Investigation of Cod Hatchery Techniques

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SEAFISH

Seafish Aquaculture

Marine Farming Unit, Ardtoe

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A report prepared for Highlands and Islands Enterprise

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Authors: B.Gara & R.J.Shields

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Summary

This report details the research work carried out on intensive cod cultivation techniques at Seafish Aquaculture during 1997. The report provides technical details of rearing methods and results, discussed in the context of previously reported work and with a view to future prospects for economically viable cod farming in the UK. Since the project focussed on hatchery rearing procedures, the live feeding and weaning phases are covered comprehensively, while broodstock maintenance and spawning are discussed in less detail.

The collection of fertilised eggs from broodstock cod, spawning naturally in tanks, was found to be relatively straightforward by means of overflow collectors. Observations confirmed published reports that captive cod can reach maturity in their second year, at a weight of approximately 2kg, under suitable nutritional and environmental conditions. This potential for rapid recruitment, together with the huge numbers of viable eggs produced, (>20,000,000 in this study) suggests that, unlike other important commercially farmed marine fish species, gamete supply is unlikely to be a limiting factor to expansion of a cod farming industry. Furthermore the egg incubation phase was also found to be straightforward, with numbers of hatched larvae greatly exceeding the requirements of the 1997 rearing trials.

Live feeding methods developed for turbot and halibut larviculture were tested for rearing the cod larvae. Feed uptake was extremely high when enriched rotifers (*Brachionus plicatilis*) were used as a start feed and high survival and growth rates of the cod larvae were initially observed. However, diet-dependent differences in survival subsequently emerged. In particular, a protein-based enrichment product was deemed to be unsuitable for the young cod. Cod larvae were transferred from rotifer diets to brine shrimp, *Artemia*, at an earlier developmental stage (15 days post-first feeding) than previously reported. Uptake of the

Artemia nauplii and, later, enriched *Artemia* was high. Comparison of larval growth rates under this regime with previous published reports indicated, however, that the transfer to *Artemia* may have been undertaken at too early a stage.

All groups of larvae exhibited an abnormal swim bladder phenomenon from approximately day 25 post-first feeding. Characteristically, the swim bladder became over-inflated, trapping the larvae at the water surface, and this was accompanied by high mortality rates (exceeding 50%). Histological examination of larvae at this stage demonstrated that neural degradation had occurred in the affected animals. The extent of the swim bladder syndrome varied according to diet. Those animals receiving protein-enriched *Artemia* succumbed to the greatest extent. A hypothesis was developed linking the syndrome to larval nutritional deficiencies and it is recommended that further research should be carried out on this topic. The highest survival rate through the larval rearing phase (start-feeding to onset of weaning) was 9.4%, similar to values previously published for cod.

3,711 surviving cod fry were transferred to weaning tanks and there was generally a good acceptance of formulated dry feed. Automatic feeders were found to be necessary to meet the high intake requirements of the fry. Weaning was initiated from day 45 post-first feeding for the largest animals, up to day 70 for smaller, slower growing groups. A high level of aggressive behaviour, including cannibalism, was observed during the weaning phase. Large individuals could be removed straightforwardly from the weaning tanks, but it was not possible to achieve rigorous grading of the remaining population, due to the schooling behaviour of the fish and their fragility. In any case, aggressive behaviour was commonly observed between equal-sized fry, pointing to factors other than size discrepency in trggering the aggressive response. Further research should seek to devise suitable mechanical grading systems for this developmental stage and investigate the use of alternative feeding regimes for reducing aggression. Contrary to previous reports, the aggressive behaviour was relatively short-lived (10- 14 days) and did not account for an acute level of mortality. Survival through the weaning phase was 33.4%. Over 1200 weaned fry were produced and most of these are scheduled for transfer to industry partners during 1997.

Morphological measurements of post- weaned fry suggested that the animals undergo a period of compensatory growth, wherein the fish recover from energetic deficiencies during the late live feeding phase. It is proposed that the manipulation of dietary fat level (increased fat) during early weaning may reduce the duration of this compensatory phase and lead to faster growing, fitter fish.

Preliminary modelling of the production costs of cod fry incorporating the rearing techniques used in this study and the survival rates obtained, indicate that a fry cost of below

£0.65 is currently achievable. Future increases in larval survival are likely to lower production costs to below £0.40 per 5g fry, with a concommitant increase in fry numbers.

List of Figures and Tables

Glossary of Terms

A2	A high protein particulate *Artemia* enrichment developed by the UK turbot industry in the 1980's
AM	AlgaMac2000- a commercially available spray dried cell, rich in essential fatty acids, used as a rotifer or *Artemia* enrichment.
Condition factor	Weight/ length^3 - used to measure the physical condition of a fish.
DHA (22:6 n3)	Docosahexaenoic acid- an omega 3 fat that has an important role in both neural and visual tissues.
EPA (20:5 n3)	Eicosapentaenoic acid- an omega 3 fat that has an important role in neural tissue and metabolism.
LSI (Liver Somatic Index)	Liver weight/ body weight X 100 indicates the percentage of the whole body weight that the liver makes up.
PFF	Days Post First Feeding- used to represent larval age from a set time point.
PUFA	Polyunsaturated Fatty Acid eg. DHA or EPA.
SBSS	Swim Bladder Stress Syndrome- a loose term for a range of symptoms shown by fish that include over- inflation of the swimbladder, and are believed to have a nutritional basis.
SS	Super Selco- a commercially available lipid emulsion, used as a rotifer or *Artemia* enrichment.
TOO	Tuna Orbital Oil- a high grade fish oil extracted from Tuna processing waste, that has high levels of omega 3 fats, especially DHA.
TOO/AM	A mix of Tuna orbital oil and AlgaMac 2000.

Units of Measurement

mg (milligram)	One thousandth of a gram
ug (microgram)	One millionth of a gram
µm (micrometer/ micron)	One millionth of a metre
Joule	4.2 Joules = 1 calorie

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1.0 Introduction

1.1. Background to the present study

This hatchery study was undertaken on behalf *of Highlands and Islands Enterprise* to provide preliminary information on the farming prospects for Atlantic cod *Gadus morhua* in the Scottish Highlands and Islands. Considerable interest has been generated in farming this economically important species in recent years, in view of declining stocks and fishery limitations. The economic potential of cod farming in the UK has been investigated in several recent desktop studies (Whitmarsh & Pickering, 1996; "Nautilus Report", 1997) and these have pointed to the important requirement for achieving low cost fry production. Cod rearing trials were therefore undertaken during 1997 at Seafish Aquaculture, Ardtoe, to test the effectiveness of existing intensive rearing methods that have been developed in the UK for marine flatfish species (turbot and halibut). The broad objectives of the work were to provide preliminary figures on cod survival rates through the hatchery production cycle and to identify priority areas for further research.

Recent economic studies of the potential for cod farming in the UK have rightly pointed to historical, or ongoing research and commercial operations in Norway and Canada as models. It has been stated in some cases that hatchery production technologies for cod are available "off the shelf" from these countries. However, while there is undoubtedly much practical information to be gained from the

Norwegian and Canadian experiences, we submit, from experience with flatfish, that the relatively complex hatchery rearing process should be tailored to the attributes of UK facilities and personnel. Furthermore, on examination of the published record, there is actually very little detailed guidance available on hatchery rearing protocols for cod and some of this is out-dated. The intensive cultivation of cod fry has only been reported in any detail twice, once in the early 1980's on a laboratory scale (Howell, 1984) and then in Norway in the 1990's (Rosenlund *et al*, 1994). At the other end of the rearing spectrum, extensive methods involving outdoor lagoons, as previously used for cod rearing in Norway, have now fallen from favour in that country, due to the difficulty of providing adequate system control and because of pathogen and parasite risks. This should be borne in mind in devising UK strategies for cod larval rearing.

Having inserted these notes of caution about the ease of establishing UK cod rearing methods, it is inevitable that experiences of cod farming in other countries will prove invaluable to the UK effort and these sources should be pursued vigorously. Also, there should be scope for considerable "read over" from other farmed marine fish species such as sea bass and sea bream, in terms of feeding technology.

1.2. Aims of the present study

Against this background, the aims of the present study were to test the effectiveness of currently available UK larviculture techniques for the intensive cultivation of cod fry on a pilot scale. Two broad larval nutrition approaches were tested, (1) live feed enrichments used in the turbot industry, and (2) enrichments that are currently being developed for the halibut industry. Recent advances in weaning marine fish larvae were also applied in an effort to reduce the expected losses of cod due to cannibalism at this developmental stage.

2.0 Materials and methods

2.1. Broodstock management and egg supply

2.1.1. Egg collection

Fertilised cod eggs were obtained from the naturally spawning Ardtoe cod broodstock. The broodstock were held in a 150m^3 tank and consisted of twelve wild caught fish (six each of 4 and 9 year old fish). The sex ratio was 1:1 in the tank. The egg collection method followed that used in a preliminary trial in 1996 i.e. the water from the tank was directed through a 100um plankton net suspended in a 2m^3 tank. The buoyant eggs were concentrated in the plankton net, and could be skimmed off the water surface. A problem noted in 1996 however, was that the egg accumulation was not fast enough to collect one discrete batch of eggs. This caused problems for egg incubation because the oldest eggs could hatch up to seven days before the youngest, so preventing exact determination of first feeding. A modification, in an attempt to collect discrete batches of eggs, was therefore tried in the 1997. The collector net was removed and the flow into the tank was increased to 70- 80 litres per minute in the morning and left on for 4- 5 hours. The eggs in the tank were not collected, and were allowed to drain to sea. The flow was then set back to the original, low flow rate and the collecter net put under the overflow. The eggs spawned that night could then be collected, with little "contamination" from older eggs.

Collected eggs were removed from the plankton net by concentrating them in a fine, soft hand net and placing them in a twenty litre polyethylene fermenting bin filled with seawater. Enough brine (at 150ppt) was added to raise the salinity in the bin to 35.5ppt. This caused the fertilised eggs to rise to the water surface and the dead eggs to sink to the bottom, where they could be siphoned off. A sample of eggs were then taken at this stage to assess the quality and developmental stage (see section 2.1.4).

2.1.2. Egg sterilisation and counting

Egg batches were routinely sterilised using the method of Kristjansson (1995) before being incubated. Eggs were gently scooped up into a soft, fine mesh hand net and held in the air until most of the water had drained off. The net was then dipped into a bucket containing 10litres of seawater, too which 40 mls (a 1: 250 dilution) of Kick Start ™ (a proprietary sterilising

solution, containing an equilibrium mix of acetic acid, peracetic acid and water) had already been added. The eggs were held in the solution for 1 minute and then lifted out and placed into a bucket containing 10 litres of clean UV sterilised and filtered seawater. Ten litres of clean sterilised seawater were then poured over the eggs to rinse them of any remaining Kick- Start solution. Finally the eggs were gently tipped out of the net into a 12 litre acrylic container (petcraft), filled with 8 litres of clean UV sterilised and filtered seawater. The volume of water in the petcraft was then made up to 10 litres and the salinity raised to 35ppt. Eggs that had died during the sterilisation procedure turned opaque and sank to the bottom of the petcraft within 15 minutes and were siphoned off. The water volume was then made back up to 10 litres and a small air stone was placed under the water to prevent the eggs from rafting at the surface.

Two methods of counting were employed; 1. gravimetrically and 2. volumetrically. The first method was used immediately after egg sterilisation. Before starting the sterilisation procedure the hand net was placed on top of a two litre jug and both the jug and net were tared on an electronic balance. After the eggs had been sterilised and rinsed the net was lifted out of the water and blotted dry with tissue paper. The net was then placed back on top of the jug allowing the weight of eggs to be recorded. The number of eggs could then be calculated;

Number of eggs in net = Weight of eggs in net X number of eggs per gram (645 eggs/g^{-1})

If there were a lot of dead eggs after the sterilisation procedure the dead egg could be siphoned off through the hand net, and this could be reweighed to account for the loss.

The second method for counting the number of eggs was as follows. Once the petcraft volume had been made up to 10 litres and the dead eggs removed, the air supply to the air stone was turned up to mix the eggs and water. A 1ml pipette holder with a Pasteur pipette on the end was then used to obtain a 1 ml sample of the mixed eggs and water. By holding the pipette to the light the number of eggs per ml could be counted. This was repeated three times and the average egg count per ml obtained. The number of eggs in the petcraft could be calculated from;

Number of eggs in petcraft = average number of eggs in 1 ml X 10,000

2.1.3. Egg incubation

After being sterilised the eggs were stocked in egg incubators. These had a 250 litre capacity and were made of white MDPE (see figure 1.). Water inflow (2 lpm) was from a narrow inlet at the bottom of the tank. The outflow went through a 15 cm "banjo" filter covered in 250um nylon mesh. Two air stones were used for each incubator. One was positioned at the bottom of the tank over the inlet to prevent eggs sinking to the bottom and becoming trapped in the inlet during periods of reduced ambient salinity. The second air stone was positioned directly below the banjo filter, to prevent the bouyant eggs becoming trapped on the mesh and blocking the filter. Up to 500,000 (2/ml) eggs were stocked in each incubator without any discernible problems. Dead eggs could be removed by switching off both the aeration and the water supply at the inlet valve. After 10- 20 minutes the dead eggs sank to the bottom, where they could be removed by opening the drain valve. These eggs could then be weighed to estimate mortality.

2.1.4. Staging of larvae

All larval batches used in egg incubation were examined for developmental stage according to criteria described by Makhotin *et al*, 1984, and Apstein (In Russell, 1974). Time to hatch was predicted by comparing developmental stages between the present work and to previous reports.

Table 1. Average duration (days) to hatch for artificially fertilised cod eggs at different temperatures (from Dannevig, in Russell 1974).

Temperature	-1^{0C}	3^{0C}	4^{0C}	5^{0C}	6^{0C}	8^{0C}	10^{0C}	12^{0C}	14^{0C}
Days	42	23	20.5	17.5	15.5	12.75	10.5	9.67	8.5

Figure 1 Cod egg incubator

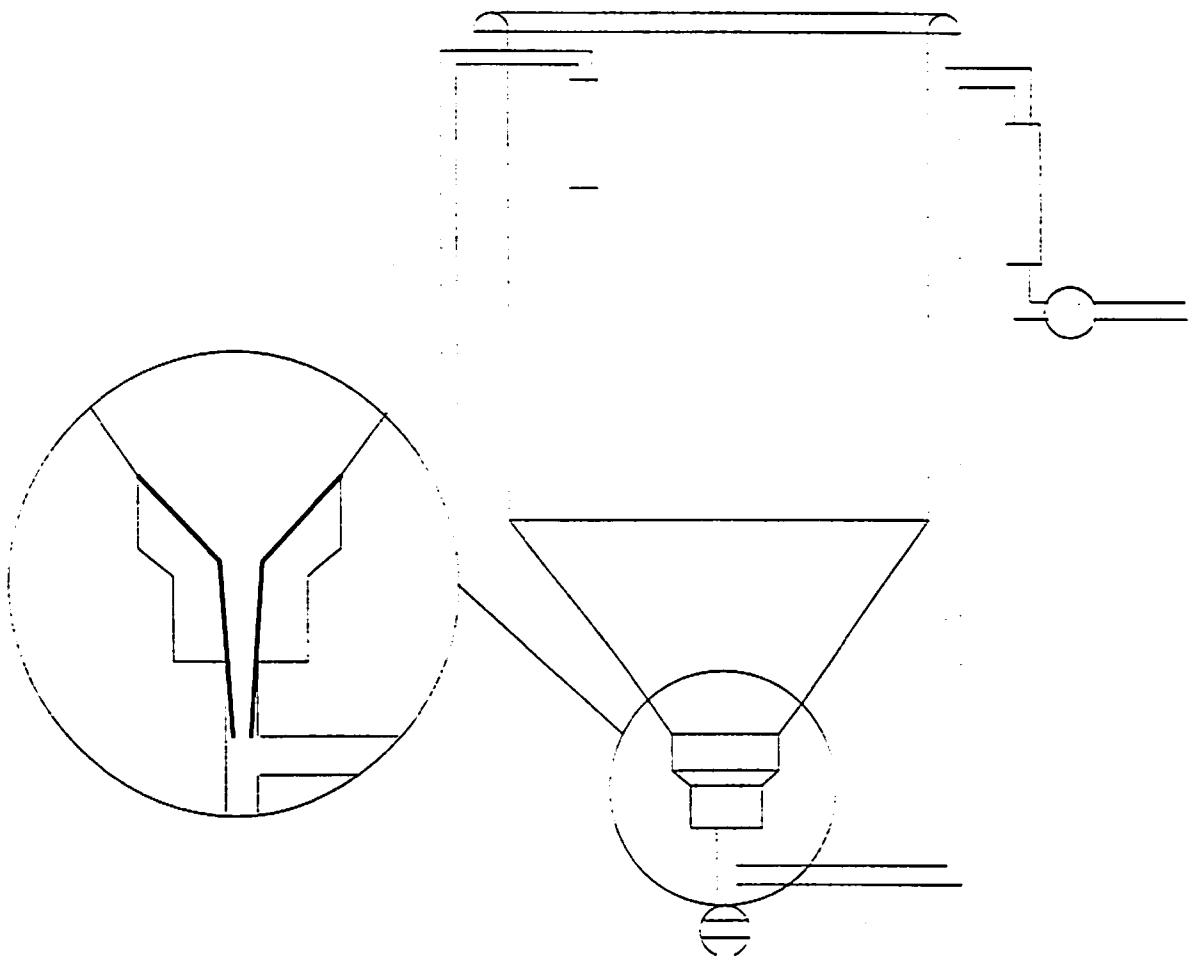


Figure 2 Cod larval rearing tank

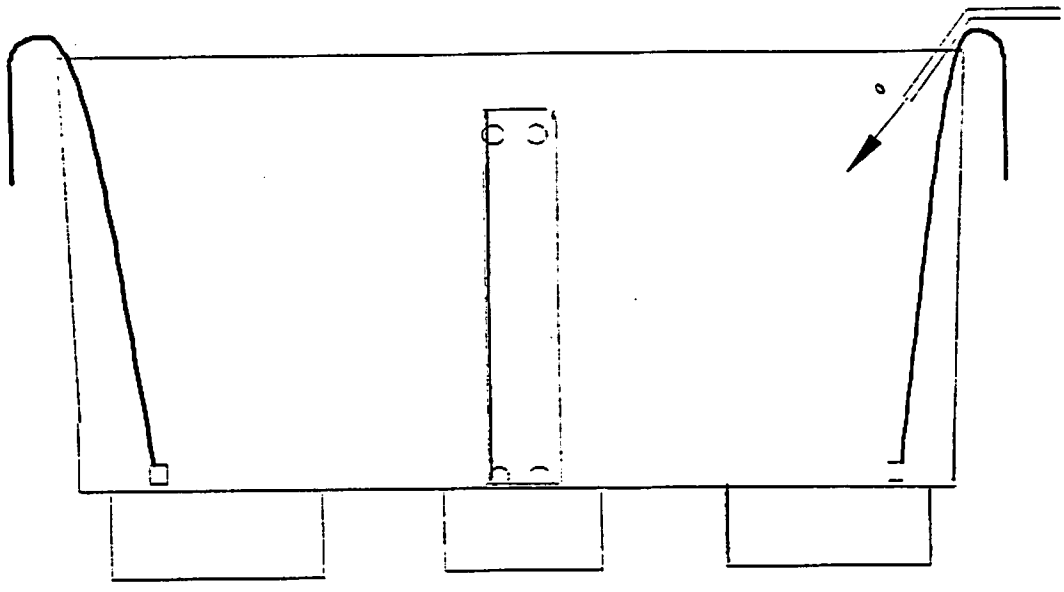
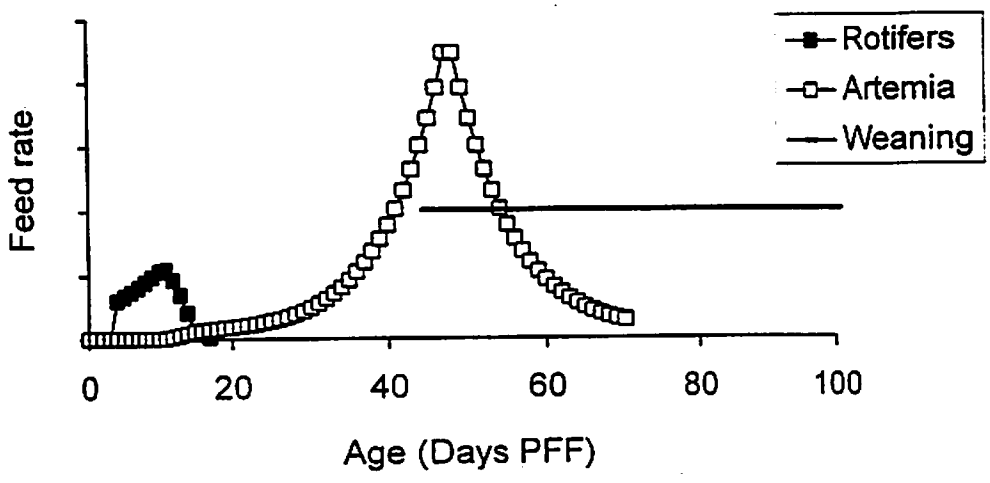


Figure 3. Generalised larval rearing feed types used in 1997



2.2. Hatching of eggs and transfer to larval rearing tanks

2.2.1. Hatching

Cod eggs were hatched *in situ*, and were transferred to larval rearing tanks 2 days after 50% hatch. Bouyant larvae were skimmed off the water surface using a two litre jug and poured gently into a clean 10 litre petcraft containing at least 2 litres of clean UV sterilised and filtered seawater. The petcraft was then gently aerated using one airstone.

The third and fourth larval batches were removed prior to hatch and stocked into a clean larval rearing tank, where hatching occurred. The reasons for this are described in section 3.1.2.

2.2.2. Counting and transfer

Larvae were counted by volumetric methods. 50 ml samples of larvae and water were taken from the petcraft and poured into a jug containing at least 1 litre of clean seawater. This was then slowly tipped out into a basin, and the number of larvae counted. This procedure was repeated at least three times and the average number of larvae per 50mls was calculated. This could then be used to calculate the number of larvae per litre;

No. of larvae per litre = Average no. per 50ml sample X 20

The water volume from the petcraft required to give the correct number of larvae for each rearing tank (16 per litre/ 16000 larvae per 1000 litre tank) could be calculated by;

$$\textbf{Litres of petcraft water (containing larvae)} = \frac{\text{No. of larvae required}}{\text{No. of larvae per litre}}$$

The volume of water required was then taken from the petcraft with a 1 litre jug and poured into another petcraft and the water volume made up to 10 litres. This was floated in the rearing tank until any slight temperature differences had equalised. The petcrafts had a small airline and a low

aeration in them to prevent any stress associated with low dissolved oxygen levels.

After the crash of one larval population three days after transfer to larval rearing it was hypothesised that high larval densities in the egg incubators could reduce larval viability, by increasing either the transmission of bacteria between the two systems or by the continuos exposure to high levels of hatching enzymes, released from the egg shell.

To remedy this, eggs destined for larval rearing were skimmed off the surface of the water using a soft fine hand net and transferred to a clean bucket. The eggs were then transferred to a clean 1000 litre rearing tank filled with UV sterilised filtered water. A flow of 0.5- 1 litre per minute was maintained in the tank to prevent temperature fluctuations and water quality problems from occurring. Two days after the larvae had reached 50% hatch the larvae were skimmed off the water surface and counted as in the previous section. Transfer and stocking to larval rearing tanks was the same as described above.

2.3. Early larval rearing

2.3.1. Initial tank set- up

Early larval rearing was performed in two types of tank;
1. 1000 litre deep tanks (1m deep)
2. 500 litre shallow tanks (0.5m deep).

The two types of tank were chosen because it was thought that better cleaning of the tanks could be achieved if a wider and shallower tank design was used. All tanks had ambient seawater (31- 35ppt) and air supplies (see figure 2). Four airlines and stones were used per tank to give optimal mixing of the tank water and to prevent acute drops in dissolved oxygen levels. Each tank had an overhead tungsten floodlight connected to an individual dimmer switch. Light intensities could be varied from 30 lux at the water surface to approximately 1500 lux. The tank outflow was by a central standpipe, surrounded by a wider bore pipe collar into which several large holes had been drilled. These were covered with a 150um nylon mesh. Two sets of holes, at the base of the pipe and at the waters surface were used. This enabled water flow from both the water surface and the tank bottom. A surface outflow was considered important, to prevent the formation of an oil layer (caused by the release on capture of oil from enriched rotifers) at the

water surface, which has been implicated in the failure of physoclistous fish (eg. seabass) to inflate their swimbladder.

Immediately prior to stocking with larvae, each tank was filled with clean UV sterilised and filtered sea water and 20 - 40 litres of *Nannochloris atomus*. The light intensity was lowered to approximately 50 lux at the waters surface, and aeration was kept fairly low. Larvae were stocked at 16 larvae per litre.

2.3.2. Rearing regime

(Detailed descriptions of the rearing strategies used in paticular batches and tanks are described and discussed in detail in the results and discussion section.)

The general rearing regime was as follows (refer to figure 3 for digrammatic overview). After stocking the tanks with 2 day old larvae, no water flow was introduced into the tanks until day 2. Water flow was initiated at 0.25 litres per minute (LPM), rising to 0.5 LPM at day 6, then 1 LPM at day 14 post stocking. Rotifers were introduced on the day after stocking at the rate of 1-2/ml. The same quantity was added every day for the first three days at which time feeding rate observations could be started. From this point, rotifers (up to 3 million per tank per day) were added in line with the feeding rate i.e. enough rotifers to ensure that the larvae had consumed almost all by the next day. Algae (*Nannochloris*) was added every other day to keep the tank water "green" and to provide food for the rotifers. The algae addition was stopped after day 20, due to the high water flows being used, and the dominance of *Artemia* feeds rather than rotifers.

Artemia nauplii were added at day 10, and at day 14 -15, ongrown *Artemia* was added. Rotifers were gradually phased out after day 16. *Artemia* nauplii were phased out after day 25. *Artemia* feed rates were again determined by larval feed rates i.e. enough so that the tank was not totally clear in the next morning. Rotifers and *Artemia* nauplii were fed first thing in the morning, and A2 enriched Artemia in the afternoon, until enriched *Artemia* was the dominant feed, when it was fed both morning and afternoon. Super Selco and TOO/AM enriched *Artemia* were fed in the morning.

2.4. Live feeds

2.4.1. Algae

Production of algae used the standard Ardtoe method of 100 litre plastic bag cultures. These bags are stocked from algae cultured in smaller (1- 5 litre) flasks. Generally only the green flaggellate *Nannochloris atomus* is cultured, due to its ease of culture. However, because of the the low levels of polyunsaturated fatty acids (PUFA) present in this species, two other algal specioes were grown; *Pavlova lutheri* and *Isochrysis* Tahitian strain (T-Iso). Both these algal species have much higher levels of PUFA and espeically Docasahexaenoic acid (DHA) present (*see section 3.2.4. for discussion of the role of DHA in larval development*). Unfortunantly the culture of these two algal species was not wholly successful and the limited quantity available meant that the algae could only be used for the final enrichment of the rotifers (see section 2.4.2.2.).

2.4.2. Rotifers

2.4.2.1. Culture

Rotifers were cultured in white 80 litre volume cylindro- conical MDPE containers. Each container was fitted with one airline and air stone and a 150 watt aquarium heater. Water temperature was maintained at 22°C, and salinity was increased from 17ppt to 35ppt as the culture and the water volume increased. Each tank culture was stocked iniatilly with 3 million rotifers in 30 litres. Every second day the water volume was increased with the addition of clean seawater and algae (*Nannochloris*). Dried bakers yeast was added every other day at the rate of 1g/ million rotifers.

2.4.2.2. Enrichment

A separate enrichment was performed on rotifers that were to be fed to larvae. The next days rotifer requirement was harvested from one or more rotifer cultures, depending on the numbers present in the tanks. These rotifers were harvested through a 100um mesh and gently rinsed with clean seawater. The rotifers were then set up in a clean 80 litre paxton at 200,000 rotifers per litre. Water temperature was increased to 22°C. The enrichment of AlgaMac 2000 was added

at 1500 hours at the concentration of 0.15g/litre. The following day at 0900 the rotifers were harvested by concentrating them in a 100um mesh and rinsed with clean sewater. The rotifers were transferred to a 10 litre bucket at a density of 1,000,000 per litre. The AlgaMac enrichment was chosen as;

1. it was a clean enrichment i.e. there was no oil emulsion requiring excessive rinsing.

2. published information (Barclay and Zeller, 1995) had shown that rotifers enriched with AlgaMac had very high levels of fat and high ratios of long chain fatty acids.

Prior to feeding to the larvae, 2 litres of either, Tahitian strain *Isochrysis*, or *Pavlova* was added to the rotifer bucket, and left for at least ½ hour.

An additional enrichment using the *Artemia* A2 enrichment was also tried with the AlgaMac enriched rotifers. The A2 enrichment was originally developed by the UK turbot industry and has a very high protein content. After being harvested and rinsed as already described, the required number of rotifers were set up in a 10 litre bucket and were then enriched for a further hour with a mixture of A2 (1.0 g/million rotifers) and canthaxanthin (Carophyll red™, Roche) (0.5g/10 litres), blended in warm fresh water. After 1 hour the rotifers were harvested and concentrated through a 100um mesh and rinsed with at least 20 litres of clean seawater, to remove excess pigment and A2.

2.4.3. *Artemia*

2.4.3.1. Hatching

Decapsulated Great Salt Lake strain, *Artemia* cysts (Aquafauna Biomarine Inc) were used for all cod rearing trials. *Artemia* cysts were hatched in 80 litre white cylindro- conical containers for 18 hours at 28°C. Once hatched the nauplii were light- seperated to remove hatching debris and unhatched cysts, and then rinsed in clean UV sterilsed and filtered seawater through a 100um mesh. *Artemia* were counted using a dilution method, where 50 mls of a known

volume of *Artemia* are diluted in 950 mls of seawater. This is then aerated and at least three 1 ml counts are made of the number of *Artemia* per ml. The number of *Artemia* is then calculated by;

No. *Artemia* in container = Average count in 1ml *x* 20 *x* Volume in container

Freshly hatched nauplii was stored in buckets at a density of 1 million nauplii per litre in a cold room (<10^{0}C) until fed to the larvae. In addition, ice packs were also placed in the bucket to reduce the temperature even more. This has the effect of stopping the *Artemia* growing and using their endogenous energy reserves for growth.

2.4.3.2. Enrichment

Artemia enrichments were carried out in the same kind of tanks as described in the previous section. The required number of *Artemia* for the next days feeding were stocked into these tanks at the density of 150,000 *Artemia* per litre. The enrichments used were as follows;

Super Selco™- (Inve Aquaculture) a proprietary lipid emulsion containing marine oils, vitamins, antioxidants and emulsifiers. This enrichment has been widely used for improving the fat levels and fatty acid ratios of *Artemia*, prior to feeding to marine fish larvae.

AlgaMac 2000 ™- (Aquafauna Biomarine) a spray dried single celled marine protist. Again published information had shown that high levels of long chain fatty acids could be incorporated into *Artemia* with this product. AlgaMac had also been used with considerable success within the UK halibut research program eg. Gara & Shields (1997).

A2- a high protein *Artemia* enrichment originally developed by the UK turbot industry. The exact formulation of this enrichment was not available, as each UK hatchery makes slight changes to the exact constituents.

TOO/AM- a mixture of 75% AlgaMac and 25% Tuna orbital oil, an enrichment used in UK halibut culture.

Super Selco and AlgaMac were enriched at the rate of 0.6g of enrichment for every litre of culture water, at 27°C for 18 hours (overnight). *Artemia* for the AM/TOO and A2 enrichments were starved overnight at ambient temperature (15-17°C), then fed the enrichment at a rate of 0.5g/ million *Artemia* for 1½ hours. All enriched *Artemia* were rinsed well in copious amounts of clean, UV sterilised and filtered water before being fed to the larvae. Excess *Artemia* was again stored in a cold room until it was required.

2.5. Weaning

2.5.1. Tank and autofeeder design

At between day 45 and day 65 PFF, the surviving cod were transferred to weaning tanks. This was achieved by jugging the larvae, rather than netting them. The larvae were not resilient enough to handle the stress associated with hand netting. The first tanks to be used were 70 cm X 70 cm X 40 cm black GRP rounded square tanks. Up to 800 larvae per tank were stocked and weaning was intiated. It soon became apparent that it would be difficult to maintain adequate food levels and keep the tanks clean. The larvae were roughly graded into large and small sizes by length and moved into 500 litre black round GRP tanks. A "spray bar" was used to creae a slow circular flow. Automatic feeders (Norfab Ltd.) were installed above the tanks, feeding for 5 seconds every hour. Size grading of fish within the three tanks occurred after the second week.

2.5.2. Weaning diets

Fry were initially weaned onto a dry pellet (BOCM Pauls "Keystart hatchery") with a 12% oil content. As the fry grew the size of the pellet was increased from 300- 500um, to 400-700um and then 500- 1000um.

2.6. Sampling

2.6.1. Egg and larval dry weights

Samples of eggs and larvae were taken regularly for dry weight analysis. Larvae were rinsed in distilled water before being placed in preweighed "cryovials" and freeze dried for 24 hours. The vials were then weighed again on a Sartorius electronic balance to the nearest mg.

2.6.2. Length/ weight samples and analysis of liver weights

A sample of thirty five cod fry (100 days PFF) were sampled randomly from the three weaning tanks. Fry were anaesthetised in MS 222 before being processed. Whole body, blotted wet weight and standard length (tip of bottom jaw to the tail fork) were measured. The liver was then dissected out of the specimen and placed in preweighed cryovials, which were then re-weighed. The Liver: Somatic index (LSI or Hepatosomatic index, HSI) was calculated by;

$$\mathbf{LSI} = \frac{\text{Weight of liver}}{\text{Weight of body}} \times 100$$

This gives the proportion of the body weight that the liver makes up.
The condition factor (difference in body weight for a given length) was calculated by the following equation;

$$\mathbf{CF} = \frac{\text{Weight (g)}}{\text{Length (mm)}^3} \times 1{,}000{,}000$$

The length/ weight data was used to calculate the length/ weight relationship for cod fry. Principal Component Analysis (PCA) of parameters measured was also used to describe trends. Minitab software was used for all analyses.

3.0 Results and discussion

3.1. Broodstock management and egg incubation

3.1.1. Egg collection

The Ardtoe cod broodstock spawned from the 22nd of January to 26th of March 1997. 32Kg (>20,000,000) of eggs were collected (see Table 2). All egg batches had been fertilised to some degree and 6 batches of eggs were selected for incubation. In addition, a group of wild caught, two year old fish spawned for the first time in a separate tank. These egg production figures confirm that gamete supply is unlikely to be a limiting factor for commercial scale hatchery rearing of cod fry.

The good spawning performance is also encouraging in terms of broodstock nutrition. The 6 and 9 year old broodstock used for the 1997 rearing trials had been captured as 0-Group animals and had therefore received moist, formulated diets for most of their nutritional history. It appears that the broodstocks' nutritional requirements for good quality egg production were well met by the diet used. Kjorsvik and Holmefjord (1995) reported that the duration to sexual maturity in cod is influenced by both the nutritional history and the condition factor of the fish. Considering this, together with the known first spawning age of the Ardtoe stock (2 years age), it can be concluded that the diet and environmental conditions used were conducive to early maturation. These characteristics will enable a cod farming industry to recruit hatchery-reared, or wild-caught juveniles as spawners within a short space of time.

The implications for ongrowers of animals maturing at around 2Kg should be borne in mind, in view of reduced food conversion efficiencies following maturation. Ahmed (1995) and the Nautilus report (1997) calculated that a cod farm could obtain reasonable returns by selling 2Kg animals, however it was preferable to sell animals over the size range 2 to 4Kg.

Table 2. Quantity of cod eggs collected and incubated at Seafish Ardtoe during the 1997 spawning season.

	No. batches	Weight of eggs	Eggs/g^{-1}	No. of eggs
Collected	N/A	32 Kg	645	> 20,000,000
Incubated	6	2.7 Kg	645	1,650,000

The 1997 modification to the egg collection technique improved the efficiency of collecting discrete, similar- aged egg batches. Removal of single batches of eggs could be further improved in future by reducing the size of the broodstock tank, enabling greater rates of water exchange. It has been reported, however, that reducing the size of a tank below a minimum threshold can stress the broodstock cod and adversely affect regularity of spawning, fertilisation rate and embryo development (Kjesbu, 1989; P. Smith pers. com.).

Rosenlund *et al* (1993) stated that the collection of eggs twice a week was sufficient to maintain synchrony of first feed requirements in batches of eggs. This suggests that there was little interest in the reported Norwegian trials in capturing discrete batches of eggs and being able to trace the exact parentage of a particular batch of eggs. Separate research has been carried out on individual cod spawners to identify and quantify timing of spawning, fecundity and egg quality (see Kjesbu, 1993; and Kvorsvik, 1994 for reviews). However, this approach is unlikely to be necessary, or feasible in practice, due to the natural spawning of mixed groups and the huge numbers of eggs available. It is likely that other selection criteria, for example batch size, buoyancy of eggs, percentage fertilisation and blastomere morphology will be used to screen batches for incubation (see reviews by Kvorsvik *et al*, 1990; Bromage *et al*, 1994 and Shields *et al* 1997).

It is unclear whether the increased number of dead eggs collected in the overflow meshes later in the season was a natural phenomenon or as a result of having adopted a larger mesh size. Cod egg quality, as with other batch spawning fish has been shown to decrease towards the end of the spawning season (Kvorsvik 1994). Also, the resistance of eggs to mechanical stress has been shown to decrease at the end of the season (in Kjesbu, 1993). These two factors may have been working together i.e. poor quality or dead eggs were collected. which then could not stand up to the rigours of being concentrated in any mesh. It would be necessary to collect early- and mid-season eggs using the larger sized mesh to test which factor is the most important. If late

eggs are indeed of poorer quality, this should be taken into account in planning the seasonal production cycle of a commercial cod hatchery, with greatest emphasis on fry output during the early and mid- season.

Late season cod eggs also had white, opaque chorions when collected. At first this was assumed to be a fungal or bacterial growth on the chorion resulting from physical damage in the collector net. The phenomenon was observed, however, in egg incubators stocked with sterilised eggs and incubated in UV sterilised and filtered seawater. It was therefore reasoned to be:

1. Already present in the incoming seawater supply to the site, hence it's presence on eggs collected from the broodstock tanks, and
2. Present on eggs transferred to egg incubation, suggesting that the method of egg sterilisation was not adequate.

Further work would be required to identify the overgrowth and to optimise sterilisation parameters for the cod i.e. egg density, duration and disinfectant concentration.

3.1.2. Egg incubation

Six groups of cod eggs were selected for incubation (see Table 2). Incubation was successful with high stocking densities achievable (up to 2000 eggs per litre). Survival rates were not monitored closely, due to the huge numbers of eggs available, although survival to hatch was estimated to be around 60-65% on average. The high stocking densities, along with the minimal water flow rates of ambient seawater required, effectively reduce the cost of this production stage to an absolute minimum. Most egg batches collected were at stage 5-8 (Apstein, 1909, in Russell) giving an approximate duration to hatch of 12 days. Using this information the live feed and algae production could be tailored to meet the requirements at hatch.

There did not seem to be any deleterious effect of this stock density during egg development. However, possible deleterious effects on the hatched larvae were noted following the crash of batch II, 5 days after stocking in the larval rearing tanks. It was hypothesised that the release of hatching enzymes into the water, combined with the long hatching period (several days from first eggs to last), may have adversely affected the oldest larvae by the time of transfer. Batch III was therefore removed from the egg incubator prior to hatch and stocked into a clean larval rearing tank, in order to avoid this possibility. After hatching had occurred, the larvae were skimmed off and

counted as in section 2.2.2. A further step that could be performed at this stage in the future would be an additional sterilisation of the eggs to totally prevent cross contamination from egg incubators into larval rearing tanks.

3.2. Larval rearing

3.2.1. Experimental design.

Seven larval rearing tanks were stocked with a total of 84,000 newly hatched cod larvae in 1997. The broad aims of the rearing trials undertaken were to provide preliminary information on effective tank volume/ dimensions and appropriate combinations of rotifer and *Artemia* enrichments. It was originally hypothesised that the inclusion of a high protein A2 type enrichment, as used in the UK turbot industry may be beneficial to the cod larvae, as cod are known to require a high dietary protein content in their juvenile and mature stages.

Details of each individual tank feeding regime are given in appendix 1.

3.2.2. Growth of cod larvae

All batches of larvae showed extremely rapid uptake of feed, with larvae accepting rotifers within half an hour of the prey being added to the tanks. Consumption of rotifers was high, with generally 3-4 million rotifers added per 1000 litre tank per day. Larval growth rates during the rotifer phase were generally high (>10% day), however the extra enrichment with the A2 formulation did not seem to confer any advantage (see table 3.)

Table 3. Specific growth rates of cod larvae from day 0- 10.

Rotifer enrichment	
AlgaMac/ T-Iso + A2	AlgaMac/ T-Iso
10.9	15.9
3.6	13.7
	14.9
	21.7

SGR= (Ln (weight at end)- Ln (weight at start))/time *100

In comparing these observations with published information it is surprising that an extra high protein enrichment was not beneficial to the cod larvae. Fyhn (1989) has suggested that free amino acids (FAA) are an important energy source during the early life history of cod and Opstad *et al* (1989) demonstrated improved larval growth in cod when they were fed rotifers

enriched with fishmeal rather than algae. Although only two batches of larvae in the present study received the A2 enrichment, it is still striking that growth rate was much lower, considering that the rotifers also received the AlgaMac enrichment regime. Comparison of larval growth rates within batches also showed a lower growth rate when using A2-enriched rotifers. It can be tentatively concluded that the extra enrichment with A2 was unnecessary and possibly harmful to the cod larvae, although replicated trials would be needed to confirm the observed 1997 trend. Also, it is acknowledged that, since proximate analyses were not undertaken of the rotifers or larvae, dietary differences in protein intake cannot be quantified.

The dry weight of the cod larvae increased from a mean of 0.11mg at day 1 post hatch to 1.28mg at day 40 post first feeding, although the results were highly variable for all groups of larvae. Figure 4 shows the increase in dry weight through development. It is possible that the weights presented are an overestimation, due to size-specific mortality (ie greatest mortality amongst small larvae), especially at the time of transition between different live feed types. As prey size is increased, larger larvae within the population are better able consume the new prey than smaller individuals.

Figure 5 shows the mean specific growth rates of cod larvae for all diet treatments at different ages. The larvae showed a very high growth rate (13.5%) during the early rotifer phase (day 0- 10), which then fell to 1.7% over the next 10 days. This is thought likely to be due to the introduction of *Artemia* during this time period. *Artemia* were introduced at an early stage due to their greater calorific value relative to rotifers (a comparable feeding strategy to that used for turbot rearing). However, there was possibly a time delay before the *Artemia* were being caught efficiently, causing a reduction in growth rate. In addition, the larval digestive system may not initially have been sufficiently developed to digest the *Artemia* fully.

Existing published studies on intensive cod culture have used more extended periods of rotifer feeding, with variable results. For example Howell (1984) used rotifers (enriched with algae) for 25 days prior to presenting *Artemia* metanauplii. Opstad *et al* (1989) fed rotifers enriched with fishmeal until day 30 PFF, however the larval dry weight at day 30 was only 0.2mg, whereas the larvae in the current study were approximately 0.9mg at that age. Rosenlund *et al* (1993) fed cod larvae Super Selco™ and DHA Selco™ enriched rotifers for only 20 days and achieved high dry weights of between 1.5 and 2mg.

In summary, no clear consensus has emerged regarding suitable durations for rotifer feeding of intensively reared cod larvae. Appropriate timings will undoubtedly vary according to the nutritional quality of the rotifers and the high growth results of Rosenlund *et al* indicate that expensive oil enrichments may be advantageous in this respect. However, it should be noted that comparisons between studies are limited by differences in the weighing methods used and replicated experiments using single batches of larvae will be required to more accurately define suitable regimes.

Mean specific growth rates of the larvae were seen to increase again from day 20-30. Size-specific mortality may again have played a part in this observation. In all but two of the rearing tanks rotifer feeding was stopped at day 16, as the proportion of *Artemia* was increased. Those small larvae that were unable to fully utilise the *Artemia* would have starved and died, resulting in an increase in the mean weight of the remaining population. In addition, those larger larvae that successfully transferred to *Artemia* nauplii and ongrown *Artemia* would have benefited from the greater calorific content of the *Artemia* relative to rotifers (approximately 0.044 and 0.0033 joules per individual respectively; T. Van der Meeren pers.com.), so they would have more energy available for growth.

The reduction in population specific growth rate from day 30 to day 40 is again considered to be due to a combination of selective mortality and actual changes in growth rate. The larger, faster growing fish within each population were the first to succumb to mortality associated with hyperinflation of the swimbladder (see section 3.2.4.2.), reducing the mean weight of the remaining population. Also, Thorisson (1994) reported that a major cause of larval cod mortality is an apparent energy crisis at metamorphosis. Future research should therefore investigate methods of satisfying the energetic requirements of the cod fry at this critical stage, either through early weaning onto dry diets, or via better *Artemia* enrichments and presentation methods.

3.2.3. Diet treatments

As discussed previously (section 3.2.2.) the use of A2 as a rotifer enrichment did not confer any advantage to the cod larval growth rates. This negative effect of A2 was also observed during the *Artemia* feeding phase (see Table 5). When A2 was used as the dominant *Artemia* enrichment, larval survival was consistently lower than with the TOO/AM diet regime (see section 2.4.3.2. for enrichment details).

Table 4. *Artemia* enrichment combinations used for cod cultivation trials with larval survival.

Larval batch	Rotifer enrichment	*Artemia* enrichment	% Survival
1	AM- TISO	SS & A2	0.00
1	AM- TISO	SS & A2	0.00
2	AM- TISO	SS & A2	0.85
3	A2 & AM and AM & TISO	SS & TOO/AM	4.00
3	A2 & AM and AM & TISO	SS & TOO/AM	8.95
3	AM- TISO	SS & TOO/AM	12.15

It can be seen that, amongst those tanks fed TOO/AM and Super Selco, the best survival (tank 5) was achieved when the Super Selco was fed for the longest duration i.e. day 21- 50 rather than day 35 - 50 as in tanks 1 & 4 (refer to appendix 1 for detailed timings of different feeds). The limitations of these observations are acknowledged in terms of single runs using different batches of larvae, nonetheless the trends are striking.

In a separate study on larval halibut, Gara & Shields (1997) reported increased mortality among larvae fed A2 & Super Selco enriched *Artemia*. It was demonstrated that larvae fed combinations of these two enrichments had a reduced level of Triacylglycerol (TAG) in their livers. Liver TAG levels have been used as indicators of nutritional status in fish larvae (Fraser *et al*, 1987). Larvae fed TOO/AM, however had very high levels of TAG in their livers indicating a superior nutritional status. It is hypothesised, then, that one of the reasons for the poorer survival in A2 fed cod larvae is that they were receiving insufficient amounts of fat in their diet.

3.2.4. Problems associated with larval rearing

3.2.4.1. Swimbladder inflation

Cod posses a swimbladder (used for buoyancy control) described as physoclistous, meaning that the bladder is initially connected to the gut, but later in larval development the connection closes and regulation of the gas content is mediated by a specialised organ called

the *rete mirabilis*. An important step in the development of physoclistous species is the initial inflation of the swimbladder, which is achieved firstly by gulping air at the water surface.

Without proper inflation of the swimbladder, larvae can still survive and grow but are likely to suffer from various skeletal defects and other problems e.g. lordosis (vertical curvature of the spine), scholiosis (horizontal curvature of the sine) and reduced growth rate due to a higher energy expenditure in maintaining their position. These traits can have serious economic effects on farmed marine fish species, as was experienced with seabass and sea bream in the Mediterranean, and their incidence should be reduced as much as possible. Divanach *et al* (1996) reviewed the occurrence of this problem and stated that in the 1970's and 80's up to 90% of cultured Mediterranean marine fry had to be discarded through such anomalies.

Improvements in overcoming this problem have been made by fitting "surface skimmers" to the rearing tanks, to remove oily films at the water surface which prevent the initial gulping (see Chatain & Ounais- Guschemann, 1990 for review), and by reduced water currents (allowing the larvae to reach the surface) and improved nutrition (see Kitajima *et al*, 1994 for review).

To avoid the build up of an oil layer on the water surface of the cod rearing tanks the present study used AlgaMac2000 (a spray dried single cell) as the dominant rotifer enrichment. Rotifers enriched with this product could be rinsed easily and quickly. The use of surface skimming via the central standpipe in the tanks also helped reduce any build up on the water surface. The cod in all tanks seemed to have no major problems in inflating the swimbladders, however as the fry metamorphosed and reached approximately 0.5-1g wet weight, an incidence of lordosis became apparent. At the time of writing, the proportion of the juvenile population suffering from lordosis is judged to be approximately 10-20%.

3.2.4.2. Swimbladder over- inflation

By far the most significant mortality of cod larvae experienced in this study, which affected all batches of larvae, was linked to the

appearance of hyperinflated swimbladders. When the larvae reached an age of approximately 25 days post-first feeding (PFF) they began to 'pop' to the surface and were unable to swim back down. The swimbladder was observed to be over-inflated, crushing the gut and rendering it useless. The duration of the phenomenon was only approximately 10 days for each batch of larvae, however high mortality rates were encountered during that period. The worst hit tanks were those receiving the A2 *Artemia* enrichments. Initial causes were speculated to be supersaturation of the incoming water, or bacterial degradation of the gas gland. Analysis of the incoming water indicated that gas supersaturation was unlikely to be a significant factor. Samples of affected and non- affected larvae were collected and fixed for histological examination, in order to describe the condition more fully. Affected fish exhibited marked degenerative changes in the brain which may have originated from a nutritional deficiency. Neural degradation would reduce the likelihood of the fish being able to control their gas gland (under nervous and hormonal control), potentially leading to over-inflation.

Reviewing the published literature, there are several similar incidences of this phenomenon, and it has been loosely termed Swimbladder Stress Syndrome (SBSS) (Bagarinao & Kungvankij 1986). It has been reported in Asian sea bass, *Lates calcifer* (Bagarinao & Kungvankij 1986), Mediterranean sea bass, *Dicentrarchus labrax* (Johnson & Katavic, 1984; Katavic, 1986; Nehr *et al*, 1996) and red sea bream *Pagrus major* (Foscarini, 1988) and the causes have been reviewed by Divanich *et al* (1996). A major incidence of this over-inflation occurred in the current study after grading one tank of larvae into another clean tank, with the appearance the following day of both many mortalities and 'floating' larvae.

If, as speculated, the cod larvae had degenerative brain tissue which predisposed them to SBSS, then the most likely candidate for a cause is the enrichments of the live feed. Marine fish larvae are unable to convert short chain fatty acids into the long chain polyunsaturated fatty acids (PUFA) that are essential for proper development of nervous tissue (Sargent *et al*, 1995). Rotifers and *Artemia* have to be enriched with these specific lipids in order to meet the requirements of the developing larvae. Increasing the level of these lipids and

particularly Docosahexaenoic acid (DHA, 22:6*n*3) in the *Artemia* enrichment has been shown to increase the resistance of fish larvae to stress (Dhert *et al*. 19990; Ashraf *et al*, 1993; Tuncer *et al*. 1993 and Ako *et al*, 1994). Several of the enrichment products used in the present study were selected on the basis of their good fatty acid profiles, as reported in previous Ardtoe-based nutrition work on halibut larvae (for example, Gara & Shields, 1997). In particular, tuna orbital oil (TOO) and AlgaMac 2000 impart relatively high levels of dietary DHA to the fish larvae via enriched *Artemia*. A2-enriched *Artemia*, as used in the present study, are known to contain much lower levels of DHA than TOO/AM-enriched *Artemia* and this may partially account for the different incidences of SBSS among cod batches in the current study.

Despite the adoption of enrichments with good fatty acid profiles in this study, SBSS was encountered in all diet treatments, indicating that the nutritional requirements of the cod larvae were not being fully met in any case. Nonetheless, the observed diet-dependent differences in the extent of the syndrome (greatest amongst larvae fed A2-enriched rotifers/*Artemia*) support a nutritional basis. Comparison of growth rates in this study with those reported by Rosenlund *et al* (1994) indicate that the greater use of enrichments based on oil emulsions may be more beneficial to the cod larvae. Dhert and Naess (unpublished data) report that DHA Selco, as used in the Norwegian study, imparts a 3-fold increase in the levels of essential n-3 PUFA in *Artemia*, relative to "conventional" enrichment methods.

Another important dietary factor that must be considered is the initial presentation of *Artemia* nauplii, rather than enriched *Artemia*, to the cod larvae. Although *Artemia* nauplii may be beneficial to small start-feeding larvae in terms of their smaller size and higher energy content, it is not possible to manipulate the nutritional composition of the nauplii because they do not have a functional digestive tract. Nauplii therefore have a poor PUFA profile relative to enriched *Artemia* and this may be deleterious if the nauplii are fed to excess, or are supplied to the cod larvae for too long a period. Several studies have shown that the survival of fish larvae reared on *Artemia* nauplii depends on the *Artemia* batch used. For example Howell (1984) reared cod larvae on *Artemia* from San Francisco Bay (SFB) and

Brazil, and found that SFB *Artemia* were unsuitable for rearing unless they had been fed the microalgae *Isochrysis* for 2 days prior to feeding. Leger (1986) showed that the survival of various marine fish larvae was influenced by the level of Eicosapentaenoic acid (EPA, 20:5*n*3) in unenriched *Artemia*. This was later confirmed in striped bass *Morone saxatilis* (Webster & Lovell 1990) and sea bass *Dicentrarchus labrax* (Sorgeloos *et al*, 1988). When considering these limitations of the naupliar stage, it is possible that in the current study *Artemia* nauplii were presented to the cod larvae in excess, or for too long. It is notable that Rosenlund *et al* (1994) did not report any feeding of nauplii in Norwegian intensive cod rearing trials, only enriched *Artemia*.

Aside from the nutritional factors noted above, it is possible that the feeding strategy adopted (continuous high prey densities) resulted in excessive depletion of the dietary fatty acid profiles while the rotifers/*Artemia* resided in the larval rearing tanks. Also, the tank husbandry procedures used in 1997 may have subjected the cod larvae to chronic stress that was expressed in the form of SBSS, with those larvae reared on A2-enriched *Artemia* being more susceptible to the condition. Future research should therefore encompass both the environmental and nutritional requirements of the animals. In particular, diet enrichment procedures and methods of diet presentation should be refined to reduce the occurrence of this major mortality factor.

Figure 4. Cod larval dry weights with age post first feeding. (Three populations used for weights)

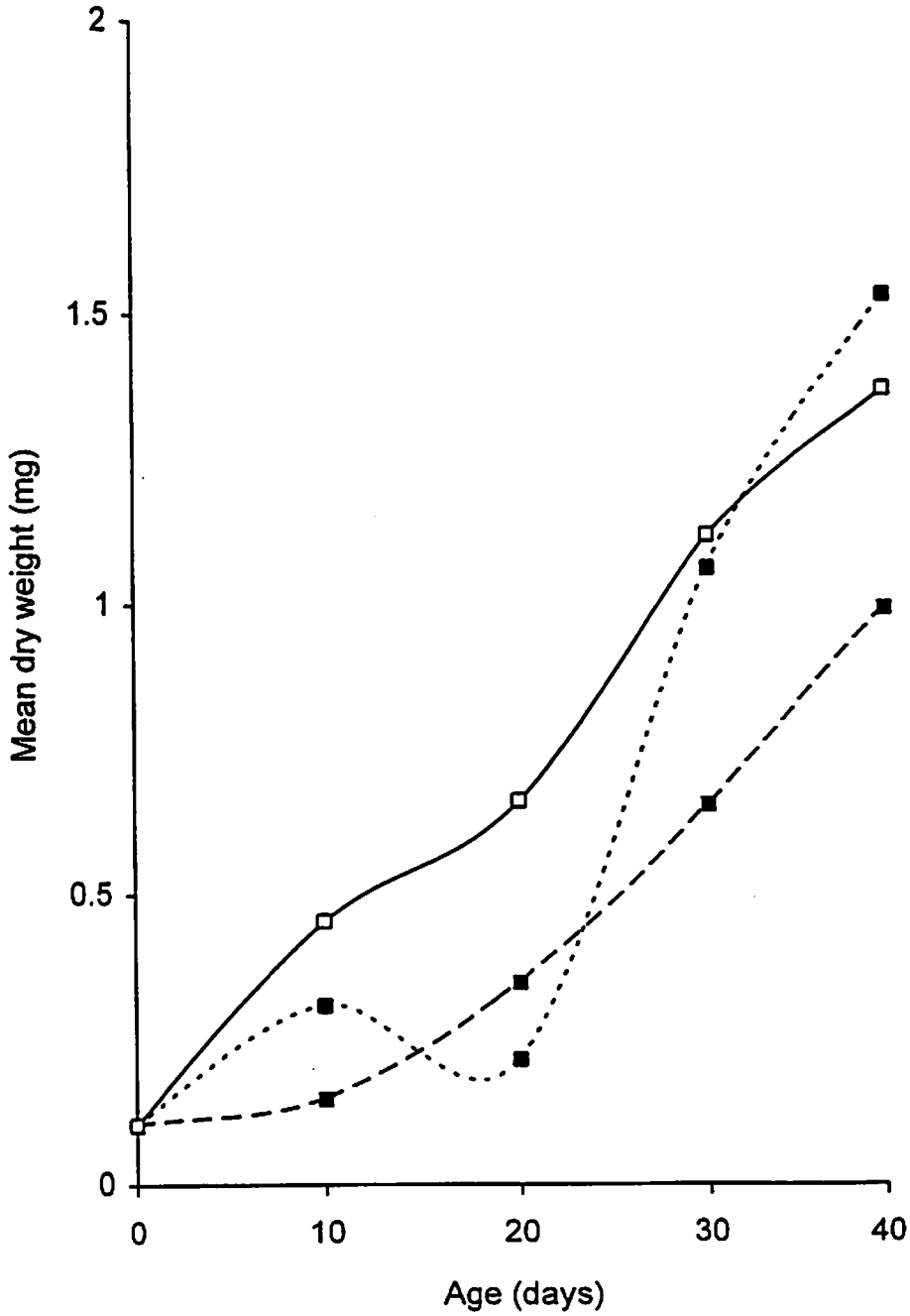
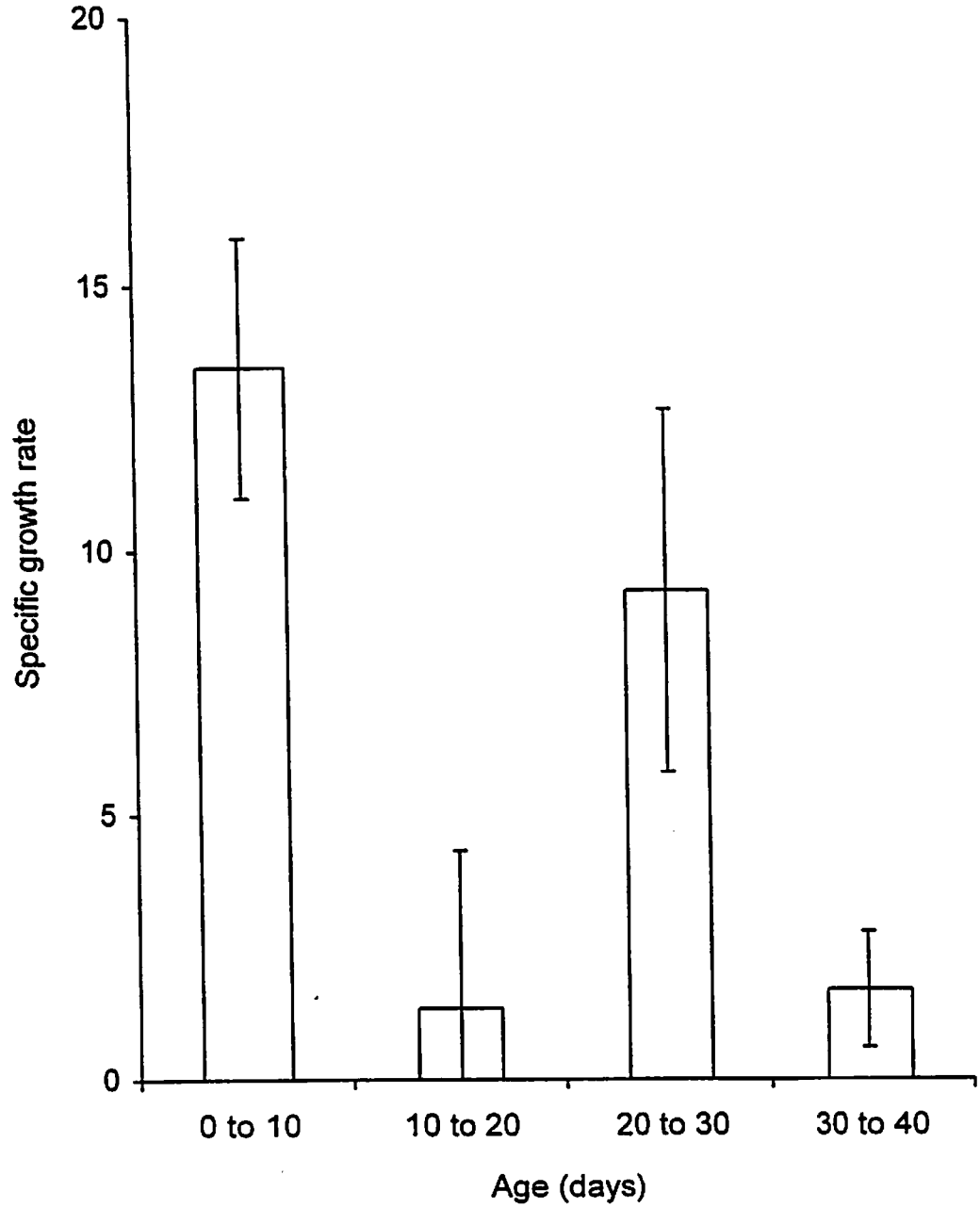


Figure 5. Mean specific growth rates of cod larvae (+/- SEM)



3.3. Growout

3.3.1. Weaning

A total of 3,711 surviving cod fry from the larval rearing trials were graded into shallow tanks according to size, in preparation for weaning. The separate groups of fry were amalgamated at this stage, therefore it is not possible to discern the effects of different rearing histories on weaning success. The largest fish began weaning at age 45- 55 post first feeding. The smaller fish were held in smaller tanks and maintained on *Artemia* until day 65-70 PFF, when they were judged to have reached an appropriate size for weaning. The fry accepted the formulated dry diet readily, although the animals were gradually weaned off *Artemia* over a period of at least two weeks and, in the case of the smallest fish, up to 1 month.

By reference to published reports, cannibalism was expected to be a significant problem at this developmental stage. Aggressive behaviour, including cannibalism, was indeed pronounced during the weaning period. However, the rates of mortality experienced through aggression were small in comparison to the earlier phenomenon of over-inflated swim bladders. The duration of the aggressive stage was approximately two weeks and overall survival rate through weaning was 33.4%

Population size variation in the weaning tanks increased rapidly, possibly due to the more effective uptake of dry diet by a few individual fish. These large fish were noticeably aggressive to conspecifics, with sudden lunges at smaller fish causing a "panic wave" to spread round the weaning tank. Large size variations in fish populations have previously been shown to have a significant effect on the incidence of cannibalism (Folkvord & Ottera, 1993), with the smallest fish suffering the highest mortality. Mortality rate has also been shown to rise due to deaths stemming from non-fatal attacks and this was observed in the present study. It was found that the obvious large aggressive cod could be removed by hand grading. However it was very difficult to effectively grade the remaining population, due mainly to the schooling behaviour of the fish. It is clear that mechanical grading devices will be required for a commercial scale cod hatchery, but it was beyond the scope of this preliminary study to test the effectiveness of such systems. In any case, aggressive behaviour was frequently observed between equal-sized animals, pointing to factors other than population size variation in the aggression response.

Folkvord (1991) showed that the availability of food is another significant stimulus to the onset of cannibalism. Feeding frequency and ration level have been suggested as factors that may influence the rate of cannibalism (see Hecht & Pienaar, 1993 for review), however there are many contrasting results. Katavic *et al* (1989) found that the rate of cannibalism in sea bass increased when the fry were fed only once a day, but the rate was lower when feed was available three or six times a day. Folkvord & Ottera (1993) however, found no increase in cannibalism when the feeding frequency was reduced from once a minute to only four times a day. Interestingly they observed no increase in growth rate at the higher feeding frequency. Feeding regime during weaning should certainly be the focus of future cod research. The effect of density on cannibalism has been reviewed by Hecht & Pienaar (1993), and in general there exists a positive density- dependent effect on the incidence of cannibalism. Relatively low densities of cod fry were used in the current study, due to the limited numbers of animals available and this is viewed as an area requiring further work. It is likely that the economic advantage of highly stocked weaning tanks will encourage the adoption of greater stocking densities in order to maximise the tank volume in a hatchery and reduce overall costs of equipment.

3.3.2. Growth and condition parameters of weaned cod

Growth and survival of the cod fry on the commercial weaning diet formulation was encouraging. The mean weights of fry in the four tanks at day 100 PFF are shown in table 4. Large weight variations within tanks were recorded, as illustrated by the large standard deviations and coefficients of variation. The biomass of fish (calculated from the mean weights) was low in all tanks, due to the restricted total numbers of fish. As stated previously, it is envisaged that higher stocking densities will be required for viable commercial operation in future. The coefficients of variation were observed to increase according to size grade for the day 100 weight measurements, pointing to hierarchical effects occurring in the tanks. This may be due to dominant fish consuming a larger proportion of the available feed and increasing their growth relative to conspecifics. Also, the presence of dominant fish in tanks has been shown to reduce the growth rate of subordinates via other factors such as stress (Jobling, 1995).

Table 5. Wet weight and growth parameters of cod fry at day 100PFF.

	W19	W14	W15	W20
Mean wet wt (g)(+/- SD)	0.45 (0.13)	0.92 (0.21)	1.17 (0.86)	2.42 (1.00)
CV	29.7%	22.4%	73.4%	41.2%
number of fry	455	378	473	35
Biomass (g)	206.4	349.6	554.2	84.7
Density (Kg/m^3)	0.41	0.70	1.11	0.42

Coefficient of variation = (Standard deviation/ mean)* 100%

The length/weight relationship for cod at 100 day PFF is shown in Figure 6. As expected, there was an exponential rate of weight increase with increasing length. The wet weight of the liver increased linearly with whole body wet weight (figure 7). When expressed as Liver: Somatic index (LSI), the liver size of the fry was not seen to reach a level indicative of obesity. When Liver: Somatic index was plotted against wet weight, a highly significant regression was obtained using a natural logarithmic relationship ($F_{1,28}$= 21.93, $p<0.001$) (see figure 8). This shows that the rate of increase in LSI decreased with increasing fish weight, reaching an asymptote at a value of 10%, for fry of approximately 5g wet weight.

Previous studies have suggested that LSI values in excess of 9% are indicative of obesity in farmed cod, while LSI values of between 2% and 5% have been recorded in wild cod (Jobling, 1988). High levels of fat in the liver (indicated by high LSI values) can lead to reduced liver activity and eventually death. This is extremely important in white fish such as cod, where most of the lipid in the animal is stored in the liver, with muscle storage only accounting for 1% of the total (Love, 1980). Jobling (1988) reviewed the influences on LSI in cod and found that growth rate was positively related to LSI. This was also evident in the present study, with the largest size grades (having highest growth rate) exhibiting the highest LSI values. However, as referred to above , the LSI levelled out above the weight of 5g.

When condition factor was plotted against length, a U shaped curve was found (figure 9), which suggests a compensatory growth response by the fry following transition from live feed to the more energetically favourable formulated weaning diet, ie the cod were nutritionally deficient during the

latter stages of live feeding. Further research is required to confirm this suggestion, involving the sequential sampling of animals during the weaning process.

It is expected that further refinements to the live feeding regimes for cod will act to reduce the compensatory growth response during this developmental stage. Also, it is possible that weaning diets containing higher lipid contents (eg 16%, rather than the 12% used in this study) could be applied for a short period, in order to speed the growth of the fry through the compensatory phase. The short term use of dry diets containing increased levels of PUFA may also help the fry 'recover' from deficiencies during live feeding, as has been shown for turbot fry (Mourente & Tocher, 1992). In considering these possibilities, it is recognised that LSI in cod increases in response to the lipid content of the diet (Lie *et al*, 1986), therefore feeding strategies during weaning would need to balance short term growth advantages against the risk of excessive lipid accumulation in the liver.

Figure 6. Length/weight relationship for cod fry (100 days PFF)

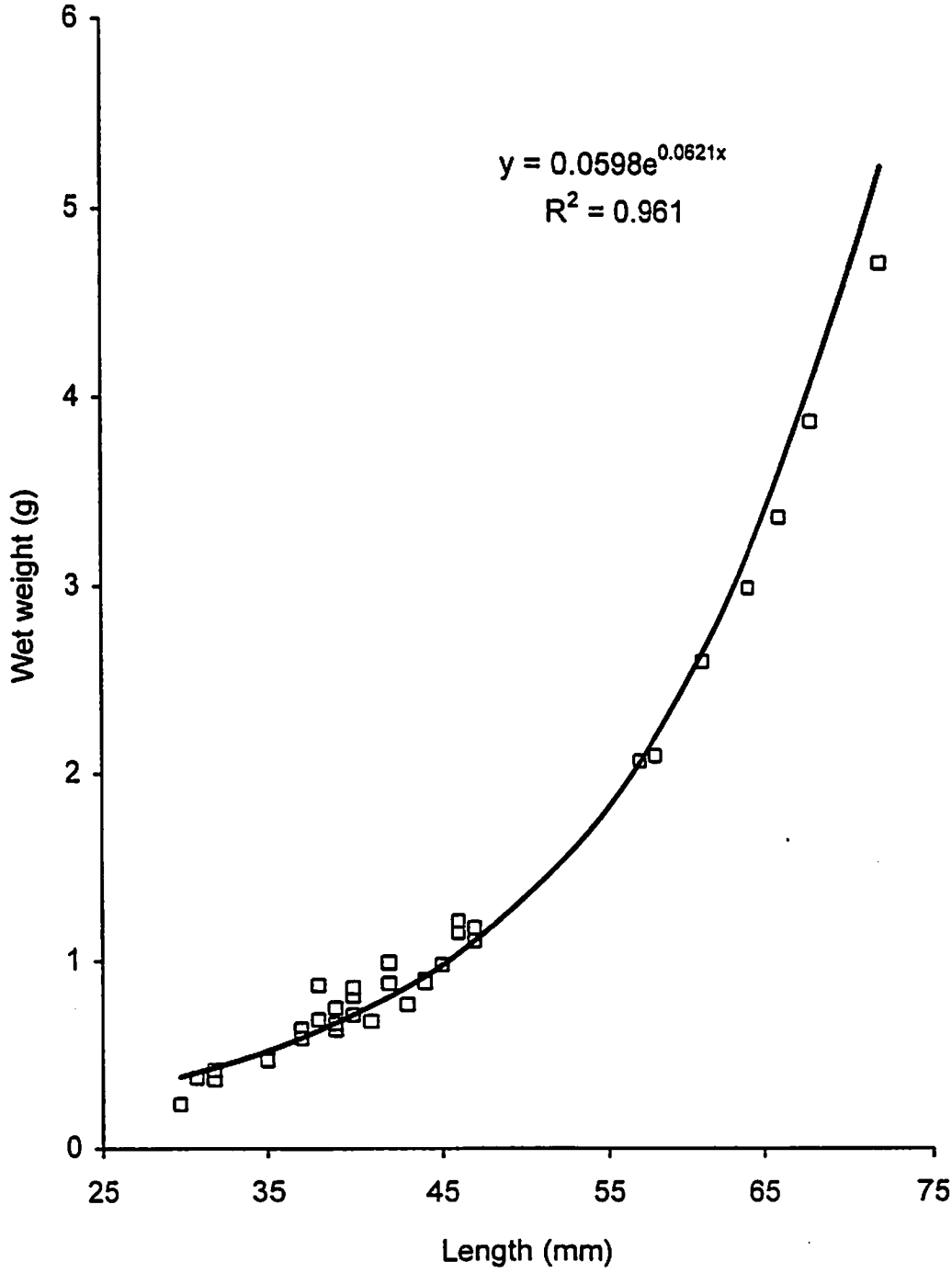
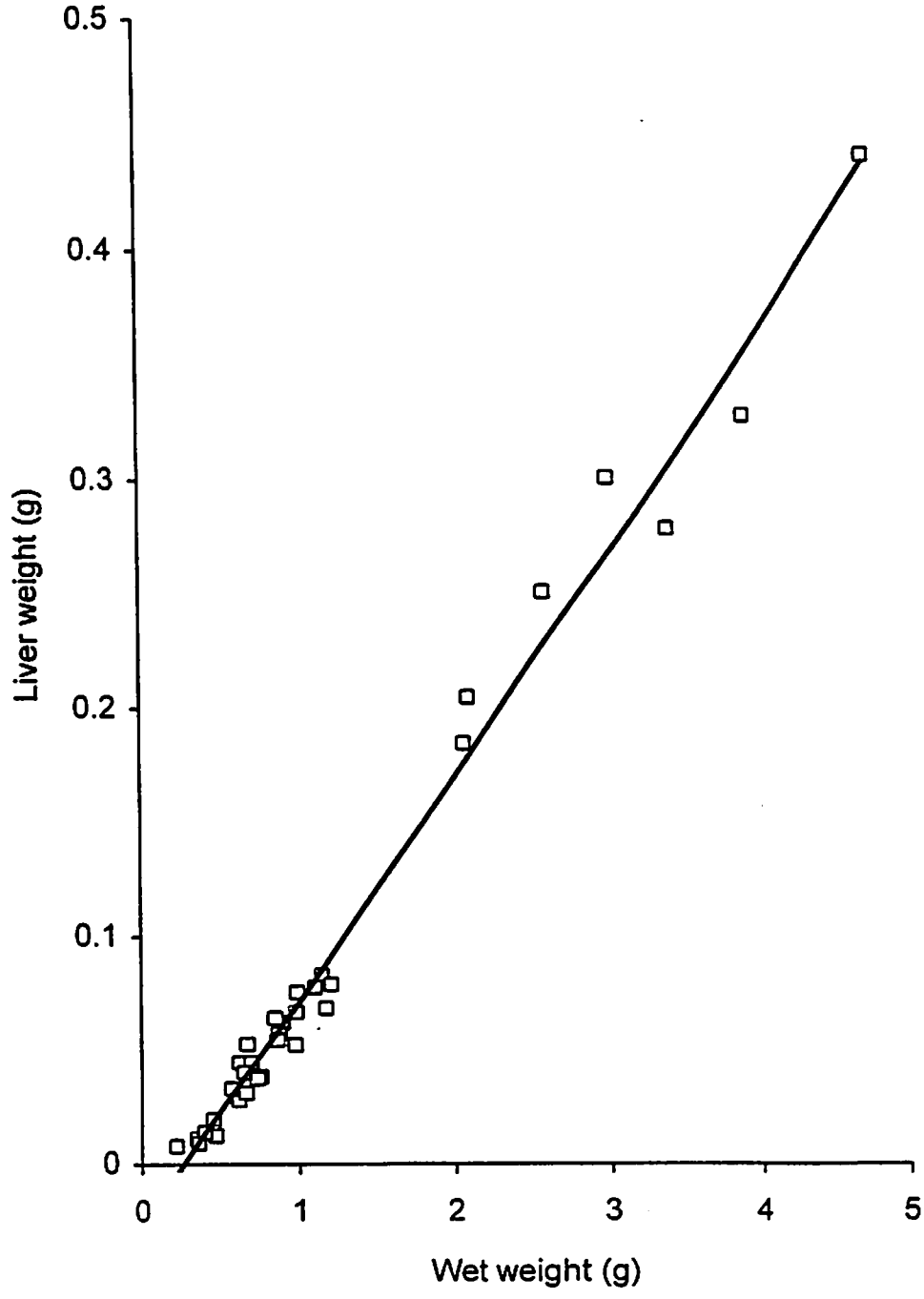


Figure7. Plot of liver wet weight against wet weight of cod fry (100 days PFF)



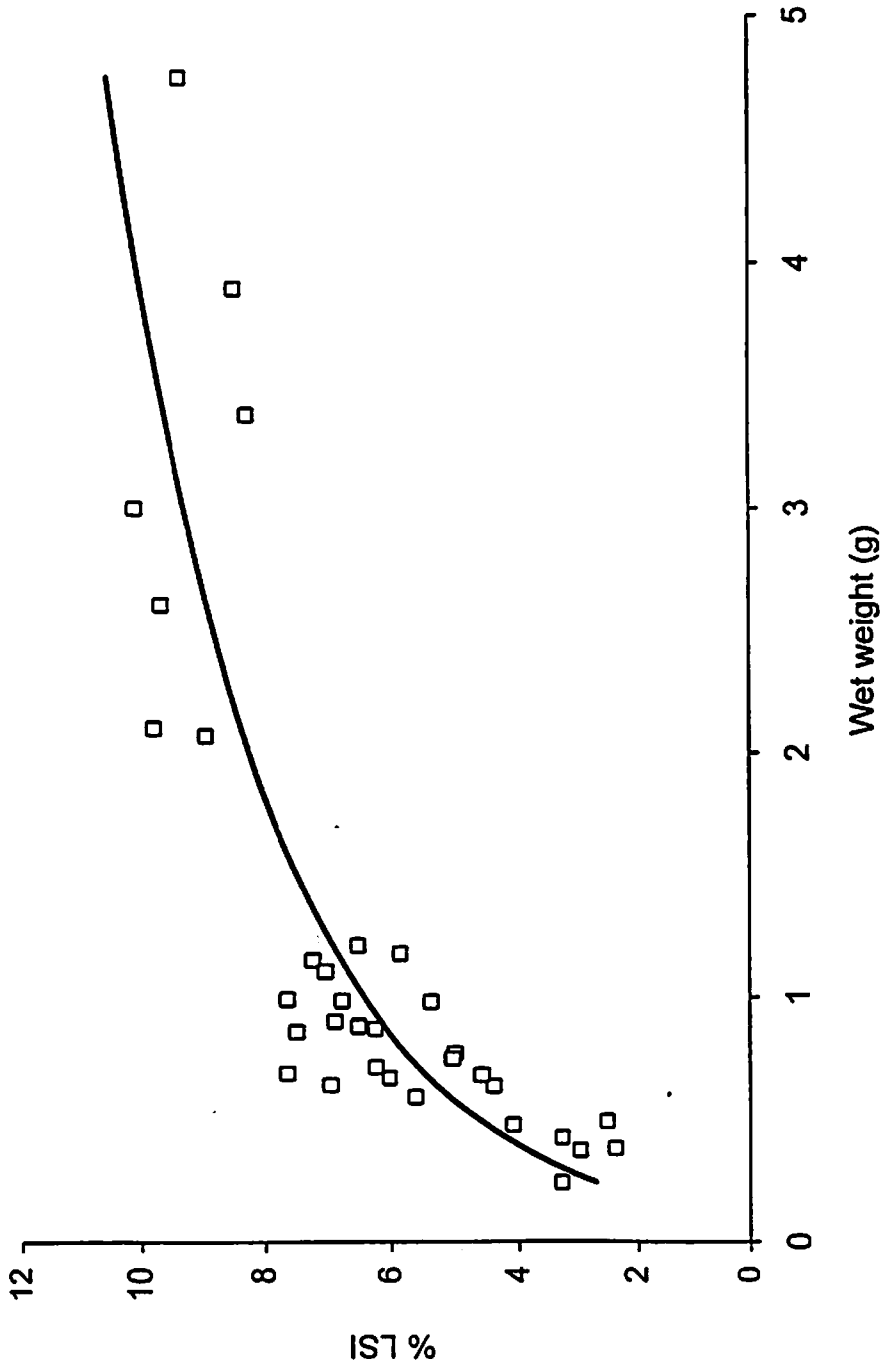
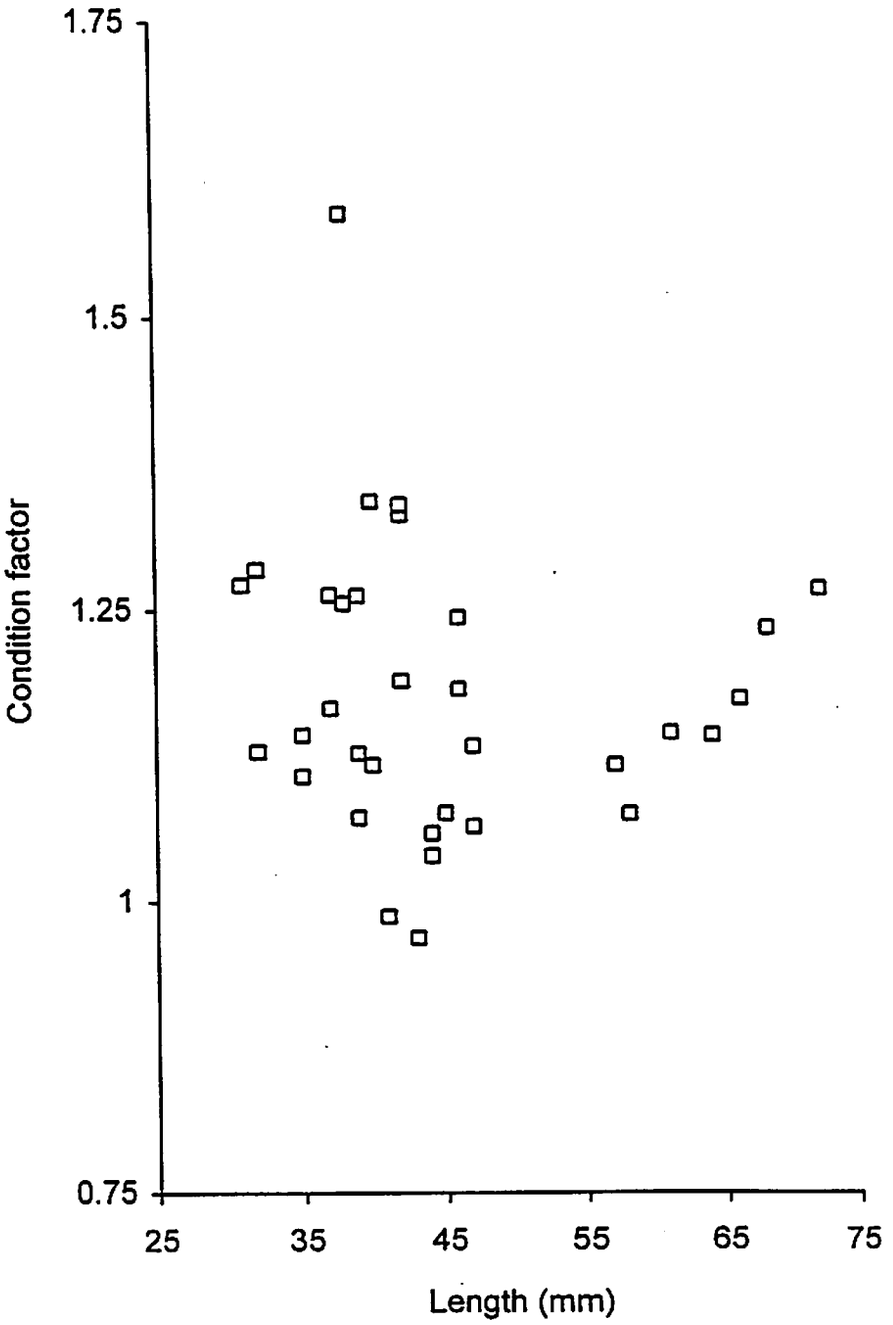


Figure 8. Relationship of Liver Somatic Index and wet weight of fry (100 day PFF)

Figure 9. Relationship of condition factor with length of fry (100 days PFF)



4.0 General Discussion

This preliminary study has confirmed the feasibility of rearing Atlantic cod using UK intensive larviculture techniques. The production of more than 1,200 weaned fry from the small scale 1997 rearing trials is very encouraging for the expansion of cod production to commercial levels over the time scale envisaged by interested UK parties. As expected, egg supply was not a limiting factor and satisfactory survivals were achieved through the egg incubation phase. However, clear bottlenecks to cost efficient hatchery production were identified during the cod live feeding and weaning phases and these should be the focus of future research. Some of these bottlenecks were predicted from previous cod rearing reports, while others emerged specifically within this project.

The results showed that, under the best 1997 rearing regime, an average survival of more than 9% through larval rearing (start-feed to onset of weaning) could be achieved (Table 6). All groups of larvae exhibited high rates of feed uptake and initially high growth rates, however survival to weaning varied greatly between groups, dependent on nutritional history. The over-inflated swim bladder syndrome was the major mortality factor during the live feeding phase, accounting for more than 50% losses over a period of approximately 10 days. Aggression and cannibalism amongst the surviving fry were subsequently observed, again over a relatively short time period. Losses due to aggressive behaviour were relatively low compared to the earlier swim bladder syndrome and survival through weaning exceeded 33% (Table 6). Nonetheless, it is recognised that suitable handling and feeding strategies need to be devised for this aggressive stage, in order to avoid significant losses in commercial scale operations. The formulated dry feeds used during the early nursery phase elicited a good growth response by the cod and liver indices did not rise to critical levels using a diet containing 12% fat. It will be necessary to continue monitoring the farmed 1997 stock to determine if these encouraging traits continue during the growout phase.

In comparing the overall rearing performance from the 1997 UK pilot trials with other reports, it can be seen that survival rates are on a par with reported Norwegian and Canadian results and could be significantly improved if mortalities associated with over-inflated swim bladders can be reduced or eradicated. Norwegian workers have reported pre-weaning survivals of between 5 and 20% (Folkvord, 1991) using extensive systems. Weaning mortalities were very much higher than the figure obtained in this study (1- 2.5% per day), largely due to the wide size ranges of fry present in the lagoons and the inefficient presentation of dry feed. Howell (1982) reported larval survivals between 2 and 5% for cod reared in experimental scale intensive systems. Although numbers of fry were not presented, Howell stated that cannibalism is likely to be an important problem to intensive culture. Rosenlund *et al* (1994), in larger scale intensive systems, recorded larval rearing survivals of up to 20%, yet extremely low (0.5%) weaning survivals were reported: of 20,000 fry

reaching weaning only 1,000 survived this phase. Reports of cod survival from Canada are not yet widely available, with information being based on press articles and personal communications. The 1997 Nautilus report prepared for HIE stated that an overall figure of 10% from larva to weaned fry is a realistic figure. Initial work at St Johns, Newfoundland demonstrated a weaning survival of 16% (Anon). It is known that 20,000 weaned fry were produced at the Canadian Sea Forest Plantations hatchery in 1996, therefore experience has been generated in handling large numbers of larvae and fry and advice should be sought from this source for future, larger scale UK work.

Regarding the economic costs of intensive hatchery production, preliminary calculations using a straightforward hatchery model indicate production costs for larger numbers of fry (32,000 animals) as low as £0.65 per 5g cod, using survival rates from the current project and Ardtoe feed costs. This cost could be significantly reduced in future by adopting more efficient food production techniques from the European sea bass and sea bream industries. The desired lower costs for viable hatchery production of cod therefore look to be achievable and several key research topics have been identified within this project, aimed at reducing production costs. Clearly, the nutritional requirements of cod larvae at both the rotifer and *Artemia* phase are not fully understood and better feeding strategies should enable significant reductions in mortality during the late larval phase. Also, husbandry procedures will need to be devised to handle greater stocking densities during larval rearing and, especially, the weaning phase, in order to maximise the efficiency of use of the hatchery infrastructure.

Table 6. Summary table of larval rearing survivals in 1997.

Larval batch	Tank Volume	No. stocked	No. to weaning	Age	% Survival to weaning
1	500 Litres	7500	0	50	0
1	1000 Litres	15000	0	50	0
2	500 Litres	7500	64	48	0.9
2	1000 Litres	15000	0	8	0 crashed
3	500 Litres	7500	300	45	4
3	1000 Litres	15000	1343	45	9
3	1000 Litres	16500	2004	45	12
Total		**84000**	**3711**		**4.4**
'best' case	**AlgaMac**	**39000**	**3647**		**9.4**

No.s of fry to weaning	No.s post weaning (- sampled fish)	Survival through weaning
3711	1240	33.4%

Overall survival from larva to weaned fry= 3.3%

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