SAMSardtoe

Cod hatchery investigations 2001-2002

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Seafish Aquaculture undertakes research to identify best aquaculture systems and methods to be applied in the cultivation of marine fish and shellfish. We also support, through advice and consultancy, the development of the aquaculture industry and the generic promotion of fish and shellfish products.

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1. Introduction

Since 1997 there has been renewed interest in the commercial development of farmed Atlantic cod *Gadus morhua*. This is primarily due to supply from wild cod stocks, which have declined dramatically since 1997. Stock decline has resulted in large price increases; for example, price rose 22 % from 2000 to 2001. Despite the increasing price, the demand for cod in the UK remains stable at 170,000 tonnes per annum. Of this demand, 110,000 tonnes are imported into the UK. Over 50 tonnes per week of large, high quality fillets are being delivered by air from Iceland at a price of £5 to £10 per kg. A number of economic summaries have concluded that efficient, large-scale production of high quality farmed cod would now be economically viable. With the economic threshold for cod farming becoming realised, a number of aquaculture companies have begun to look in more detail at the development of a marine farming industry in the UK.

Modest numbers of farmed cod have been successfully produced to harvest weight in the UK (100 tonnes in 2002, compared with 10 tonnes in 2000). This growth is due in part to the completion of the Cod Farming Demonstration Project by Seafish Aquaculture in 2000. During this project a number of hatchery bottlenecks were identified that would prevent a rapid and large-scale development of a cod farming industry. Further improvements in a number of general husbandry methods would provide valuable information, maximise efficiency and facilitate the development of the industry. In addition, the development of year round spawning stocks would provide the necessary egg material and flexibility for economic development.

Project objectives:

Seafish Aquaculture investigated and developed techniques for improving performance and reducing costs of hatchery production. Cod juveniles produced were transferred to industrial on-growers in the UK.

The detailed objectives of this project were:

1. To study the effects of algal concentration for cod larvae cultivation from the time of hatching until the end of first-feeding.
2. To study the effects of aeration/ turbulence in promoting foraging in commercial scale tanks from the time of hatching until the end of first-feeding.
3. To investigate the effect of salinity on cod swim bladder inflation and survival.
4. To optimise the feeding protocols of cod larvae with enriched rotifers and *Artemia*, using an extensive range of commercial proprietary enrichment products.
5. To optimise the protocols for transfer of cod larvae from live food onto inert weaning diets.
6. To culture cod larvae from out-of-season spawning stocks, and investigate their performance.

Expected project outcomes:

- A number of hatchery operators are turning to cod juvenile production as an alternative species for aquaculture. Optimisation of hatchery protocols will help the reliable and economic supply of juveniles to the industry.
- Reports will be produced on each of the research areas outlined above and refined rearing protocols and hatchery techniques will be transferred to the industry.
- Growing larvae from out-of-season broodstock will help a faster development of the farm on-growing sector, which will play an increasing role in the supply to the processing and retail sectors.

2. The effects of different algae concentration on early rearing stages in the production of cod juveniles

2.1 Introduction

As with many marine species, the addition of algae to the rearing tanks (green water technique) is widely regarded as essential for the early first feeding stages of larval cod. It is therefore common practice to add algae on a daily basis to larval rearing tanks from day 1 *post* hatch. The specific benefit of the algae is currently thought to be a combination of adjustment of the physical environment e.g. light attenuation; an overall improvement of water quality (for example, dissolved oxygen maintenance); and as a possible stimulant to the development of the larval digestive system to enable successful first feeding.

Currently the quantity of algal addition is determined largely by qualitative assessment. However the production of sufficient algae on a commercial scale is a significant investment both in terms of manpower and direct cost and a more quantitative determination of optimum algal requirements would be of significant benefit to industry.

2.2 Materials and methods

The trial was conducted in replicated 100L tanks in the Seafish hatchery at Ardtoe. The trial was run for 20 days *post* hatch with three treatments consisting of different quantities of daily algae (*Nannochloris atomus*) addition plus a control treatment with no algal addition:

- Control: Control with no algal addition.
- Treatment 2: Standard addition as used in current "best practice" of 2 L of algae per 100 L (mean final concentration = 280 000 cells ml^{-1}).
- Treatment 3: Double standard addition (mean = 570 000 cells ml^{-1}).
- Treatment 4: Three times standard addition (mean = 950 000 cells ml^{-1}).

16 tanks occupying a temperature/light controlled room were randomly allocated to the different treatments (four replicates per treatment). All other parameters conformed to "best practice" larval rearing requirements to date.

Daily algal cell counts per tank were carried out indirectly by using a turbidimeter. Turbidity readings (nephelometric turbidity units [NTU]) were then correlated with standard concentrations of algal cells per ml and algal concentration per treatment was calculated. Percentage survival and growth rate was assessed at the end of the trial.

2.3 Results and conclusions

Mean daily turbidity readings for each treatment are presented in Fig. 2.1*a*. Corresponding mean algal cell concentration per ml is presented in Fig. 2.1*b*. Algal cell counts differed significantly between treatments for the duration of the experiment ($F_{3,19} = 214.628$, $p < 0.001$; Fig. 2.1*b*).

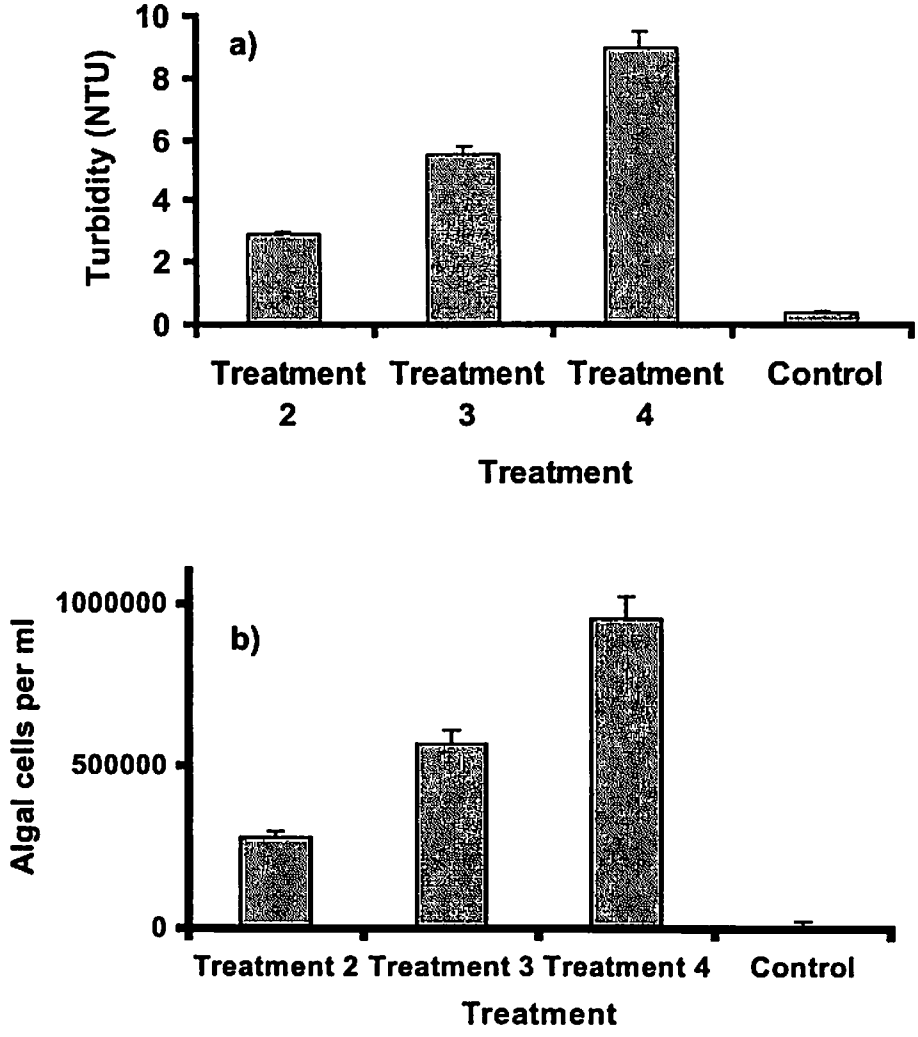


Fig. 2.1: Tank algal cell concentrations in four different algal dosing treatments measured as both *a)* NTU's and *b)* algal cells per ml. Error bars denote standard error. See text for statistical analysis and algal quantities within treatments.

Mean larval growth did not differ significantly between algal dosing treatments (mean specific growth rate = 5.95±0.94 (= S.D.). There was an almost significant effect of algal treatment on survival (One-way ANOVA: $F_{3,16} = 2.772$, $p = 0.08$), high algal treatments doing less well than the control and treatment 1 (Fig. 2.2). Data analysis showed an unforeseen room effect on larval survival, tanks on one side of the room surviving better than the other ($F_{1,18} = 5.153$, $p < 0.05$). This was associated with two separate water lines supplying water to different groups of tanks at either side of the room. This effect can be controlled for by using a General Linear

Model (GLM) , which can disassociate the effects of one factor (e.g.: algal treatment) from the overlying effects of another (e.g.: water line). By controlling for this effect, a significant effect of algal treatment could be discerned ($p < 0.05$). For clarity, Fig. 2.2 presents survival data with regard to experimental treatment from the side of the room with most tanks (12 out of the 20 tanks).

Table 2.1: Results of a General Linear Model for factors affecting survival in the algal concentration experiment.

Factor	DF	Seq SS	Adj SS	Adj MS	F	*P*
Water line/room side	1	77.152	85.546	85.546	**9.00**	**0.009**
Algal concentration	3	126.937	126.937	42.312	**4.45**	**0.020**
Error	15	142.563	142.563	9.504		
Total	19	346.652				

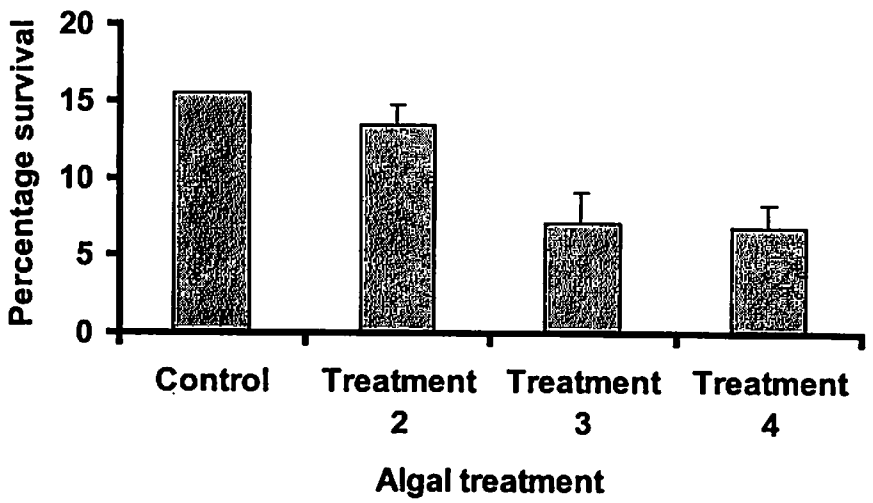


Fig. 2.2: The relationship between algal treatment and percentage survival at 20 days *post*-hatch for larval cod. Error bars denote standard error. See Table 2.1 for statistical analysis, and text for algal quantities within treatments.

Algal concentrations above 600,000 cells per ml produced lower survival at the end of the experiment. Furthermore, survival in the low algal concentration treatment did not differ significantly from the control (no algal addition). It therefore appears that algal addition above 600,000 cells/ml in cod larviculture has no discernible benefits.

The above results are interesting in the light of similar work carried out at Ardtoe on halibut (*Hippoglossus hippoglossus*) larvae. In the case of halibut, high levels of algal inoculation clearly improved both larval survival and growth, the exact opposite of the results presented here. *Nannochloris* was also identified to provide better results when compared to *Isochrysis*, *Rhynomonas* and *Pavlova* (Seafish &

SAMS, unpublished results). This highlights the risks implicit in transferring similar husbandry protocols between marine fish species in aquaculture, and the necessity of elucidating such protocols specific to each species.

On a similar experiment on cod recently carried out by workers in Norway (T. van der Meeren & A. Mangor-Jensen), the authors reported better survival in green water, using the algae *Isochrysis*, versus a control of clear water. However, the algal density used in this experiment was 100 - 180000 cells.ml^{-1}, under half as much as the lowest algal concentration used in the Seafish trial. Larval cod are highly visual foragers; their eyes are very large in proportion to their head morphometrics. For this species, high turbidity may well compromise their ability to search for prey. Low levels of algae may be beneficial, perhaps through maintenance of the nutritional value of rotifers grazing on the algae. Considering the findings of both pieces of work in combination, we can conclude that addition of moderate concentrations of algae (between 180000 cells.ml^{-1} of *Isochrysis*, and below 300,000 cells.ml^{-1} of *Nannochloris*) can improve cod larval performance.

3. The effects of turbulence on larval cod behaviour

3.1 Introduction

The correct parameters to maximise foraging ability, survival, and growth are essential in successful cod (*Gadus morhua*) larviculture. Furthermore, environmental parameters immediately *post*-hatch must be correctly evaluated in order to promote survival and subsequent performance. Larval fish can be compromised by inappropriate levels of turbulence in tanks, mediated by aeration, tank volume and tank configuration. This can be due to either insufficient movement of prey items relative to the larvae, so they are simply not coming into contact with prey at a maximal rate, or to physical damaging of the larvae by excessive turbulence, which would also compromise foraging ability. This study assesses a range of different aeration (resulting in turbulence) rates in production scale tanks in order to promote maximal foraging ability, which in turn may lead to improved growth and performance.

3.2 Materials and methods

Six 1300 L tanks were stocked with 100 000 stage V cod eggs each. Routine husbandry and feeding regimes were established immediately *post*-hatch (Cutts & Shields, 2001) with the exception of aeration. Aeration was applied centrally to each tank, via an air-collar at the base of each standpipe. Aeration regimes were set at 0.10, 0.35 and 0.50 L min^{-1} (low, medium and high treatments), with two tanks per treatment. This provided different levels of turbulence. The experiment was terminated at 13 dph (days *post*-hatch), with air inflow rates reset to 0.35 L min^{-1}.

Larval behaviour was assessed on four separate occasions: at 9, 12, 17 and 25 dph. This provided two sets of measurements during the experimental aeration period and two sets during normal husbandry practice (*post* day 13). Five larval cod were randomly selected from each of the six tanks, and their behaviour assessed. This involved observing each larva for one minute, and counting the number of occasions when the fish was actively foraging. This was characterised as any directional movement, and was distinct from holding station or buffeting from turbulence. Duration of foraging within one minute was not assessed, but absolute number of directional swimming activities was deemed a suitable index of foraging intent, if not foraging success.

Larval dry weight (at day 16) and percentage swim bladder inflation (at days 12 and 16) were assessed as measures of larval performance.

3.3 Results and conclusions

Figure 3.1 shows the differences in foraging activity between the three treatments from 9 to 25 dph. At 9 dph, larvae in the medium aeration regime foraged at a significantly greater intensity than those in the high aeration regime (One-way ANOVA: $F_{(1,19)} = 6.68$, $p < 0.05$). Larvae from the low aeration regime performed at a midpoint between the high and medium regimes. Significant differences persisted at 12 dph, with larvae in the medium regime again perfoming at a greater intensity ($F_{(2,28)} = 4.14$, $p < 0.05$). However, there were no significant differences in foraging activity

during the last two sampling periods (17 and 25 dph), since aeration had been restored to a constant value between treatments at 13 dph.

Percentage swim bladder inflation was not significantly different at either sampling point (day 12: 54±23 (= S.D.), 88±18 & 55±17; day 16: 77±10, 61±22 & 70±8 % for the low, medium and high regimes respectively). Furthermore, there was no difference in larval dry weight at day 16: 250±70, 220±20 & 240±40 µg for the low, medium and high regimes respectively.

The above results show that turbulence strongly affected the ability to forage in larval cod. Larvae in the medium aeration regime foraged at a consistently higher rate than the other two treatments, which may affect subsequent growth and survival. The larvae in the low and high aeration regimes foraged at consistently lower rates. This may be due to two distinct reasons: in the low aeration treatment, there may have been insufficient turbulence to increase encounter rates between predator and prey. Conversely, in the high aeration regime, higher turbulence may buffet larvae to such an extent that they could not forage effectively. Although not measured, this was observed in the two high aeration tanks.

Although different air inflow rates did have a pronounced effect on directional movement, it is important to note that those effects were mediated *via* turbulence. Turbulence was not measured directly, and it will be affected not only by air inflow (measured here) but also by water inflow, tank configuration and tank volume. By maintaining all the other parameters in this experiment equal, and by ruling out any differences in oxygen concentration in the tanks, the experiment gave a good indirect measure of the effect of different turbulence levels on foraging behavior and success for larval cod.

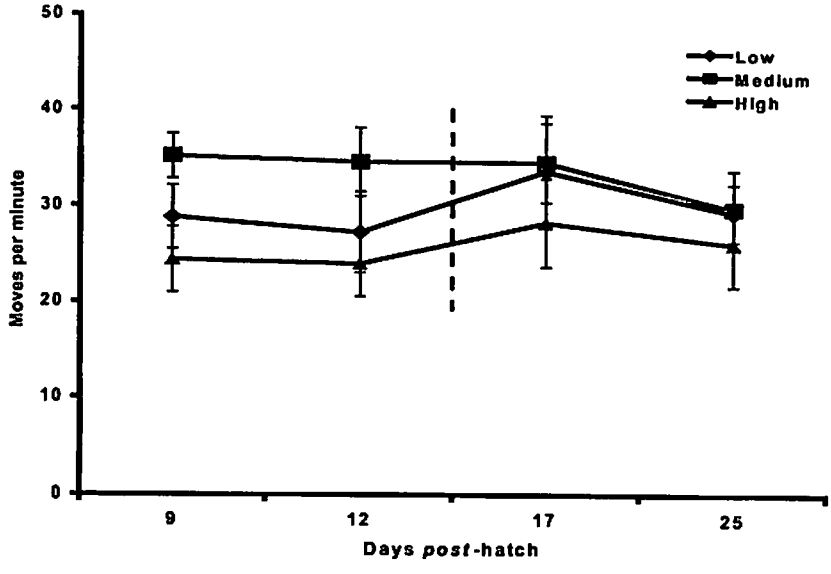


Fig. 3.1: Mean foraging activity (measured as directional moves per minute) of cod larvae in three aeration treatments from 9 to 25 dph. Differences in aeration were maintained until 13 dph (dashed line), with a single aeration regime thereafter (0.35 L min^{-1}). Mean values are derived from ten larvae per treatment. Error bars denote standard error. See text for statistical analysis.

4. The effects of salinity on larval cod performance

4.1 Introduction

In addition to environmental parameters *post* first-feeding, the optimal conditions *pre* first-feeding must also be determined. Immediately *post*-hatch, cod larvae are known to float on the water surface, bound by the buoyancy of the yolk-sac. Previous work at Ardtoe found that surface-bound larvae had significantly larger yolk-sacs than larvae with the ability to swim through the water column. This may compromise their ability to feed, as cod larvae will prey on food items before full yolk-sac absorption, and barriers to this learning behaviour may compromise future performance. A preliminary experiment was set up in 2001, with the aim to investigate whether lower salinity would decrease the buoyancy of surface-bound larvae, allowing them to move into the water column and experience prey items. A second more rigourous and replicated trial was carried out in 2002.

4.2 Materials and Methods

In 2001, three 100 L tanks were each stocked with 1000 Stage V cod eggs. Routine husbandry procedures were established immediately *post* hatch. However, each tank was kept at a different salinity by the daily addition of distilled fresh water. Salinities tested were 30, 32 and 34 (ambient) ppt. The experiment was terminated after 13 days of husbandry and feeding with rotifers. Survival, swim bladder development and feeding incidence were all assessed.

In the 2002 trial, eight 100 L tanks were each stocked with 3000 Stage V cod eggs each. Husbandry procedures were identical to the previous year's trial. However, four tanks were maintained at ambient salinity (34 ppt) with the remaining four maintained at 30 ppt by the daily addition of distilled fresh water. The trial was terminated at 20 days *post*-hatch, and the above parameters were assessed.

4.3 Results and conclusions

Table 4.1 summarises the performance results at 13 days *post*-hatch for the 2001 trial:

Table 4.1: Performance of larval cod exposed to three test salinities

Salinity (ppt)	% survival	% swim bladder inflation	% feeding incidence
30.0	8.5	60	70
32.0	3.9	30	100
34.0	14.4	20	90

Although a highly preliminary experiment, the low salinity treatment did show the highest degree of swim bladder inflation. Although the high salinity treatment did show the best survival, the very low degree of swim bladder inflation may result in an actual survival of 2.9 %, since larvae with non-inflated swim bladders will not be

viable. Conversely, the high degree of swim bladder inflation in the low salinity treatment may result in an actual survival of 5.1 % for that tank.

However, the following year's replicated experiment did not show any advantage in terms of larval performance. Percentage survival (mean = 11.7±3.3 [= S.D.] %), percentage swim bladder inflation (mean = 49.2±11.4 %) and specific growth rate (mean = 6.1±0.8 %) did not differ significantly between treatments ($F_{1,6}$ = 0.058, 0.000 & 0.506; p = 0.818, 0.998 & 0.503 respectively).

Nevertheless, daily observations of each tank demonstrated that larvae in the low salinity tanks were much better distributed throughout the water column. This circumvented the common problem in cod larviculture of larvae 'rafting' on the surface and quickly becoming moribund. This was thought to be due to the low relative density of a large proportion of freshly hatched cod larvae preventing them from moving through the water column and accessing prey (Cutts & Shields, 2001). Further work would be needed to elucidate the benefits of salinity manipulation in cod larviculture.

5. The effects of live feed enrichments and weaning date on growth and survival in larval cod

5.1 Introduction

The correct nutritional parameters in live feed are essential for larval cod performance. However, with species new to aquaculture, a relatively narrow range of existing commercial proprietary live-feed enrichments must be used for economic viability, and to elucidate the optimal composition of future products. Therefore, an experiment was carried out to investigate the influence of different live feed regimes on larval performance. Different blends of AlgaMac 2000 and Super Selco were used, since they are ubiquitous live feed enrichments in cold water larviculture. Fatty acid analysis was also carried out on larvae from each treatment, in order to precisely determine the nutritional requirements for maximising larval survival and growth. Previous studies have highlighted the importance of three essential acids in larval nutrition: docosahexaneoic acid (DHA), eicosapentaneoic acid (EPA) and arachidonic acid (ARA). Both the absloute levels and ratios between these three fatty acids have been extensively studied with regard to larviculture, and are examined in depth here.

Furthermore, the influence of previous live-feed nutritional history on ability to wean was also investigated. There is an economic incentive in reducing the live-feed period and it's associated labour costs, so inert diet was experimentally introduced at three distinct times. The effects on different weaning points on larval survival and growth were compared.

5.2 Materials and methods

Twenty seven 100 L tanks were each stocked with 3000 stage V cod eggs. Routine husbandry regimes were established immediately *post*-hatch (Cutts & Shields, 2001) with the exception of live-feeding regime. Three live-feeding regimes were administered, but in each tank rotifers were fed until 250 °d (degree-days) *post*-hatch, when *Artemia* was added thereafter. The regimes differed in terms of live-feed enrichment: nine tanks were fed 100 % prey items enriched with Super-Selco, nine with 50 % prey items enriched with Super-Selco and 50 % with AlgaMac 2000, and the remaining nine tanks were fed with 60 % prey enriched with Super-Selco and with 40 % AlgaMac 2000.

Weaning date was also varied within each group of nine tanks: three tanks from each live-feeding regime were offered inert diet (Nippai weaning powder) at 400 °d, a further three at 500 °d, and the remaining three were maintained on *Artemia* until the termination of the experiment at 570 °d. Tanks receiving inert diet were co-fed with *Artemia* for the remainder of the trial.

Each tank was sampled for dry weight, feeding and swim bladder inflation at 140, 250, 400, 500 and 570 °d. In addition to these parameters, mean length was also measured at the end of the experiment. Furthermore, the bottom of the tanks were also thoroughly siphoned at regular intervals in order to estimate mortalities in each tank: subsamples of detritus collected from the tank bottoms were examined under a microscope, mortalities counted, and total mortality assessed. Samples of cod larvae at hatch were also taken for dry weight analysis, in order to estimate overall growth.

Larval samples were taken at day 15 *post*-hatch and preserved in chloroform:methanol (2:1) for fatty acid analysis. Lipids were extracted from the

samples, and fatty acid methyl esters (FAME) were prepared from the extract and purified using thin layer chromatography (TLC) before analysis by gas-liquid chromatography (GLC). This determined a quantitative profile of the fatty acids present in the larvae. All fatty acid analysis was carried out at the Institute of Aquaculture, University of Stirling.

5.3 Results and conclusions

Regular siphoning provided an accurate assessment of ongoing larval mortality throughout the trial. Percentage survival at 14 dph (~ 140 °d) was a significant predictor of survival at the end of the trial ($F_{(1,26)} = 4.48$, $p < 0.05$, $r^2 = 0.12$). Furthermore, a multiple regression model of percentage survival (estimated by siphoning) at three separate sampling periods versus end survival improved predictability considerably ($F_{(3,26)} = 3.37$, $p < 0.05$, $r^2 = 0.22$), demonstrated by the increased correlation coefficient. However, a stepwise multiple regression showed that percentage mortality at 14 dph was the best predictor of end survival ($p < 0.01$).

Early survival at 14 dph was highly variable throughout the experimental treatments: mean = 60 ± 2.43 (= S.E.) %, with a minimum and maximum of 40 and 89 % survival respectively. The different live feeding regimes had no significant effect on survival *pre*-weaning. Nor did they have any significant effect on feeding incidence and swim bladder inflation. However, there was a slight effect of feeding regime on growth *post Artemia* introduction ($F_{(2,26)} = 3.00$, $p = 0.06$; Fig 5.1), with larvae fed on the 50/50 Super Selco/ AlgaMac regime growing slightly better (although the result was not quite significant). There was no effect on growth during the rotifer phase of feeding.

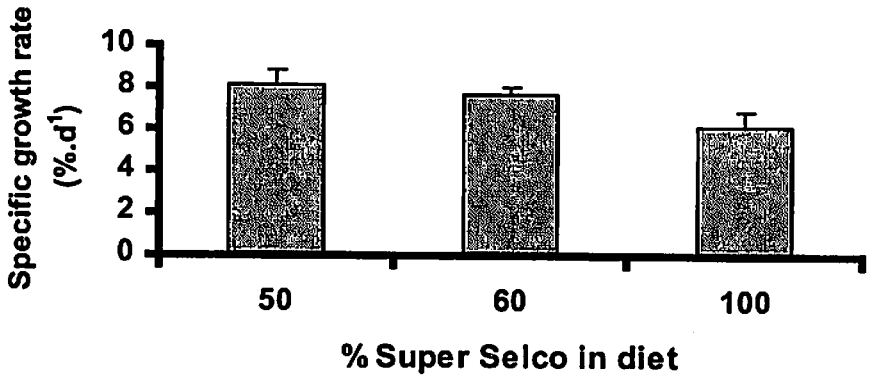


Fig. 5.1: The specific growth rate of larval cod during the *Artemia* phase of live-feeding for three different *Artemia* enrichment regimes: 50, 60 and 100 % Super Selco (error bars denote standard error). See text for statistical analysis.

Although enrichment regime had no effect on early mortality, and there was the slight (but not significant) effect of feeding regime on growth (Fig. 5.1), there was a pronounced effect of early mortality on subsequent growth of surviving larvae ($F_{(1,26)} = 4.43$, $p < 0.05$, $r^2 = 0.12$; Fig. 5.2).

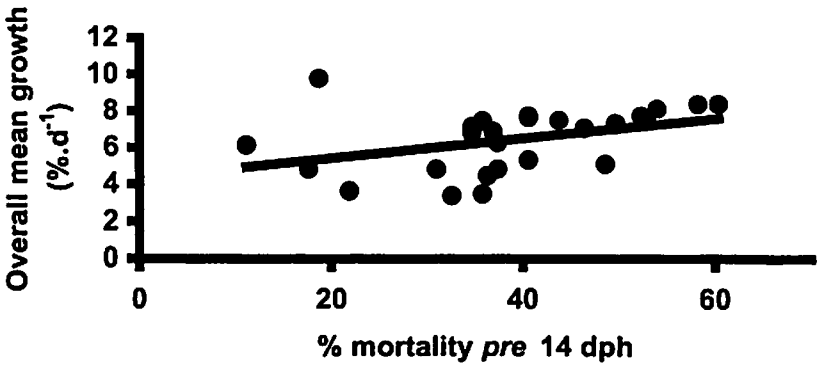


Fig. 5.2: The relationship between percentage mortality from 0 – 14 days *post*-hatch and subsequent mean specific growth rate (% d^{-1}) in larval cod (see text for statistical analysis).

The biggest influence on growth was therefore initial mortality. Tanks that suffered high mortality in the first two weeks *post*-hatch experienced better growth thereafter. The likely cause is that differences in larval density allowed larvae in low density tanks to grow better.

Date of weaning had a significant effect on larval survival *post*-weaning. Tanks co-fed with inert diet and *Artemia* at 400 °d suffered higher mortality ($F_{(2,26)}$ = 3.67, $p < 0.05$; Fig. 5.3). There was no significant difference between tanks co-fed at 500 °d and the unweaned control. There was no significant effect on growth.

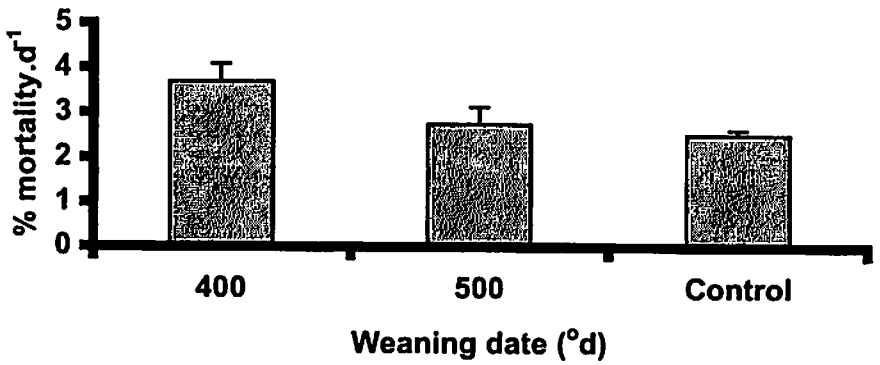


Fig. 5.3: The effects of weaning date (400, 500 °d & unweaned) on subsequent survival (% mortality d^{-1}) (error bars denote standard error). See text for statistical analysis.

In addition to differences in mortality brought about by weaning date, there were also significant differences in the size distribution of juvenile cod at the end of the experiment. Size distribution is measured as a coefficient of variation (= mean length / S.D.), and was significantly greater in the group weaned at 500 °d ($F_{(2,25)}$ = 4.04, $p < 0.05$; Fig. 5.4).

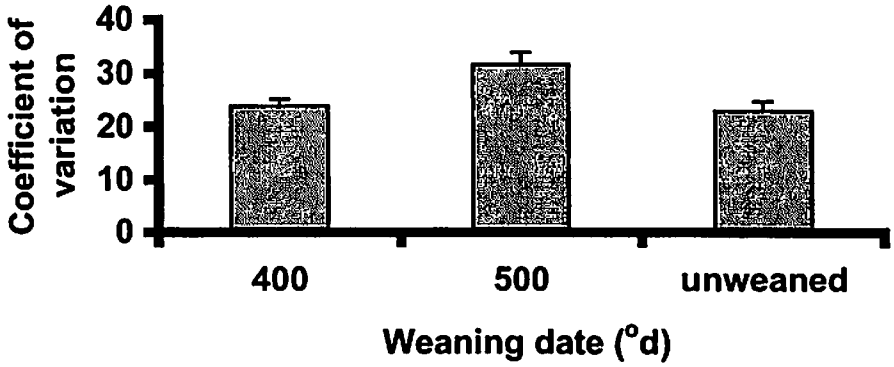


Fig. 5.4: The effects of weaning date (400, 500 °d & unweaned) on the mean coefficient of variation in length (mm) (error bars denote standard error). See text for statistical analysis.

The groups weaned at 400 °d and those fed on live feed throughout both exhibited less size variation than the group weaned at 500 °d. This may have been due to size-selective mortality in the 400 °d and unweaned groups: although growth was determined more by random initial mortality than experimental treatment, mean end size (length; mm) was significantly lower in the 500 °d weaning treatment ($F_{(2,75)}$ = 10.40, $p < 0.001$; Fig 5.5).

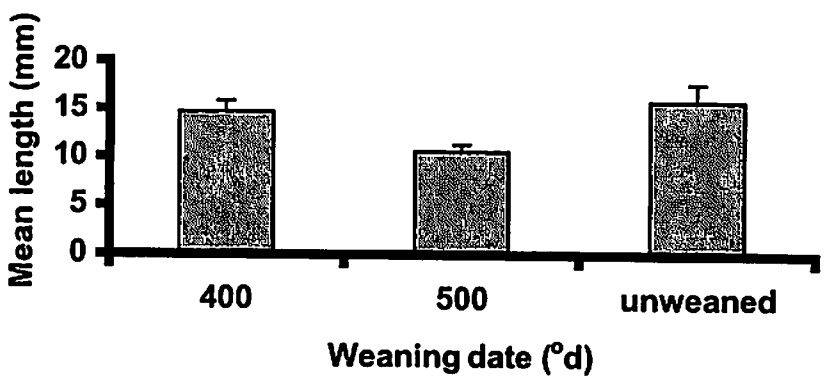


Fig. 5.5: The effects of weaning date (400, 500 °d & unweaned) on mean length (mm) in juvenile cod (error bars denote standard error). See text for statistical analysis.

The differences in length, coefficient of variation and *post*-weaning mortality between the experimental treatments suggest that mortality was selective in the treatments: the 400 °d weaning treatment suffered higher mortality, with a significantly narrow size range of larger fish surviving by the end of the experiment. This suggests that smaller fish were dying *post*-weaning. Conversely, fish maintained on *Artemia* were also relatively large within a narrow size range, but without the significant mortality over the same time period as mortality in the former treatment.

This suggests that very early weaning with the test diet does not provide any advantages in terms of growth for the duration of the experiment. However, the larger size variation but overall smaller size of the 500 °d weaning treatment may exacerbate cannibalism later on, although mortality did not increase for the remainder of the experiment.

5.3.1 Fatty acid analysis

Being the objective of this study to provide with an "of-the-shelf" dietary protocol, the different dietary treatments differed widely in composition. However, the major fraction of the different enrichment diets is constituted by lipids, and the main benefit claimed by the different commercial products is their contribution of dietary essential fatty acids (mainly DHA, but also EPA and ARA). Thus, some notes on the analysis of the EFAs content of the larvae from the different dietary treatments, and its relation to growth and survival, could be of interest.

The levels of DHA in larvae at day 15 did not differ significantly between treatments ($F_{(2,21)} = 2.17$, $p = 0.189$): mean percentage of total fatty acids was 24.7 %. However, there was significantly more EPA in larvae from the 100 % Super Selco treatments than from both the 50 % and 60 % treatments: 7.89 ± 0.28 %, 6.81 ± 0.27 % and 6.55 ± 0.16 % respectively ($F_{(2,21)} = 8.54$, $p < 0.005$). Conversely, there was significantly less ARA in larvae from the 100 % Super Selco treatment than in the 50 and 60 % treatments: 3.80 ± 0.19, 4.24 ± 0.12 and 4.43 ± 0.08 % respectively ($F_{(2,21)} = 5.28$, $p < 0.05$).

Although DHA levels did not differ significantly in larvae from different treatments, the ratio of DHA to EPA did. Both the 50 and 60 % Super Selco treatments produced larvae with a significantly higher DHA/EPA ratio than the 100 % treatment ($F_{(2,21)} = 7.68$, $p < 0.005$; Fig. 5.6). Conversely, the EPA/ARA ratio was significantly higher in the 100 % Super Selco treatment, compared to both the 50 and 60 % treatments ($F_{(2,23)} = 39.59$, $p < 0.0001$; Fig. 5.6).

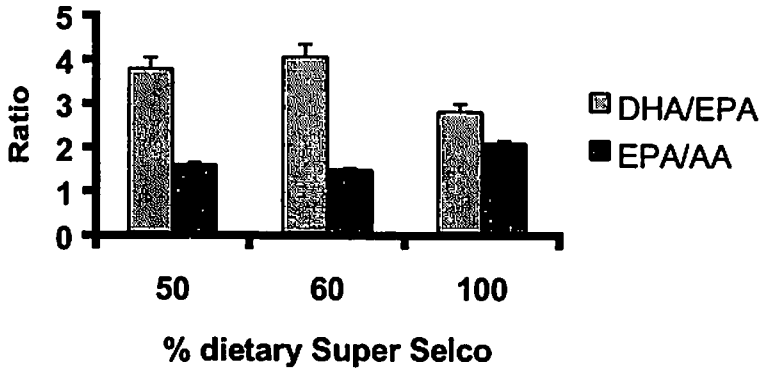


Fig. 5.6: The effect of differing percentages of dietary Super Selco on the ratios of DHA to EPA and EPA to ARA in larval cod somatic tissue. Error bars denote standard error. See text for statistical analysis.

Therefore the different dietary treatments had a profound influence on the essential fatty acid content of the larvae. Due to the large variation in larval fatty acid content between treatments, regression analysis was used to investigate the effects of nutritional history on survival and growth in individual tanks. Neither absolute levels of EPA or ARA had significant effects on survival at 15 days *post*-hatch. However,

absolute levels of DHA had a pronounced effect on survival ($r^2 = 0.16$, $p < 0.05$; Fig. 5.7). This effect on survival persisted until day 40, but was not quite significant during this later period ($r^2 = 0.09$, $p = 0.08$).

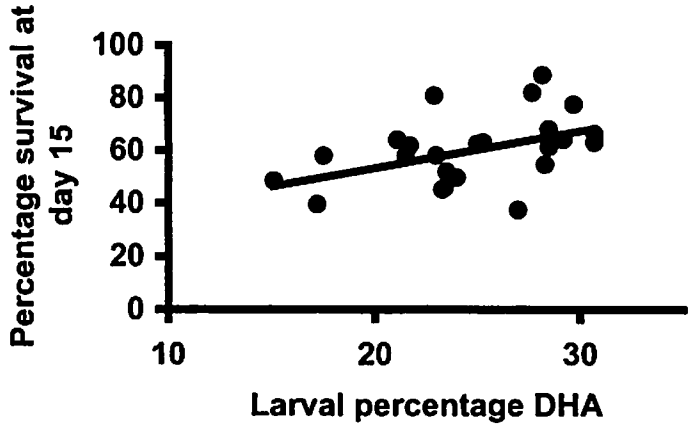


Fig. 5.7: The effect of percentage DHA in larval somatic tissue on survival at day 15 *post*-hatch. See text for statistical analysis.

As mentioned above, initial mortality had profound effects on subsequent growth of the survivors, potentially obfuscating any effects of diet itself on growth. Such effects could be controlled by regressing mean specific growth rate against initial mortality (and hence subsequent larval density), and analysing the residual growth rates (rSGR) from the regression. This technique controls for differences in mortality, by calculating the difference between the actual mean growth rate and the growth rate predicted by the relationship between growth and early mortality. This analysis highlights whether larvae are performing better or worse than expected, given the initial mortality in the tank, and removes the effect of *inter*-tank variability in density. The regression equation used was:

$$\text{Mean SGR} = 7.33 - 0.139 \quad (\% \text{ survival at day 40})$$

($r^2 = 0.35$, $p < 0.005$).

There was a significant polynomial relationship between residual SGR and both absolute levels of DHA and DHA/EPA in larval cod somatic tissue (DHA: $r^2 = 0.68$, $p < 0.0001$; DHA/EPA: $r^2 = 0.36$, $p < 0.005$; Fig. 5.8). Levels of EPA, ARA or EPA/ARA had no effect on residual growth.

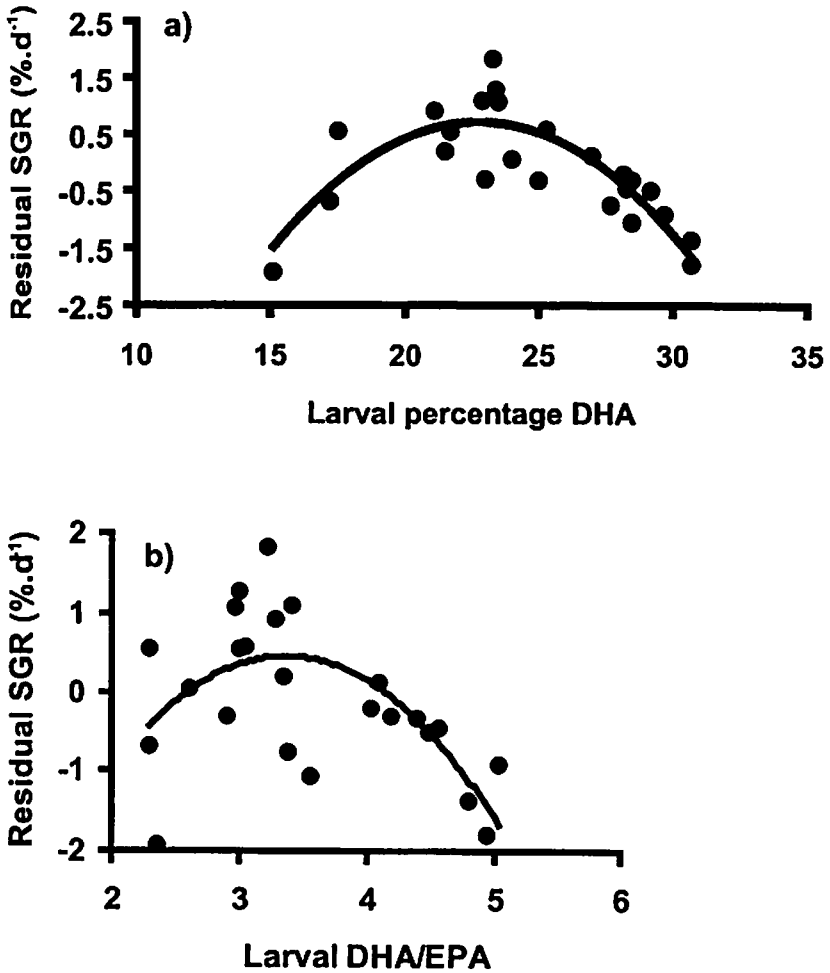


Fig. 5.8: The effect of *a)* larval DHA (as a percentage of total somatic fatty acids) and *b)* larval DHA/EPA on residual specific growth rate (%.d^{-1}). See text for statistical analysis.

Although percentage levels of DHA in the larvae did not differ significantly between treatments, there was more EPA present in larval cod from the 100 % Super Selco dietary treatment. Due to this, DHA/EPA ratios were significantly lower in that treatment. Furthermore, there was also significantly less ARA in larvae from that treatment, with a concomitant increase in EPA/ARA levels.

Although nutrition had a profound effect on the fatty acid make-up of larvae, only percentage levels of DHA had any positive influence on survival. However, this relationship was pronounced. The result complements previous research, which states that DHA is particularly important for the development of normal cellular functions. Low levels of DHA have been linked to underdeveloped swimbladders and deformity, although there was no such relationship in this study. Furthermore, fish eyes contain high levels of DHA and cod are primarily visual foragers. Therefore, low levels of DHA in the diet may result in poorly developed eyes and in turn reduce feeding success.

Moreover, the increasing incidence of DHA in larval tissue matched an improvement in growth (after controlling for the effect of initial mortality). However, this relationship showed an optimum value of *approx.* 22 % DHA (of total fatty

acids). Thereafter, performance rapidly decreased. This is reflected in the similar relationship between residual growth and larval DHA/EPA. This relationship showed an optimal ratio of *approx.* 2.5 in terms of growth. This suggests that too much DHA can be just as deleterious as too little. An excess of DHA in relation to EPA may upset cell membrane fluidity, leading to poor performance.

Prey items, either naturally or through enrichment techniques should not only deliver the necessary fatty acids total levels, but also in appropriate relative concentrations. Including AlgaMac in the dietary treatment improved the DHA/EPA ratio in the larvae, but only through modulation of absolute levels of EPA. All treatments showed DHA/EPA ratios in excess of the suggested optima of 2.5, with the 100 % Super Selco treatment having the value nearest the suggested optima. Enrichments with high levels of DHA should be tested; these treatments should also have high levels of EPA (resulting in a ratio of approx. 2.5).

6. The effects of a range of commercial live feed enrichments on cod (*Gadus morhua*) larval performance

6.1 Introduction

The correct nutritional parameters in live feed are essential for larval cod performance. However, with species new to aquaculture, a relatively narrow range of existing commercial proprietary live-feed enrichments must be used for economic viability, and to elucidate the optimal composition of future products. Section 5 detailed an experiment using the two products typically used at Ardtoe, with some promising results. This section expands and elaborates on that work with a greater range of commercial enrichments.

An experiment was carried out to investigate the influence of different live feed regimes on larval performance. A control diet of AlgaMac 2000 and Super Selco was used, since they are ubiquitous live feed enrichments in cold water larviculture and constituted current Ardtoe best practice, and was compared with larval performance under two other dietary regimes. Essential fatty acid (EFA) analysis was also carried out on larvae from each treatment and on the diets themselves, in order to precisely determine the nutritional requirements for maximising larval survival and growth. Previous studies have highlighted the importance of three essential acids in larval nutrition: docosahexaneoic acid (DHA), eicosapentaneoic acid (EPA) and arachidonic acid (AA). Both the absloute levels and ratios between these three fatty acids have been extensively studied with regard to larviculture, and are examined in depth here. Due to the greater range of commercial enrichments tested compared to the previous section, EFA levels in the cod larvae are compared in detail with EFA levels in corresponding dietary treatments. This allowed us to elucidate which enrichments were most effective in transferring EFA levels appropriate to good performance from the prey to the larvae.

6.2 Materials and Methods

Twelve 100 L tanks were each stocked with 3383 disinfected stage V cod eggs. Routine husbandry regimes were established immediately *post*-hatch (Cutts & Shields, 2001) with the exception of live-feeding regime. Samples of cod larvae at hatch were taken for dry weight analysis, in order to estimate growth. The mean temperature throughout the experiment was 10.5 °C, and mean salinity was 33.8 ‰.

Three live-feeding regimes were administered. Rotifers were fed until 25 days *post*-hatch, when *Artemia* was added thereafter. The regimes differed in terms of live-feed enrichment: four tanks were fed with rotifers enriched with DHA Protein Selco from day 1 until day 24 and then DC DHA Selco enriched *Artemia post* day 25 (**Treatment II**); a further four tanks were fed with Protein Selco rotifers and DC Selco *Artemia* (**Treatment III**). The remaining four tanks were fed with rotifers enriched with AlgaMac 2000 until day 10, rotifers enriched with Super Selco (60 % of prey items) and AlgaMac 2000 (40 % of prey items) until day 25, and *Artemia* enriched with Super Selco (60 %) and AlgaMac 2000 (40 %) thereafter. The latter treatment was the standard Ardtoe treatment, and acted as the **Control**.

Larval cod from each tank were sampled for length, dry weight, feeding and swim bladder inflation at 10, 24 and 51 (termination of the experiment) days *post*-

hatch. Furthermore, the bottom of the tanks were also thoroughly siphoned at regular intervals in order to estimate mortalities in each tank: subsamples of detritus collected from the tank bottoms were examined under a microscope, mortalities counted, and total mortality assessed. End survival was assessed at day 51 by the removal and counting of cod juveniles from each tank.

Larval samples were also taken at days 10, 24 and 51 *post*-hatch and preserved in chloroform:methanol (2:1) for fatty acid analysis. Lipids were extracted from the samples, and fatty acid methyl esters (FAME) were prepared from the extract and purified using thin layer chromatography (TLC) before analysis by gas-liquid chromatography (GLC). This determined a quantitative profile of the fatty acids present in the larvae. All fatty acid analysis was carried out at the Institute of Aquaculture, University of Stirling.

6.3 Results

There were no differences in survival between treatments at the termination of the experiment at day 51 *post*-hatch (mean survival = 5.81±2.39 (= S.D) %; $F_{(2,9)}$ = 0.408, p = 0.677). In addition, there were no significant differences in swim bladder development at day 10 (mean = 39.92±21.84 %; $F_{(2,9)}$ = 1.668, p = 0.242) or day 24 (mean = 80.58±14.76 %; $F_{(2,9)}$ = 2.436, p = 0.143). However, larvae fed the control diet grew significantly faster during the rotifer feeding stage (up to 25 days *post*-hatch) than the experimental treatments ($F_{(2,9)}$ = 6.382, p < 0.05; Fig. 6.1). Overall mean specific growth (SGR) did not differ significantly between treatments (mean = 6.99±0.70 %, $F_{(2,9)}$ = 2.951, p = 0.103), since there were no significant differences in growth during the *Artemia* feeding stage (mean = 13.05±1.45 %, $F_{(2,9)}$ = 0.386, p = 0.691).

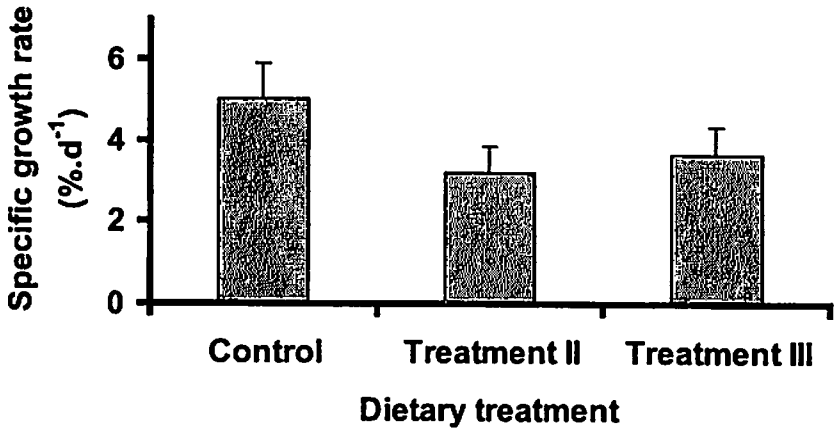


Fig. 6.1: The relationship between three dietary treatments and subsequent specific growth rate (% d^{-1}) during the rotifer feeding stage (hatch to day 25) in larval cod. Error bars denote standard deviation. See text for statistical analysis.

Although there were few significant differences between dietary treatments with regard to larval performance, the mean characteristics of surviving larvae (e.g. fatty acid content) from tanks across all treatments can be compared with the

performance parameters of those tanks using regression analyses. For example, percentage survival at day 51 *post*-hatch correlated strongly with DHA content of the surviving larvae (measured as a percentage of total fatty acid content; Spearman's R = 0.669, $p < 0.05$; Fig. 6.2*a*). There was no such correlation for percentage EPA, although there was a significant relationship between the DHA/EPA ratio of surviving larvae and percentage survival (Spearman's R = 0.573, $p = 0.05$), due to increasing amounts of DHA promoting survival. Furthermore, there was a significant positive correlation between levels of percentage AA and survival at day 51 (Spearman's R = 0.605, $p < 0.05$; Fig. 6.2*b*), although this relationship was weaker than that of DHA.

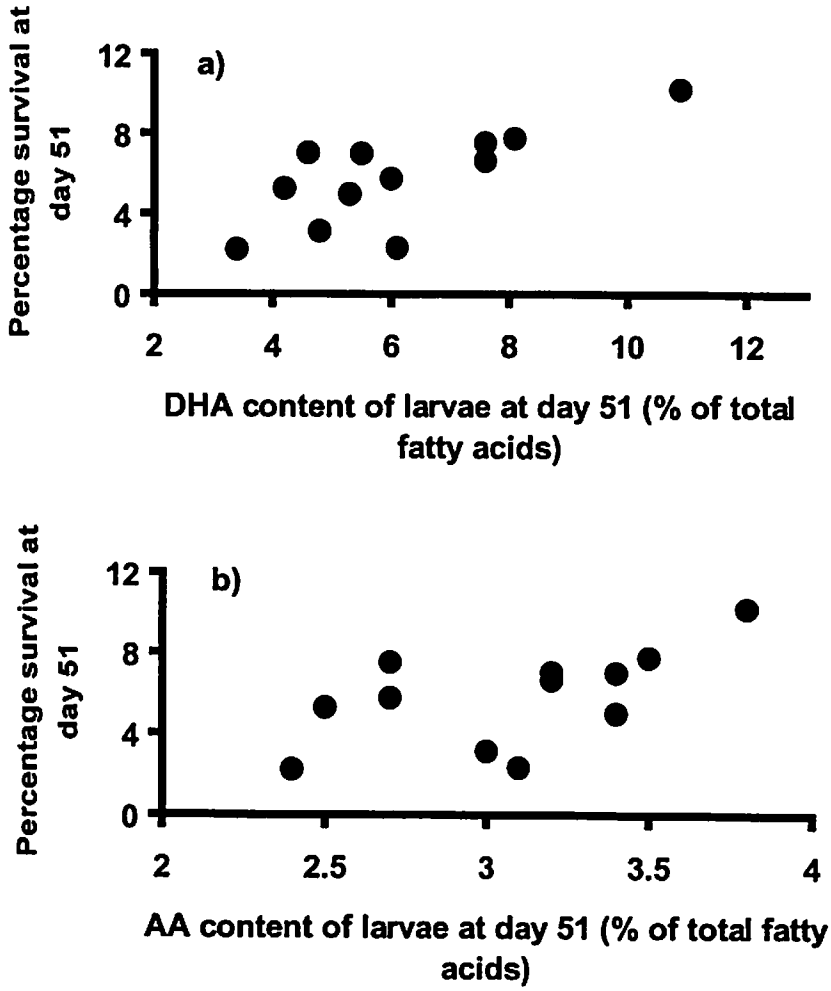
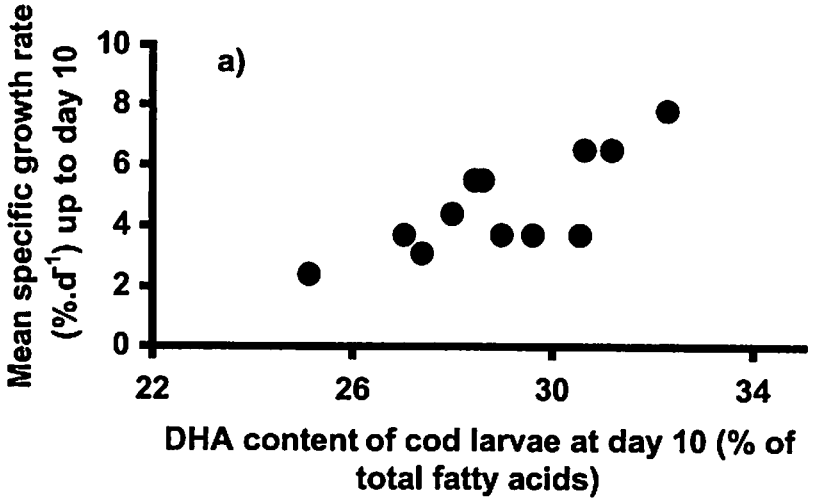


Fig. 6.2: The relationship between percentage survival at day 51 post-hatch and *a)* mean docosahexaneoic acid (DHA) content of surviving cod larvae and *b)* mean arachidonic acid (AA) content of surviving cod larvae. Both EFA levels are expressed as a percentage of total fatty acids. See text for statistical analysis.

In addition, specific growth rate also correlated positively with DHA and AA levels (sampled at day 10) during the first ten days of feeding, but at no stage thereafter (DHA vs. SGR: Spearman's R = 0.736, $p < 0.01$; AA vs. SGR: Spearman's R = 0.675, $p < 0.05$; Fig. 6.3). However, it is worthwhile noting that mean growth rate (over the whole trial period) correlated negatively with the DHA

content of surviving larvae at the end of the trial (Spearman's R = -0.588, $p < 0.05$). This was not due to concomitant increased survival at high DHA levels compromising growth, since there was no correlation between survival and mean growth (Spearman's R = -0.238, p = 0.457).



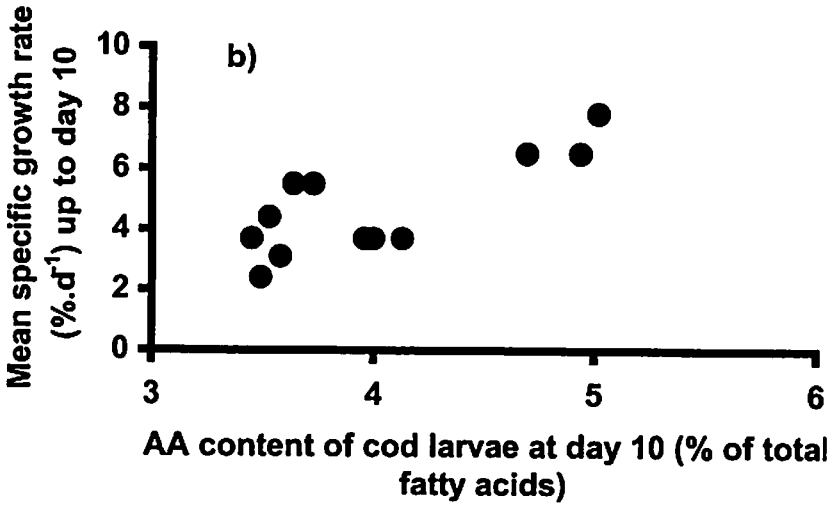


Fig. 6.3: The relationship between mean specific growth rate (% d^{-1}) from day 1 to day 10 *post*-hatch and *a)* mean DHA content of cod larvae at day 10 and *b)* mean AA content of larvae at day 10. Both EFA levels are expressed as a percentage of total fatty acids. See text for statistical analysis.

However, it is unclear whether higher levels of DHA and AA contribute to faster growth, or larger, faster growing fish have simply incorporated higher levels of DHA and AA over a set period of time, in association with faster deposition of somatic tissue. Figure 6.1 showed that larvae fed the control diet grew significantly faster during the rotifer phase of feeding. It was found that the control diet had

significantly higher levels of DHA than the other two diets ($F_{(2, 9)}$ = 8.648, $p < 0.01$; Fig. 6.4*a*), but there was no significant difference in AA levels ($F_{(2, 9)}$ = 0.482, p = 0.704; Fig. 6.4*b*). Furthermore, larvae fed on the control diet and sampled at day 10 had higher levels of DHA than larvae from the other two treatments ($F_{(2, 9)}$ = 4.027, p = 0.05; Fig. 6.4*a*), although this result was weakly significant. In addition, although there were no significant differences in AA levels between diets, larvae fed on the control diet had significantly higher levels of AA by day 10 than the other two treatments ($F_{(2, 9)}$ = 16.006, $p < 0.005$; Fig. 6.4*b*).

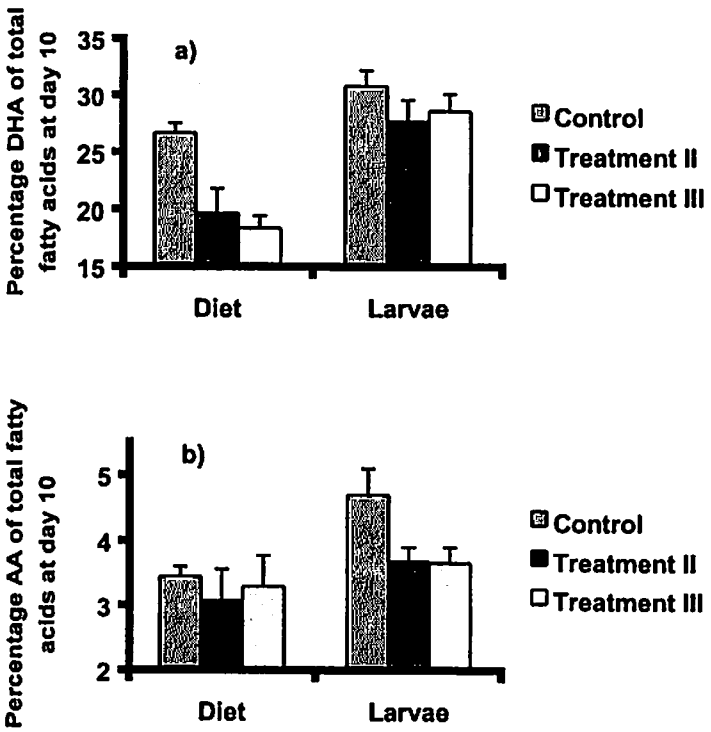


Fig. 6.4: Levels of essential fatty acids (expressed as a percentage of total fatty acids) in three rotifer diets enriched with different products and in the cod larvae fed the diets (sampled at 10 days *post*-hatch) for *a)* DHA and *b)* AA. Error bars denote standard deviation. See text for statistical analysis.

Fig. 6.4*a* shows that larvae from all dietary treatments were capable of incorporating DHA by day 10 in significantly greater amounts than present in the diet ($F_{(1,6)}$ = 20.332 [control], 27.126 [treatment II] & 99.017 [treatment III]; $p < 0.01$, 0.005 & 0.001 respectively). Although larvae from the control diet had significantly more percentage DHA than larvae from the other two treatments, this was only just significant, and larvae from treatments II and III were able to incorporate similar amounts from diets with much less DHA.

Conversely Fig. 6.4*b* shows that (in addition to no significant difference in AA content between the diets but a significant difference in AA content between larvae fed the three treatments) larvae from treatments II and III did not show significant incorporation of AA (differences between dietary and larval levels: $F_{(1,6)}$ = 5.371, p = 0.07 & 1.834, p = 0.234 for treatments II and III respectively). However larvae fed the

control diet exhibited significantly higher AA levels than reflected in their diet ($F_{(1,6)} = 25.220$, $p < 0.005$).

Fig. 6.2 showed the relation of both DHA and AA levels in larvae at the end of the experiment with larval survival in individual tanks. Therefore it is important to study any relationship between larval EFA levels and the corresponding dietary EFA levels in the *Artemia* feeding phase of the experiment. Fig 6.5 shows that the percentage of DHA of total fatty acids in larvae had been considerably reduced since day 10 *post*-hatch (mean = 6.18±2.07 *cf.* 28.98±2.00 %), whereas AA percentage remained similar (mean = 3.08±0.43 *cf.* 4.01±0.57 %). This coincides with reduced concentration of DHA in the *Artemia* (circa 6% of total fatty acids) with respect to that found in the rotifers (20-30%).

Figure 6.5*a* shows that DHA levels did not differ significantly between experimental diets during the *Artemia* pahse ($F_{(3,11)} = 2.346$, $p = 0.149$), whereas there was a significant difference in levels between larvae fed the different treatments ($F_{(2,9)} = 4.680$, $p < 0.05$). This was most pronounced between the control and treatment III. However, none of the differences between DHA content of the diet and the larvae fed the diet were significantly different ($F_{(1,9)} = 0.628$, 1.812 & 0.024; $p = 0.451$, 0.236 & 0.884 for the control, treatments II & III respectively).

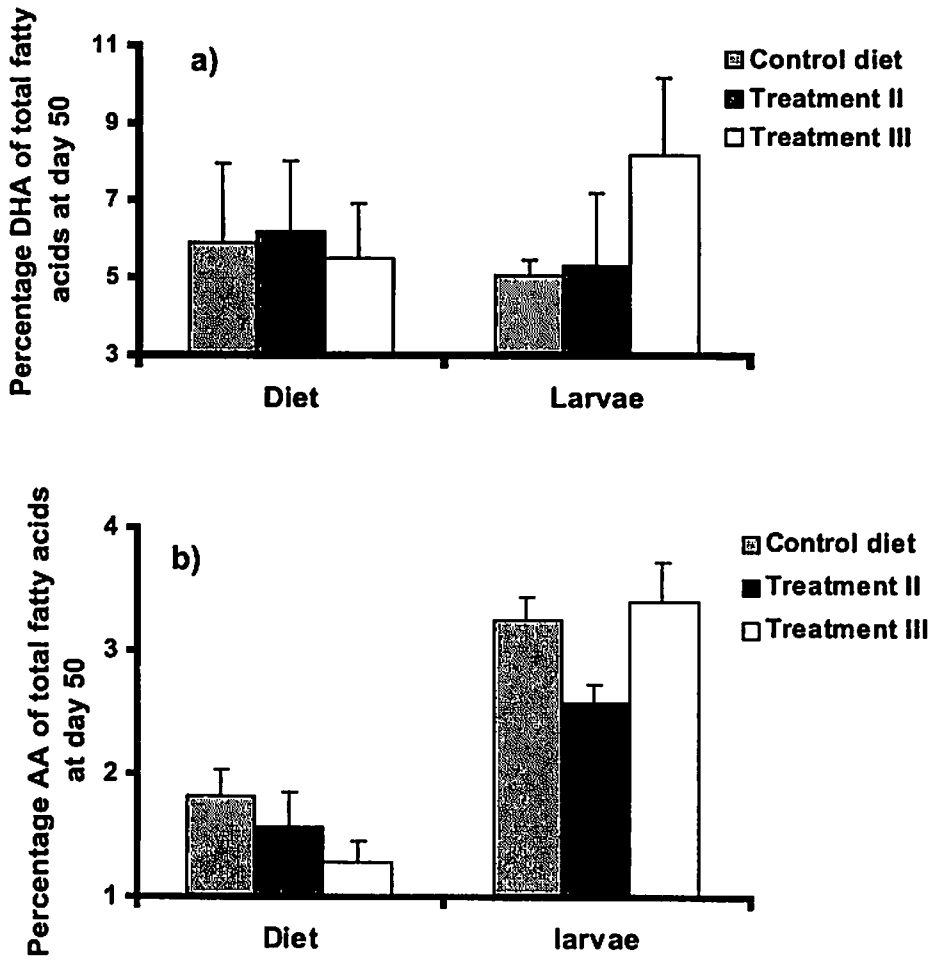


Fig. 6.5: Levels of essential fatty acids (expressed as a percentage of total fatty acids) in three *Artemia* diet treatments and in the cod larvae fed the diets (sampled at 50 days *post*-hatch) for *a)* DHA and *b)* AA. Error bars denote standard deviation. See text for statistical analysis.

AA concentration was also lower than during the rotifer phase, with the control diets exhibiting higher levels than treatment III, although this was not quite significant ($F_{(3,11)} = 2.846$, $p = 0.069$). However, cod larvae had higher levels of AA compared to their diet by day 50 *post*-hatch (Fig. 6.5*b*). These levels were significantly higher for each treatment ($F_{(1,9)} = 112.403$, 63.195 & 125.304; $p < 0.0001$, 0.005 & 0.0001 for the control, treatments II & III respectively). In addition, AA levels in larvae fed the control and treatment III diets were significantly higher than in treatment II fish ($F_{(2,9)} = 14.560$, $p < 0.005$).

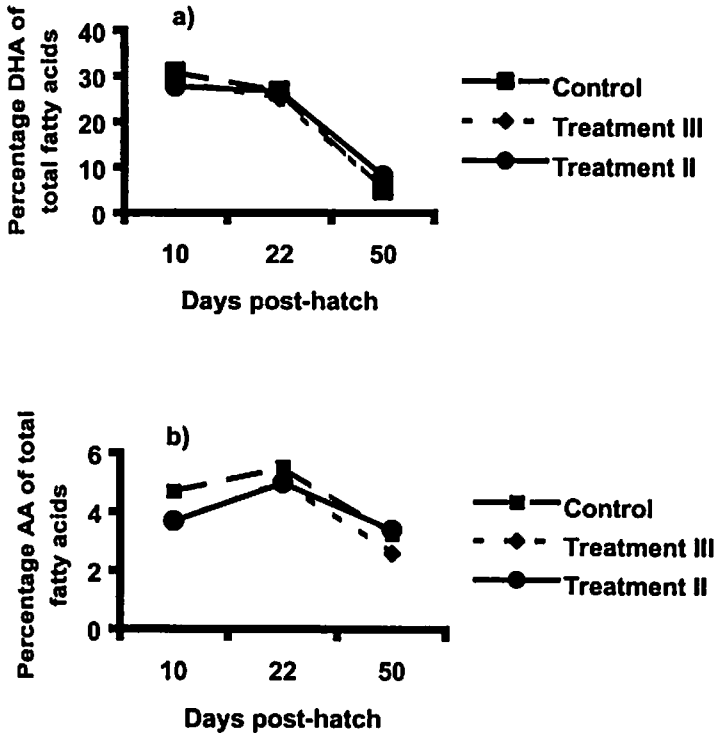


Fig. 6.6: Levels of *a)* DHA and *b)* AA (expressed as a percentage of total fatty acids) in larval cod somatic tissue under three different dietary treatments at 10, 22 and 50 days *post*-hatch.

Fig. 6.6 shows the changing levels of DHA and AA for the 51 days of the trial duration.

There were marked changes in larval DHA levels during the trial (Fig. 6.6*a*). In comparison to day 50, cod DHA levels were approximately six times higher at days 10 and 22, when the larvae were still fed rotifers. These high levels were largely due to higher levels of DHA in all three rotifer diets and greater incorporation of DHA by the larvae. By day 50, DHA levels in the larvae had dropped to similar levels to those in the *Artemia* diet, with much reduced DHA concentration.

AA levels appeared to be far more conservative than DHA levels throughout the trial (Fig. 6.6*b*), reflecting the lesser change of content in the two prey items. By day 10 *post*-hatch, all larvae with the exception of control fish reflected their diet with regard to percentage AA (control fish were able to incorporate AA to nearly twice that

of their diet; Fig. 6.4*b*). Despite the levels in the *Artemia* diets being substantially lower than in the rotifer diets (1.5 *cf.* 3.0 % on average; Figs. 6.4*b* & 6.5*b*), by the end of the trial fish from each group were all exhibiting incorporation of AA, since their levels were approximately similar to throughout the trial.

6.4 Discussion

There was a marked diet difference between the rotifer and the Artemia phase, substantially due to the differences in final concentration of EFAs in the two prey items. Larvae fed the control treatment grew faster than larvae in the other two dietary treatments, but only during the rotifer feeding phase (hatch to 25 days *post*-hatch). However, due to similar growth rates between treatments during the *Artemia* feeding phase, there were no significant differences in overall growth. During the rotifer feeding phase, it is therefore worthwhile noting that growth rate correlated positively with both larval DHA and AA levels when measured at 10 days *post*-hatch. As noted before, it is hard to disassociate cause-and-effect with these two correlations: the larvae may grow faster due to elevated levels of both DHA and AA, or they may have elevated levels due to being bigger and incorporating DHA and AA at a greater rate. However, larvae in the control treatment grew faster during the first 25 days, and the control diet had significantly higher levels of DHA, but not AA. Furthermore, this was reflected in the DHA levels of control larvae at day 10; they had significantly more percentage DHA than the two experimental treatments. Although control larvae had significantly more DHA than the other two treatments, treatments II and III were able to incorporate comparable levels using diets with significantly less DHA than the control. However, there was the slight trend of enhanced DHA levels in the control diet promoting DHA levels in the faster growing control larvae during the rotifer phase. Therefore, providing adequately high levels of DHA may well promote growth.

As with DHA, there was also a positive correlation between growth and AA percentage levels at day 10, albeit weaker. However, unlike DHA levels in the three different rotifer diets, AA levels did not differ significantly between dietary treatments. Despite this, larvae from the control group showed significant incorporation of AA, exhibiting levels significantly higher than both their diet and larvae from the two experimental treatments. Conversely, larvae from the two experimental treatments did not exhibit significant incorporation of AA over and above levels in their diet. Since control larvae grew faster, showed higher levels of both DHA and AA, and the control diet contained significantly more DHA (but not AA), higher levels of DHA and AA in the diet may improve larval growth during this period. However, other factors unmeasured in this study, such as dietary total energy content and digestibility, will also influence growth.

Nevertheless, the control diet during the rotifer feeding phase seemed to promote growth, and also displayed enhanced levels of DHA, although larvae from all treatments were able to incorporate DHA at significant rates. In addition, although AA levels did not differ between diets, the ability to incorporate AA was markedly higher in the control diet.

The dynamics of EFA supplementation and uptake were markedly different between the two prey items. Percentage levels of larval somatic DHA had markedly dropped during the *Artemia* phase from 28.98 to 6.18 %. This was a reflection of much lower DHA levels in *Artemia* from all dietary treatments, with none of the larvae from each treatment expressing incorporation of DHA. Thus, *Artemia* enriched

in the conditions of this experiment seemed to be unable to meet the requirements of larvae for DHA However larvae in treatment III did show significantly more DHA than control larvae, although this was due to low levels in the control group rather than incorporation of DHA in treatment III.

Conversely, larvae from each group retained the ability to incorporate AA from lower levels in the *Artemia*. This was done to such an extent that larval AA levels at the end of the experiment were comparable to levels during the rotifer feeding phase. Moreover, control and treatment III levels accumulated AA to a greater extent than treatment II fish. The inefficiency of using *Artemia* as a conduit for transferring EFA's from enrichment emulsions to larval fish is well known: *Artemia* catabolise EFA's themselves, converting them into triacylglycerols, which are of much less use to fish larvae. Furthermore *Artemia* store a large proportion of their EFA's in their neutral lipids as opposed to polar membrane lipids. This makes them more indigestible, since it is polar lipids that form lipid emulsions in the larval gut, facilitating lipid digestion.

In addition to the significant effects of dietary and larval EFA's on larval growth, mean larval levels of both DHA and AA also correlated positively with individual tank survival at the end of the trial. This again demonstrates the importance of ensuring high levels of DHA and AA in cod larvae for general good performance.

Despite the inefficiency of *Artemia* as a live food, and acknowledging the importance of both DHA and AA, this study suggests purely in terms of larval EFA composition that treatment III is the highest performance diet during the *Artemia* phase. This recommendation is based on the finding that fish from this treatment exhibited higher levels of DHA than control fish, and showed AA levels comparable to control fish, although AA levels in their diet were almost significantly lower.

7. Assessment of out of season cod production (photoperiod manipulated to 8. spawn in November) and larval performance

Peter Smith, Jon Sherwood, Jim Treasurer

7.1 Introduction

Out of season production in marine finfish hatcheries is a relatively new procedure enabling spawning of broodstock and rearing of larvae throughout the year. Photoperiod is used to simulate short daylengths followed by long daylength to encourage salmonid fish to spawn at different times. In marine species farming the requirement for all year round production is also desirable and can be achieved by adjusting daylength and chilling water to delay or advance spawning. Although spawning of halibut in the UK has been adjusted in several hatcheries to produce all year round spawners, year round cod culture is a recent development. Two marine hatcheries have been reported to hold photoperiod stock that spawn in October-December but there have been no published reports on the performance and survival of cod larvae produced in late autumn. The objective is to provide eggs in any season to optimise the use of marine hatcheries, to spread the workload, and to utilise effectively limited live feed resources.

The present trial utilised eggs collected in November at a farm in Orkney. These were hatched and reared according to the best practice achieved at Ardtoe in the normal spring production. The objective was to determine whether rearing of out of season stock is possible and whether performance of out of season production is comparable with ambient spawned eggs, particularly survival to weaning, growth, and incidence of swim bladder development, an indicator of larval quality and survival.

7.2 Methods

7.2.1 Egg stocking density.

The eggs from Orkney were stocked at Ardtoe on 11 December 2002 in 3 x 1.3 m^3 volume tanks in an environmentally controlled room. Eggs were stocked at 50,000 per tank=38.5 l^{-1}.

7.2.2 Rotifer feeding

The husbandry recommendations for cod from trials in 1999/2000 were followed (Seafish Report number 484), with the following amendments:

Rotifers enriched with Pavlova were also used as this keeps the water surface clean for swim-bladder inflation. An additional pointer was that Pavlova enrichment was recently used successfully for rearing haddock. Average prey density was maintained at 5 per ml during the rotifer feeding phase (as per Canadian publication, Puvanendran and Brown, 1999).

Pavlova was the enrichment used for rotifers for the first 3 days post hatch (dph), and also for all morning feeds and evening feeds from day 10 to day 31. For afternoon

feeds rotifers were enriched with Protein Selco from day 4 to the end of rotifer feeding (day 40)

7.2.3 Artemia feeding

Artemia were fed from day 25 and these were enriched with DC Protein Selco (Inve) rather than Algamac and Super Selco as it represents a recent advance in enrichments and was recommended from experimental trials, see bin room trials (Cutts *et al.*, 2002).

7.2.4 Dry diets

The dry diet offered was Lansy 00 and 01 from 65 days ph and, as there was poor acceptance, this was followed by Nutra 00, before returing to Lansy.

7.3 Results

7.3.1 Rotifers

There was high early larval survival and first feed acceptance due to high rotifer density. With "traditional" rotifer feeding of gadoids (i.e. feed sufficient to leave a residue of 0.3 per ml next morning), there is high mortality around 160^0 days, when larvae with no swimbladders or with deformities do not survive. In this trial, this mortality was not very pronounced (see notes day 18 + 21, Table 1), but instead the poor larvae died gradually (estimated 10,000 larvae in total) from day 31 – day 53, giving a continuous hygiene problem. Also, the larvae were noted as "lethargic" from day 31, and this has been seen in subsequent reared batches of gadoid larvae. It was therefore concluded that, although Pavlova is a good early enrichment, it was not sufficiently nutritious from day 25 onwards.

7.3.2 Artemia

The experience at Ardtoe with cod rearing is that the Artemia phase has always been problematical, and was so in this trial, and in later batches. Different enrichments/rates were tried, and many husbandry factors were examined, but there were still continuous mortality and "floaters" (suggested as a stress reaction) amongst the feeding fish. Perhaps the high mortality (Table 1) simply reflected the poor egg quality.

Although the cod accepted Artemia enriched with D.C. Protein Selco in preference to rotifers, feeding rates were not high and a much better feeding response was produced using the oil-based DC Selco product. Effectively, the fish received a setback of 12 days. However, the DC Selco, although less oily than the "traditional" Super Selco, proved to be too rich, evidenced by the rapid appearance of "floaters" with full guts. Consequently the Artemia enrichment was reduced to once per day, instead of twice, and there was a brief improvement. However, 12 days later, on day 65, there was the first significant sinking mortality of fish with swimbladders, and these were "thin", as were the surviving fish. This prompted the start of feeding dry diets, but also returning to double-enriching the Artemia, but, on this occasion, at half the recommended dose.

When the "floaters" were at their peak, 300 were sent to the Fish Vet Group, and they identified 3 types of Vibrio from them. This led to treatment of one of the tanks on day 61 post hatch with the antibiotic Oxytetracycline 50 ppm for 30 mins, but the only obvious effect was to put that stock of fish off feed for 36 hours. A second tank received a tentative dose of Chloramine T, but that did not even kill the ciliates. Escalating mortality appeared to be controlled when all tanks were treated with oxytetracycline on days 75 and 78. For this reason perhaps regular antibiotic treatment of the Artemia should be used in cod rearing.

During this period, on day 63, the fish reached 550^0 days, which has "traditionally" been a landmark, when they can be netted and graded on a 3mm grid. However, the fish in this trial were obviously not big or robust enough for handling. Growth rate would have been slowed down by the setback of feeding Protein Selco for 12 days, and also probably by the high early densities, and the low winter (out of season) water temperatures, which were around 9^0C, but dipped to around 7.5^0C on days 53/54 and days 64-72. Heaters were installed in two of the tanks on day 66, to hold the water temperature at 10^0C, but this had no obvious beneficial effect, athough this may have been effective if used earlier.

7.3.3 Weaning

Nutra Dry diet was offered from day 65 (580 degree days) and feeding of Artemia was continued until after grading (Day 98). Therefore Artemia feeding duration was more than 65 days.

Lansy diet (Inve) was used but on day 73, there had been no sign of acceptance of the Lansy diet. Therefore, the diet of fish in one tank (H2) was changed to Nutra diets, and subsequently feeding fish were noted within 2 days. On day 84, the fish in the other tanks were also transferred to Nutra feeds, with the same result.

7.3.4 Survival data

Survival here was 2.93%, compared with 4.2%, 7%, 8.5%, 11.3% and 11.4% in 5 tanks reared in the same year under ambient water temperature and seasonal conditions.

7.3.5 Growth

Growth expressed as dry weight was equivalent to ambient spring production (see both growth curves, Fig. 1) up to 400 dd but was lower until 600 dd. This coincided with the problems encountered with poor acceptance of the Artemia enriched with DC Protein Selco and problems with ciliate growth in the tanks. The experiences from Artemia enrichment with the out of season batch were utilised in the later ambient production and it appears that the benefits in survival and growth were derived because of this.

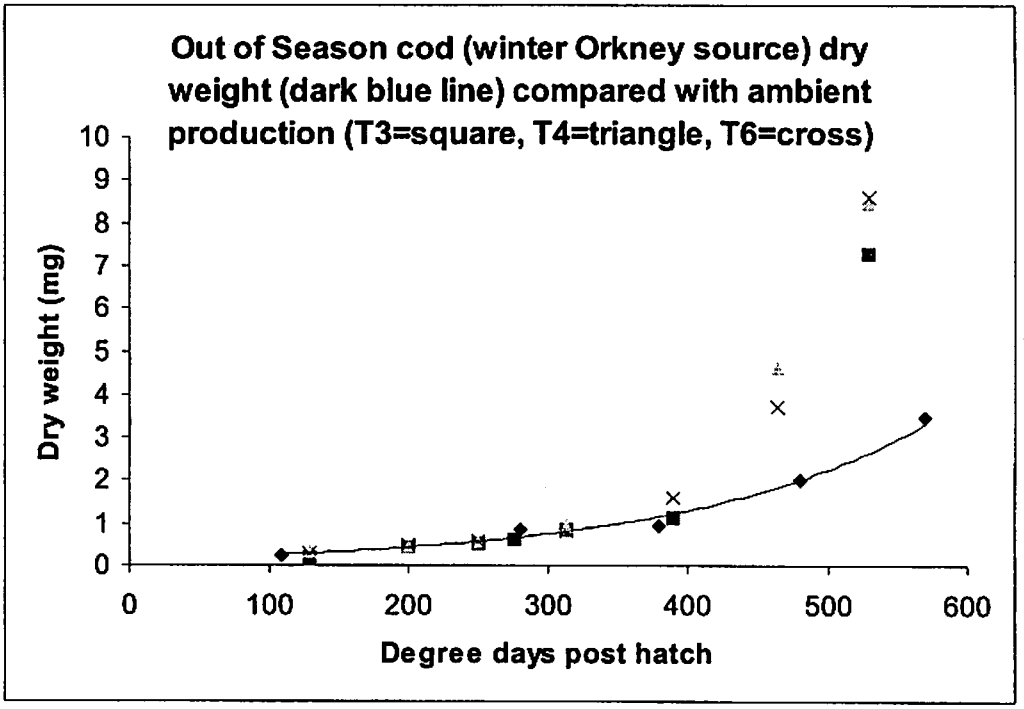


Fig. 1. Growth as dry weight of cod larvae reared in December-January compared with ambient production in March-May

7.3.6 Swimbladder inflation

The range in incidence of larvae with successful swimbladder inflation was wide from 11.1% to 83.3% (n=20 each batch of measured larvae) but the overall average of 45.7% (9 measured batches at 28 dph) was low compared with the mean of 78.2% (6 batches measured) for ambient production.

7.4 Grading and Moving

From day 64 from first offering of dry feed, and despite the non-acceptance of the weaning diet, the cod were growing on the Artemia. There was considerable size variation and consequent aggression. An attempt was made to grade fish on day 82 but was curtailed when 50% of juveniles died from the effects of netting.

An attempt was made to grade the most advanced fish (H2 = early Nutra) on day 87, and the mortality due to netting was only 15%. Therefore, they were graded on a 4 mm grid on days 90/91 (with c.13% mortality) and again at 4 and 5mm on days 96/97. The other 2 tanks were graded on days 97 and 98 with c.6.0% and c.10.5% netting mortality.

Total survival for the three tanks was 4,400 cod juveniles (the smallest grade was not accurately counted, to minimise stress) = 2.93% of the eggs stocked, although, in addition, 8,000 were removed for a research trial on day 42.
After grading, the stock was transferred back to Lansy diets and, although there was extra aggression and mortalities, most converted to the new diet.

7.5 Discussion and Conclusions

A 3% survival from eggs to weaned juveniles of 1 g achieved here for out of season cod production
is a figure lower but comparable with the lower end of survival of cod in spring production (pers.obs.). This was also reasonable given an apparent suboptimal parentage and subsequent poor egg quality. In contrast, the hatchery supplying the eggs failed to achieve any survival in commercial production with eggs from the same stock of fish.

High rotifer density gave high first feed acceptance and early survival, but the prolonged use lead to poor tank hygiene and this was illustrated with: high mortalities, observed ciliates and elevated ammonia levels. Pavlova was not a sufficiently nutritious enrichment for rotifers from day 25 post hatch.

The present trial highlights the requirement for further research in the Artemia feeding phase to increase cod survival, perhaps with improved enrichments and the use of antibiotics to counter *Vibrio* species carried by the Artemia (external laboratory bacteriology tests on larvae were positive for *Vibrio* species).

When stocking photoperiod eggs, temperature control is required to keep the larvae at approx 10^0C as survival can be maximised and development time is reduced.

Although cod juveniles grew well on Lansy diets, the larvae would not accept the diet as a first inert feed.

7.6 References

Puvanendran, V. and Brown, J.A. (1999). Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. *Aquaculture* 175, 77-92.

Sea Fish Industry Authority. (2001). Investigation of Cod Hatchery Techniques and Demonstration of Farming prospects SR 484.

Table 1. Out of season cod production: notes												
STOCKED as eggs	11th December 2002		Dark Static									
Hatched	13th		LUX 90, Flow + Air, Algae									
	14th	Day 1	Pav Rots @ 5/ml - 1 feed									
	15th	Day 2	Pav Rots @ 5/ml - 2 feeds			Skimmers on						
	17th	Day 4	Prot Selco pm									
	23rd	Day 10	Pav Rots @ 5/ml - 3 feeds									
	31st	Day 18	Pav Rots @ 5/ml - 3 feeds			Lots of live No S/Bs/nonfeeders near floor, Ciliates, so FORMALIN						
	3rd Jan	Day 21	Pav Rots @ 5/ml - 3 feeds			Morts siphoned out						
	10th Jan	Day 28	Pav Rots @ 5/ml - 3 feeds			Siph, few morts, but live caught No S/Bs						
	13th Jan	Day 31	Pav Rots @ 5/ml - 3 feeds			Lots dead & dying - lethargic, sparser						
	14th Jan	Day 32	Pav Rots @ 5/ml - 2 feeds			Either tiny S/Bs or none in morts						
(298^{0} Days)	15th Jan	Day 33	FIRST ART - DC Selco Oops									
	16th Jan	Day 34	The P.Selco									
	17th Jan	Day 35				Siphoned - 2000 dead and dying				No S/Bs & Deformed		
	21st Jan	Day 39				Siphoned - 1500 dead & dying - No S/Bs & Deformed						
	22nd Jan	Day 40	Last rots (8 day overlap)									
	23rd Jan	Day 41	2 feeds/day (both P. Selco)									
	24th Jan	Day 42	8,000 to trial - Next day stress/floaters.				700 dead and dying No S/Bs and deformed					
	26th Jan	Day 44					600 dead and dying No S/Bs and deformed					
	28th Jan	Day 46	Cilliates. Very little Artemia being consumed. Fish thin, empty guts									
			lethargic. Change to DC Selco (oil) = better feeding									
	29th	Day 47	FORMALIN									
	31st Jan	Day 48				Floaters off - Siphoned 800 morts - No S/Bs						
	2nd Feb	Day 51				Floaters off - Siphoned 1000 morts - No S/Bs						
	3rd Feb	Day 52				Loads of floaters fewer H2 - Aeration ? Floaters full guts						
						Selco too rich ? Cut enrichment						
						300 -> to fish vet = 3 types vibrio on 11th Feb						
	4th Feb	Day 53	DC Sel no 2nd enrichment			Est 1,600 morts Mostly No S/Bs + zig-zags						
						A few (50) Big H2 - full guts + S/Bs						
	5th Feb	Day 54				Mostly full guts - few morts or floaters						
	10th Feb	Day 59		few morts or floaters - Most nohopers Some good S/Bs. Capsules								
	12th Feb	Day 61				H3 50ppm Oxytet (put fish off feed)						
	Feb 13th	Day 62				H2 10ppm Oxychlor (didn't kill ciliates)						
	Feb 14th	Day 63	= 550 degree Days - should be grading at 3mm - nowhere near - lots of ciliates									
	15th Feb	(Sat) 64	FORMALIN (survivors back from bin room Mostly No S/Bs. All died in transfer)									
	16th Feb	Day 65	1,000 Morts - half had S/Bs - first significant numbers "Thin" 7^{0}C. START 00									
	17th Feb	Day 66	00 + Lansy 2/4 Heaters H2 + 3 = 10^{0}C									
	18th Feb	Day 67	Just Lansy. 1,400 Morts									
	Feb 19th	Day 68	Just Lansy 4/6									
	Feb 20th	Day 69		1,400 Morts			pm Selco back to double enriched					
							Algamac	am to H3 only from today				
	Feb 22nd	Day 70		2,100 morts - lots of rollers								
	Feb 24th	Day 73	No sign of Lansy acceptance. H2 only back to Nutra 00 + 01									
	Feb 25th	Day 74	(600 Morts) Selco double enriched, but half rate									
	Feb 26th	Day 75	Oxytet all tanks., H2 - fish seen taking 01. Cannibalism H3									
	Feb 27th	Day 76	1,100 morts v.few ciliates after Oxytet									
	Feb 28th	Day 77	Lancy 6/8 -> H1 + 3 (Noisy Pump off)									
	March 1st	Day 78	Oxytet all tanks.									
	March 2nd	Day 78	293 Morts Autofeeders on - 2am -> 10am									
	March 4th	Day 80	230 Morts Aggro H1									
	March 5th	Day 82	New Nutra 00 + 01 on site therefore to H2 (+4)									
			H1 tried grading a netful = 50% mort									
	March 6th	Day 83	just pm Artemia feed from today									
	March 7th	Day 84	530 morts. Nutra to All tanks									
	March 8th	Day 85	360 morts	H2 now feeding well on powder - "healthy aggressive"								
			Some feeders other tanks									
	March 9th	Day 86	300 morts + floaters - due to aggro ?									
	March 10th	Day 87	300 morts - Grading tried H2 - 15% mort - survivors all fit 24hrs later									
	March 11th	Day 88	350 morts - H2 lots of cannibalism									

	March 12rh	Day 89	300 morts - Aggro H3	
	March 13th	Day 90	220 morts - H2 over 1,000 graded @ 4mm 218 killed by net	under 4mm H5
	March 14th	Day 91	130 morts - H2 rest 1,500 graded @ 4mm 109 milled by net	683 over 4mm 1D
				regraded 19/20th
	March 20th	Day 97	H3 (E) 17 over 5mm 585 @ 4-5mm 1,200 under 4mm 112 killed by net	
	March 21st	Day 98	H1 (E) 12 over 5mm 368 @ 4 - 5mm 700 Under 4mm 126 killed by net	
			TOTAL 50 over 5mm 1,941 @ 4-5mm 2,400 under 4mm = 4,400	

8. Acknowledgements

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9. Summary

Despite increasing volumes of cod being produced in the UK annually, several bottlenecks to large-scale production, especially at the hatchery stage, require research. To maximise efficiency appropriate larval husbandry, nutrition and environmental parameters must be met.

Algal inoculation regimes in larvae rearing tanks were investigated. Algal concentrations below 300,000 cells/ml of *Nannochloris atomus*, giving a turbidity of less than 3 NTU were found to promote larval cod survival. Concentrations of algae above 600,000 cells/ml produced lower larval survival; this is in stark contrast to larval halibut husbandry. This study was compared to complementary work carried out in Norway. Considering the findings of both pieces of work in combination, we can conclude that addition of moderate concentrations of algae (between 180000 cells.ml^{-1} of *Isochrysis*, and below 300,000 cells.ml^{-1} of *Nannochloris*) can improve cod larval performance. Low levels of algae may be beneficial, perhaps through maintenance of the nutritional value of rotifers grazing on the algae

Levels of turbulence mediated by aeration were also investigated, and an optimal level for a particular tank configuration was found. Foraging behaviour was compromised if turbulence was too low (possibly by reducing the rate of predator-prey interactions) and also if it was too high (whereby the larvae were excessively buffeted and unable to forage).

In addition to the algal and turbulent environment of cod larvae, the rearing salinity may compromise survival. Freshly hatched larvae may become surface-bound and unable to experience prey items, since their large yolk-sacs make them too buoyant to swim through the water column. Lowering the salinity may help prevent this phenomenon of surface-bound 'rafts' of larvae. Both a pilot and a more extensive replicated experiment were inconclusive, or with no significant difference in larval performance, but it was observed that larvae in the low salinity treatment were much better distributed through the water column. More work needs to be done to discern the effects of salinity control during cod larval rearing.

Larvae appeared to exhibit density-dependent growth: tanks with high early mortality and subsequent low density tended to grow faster overall.

There was a positive relation between survival and growth and larval docosahexaneoic acid (DHA) content (around 20-30% EFAs), and between survival and larval arachidonic acid (AA) content (around 3-4%). Rotifers were a fairly efficient "bio-capsule" for transferring EFAs to the larvae. DHA concentrations of around 20-28 % and AA of around 3.5% of total fatty acids were consistently found in the analysis of enriched rotifers. AlgaMac enriched rotifers had significantly higher percentages of DHA than the other diets tested. *Artemia*, on the other hand, rapidly processed DHA. Concentration of DHA in *Artemia*, as percentage of total fatty acids, was around 5-6 %. Concentration of AA was also reduced from levels in rotifers (1-2 %). Besides the many other differences between the two preys, the different EFAs contribution of each prey and the much lower DHA concentration in larvae fed *Artemia* must be an important contributory factor to the decline in survival during this dietary phase. By 10 days *post*-hatch larvae were able to incorporate high levels of

DHA (around 30 % of total fatty acids) to concentrations higher than those in the diets, although those fed on AlgaMac-enriched rotifers did exhibit slightly higher levels. By the end of the *Artemia*-feeding phase, larvae DHA concentration was much lower, between 5 and 10 %. Thus, *Artemia* enriched in the conditions of this experiment seemed to be unable to meet the requirements of larvae for DHA.

The second EFA effective in promoting growth and survival (AA) was also analysed in depth. During the rotifer phase, there was no significant difference in AA levels between the diets, but only the control diet (AlgaMac rotifers only at day 10) appeared to allow larvae to incorporate significantly greater amounts than present in the diet. Arachidonic acid larval levels changed little throughout the experiment. Dietary levels of AA were lower in *Artemia* than rotifers, but unlike DHA, larvae from each dietary treatment seemed able to incorporate significantly higher levels. This maintained their percentage AA at similar levels to those at the start of the experiment. However, larvae from Treatment II (DC DHA Selco *Artemia*) still had significantly lower AA levels than the other two treatments.

Date of weaning can also have a pronounced effect on survival. Larvae weaned onto inert diet at 500 °d presented similar survival to those kept on live prey until 570 °d. Larvae weaned onto inert diet early (400 °d) suffered significantly higher mortality.

Out of season photoperiod larval production in cod was examined to assess whether performance was comparable with ambient produced eggs with reference to survival to weaning, growth, and success of swimbladder development. Poor genetic quality of the egg material was suspected but, nevertheless, survival of 3% from eggs to weaned juveniles of 1 g was attained, comparable with the lower end of survival of eggs spawned in spring. When rearing larvae from photoperiod egg production temperature control is required to rear larvae at 10°C to maximise survival and reduce development time. Prolonged use of high rotifer levels reduced tank hygiene. Action is required to improve survival in the Artemia feeding phase, perhaps related to enrichments and problems with Vibrio disease.