Preliminary Evaluation of the *Schizochytrium-Based* Products, Algamac-3010 and Schizotein, as Diet Enrichments for Atlantic Halibut Larvae.

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SUMMARY

A trial was conducted to evaluate two *Schizochytrium-based* test products (Algamac-3010, Schizotein) as *Artemia* enrichments for rearing Atlantic *halibut (Hippoglossus hippoglossus* L.) larvae. The rearing performance of halibut (in terms of survival. growth and metamorphosis) receiving the test diets was compared to those receiving a UK control feeding protocol (Algamac-2000-enriched and Super Selco enriched *Artemia,* fed in a 1: I ratio). The 3 experimental enrichments comprised Schizotein-only (at 0.6g/l), Algamac-3010 only (at 0.6g/l) and Algamac-3010 (0.6g/l) + Super Selco. Four replicate tanks per diet (110 larvae per tank) were established on the experimental diets on day 9 post-first feeding (307° days post hatch) and run until day 37 PFF (626°d). At day 37 PFF the replicated groups were pooled into two tanks and the larvae continued to receive the experimental diets for a further 15 days, until 54 PFF (839°d). No statistics were applied to the data from the final, non-replicated phase of the trial (days 37-54 PFF). Larvae were sampled for weight measurement on days 9, 26, 37 and 54 PFF. All surviving halibut were scored for eye migration and pigmentation characteristics on day 54 PFF. For data pooled from all treatments, the average survival rate over the period day 9-37 PFF was 52.6% and for the period 38-54 PFF, 98.4%. Diet treatment exhibited clear effects on the growth rates and metamorphosis characteristics of the halibut larvae. The mean dry weight of all halibut at the outset of the study was 1.24±0.06mg and at the conclusion was 37.12±16.55mg, an overall specific growth rate of 7.481%/day. All groups showed a reduction in specific growth rate during the final phase of the trial (days 38-54 PFF), however this was particularly

marked in the Schizotein treatment. Likewise, Schizotein larvae had poorer eye migration and a greater proportion of ambi-colouration than all other treatments on day 54 PFF. Larvae receiving Algamac-3010, (either in isolation, or together with Super Selco) exhibited better eye migration and pigmentation characteristics than all other treatments. The Algamac-3010 only treatment contained the highest proportion of "perfectly" pigmented halibut (65.9%). However, a deceleration in growth rate was observed within this treatment during the latter part of the trial (days 38-54 PFF), which may have been indicative of lipid shortage during metamorphosis.

BACKGROUND

Schizochytrium-based products are now widely used by commercial UK marine fish hatcheries for the enrichment of live prey. As reported by Gara et al (in press), a standardised feeding protocol for larval Atlantic halibut is currently in use, involving the presentation of Algamac-2000™ enriched *Artemia* in combination with *Artemia* enriched using proprietary lipid emulsions, such as Selco™ products (Inve Aquaculture NV). The rationale for this dual enrichment approach is to combine the DHA-boosting attributes of Algamac-2000 with high energy provision *via* the lipid emulsion (metamorphosis having been identified as an energy-demanding process for halibut). However, limitations have been identified with the method, namely the association between prolonged presentation of lipid emulsions and albinism of the halibut juveniles (Gara et al, in press).

Methods are therefore being sought to avoid the pigmentation problems associated with the available emulsions, without incurring the mortality penalty that occurs if emulsions are completely withdrawn. In this respect, the development by Monsanto of new *Schizochytrium-based* products, containing higher lipid contents, provides an opportunity to simplify the halibut feeding regime (ie: requirement for only one enrichment type). Such products may also enable better pigmentation characteristics to be achieved, through greater dietary input of DHA. In conjunction with these new lipid-rich products, the availability of de-fatted cell preparations may enable both the protein and lipid components of live prey to be manipulated.

The aim of the present study was to determine whether the rearing performance (survival rate, growth rate, metamorphosis attributes) of Atlantic halibut could be improved by using a *Schizochytrium-based* preparation (Algamac-3010), with higher lipid content than the commercially available product, Algamac-2000. The trial also incorporated an experimental de-fatted cell preparation (Schizotein), to evaluate whether it is beneficial at any stage of the feeding larval phase to restrict dietary lipid input. The test products supplied by Aquafauna Bio-Marine were compared against a standardised halibut feeding protocol. In the absence of any information on preferred enrichment protocols for these test products, we adopted the basic technique developed for Algamac-2000. Algamac-3010 was tested both alone and in combination with a lipid emulsion (Super Selco™), to investigate whether the lipid content of the experimental *Schizochytrium* product was sufficient to sustain halibut larvae through metamorphosis.

METHODS

On 12th May 1998, 110 Atlantic halibut larvae (9 days post-first feeding, 307° days post-hatch) were stocked into each of sixteen experimental rearing tanks (4 tanks per treatment) containing 801 5*u*m-filtered, UV sterilized sea water and microalgae, *Nannochloris atomus*. The larvae were retained in this replicated tank system for 28 days (37 PFF, 626°d). During this period, 20% of the tank water was exchanged, via a surface inflow, on alternate days and microalgae was added after each exchange. On day 28, the replicate tanks from each treatment were pooled into single tanks (501) and the larvae were reared for a further 15 days, until day 54 PFF (839°d). During this phase of the trial the larvae received a continuous water flow and microalgae was withdrawn. Salinity, oxygen levels and water temperature were monitored daily. The mean temperature (± standard deviation) over the period from 9 to 37 PFF was 11.8±0.1°C and from 38 to 54 PFF was 14.1±0.1°C.

The following experimental diets were presented to the larvae twice daily throughout the trial:

- Ardtoe Control (AlgaMac-2000 & Super Selco-enriched *Artemia,* 1:1 ratio).
- AlgaMac-3010 & Super Selco, 1:1 ratio.
- AlgaMac-3010
- Schizotein

Daily ration levels were adjusted according to consumption, with the aim of minimizing levels of residual prey.

Artemia preparation and enrichment protocols were as follows:

Brine shrimp *Artemia* cysts (EG grade, Inve Aquaculture NV) were decapsulated and incubated for 18 hours in aerated sea water (27°C, 34°/oo), that had been filtered to 5*u*m and UV sterilized. After harvesting, the newly hatched nauplii were set up in aerated seawater in 5 liter polyethylene containers, at a density of 150,000 individuals per liter, at 27°C. These nauplii were retained in sea water only until 18 hours prior to use. Enrichments were blended in freshwater for 1 minute before adding to the nauplii in the 5 liter containers. AlgaMac-2000 was applied in a single dose at a concentration of 0.5g/l, 18 hours prior to feeding. AlgaMac-3010 and Schizotein were applied at a concentration of 0.6g/l, 18 hours prior to feeding. Super Selco was applied in two doses of 0.3g/l, 18 hours and 12 hours prior to feeding.

Samples of enriched *Artemia* (25,000 per sample) were collected on three occasions during the trial and stored in liquid nitrogen, awaiting lipid analysis.

A sample of forty halibut larvae was taken from the source population at the start of the trial (9 PFF, 307° days) for analysis of wet and dry weights. The mean wet weight of these larvae was 15.93±1.62mg (n=40). On day 17 (26 PFF, 507°d) five larvae from each experimental tank were removed for measurement of wet and dry weights, while on day 28 (37 PFF, 626°d) 10 individuals per tank were removed for this purpose. Weights of halibut in the two treatment groups were compared directly on each sampling day. Specific growth rates (SGR, %/d) were calculated according to the equation:

$$SGR = 100 \times (1n(w_i) - 1n(w_t)$$

Where w_t is the final weight (at time t) , w_i is the initial weight and t is the time interval in days.

Within-group growth variability was measured using the coefficient of variation for wet weight (%), which was calculated for each sampling day according to the equation:

$$CV = 100 \times s.d./x$$

Where s.d. is the sample standard deviation and x is the sample mean.

At the conclusion of the trial (54 PFF, 839°D) eye migration and distribution of pigment were classified for individual fish using the methods described by Gara *et al* (in press). Each fish was examined and scores relating to eye migration and pigmentation were assigned by visual inspection. Eye migration was assessed by laying the larva flat on it's blind side and awarding a score as follows:

0: Blind side eye not visible.
I: Blind side eye just visible.
2: Full diameter of blind side eye visible, but lens not visible.
3: Complete migration, both eyes on ocular side with lenses visible.

Pigmentation was scored separately for the ocular and blind sides of the body using a 5 point scoring system.

RESULTS

- **SURVIVAL**

The survival rates of halibut larvae from the 4 treatment groups, measured on day 37 PFF and day 54 PFF are shown in Table 1. The mean survival rate (±st. dev.) during the 28 day period from days 9 to 37 PFF was 52.61 ± 16.68%. No significant variation was seen in percentage survival over this period ($F_{3:12}$=0.33, p>0.05), although those reared on Schizotein showed a lower rate than all other treatments. From days 38 to 54 PFF a mean survival rate of 98.45% was seen. and this was similar in all groups (Table 1).

Table 1. Percent survival (±st. dev.) of halibut larvae reared on 4 experimental diets, measured on days 37 and 54 post first feeding (626' and 839d).

DIET	Survival (%) 9-37 PFF	Survival (%) 38-54 PFF
Schizotein	45.00 ± 17.02	100
AlgaMac 2000 + Ss	56.59 ± 10.51	98.8
AlgaMac-3000 + SS	54.76 ± 10.05	97.5
AlgaMac 3000	54.32 ± 18.08	97.5

- **GROWTH PERFORMANCE IN TERMS OF WET WEIGHT**

Wet weight data are presented for the benefit of commercial hatchery operators, who are not equipped to carry out dry weight measurements. The mean wet weight (± st. dev.) of halibut larvae at the beginning of this trial (day 0) (9 PFF, 307°d) was 15.93±1.62mg. On day 17 (26 PFF, 507°d) this had increased to 44.92±19.20mg. At this time there were significant differences between the weights of halibut in the different treatment groups ($F_{3:10}$=5.74, P<0.05) with larvae reared on the Algamac-3010 + SS regime having the highest mean weight (Fig. 1). The increase in wet weight over this period represents a mean specific growth rate (SGR) of 6.15±0.65%/day and there were no significant differences between treatment groups ($F_{3:10}$=5.74,P>0.05). SGR values for each treatment group are shown in Table2.

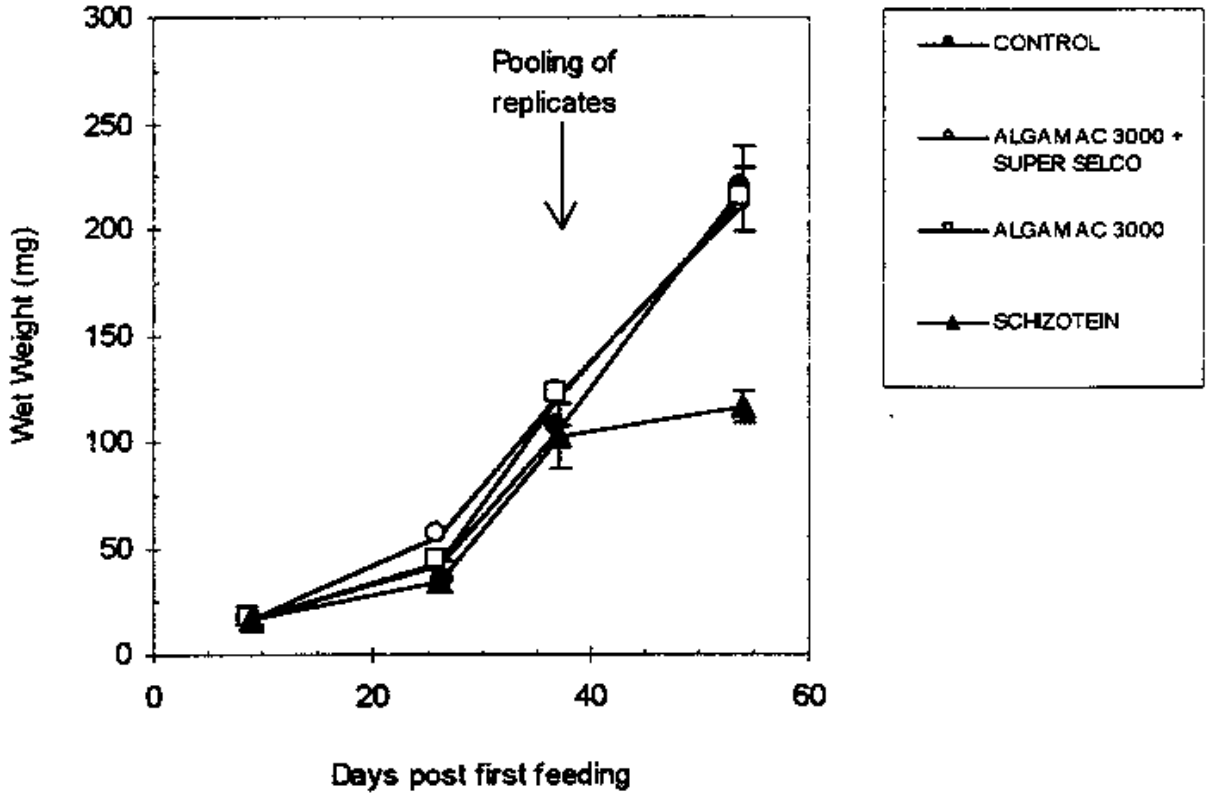

Figure 1. The increase in mean wet weight (± standard error) of halibut larvae reared on 4 experimental diets over the trial period, from 9 to 54 days post-first feeding.

Table 2. Mean wet weight specific growth rates (%/d) (± standard deviation) of halibut larvae reared on 4 experimental diets during the first two time intervals in this study, and from 9 to 37 PFF.

DIET	9-26 PFF	27- 37 PFF	Overall 9-37 PFF
Schizotein	4,54 ± 0.28	9.35 ± 0.89	6.62 ± 0.38
AlgaMac 2000 + SS	5.67 ± 0.76	9.66 ± 2.07	7.33 ± 0.26
AlgaMac 3000 + SS	7.19 ± 1.49	7.81 ± 2.58	7.30 ± 0.23
AlgaMac 3000	7.19 ± 1.52	9.20 ± 5.31	7.60 + 0.43

On day 28 of this trial (37 PFF, 626°d) the mean wet weight of all experimental halibut was 119.79±16.Omg. The weights of halibut in the 4 diet treatments at this time are shown in Fig. 2. The wet weight specific growth rate (%/day) of experimental halibut between days 27 and 37 PFF was similar in all treatment groups ($F_{3:10}$=0.29, P>0.05). However, the SGR over the entire replicated trial period, from days 9 to 37 PFF,

showed significant variation among treatment groups ($F_{3:10}$=5.70, P<0.05), with halibut receiving the Schizotein diet having significantly lower growth rates than those on the Algamac-3010 diet (Table 2).

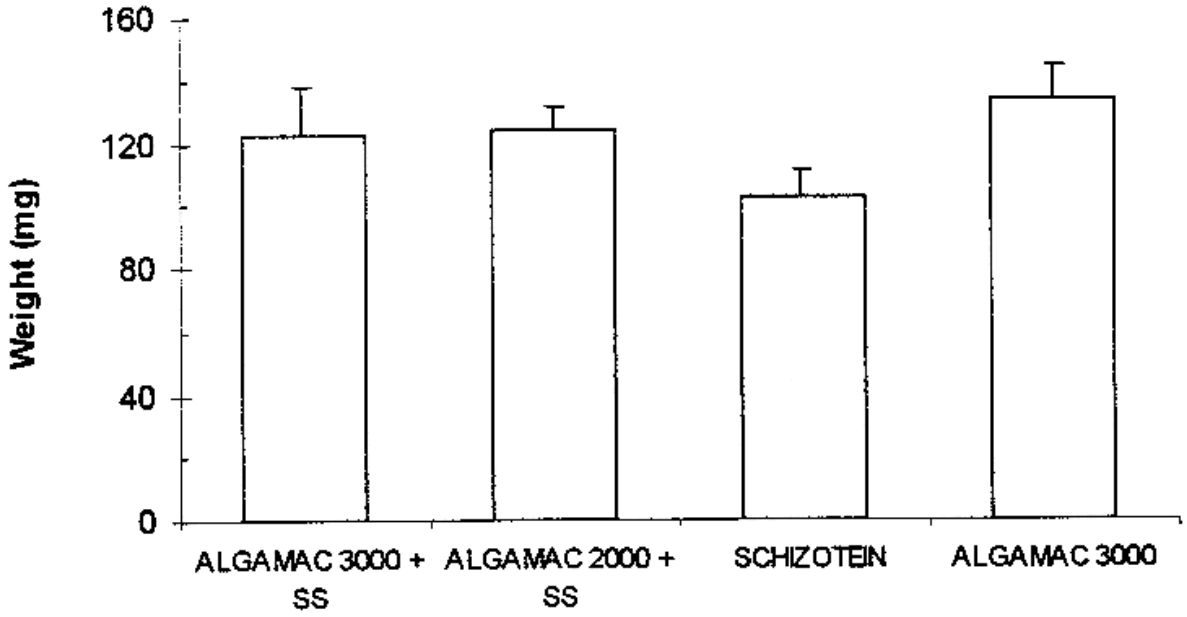

Figure 2. Mean wet weight of halibut larvae reared on 4 experimental diets on day 28 (37 PFF, 626°d). *Vertical bars represent one standard deviation.*

On day 54 PFF (839°d) the mean wet weight of experimental halibut was 190.18±79.16mg. Table 3 presents the mean weights of halibut from each treatment, the corresponding SGR values for the period 38-54 PFF, and SGRs for the entire trial period day 9 to 54 PFF. On day 54 PFF the wet weights of halibut reared on Schizotein-enriched *Artemia* were highly significantly lower than those of all other treatment groups ($F_{3:76}$=10.51, P<0.001, Fig. 1). In this case the individual fish was treated as the replicate for the purpose of analysis as there was only one tank per treatment.

Table 3. Mean wet weights on day 54 PFF of halibut larvae reared on 4 experimental diets, specific growth rates (%/d) over the final non-replicated period of the trial (days 38-54 PFF) and over the entire 45 day study period. *Standard deviations in parenthesis.*

DIET	Mean weight (mg), day 54 PFF	SGR (%/day) 38 - 54 PFF	SGR (%/day) 9 - 54 PFF
Schizotein	116.57 (35.32)	0.78	4.42
AlgaMac 2000 + SS	219.04 (90.60)	3.34	5.82
AlgaMac 3000 + SS	211.12 (70.16)	3.17	5.74
AlgaMac 3000	213.98 (63.52)	2.73	5.77

Within-group growth variability, as assessed using the coefficient of variation for wet weight, increased substantially in all treatments during the first time interval of the trial, days 9-26 PFF, (Figure 3). Larvae in the Schizotein treatment exhibited the highest growth variability on day 26 PFF, while those in the control treatment (Algamac-2000 + SS) had the lowest. Coefficient of variation had decreased overall by the end of the replicated phase of the trial, on day 37 PFF, although the inter-treatment differences in CV were greater at this time (Figure 3). The highest growth variability was seen in the Algamac-3010 + SS treatment group, while the Algamac-3010 and Algamac-2000 + SS groups had similarly low CVs.

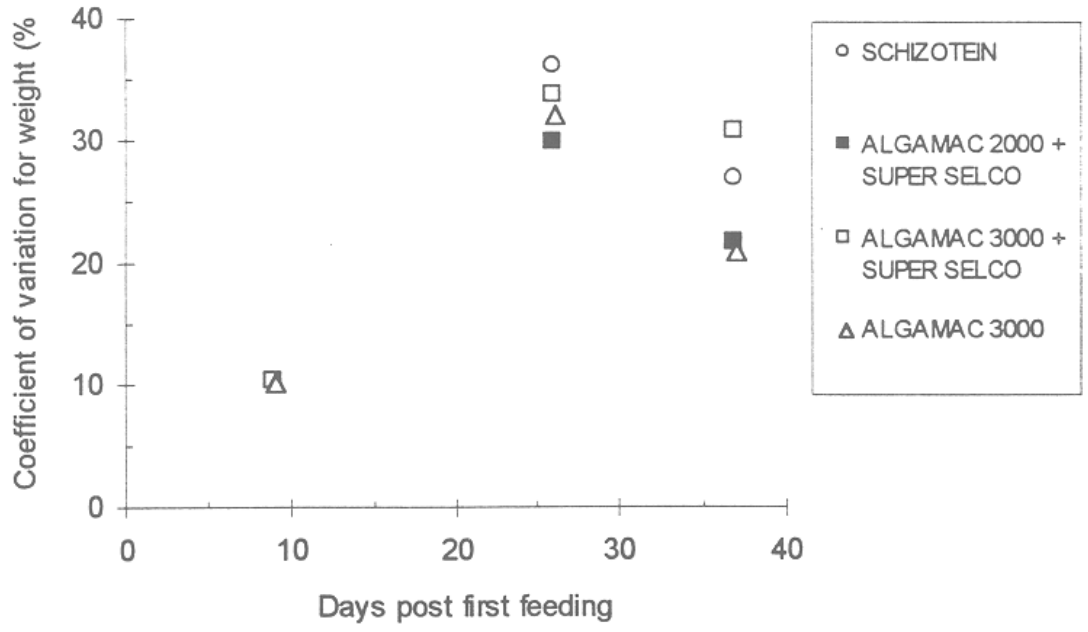

Figure 3. The coefficient of variation for wet weight (%) in the four treatment groups of halibut from day 9 to 37 post first feeding.

- **GROWTH PERFORMANCE IN TERMS OF DRY WEIGHT**

The mean dry weight (±st. dev.) of halibut on day 0 (9 PFF, 307°d) was 1.24±0.06mg. By day 17 (26 PFF, 507°d) the mean weight had increased to 5.97±2.82mg. There were no significant differences between the dry weights of halibut in the various treatment groups at this time ($F_{3:12}$=2.11, P>0.05). The mean specific growth rate over this period was 9.01 ± 1.71 %/d. The mean dry weight (±st. dev.) of all experimental halibut on day 28 (37 PFF, 626°d) was 20.11±3.O1mg. At this time halibut reared on the Schizotein diet exhibited the lowest mean dry weight (Figure 4), however the differences between the 4 treatment groups were not statistically significant ($F_{3:10}$=2.24, P>0.05). On day 45 (54 PFF, 839°d) the mean dry weight of experimental halibut was 37.12±16.55mg. The mean weight of halibut reared on the Schizotein diet was at this time highly significantly lower than all other treatment groups ($F_{3:76}$=8.85, P<0.001); there were no differences between the remaining 3 treatment groups.

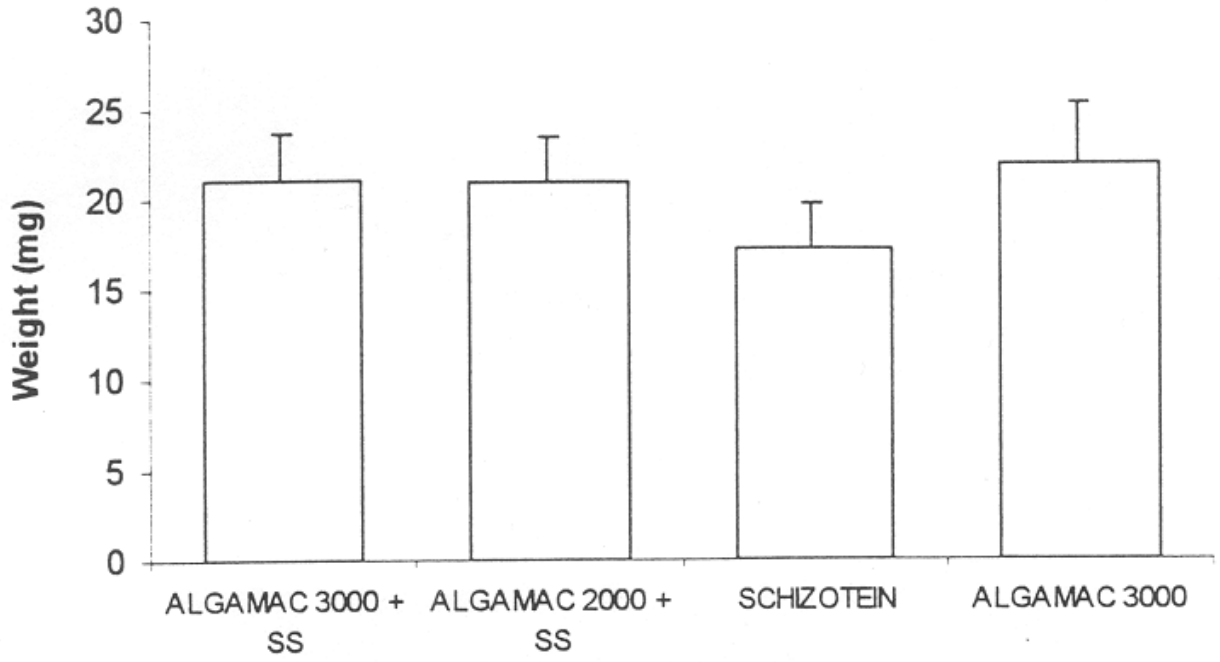

Figure 4. Mean dry weights of halibut from the 4 treatment groups on day 37 PFF (626°d). *Vertical bars represent one standard deviation.*

The dry weight SGRs of halibut in the four treatment groups over each growth period and over the entire experimental period are shown in Table 4.

Table 4. Dry weight specific growth rates (1/o/d) (± standard deviation) of experimental halibut over the first three time intervals of this study and over the entire study period from 9 - 54 days PFF.

DIET	9 - 26 PFF	26 - 37 PFF	38 - 54 PFF	9 - 54 PFF
Schizotein	6.92 ± 2.01	13.17 ± 1.79	1.63	6.47
AlgaMac 2000 + SS	9.20 ± 1.17	13.31 ± 3.96	4.27	7.9
AlgaMac 3000 + SS	9.81 ± 1.30	10.01 ± 2.70	3.74	7.8
AlgaMac 3000	9.44 ± 1.38	10.98 ± 0.96	2.8	7.76

Between-treatment differences in dry weight SGR followed the same trends as the wet weight SGR data. The Algamac-3010 and Schizotein teatmcnts experienced particularly marked reductions in SGR over the final time interval, days 38-54 PFF

- **METAMORPHOSIS**

As final metamorphosis data was collected at the conclusion of this trial, following the pooling of all replicate tanks on day 37 PFF, there is no data on the variability in the parameters under investigation and no statistical analyses were undertaken. Eye migration at the conclusion of the trial (54 PFF, 839°d) was much poorer for halibut reared on the Schizotein diet than for those from all other treatments (Figure 5). The two Algamac-3010-based diet treatments had equally high eye migration scores, while the Algamac-2000 + SS treatment was intermediate in this respect. The pigment category results paralleled the findings on eye migration (Figure 6). The Schizotein treatment contained the greatest proportion of ambi-coloured halibut and the lowest proportion of fish with correct pigment distribution (35.4%). The Algamac-3010 based treatments contained the highest proportions of correctly pigmented halibut (65.9% for Algamac-3010 only), while the Algamac-2000 + SS contained an intermediate proportion of fish with correct pigment distribution.

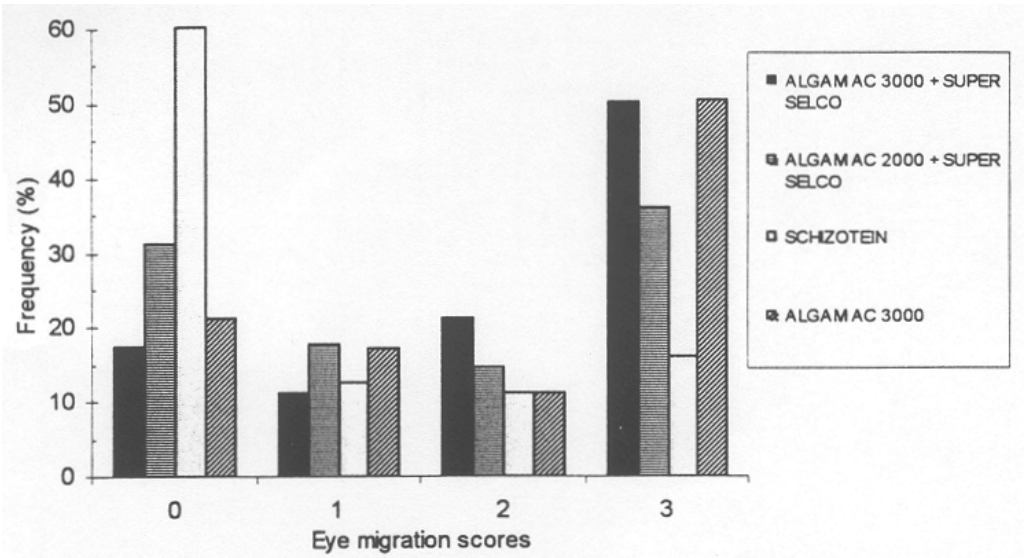

Figure 5. Percent frequency distribution of halibut eye migration categories (54 PFF, 839°d). 0: blind side eye not visible; 1: blind side eye just visible; 2: full diameter of blind side eye visible, but lens not visible, 3: complete migration, both eyes on ocular side with lenses visible.

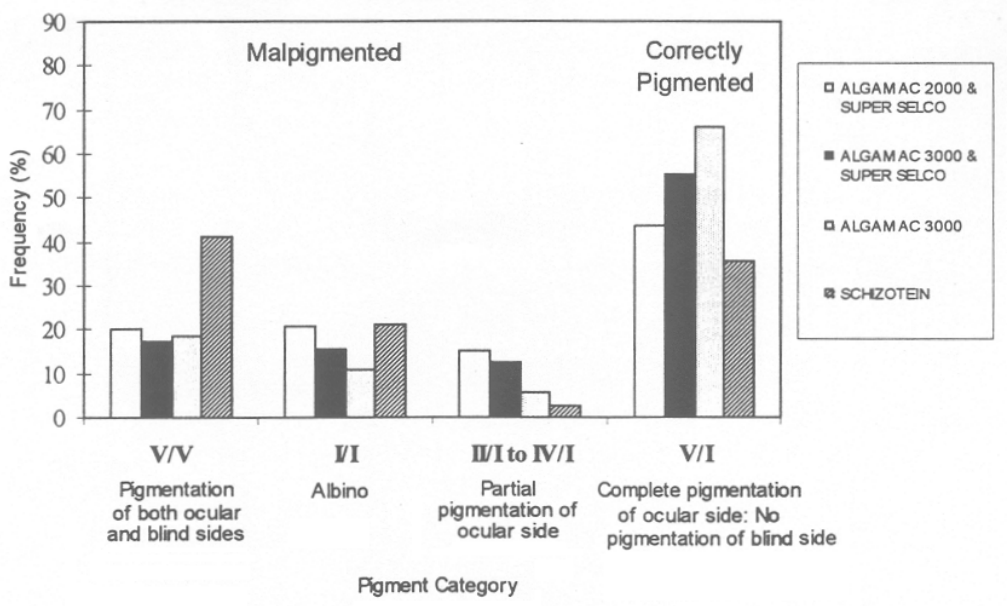

Figure 6. Percent frequency distribution of halibut pigment categories (54 PFF, 839°d).

DISCUSSION

Artemia enrichment using the *Schizochytrium*-based test products resulted in significant differences in halibut rearing performance, relative to the control feeding regime. In discussing the observed diet-related differences in rearing performance, it is most straightforward to differentiate the Schizotein treatment from the 3 remaining treatments. Schizotein was clearly inferior as an *Artemia* enrichment in terms of halibut growth rate and metamorphosis attributes (eye migration and pigment distribution). The specific growth rate of Schizotein larvae was relatively low during the replicated phase of the trial (days 9-37 PFF), but more particularly during the final period, from days 38-54 PFF. Related to this, these larvae also exlubited much poorer eye migration and a higher proportion of abnormal pigmentation (ambi-colouration) on day 54 PFF. The Schizotein-fed larvae furthermore suffered a substantially higher mortality during the replicated phase of the trial.

The growth and metamorphosis attributes of the Schizotein larvae are comparable to those previously reported by Shields and Bell (1995), for halibut reared using either Super Selco or Algamac-2000 enriched *Artemia.* The low growth rates in that study resulted from insufficient prey ingestion rates and, in our subsequent diet experiments, it has become standard practice to feed halibut larvae to satiation. Despite adopting that approach in the present study, the Schizotein larvae grew much less rapidly than larvae from all other treatments. We interpret this as a nutritional deficiency in the enriched *Artemia,* rather than reduced prey ingestion by the Schizotein larvae. It should be emphasised that, at the outset of this study, the Schizotein preparation was an "unknown quantity", both to ourselves and to Aquafauna Bio-Marine. It was not feasible to carry out enrichment optimisation trials with the product in advance of the halibut feeding trial, therefore the adopted protocol may have been incorrect. This situation will be clarified once lipid analyses of the enriched *Artemia* have been completed. The results on rearing performance indicate that the lipid requirements of halibut larvae exceed the levels that can be inputted via de-fatted cells, nonetheless there may be scope for using such preparations in conjunction with other types of enrichment product.

The results achieved with Algamac-3010 were, by contrast, superior to the control regime in most respects. When offered either as the sole enrichment, or in conjunction with Super Selco, Algamac-3010 produced the best eye migration and pigmentation characteristics of any of the diet treatments (although, no statistical comparisons were possible, due to pooling of replicate groups on day 37 PFF). Growth rates were also initially the highest (day 9-26 PFF), although the SGRs fell below that of the control treatment (Algamac-2000 + Super Selco) from day 27 PFF onwards. This deceleration in growth rate was particularly marked in the Algamac-3010 only treatment, possibly due to insufficient dietary input of lipid during metamorphosis.

In conclusion, the Algamac-3010 test product has shown good promise as an *Artemia* enrichment for rearing Atlantic halibut larvae. We were particularly encouraged that the

positive effects on rearing performance were achieved in the absence of preliminary enrichment optimisation trials, although such trials are surely needed. Despite the product's higher lipid content relative to Algamac-2000, there was some evidence that lipid input becomes limiting for halibut larvae during metamorphosis, when supplied only with Algamac-3010 enriched *Artemia.* In this respect, it would be valuable to test the next generation *Schizochytrium* product, Algamac 3010 in a future diet trial. As for the Schizotein product, we conclude that lipid content was too low to provide energy for sufficiently rapid growth of halibut larvae, when used in isolation.

ACKNOWLEDGEMENTS

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