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PhD studentship
The Development of Larval Cod Head Morphology and Aggression and Boldness in Cod

Year 3 Annual Report

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Contents

1. Introduction

This report details experiments carried out in 2004 and 2005 and summarises some of the key results from these experiments. In most studies, data analysis is still underway. Each section of this report will form a chapter of the final thesis, together with a general introduction and general discussion. A summary of the objectives for future experiments is detailed at the end of the report.

2. The Development of Larval Cod Head Morphology

2.1 Material and Methods

The study was carried out at SAMS Ardtoe marine laboratory, Argyll, Scotland. In May 2004, 65,000 cod eggs obtained from broodstock at this facility were stocked in a 35-litre production tank. The resulting larvae were then reared following standard protocol and fed Algamac or DHA Protein Selco (DHAPS) enriched rotifers (*Brachionus plicatilis*) from 1 day post-hatch (dph) to 32 dph, Algamac or DC Selco enriched brine shrimp (*Artemia)* from 27 to 60 dph and gradually increasing sizes of the inert feed, Aglonorse, from 51 to 78 dph.

Each week, 20 larvae were removed from the tank for morphometric analysis and killed using an overdose of anaesthetic (MS222). They were then photographed using a digital camera fitted to a dissecting microscope. A number of variables were then measured using the image analysis programme, Image Pro Express. These measurements are detailed in Appendix 1. In order to correct head morphometric data for an effect of size, residual scores were obtained from regression analysis of each variable against standard length. Principal Components Analysis (PCA) was then carried out on each set of residuals.

2.2 Results

Analysis of residuals obtained from regression against standard length identified a pattern of growth that was consistent between most of the head variables measured. With the exception of jaw width and medulla width, there was negative allometric growth of head dimensions in the first 4 to 5 weeks, which gradually switched to positive allometric growth in the following 4 to 5 weeks, before gradually reverting back to negative allometric growth in the final two weeks (Fig. 1). Conversely, there was positive growth of the post cranium in the first 4 weeks, which gradually switched to negative allometric growth in the following 6 weeks, before reverting back to negative allometric growth in the final 2 weeks (Fig. 1). Thus, during the first few weeks of development, the greatest amount of growth took place in the tail (hatching to approx. 34 dph), before gradually switching to the head (approx. 35 to 69 dph) and then gradually switching back to the tail (approx. 70 dph onwards). Periods of increase in the growth of the head corresponded with periods when the snout was more pointed (acutorostral), while periods of growth of the post cranium corresponded with periods when the snout was more blunt (obtusorostral) (Fig. 2).

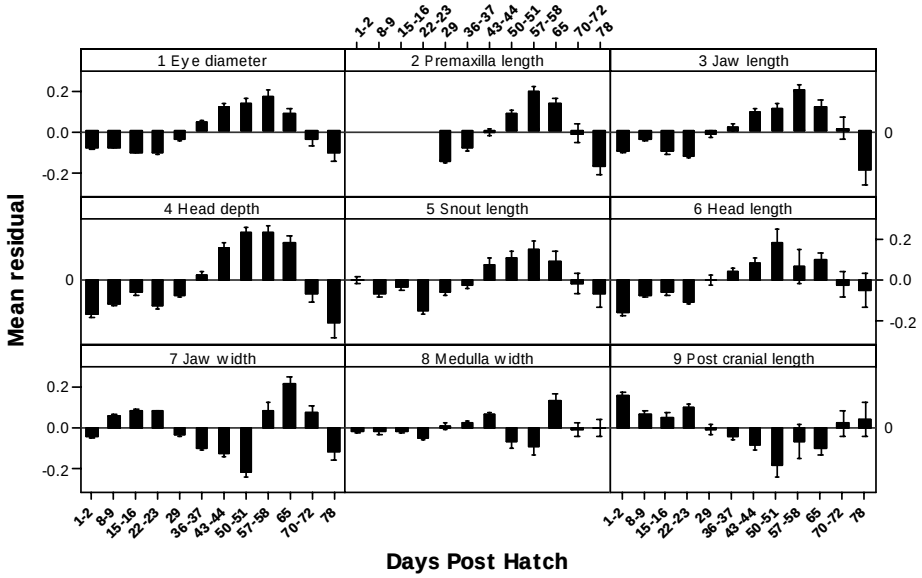

Fig 1: Mean residuals from regression of nine cod measurements against standard length by days post-hatch

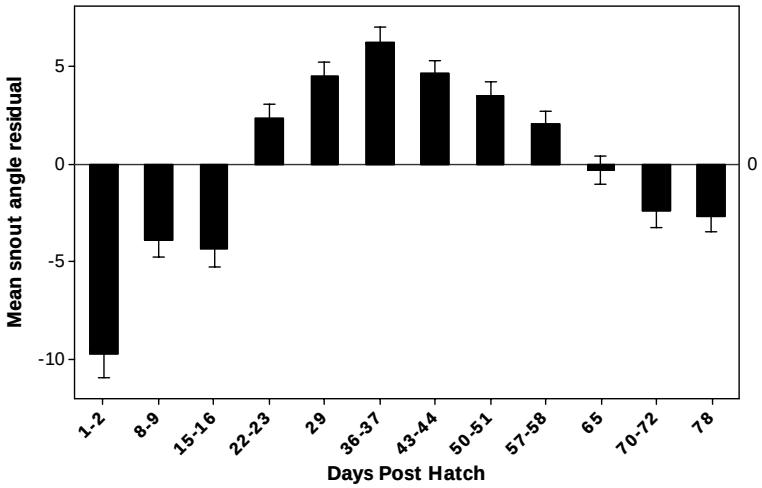

Fig 2: Mean snout angle residuals by days post-hatch

3. The Effect of Prey Size on the Development of Larval Cod Head Morphology

3.1 Materials and Methods

The study was carried out at SAMS Ardtoe marine laboratory, Argyll, Scotland. In June 2004, cod eggs obtained from broodstock at this facility were stocked in twenty black 100-litre tanks at a density of 3000 eggs per tank. The resulting larvae were then reared following standard protocol and fed Algamac or DHAPS enriched rotifers from 1 to 31 dph. From 26 dph to the end of the experiment (52 dph), larvae were fed on one of five different diets, with four replicates per diet. These diets consisted of Algamac enriched rotifers, fresh-hatch *Artemia*, Algamac enriched *Artemia* (control), Prolon enriched *Artemia* or both rotifers and Algamac enriched *Artemia* (extended cofeeding). Each tank was offered prey at densities that ensured that prey biomass was consistent between treatments. Under normal conditions, a new prey species is cofed with rotifers for a period of one week. Consequently, those larvae

fed fresh-hatch *Artemia*, Algamac enriched *Artemia* or Prolon enriched *Artemia* were also cofed 300,000 rotifers per tank from 26 to 31 dph.

Each week, following the commencement of individual feeding regimes, two larvae from each tank were photographed for morphometric analysis. At the end of the experiment, over a two-day period (51 to 52 dph), ten larvae from each tank were photographed for analysis. The morphological measurements taken are detailed in Appendix 1. All remaining larvae were removed from the tanks and sampled in order to obtain an estimate of survival. In order to correct head morphometric data for an effect of size, residual scores were obtained from regression analysis of each variable against head length. Principal Components Analysis was then carried out on each set of residuals. Data was analysed as four sets of one-week data.

3.2 Results

Principal components analysis identified three components that accounted for between 63.2 to 73.8% of the total variance of the characters measured (Table 1). PC1 in all weeks reflected the size of the larval head, specifically smallness. All characters loaded negatively, with the exception of the dimensional character, snout angle in weeks 5, 6 and 7, and upper jaw width in weeks 5 and 7. Principal components 2 and 3 were not consistent in character loadings between weeks. These components are discussed further below.

Table 1. PC1, 2 and 3 loadings for PCAs of 9 head measurement residuals

	Week 5 (29-30 dph)			Week 6 (36 dph)			Week 7 (43-44 dph)			Week 8 (51-52 dph)		
Character	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Eye diameter	-0.402	0.115	-0.287	-0.435	0.177	-0.132	-0.396	-0.268	-0.333	-0.246	-0.578	0.270
Premaxilla length	-0.349	-0.107	-0.064	-0.341	-0.206	-0.459	-0.408	0.005	-0.303	-0.336	-0.082	0.218
Lower jaw length	-0.342	-0.350	-0.186	-0.397	-0.363	-0.007	-0.409	0.178	0.173	-0.302	0.235	0.336
Head depth	-0.374	-0.212	-0.228	-0.462	-0.121	0.049	-0.370	0.379	-0.044	-0.481	0.110	-0.119
Snout length	-0.352	0.339	-0.367	-0.405	0.255	-0.196	-0.421	-0.304	-0.136	-0.323	-0.482	-0.256
Snout angle	0.167	-0.740	-0.042	0.173	-0.645	-0.123	0.228	0.420	-0.410	-0.091	0.290	0.707
Jaw width	0.291	0.061	-0.659	-0.094	-0.518	0.144	0.036	0.594	-0.244	-0.204	0.481	-0.407
Medulla width	-0.354	-0.300	0.198	-0.149	-0.109	0.721	-0.311	0.273	0.005	-0.372	-0.045	-0.057
Gill width	-0.315	0.227	0.466	-0.314	0.136	0.419	-0.214	0.239	0.721	-0.460	0.203	-0.134
Eigenvalue	4.674	1.112	0.854	3.999	1.446	1.192	3.600	1.604	1.000	2.901	1.566	1.222
% of variance	51.9	12.4	9.5	44.4	16.1	13.2	40.0	17.8	11.1	32.2	17.4	13.6

There was no significant difference in PCs 1, 2 and 3 scores for larvae fed different diets during weeks 5 to 7, but highly significant differences did emerge during week 8 (51-52 dph). Larvae fed the smallest prey item, rotifers, scored highest for PC1 and thus had the smallest heads (Fig 3: PC1: $F=19.93$, $DF=197$, $P<0.001$). Larvae fed fresh-hatch *Artemia*, had a slightly bigger head than those fed rotifers, while larvae fed Algamac *Artemia* or cofed had a larger head than those fed fresh-hatch *Artemia*. Larvae fed the largest prey item, Prolon *Artemia*, scored lowest for PC1 and thus had the largest heads.

In week 8, PC2 reflected contrasts in the dimensions of larval head shape (Table 1). Eye diameter and snout length loaded heavily and negatively on the second component, while jaw width loaded heavily and positively. Larvae fed Algamac *Artemia* or Prolon *Artemia* scored significantly higher for PC2 than larvae fed fresh-hatch *Artemia*, rotifers or cofed (Fig. 4: $F=17.74$, $DF=197$, $P<0.001$). Larvae fed the largest prey items thus had the smallest eyes, shortest snouts and widest jaws. The relationship between PC1 and PC2 and the clear distinction between morphological variables that these principal components represent is illustrated in Figure 5. PC3 was defined by a single significant loading factor, snout angle (Table 1). This variable loaded positively, reflecting a pointed (acutorostral) snout. Larvae cofed rotifers and Algamac *Artemia* scored significantly higher for PC3 than larvae fed any of the other diets (Fig. 6: $F=22.24$, $DF=197$, $P<0.001$), indicating that these larvae had more pointed snouts than all other larvae.

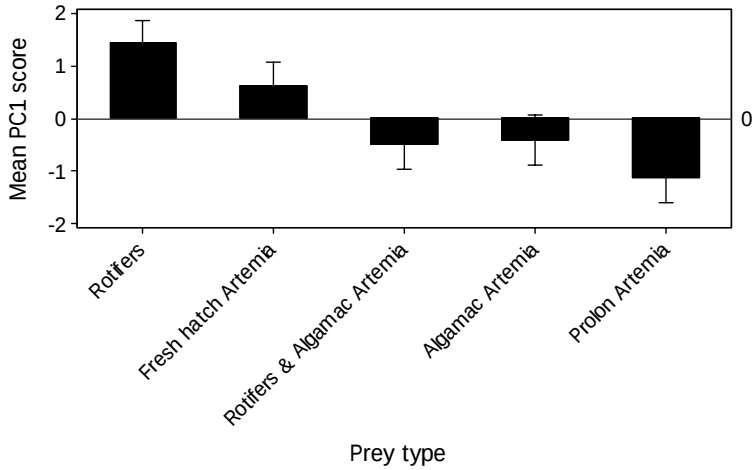

Fig. 3. Mean week 8 score for PC1 of 9 head measurement residuals, by prey type

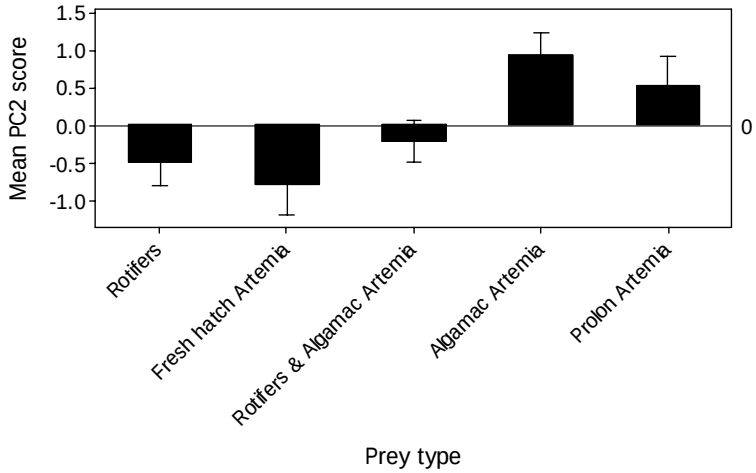

Fig. 4. Mean week 8 score for PC2 of 9 head measurement residuals, by prey type

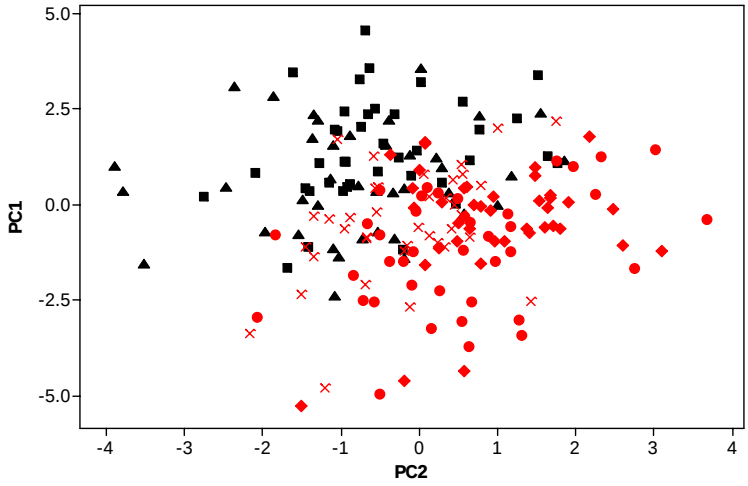

Fig. 5. Scattergram of week 8 scores for principal components 1 (PC1) and 2 (PC2) of 10 head measurement residuals, ■ = rotifer diet, x = rotifers and Algamac *Artemia*, ▲ = fresh-hatch *Artemia*, ◆ = Algamac *Artemia*, ● = Prolon *Artemia*.

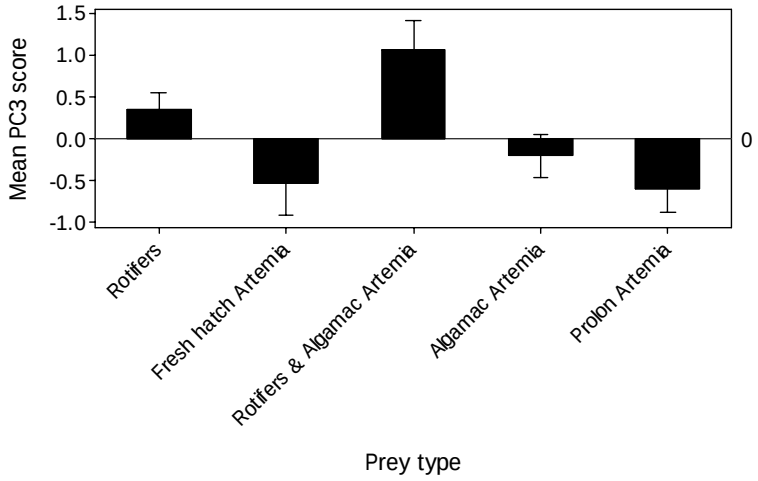

Fig. 6. Mean week 8 score for PC3 of 9 head measurement residuals, by prey type

4. Aggression in Juvenile Cod and the Effect of Prey Density on Aggression

4.1 Materials and Methods

This study was carried out at SAMS Ardtoe marine laboratory, Argyll, Scotland in March 2005. Thirty-five day old larvae were removed from production tanks and placed in twelve 10-litre aquaria. A total of 225 larvae were stocked in each aquarium. Algamac enriched *Artemia* were offered to each aquarium at four different densities, 5%, 10% (control), 15% and 20% of fish biomass, with three replicates per density. Daily feeds were split into two and *Artemia* offered once in the morning and once in the afternoon from 36dph to 60 dph.

Direct observations of fish behaviour commenced at 36 dph and took place 1 hour, 3 hours and 5 hours after the morning feed and prior to the afternoon feed. Observations rotated round tanks, so that all tanks were viewed each day. On each occasion, one tank from each treatment was viewed for 10 minutes, and any aggressive behaviour noted. Following this procedure, it was possible to clearly identify a point at which aggressive behaviour, largely exhibited as nipping conspecifics, increased from zero or one incident in each 10 minute period, to at least 3 or more incidents in each 10 minute period (with the exception of the tanks receiving prey at 20% fish biomass). Thereafter (45 to 60 dph), direct observations ceased and fish behaviour was recorded on video. Each day, one tank from each treatment was recorded for a period of one hour, resulting in a total recording time of 4 hours each day. Prey were offered to each tank exactly halfway through this period of recording. This permitted observations of larval behaviour several hours following a feed and for 30 minutes immediately after a feed. Recordings always took place in the morning and prior to the afternoon feed in order to maximise the time since the fish were last offered food.

Videos analysis was carried out at the University. Unfortunately, the first 5 days (45 to 49 dph) of recordings were faulty and consequently video analysis was of fish aged 50 to 60 dph. The first 2 minutes of every 5 minutes of footage were analysed, with the exception of the first 2-minute interval when fish may still have been influenced by movements associated with the movement of the camera, and the first two-minute interval post feed when the addition of feed often blurred the screen. Only one quarter of the video screen was viewed, in an area to the left of the screen, but vertically central. The rest of the screen was blocked from view using black cardboard. The number of fish at the start and end of each two-minute observation was recorded. Behaviour was recorded as the number of times in each two-minute period that nipping occurred. When possible, the on screen lengths of both the predator and prey were also recorded. Nipping behaviour generally induced a darting escape response from the prey. Darting behaviour also occurred without any obvious nipping having taken place, or

in response to a perceived threat which the prey successfully managed to avoid. These behaviours were also recorded.

4.2 Results

The incidence of nipping among fish did not vary significantly between fish fed different densities of prey (H=1.29, DF=3, P=0.732). However, the difference in the incidence of nipping prior to and following a feed was highly significant (Fig 7: W=972.5, N_1=42, N_2=42, P<0.0001). In total only 27 nips were recorded post feed throughout the study. This difference in the incidence of nipping behaviour pre and post feed was consistent within feeding regimes. There was no difference in the incidence of nipping over time either prior to or post feed (Pre feed: H=0.77, DF=4, P=0.943; Post feed: H=4.78, DF=4, P=0.310). The absence of an effect of time was consistent within feeding regimes. Over the 11 days that behaviour was recorded, the age of the fish had no effect on the number of nips recorded.

Darting behaviour was most prevalent in fish fed the higher densities of prey (15 and 20% fish biomass) (Fig 8: H=8.52, DF=3, P=0.036). In contrast to the marked difference in the incidence of nipping behaviour prior to and following a feed, the number of darts did not differ between the pre and post-feed periods (W=1953, N=84, P=0.134). The absence of an effect was consistent within feeding regimes. In the half hour prior to a feed, the number of darts did not vary significantly with time (H=0.14, DF=4, P=0.998). However, in the post feed period, darting behaviour increased as the time since the feed took place increased (Fig 9: H=13.84, DF=4, P=0.008). This pattern was more pronounced in fish fed the smaller prey densities (5 and 10% fish biomass). The number of darts also increased with the age of the fish (H=57.33, DF=10, P<0.0.001).

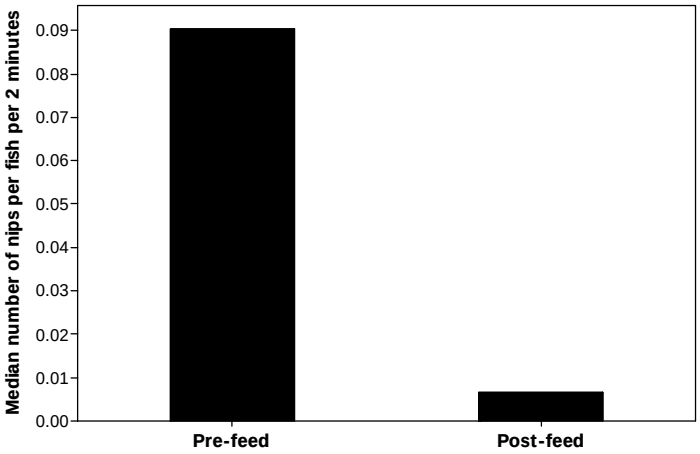

Fig. 7: Median number of nips per fish per 2 minutes prior to and following a feed

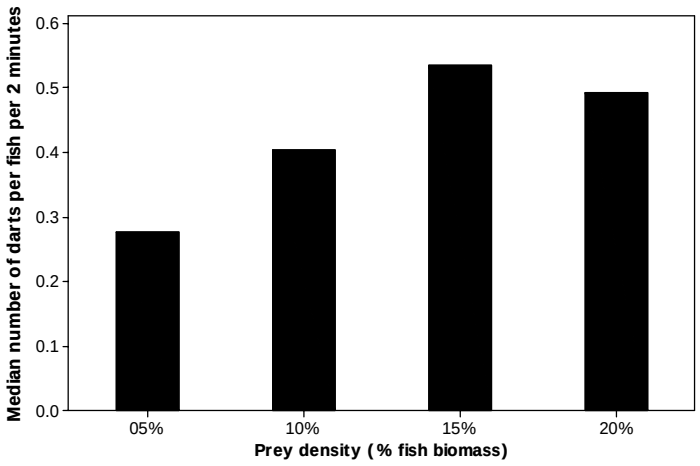

Fig. 8: Median number of darts per fish per 2 minutes by prey density

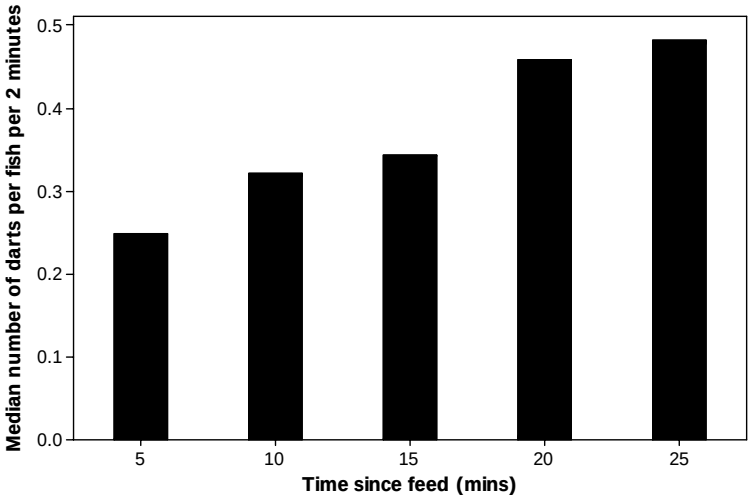

Fig. 9: Median number of darts per fish per 2 minutes over time since feed

5. Variable Boldness in Cod

5.1 Materials and Methods

This experiment was carried out the Aquaculture Research Station, Tromso, Norway in July 2005. The 170 cod used in this study were hatched at the station in Spring/Summer, 2004, using eggs from wild Arctic or Northeastern coastal cod. All cod had previously been fitted with PIT tags so that their genetic family and stock origin could be identified.

Two adjacent experimental arenas were used, situated in isolation in a room at the research station. Each arena consisted of two adjacent chambers, one with a removable cover, and one uncovered, with a sliding door linking each chamber. A flow through system operated in each chamber, with ambient water passing from the nearside of a chamber to the farside (perpendicular to the door). A scent cue was suspended centrally at the farside of each uncovered chamber (near the outflow). This cue consisted of a globe shaped tea strainer, containing a single, defrosted capelin. Two visual cues were also suspended adjacent to each scent cue in the form of a red lure and a 5 kroner coin. The two arenas were separated by a wall and net.

Two cameras were fitted above each arena and linked to two VCRs and monitors and a PC that operated the EthoVision programme. EthoVision is an integrated system that permits automatic detection, recording and analysis of animal behaviour. This system was set to simultaneously record the movements of fish in both arenas, relative to four predetermined 'zones'. These zones were the undercover zone, the danger zone (in the open but not in the food or door zone), the food zone (35 x 35 cm, with the capelin in the centre) and the door zone (an area just extending beyond the area occupied by the door, as viewed on screen).

Prior to a trial commencing, EthoVision was calibrated and pre-trial information was recorded. Two fish were then randomly sampled from a holding tank, transferred to individual buckets and carried to the experimental arena. Each fish was carefully released from the bucket into one of the two undercover chambers, the cover replaced and curtains surrounding the arenas closed. After 5 minutes, the doors in each arena were opened and fish movements thereafter recorded digitally by EthoVision.

After 25 minutes, the door in each arena was closed and recording ceased. Water flows were increased to maximum in order to flush out scents prior to commencement of the next set of

trials. Each fish was then removed and anaesthetised. Once sedated, the fish were identified using a PIT tag reader. Weight and fork length were recorded and a fin clip of the second dorsal fin removed for DNA analysis. The fish were then released into a recovery tank and the information collected entered into EthoVision.

During earlier trials, it was observed that fish entering the uncovered ('danger') zone exhibited one of two behaviours. Fish appeared to be either 'foraging', characterised by a regular slow swimming speed and wide coverage of the uncovered chamber or 'escaping', characterised by an irregular swimming speed and prolonged periods in the corner of the uncovered chamber, attempting to exit the arena. In order to identify the type of behaviour that each fish exhibited, 51 of the total of 170 fish, were also manually observed via monitors and behaviour recorded as 'foraging' or 'escaping'. Using a discriminant function analysis of the movements of these fish, as provided by Ethovision, it was then possible to also classify the behaviour of fish not manually observed as either 'foraging or 'escaping'.

5.2 Results

There was a significant stock effect on the degree to which fish were prepared to exit the covered area of the arena and forage (Fig 10: $Chi^2 = 10.18$, DF=1, P=0.001). Of the migratory fish, 65% emerged at least once to forage, while the comparable figure for the coastal fish was only 40%. All other fish either remained undercover or only emerged to attempt to escape from the arena (together defined as 'avoidance'). In total, only 8% of all the migratory fish remained undercover, while 19% of the coastal fish remained undercover. Foraging and avoidance behaviour did not vary between families of migratory fish (Chi^2=13.18, DF=9, P=0.154), but a weakly significant difference did exit between families of coastal cod (Chi^2=15.5, DF=9, P=0.078).

Behaviour during foraging did not vary significantly between stocks for any of the parameters measured (e.g. time to emerge from undercover, time spent foraging, time in the food zone). However, several of these parameters varied significantly between families of coastal cod such as the total time spent foraging (Fig 11: H=20.40, DF=9, P=0.016) and total time in the food zone (H=19.23, DF=9, P=0.023). There was no significant difference in the behaviour of migratory fish while foraging for any of the parameters measured.

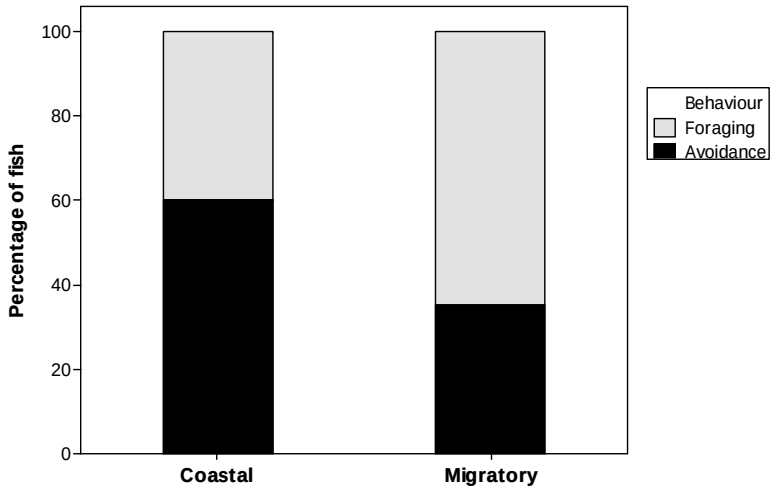

Fig. 10: Percentage of fish that exhibited foraging or avoidance behaviour.

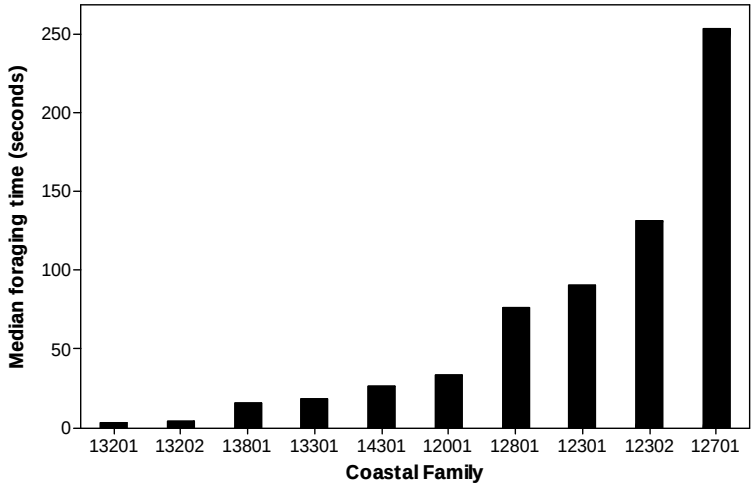

Fig. 11: Median foraging time in coastal fish by family

6.Things Still To Do and Further Study

Data analysis has still to be completed for the boldness and aggression studies. Further video analysis has also to be carried out in the boldness study, to assess the behaviour of attackers prior to, during and post attacks and to assess the condition of attackees. These studies will then each be individually written as papers and combined to form part of the final thesis.

In addition, the following studies have still to be carried out:

Genetic Differences in Cod Head Morphology

During the boldness study, pictures were taken of the heads of 200 cod, including the 170 cod used in the study. Approximately half of these fish were reared from the eggs of migratory broodstock and half from coastal stock. Analysis of the head morphology of these cod will be carried out in an attempt to identify differences in the head morphology between stocks of cod and/or families of cod. The measurements taken will be similar to those variables measured in the earlier two morphology studies described in this report.

Further work on Cod Head Morphology and Diet: Analysis of the Effect of Selective Mortality

This proposed experiment is a follow on from the experiment that examined the effect of prey size on larval cod head morphology and which suggested that larger prey sizes promoted the development of larger jaws and smaller eyes in larval cod. In this study it was impossible to know to what extent the observed effects were a result of developmental plasticity or selective mortality (e.g. that fish that had smaller jaws could not feed on larger prey items and died). Consequently this new experiment will examine the morphology of both live and dead cod reared on different prey sizes in an attempt to address this question.

This experiment will be carried out at Viking Fish Farms, Ardtoe, Argyll (formerly SAMS Ardtoe) in April 2006. Eight 100-litre tanks will each be stocked with 25 dph cod larvae, sampled from production tanks at the site. Larvae in four of these tanks will be fed rotifers only. Larvae in the other four tanks will be fed rotifers and Algamac enriched *Artemia* until 31 dph, and Algamac enriched *Artemia* only thereafter. Once each week from 32 dph onwards, mortalities will be counted and five mortalities from each tank photographed for subsequent analysis of morphology. At the end of the experiment (52 dph), 120 fish (20 from each tank) will be photographed for subsequent analysis.

Appendix 1: Morphometrics Analysis

The following variables were measured using the image analysis programme, Image Pro Express: standard length, horizontal eye diameter, length of the premaxilla (in larvae older than 28 dph), length of the lower jaw (Meckel's cartilage), head depth at the posterior edge of the eye, length of the snout (from the anterior tip of the snout to the intersect with the line defining head depth), angle of the snout (all lateral view, Fig.A1a); medulla width, upper jaw width (maximum distance between maxillae), head length (from the anterior tip of the snout to the point midway between the operculae) (all dorsal view, Fig.A1b); the maximum distance between the gills (prey size experiment only) (ventral view, Fig.A1c).

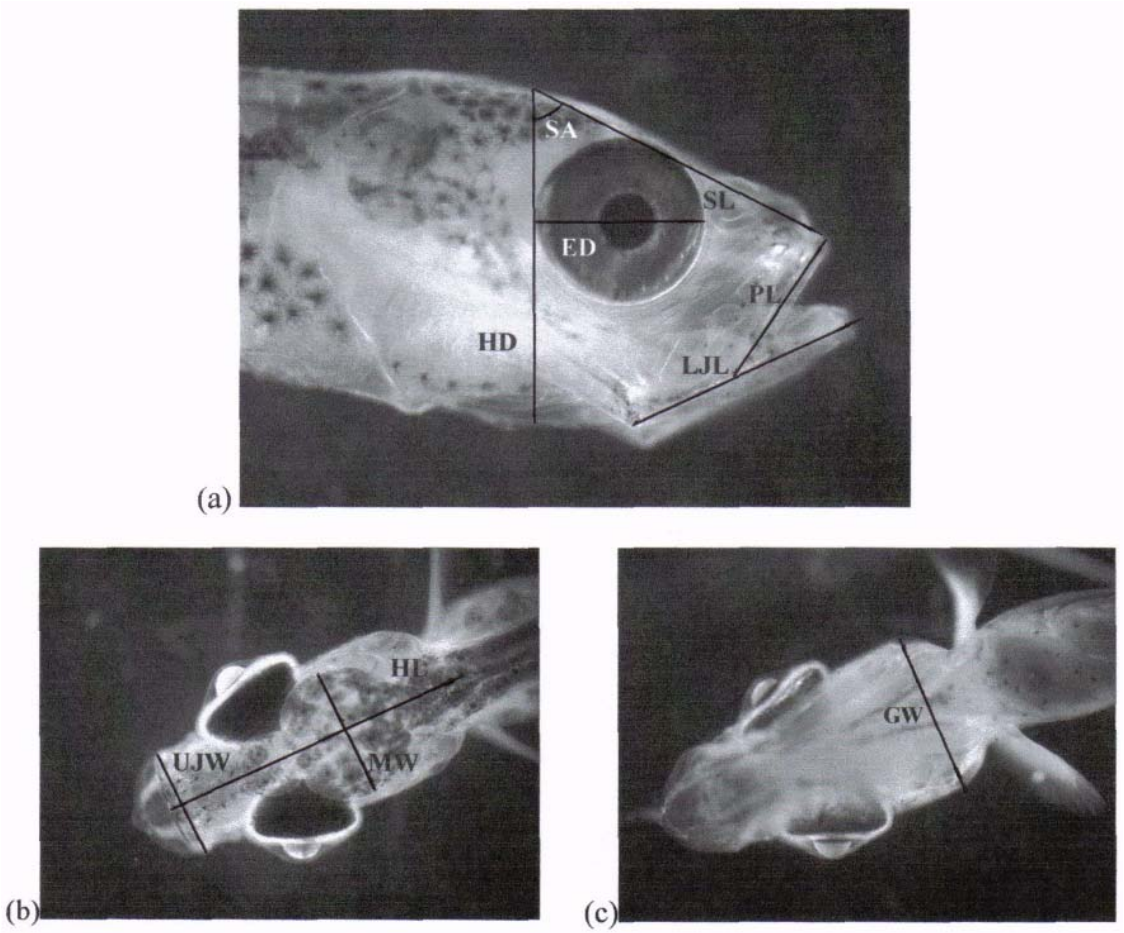

Fig.A1. Photographs of a 36 dph cod larva showing morphometric head variables measured: (a) lateral view, ED = eye diameter, PL = premaxilla length, LJL = lower jaw length, HD = head depth, SL = snout length, SA = snout angle; (b) dorsal view, UJW = upper jaw width, MW = medulla width, HL = head length; (c) ventral view, GW = gill width.