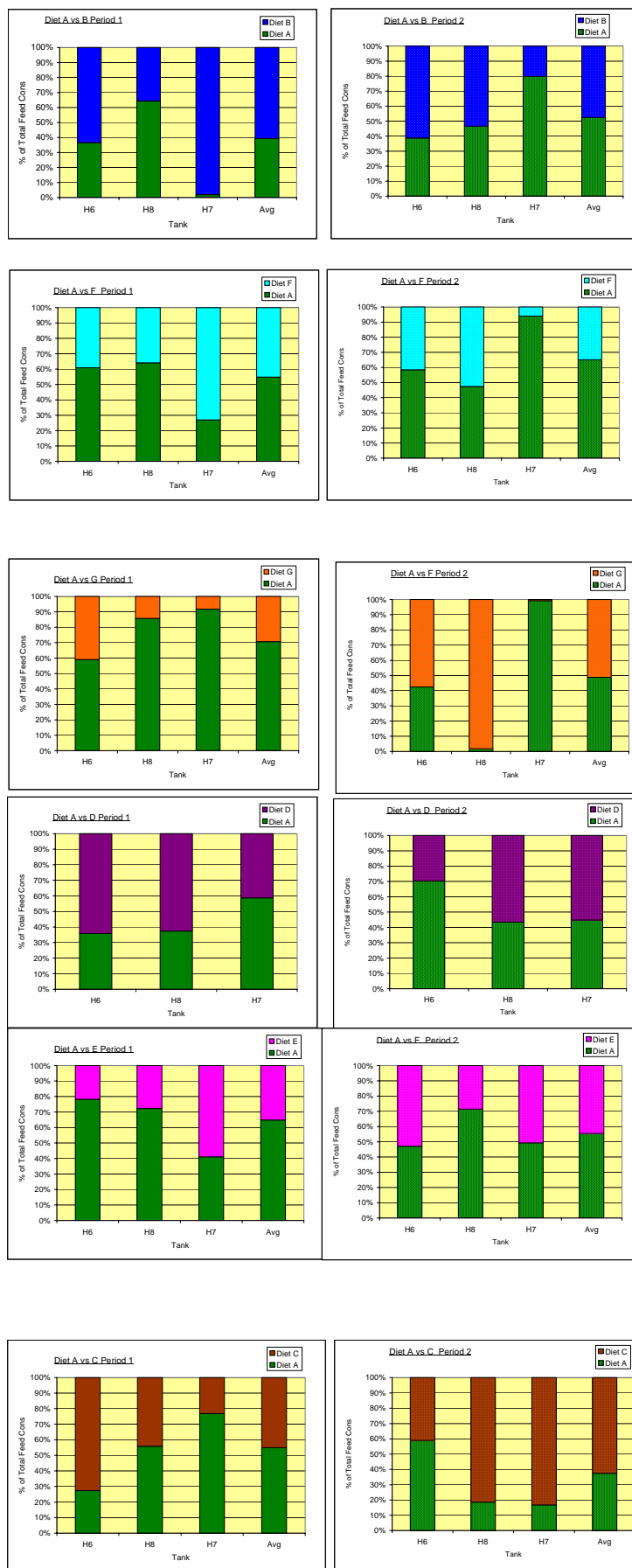


Fig. 17. The percentage of each diet taken in the feed of cod from two demand feeders in each of triplicate tanks, with average values. The results of each duplicate 3 week trial is shown on the right of each initial trial. Results of statistical tests are given in the text. The respective diets are described in section 2.4, viz. Diet A: Control Diet, Diet B: Standard Cod recipe with 10% addition of Krill Meal, Diet C: Attractant diet with crustacean extract, Diet D: Salt Diet, Diets E and F: High Moisture diets, Diet G: Smaller pellets.

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