The effect of secondarily enriched rotifers on growth and survival of marine fish larvae

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for a degree of Doctor of Philosophy

by

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ABSTRACT

Nutritional aspects of three species of marine fish larvae, herring Clupea harengus L., plaice Pleuronectes platessa L. and turbot, Scophthalmus maximus L. were examined. Larvae were fed three different diets all of which were derived from rotifers Brachionus plicatilis (O.F. Müller). These rotifers had been mass-cultivated on baker's yeast and then enriched with baker's yeast, or with one of two unicellular algae, Isochrysis galbana Park or Nannochloropsis (Nannochloris) oculata (Droop) Hibberd.

The biochemical changes that these enrichments conferred on the rotifer were examined. There was little difference in proximate and amino acid compositions. Dry weights, and calorific contents of the rotifers fluctuated according to the type, temperature and duration of enrichment, with the yeast enriched rotifers tending to weigh less (200-320 ng) than their algal counterparts (250-370 ng). Major differences were found in the total fatty acid profiles of the rotifers. Yeast-enriched rotifers had no 18:3n-3 or 20:5n-3 and only trace amounts of 22:6n-3 fatty acids. The Nannnochloropsis-enriched rotifers had substantial amounts of n-3 fatty acids though only of 20:5n-3 (11-14%), while rotifers enriched with Isochrysis had only trace amounts of 20:5n-3 (2-4%).

All three species of fish responded similarly to the yeast-enriched rotifer diet. Their growth, in terms of length and weight, was minimal and they never developed any fin ray elements. Both flatfishes developed a looped gut in the short growth duration on this diet and some turbot (20%) inflated their swimbladder. This retardation of growth was attributed to the lack of long chain highly unsaturated n-3 fatty acids (n-3 HUFA) in the diet.

During one month feeding trials it was shown that herring and plaice were able to exploit both algal-enriched rotifer diets equally well. Turbot, on the other hand grew better, over 14 days, on rotifers enriched with Isochrysis compared with Nannochloropsis. The reaction of the different species was linked to the fatty acid profiles of the diet. Hence, turbot require a dietary input of 22:6n-3 while herring and plaice thrived equally well on diets containing only 20:n-3.

Juvenile plaice consistently exhibited improved pigmentation when Isochrysis compared to Nannochloropsis were used as the rotifer enrichment. The effect was attributed to specific nutrients particular to algal chloroplasts, which need to be present in the diet of the larvae up to metamorphosis.

Assessment of daily rations, employing the rotifer enriched with Isochrysis diet only, showed herring to reduce their food intake from 46 to 19% body dry weight/d between days 10-21 post hatch while turbot consumed between 34-169% body dry weight/d with no apparent age related effects. The gross growth efficiencies (K1, Brett & Groves, 1979) for both species were seemingly unaffected by age related processes and fluctuated between 15-50% in turbot and 20-61% in herring according to fluctuations in daily food intake. Ingestion is further described for both species with a linear model that related food intake to larval weight. The weight-specific ingestion was found to be a constant 13% and 43% of body dry weight in herring and turbot respectively. The weight-specific ingestion and specific growth rate estimates were combined with other data available from the literature, revealing that larvae tend to consume food (in dry weight) at a level equivalent to approximately twice their daily dry weight increase.

INTRODUCTION

One of the main obstacles facing the current expansion of marine fish farming, excluding salmonids, is the availability of weaned juveniles for stocking the ongrowing facilities. European hatchery production during 1985 has been estimated at 6.5 million juveniles of sea-bass (Dicentrarchus labrax), gilt head bream (Sparus aurata), and turbot (Scophthalmus maximus) according to J. Olsen, (1986) and by 1995 the Norwegians alone predict an annual production of 120,000 tons of marine fish (Oiestad, 1987), a probable requirement of 80-100 million juveniles. In Japan the current annual production stands at 300 million juveniles (Kanazawa, 1985). The provision of these large numbers of juveniles is beset by problems of mortalities in the larval phase and the need to provide most species with vast quantities of very small food items at their early feeding stages. Mortalities could also be reduced by enhancing egg quality through brood stock nutrition (Watanabe, 1985), providing an optimal physico-chemical environment for the larvae, and determining their dietary requirements. It is this last issue that the present study addresses.

Most marine fish larvae are planktivorous and, in the wild, feed on small crustaceans or other zooplankters. Unless exogenous feeding is initiated at, or closely following, yolk absorption, their condition rapidly declines so that they reach what has been termed the point-of-no-return (PNR, or irreversible starvation). At this point the larvae are still alive but too weak to feed if food becomes available (Blaxter & Hempel, 1963). First feeding success has an immediate impact on the potential number of larvae surviving to

metamorphosis in the hatchery and depends on the species, size and density of prey they are offered (reviewed by Hunter, 1980).

Zooplanktonic prey are usually offered to artificially reared larvae at densities of 5-10 individuals/ml, which is a daily requirement for 5-10 million zooplankters/1 m^3 larval tank. Such quantities make feeding on sea-caught zooplankton, in most cases, a practical impossibility. As mass cultivation of naturally encountered prey such as copepods has had only limited success (Kahan et al., 1982; Kuhlmann et al., 1981), aquaculturists have resorted to the use of readily available food organisms. There are two main species currently employed, the first is a rotifer Brachionus plicatilis (O.F. Müller) commonly offered to first-feeding larvae over the first 7-10 days of exogenous feeding, followed by nauplii of the brine shrimp Artemia salina. A few species, such as herring Clupea harengus, plaice Pleuronectes platessa, and sole Solea solea, have large enough mouths to take brine shrimp without the initial rotifer phase.

Rotifer mass culture was initiated in Japan in the early sixties (Ito, 1960), introduced to the West by Theilacker & McMaster (1971), and has since undergone considerable technological refinements. Rotifers are raised on a variety of microalgae or yeast, at densities of 200-600 individuals/ml with daily yields of 10-40% of the standing stock (Lubzens, 1987). B. plicatilis is found in a range of genetically determined sizes from which the aquaculturist can choose a prey size to suit the requirements of the species of fish being reared (Fukusho & Okauchi, 1982; Snell & Carrillo, 1984). Artemia nauplii are readily obtained from hatching of commercial dried cysts which in 1975 were estimated to be used by 99% of mariculturists (Sorgeloos & Persoone, 1975).

Larval culture techniques could be greatly improved by introducing inert larval diets, thereby eliminating the need for mass cultivation of live food. Finely ground microparticles and microcapsulated diets have already been tried with some success on larvae of plaice (Adron et al., 1974), sole (Appelbaum, 1985), Atlantic silverside Menidia menidia (Beck & Bengston, 1979) and sea bass (Barnabe, 1976), although growth and survival were inferior to those obtained when using live feed.

Formulated diets can also be employed when investigating specific dietary requirements in fish larvae, as mentioned by Kanazawa (1985) for the phospholipid requirements of red sea bream Pagrus major, and ayu Plecoglossus altivelis. Although the composition and size of inert diets can be predetermined their use has mostly been unsuccessful (Hofer, 1985). This is probably due to a combination of factors: rapid sedimentation, leaching, a limited capacity to stimulate larval feeding and the digestive potential of larvae. It is this last factor which seems to be the most troublesome. Marine fish larvae at first-feeding possess a digestive tract divided into fore, mid and hind-gut regions separated by sphincters, an arrangement which remains basically unchanged up to metamorphosis when the functional stomach (involving acid secretion) and pyloric caeca start to develop (Tanaka, 1973; Govoni et al., 1986). Although a variety of active digestive enzymes and a functional pancreas and liver have been observed in first-feeding larvae (Tanaka, 1973; Govoni, 1980; Govoni et al., 1986; O'Connell, 1981; Cousin et al., 1987), in some instances their activity levels have been reported either to increase (Kawai & Ikeda, 1973b) or fluctuate (Hjelmeland et al., 1983) throughout larval life. For example in lipid digestion only esterase activity has been observed

in first-feeding pike Esox lucius (Szlaminska, 1980) and turbot, whilst lipase activity has only been reported in turbot at a stage near metamorphosis (Cousin et al., 1987). The mechanism of protein digestion in larvae is markedly different from that observed in adult forms. In larvae it is intracellular following pinocytosis of the macromolecules in the hind-gut (Watanabe, 1981, 1982a, 1984a), while normal extracellular peptic digestion is initiated after functional stomach formation at metamorphosis. Such factors might also explain the increasing success reported for weaning larvae on to inert diets as they near metamorphosis (Bromley & Howell, 1983; Bromley & Sykes, 1985).

The second major problem in deploying inert diets is the lack of information on dietary requirements of larvae. At present these can only be inferred from studies on adults or larger freshwater forms, such as carp, salmonids, or coregonids. Moreover the specific requirements for minerals, vitamins or amino acids could change throughout ontogeny (Cowey, 1979; Dabrowski, 1986).

Many of the problems of inert diets do not exist with live feeds; in fact living prey may contain enzymes which complement and enhance the digestive processes of the predator (Lauff and Hofer, 1984). On the other hand an ability to manipulate the biochemical composition of live prey, which is a prerequisite in nutritional experiments, is limited in comparison with what can be done to inert diets. Nevertheless, it is still possible to alter some of the biochemical components of live food, and rotifers in particular, as has been shown in the past (Scott & Middleton, 1979; Watanabe et al., 1983a), and in the present study.

The dietary value of rotifers, as reflected in larval survival and growth, is known to depend on the food used for cultivation or

enrichment immediately prior to feeding to the larvae. Raising rotifers on baker's yeast, a practice initiated in Japan in the early seventies, produces consistently poor growth and high mortality rates in larvae. On the other hand using algae in conjunction with yeast or as an enrichment eliminates this problem (Watanabe et al., 1983b). Not all algae, however, confer good growth results on marine fish larvae and it has been found that in beneficial algae such as Chlorella sp. (Watanabe et al., 1983b), Tetraselmis tetrathele (Fukusho et al., 1984) and Isochrysis galbana (Scott & Baynes, 1979) it is the highly unsaturated fatty acids (HUFA) 20:5n-3 and 22:6n-3 which determine their value.

The highly unsaturated fatty acids required by teleosts differ from those of terrestrial animals in having their first double bond at the n-3 compared with the n-6 position. Since these fatty acids are incorporated into the phospholipid fraction of bio-membranes, the above difference is assumed to stem from the need in aquatic poikilotherms for fatty acids with lower melting points. Consequently, these bio-membranes will retain their physiological activity at lower temperatures (Castell, 1979; Cowey and Sargent, 1979).

HUFA are most abundant in the structural phospholipid fractions of tissues and in juvenile red sea bream radioactive 20:5n-3 was shown to be incorporated into physiologically active tissues such as the gills, alimentary canal, swimbladder, gall-bladder, liver, pyloric caecae, skin and muscle (Kanazawa et al., 1983). Unsaturated fatty acids are important for the stabilization of lipid-protein complexes that are formed from fatty acidsfollowing their absorption across the intestinal mucosa. In their absence the transport of lipids from their absorption site into

the body will be impaired (Castell, 1979). Although there are suggestions that dieteary HUFA deficiencies cause the deterioration of the alimentary canal and associated organs in larval turbot (Cousin et al., 1986), such cause-effect relations have so far not been systematically studied in marine fish larvae. On the other hand it has been demonstrated that juvenile turbot (4.9 g) could not grow on diets deficient in HUFA, and developed a pathological condition culminating in the degeneration of their gill epithelium (Bell et al., 1985). In salmonids a variety of pathological conditions, such as fatty livers, increased water content and reduced feeding efficiency have been associated with a deficiency of dietary linolenic acid (18:3n-3) (Castell et al., 1986).EFA deficiencies are therefore likely to impair lipid transport and cause structural, energetic and enzymological imbalances that will undoubtedly suppress the ontogeny of the rapidly developing larvae.

Ripe roes of marine fishes are rich in HUFA. Atlantic silverside (Menidia menidia) have 40% HUFA of the n-3 isomers in their eggs (Schauer & Simpson, 1979). In herring, it has been shown that 47% of fatty acids of the phospholipids, and 31% of the fatty acids of neutral lipids, are polyunsaturates, mainly (97%) of the n-3 isomers (Tocher & Sargent, 1984). In turbot eggs n-3 HUFA have been estimated from 26% (Witt et al., 1984) to 38% (Scott & Middleton, 1979) of total fatty acids. HUFA are mostly retained throughout embryogenesis so that newly hatched larvae have HUFA levels very similar to those of the eggs, i.e. 22-32% in turbot (Scott & Middleton, 1979) and 49%, and 27% of phospho- and neutral lipids respectively in herring (Tocher et al., 1985a,b). This selectivity in retention of HUFA, mainly 22:6n-3, has been demonstrated to continue for at least the first 14 days after hatching of herring

larvae (Tocher et al., 1985a). A similar process seems to be taking place in turbot larvae as attested by the slow decline of 22:6n-3 during 20 days of rearing on a diet deficient in this fatty acid (Scott & Middleton, 1979). Selective retention of HUFA might also take place in developing rainbow trout, Salmo gairdneri, (Hayes et al., 1972) and Atlantic silverside (Schauer & Simpson, 1979).

Dietary requirements for essential fatty acids in fish usually range between 0.5-1% of dry diet (Kanazawa, 1985), and in fresh water forms, eels and salmonids consist mainly of linolenic acid (18:3n-3). Trout can readily bio-convert 18:3n-3 to eicosapentaenoic (20:5n-3) and decosahexaenoic (22:6n-3) acids. True marine forms, such as turbot and red sea bream, however, have very poor abilities for such bio-conversion and therefore require 20:5n-3 and 22:6n-3 in their diet (Owen et al., 1975; Yamada et al., 1980; Castell, 1979).

Essential fatty acid requirements of turbot larvae have received considerable attention due to the commercial importance of this species. One of the major breakthroughs in rearing of turbot larvae and to the general understanding of dietary EFA requirements of marine fish larvae was made by Scott & Middleton (1979). These authors fed the larvae on rotifers enriched with a variety of unicellular algae and showed that stunted growth and high mortalities occurred when Dunalliela tertiolecta was used as an enrichment. If, however, Pavlova lutheri, Isochrysis galbana or Phaeodactylum tricornutum were used, larvae grew and developed well. The differences were attributed to the high levels of 20:5n-3 and 22:6n-3 in the latter algae, acids which are present only in trace quantities in the former. These results explained an earlier observation by Howell (1979), who showed a better growth of the larvae in the presence of I. galbana compared with D. tertiolecta.

Witt et al. (1984) arrived at a similar conclusion after comparing growth of the larvae on either the rotifer-Artemia system with the rotifers enriched with Nannochloris sp. or on different copepod stages of Eurytemora affinis. Larvae grew better on the copepod diet due to the earlier initiation of feeding and the significant quantites of 22:6n-3 present (5-11% of total FA). An analysis of the fatty acids in the rotifers and Artemia revealed them to be devoid of 22:6n-3 though they did have appreciable quantities of 20:5n-3. HUFA can be synthesized to a limited extent by the rotifer (Lubzens et al., 1985) but at a low level which necessitates their introduction into the rotifer using the algae. HUFA can also be incorporated directly into the rotifers by soaking them in emulsified marine lipids such as cuttlefish liver oil, before offering them to the larvae (Watanabe et al., 1983a). Direct enrichment of rotifers by algal substitutes has also been explored by Gatesoupe & Luquet (1981) and Teshima et al. (1981).

A further factor which is associated with the success of rearing marine fish larvae is the presence of microalgae in the rearing tanks. This method commonly referred to as the "green water" technique has been reported to be used in the rearing of a considerable number of fish such as: flounders, Limada feruginea (Smigielski, 1979) and Platichthys stellatus (Policansky & Sieswerda, 1979), turbot (Howell, 1979), mullets, Mugil curema (Houde et al., 1975) and M. cephalus (Nash et al., 1974), sea-bream Archosargus rhomboidalis (Stepiens, 1976) and the anchovy Engraulis mordax (Hunter, 1976; Moffat, 1981). The method is routinely employed in Japanese fish hatcheries where Chlorella sp. is the main algae in use.

The green water effect is known to operate via the enhancement of the rotifers, or other zooplankters, nutritional quality whilst residing in the larval tanks (Scott & Baynes, 1979). Large algae might also be utilized directly by some larvae, as in the case of Engraulis mordax, actively feeding on Gymnodinium splendens (Scura & Jerde, 1977). Smaller (< 10 µm) forms such as Chlorella can passively enter the gut (Howell, 1979; Moffat, 1981) either during feeding on zooplankton, or drinking (Mangor-Jensen and Adolf, 1987), and once inside could (depending on digestibility) contribute to the nutrition. Through their metabolic activities algae might also recycle nitrogenous waste and raise dissolved oxygen levels in the rearing tanks.

The present experiments were aimed at deepening our knowledge of larval nutrition, using techniques of enriching the live food. For control purposes rotifers raised on baker's yeast were fed directly to larvae of herring (Clupea harengus L.), plaice (Pleuronectes platessa L.) and turbot (Scophthalmus maximus L.). The same rotifers were also tried out after a short enrichment with either Isochrysis galbana, commonly regarded as a beneficial algae in mariculture systems for both molluscs (Epifano, 1979) and fish larvae (Scott & Baynes, 1979), or Nannochloropsis oculata, an as yet unexplored alga. The three diets were then compared at the biochemical level. The comparative aspect of this study could therefore provide some insight as to the diversity of dietary requirements among marine fish larvae. Furthermore, comparing the diets on the biochemical level should enable the singling out of possible dietary components required by larvae, these could then be tested using direct rotifer-enrichment techniques. Finally, it was hoped that in conjunction with measurements of daily food intake,

the information on the dietary requirements could be assessed not only qualitatively but also quantitatively.

MATERIALS

A. Herring

Methods for collection and incubation of herring gametes have been developed by Blaxter (1986). Ehrlich (1972) provides a detailed account of the procedures employed for this study. Spawning fish were caught by trawl in the Clyde and their gonads dissected and transported on ice to Dunstaffnage. The eggs were then spread on to glass plates, to which they adhered and the plates with the eggs were dipped in milt for 10 min. Incubation was carried out in a running sea water bath, in darkness, at 7.5-8.5°C, up to hatching 13-14 days later.

B. Plaice

Maturing plaice brood stock, of Clyde origin, were transported back to Dunstaffnage where they were held in large circular tanks. Female maturation was evaluated on the basis of the degree of swelling and softness of the abdomen. Eggs were stripped from each female on a few occasions by applying pressure to the abdomen. These eggs were collected into a glass beaker and fertilized with milt obtained similarly from the males. Sea water was then added to the beaker and about half an hour later eggs were examined for fertilization. The floating fertilized eggs were poured into a 100 l circular tank provided with running sea water and an air stone. Incubation temperatures ranged from 7.5 to 11°C according to the time in the season. Hatching took place 12-14 days later at

which time aeration and water exchange rates were slowed to a minimum.

C. Turbot

Larvae on their day of hatching were purchased from the Golden Sea Produce hatchery at Hunterston. They were transported to Dunstaffnage by road (2-3 h) in 3-5l of sea water in a plastic lined, insulated and sealed box. On arrival at the laboratory they were transferred immediately to the experimental tanks.

METHODS

A. Rotifer mass culture

1. Rotifer strain

The two strains of Brachionus plicatilis (O.F. Müller) which were tested for suitability of cultivation originated from the laboratory's collection (by courtesy of Mr J.M. Scott) and from the SFIA research unit, Ardtoe (by courtesy of Mr J. Dye). The former strain was used when evaluating the optimal dry yeast ration for cultivating the rotifer while the latter was employed in the mass cultures. Both strains are of L-type with a typical lorica length of 226 ± 14 µm $\pm$ S.D. During the 1987 season a smaller S-type rotifer lorica length (187 ± 9.5 µm) invaded the cultures and was used for both the ingestion rates experiments and the proximate analysis of Isochrysis-enriched rotifers. The above classification of L and S types are according to Fukusho and Okauchi (1983).

2. Production requirements and feeding

The maximal rotifer requirement in a larval feeding experiment was calculated at 6×10^6 individuals/day based on a daily stocking level of 5 rotifers/ml in 12 x 100 l rearing units.

For reasons explained in the introduction it was essential that the rotifers be cultivated on baker's yeast, Saccharomyces cerevisiae alone. Mass culture techniques using this feed are well established (Hirata, 1980), and utilize fresh baker's yeast at a daily ration of 1 g/10^6 rotifers. The

absence of a regular supply of fresh yeast made it necessary to resort to a dried commercial form produced by the Distillers Company, Yeast Ltd., Scotland, which was available locally. In this form the yeast requires activation before being fed to the rotifers by adding sugar to the weighed ration of yeast at approximately 1:10 sugar:yeast, mixing in a small volume of lukewarm tap water and allowing to stand in a warm location for 15-30 min or until a thick layer of froth is formed.

2a. Feeding rations

Evaluating the optimal feed ration was done in a two- stage experiment.

Method

In the first, four food rations were tried: 2.5, 1.0, 0.7 and 0.5 g dry yeast/10^6 rotifers/d, henceforth called the 2.5, 1.0, 0.7 and 0.5 rations. In the second, food rations of 0.5 and 0.3 g dried yeast were tried and compared with growth on 1.0 and 0.5 g fresh yeast/10^6 rotifers/d.

Experiments were carried out in 1 l Ehrlenmeyer flasks with 500 ml sea water (Sal ≈ 32 ppt) filtered through a Whatmans GF/C filter paper. A single population of rotifers was divided equally among the flasks (2 per treatment) at an initial density of 52 individuals/ml for the first and 100 individuals/ml for the second experiments. Incubation took place in a constant temperature room (25°C) at constant illumination with continuous aeration. Population growth was recorded daily by withdrawing 3 x 1 ml samples from each flask into a pipette and counting the number of females and eggs under a dissecting microscope. Cultures were rinsed once

throughout each experiment (day 4) by collecting the rotifers on a 60 µm sieve and resuspending in fresh pre-heated sea water. Experiments were terminated after nine days.

Results

Growth as observed in the various treatments had an oscillating component (Figs 1, 2). The only ration to cause an actual decline in the population was the 2.5 g yeast/d. Growth at all other food rations was analyzed using the exponential growth model

$$N_t = N_o e^{r.t}$$

where: N_t = number of individuals at time t, N_o = their initial number and r = the population's intrinsic growth rate. Pooling the data for each treatment, from both experiments (Fig. 3) showed the 0.3, 0.5 and 0.7 rations to produce r values of 0.148, 0.145 and 0.145/d respectively. However, the 0.3 g ration was inferior to the other two as the population reaches an asymptote with the former at approximately 140 individuals/ml whereas it continues to increase up to 300 individuals/ml on the latter rations. An analysis of covariance comparing the intrinsic growth rates showed the 0.5 and 0.7 rations to be identical and to differ significantly from the r = 0.07 produced by the 1.0 ration ($F = 4.08$, d.f =2; 45).

Growth rates on dry and wet yeast were identical for the 0.5 dry and 1.0 fresh rations, r values being 0.14/d. A cell count using a haemocytometer showed that suspending the above rations of yeast in equal volumes of water resulted in equal cell concentrations, e.g. 1 g of wet yeast equalled 1.612×10^{13} cells/ml.

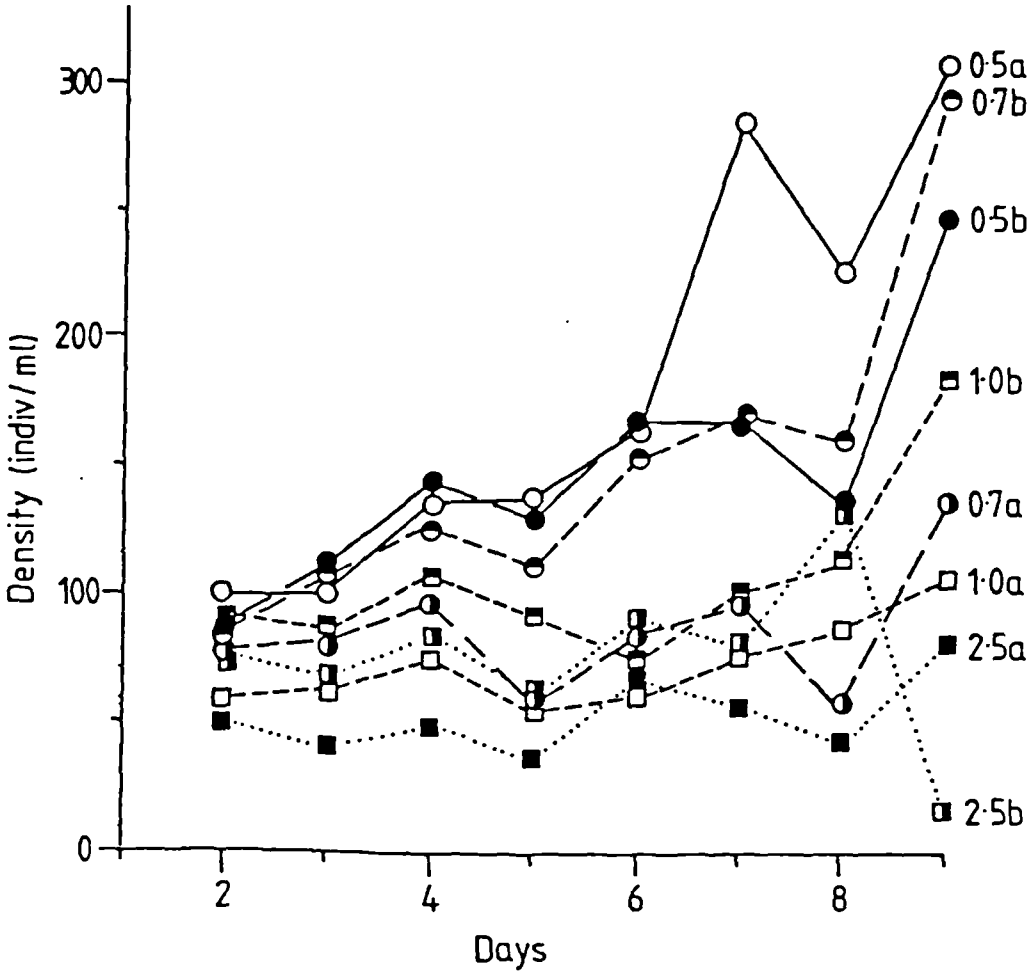


Fig. 1: Growth of rotifers in 500 ml sea-water (32 ppt), at 25°C and constant illumination. Numbers denote food rations, in g/million rotifers, of dried yeast added daily, a and b are replicates.

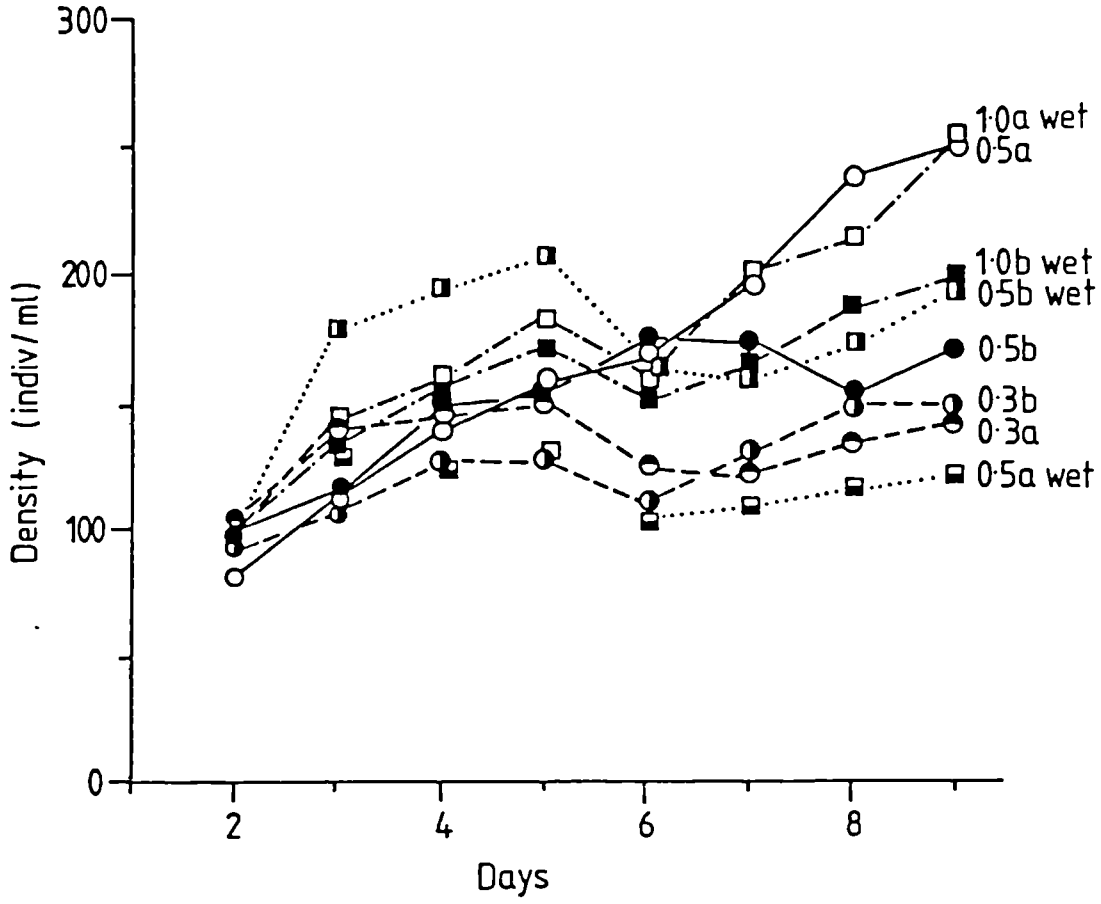


Fig. 2: Growth of rotifers in 500 ml sea-water (32 ppt), at 25°C and constant illumination. Numbers denote food rations, in g/million rotifers, of wet (wet) or dry yeast added daily, a and b are replicates.

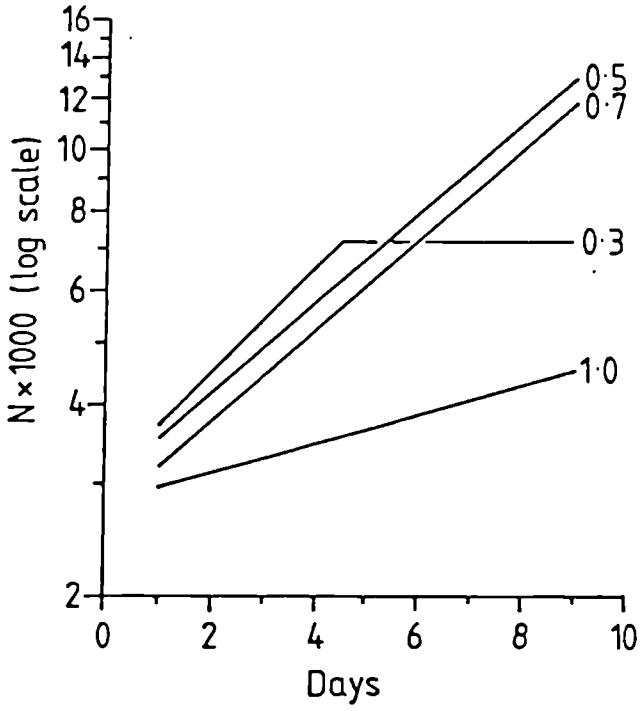


Fig. 3: Daily increase in numbers of rotifers from the experiment on effects of dry yeast rations on population growth depicted in Fig. 1 and Fig. 2. Duplicates were combined to form each of the regression lines.

Conclusions

As a result of these experiments the 0.5 ration was chosen as standard feed for mass culturing purposes. Both the intrinsic growth rate and cell count of this ration are equivalent to those obtained when using fresh baker's yeast (Hirata, 1980; Lubzens, 1985).

2b. Automatic feeders

For the second year an automatic food dispenser was installed. This unit consisted of a peristaltic pump connected to a timer which transferred 250 ml of the suspended yeast from 2 l plastic soft drink bottles to the rotifer tanks four times a day. Yeast was kept suspended in the bottles with vigorous aeration. A noticeable advantage of the system was that the daily yeast ration could be reduced and was most likely a consequence of the more constant and even distribution of the yeast in the tanks; doing so empirically resulted in a reduction of the ration by half to 0.25 g yeast/10^6 rotifers/day. As the feed bottle capacity was 2 l they needed replacing only once every 48 h so that the new calculation for feed was 0.5 g yeast/10^6 rotifers over two days plus 25% which allows for population increase within that time.

3. Mass culture unit

3a. General conditions and equipment

Rotifers were grown in up to three round 90 l polyethelene hopper units with a deep conical base to which 1.2 cm outlet ball valves were fitted. Sea water from the institute's central

water supply was filtered on line by consecutive cartridge filters, the first an IMI polypropylene filter and the second a Millipore type CP15. This should have eliminated all particles down to 0.22 µm. Flagellates and other protozoa developed quite regularly, however, in cultures maintained for over seven days in the same medium. Water temperature was kept between 23-25°C using 300 W aquarium type heaters. Vigorous aeration was provided from a single centrally positioned air stone.

3b. Building up the population

Culture units were inoculated with rotifers grown in a variety of receptacles such as Ehrlenmeyer flasks or 10 l buckets, at an initial density of at least 100 individuals/ml. Initial culture volumes were usually low (ᔕ 30 l) and built up in accordance with the rise of the rotifer populations. An example for this is drawn from the build-up of cultures prior to the 1986 herring season (Fig. 4a, 4b). Initially two hoppers (1 and 2) were each stocked with 4-5 x 10^6 rotifers in 30 l. After three days rotifer density reached 230-250 individuals/ml and the culture volume was increased to 50 l. The final volume of 80 l in hopper 1 was reached on day 9. Subsequently the volume was held constant while the rotifer density increased to 600 individuals/ml on day 22 when the culture was first harvested for feeding to fish larvae. Growth in hopper 2 did not proceed so smoothly and after a premature increase of volume to 90 l on day 7, it was reduced to 50 l on day 9 and finally stabilized at 80 l on day 12. Hopper 3 was introduced at 50 l on day 14

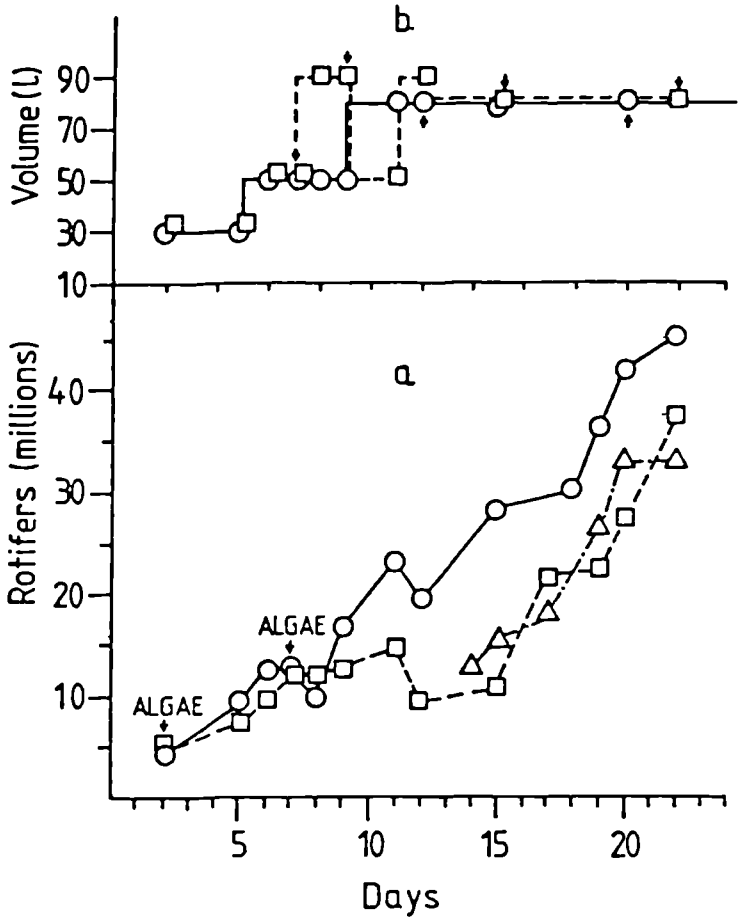


Fig. 4: The build-up of rotifer stocks in three 90l hopper units, at 20-25°C feeding on dry baker's yeast rations of 0.5 g yeast/million rotifers/day. Fig. 4a shows the population growth. Arrows denote addition of algae to the culture. Fig. 4b shows the increase of culture volume in two of the units. Arrows denote rinsing and resuspension of the entire population in fresh sea-water.

—○— Hopper 1, -□- Hopper 2, —△— Hopper 3.

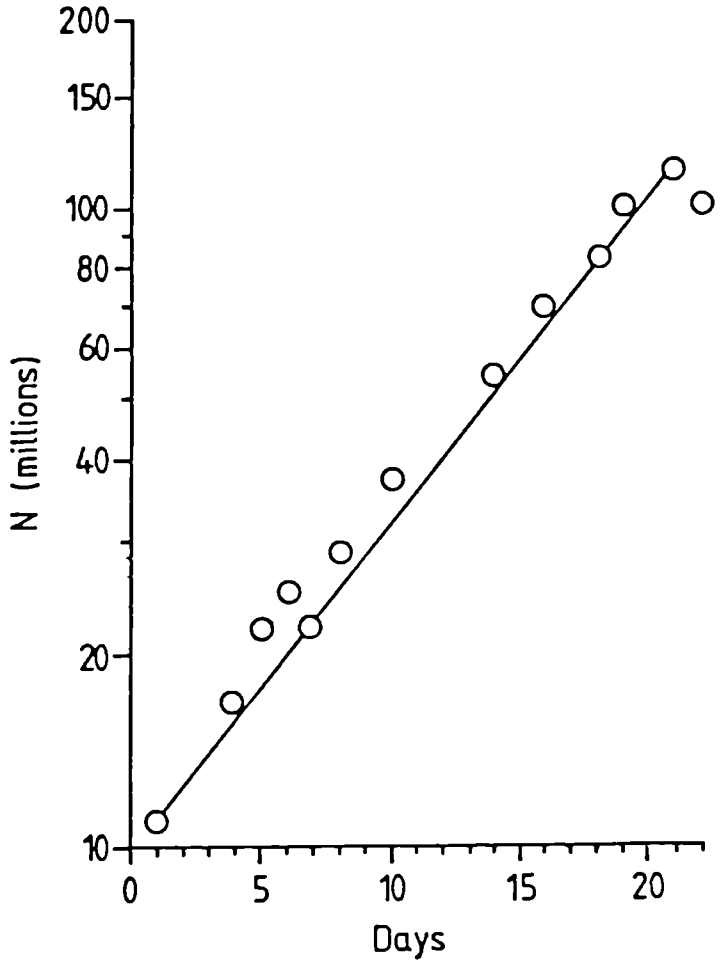


Fig. 5: The cumulative increase of the entire (hoppers 1, 2 and 3) rotifer population depicted in Fig. 4.

and increased to 80 l on day 17. The development of the total population (Fig. 5) was exponential and a ln transformation showed the relation of numbers and time to be linear ($r = 0.96$), the intrinsic growth rate $g = 0.11$ being close to that found in the feeding experiments (Section 2a). Doubling time for the entire population was 6.3 days.

3c. Management during harvesting

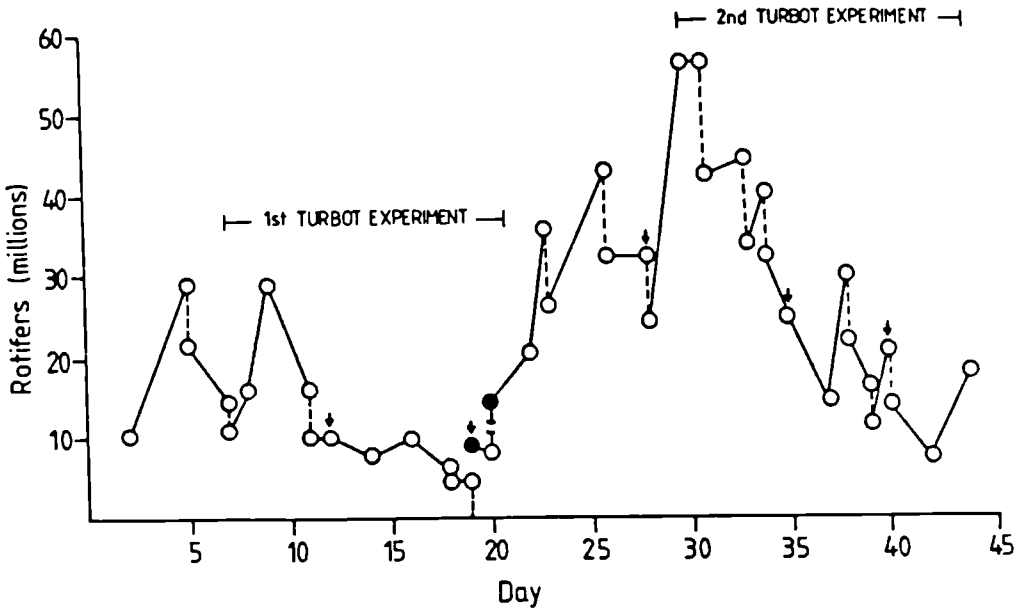
The cultivation method can be considered as "semi-continuous" with harvests regulated by demand as well as by the growth of the rotifer population. Each culture was transferred regularly (5-7 days) to fresh pre-heated sea water by filtering through a 200 µm sieve into a double bag of 60 µm plankton netting. During such filtration special care was taken to keep the rotifers wet by keeping the filters suspended in water.

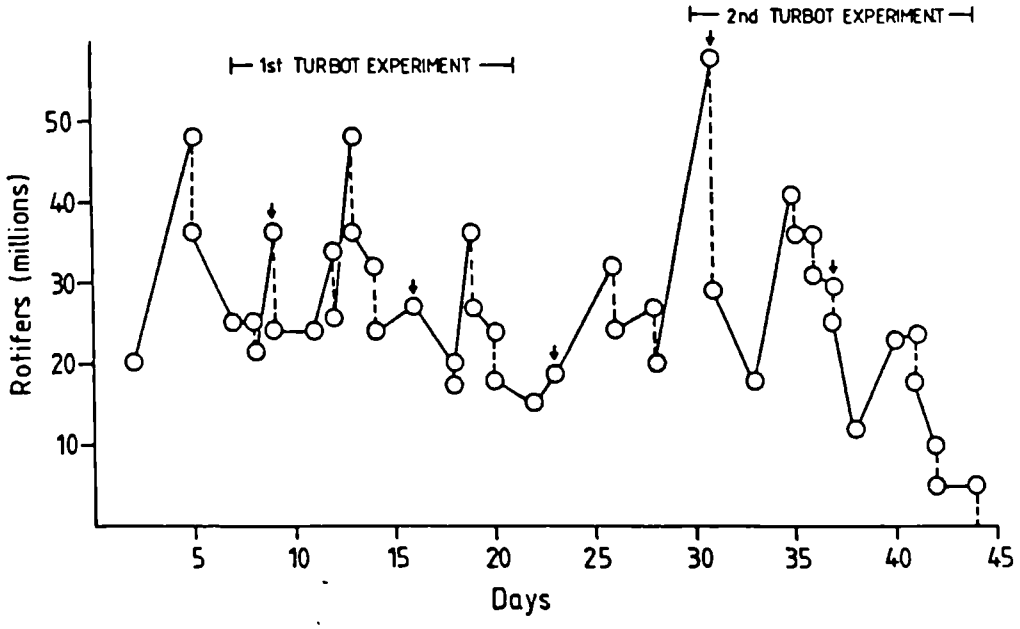
Management of mass cultures with regular harvests is illustrated in Figs. 6, 7. Data were taken from cultures used during two experiments on turbot larvae, in February 1986, which required 4.5×10^6 rotifers daily. Surplus rotifers produced during this time were either discarded or transferred to a third back-up hopper. An attempt was made to stabilize the population in each hopper at 250 individuals/ml, and whenever possible to stagger the harvests between the two hoppers. Following each harvest hoppers were topped up with an equal volume of fresh sea water.

The first experimental harvests were on day 8 from hopper 2 and on day 31 from both hoppers for the 1st and 2nd experiments respectively. A relative state of equilibrium was achieved in hopper 2 up to day 28. Hopper 1 underwent a slow

Fig. 6: Management of rotifer mass culture hopper 1, during regular harvests (denoted by the broken lines). For growth conditions see Fig. 3. Arrows denote complete change of growth medium. Black circles show restocking using surplus rotifers from the hopper depicted in Fig. 7.

Fig. 7: Same as Fig. 6, but for mass culture unit 2.


2nd TURBOT EXPERIMENT
1st TURBOT EXPERIMENT
Rotifers (millions)
60
50
40
30
20
10
5
10
15
20
25
30
35
40
45
Day


2nd TURBOT EXPERIMENT
1st TURBOT EXPERIMENT
Rotifers (millions)
50
40
30
20
10
5
10
15
20
25
30
35
40
45
Days

decline from day 12 and was restarted with rotifers from hopper 2 on days 19, 20. It is noticeable that the populations of both hoppers underwent a rapid increase from day 20-22 until day 31 where the high densities (600-700 individuals/ml) necessitated a heavy cropping, following this the population gradually diminished. One factor which could have had a significant impact on this decline was the lack of algal supplements in the diets for a period exceeding 60 days. Algal supplements should be administered every 40-60 days to replenish nutrients such as vitamin E and B_{12} (Scott, 1981) which are essential for rotifer growth.

3d. Production figures

The average standing stock during this 42 day period was 22.8×10^6 and 28.2×10^6 rotifers/d in hoppers 1 and 2 respectively. Average volume was 80 l/hopper, the respective population densities ($\bar{x} \pm$ S.D.) were 308 ± 187 and 259 ± 144 ml^{-1} from which a total of 102.6 and 101.7 million rotifers were cropped at 14 harvests/hopper. The total number of rotifers raised in this period was 2.1×10^9 rotifers which yielded 204.3×10^6 rotifers, i.e. a 10% yield.

In terms of feed 535 g of dried yeast were used and were converted to 61.29 g dry weight of rotifers (1 rotifer = 0.3 µg). The conversion efficiency was therefore $\frac{61.3}{535} = 0.114$ or 11.4%. The production values obtained from this system compare well with those published in the literature (reviewed by Lubzens, 1987).

4. Enriching rotifers

Before feeding to larvae the required quantity of rotifers harvested from the mass culture was divided into three lots and resuspended at densities of 500-850 individuals/ml in fresh sea water containing algae or yeast at cell to rotifer rations of 6×10^3 for Isochrysis, 3.5×10^6 for Nannochloropsis and for yeast 0.5 g/10^6rotifers. Enrichment containers were immersed for 16-18 h in 20 ± 1°C constant temperature baths and vigorously aerated. Debris was separated from the enriched rotifers prior to feeding to larvae by sieving through a 200 µm filter.

B. Algal mass culture

Two algal species, Isochrysis galbana (SMBA Cat. No. 58, CCAP 927/1A) and Nannochloropsis oculata (SMBA Cat. No. 66, CCAP 849/1A) were used in the experiments.

The algal culture unit was designed to provide a minimum of 10 l of algae/day, 5 l from each algal species. Within the system algae were grown under sterile or semi-sterile conditions in either glass carboys for the former or polypropylene bags for the latter. The facilities included a vertical bank of 125 W, white, fluorescent lights attached to the back of a bench and shelf unit. Air was passed through a grade 12-03 Whatman's Gamma 10 filter unit before being introduced into culture vessels. Temperature was ambient (usually 13-15°C during the larval season) and mass culture

units were immersed in water baths (ca 13°C) if air temperature rose above 20°C.

The growth medium used was the "MA" salt solution (devised by Dr M.R. Droop) and consisted of 100 g KNO_3, 10 g K_2HPO_4, 20 g EDTA, 2.5 g $FeSO_4$ $7H_2O$, 0.25 g $MnSO_4$ $4H_2O$, 50 µg Vitamin B_{12} and 6.0 mg Thiamine, in 1 l H_2O from which 1 ml was added to each 1 l sea water growth medium prior to autoclaving (15 min at 7.7 kg/cm^2).

Mature seed cultures (100 ml) were used to inoculate 1.5 l culture flasks which in turn were used to start 20 l mass cultures in pyrex carboys. Once cell density reached a value of approximately 8 x 10^6 cells/ml for Isochrysis and 25 x 10^6 cells/ml for Nannochloropsis the cultures were harvested on a regular basis.

The exponential growth coefficient (r) for Isochrysis was on average 0.225 ± 0.045/d ranging from 0.175 to 0.26/d. Doubling time was 3.1 days, and average cell density was 8.5 x 10^6 cells/ml. This enabled 5 l to be harvested daily out of one of three cultures running simultaneously. After harvesting, carboys were refilled with fresh, autoclaved medium. No major problems were experienced in keeping such cultures running for over 60 days.

The exponential growth coefficient of Nannochloropsis was on average 0.265 ± 0.082/d ranging from 0.13 to 0.34/d. Doubling time was 2.6 days and average cell density 25 x 10^6 cells/ml, though this could reach 70 x 10^6 cells/ml depending on harvesting regime.

Five litres Nannochloropsis from the sterile carboy culture were used to inoculate a bag culture (23 cm "Layflat"

tubing) of approximately 25 l once every five days. The medium in the bag was only filtered (Millipore 0.22 µm) before addition of salts and starter culture. Cell density in these cultures reached an average of 70 x 10^6 cells/ml. This higher cell density is probably due to the larger surface to volume ratio. Bags were harvested at the rate of 5 l/day from day 5. After 10 days the bag-cultures had always become contaminated, causing their rapid collapse.

C. Larval rearing unit

The unit was set up in a constant temperature room equipped with both heating and cooling facilities. Sea water from the main supply was filtered on line to the header tanks. The filter employed aquaria wool rolled into a tight cartridge with nylon mesh and eliminated all zooplankton from entering the system.

In the 1986-7 experiments water flowed through three 40 l header tanks connected by 2.5 cm diameter pipes in which N_2 degasing (Ehrlich, 1972) took place by vigorous aeration. Each tank was fitted with a 500 W immersion heater joined through a relay to a contact thermometer enabling temperatures to be controlled above ambient. From the third header tank water flowed by gravity through 2.5 cm diameter pipes (ABS) towards the rearing tanks. Twelve 100 l black polypropylene (cold water cistern) tanks set on three benches were used for rearing larvae. Water entered these tanks at the surface, and parallel to it, through two glass (5 mm diameter) tubes set on either side (Fig. 8) causing water to

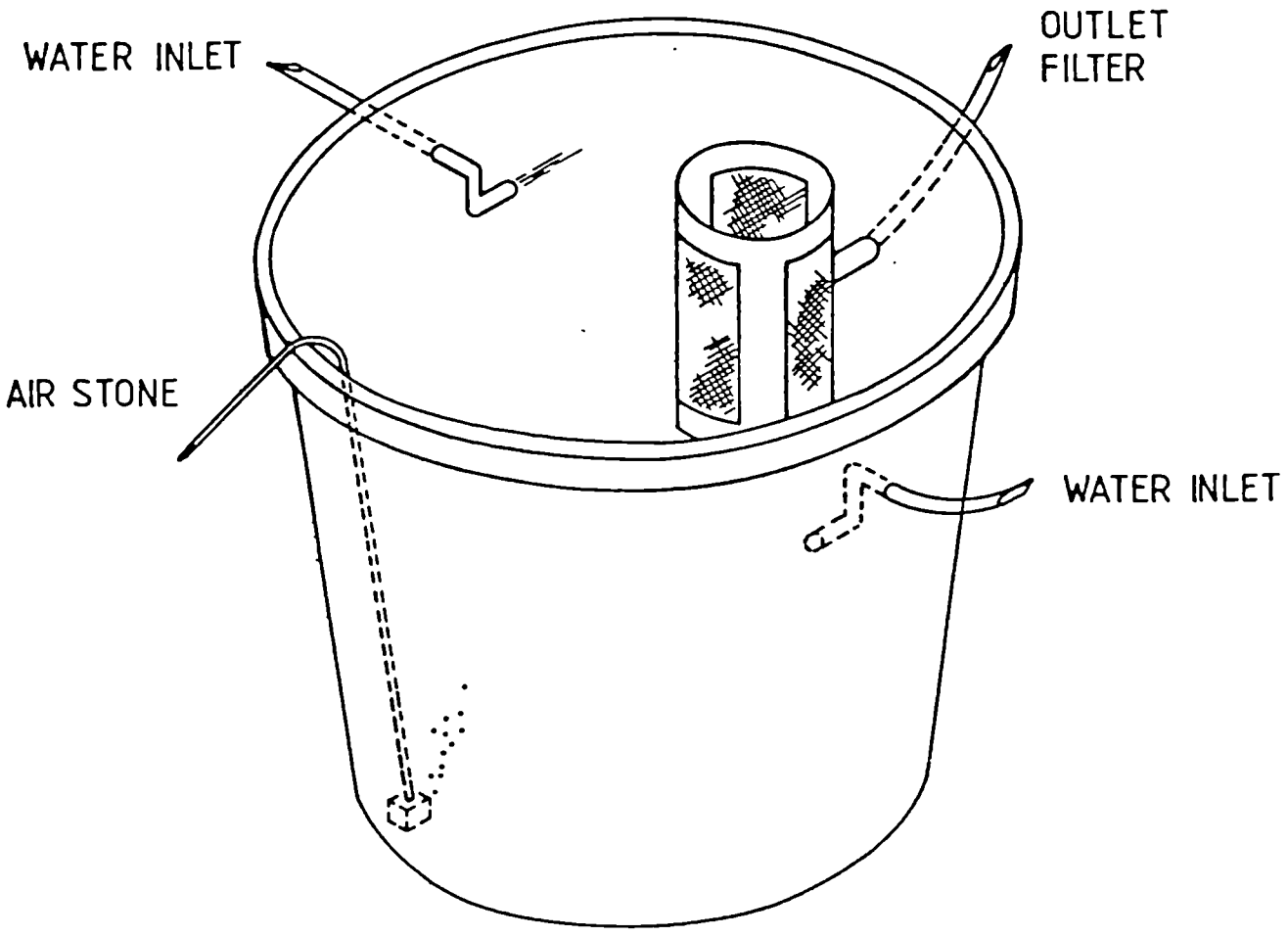


Fig. 8. Diagram of a 100 l fish larval rearing tank.

circulate around the tank. Inflows were controlled at the connection to the main pipes by 6 mm diameter stopcocks. Daily adjustments were made manually and exchange rates ranged from two to three tank volumes a day. Outflows at the water surface were protected by a plastic cylinder with a 200 µm screen stretched over the bottom and the three vertical windows cut into its sides thereby enabling drainage of water from both surface and subsurface layers.

Mixing was achieved by gentle aeration with air-stones or diffusers set on the tank bottom and close to the tank wall opposite the outflows, aeration being provided centrally from a Compton 2D351VM pump.

Illumination was provided by either three twin fluorescent tubes set in the ceiling of the room or by supplementing other fluorescent tubes at a height above the tanks adjusted so as to provide the required light intensities. Photoperiod was controlled either manually or by a time clock.

In the 1985 experiment each row of four tanks was connected to one 40 l header tank which had no temperature controlling facilities. Aeration was not provided to larval units and instead of the twin surface water inlets there was only one inlet at the base of each tank.

D. Nutritional experiments

1. General procedures

Larvae were counted from their hatching facilities into rearing tanks using either a wide bore (> 5 mm) pipette, with the opening smoothed by heating in a flame, or a beaker.

Stocking proceeded sequentially in lots of 100-200 larvae until all tanks received their final complement of larvae.

Feeding was initiated once observations showed larvae to have open mouths. The larvae were offered rotifers which had been pre-fed with yeast or yeast plus algae (see section A.4) and these algae, though not the yeast, were also added daily to each 100 l rearing tank in the quantities shown below:

Treatment	Rotifers	Algae	Ration
	Number/d	Cells/d*	Algae/rotifer
I. galbana	5×10^5	8×10^9	1.6×10^4 cells/rotifer
N. oculata	5×10^5	4.5×10^{10}	9×10^4 cells/rotifer
Yeast	5×10^5		

*One litre of culture added daily. Numbers given are averages.

Routine tank maintenance, which preceeded feeding, included the siphoning of debris from the bottom, the skimming of surfaces, rinsing outlet filters and adjustment of water inflows. Tank and room temperatures were recorded daily.

Experiments were terminated by individually collecting and preserving 100 larvae from each tank following which the water was slowly siphoned out. The siphon inlet was protected by a 200 µm net so that all larvae were retained in the tanks. Once the water level was low enough, larvae were scooped out and counted for final survival estimates.

2. Sampling procedures

2a. Larvae: Thirty larvae were sampled from each tank on three to four occasions throughout each experiment. Larvae were removed individually by pipette (5 mm diameter) in the late afternoon to ensure that all specimens had food in their guts. They were anaesthetized in benzocaine (1:2000) and immediately transferred to 4% formalin in half strength sea water (Blaxter, 1971).

2b. Gut contents: To check whether the type of rotifer enrichment affected the larval feeding response, gut contents were examined. For this purpose two to three larvae were removed from each treatment tank, briefly anaesthetized in benzocaine and immediately transferred through a clean sea water rinse onto individual microscope slides. The rapidity of the process ensured that peristaltic gut evacuation was minimal. Once on the slide they were measured and staged (see Section E.1) following which a cover slip was placed over them. This pressure caused the gut to rupture and the number of rotifers in it was estimated by counting the chitinous trophic region of the rotifers (mastax). This was repeated for at least ten larvae per tank.

2c. Rotifer residue: On a few occasions 100 ml water samples were taken from the water column of each tank before daily cleaning. Rotifers from these samples were concentrated on a 60 µm filter, resuspended in a small volume of sea water and

counted under a dissecting microscope following the addition of a few drops of formalin.

2d. Mortalities: Dead larvae were removed and counted each morning except for turbot whose small initial size only allowed counting from seven days post-hatch.

3. Experimental conditions

3a. Herring: Two experiments were done, one in 1985 and one in 1986. In 1985 each of the three treatments was allocated to one row of four 100 l tanks with 1,000 larvae/tank. A further tank was run as an Artemia control although its initial larval density was not known. Larvae were transferred to the tanks on days 2 and 3 post-hatching. Food was first introduced on day 5. Water was continuously circulated (2-3 change/day) at ambient temperatures which ranged from 9.5°C-11.4°C. Illumination was 200-300 lux at the water surface with a photoperiod L:D of 16:8. Rotifer residue in tanks was sampled when possible on a daily basis. Larvae samples were taken on days 4, 9, 12 and at termination on day 15. Gut contents were analyzed on day 11.

In 1986 the same numbers of larvae and replicates were employed but the replicate tanks were not distributed throughout the room in blocks but in such a way that would spread the possibility of a statistical bias caused by their location in the room, evenly across the treatments. Transfer to rearing tanks took place on days 2, 3, 4 post-hatch, food was first introduced on day 4 and first-feeding larvae observed

on day 6. Temperatures were increased from ambient on day 4 to a controlled 11 ± 1°C by day 5. The photoperiod was L:D 24:0 up to day 11 when it was changed to 19:5. Rotifer residue was sampled seven times on days 15 to 18 and 22 to 24. Larvae were sampled on day 4 (from stock tanks) 12, 19 and at termination on day 28. Gut contents were analyzed on days 16, 17 and 22. Due to a shortage of I. galbana all algal supplements to the rearing tanks (but not the rotifer enrichment) were stopped on day 11.

3b. Plaice

For the first experiment (1985) three black round polycarbonate 30 l tanks were set up in the institute's main aquarium. There were no replicates with 700 larvae per treatment and feeding levels and algal supplements were adjusted proportionally to tank volume. Larvae were transferred to rearing tanks on day 3 post-hatch. Food was first introduced on day 4 post-hatch. Temperatures were ambient and rose (subject to fluctuations) from an initial 9.8°C to 12.5°C at termination. Light intensity was 200-300 lux at the water surface and the photoperiod was L:D 16:8. Samples of larvae were taken on days 8, 16, 23, 29 and at termination on day 31. Some of the larvae from both algal treatments were reared on to metamorphosis in their respective tanks on a diet of Artemia nauplii only. After metamorphosis their pigment pattern was recorded to evaluate if the initial diet influenced this pattern.

In 1986 there was again a scarcity of gametes and the experiment was confined to three tanks with one tank of 350

larvae per treatment. However these experiments were carried out in 100 l tanks in the larval rearing unit. Transfer to rearing tanks took place on day 4 post-hatch when first food was also introduced. Temperature was controlled at 12 ± 1°C, light intensity was 200-300 lux and the photoperiod was L:D 18:6. Larvae were sampled on days 4, 10, 18 and 25, rotifer residue on days 17 and 21 and gut analysis on days 5, 7. At termination algal treatments were grown on to metamorphosis using Artemia nauplii only for determination of pigmentation pattern.

3c. Turbot

Two experiments were carried out, the first in February and the second in March, 1986. The first one did not produce any results but following it the rearing conditions were corrected so as to cater for the specific needs of these larvae.

The second experiment was carried out in nine 100 l tanks which were divided randomly among the three treatments. Larvae were stocked at a density of 1,250 larvae per experimental tank on the first day post-hatch. Food was first introduced on the evening of day 3 and first-feeding observed on the following day. Temperatures were controlled at 18 ± 1°C. Illumination was 1,500-3,000 lux at the water surface and the photoperiod was L:D 24:0. Rotifer residue in tanks was sampled on days 5, 6, 7 and 14. Samples of 30 larvae were taken on days 3 (a grouped sample from all tanks), 5, 8, 12 and at termination on day 14 when 150 larvae were collected from each tank. Gut contents analysis were performed on day 4 and 5 (1st and 2nd days of feeding) and a

check for swimbladder inflation was done on 25 larvae from each tank on day 12.

E. Morphometric estimates

Prior to analysis larvae were kept for over three months in formalin allowing for maximal shrinkage to take place. Twenty to 24 individuals from each sample were rinsed three times in distilled water and then placed individually in 3 mm-deep wells in perspex multiwell trays. Preliminary trials showed that after oven-drying the formalin-fixed larvae could be picked off perspex using fine forceps which facilitated their removal for weighing. Numbering the wells made it easy to follow each larvae individually through the analysis.

1. Development: This was evaluated according to the staging systems of Doyle (1977) for herring, Ryland (1966) for plaice and Al-Maghazachi and Gibson (1984) for turbot. As herring larval development proceeded slowly relative to the experimental period, a further characteristic, that of fin ray development, was also recorded.

2. Lengths: TL, tip of snout to tip of tail, and SL, tip of snout to end of notochord, were measured with the ocular micrometer of a Wild M5 dissecting microscope.

3. Weights: Dry weights in µg were recorded in a desiccated weighing chamber of a Perkin-Elmer ZH Autobalance. A preliminary investigation showed that a drying time of 24 h at 70°C and an equilibrium period of 60 s for cooled samples on the balance gave constant results. Dried samples were either weighed immediately or stored for later weighing in a desiccator.

F. Shrinkage

Formalin fixation results in reduction of larval lengths (Blaxter, 1971; Hay, 1981) and weights (Hay, 1984). The extent of this depends on salinity, formalin concentration, initial body size and the duration of fixation.

For calculating fresh lengths and weights of larvae in the current experiments it was necessary to evaluate shrinkage with respect to both duration of fixation and initial size of the diverse larvae used.

Samples of larvae were collected for shrinkage analysis using the same procedures employed when collecting larvae for growth analysis. One group of larvae (usually 10-20 specimens) was always measured immediately after anaesthetizing in benzocaine, for length (TL,mm) and dry weight. Fresh larvae tended to adhere to the perspex surface commonly employed when drying formalin fixed larvae (see section E) and were therefore dried on a glass slide from which they were transferred to a pre-weighed aluminium foil boat for weighing. Larvae were routinely preserved in 4% formalin, half strength sea water and stored at room temperature, in darkness.

Herring were tested for effects of both duration and initial size (TL) on shrinkage. In 1985, a group of 50 13-day old larvae were taken from a general stock tank, and after recording fresh lengths they were divided among three sample vials to be re-measured after being preserved in formalin for 10, 64 and 104 days. The greatest reduction in length (11% shrinkage) occurred within the first 10 days after which only minimal (a further 2-3%) changes took place (see Table 1). Following this it was decided to use a three-month period as the minimal preservation time.

The effect of larval age on length (TL) and dry weight loss after formalin fixation was determined on two duplicate samples (n = 20) of 10- and 21-day old herring from the 1986 experiment. One sample was measured when fresh and the other following 240-250 days in formalin. It was found (see Table 1) that during this preservation period larvae of either age group lost about 33% of their dry weight. This may be compared with Hay (1984) who found 25% for newly hatched larvae. The initial fresh length of the larvae seemed to affect their length following preservation, with larger larvae tending to shrink less than the smaller ones. For lengths (TL) the percent shrinkage found in both the 1985 and 1986 experiments were combined and regressed on the preserved length (in microscope units). The shrinkage factor K=21.29 - TL x 0.956, (TL = microscope units) was thus employed for converting preserved to fresh larval lengths.

Plaice were analyzed for shrinkage on duplicate samples of n = 24, 12- and 32-day old larvae from the 1986 experiment. One sample from each age group was measured fresh and the

Table 1. Percent loss of length (TL) and dry-weight of larvae preserved in 4% formalin, half strength sea water.

Year	Species	Initial TL mm ± S.D.	Initial dry-weight µg ± S.D.	Days preserved d	Length loss %	Weight loss %
1985	Herring	13.86 ± 0.54		10	11.0	
1985	Herring	13.86 ± 0.54		64	13.9	
1985	Herring	13.86 ± 0.54		104	12.8	
1986	Herring	11.16 ± 0.4	137.0 ± 50.0	251	15.9	32.7
1986	Herring	13.67 ± 1.16	259.6 ± 80.5	240	14.6	33.5
1986	Turbot	4.44 ± 0.83	64	155	8.2	21.0
1986	Turbot(1)	4.70 ± 0.27		155	10.2	
1986	Turbot(2)	7.04 ± 0.62		155	5.2	
1986	Turbot(3)	6.15 ± 0.59		155	6.8	
1986	Plaice	7.99 ± 0.46	147.8 ± 45.5	110	6.7	30.0
1986	Plaice	10.46 ± 0.65	775.8 ± 212	90	7.32	20.4

(1) Turbot larvae feeding on rotifers enriched with yeast
(2) Turbot larvae feeding on rotifers enriched with Isochrysis
(3) Turbot larvae feeding on rotifers enriched with Nannochloropsis

other following 90-110 days in formalin. The shrinkage in percent length (TL) was not found to vary between the two age groups (Table 1) and a 7% correction factor was used for all fresh length calculations. Weight loss was higher for younger than older larvae. However, an average 25% was used as the correction factor irrespective of larval age.

Turbot were analyzed using four duplicate samples of 15 larvae; one duplicate was from a stock tank of 6 day old larvae, the others were from either the yeast, the Isochrysis or the Nannochloropsis treatments on day 13. Regressing percent shrinkage as determined after 155 days in formalin on preserved length gave the correction factor $K = 16.4 - 1.39\ TL$ ($r = -0.99$; TL = microscope units) which was used for calculating fresh lengths. The sample of larvae feeding on yeast-rotifers was not included in the above regression, these larvae exhibited a higher level of shrinkage compared with the larvae receiving an algal supplement in their diet and the shrinkage factor of 10.2% was used in this treatment for calculating fresh weights. This probably resulted from the poor condition of these larvae and parallels the higher shrinkage levels reported for starved larvae (Hay, 1984). Weight loss in turbot was only recorded for the first sample. However, as the length loss in turbot was of the same order as that found in plaice, and as there seemed to be some similarity in the weight loss factors it was decided to use the 25% weight loss factor for calculating fresh weights in turbot. This is also justifiable when one takes into consideration the morphological similarities between the larvae of these two species.

G. Ingestion rates

Daily ingestion (24 h) for turbot and herring were estimated using the following procedure: Duplicate 100 l stock tanks of larvae were grown from first-feeding on rotifers enriched with Isochrysis using the standard procedures (Sections D.3a for herring and D.3c for turbot). From each tank larvae were removed using a wide bore pipette into duplicate two litre pyrex beakers on days (post-hatch) 10, 13, 18, 21 for herring and 3, 5, 8, 10, 17 for turbot. Transfers were initiated 1-2 h after the stock tanks were supplemented with the daily ration of rotifers. The number of larvae in the beakers was set at levels approximating the survivals recorded in the nutrition experiments (algal treatments only) which were 30, 30, 20, 20 and 10 individuals for the corresponding days in turbot and 20, 20, 12 and 10 for herring. Each beaker received ∿ 10,000 rotifers enriched with Isochrysis and 20 ml of the algae. Two control beakers were stocked with rotifers and algae only. All the beakers were floated in a single 100 l tank kept at the appropriate rearing conditions. After 24 h (at constant illumination) fish were pipetted out, the number of actively feeding, moribund and dead larvae recorded and excess water returned to the beaker. The contents of the beakers were then sieved through a 60 µm mesh, the retained rotifers resuspended in 50 ml sea water and their number estimated by counting five one-ml subsamples.

Larval fresh lengths (TL) fresh dry weights (µg) and development stage were estimated from nine larvae taken from each stock tank on each experimental day. Only on one

occasion, day 17 of the turbot experiment, were larvae from the experimental beaker measured directly at the end of the 24 h feeding period. These data were used for establishing the length-weight relationship (using log/log transformation) and the specific growth rates (SGR) experienced between any two experimental days

$$SGR\% = (\ln W_2 - \ln W_1) / \Delta t \times 100; \quad \text{(Ricker 1979)}$$

Rotifer dry weights were recorded on each experimental day using the procedure outlined in section H.2.

The changing relation of gut contents to growth was estimated by removing larvae from their stock tanks on several occasions throughout the experiment and counting the number of rotifers in the guts as described in Section D.2b.

Daily ingestion, I, in dry weight was calculated according to:

$$[\,1 / 2\, R_o + (R_{c1} + R_{c2}) / 4 - R_{ti}\,] * K / N_t$$

where

R_o = 10,000 (initial number of rotifers in a beaker)

R_{ti} = Final number of rotifers in beaker i

R_{c1} and R_{c2} = Final number of rotifers in control beakers 1 & 2

K = Dry weight of an individual rotifer in µg

N_t = The number of active larvae at t.

The conversion efficiency K_1 (Brett & Groves, 1979) was estimated according to:

$$K_1 = (SGR_t \times W_t) / I \times 100$$

where

SGR_t = specific growth rate at time t.

W_t = Average dry weight of larvae at time t.

I = Ingestion in µg

H. Rotifer biochemical analysis

Samples of rotifers used for analyzing the biochemical composition of larval diets were not collected at the time of larval rearing trials, but at a later date when, apart from the presence of larvae, the experimental conditions were simulated.

1. Preparing samples for proximate and amino acid analysis

The three main treatments, i.e, rotifers enriched on yeast or one of the two alga, were carried out at enrichment/rotifer ratios proportional to those used in the experiment. Samples were not prepared simultaneously, the yeast and Nannochloropsis being prepared within a week of each other and Isochrysis at a much later date. For all the enrichments freshly harvested algae during the logarithmic growth phase were used. The procedure started with harvesting the rotifers (approximately 6×10^6/treatment) from the mass culture, rinsing in clean sea water and resuspending in 30 l at 20°C with the appropriate enrichment. After about 18 h the rotifers were harvested, rinsed, resuspended in 900 ml sea water and divided into three lots. Two of these underwent a further 24 h enrichment in 30 l at either 10°C (an approximation of experimental temperatures for plaice and herring) or 20°C (for turbot), further algae being added only to the rotifer cultures which had previously been enriched with algae. Yeast was not added at this phase to the non-enriched rotifer culture. The third lot was resuspended in a 2 l beaker allowing for settlement of debris; from this a small volume

was set aside in an ice bath for dry weight and calorific estimations for which they were prepared within 1 h. The rest were decanted onto a 60 µm filter, rinsed with sea water followed by distilled water and flushed with a small volume of distilled water into a sample vial. Vials were stored at -20°C for up to four days following which they were freeze-dried. The other two batches which underwent the 24 h larval tank simulation were collected and prepared in the same manner. Freeze-dried samples were stored at -20°C up to analysis.

2. Dry weight determination

The density of rotifers in the sub-sample set aside for dry weight determination was readjusted to 200-300 individuals/ml. Five 1 ml aliquots were counted for females and eggs over a grid under a dissecting microscope after being killed with a few drops of 4% formalin. The quantity of rotifers in one dry weight measurement was taken as the nearest whole number of ml to give about 1,000 rotifers.

Preparations and weighing were similar to those described in Strickland and Parsons (1968) for zooplankton. GF/A filters were cut into 14 mm diameter discs rinsed in distilled water, oven-dried for 1 h on large GF/A discs at 70°C, numbered with waterproof ink and weighed ($\pm$ 1 µg) in a Perkin-Elmer Autobalance. They were then set in the holder of a filtering apparatus (provided by Mr J.M. Scott) made from an inverted 5 ml syringe set in the bung of a Buchner suction flask. The sample was introduced, suction applied and the rotifers rinsed twice under suction with distilled water on the filter. Special care was made to rinse salts from the margins

of the filter from salts. The filters were then oven-dried for 1 h on a non-stick surface (large GF/A filters) at 70°C, cooled in a desiccator and weighed. An average of seven measurements and three blanks (sea water only with distilled water rinse) were made per treatment.

Dry weights were adjusted for the presence of eggs using the observed egg/female ratios in a sample and the dry weight ratios female:egg 4:1 as estimated for rotifers by Dumont (1977) and Leimenth (1980).

3. Calorific values

The same size samples of rotifers used for dry weights were filtered onto GF/A discs (14 mm) which previously had been subjected to ½ h at 500°C in a muffle furnace. After 24 h drying in a desiccator they were either measured directly on a microcalorometer (Scott and Marlow, 1982) or stored in the desiccator wrapped in aluminium foil for up to 1 month prior to calorific determination. Calorific values were averaged from three to five such discs with two to three readings per disc. The results were corrected for the egg/female ratio of a given sample as in the dry weight estimates as well as for the ash content using the 7.8% ash/dry weight determined by Theilacker and Kimball (1984).

4. Proximate analysis

Carbon and Nitrogen were determined using a Perkin Elmer model 240 Carbon:Hydrogen:Nitrogen analyzer (operated as

per manufacturer's instructions). Two mg samples in triplicates were analyzed on platinum boats with boats only used for blank determination. Calibration was done with acetalamyde as standard. Nitrogen was converted to total protein by multiplying by a factor of 6.25.

Crude lipid was measured using the Korn & Macedo (1973) method. Five 50 mg samples were averaged for each determination. Samples were homogenized, weighed on plastic weighing trays, mixed with a liberal amount of sodium sulphate (anhydrous) and poured into a Pasteur pipette with a glass fibre plug at its tapering end. Pre-weighed aluminium trays were placed beneath the pipettes and the lipid extracted by rinsing 2-3 times with freon (trichlorofluoromethane) in a ventilated chamber. One reagent blank was done for each three samples. After all the freon had evaporated the aluminium trays were re-weighed and their increase in weight was calculated as percent crude lipid in the original sample.

5. Amino Acids

Analysis was done on an amino acid analyzer LKB Biochrom, Model 4151 by Mr A. Porter of Stirling University's Institute of Aquaculture.

6. Fatty Acids

Samples from all three treatments were prepared from one batch of rotifers though not from the same batches used for

proximate analysis. The minimal dry weight required for one analysis was 20 mg which at an average rotifer weight of 300 ng comprises 60,000 individuals. To provide a safety margin 1×10^5 rotifers were used per sample.

Rotifers harvested from the mass culture were rinsed and resuspended in a clean volume of sea water to be divided equally among the three main treatments. The first enrichment at 20°C lasted for 19 h, following which the rotifers from each treatment were filtered through 200 µm mesh, collected on a 60 µm mesh, rinsed thoroughly with fresh filtered sea water, resuspended and divided into three groups. One group was filtered and rinsed twice on 60 µm mesh and then washed into glass sample vials with a small volume of GF/C filtered sea water. An equivalent volume of analytical grade chloroform ($CHCl_3$) was added to this, air was replaced with N_2, the vials were then stopped with teflon and placed in -20°C. The other two groups underwent a further 24 h period under simulated larval tank conditions and were then collected and stored as described above. Two control samples were prepared, one of 10^5 rotifers from the mass culture and one of filtered sea water only.

Following preparation of all these samples they were removed from the freezer and transferred to Stirling University's marine biochemistry laboratory where they were stored at -80°C for one month prior to extraction.

Extraction and fatty acid analysis was done by Mr Paul Hodgeson using the procedures outlined in Appendix I.

RESULTS

A. Nutritional experiments

1. Herring

1a. 1985

Throughout this experiment no major mortality was observed. Survival (Table 2) was high (80-100%) in those tanks in which the final number of larvae was counted, with no significant effects of the type of rotifer enrichment as ascertained by an analysis of variance (ANOVA, $F = 0.95$, d.f. = 2; 6).

Effects of diets on growth rates in TL (mm/d) were tested by linear regressions (Table 3). Growth rates ranged from 0.3 mm/d in the Artemia treatment to an average 0.4 mm/d for rotifer treatments. Between the rotifer treatments growth rates were not significantly different (ANOVA, $F = 0.2$, d.f. = 2; 9). However, pooling the regressions for the rotifer treatments showed that larvae fed this diet grew significantly faster than when fed Artemia (analysis of covariance, ANCOVA, $F = 11.15$, d.f. = 1; 906).

The same trend was observed when comparing growth in terms of the logarithm of the daily weight increase (Table 4). The pooled slope obtained for the rotifer treatments, which were not statistically different (ANOVA, $F = 0.31$, d.f. = 2; 9), differed significantly from that obtained for the Artemia treatment (ANCOVA, $F = 5.5$, d.f. = 1; 906).

At termination (day 15) larvae in all treatments had reached development stage 2a (Table 5) in which dorsal fin elements begin forming. In a number of larvae the posterior

Table 2: Percent survival of herring fed rotifers enriched with either Isochrysis, Nannochloropsis or baker's yeast, during the 1985 experiment. A correction was made for larvae removed as samples by subtracting the total number sampled from the initial number stocked.

	Isochrysis	Nannochloropsis	Yeast
1	90.5	78.2	97.6
2	89.8	94.3	86.6
3	89.6	91.0	100
4	n.c.*	n.c.	n.c.
Average	89.9	87.8	94.9

*n.c. = not counted.

Table 3. Relationship between TL(mm) and age (t, days post-hatch) of herring during the 1985 experiment on rotifers enriched with either Nannochloropsis (Nanno), Isochrysis (Iso), baker's yeast or newly hatched Artemia nauplii.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	1	TL=.37t+11.0	71	.030	.82
	2	TL=.42t+10.4	71	.022	.92
	3	TL=.41t+10.4	71	.026	.89
	4	TL=.41t+11.8	71	.024	.90
Nanno	1	TL=.36t+11.0	71	.029	.82
	2	TL=.56t+10.0	71	.019	.95
	3	TL=.38t+10.8	71	.022	.90
	4	TL=.41t+10.6	71	.020	.92
Yeast	1	TL=.42t+10.4	71	.019	.94
	2	TL=.40t+10.3	71	.032	.83
	3	TL=.36t+10.8	71	.030	.82
	4	TL=.38t+10.7	71	.026	.87
Artemia		TL=.30t+11.1	71	.026	.81

*Sb = standard error of the regression coefficient.

Table 4. Relationship between dry weight (µg) and age (t, days post-hatch) of herring larvae during the 1985 experiment. For abbreviations see Table 3.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	1	logW=.018t+2.24	71	.003	.57
	2	logW=.020t+2.22	71	.002	.72
	3	logW=.021t+2.21	71	.003	.71
	4	logW=.019t+2.24	71	.002	.71
Nanno	1	logW=.016t+2.24	71	.003	.61
	2	logW=.022t+2.21	71	.002	.82
	3	logW=.021t+2.21	71	.002	.83
	4	logW=.021t+2.21	71	.001	.85
Yeast	1	logW=.022t+2.21	71	.002	.84
	2	logW=.019t+2.21	71	.003	.55
	3	logW=.018t+2.03	71	.002	.85
	4	logW=.016t+2.22	71	.002	.64
Artemia		logW=.013t+2.19	71	.002	.52

*Sb = Standard error of the regression coefficient.

Table 5. Development expressed in stages (Doyle, 1977) and the number of dorsal fin elements in 14 day old herring larvae, 1985. See Table 3 for abbreviations.

Treatment		Development stage	Dorsal fin elements Average	s.d.
Iso	1	2a	8.7	1.8
	2	2a	9.7	1.42
	3	2a	9.7	.98
	4	2a	10.25	1.07
Nanno	1	2a	8.8	1.7
	2	2a	9.4	1.04
	3	2a	9.95	1.1
	4	2a	10.0	.97
Yeast	1	2a	9.4	.88
	2	2a	8.5	2.04
	3	2a	8.45	3.03
	4	2a	9.4	.94
Artemia		2a	7.7	1.17

margin of the dorsal fin was observed to be separating from the primordial fin-fold at early stage 2b. However, this separation was never complete so that no larvae could be classified as stage 2b proper. The number of dorsal fin elements (Table 5) averaged 9.5, 9.5 and 8.9 rays for Isochrysis, Nannochloropsis and yeast treatments respectively. These values were not significantly different (ANOVA, $F = 1.54$, d.f. = 2; 9) and were higher than the 7.7 elements observed on average in those larvae grown on Artemia.

1b. 1986

Average survival levels in this experiment, at the end of 28 days of rearing (Table 6) were 50 $\pm$ 13%, 38.7 $\pm$ 18.2% and 16.2 $\pm$ 4.4% (average $\pm$ S.D.) for Nannochloropsis, Isochrysis and yeast treatments respectively. An ANOVA, performed on the number of larvae surviving in the individual replicates presented in Table 6, showed that both algae enriched rotifers conferred higher larval survival levels than yeast-rotifers ($F = 6.8$, d.f. = 2; 9). Between algal treatments, however, there were no significant differences ($F = 1.01$, d.f. = 1; 6).

In all rearing units the mortality patterns recorded (Fig. 9) showed a gradual slope up to day 14 followed by a major mortality event which was terminated by day 19. From day 20 onwards very little mortality was recorded. There was no variation in t_{50} (the days to 50% of the survival levels recorded on day 28) and the average t_{50} value for all treatments was calculated as 16.2 $\pm$ 0.7 (d $\pm$ S.D.).

Larvae grew from an initial 9.8 $\pm$ 0.4 (mm $\pm$ S.D.), TL, to average lengths of 15.4 $\pm$ 0.7 mm, 15.4 $\pm$ 0.3 mm and

Table 6. Growth and survival of herring larvae during the 1986 experiment. Larvae fed rotifers enriched with either Isochrysis, Nannochloropsis or baker's yeast.

	Initial (day 4)	Final (day 28)		
Parameter		Isochrysis	Nannochloropsis	Baker's yeast
TL (mm ± S.D.)	9.8 ± 0.44	16.23 ± 1.31	15.72 ± 1.03	13.46 ± 0.75
		15.82 ± 0.99	15.74 ± 1.25	13.38 ± 0.72
		14.82 ± 0.99	15.15 ± 0.95	13.65 ± 0.51
		14.98 ± 0.87	15.10 ± 0.95	13.69 ± 0.6
Dry weight (µg ± S.D.)	148.1 ± 15.6	499.5 ± 179.0	427.8 ± 116.7	215.8 ± 51
		434.0 ± 134.2	444.0 ± 166.3	203.4 ± 47
		326.0 ± 116.1	372.6 ± 101.2	214.0 ± 38
		335.8 ± 112.7	352.3 ± 109.3	228.5 ± 54.7
SGR (% dry weight)		5.06	4.42	1.5
		4.48	4.57	1.3
		3.2	3.84	1.5
		3.4	3.61	1.8
Survival (%)		63.3	37.0	18.0
		41.4	65.3	11.0
		28.2	41.7	21.2
		22.0	56.0	14.6

* SGR = ($\Delta \ln W / \Delta t$) x 100

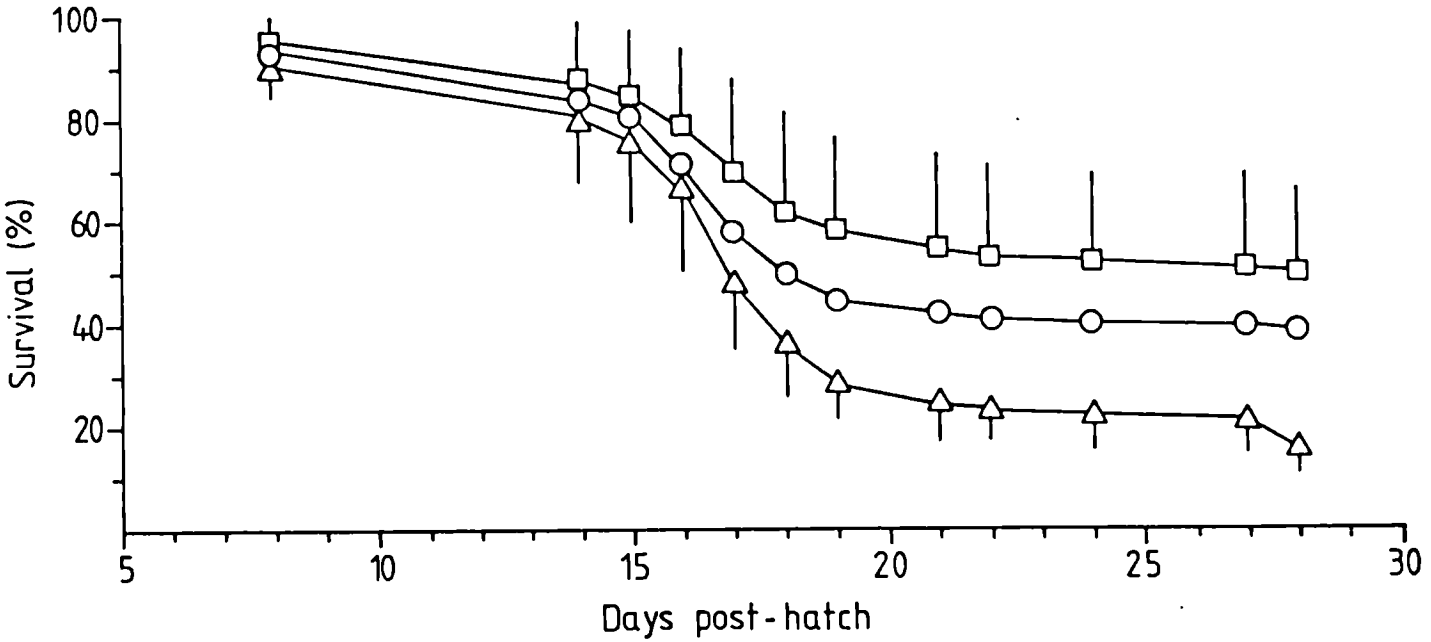


Fig. 9: Survival curves for herring larvae during the first 28 d post-hatch in the 1986 experiment. Points are averages of 4 replicates. Larvae feeding on rotifers enriched with: O Isochrysis, □ Nannochloropsis, Δ baker's yeast. Bars represent the 95% confidence limits and are omitted from the Isochrysis treatment due to overlapping.

13.5 ± 0.1 mm on Isochrysis, Nannochloropsis and yeast treatments respectively. An ANOVA performed on the values for the individual replicates presented in Table 6 showed the yeast treatment to differ significantly from algal treatments ($F = 24.12$, d.f. = 2; 9).

Growth rates (mm/d) of larvae fed with algae enriched rotifers were linear throughout the experiment (Table 7, Fig. 10). Larvae fed yeast-enriched rotifers grew at the same rates as the algal treatments up to day 12 following which their growth rates declined. Within the Nannochloropsis treatment there were no significant differences in the rate of growth (ANCOVA, $F = 2.0$, d.f. = 3, 376), though significant differences were recorded within the Isochrysis treatment (ANCOVA, $F = 8.6$, d.f. = 3; 376). Comparing the two algal treatments revealed that both the fastest and slowest growing larval populations had been reared on Isochrysis enriched rotifers, tanks 1 and 3 respectively (Table 7). Between the other two Isochrysis replicates and the four Nannochloropsis replicates there were, however, no significant differences (ANCOVA, $F = 2.0$; d.f. = 5, 564).

Larvae exhibited a loss in weight following yolk absorption, but had more or less recovered from this loss by day 12 post-hatch (Fig. 11). At termination (Table 6) larvae grown on algal enrichments had significantly higher average weights of 399 ±83 and 399 ± 44 (µg ± S.D.) for Isochrysis and Nannochloropsis enrichments respectively, compared with 215 ± 10 µg for the yeast treatment (ANOVA, $F = 15.07$, d.f. = 2; 9). Growth rates for algal treatments between days 12 and 28 were exponential while those for yeast were linear.

Fig. 10. The relationship of length (TL, mm) and time (d) of herring in the 1986 experiment. Points are averages of 4 replicates with n=24 samples in each. Bars are 95% confidence limits. Full circle represents initial length for the population. Larvae feeding on rotifers enriched with:

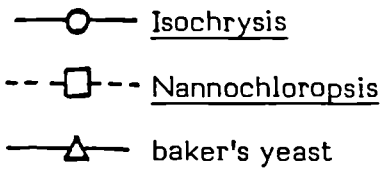
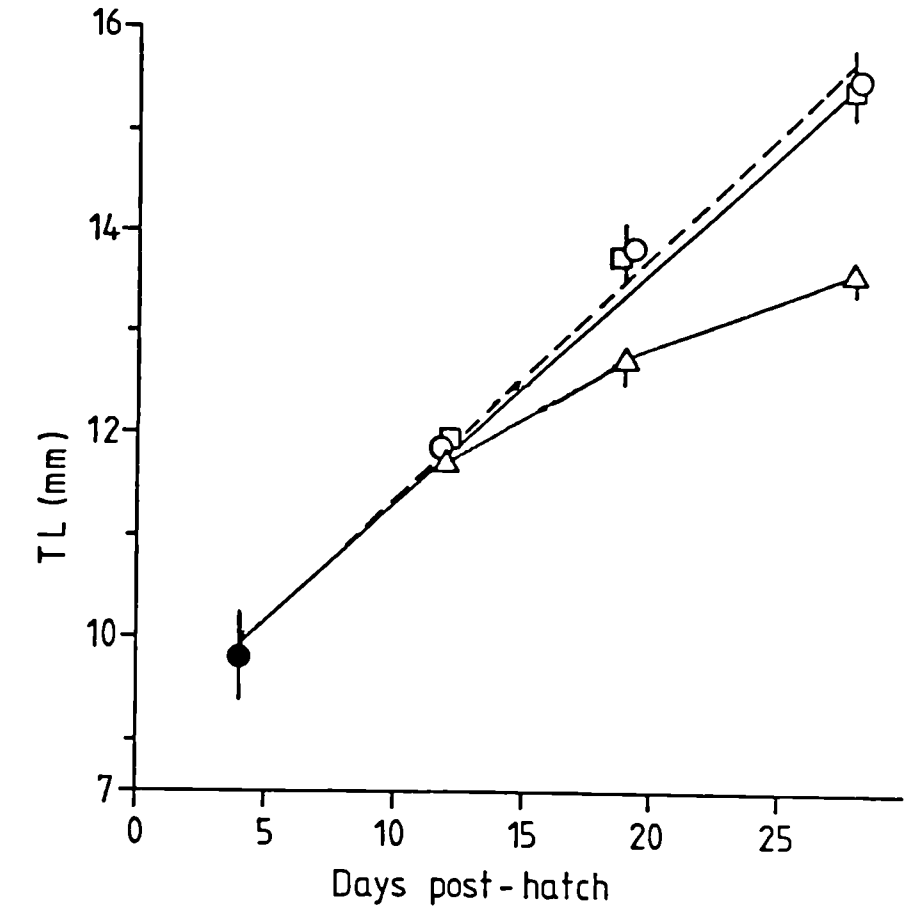


Fig. 11. The relationship of dry weight (µg) and time (d) of herring larvae in the 1986 experiment. Symbols and treatments as in Fig. 10.


16
14
12
10
7
TL (mm)
0
5
10
15
20
25
Days post-hatch

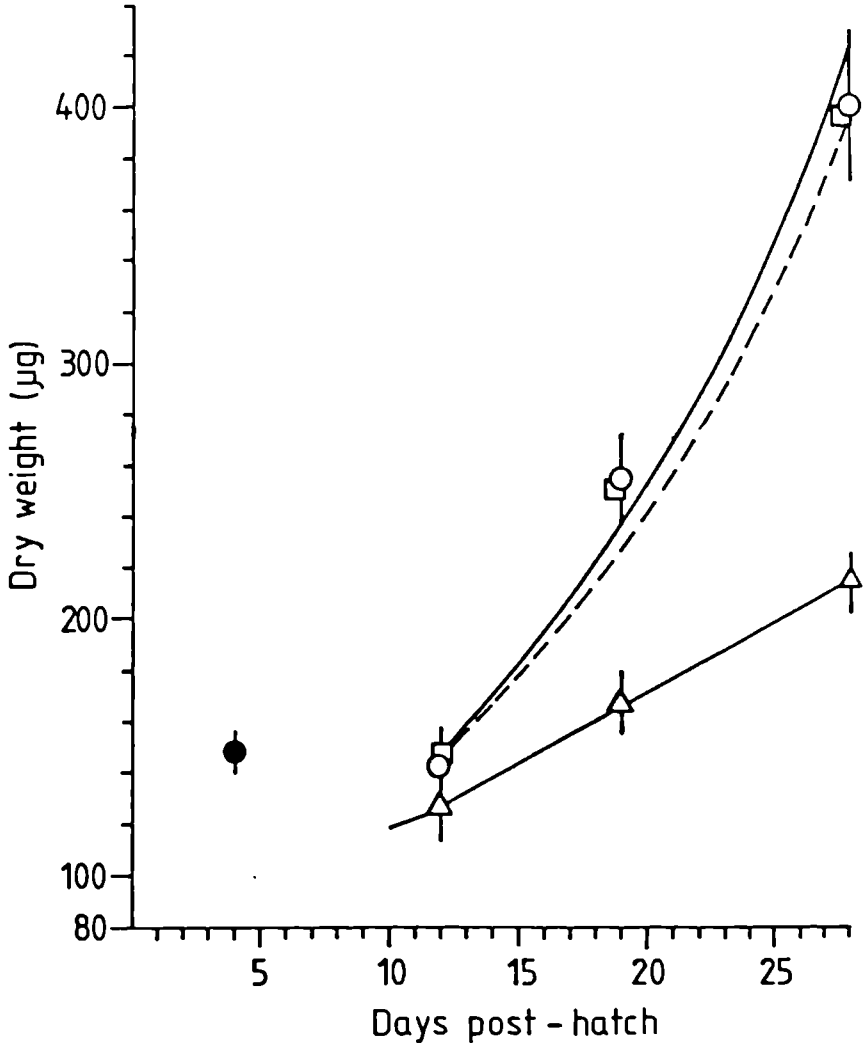

400
300
200
100
80
Dry weight (µg)
5
10
15
20
25
Days post-hatch

Table 7. The relationship between TL(mm), and age (t, days post-hatch) of herring during the 1986 experiment. Larvae fed rotifers enriched with either Isochrysis (Iso), Nannochloropsis (Nanno) or baker's yeast.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	1	TL=0.27t+8.8	96	.011	.93
	2	TL=0.25t+8.8	96	.011	.93
	3	TL=0.21t+9.2	96	.009	.92
	4	TL=0.22t+9.1	96	.009	.94
Nanno	1	TL=0.24t+9.1	96	.009	.94
	2	TL=0.25t+8.9	96	.01	.93
	3	TL=0.23t+9.1	96	.01	.93
	4	TL=0.22t+9.1	96	.009	.93
Pooled		TL=0.23t+9.0	384	.005	.93
Yeast	1	TL=0.15t+9.4	96	.009	.88
	2	TL=0.14t+9.5	96	.008	.88
	3	TL=0.16t+9.4	96	.007	.91
	4	TL=0.16t+9.4	96	.008	.90
Pooled		TL=0.15t+9.4	384	.004	.89

*Sb = Standard error of the regression coefficient.

Yet again, the differences in growth rates (µg/d) within the Isochrysis treatment were significant (ANCOVA, F = 3.8; d.f. = 3; 280) while within the Nannochloropsis treatment there were no significant differences (ANCOVA, F = 1.7; d.f. = 3; 280). Comparing all the algal-enriched treatments showed the Isochrysis enriched rotifers to produce both the fastest and slowest growing larval populations, while between the other two Isochrysis populations (tanks 2 and 4) and all the Nannochloropsis replicates there were no significant differences (ANCOVA, F = 2.0; d.f. = 5; 360).

Specific growth rates (SGR in %/d) (Table 6) were significantly higher in the algal than the yeast treatments (ANOVA, F =25.1, d.f. = 2; 9) averaging 4.03 and 4.11%/d for Isochrysis and Nannochloropsis respectively and 1.5%/d for yeast.

In all treatments there was an initial drop in condition factor (CF) during the final stages of yolk absorption and transfer to exogenous feeding (days 4-12, Fig. 12). From day 12 onwards the condition factor slowly improved, though by the end of the experiment yeast treatments had significantly lower CFs than those calculated for algal treatments (ANOVA, F = 17.3, d.f. = 2; 9).

There was a significant correlation in all treatments for the regression of log weight (µg) on log TL (mm) and the equations for the pooled regressions were:

log W = 3.92 log TL - 2.07 (r = 0.98) for Nannochloropsis,

log W = 4.007 log TL - 2.18 (r = 0.98) for Isochrysis,

and log W = 3.68 log TL - 1.83 (r = 0.93) for yeast.

Algal treatments differed significantly with respect to their slopes from the yeast treatment (ANCOVA, F = 6.2, d.f. = 2; 858).

Table 8. The relationship between dry weight (µg) and age (t, day post-hatch) of herring larvae (1986). Larvae fed rotifers enriched with either Isochrysis (Iso), Nannochloropsis (Nanno) or baker's yeast.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	1	logW=.031t+1.83	72	.003	.79
	2	logW=.029t+1.80	72	.002	.82
	3	logW=.021t+1.92	72	.002	.72
	4	logW=.025t+1.79	72	.002	.80
Nanno	1	logW=.026t+1.88	72	.002	.84
	2	logW=.028t+1.83	72	.002	.82
	3	logW=.026t+1.85	72	.002	.81
	4	logW=.024t+1.85	72	.002	.83
Pooled		logW=.028t+1.84	288	.001	.80
Yeast	1	W=6.12t+45.2	72	0.26	.68
		W=4.3t+73.4	72	0.25	.56
	3	W=5.6t+58.5	72	0.21	.71
	4	W=6.45t+53.1	72	0.26	.70
Pooled		W=5.6t+58.3	288	0.04	.70

*Sb = Standard error of the regression coefficient.

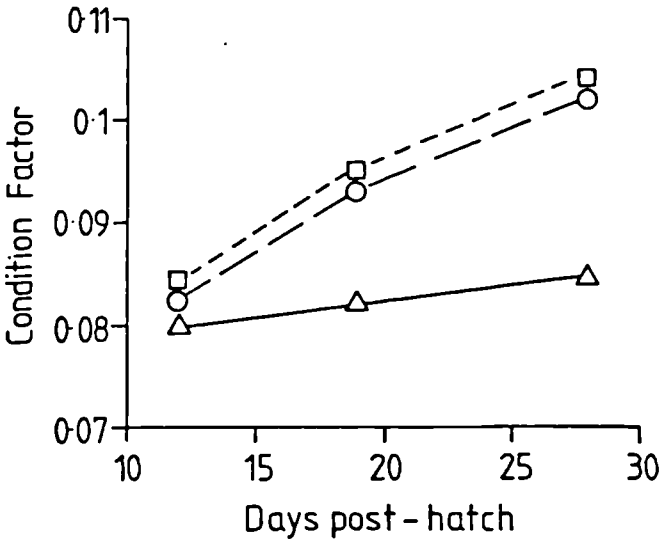


Fig. 12. The relationship between the condition factor ($\mu g/mm^3$) and time (d) for herring larvae in the 1986 experiment from day 12 post-hatch. Symbols and treatments as in Fig. 10.

Larvae grown on rotifers enriched with algae reached a higher degree of development by the end of the experiment (Table 9). At this time very few of the larvae fed on yeast rotifers had developed any dorsal fin elements and at most 4-5 rudimentary elements could be found in these larvae. On the other hand a large number of larvae receiving the algal supplemented diets had fully formed dorsal fins which had separated completely from the primordial fin-fold (Stage 2c). Comparison of the average number of fin elements observed on day 28 in the dorsal, anal and caudal fins of larvae (Table 9) did not reveal any significant statistical differences between the algal treatments, F values ranging between 0.8-0.9 for any of the above parameters (d.f. = 95; 95).

All replicates were further analyzed for effects of growth rates on survival at termination. Survival levels were found to be higher in tanks with higher specific growth rates (SGR). The relation between survival and SGR on day 28 (Fig. 13) was best described by the equation:

$$Y = 11.8X - 3.15 \ (r = 0.85, p < 0.001)$$

where Y is the percent survival and X the SGR (%/d) during this period.

Density of rotifers, in the larval rearing units, was dependent on the level at which they were stocked daily, the rates at which they reproduced, and the rates at which they were consumed by the larvae and washed out of the tanks due to the continuous sea water exchange. Monitoring the rotifer population was therefore important for ensuring that the

Table 9. Development stage (according to Doyle, 1977), and the number of elements present in the fins of herring larvae from the 1986 experiment.

Treatment		Development		Fin rays (av. ± s.d.; % Positive*)		
		Stage	Range	Dorsal	Anal	Caudal
Iso	1	2b	2b-2c	11.4 ± 2.4;100	5.4 ± 3.4;79	8.0 ± 6.3;66
	2	2b	2b-2c	11.5 ± 1.7;100	4.2 ± 3.5;71	6.3 ± 5.7;62
	3	2b	2a-2c	9.2 ± 2.0;100	2.0 ± 3.1;37	3.0 ± 5.5;33
	4	2b	2a-2b	8.9 ± 2.5;100	1.3 ± 2.2;29	2.0 ± 3.5;29
pooled		2b	2a-2c	10.3 ± 2.6	3.2 ± 3.6	4.7 ± 5.6
Nanno	1	2c	2b-2c	11.8 ± 1.8;100	4.9 ± 3.4;79	7.5 ± 5.5;75
	2	2b	2b-2c	11.1 ± 2.3;100	4.0 ± 3.4;66	5.8 ± 5.6;62
	3	2b	2b-2c	10.3 ± 2.7; 96	2.7 ± 2.7;58	4.1 ± 4.5;50
	4	2b	2a-2c	10.5 ± 2.1;100	3.2 ± 2.9;58	4.4 ± 5.1;50
pooled		2b	2a-2c	10.9 ± 2.3	3.7 ± 3.1	5.4 ± 5.3

* % Positive = The percent of larvae from a sample of n=24 having at least 1 element of the relevant category.

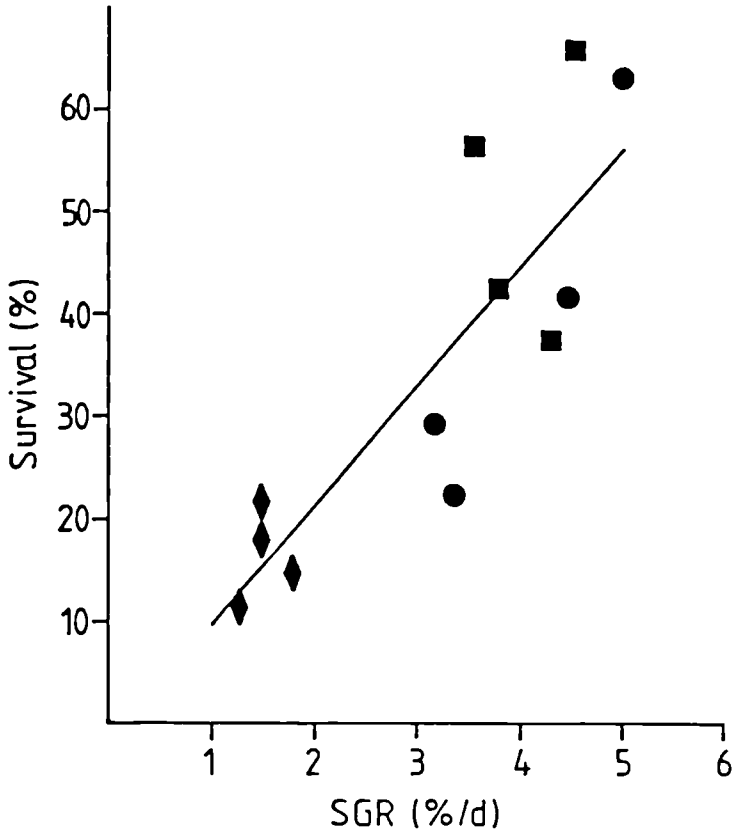


Fig. 13: The relation of survival on day 28 of the 1986 experiment to the specific growth rates of herring larvae fed rotifers enriched with: ● Isochrysis, ■ Nannochloropsis, ♦ baker's yeast. y=1.18x-3.15, , r=0.85; $p < 0.001$.

larvae had consistently been offered equal quantities of food in all the rearing units. Throughout the experiment no major increases in the rotifer population were observed in any of the larval rearing units. In most cases (90% of readings) the residual rotifer population, before daily feeding, was less than 0.5 individuals/ml (Fig. 14), which is less than 10% of the numbers stocked 24 h previously. No obvious pattern evolved between different treatments or in any particular tank and it can therefore be safely assumed that larvae fed mostly on fresh food daily.

The numbers of rotifers found in the guts of the larvae, on three separate occasions, are shown in Table 10. In these samples there was no apparent increase in gut contents with respect to larval age or length in any one of the treatments. An analysis of the gut contents was performed by dividing the number of rotifers by the cube of the respective length for each sampled larvae and multiplying by 10^3 for convenience of calculation:

$$GCI = (n/TL^3)\ 10^3$$

GCI. = Gut Contents Index

n = Number of rotifers

TL = Total length

These indexes were pooled for all observations in each treatment and compared by an ANOVA, showing no significance differences between the three treatments ($F = 1.5$, d.f. = 2; 82). It can therefore be assumed that

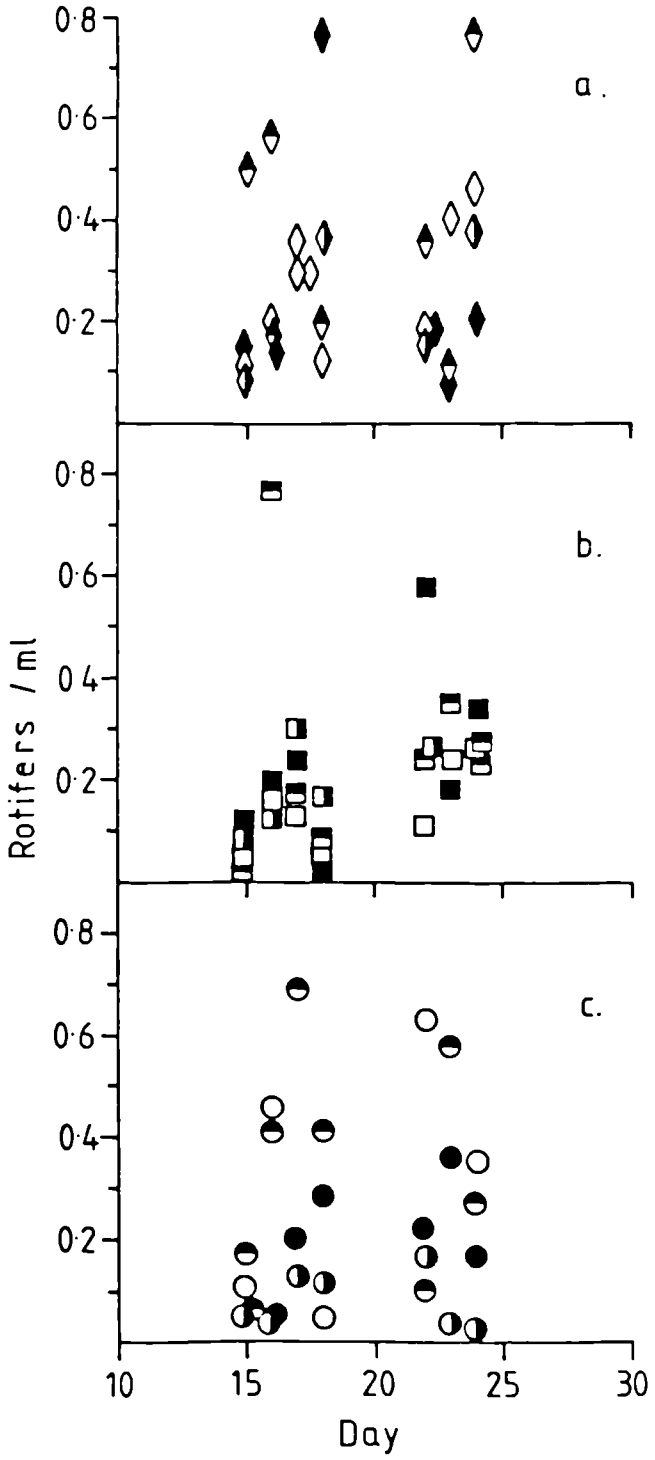


Fig. 14. Densities of rotifers in herring rearing tanks 24 h after stocking (1986 experiment). Tanks receiving rotifers enriched with: a, baker's yeast, b, Nannochloropsis, c, Isochrysis. The different shadings of each symbol denote individual replicates.

Table 10. The number of rotifers in guts of herring larvae (1986). See Table 7 for abbreviations.

Enrichment	Day	TL	Rotifers	N*
		mm+s.d.	n+s.d.	
Nanno	16	13.1+1.3	29.75+15.8	4
	17	13.4+9.6	21.9 + 9.6	10
	22	14.3+0.1	24.0 + 8.5	12
Iso	16	13.8=0.8	36.7+16.8	4
	17	13.1+1.5	20.1+11.4	15
	22	14.1+1.4	23.2+11.4	16
Yeast	16	12.6+0.5	23.6+13.0	3
	17	12.6+0.6	15.2+ 5.1	16
	22	13.6+0.1	17.8+ 7.1	12

*N = The number of larvae sampled.

Table 11. Growth and survival parameters for plaice larvae fed rotifers enriched with either Isochrysis, Nannochloropsis or baker's yeast.

(a) 1985. Conditions were 30 l tanks stocked initially with 700 larvae and grown for 31 days in ambient temperatures (10°-13°C); L:D 16:8 and 200-300 lux illumination.

(b) 1986. Conditions were 100 l tanks stocked initially with 350 larvae and grown for 30 days at 11 ± 1°C; L:D 16:8 and 200-300 lux illumination.

Parameter		Initial	Final		
			Isochrysis	Nannochloropsis	Baker's yeast
TL (mm ± s.d.)	(a)	7.00 ± 0.25	8.94 ± 0.82	8.92 ± 0.81	7.26 ± 0.4
	(b)	7.34 ± 0.23	9.82 ± 0.9	9.84 ± 0.83	7.74 ± 0.28
Dry Weight (µg ± s.d.)	(a)	88.45 ± 5.3	413 ± 187	439 ± 227	161 ± 65
	(b)	117.80 ± 6.1	569 ± 220	550 ± 212	130 ± 35
Development* (stage)	(a)	1b'	3b(2a - 4b)	3b(2a' - 4b)	2a(1d - 2a')
	(b)	1b	3b'(2b - 4b)	3b'(2b - 4b)	2a(1d - 2a')
SGR** (% dry weight/d)	(a)		5.5	5.72	2.13
	(b)		7.4	7.3	0.46
Survival (%)	(a)		42	31	14
	(b)		81.5	83.75	4.8

* Development stages according to Ryland (1966)
** SGR = $(\Delta \ln W / \Delta t) \times 100$

the type of enrichment had no significant effect on the quantities of rotifers consumed by the larvae.

2. Plaice

Actively feeding plaice larvae were observed on day 4 post-hatch of the 1985 experiment and on day 5 of the 1986 experiment, henceforth referred to as exp. 1 and exp. 2 respectively. The average number of rotifers ($\pm$ S.D.) in larval guts on day 6 of exp. 2 were 16.2 $\pm$ 6.5, 13.8 $\pm$ 9.1 and 18 $\pm$ 6.5 for yeast, Nannochloropsis and Isochrysis treatments respectively, these values not differing significantly (ANOVA, $F = 0.08$, d.f. = 2; 9). On the following day larvae of the Isochrysis treatment had significantly lower gut contents (15.8 $\pm$ 6.8) compared with Nannochloropsis (26 $\pm$ 9.5) and yeast (21.8 $\pm$ 5.7) (ANOVA, $F = 2.3$, d.f. = 2; 12).

Throughout exp. 2 no major mortalities were recorded in the algal treatments and such mortality as did occur took place between days 14 and 22. Within each experiment the final survival levels were highest for those larvae receiving an algal supplement in their diet (Table 11) with Nannochloropsis and Isochrysis treatments having similar values. Survival was higher in exp. 2 (81-84%) compared with exp. 1 (31-42%), which is probably due to the larger rearing tanks and lower stocking densities of exp. 2.

Effects of the different treatments on growth in length, weight and development are shown in Table 11. Those larvae receiving a diet not enriched with algae were significantly shorter and weighed significantly less at termination compared with those on algal diets. Between the two algal treatments,

within each of the experiments, there were no significant differences in final length, weight and development parameters. However in exp. 2 these values were higher compared with exp. 1. The final average developmental stage in exp. 2 for algal treatments was 3b' (notochord is upturned but not yet at the full 90°). The range of stages was 3a-4a, i.e. from initiation of notochord flexing to initiation of eye migration (metamorphosis) stage. In exp. 1 larvae were slightly less developed with the average stage being 3b (notochord is upturned at just over 45°) and the range was reduced so that some larvae were at the pre-notochord flexure stage (2b).

Rates of growth in length (TL, mm) and log-transformed weights for algal treatments (Figs 15a,b, 16a,b) were initially slow (up to day 9 in exp. 1 and day 14 in exp. 2) and accelerated thereafter. Linear regressions fitted to these curves (Tables 12, 13) had higher regression coefficients in exp. 2. However, there were no statistical differences between algal treatments within any one of the experiments. Yeast treatments, in both experiments, also underwent a duration of weight loss and minimal length increase followed by a minor recovery.

Using Ryland's (1966) staging system it was found that in both experiments there were no obvious differences in average levels of development for larvae feeding on either the Isochrysis- or the Nannochloropsis-enriched rotifer diets on any of the sampling days. Rates of development were estimated by transforming Ryland's staging system to a numerical one as follows: stage 1a-1, 1b-2, 1b'-3, 1c-4, 1c'-5,

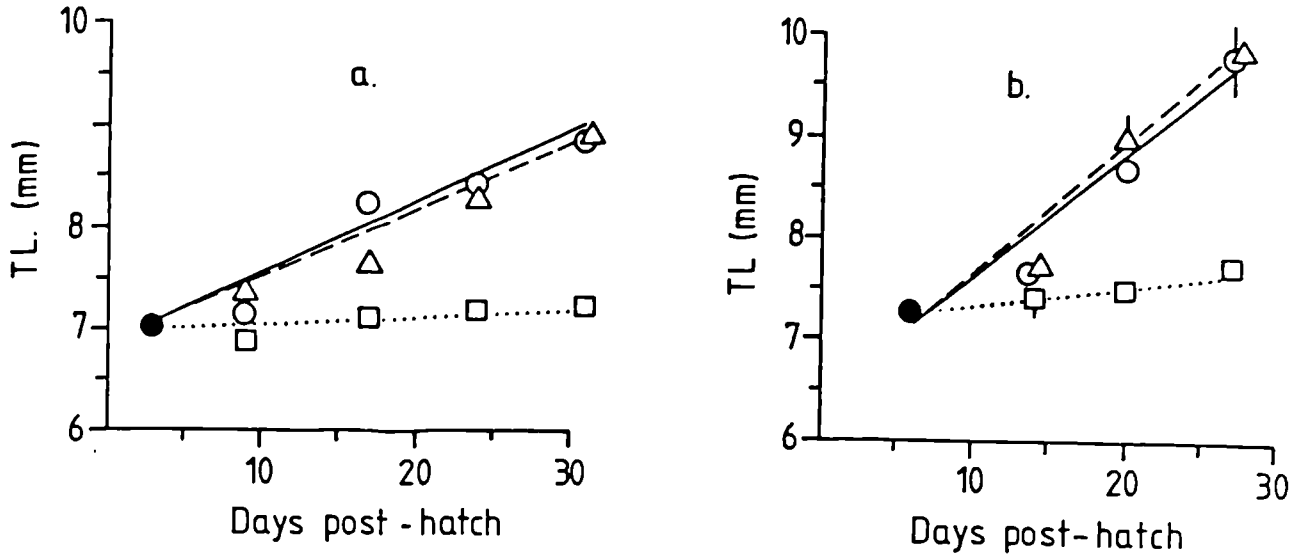


Fig. 15. The relationship between TL (mm) and age (d) of plaice larvae during: a 1985, b 1986. Points represent average of n=24 larvae, bars are 95% confidence limits. One replicate per treatment for larvae fed rotifers enriched with: ---○--- Isochrysis, —△— Nannochloropsis, ·····□····· baker's yeast.

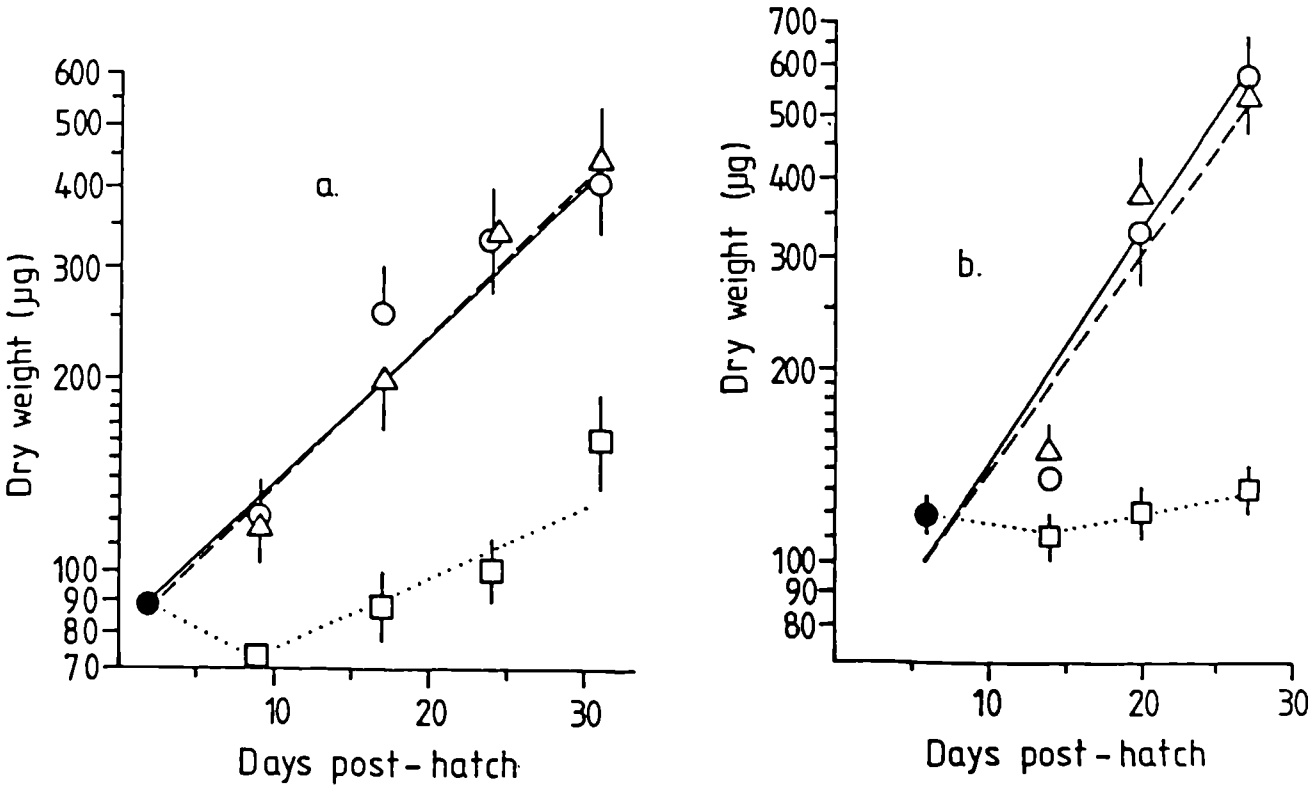


Fig. 16. The relationship between dry weight (µg) and age (d) of plaice larvae a 1985, b 1986. For details see Fig. 15.

Table 12. Relationships between length (TL,mm) and time (t, days post-hatch) for plaice larvae. Regression coefficients for each experiment compared by an analysis of covariance (ANCOVA). See Table 11 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	TL=0.074t+6.74	120	0.005	0.8
	b	TL=0.122t+6.36	96	0.009	0.82
Nanno	a	TL=0.068t+6.7	120	0.005	0.8
	b	TL=0.13t+6.36	96	0.008	0.85
ANCOVA					
	a	F = 0.5 d.f. = 1;238			
	b	F = 0.2 d.f. = 1;188			

a : Exp. 1. 1985
b : Exp. 2. 1986
*Sb, Standard error of the regression coefficient.

Table 13. Relationships between dry weight (µg) and time (t, days post-hatch) for plaice larvae. Regression coefficients for each experiment compared by an analysis of covariance (ANCOVA). See Table 11 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	logW=0.024t+1.89	120	0.005	0.81
	b	logW=0.033t+1.79	96	0.002	0.82
Nanno	a	logW=0.025t+1.86	120	0.005	0.83
	b	logW=0.033t+1.82	96	0.002	0.85
ANCOVA					
	a	F = 0.53 , d.f. = 1;238			
	b	F = 0.0003 d.f. = 1;188			

a: Exp. 1. 1985
b: Exp. 2. 1986
*Sb, Standard error of the regression coefficient.

1d-6, 2a-7, 2a'-8, 2b-9, 2b'-10, 3a-11, 3a'-12, 3b-13, 3b'-14, 3c-15, 4a-16, 4b-17, 4b'-18 and 5-19. Regressing development on age (Fig. 17a,b) showed that larvae receiving the algal-enriched diets developed faster during exp. 2 than exp. 1, the rates of development being 0.55 and 0.34 stages/d respectively.

Condition factors (CF) (Fig. 18a,b) tended to increase throughout the experiments in algal treatments as well as in the yeast treatments of exp. 1. However, during exp. 2 the CF for the yeast treatment remained unchanged throughout the experiment.

In both experiments there was a good correlation between log dry weight (µg) and log length (TL, mm) (Table 14). The Nannochloropsis treatment of exp. 1 had a higher regression coefficient compared with Isochrysis (ANCOVA, $F = 4.11$, d.f. = 1; 238). However during the second year this observation did not repeat itself.

Colour patterns of juvenile fish were recorded after a further 20 and 24 days of rearing on Artemia nauplii from the termination of rotifer feeding in exp. 1 and exp. 2 respectively. Pigmentation was classified as "normal" (as in wild fish), "mottled" which is a grouping for all partially pigmented fish, and "albino". In both experiments Isochrysis treatments had more normally pigmented fish than Nannochloropsis treatments (Table 15) but in exp. 1, 100% of Isochrysis larvae had some form of pigmentation compared with only 86% in exp. 2. This situation is reversed for Nannochloropsis treatments which had a higher proportion of pigmented fish (49.5%) in exp. 2 compared with only 8% in exp. 1.

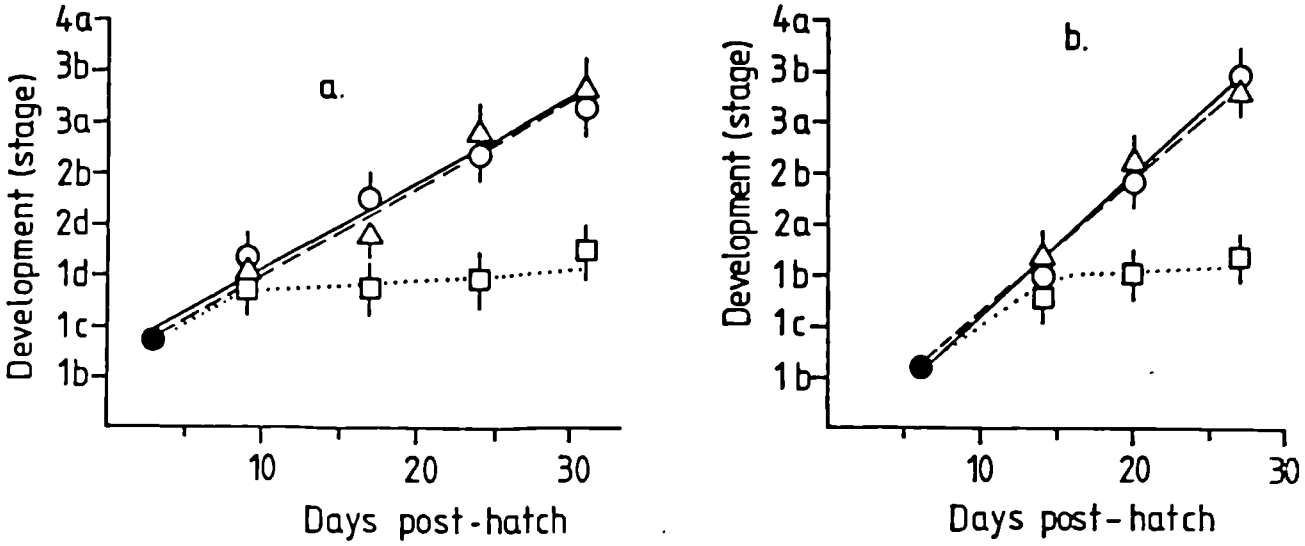


Fig. 17. Relationships between development and age (d) of plaice larvae, a 1985, b 1986 employing the staging system of Ryland (1966). Symbols as in Fig. 15.

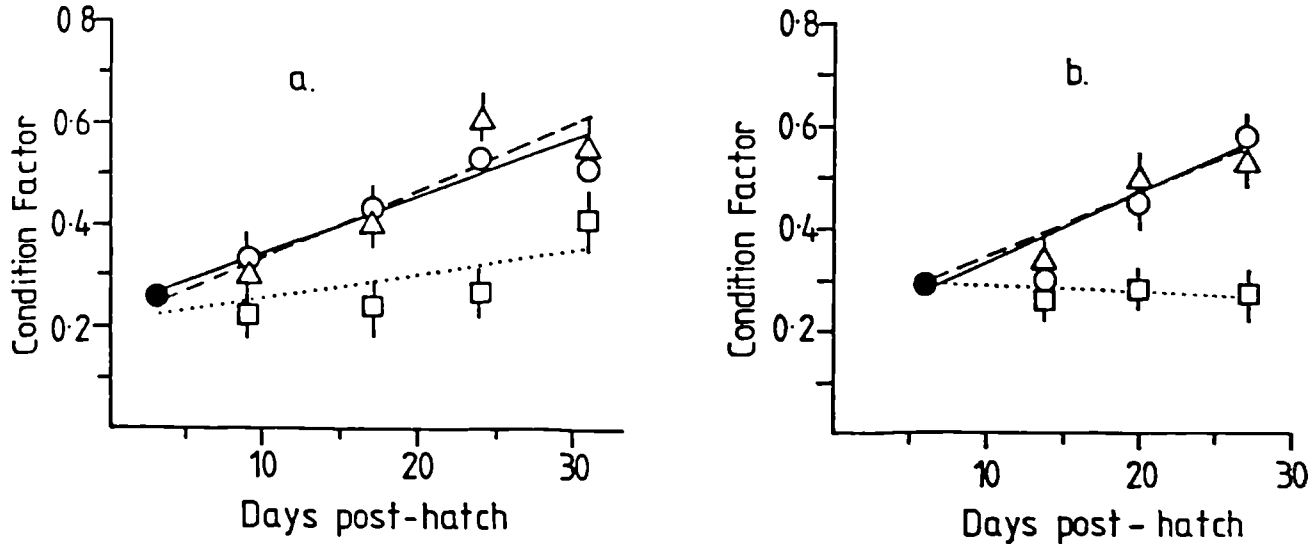


Fig. 18. Relationships between the condition factors ($\mu g/mm^3$) and age (d) of plaice larvae, a 1985, b 1986 during their development. See Fig. 15 for details.

Table 14. Relationships between dry weight (µg) and length (TL,mm) for plaice larvae. Regression coefficients for each experiment compared by an analysis of covariance (ANCOVA). See Table 11 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	logW=5.58logTL-2.72	120	0.17	0.95
	b	logW=5.25logTL-2.49	96	0.18	0.96
Nanno	a	logW=6.12logTL-3.2	120	0.22	0.93
	b	logW=5.08logTL-2.33	96	0.14	0.96
ANCOVA					
	a	F= 4.11 d.f. = 1;238			
	b	F = 0.6 d.f. = 1;188			

a: Exp. 1. 1985
b: Exp. 2. 1986
*Sb, Standard error of the regression coefficient.

Table 15. Effects of the rotifer enrichment, either Isochrysis (Iso) or Nannochloropsis (Nanno) on the pigmentation of plaice after metamorphosis.

Treatment		Pigmentation (%)			n
		Normal	Mottled	Albino	
Iso	a	76	24	0	25
	b	35	51	14	86
Nanno	a	4	4	92	25
	b	16	33.5	50.5	101

a: Exp. 1. 1985
b: Exp. 2. 1986

3. Turbot

Turbot larvae were observed to orient themselves horizontally in the tanks from the third day post-hatch and feeding behaviour was observed on day 4. On this day the number of larvae with rotifers in their guts (from ten sampled larvae) was seven for Isochrysis treatments and ten in both other treatments. The average number of rotifers found in the larval guts increased considerably between days 4 and 5 (Table 16). However, there were no statistical differences in this parameter on any one day between the treatments as ascertained by an ANOVA.

Survival levels on day 14 were lowest for yeast treatments 12.3 $\pm$ 3.8% (mean $\pm$ S.D.), highest for Nannochloropsis 43.9 $\pm$ 9% and intermediate for Isochrysis 25.6 $\pm$ 2.3% (for values of individual replicates see Table 17). Up to day 7 no appreciable numbers of dead larvae were seen on the bottom of the tanks. On the other hand, during days 4 and 5 large numbers of larvae were found floating dead on the water surface. This phenomenon disappeared completely after day 6 from those tanks to which algae-enriched rotifers and suspended algae had been added but continued for a further two days in tanks to which only yeast enriched rotifers had been added. The total number of floating dead larvae was highest for yeast, lowest for Isochrysis and intermediate for Nannochloropsis treatments, being 222, 25 and 137 respectively.

Daily mortalities, as determined by siphoning dead larvae from day 8 onwards, see Fig. 19, showed a linear relation between the percent of surviving larvae in each

Table 16. Estimates for first feeding success, gut contents (number of rotifers in the larvae gut) for turbot larvae feeding on rotifers enriched with Isochrysis (Iso) Nannochloropsis (Nanno) or baker's yeast. Ten larvae were sampled for each estimate. a, b, c -replicates.

Feed		Percent Feeding Day 4	Gut Contents Day 4	Gut Contents Day 5
Iso	a	70	17.6 ± 9	n.r.
	b	N.r.*	N.r.	35.8 ± 10.5
	c	N.r.	N.r.	29.1 ± 6.8
Nanno	a	100	15.5 ± 7	N.r.
	b	N.r.	N.r.	36.2 ± 11.8
	c	N.r.	N.r.	33.1 ± 13.1
Yeast	a	100	11.8 ± 7	N.r.
	b	N.r.	N.r.	44.8 ± 12.4
	c	N.r.	N.r.	21.0 ± 11.4

N.r.* - Not recorded.

Table 17. Final survival on day 15*, and the daily mortality rates of turbot larvae fed rotifers enriched with either Isochrysis (Iso), Nannochloropsis (Nanno) or baker's yeast.

Treatment		Survival %	Mortality (%/d)
Iso	a	24.0	1.02
	b	28.3	2.94
	c	24.5	1.64
Nanno	a	50.5	3.09
	b	33.5	1.99
	c	46.6	2.95
Yeast	a	11.5	6.8
	b	16.5	6.4
	c	9.0	7.0

*A correction was made for larvae removed as samples by subtracting the total number sampled from the initial number stocked.

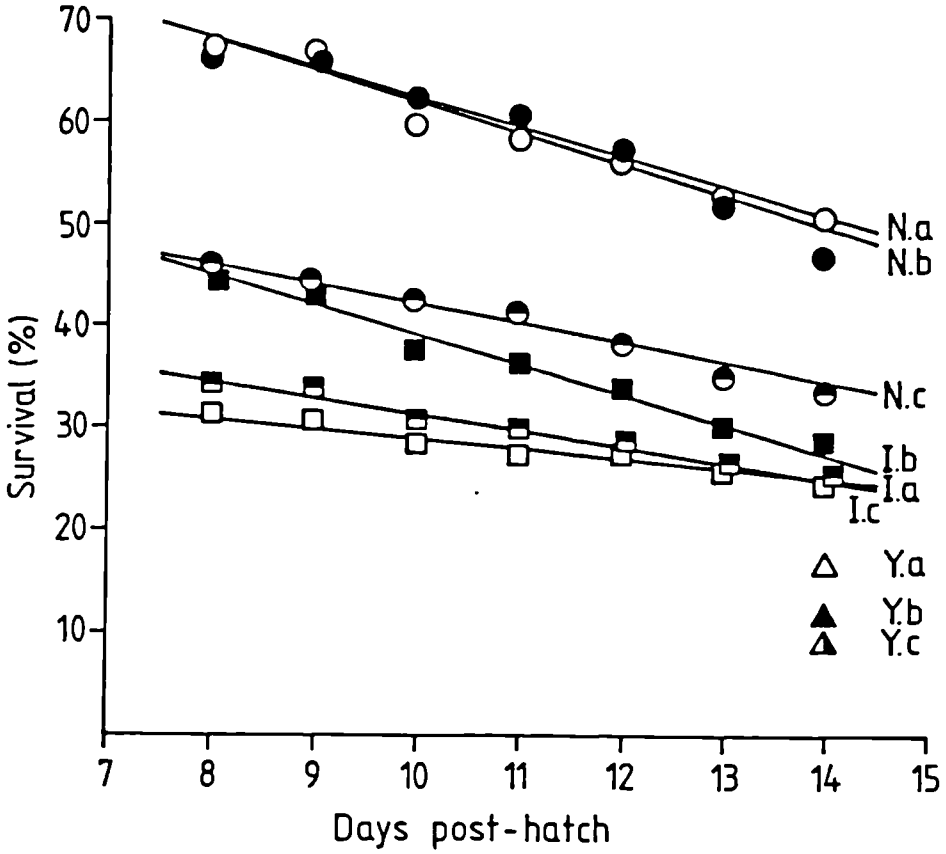


Fig. 19. The relationship between survival (%) and time (d) for turbot larvae, as recorded between days 8-15 post-hatch. Larvae were fed rotifers enriched with either Isochrysis (I), Nannochloropsis (N) or baker's yeast (Y). Replicates a, b, c.

Table 18. Growth parameters for turbot larvae reared in 100 l tanks for 14 days at 18 ± 1°C, L:D 24:0. and 2000-3000 lux illumination at the water surface. Larvae fed rotifers enriched with either Isochrysis, Nannochloropsis or baker's yeast.

	Initial (day 3)	Final (day 14)		
Parameter		Isochrysis	Nannochloropsis	Baker's yeast
TL (mm ± S.D.)	3.72 ± 0.08	7.32 ± 0.48 7.10 ± 0.59 6.83 ± 0.56	6.24 ± 0.41 6.70 ± 0.46 6.24 ± 0.37	4.83 ± 0.2 4.78 ± 0.3 4.76 ± 0.3
Dry Weight (µg ± S.D.)	33.75 ± 1.0	697 ± 234 615 ± 258 550 ± 231	367 ± 145.06 499 ± 171.6 307 ± 106.8	70.25 ± 17.31 66.50 ± 19.42 65.30 ± 18.75
SGR* (% dry weight/d)		27.52 26.3 25.3	21.9 24.5 20.0	6.6 6.1 6.0

* SGR = ($\Delta \ln W / \Delta t$) x 100

replicate and their age post-hatch. Correlation coefficients (r) of these regressions were in the range of 0.96-0.99. Mortality rates, as percent larvae dying daily, (Table 17) were higher for Nannochloropsis (2.67 ± 0.6%/d) than Isochrysis (1.86 ± 1%/d) although these differences were not statistically distinct (t-test). As sampling for daily mortalities in yeast treatments was not possible these were assumed to be linear throughout the experiment and the average calculated rate was found to be 6.7 ± 0.3%/d.

During the experiment larvae grew from 3.72 ± 0.08 (mm ± S.D.) to average total lengths of 7.08, 6.39, 4.78 in Isochrysis, Nannochloropsis and yeast treatments respectively (For values of individual replicates see Table 18). An ANOVA showed differences between treatments to be highly significant ($F = 95$, d.f. = 2; 6). At the same time larval weights increased from an average of 33.75 ± 1 (µg ± S.D.) to 620, 394 and 67 in Isochrysis, Nannochloropsis and yeast treatments respectively. These differences were significant, (ANOVA, $F = 95$, d.f. = 2; 6).

Rates of growth for both lengths and log-transformed weights were linear for algal treatments throughout the experiment (Figs 20a,b,c, 21a,b,c; Tables 19, 20). However, those larvae fed on yeast-enriched rotifers grew only up to day 8, after which no further increase in either length or weight was recorded. Growth rates of Isochrysis treatments were significantly higher for both lengths and log-transformed weights when compared to Nannochloropsis treatments using an ANCOVA (for TL/d $F = 95$, d.f. = 1; 596 and for log W/d $F = 42.3$, d.f. = 1; 596). Similarly, specific growth rates (Table 18) (SGR in µg/d) throughout the experiment

Table 19. Relationships of TL (mm) and age (t, days) for turbot larvae during the first 14 days post-hatch. See Table 17 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	TL=0.31t+2.87	100	.0024	0.96
	b	TL=0.30t+2.87	100	.0081	0.96
	c	TL=0.29t+2.99	100	.0097	0.94
Pooled		TL=0.29t+2.91	300	.0052	0.96
Nanno	a	TL=0.22t+3.25	100	.0065	0.95
	b	TL=0.26t+3.03	100	.0063	0.97
	c	TL=0.22t+3.24	100	.0058	0.97
Pooled		TL=0.23t+3.17	300	.004	0.96

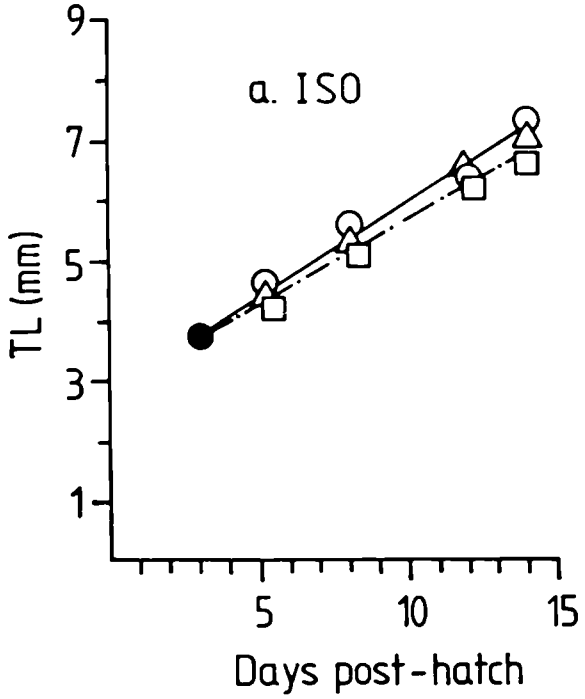
*Sb, Standard error of the regression coefficient.

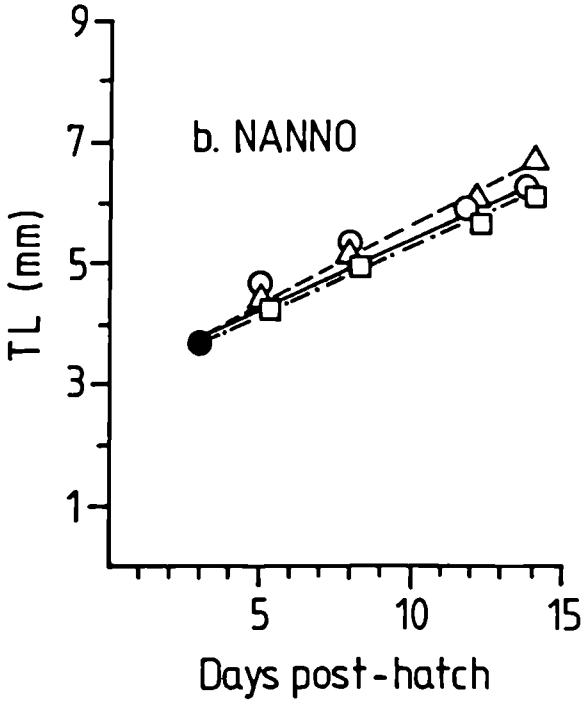
Table 20. Relationships between log-transformed dry weight (µg) and age (t, days) of turbot larvae during the first 14 days post-hatch. See Table 17 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	logW=0.12t+1.18	100	.003	0.97
	b	logW=0.11t+1.19	100	.003	0.96
	c	logW=0.11t+1.2	100	.004	0.94
Pooled		logW=0.11t+1.18	300	.002	0.96
Nanno	a	logW=0.096t+1.27	100	.003	0.95
	b	logW=0.11t+1.23	100	.002	0.97
	c	logW=0.085t+1.3	100	.002	0.96
Pooled		logW=0.095t+1.26	300	.002	0.95

*Sb, Standard error of the regression coefficient.

Fig. 20. Relationships of length (TL, mm) and age (days post-hatch) of turbot larvae feeding on rotifers enriched with either: a, Isochrysis (ISO), b, Nannochloropsis (NANNO) or c, baker's yeast (YEAST). Replicates in each treatment are presented by a different symbol. 95% confidence limits not shown as they are smaller than the symbols.


a. ISO
TL (mm)
9
7
5
3
1
5
10
15
Days post-hatch


b. NANNO
TL (mm)
9
7
5
3
1
5
10
15
Days post-hatch

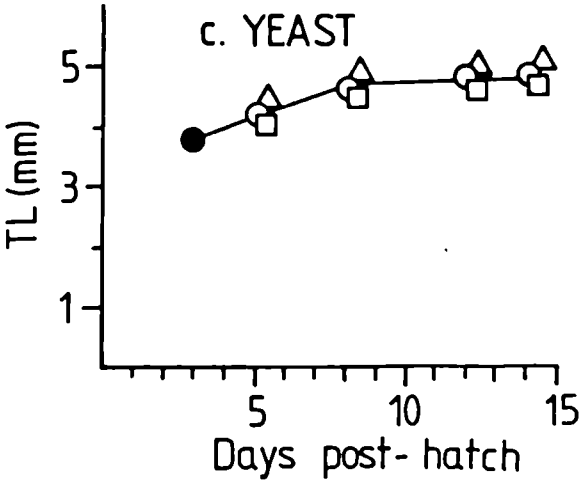
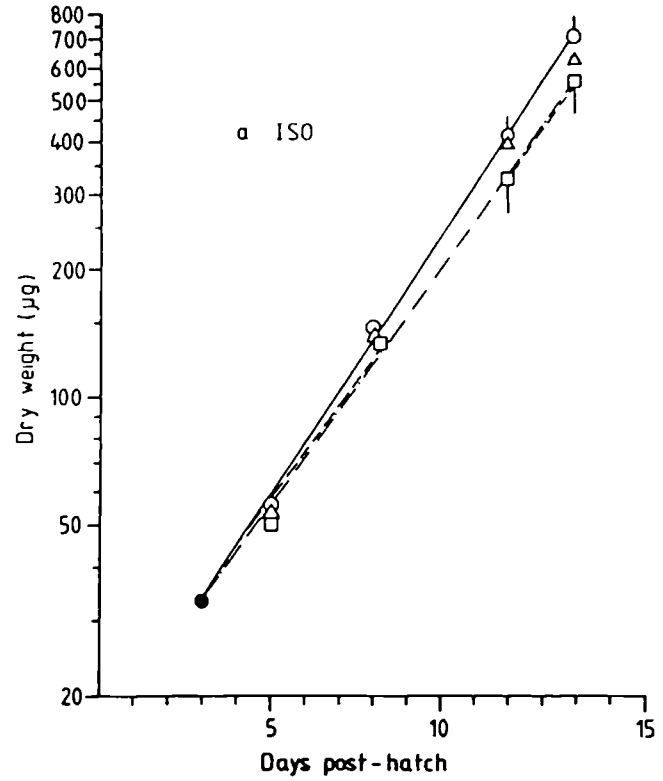
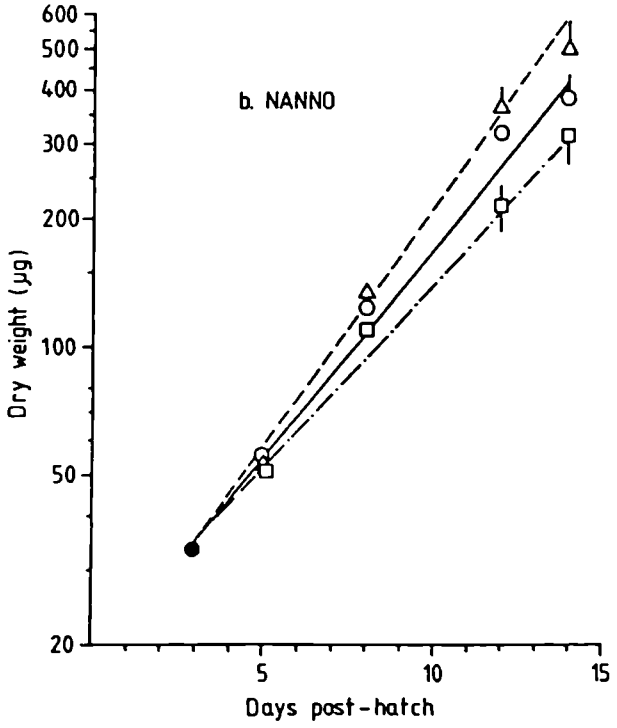
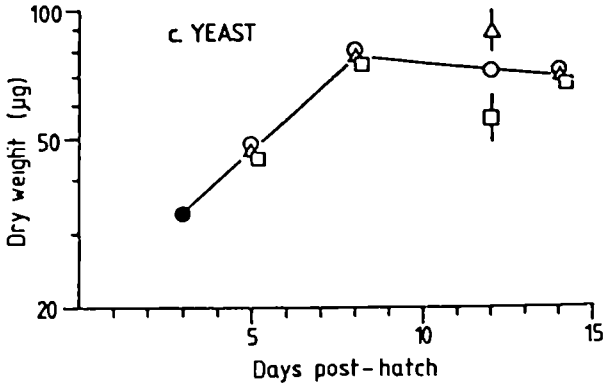

c. YEAST
TL (mm)
5
3
1
5
10
15
Days post-hatch

Fig. 21. The relationship between dry weight of turbot larvae (µg) and time (d). Larvae feeding on rotifers enriched with either: a, Isochrysis (ISO), b, Nannochloropsis (NANNO) or c, baker's yeast (YEAST). Replicates in each treatment are presented by a different symbol. Lines where shown denote 95% confidence limits.


a ISO
Dry weight (µg)
800
700
600
500
400
300
200
100
50
20
5
10
15
Days post-hatch


b. NANNO
Dry weight (µg)
600
500
400
300
200
100
50
20
5
10
15
Days post-hatch


c. YEAST
Dry weight (µg)
100
50
20
5
10
15
Days post-hatch

were significantly higher for Isochrysis compared to Nannochloropsis treatments (t-test, $\alpha = 0.05$) being 0.26 (µg/d) in the former and 0.22 in the latter.

Larval dry weights were related to lengths using log/log transformations (Table 21). A comparison of the regression coefficients between the two algal treatments showed a significant difference (ANCOVA, F = 5.37, d.f. = 1; 596).

Condition factors (CF) tended to increase linearly in algal treatments from day 5 post-hatch, whilst in yeast treatments there were no significant changes in CF throughout the experiment (Fig. 22a,b,c). Average CF at termination was significantly higher for Isochrysis (1.64 $\mu g/mm^3$) compared to Nannochloropsis (1.43 $\mu g/mm^3$).

The distribution of development stages on day 14 of 100 larvae from each of the algal treatment tanks is shown in Fig. 23. Analysis of these distributions showed that in all replicates of the Isochrysis treatments the average larva was undergoing notochord flexure and development of marginal fin rays, stages 4a-4b. However, in Nannochloropsis treatments in only one replicate was the average development stage within the notochord flexure phase (stage 4a) whilst in the other two most larvae were still at the stage of hypural element formation (3b). Another interesting feature of these distributions is that in the more developed of the Isochrysis replicates the distribution was skewed towards the higher development stages.

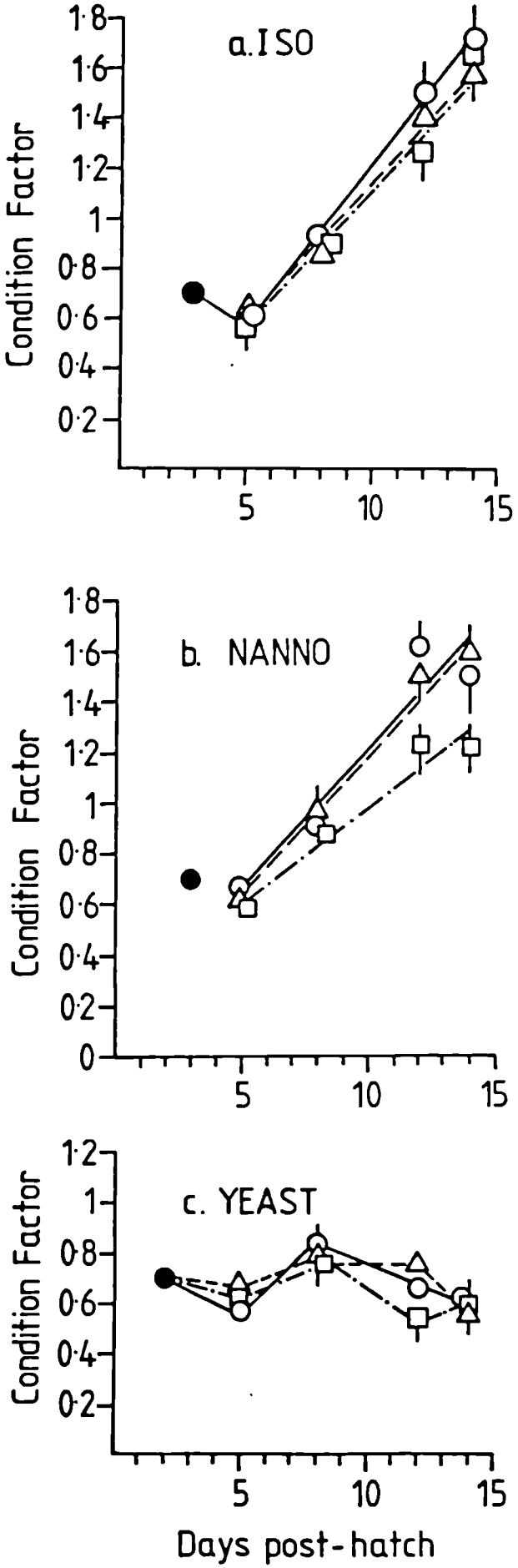
Difficulties were experienced in visually relating stages of early larval development to the criteria defined by Al-Maghazachi and Gibson (1984). This was mainly due to the

Table 21. Regressions of log dry weight (µg) on log length (TL,mm) of turbot larvae during the first 14 days post-hatch. See Table 17 for abbreviations.

Treatment		Regression	Parameters		
			n	Sb*	r
Iso	a	logW=4.79logTL-1.23	100	.055	0.99
	b	logW=4.55logTL-1.14	100	.073	0.99
	c	logW=4.63logTL-1.19	100	.076	0.99
Pooled		logW=4.76logTL-1.18	300	.038	1.0
Nanno	a	logW=4.91logTL-1.35	100	.11	0.98
	b	logW=4.77logTL-1.25	100	.077	0.99
	c	logW=4.40logTL-1.05	100	.087	0.98
Pooled		logW=4.69logTL-1.21	300	.055	0.98

*Sb, Standard error of the regression coefficient.

Fig. 22. Changes in condition factors ($\mu g/mm^3$) with time (d) of turbot larvae feeding on rotifers enriched with: a, Isochrysis (ISO), b, Nannochloropsis (NANNO) or c, baker's yeast (YEAST). Replicates in each treatment are presented by a different symbol. Lines where shown denote 95% confidence limits.


a. ISO
b. NANNO
c. YEAST
Condition Factor
Days post-hatch
1·8
1·6
1·4
1·2
1
0·8
0·6
0·4
0·2
0
5
10
15

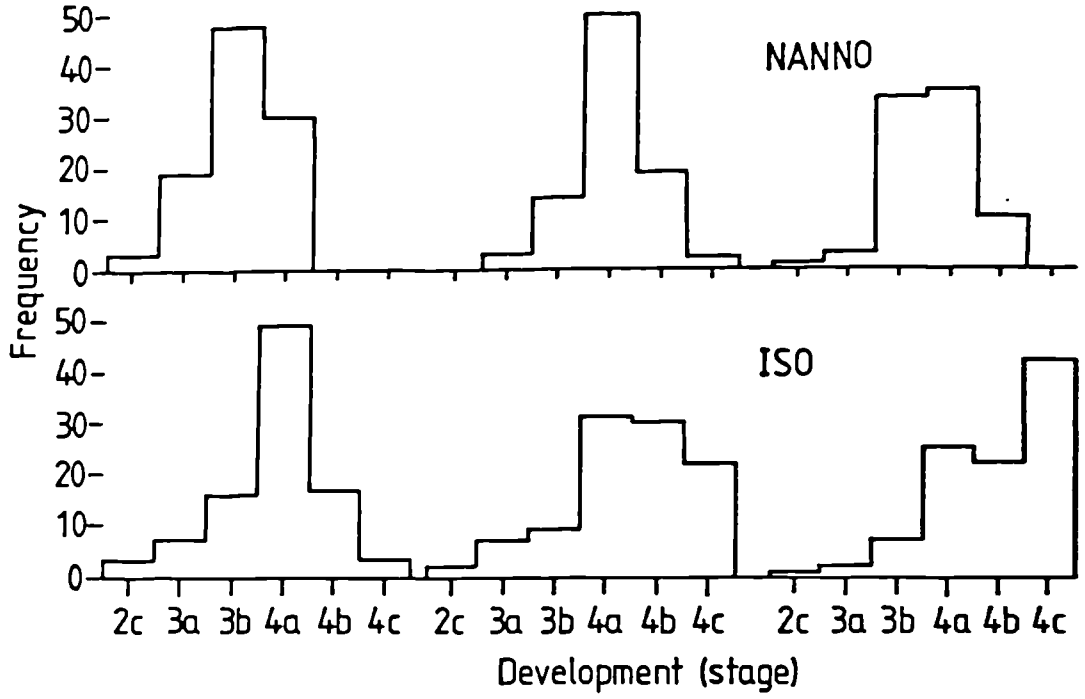


Fig. 23. Distribution of developmental stages (according to Al-Maghazachi & Gibson, 1984) of turbot larvae on day 14 post-hatch. One-hundred fish sampled from three replicates. Larvae fed on either Nannochloropsis (NANNO) or Isochrysis (ISO).

fact that these authors emphasize in their definitions the appearance of spines on the head region, which are most difficult to distinguish with conventional optics in the smaller specimen. In the present work stages 2a, 2b and 2c of Al-Maghazachi and Gibson were redefined as follows:

Stage 2ab - comprises all individuals whose mid-gut was bent ventrally but torsion was not yet complete.

Stage 2bc - mid-gut torsion complete but hypural element not yet present.

Stage 2c - hypural element present but fin rays not apparent.

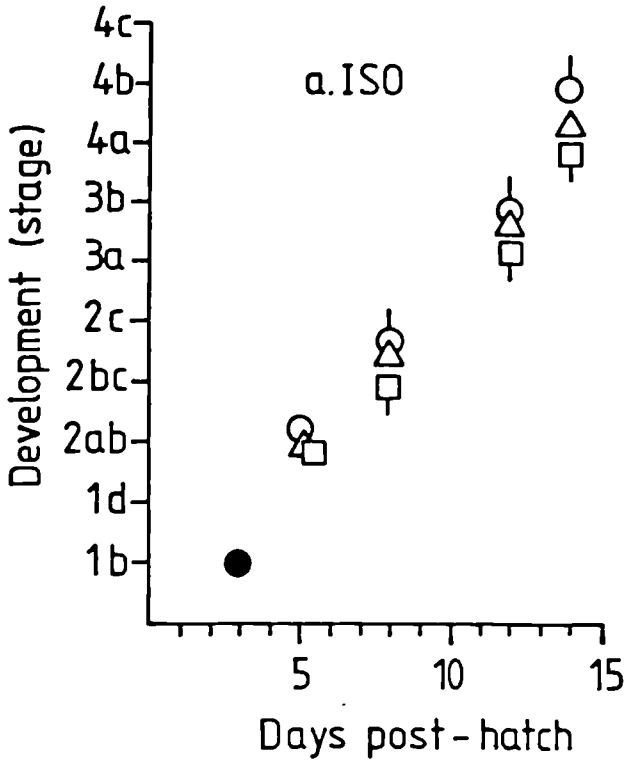
Development rates were estimated by transforming the combined staging system to a numerical one as follows:

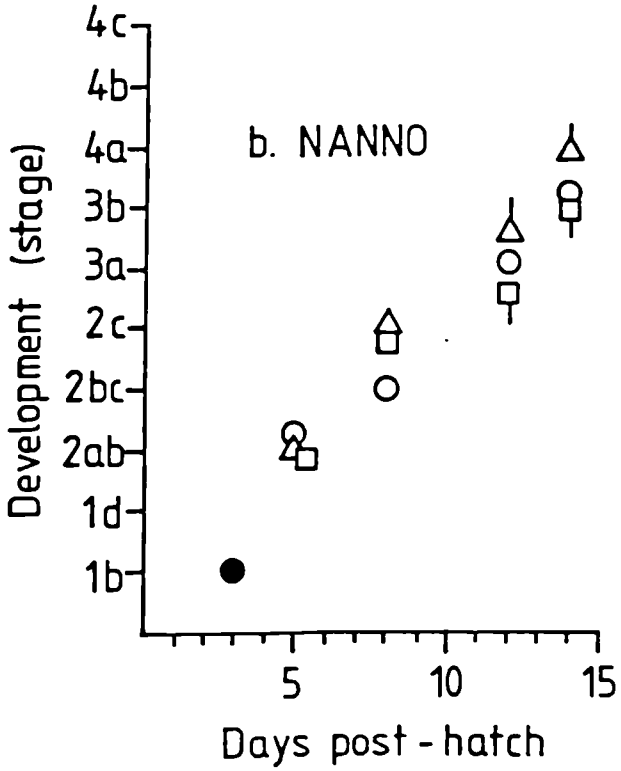
Stage 1d-1, 2ab-2, 2bc-3, 2c-4, 3a-5, 3b-6, 4a-7, 4b-8, 4c-9. Figs 24a,b,c present the average development level of larvae from the different treatment on the various sampling days. Larvae fed yeast-enriched rotifers attained development stage 2bc by day 8 and did not show any development thereafter.

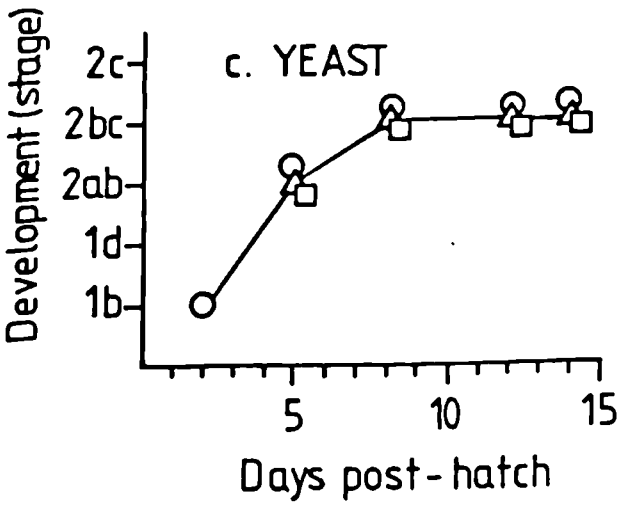
On the other hand larvae feeding on rotifers enriched with algae developed continuously, and at a constant rate throughout the experiment. There were no significant differences in the rate of development between the two algal treatments, which was 0.56 stages/day (ANCOVA, $F = 0.05$, d.f. = 1; 594).

Using the numerical conversion system it was also possible to regress log-transformed weights on development stage, which showed that for any given stage the average larva from the Isochrysis treatment had the same weight as

Fig. 24. The relation between development of turbot larvae using the staging system of Al-Maghazachi & Gibson (1984) and time (d). Larvae fed rotifers enriched with: a, Isochrysis (ISO), b, Nannochloropsis (NANNO) or c, baker's yeast (YEAST). Replicates in each treatment are presented by a different symbol. Lines where shown denote 95% confidence limits.


a. ISO
Development (stage)
4c
4b
4a
3b
3a
2c
2bc
2ab
1d
1b
5
10
15
Days post-hatch


b. NANNO
Development (stage)
4c
4b
4a
3b
3a
2c
2bc
2ab
1d
1b
5
10
15
Days post-hatch


c. YEAST
Development (stage)
2c
2bc
2ab
1d
1b
5
10
15
Days post-hatch

Nannochloropsis larvae (ANCOVA, F = 0.51, d.f. = 1; 594). The pooled regression for all algal treatments was:

log W = 0.19 st + 1.38 (W, µg; st, stage), r = 0.97; p < 0.001

Development of a functional swimbladder (filled with gas) was compared between treatments on day 12 post-hatch (Table 16) showing 20-30% inflation in yeast, compared with 60-80% in algal, treatments.

Results were also analyzed for effects of growth rates (in terms of both SGR and mm/d) on the mortality rates (% dying/d) showing (Table 17, Fig. 25) that mortality decreased linearly with increasing growth rates (F test, p < 0.001).

B. Ingestion Rates

1. Herring

Growth of herring larvae in duplicate 100 l tanks up to day 21 post-hatch is summarized in Table 22. Growth rates in total length (mm/d) were higher in duplicate b (0.41 mm/d) compared with duplicate a (0.31 mm/d). This was true also for log-transformed weights. The relation of log dry weight to log length (TL) (pooled regression for tanks a and b) was:

log W = 3.26 log TL - 1.25 (r = 0.97, p < 0.001)

Table 22. Regression length (TL,mm), and dry weight (W,µg) on time for two tank populations of herring larvae. Larvae were fed rotifers enriched with Isochrysis and used for ingestion and conversion efficiency experiment.

Duplicate	Regression	Parameters	
		r	p
a	TL=0.31t+8.72	0.83	< 0.001
	logW=0.033t+1.91	0.79	< 0.001
b	TL=0.41t+7.81	0.9	< 0.001
	logW=0.037t+1.92	0.77	< 0.001

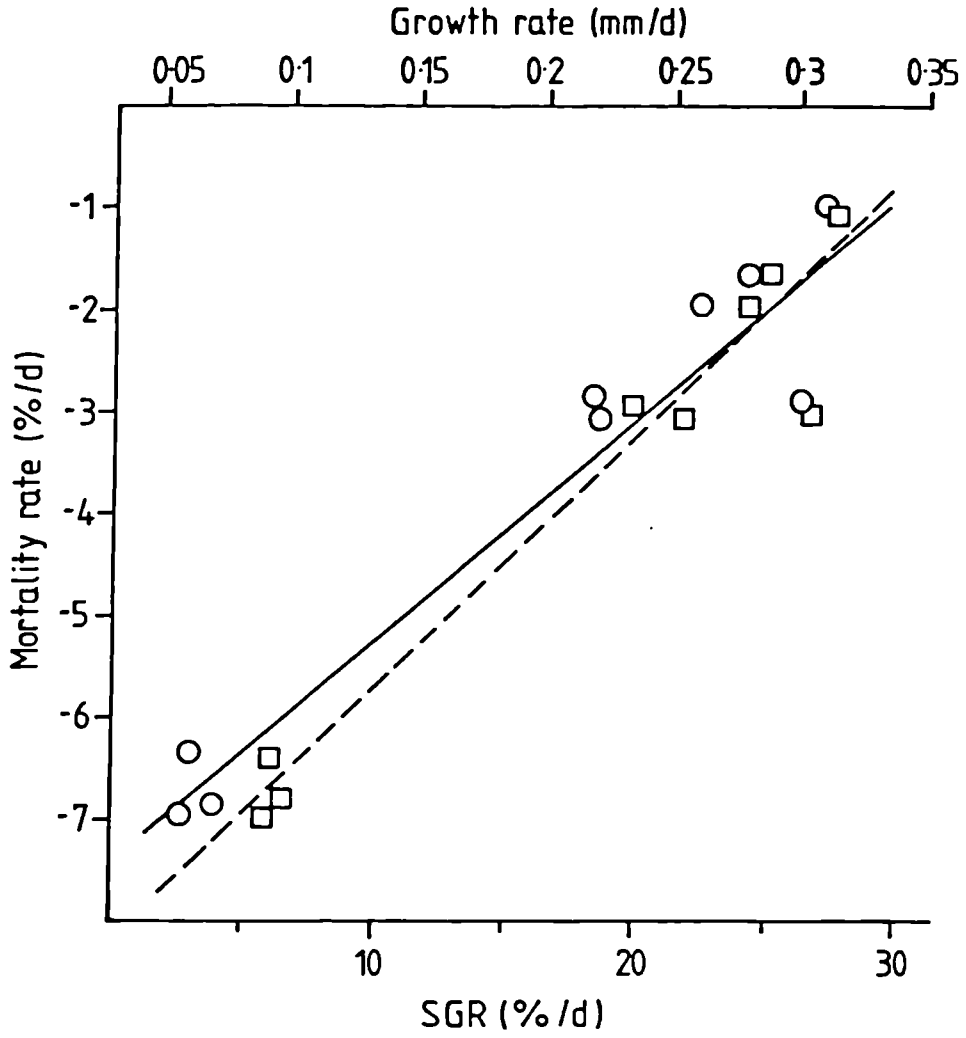


Fig. 25. The relation of mortality rates and specific growth rate for turbot larvae.

For growth in mm/d —○—

$y=21.6x-8.01$ ($r=0.96$; $p < 0.001$)

For growth in %/d (of dry weight) —□—

$y=0.245x-8.2$ ($r=0.97$; $p < 0.001$).

Gut contents analysis (Fig. 26) showed that the number and weight of rotifers found in the larval guts increased rapidly between days 7-10, from an average of 32 to 95 rotifers/larvae. After day 12 these numbers fell to around 55 rotifers/larvae by day 18. Using estimates of larval dry weights obtained by converting their measured lengths (TL) with the log/log transformation and the average dry weight of a rotifer used in these experiments, 0.17 µg, it was possible to estimate gut contents as the percent of larval dry weight. This ration tended to increase from about 3% on day 7 to 8% on day 12 and decrease thereafter to 2.2% on the final sampling day (21) (Fig. 27).

Larvae did not seem to suffer from any adverse effects due to transfer from the 100 l holding tanks to the 2 l experimental beakers. At the end of each 24 hr feeding trial all of the larvae in the beakers were alive and active. Food consumption was very high during the first two feeding trials (days 10 and 13 post-hatch) with an average 90% of the initial number of rotifers consumed in all beakers over the 24 h period. However, for the last two trials (days 18 and 21 post-hatch) this value fell down to 57%.

The quantities of food, both in numbers and in weight of rotifers, consumed daily by herring larvae, tended to increase between days 10 and 21 post-hatch (Table 23), while the daily rations (food consumed daily as percent of body weight) declined from a maximal 46%/d on day 10 to a minimal 18.8%/d on day 21 (Table 23), with an average value over the whole experiment of 30.6 $\pm$ 7.7 (% $\pm$ S.D.)/d. Ingestion (I = µg/d) was llinearly related to larval dry weight

Table 23. Specific growth rates (SGR), the number (n) and dry weight (Wr) of rotifers consumed daily, daily rations (% body dry weight consumed) and gross conversion efficiencies (K1) of herring larvae. Larvae fed rotifers enriched with Isochrysis.

Age	Tank	Larvae	SGR[1]	Ingestion		Ration	K1[2]
days		W(µg)	(%/d)	n/d	Wr(µg)	%bwt/d	(%)
10	a	172.20	9.44	450.70	79.99	46.45	20.39
		172.20	9.44	426.15	75.43	43.80	21.56
10	b	201.66	7.95	420.10	74.36	36.87	21.57
		201.66	7.95	416.19	73.67	36.53	21.77
13	a	228.60	6.92	376.00	66.55	29.11	23.77
		228.60	6.92	384.00	67.97	29.73	23.28
13	b	256.00	8.50	493.00	87.26	34.09	24.95
		256.00	8.50	380.00	67.26	26.27	32.37
18	a	323.11	11.31	552.00	97.70	30.24	37.40
		323.11	11.31	626.00	110.80	34.30	32.97
18	b	391.66	6.81	635.00	112.40	28.70	23.73
		391.66	6.81	474.00	83.90	21.42	31.79
21	a	453.00	11.31	677.00	119.83	26.45	42.76
		453.00	11.31	474.00	83.90	18.52	61.08
21	b	480.00	6.81	665.00	117.71	24.52	27.76
		480.00	6.81	585.00	103.55	21.57	31.56

(1) The SGR on any sampling day was estimated from the weight gained up to the following sampling day. SGR values of day 18 were also assigned to day 21.

(2) $K1 = (G/R) \times 100 = W \times SGR/Wr$ (Brett and Groves, 1979).

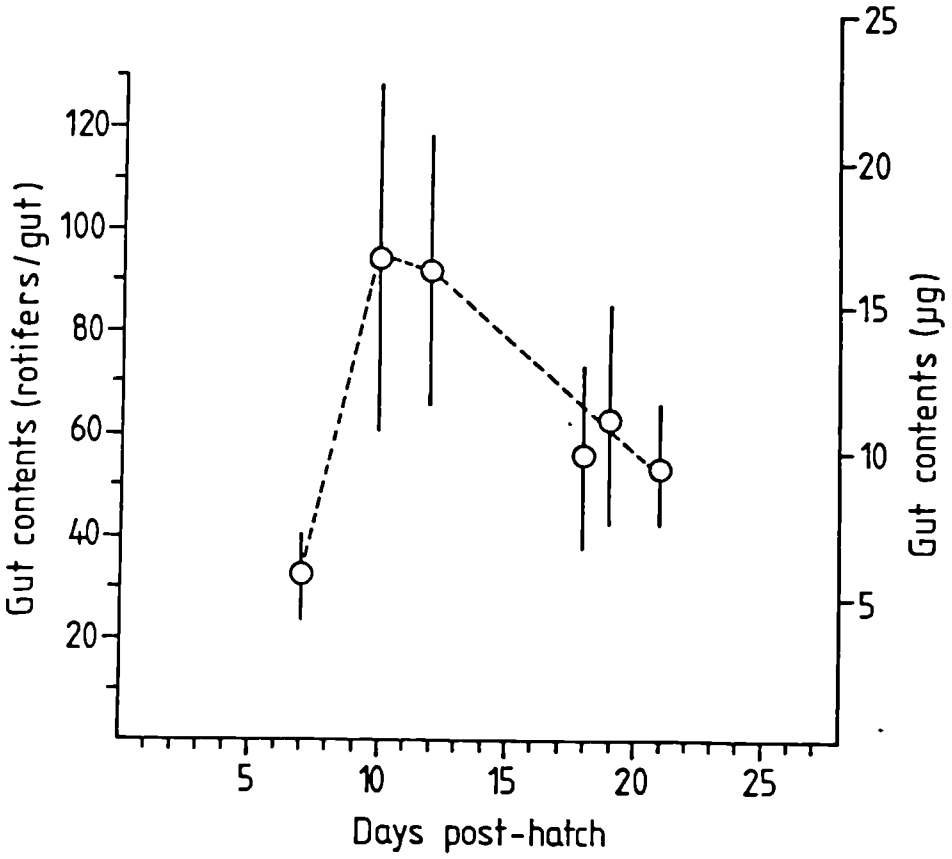


Fig. 26. The relationship between the gut contents in numbers of rotifers/gut and their dry weight/gut (1 rotifer = 0.17 µg) to time (d) of developing herring larvae.

(W = µg) (Fig. 28) and the linear relation between these two parameters was:

$$I = 0.13W + 49.67 \ (r = 0.79 \ \ p < 0.001)$$

From the equation it transpires that for a given larval weight, specific ingestion was 0.13/d. Furthermore, the regression constant has a progressively larger influence on the daily ration as larval weight decreases, which is the reason for the decline noted in the daily ration between day 10 and day 20.

The efficiency of conversion of the daily ingested food, K_1 (Table 23) ranged from 20-61% with an average value of 29.1 $\pm$ 10.9 (% $\pm$ S.D.) and tended to increase from 21% at day 10 post-hatch to 41% on day 21. Again this increase in K_1 was due to the relatively larger ingestion constant (49.67) for younger, compared with older, larvae.

2. Turbot

Growth regressions for turbot larvae in the 100 l rearing tanks throughout the feeding experiments are summarized in Table 24. Growth rates in length (TL) and log dry weights (µg) were higher in duplicate a than b. The relation of log weight to log length (pooled values for both tanks) was:

$$\log W = 4.26 * \log TL - 0.88 \ (r = 0.99, \ p < 0.001)$$

Gut contents analysis showed an exponential increase in the number (N) of rotifers found in larval guts in relation to larval length (TL). This was best described by the equation:

Table 24. Regressions for daily growth in length (TL,mm), and dry weight (W,µg) of turbot larvae in duplicate tanks. Larvae were fed rotifers enriched with Isochrysis and used for ingestion and conversion efficiency experiments.

Duplicate	Regression		Parameters	
			r	p
a	TL	= 0.28t + 2.9	0.95	< 0.001
a	log W	= 0.005t + 1.38	0.93	< 0.001
b	TL	= 0.24t + 3.02	0.94	< 0.001
b	log W	= 0.78t + 1.39	0.93	< 0.001

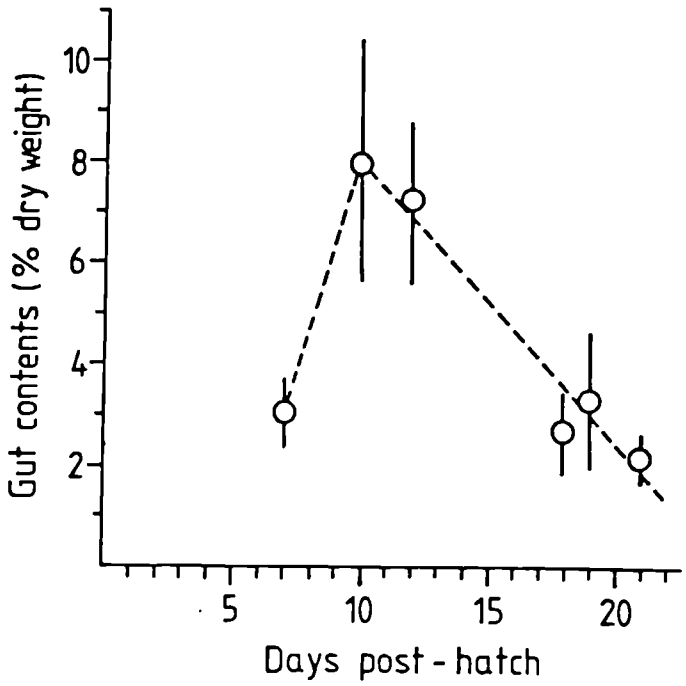


Fig. 27. The relationship of gut contents as percent dry weight and age (d) of developing herring larvae.

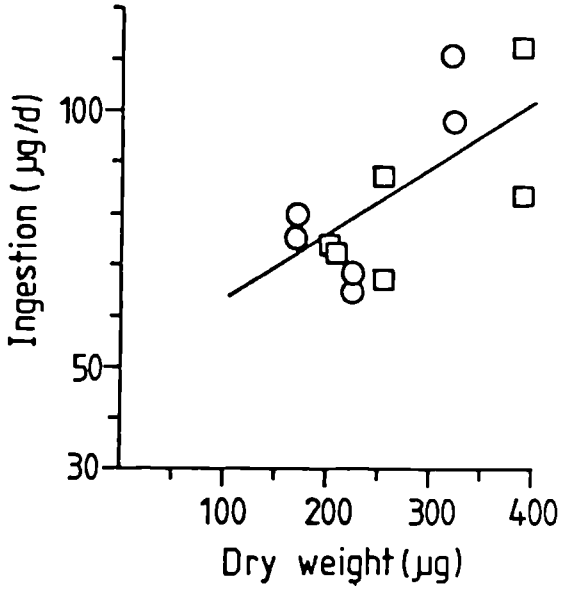


Fig. 28. Relation of ingestion to dry weight of herring larvae fed rotifers. Estimates for 24 h feeding. $y=0.13x+49.67$ ($r=0.79$, $p < 0.001$)

Replicate a, O
Replicate b, □

Table 25. Specific growth rates (SGR), the number (n) and dry weight (Wr) of rotifers consumed daily, daily rations (% body dry weight consumed) and gross conversion efficiencies (K1) of turbot larvae. Larvae fed rotifers enriched with Isochrysis.

Age	Tank	Larvae	SGR[1]	Ingestion		Ration	K1[2]
days		W(µg)	(%/d)	n/d	Wr(µg)	%bwt/d	(%)
3	a	37.50	17.86	257.14	43.71	116.57	15.32
5	a	53.60	38.67	258.02	43.86	81.83	47.25
5	a	53.60	38.67	241.62	41.07	76.63	50.46
5	b	48.37	37.66	451.59	76.77	158.72	23.73
5	b	48.37	37.66	481.35	81.83	169.17	22.26
8	a	171.00	14.16	532.00	90.44	52.89	26.78
8	a	171.00	14.16	346.25	58.86	34.42	41.15
8	b	149.70	12.18	516.67	87.83	58.67	20.76
8	b	149.70	12.18	800.00	136.00	90.85	13.41
10	a	227.00	13.85	594.15	101.01	44.50	31.12
10	a	227.00	14.19	886.44	150.69	66.39	21.37
10	b	191.00	14.23	1215.61	206.65	108.20	13.15
10	b	191.00	15.16	579.65	98.54	51.59	29.39
17	a	598.48[3]	13.85	1247.92	212.15	35.45	39.07
17	a	612.92[3]	14.19	1270.00	215.90	35.23	40.28
17	b	517.01[3]	14.23	1926.67	327.53	63.35	22.45
17	b	552.09[3]	15.16	2450.00	416.50	75.44	20.10

(1) The SGR on any sampling day was estimated from the weight gained up to the following sampling day. SGR values of day 10 were also assigned to day 17.

(2) K1=(G/R) x 100 = Wx SGR/Wr (Brett and Groves, 1979).

(3) Direct measurements on larvae from the experimental beakers after the 24 h feeding period.

$$\log N = 4.32 \log TL - 1.07 \ (r = 0.93, p < 0.001)$$

By converting larval lengths to dry weights using the log/log transformation, and rotifer numbers to dry weights using 1 rotifer = 0.17 µg as found experimentally, it was possible to describe the increase in dry weight of gut contents (GC, µg) as a linear function of larval dry weight (W):

$$GC = 0.13W - 2.28 \ (r = 0.93, p < 0.001)$$

The relation of gut contents in dry weight to larval dry weight is presented in Fig. 29.

Throughout the feeding trials an average of 50 ± 15 (% ± S.D.) of larvae stocked into the 2 l beakers survived and were active at the end of each 24 h feeding period (range (10-75%). The average number of rotifers consumed in the beakers was 50.5 ± 23 (% ± S.D.) with a range of 4-82%.

Estimates of ingestion for the individual trials are summarized in Table 25. The quantities of food consumed daily by the larvae increased from 257 rotifers (43 µg) to a maximal 2450 rotifers (416 µg) between days 3 and 17 post-hatch respectively. The daily ration estimates food consumed daily as percent of body weight tended to decline between the initial and final sampling days, averaging 77.9 ± 40 (% ± S.D.)/d. Ingestion (I = µg/d) was lilnearly related to larval weight (W = µg) (Fig. 30) and the relation between these two parameters was:

$$I = 0.43W + 38.45 \ (r = 0.83; \ p < 0.001)$$

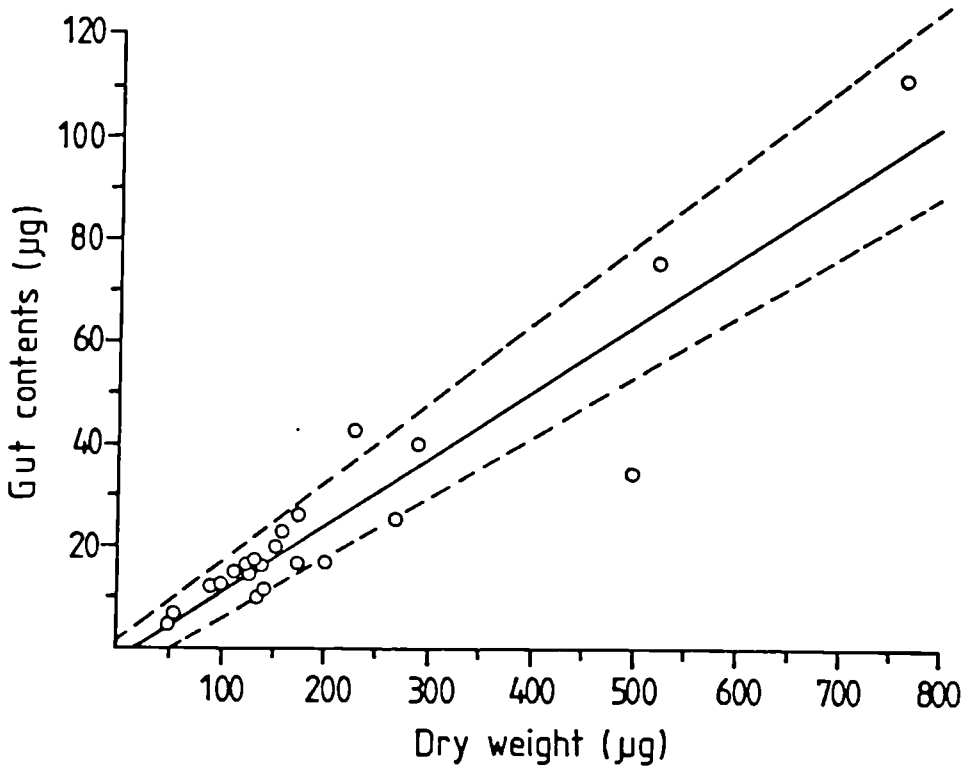


Fig. 29. Gut contents of turbot larvae in dry weight of rotifers relative to larval dry weight. Broken lines denote 95% confidence limits.

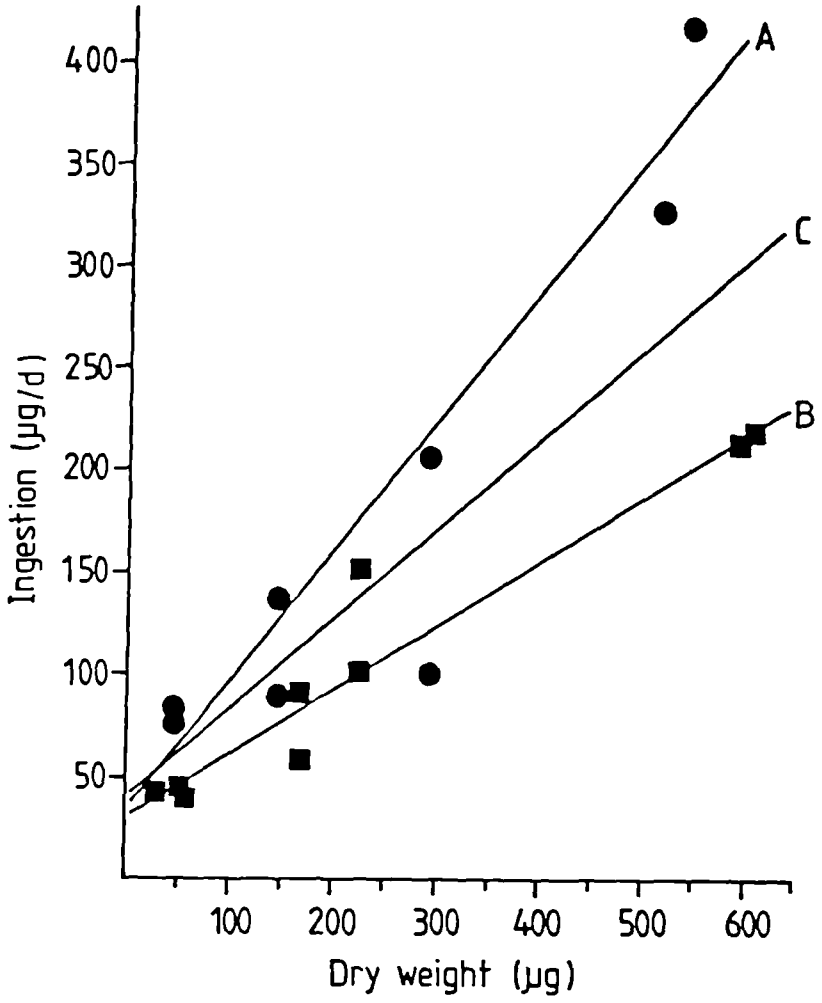


Fig. 30. Ingestion estimates, in dry weights, for turbot larvae feeding on rotifers.

For replicate A, y=0.63x+34.1, (r=0.95; $p < 0.001$) ●

For replicate B, y=0.31x+30.7, (r=0.96; $p < 0.001$) ■

C, pooled regression y=0.43x+38.45 (r=0.83; $p < .001$)

From the equation it may be shown that for a given larval weight specific ingestion was 0.43/d. As in the case of the herring larvae the regression constant had a greater effect on daily ration estimates for younger (lighter) compared with older (heavier) larvae.

Conversion efficiencies (K_1) (Table 25) were not found to fluctuate much throughout the experiment and were in the range of 15-50%, with an average ($\pm$ S.D.) of 28.5 $\pm$ 11.9%. Larvae from duplicate a exhibited higher K_1 values than those of duplicate b. This resulted from their higher daily growth rates and was particularly apparent for the very small larvae ($\sim$ 50 µg) and on the last experimental day when larvae from this duplicate weighed about 600 µg.

C. Rotifer biochemical composition

Dry weight values of rotifers corrected for female/egg ratios are presented in Table 26, column 3. Values for rotifers enriched with Nannochloropsis can be directly related to those of rotifers fed yeast as the samples were derived from the same initial population (see methods, H.1). The weight of Nannochloropsis-enriched rotifers, after the initial 24 h enrichment, was similar to that of yeast fed rotifers, 319 ng compared with 315 ng respectively. However, a further 24 h enrichment at 20°C in Nannochloropsis had a marked effect on the rotifer's dry weight which, after correction for the *egg/female ratio*, showed a 16% increase compared with its initial value. On the other hand, yeast-enriched rotifers, which were deprived of food lost about 27% of their dry

Table 26. Dry weights and calorific values of rotifers grown on baker's yeast and then enriched (a) for 24 h at 20°C with either Isochrysis (Iso) or Nannochloropsis (Nanno). Values are also shown following a second 24 h enrichment period at either (b) 20°C or (c) 10°C. (d) and (e) are yeast grown rotifers after 24 h starvation at 20°C or 10°C respectively.

Enrichment	Treatment	W ng	cal/g	cal/individual
Nanno	a	319	7301	.0023
	b	371	7539	.0028
	c	361	6197	.0022
Iso	a	270	4534	.0012
	b	332	5151	.0017
	c	315	6524	.0021
Yeast	a	316	6617	.0021
	d	204	6936	.0014
	e	230	7347	.0017

All values corrected for the presence of eggs.

Calorific values corrected for ash content estimated as 7.8% (Theilacker & Kimball, 1984).

weight after the second 24 h enrichment period at 20°C. Isochrysis-enriched rotifers also exhibited a marked increase in their dry weight during the second enrichment period at 20°C, their average weight increasing from 269 ng to 332 ng respectively.

Weight increase of algal-enriched rotifers at 10°C were not as high as that of rotifers at 20°C. Conversely, weight loss of yeast-enriched rotifers at 10°C was not as pronounced as at 20°C.

Ash and egg-free calorific values per g rotifer are presented in column 4 of Table 26 and the calorific values for individual rotifers in column 5. Isochrysis rotifers which were prepared at a date later than Nannochloropsis and yeast treatments had values somewhat lower than the other two treatments. The average value per g rotifers for unstarved treatments was 6460 $\pm$ 1025 cal/g and $(1.95 \pm 0.5) \times 10^{-3}$ cal/individual. There were no obvious changes in rotifer calorific values in relation to the type of enrichment or its duration.

Total carbon and percent protein were not markedly affected by either the type of enrichment or the enriching temperature and duration (Table 27). Average carbon content was 41.5 $\pm$ 1.95 and protein was 53.2 $\pm$ 2.3 (% $\pm$ S.D.). Average total lipid for all samples was 10 $\pm$ 2.1 (% $\pm$ S.D.). Percent total lipid showed a slight tendency to increase in algal enrichments after the second 24 h enrichment period, in particular at 10°C, where lipid values increased from around 9% to 13% of the rotifer's dry weight. Carbohydrate was calculated as the difference from 100% of the sum of lipid

Table 27. Proximate composition (% of dry weight) of rotifers grown on baker's yeast and then enriched (a) for 24 h at 20°C with either Isochrysis (Iso) or Nannochloropsis (Nanno). Values are also shown following a second 24 h enrichment period at either (b) 20°C or (c) 10°C. (d) and (e) are yeast grown rotifers after 24 h starvation at 20°C or 10°C respectively.

Enrichment	Treatment	Carbon	Protein	Lipid	Carbohydrate
		%	%	%	%
Nanno	a	41.5	52.3	8.7	31.2
	b	42.8	52.6	10.05	29.6
	c	43.7	50.0	13.5	28.7
Iso	a	42.5	54.3	9.6	28.3
	b	44.8	52.1	10.52	29.6
	c	42.03	49.7	12.39	30.1
Yeast	a	41.5	54.1	9.31	28.8
	d	38.0	53.1	6.3	32.8
	e	41.0	57.2	9.01	26.0

All values corrected for the presence of eggs.

Carbohydrates evaluated by subtraction from 100% using an ash content estimate of 7.8% (Theilacker & Kimball, 1984).

protein and ash, using an ash content estimate of 7.8% (Theilacker and Kimball, 1984). The average carbohydrate content of the rotifers was estimated at 29.6 ± 1.9%).

The absolute carbon, protein and lipid values per individual rotifer (Table 28) tended to increase with prolonged enrichments in the algal treatments, or decrease with starvation in yeast treatments, in accordance with the changes in rotifer dry weight. As an example, following a second 24 h period at 20°C, lipids increased from 26 to 36 ng/rotifer in the Isochrysis enrichments and decreased from 31 to 21 ng/rotifer in the starved rotifers.

There were no apparent differences in the amino acid composition of rotifers from the three treatments (Table 29).

Fatty acid profiles for the different treatments are summarized in Table 30. Rotifers grown on yeast were characterized by a high percentage of saturated and monoethylenic fatty acids of 16, 18 and 20 carbons. Only 2-5% of unsaturation was polyethylenic and in two of the replicates trace amounts of 22 : 6n-3 were found. In contrast, rotifers enriched with algae had high levels of polyethylenic acids (20-30%) as well as higher levels of saturated acids (37-51%). Of the polyethylenic acids 7-15% were of the n-3 series. Nannochloropsis-enriched rotifers were rich in 16 : 1 monoethylenic acids and Isochrysis-enriched rotifers in 18 : 1 and 24 : 1 acids. Of the polyunsaturated acids, Isochrysis rotifers were high in 18 : 2, 18 : 3 and 18 : 4 acids as well as containing 2-4% of 22 : 6n-3, while Nannochloropsis rotifers had small amounts of 18 : 2, no 22 : 6n-3 high amounts of 20 : 4 n-6 and very high amounts of 20 : 5n-3 (11-13%) acids.

Table 28. Proximate composition (absolute values in ng) of rotifers grown on baker's yeast and then enriched (a) for 24 h at 20°C with either Isochrysis (Iso) or Nannochloropsis (Nanno). Values are also shown following a second 24 h enrichment period at either (b) 20°C or (c) 10°C. (d) and (e) are yeast grown rotifers after 24 h starvation at 20°C or 10°C respectively.

Enrichment	Treatment	Carbon	Protein	Lipid
		ng	ng	ng
Nanno	a	136.92	172.62	28.74
	b	167.7	206.04	39.40
	c	162.49	186.00	50.22
Iso	a	116.02	148.18	26.21
	b	155.9	181.27	36.61
	c	137.03	162.45	40.52
Yeast	a	139.82	182.18	31.37
	d	78.66	109.96	13.04
	e	99.1	138.38	21.80

All values corrected for the presence of eggs.

Table 29. Amino acid composition of rotifers (in g/100g crude protein) following a 24 h enrichment at 20°C with Isochrysis (Iso), Nannochloropsis (Nanno) or baker's yeast.

Amino Acid	Nanno	Iso	Yeast
Aspartic acid	11.24	11.28	11.46
Threonine	4.58	4.46	4.63
Serine	5.36	5.26	5.43
Glutamic acid	13.58	13.19	13.95
Proline	5.83	5.06	5.32
Glycine	4.21	4.15	4.13
Alanine	5.24	5.17	4.97
Valine	6.12	6.31	5.92
Methionine	2.25	2.58	2.23
Isoleucine	4.60	5.03	4.78
Leucine	8.26	8.21	8.39
Tryosine	4.37	4.94	4.53
Phenylalanine	6.49	6.63	6.84
Histidine	1.95	1.92	2.12
Lysine	7.94	8.13	8.47
Arginine	7.97	7.66	6.83

Table 30. Fatty acid profiles of rotifers grown on baker's yeast and then enriched with either Isochrysis, Nannochloropsis or baker's yeast at 20°C for 24 h.

	Yeast			Nannochloropsis			Isochrysis		
FA	a	d	e	a*	b	c	a	b	c
14:0	1.42	1.85	1.97		5.25	5.47	12.88	13.4	15.2
15:0	0.43	0.66	0.52		0.41	0.46	0.45	0.26	0.41
16:0	9.51	11.3	9.75		16.6	19.3	14.9	10.4	9.82
16:1n-7	27.7	29.0	29.2		31.5	34.6	12.9	7.09	8.1
16:1n-5							0.3		
16:2							0.58	1.16	0.62
16:3	0.96	0.93	0.94		0.23	0.3	0.5	0.13	0.47
16:4	0.64	0.78	0.65		0.25	0.26	0.17	0.13	0.3
17:0	0.51	0.24			0.24		0.27		trace
18:0	5.81	5.77	5.23		2.25	2.49	3.57	2.2	2.12
18:1n-9	25.5	24.1	25.5		6.18	8.89	22.2	14.4	16.3
18:1n-7	10.6	10.5	9.64		5.32	5.27	5.78	4.9	3.73
18:2n-6	0.48	2.74	2.34		1.79	1.51	3.84	3.91	3.53
18:3n-6	trace				0.19	trace		trace	
18:3n-3	trace	trace			0.08	trace	3.54	3.85	3.73
18:4n-3						trace	4.0	5.09	5.28
18:5n-3									
20:0	0.1	trace	0.09		trace	trace	0.22	0.14	0.34
20:1n-9	4.61	4.39	4.26		1.63	1.79	3.67	2.57	2.53
20:1n-7	1.15	0.82	1.16		0.44	0.39	0.68	0.18	0.42
20:4n-6	0.23	0.09			3.64	3.27	trace		trace
20:4n-3					trace		1.32	2.13	1.86
20:5n-3					13.53	11.1	0.32	0.3	0.42
22:0									
22:1	1.03	0.35	0.85		0.36	0.32	0.08	0.28	0.54
22:4							0.08	trace	1.1
22:5n-3	trace				1.3	0.82			
22:6n-3	trace	0.17					1.97	4.05	3.37
24:0							0.17		
24:1n-9							1.24	19.4	10.7

a: enriched for 24 h at 20°C
a*: sample lost
b: a second 24 h enrichment at 20°C
c: a second 24 h enrichment at 10°C
d: Rotifers deprived of food for 24 h at 20°C
e: Rotifers deprived of food for 24 h at 10°C

The changes in fatty acid profiles in relation to duration of enrichment and the enrichment temperature can be seen in the Isochrysis treatment, e.g. the 16 : 1n-7 which is high in yeast-grown rotifers decreases from ~ 28% to 13% after transferring rotifers to Isochrysis for 24 h and to 7% after a further 24 h at 20°C. Likewise, 22 : 6n-3 of which only trace amounts were detected in yeast-enriched rotifers, increased to 1.97% after 24 h in Isochrysis at 20°C and to either 3.4% or 4.05% after a further 24 h at 10°C or 20°C respectively.

DISCUSSION

A. The diets

The two rotifer types, L and S, used throughout the project had characteristic widths of 151 ± 9.8 and 113 ± 6.7 (μm ± s.d.) respectively. Their respective mean dry weights, calculated according to the relation of dry weights of B. plicatilis to its width (Theilacker & Kimball, 1984):

$y = 1.4 \times 10^{-5} X^2$; (y = dry weight, μg; X = width, μm)

are 323 ng and 178 ng. These values relate exceptionally well to the dry weights experimentally determined here, which for unstarved L-type rotifers was 326 ± 33 ng and for S-type (see results section B.1) was 179 ± 15 ng. The dry weight of the S-type rotifer is identical to that employed by Tandler & Mason (1984) and Doohan (1973). Scott & Baynes (1978) reported a range of 113-650 ng for the rotifer, depending on the feeding regime. However, in improved nutritional conditions rotifers tend to increase their reproduction rates, and with it the ratio of eggs to females. The dry weight estimates of Scott & Baynes, mentioned above, did not bring this factor into consideration. As such they are only comparable to the dry weights of L-type rotifers, used in the present study, at equivalent egg/female ratios of 0.17-0.2 which they estimated at 306-340 ng.

Since rotifer eggs are surrounded by a shell containing chitin (Wurdak et al., 1978), which is most likely indigestible to fish larvae, it is essential, when using rotifers for estimates of larval nutrition, to correct for egg/female ratios. The presence of undigested rotifer eggs in the guts of the different species of larvae employed in this study corroborates this contention.

Rotifer dry weights can fluctuate by up to 30% depending on feeding regimes such as starvation or excess feeding (Scott & Baynes, 1978; Leimeroth, 1980). This does not imply a concomitant change in the size of the animal as the lorica in which it is enveloped is a rigid structure. Such effects were observed in the present experiments where rotifers from the mass culture lost 33% of their dry weight when deprived of food for 24 h at 20°C, or gained 12-23% dry weight following prolonged enrichment in the presence of algae. Interestingly, it was found that in the present experiments, proximate composition was relatively unaffected by the duration and temperatures of enrichment or food deprivation. Since fish larvae consume food in relation to availability (Boehlert & Yoklavich, 1984; Werner & Blaxter, 1981) fluctuations in the rotifer's dry weight will affect the daily ration of the larvae. This could be very significant in a mariculture facility where rotifers are introduced into the rearing systems at fixed levels. This point, which has also been stressed by Scott and Baynes (1978), is an important reason for using "green-water" in fish larval culture units since the presence of algae in the larval tanks ensures that the prey offered will have a continually high nutritive value.

Average ash-free calorific value for unstarved L-type rotifers was 6.46 cal/mg or 1.95×10^{-3} cal/individual. These values are higher than the 4.8 cal/mg reported by Theilacker & Kimball (1984) and the 5.3 cal/mg reported by Theilacker & McMaster (1971). Such differences could have arisen if the rotifer's ash content estimate employed in the present study was lower than its value in the literature (see Methods, H.3). The discrepancy can also be resolved if the calorific values of individual rotifers are calculated on the basis of their proximate composition. By using conversion factors

of Platt & Irwin (1973), where the protein, carbohydrate and lipid equal 5.65, 4.1 and 9.45 cal/mg respectively, it transpires that the average ash free calorific value of a single L-type rotifer is 1.79×10^{-3} cal/individual which agrees well with the 1.8×10^{-3} cal/individual for the same width class given by Theilacker & Kimball (1984). These authors estimated calorific value of S-type rotifers, as 0.97×10^{-3} cal. This value can be used to convert data into calorific terms in the present study whenever S-type rotifers were used.

Proximate analysis of rotifers suggests that diet has no effect on their basic chemical composition (Scott & Baynes, 1978; Watanabe et al., 1983b; Dendrinos & Thorpe, 1987). A recent publication showing wide fluctuations as a result of changes in diet (Ben-Amotz et al., 1987) most likely suffers from an artefact introduced by not rinsing salts, present in the growth medium, from the samples before drying them. The present results are in accordance with the first findings. Quantitatively they are very similar to those of Scott & Baynes (1978) with protein in the range of 50-58% and lipid as 9-16% of dry weight. They differ from results summarised by Watanabe et al. (1983a) and those of Dendrinos & Thorpe (1987) where protein and lipids were 65% and 23.5% respectively in the former and 33-42% and 14-28% in the latter. Protein requirements of adult fish are 40-60% of diet dry weight (Castell et al., 1986) and although direct comparisons between adults and larvae should be made with caution, it would seem that rotifers meet this protein requirement.

Amino acid profiles of rotifers enriched with algae or yeast were similar to each other and to other profiles reported in the literature (Watanabe et al., 1983b; Dendrinos & Thorpe, 1987). The

amino acid content of phytoplankton and consequently of zooplankton is generally regarded to be high in essential/non-essential acids for both bivalves (Epifano, 1979) and finfish (Castell et al., 1986). One can expect that they will also meet the requirements of fish larvae. However, it is also quite obvious that any differential growth patterns observed in the different larval species when fed the different diets, can not be attributed to the amino acid profiles of the diets.

Lipids are required in finfish diets both as an energy and an essential fatty acid (EFA) source. The total lipid content of the rotifers used here, 10-13%, was somewhat lower than values published by Watanabe et al. (1983b) and Dendrinos & Thorpe (1987) which were 23.5% and 14-28% respectively. The observations on rotifer lipid content in this work also suggest that, to a limited extent, lipid levels were altered in accordance with the duration and temperature of enrichment. In adult fish the ration of dietary protein invested in growth can be enhanced, to a limited extent, by increasing the lipid content of the diets (Cowey, 1979). Although it is questionable whether a similar mechanism operates in fish larvae it might be worthwhile pursuing this point. Enhancing the lipid content of the rotifer might be achieved by enriching it with algae at low temperatures (10°C, as shown in the present study) or raising them on marine algae with high lipid levels or lipid fortified yeasts such as those used in Japan (Watanabe et al., 1983a).

Of all the biochemical components analysed in this study the change in fatty acid (FA) profiles as a result of the different enrichments and their durations was the most remarkable. It is characteristic of rotifers to acquire an FA profile similar to that of their diet (Scott & Middleton, 1979; Dendrinos & Thorpe, 1987;

Watanabe et al., 1983a; Lubzens et al., 1985; Ben-Amotz et al., 1987). Furthermore the rotifer changes its FA profile within 6-24 h of having its diet changed from one food organism to another (Watanabe et al., 1979). This trend was clearly manifested in the present experiment where 24 h after being transferred from yeast to Isochrysis a major change had taken place in the rotifer's FA profile, with only minor changes taking place at longer enrichment periods. However, it should be noted that the percentage of 22:6n-3, a fatty acid considered as essential to the nutrition of marine fish, continued to increase during the second enrichment period in Isochrysis from 1.9% to 3.4% and up to 4.2%.

FA profiles of rotifers grown on baker's yeast had only trace amounts of n-3 acids. The characteristic FA profile of baker's yeast is devoid of any n-3 acids and of fatty acids with chain lengths of over 18 carbons (Lubzens et al., 1985). It is most likely therefore that the trace amounts of 18:3n-3, 22:6n-3 and of all the longer chain acids found in the rotifers resulted from de novo synthesis of these acids by the rotifer as reported by Lubzens et al. (1985).

Rotifers enriched with Isochrysis were rich in n-3 fatty acids (8-15.5%), and had both the essential fatty acids for marine fish, i.e. 18:3n-3 and 22:6n-3. This pattern is typical of rotifers enriched with this algae (Lubzens et al., 1985; Ben-Amotz et al., 1987). Scott & Middleton (1979) found an additional 3% of 20:5n-3 in rotifers raised on Isochrysis. Such variations can arise from different fatty acid profiles related to the origin of the algal strain employed (Helm & Laing, 1987), or due to contamination by other micro-organisms at the time of enrichment.

Enriching the rotifers with Nannochloropsis also raised the n-3 fatty acid levels to 12-15%. However, the situation with the fatty acids from the n-3 series was reversed, with little or no 18:3n-3 and 22:6n-3 in the rotifers and appreciable quantities of 20:5n-3 (11-13.5%). Although as yet there are no reports in the literature concerning the FA profile of N. oculata, a recent analysis of the algae (P. Hodgeson, pers. comm.) showed a similar profile with 20:5n-3 amounting to 18% of the total fatty acids. A congeneric species N. salina has been found to have a similar FA profile which manifested itself as 6.5% 20:5n-3 in the rotifer (Ben-Amotz et al., 1987). Witt et al. (1984) found that rotifers enriched with Nannochloris sp. had 21.4% of 20:5n-3, no 22:6n-3 and trace amounts of 18:3n-3 (percent of total fatty acids). This FA profile resembles that of Nannochloropsis enriched rotifers used in the present study. The genus Nannochloris has recently been reclassified as a member of the Eustigmatophyceae and the genus name changed to Nannochloropsis (Antia et al., 1975). Furthermore, as the FA profiles of algae seem to be characteristic of algal classes (Leftley, pers. comm.) it might very well be that Witt et al. (1984) have misclassified their algae.

In summary, it may be stated that the three rotifer diets employed here were unique only in their FA profiles, while being equal for all other biochemical components tested. Undoubtedly, however, other biochemical components within the rotifer would have been subject to the type of enrichment employed. These could be levels of vitamins such as E, B12 or C that are prevalent in plant matter and not in yeast, or hydrocarbons and trace elements present in the pigments of the individual algae employed.

B. The effects of the diets on growth of the larvae

The species of fish used in these experiments can be divided into two main categories according to their response to the three diets tested. The first comprised the herring and plaice which grew equally well on both of the algal enriched diets and the second, the turbot, which grew better on the Isochrysis compared with the Nannochloropsis-enriched rotifers. However, common to all three larval species was firstly, an asymptotic relation of growth to age and secondly, the arrest of ontogeny at an early developmental stage when fed the yeast-enriched rotifer diet.

The effect of the yeast-enriched rotifer diet could not be attributed to starvation. The experiments clearly demonstrated that considerable numbers of larvae, of all three species, offered these rotifers survived beyond the stage of mortality commonly associated with starvation.

Clyde herring larvae, of the same batch used in the 1986 experiment, have been shown to reach the PNR at day 12-13 post-hatch, 50% starvation mortality (t_{50}) at 16-17 d post-hatch and 100% mortality at day 22 (Yin & Blaxter, 1987). Identical values for t_{50} (16.2 d), irrespective of the diet offered to the larvae, were recorded in the present experiments. Since some larvae in all three treatments survived beyond the period of mortality associated with starvation it may be concluded that the treatment influenced the magnitude of the starvation mortality. Furthermore, on the final day of the experiment herring larvae fed on the yeast-enriched rotifer diet could not be classified as starving for two reasons. First, their dry weight had increased from 148 $\pm$ 15 µg on day 4 to 215 $\pm$ 10.2 µg on day 28 post-hatch. Second, there was a steady improvement in their condition factor, such that on day 28 the

average RCF of the surviving larvae was 0.15 (relative condition factor as defined by Ehrlich et al., 1976, employing their value of b=4.57) which is higher than the 0.11-0.13 reported by the above authors for starving specimens at PNR.

Turbot larvae did not exhibit any significant mortalities on days 6-8 post-hatch, the period associated with starvation mortality as estimated on a group of control larvae. Jones (1972) showed that at 17.5°C turbot reached their PNR at 7 days post-hatch and starvation mortality was 100% on day 9 post-hatch. Cousin et al. (1986), using histological criteria, showed that starved turbot larvae died on days 6-8 post-hatch. These authors have also shown that in larvae which start feeding, but die between days 8-14 post-hatch, the alimentary canal showed a continuing development with the coiling of the mid-gut and appearance of the swimbladder. The authors suggest that nutritional deficiencies probably cause the larvae to stop feeding and a degeneration of the alimentary canal and associated structures sets in. Similarly, in the present experiments, turbot fed yeast-enriched rotifers continued to grow beyond the yolk sac stage and up to day 8, at which time they had doubled their weight from 33.75 µg to 67.3 µg. They also exhibited the definitive ontogenetical development events mentioned before, i.e. coiling of the mid-gut and appearance of the swimbladder. However, the inflation of the swimbladder seems to have been affected by the diet since it was only inflated in 20-33% of the fish examined, compared with 70-80% inflation in those larvae receiving the algae-enriched rotifer diet. The development of these larvae terminated abruptly at the initiation of hypural element formation and the larvae ceased to thrive.

Plaice were very similar to turbot; they did not exhibit any initial starvation mortalities, which would be expected between days 12-16 post-hatch (Riley, 1966). Larvae fed the yeast-enriched rotifer diet developed a coiled mid-gut (plaice do not develop a swimbladder), and furthermore, in the 1986 experiment ontogeny ceased on day 14 and the larvae ceased to thrive.

Feeding by fish larvae is mediated by vision and can be modulated, amongst other factors, by prey characteristics such as colour and contrast with the background (Blaxter, 1980). As an example of this it has been shown that feeding intensity of Dover sole larvae can be enhanced by staining the prey, Artemia, black (Dendrinos et al., 1984). The colour of the prey in the present experiments was noticeably altered from a pale yellow in yeast to green after enrichment with Nannochloropsis or greenish brown with Isochrysis. It is likely that these changes in colour altered the contrast between the rotifer and the black background provided by the rearing tanks. Such changes might have influenced feeding intensities on different prey so leading to different growth rates. Other factors which might have influenced feeding intensities are olfactory and gustatory responses (Dempsey, 1978; Appelbaum et al., 1983; Iwai, 1980). These factors, however, did not seem to effect initial feeding success as ascertained in the turbot and plaice experiments, or feeding intensities as indicated by absence of any significant variation in gut content indexes during the first feeding days of the various species.

The most likely explanation for the stunted growth and heavy mortalities, experienced by the larvae feeding on yeast-enriched rotifers only, is that the diet was deficient in a nutritional component, namely the highly unsaturated fatty acids (HUFA) of the

n-3 series. Similar growth and mortality responses by marine fish larvae to an identical diet have previously been shown for red sea bream (Watanabe et al., 1983a), and black sea bream Mylio macrocephalus (Lee et al., 1981). The inadequacy of such rotifers was related to the deficiency of n-3 fatty acids (18:3n-3, 20:5n-3 and 22:6n-3) and the condition could be rectified by soaking the rotifers in emulsified marine lipids rich in HUFA before introducing them into the larval tanks (Watanabe et al., 1983). In the case mentioned above cuttlefish (squid) liver oil was used; other oils such as pollock or cod liver oils are, however, just as suitable.

The limited growth and development which did occur in the present experiments, when larvae were fed the yeast-enriched rotifer diet, can be explained on the basis of conservation and differential utilization of the n-3 fatty acids that had been incorporated into the larvae from the egg. This process of selective retention of HUFA during embryogenesis and early larval development has been demonstrated in herring (Tocher & Sargent, 1984; Tocher et al., 1985a,b) and may be of wide occurrence in marine fish larvae. It will most certainly prevent the loss of these essential body constituents during short food deprivation periods, such as those related to the availability and distribution of zooplankton in the sea. Furthermore, this capacity will enable the larvae to subsist or even grow for an interim period, when faced with a nutritional deprivation, in the anticipation of obtaining the suitable diet. The duration that a larva will withstand a dietary HUFA deprivation will most likely be related to its potential specific growth rate at the pertaining environmental conditions in the presence of a diet containing the full complement of fatty acids. Accordingly, herring larvae which had slow specific growth rates on

the HUFA rich diets (3-5%/d), in the present experiments,grew during the 23 days from first feeding at a steady 1.3-1.8%/d on the HUFA-deficient diet; plaice with higher specific growth rates (7.5%/d) grew at a slower 0.46%/d on the deficient diets. Turbot which exhibit very high specific growth rates (20-27%/d) grew at higher rates on the deficient diet, 6.6%/d, though only for a short duration (8 days) following which not only did growth cease but their weight declined.

The finding that all three larval species, when fed the yeast-enriched rotifer diet, ceased to develop at commencement of fin development is probably the result of the disruption of metabolic processes in the larvae rather than absence of particular nutrients required for fin formation in the diet.

Turbot larvae have been shown to have a dietary requirement for both 20:5n-3 and 22:6n-3 HUFA (Scott & Middleton, 1979). Witt et al. (1984) have further suggested a preferential requirement of 22:6n-3 over 20:5n-3 by these larvae. The growth results obtained for turbot larvae in the present experiments are in agreement with the above reports. Namely, larvae fed rotifers enriched with algae grew better due to the input of HUFA into the diet. Furthermore, the higher growth rates recorded when I. galbana was used for enriching the rotifers, was a direct result of their 22:6n-3 content, compared with its absence from N. oculata which contains only 20:5n-3. Although the present results demonstrate unequivocally preferential requirement for 22:6n-3 compared with 20:5n-3, they also show that turbot can attain a high degree of development even in the absence of the former fatty acid. Superficial observations also did not show any long term effects of the lack in 20:5n-3 during the initial feeding stages. Once these larvae were transferred to an

Artemia nauplii diet at least 25% of the larvae, irrespective of the initial treatment, metamorphosed.

According to the literature, and results presented here, there do not seem to be any qualitative differences between dietary EFA requirements of larvae, juveniles or adult turbot. Growth of adult turbot is better on a diet containing lipids of marine origin rich in n-3 HUFA, than on n-6 HUFA from corn oil or saturated fats from coconut oil (Cowey et al., 1976). This requirement for n-3 HUFA is only satisfied if both 20:5n-3 and 22:6n-3 are present in the diet since these fish lack the enzymatic capacity for converting 18:3n-3 to C:20 and C:22 HUFA (Owen et al., 1975; Cowey et al., 1976). This capacity seems to be lacking in larvae as well, as shown by the results of Scott & Middleton (1979) and Witt et al. (1984). The quantitative requirement of 20:5n-3 plus 22:6n-3 is 0.8% of the dry diet for adults (Gatesoupe et al., 1977a), while larvae seem to require higher levels of these lipids, 1.3% of the dry diet (Le-Milinaire et al., 1983).

Wild adult plaice have the high levels of phospholipid C:20 and C:22 n-3 fatty acids characteristic of marine fish (Owen et al., 1972). They have the capacity for selectively retaining these fatty acids in their phospholipids if deprived of such a dietary input and probably lack the ability for elongation and further desaturation of dietary linolenic (18:3n-3) and linoleic (18:3n-6) acids. The picture that emerges from the present experiments is that the larvae can equally exploit dietary 20:5n-3 (present in N. oculata), or 22:6n-3 plus 18:3n-3 (as in I. galbana). In other words, larval plaice do not have an apparent EFA requirement for 22:6n-3 throughout most of their early life history. Furthermore, plaice larvae can be reared through to metamorphosis on Artemia alone (e.g. Riley, 1966 and

the pigmentation experiments of this work), an organism lacking the 22:6n-3 fatty acid (Leger et al., 1984). This observation might imply that plaice eggs contain sufficient 22:6n-3 for getting the larvae through to metamorphosis, a most unlikely explanation in view of the five-fold increase in larval weight in its first 30 days alone. It might mean that larval phospholipids containing C:20 or C:22 HUFA are physiologically compatible within the environmental conditions experienced by the larvae in the experiments. Alternatively, contrary to what has been suggested for the adults (Owen et al., 1972) it might be that larvae can convert C:18 and C:20 to C:22 HUFA at a rate which precludes any deficiency symptoms from being manifested. This point can only be evaluated by comparison of larval phospholipid fatty acids with that of their diet and subsequent radio-labelling of the suspected C:22 HUFA precursors. Ability for bio-conversion of C:18 and C:20 to C:22 HUFA has been suggested on the basis of fatty acid analysis for Atlantic silverside (Schauer & Simpson, 1979). It has also been demonstrated conclusively for a number of teleosts though its level is usually lower in marine compared with freshwater forms (see Kanazawa, 1985).

Composition of phospholipid fatty acids from cultured adult herring shows very high levels of 22:6n-3 and 20:5n-3 (Owen & Middleton, 1977). This suggests that the capacity for preferential HUFA conservation observed in embryos and early (non-feeding) larvae of this species (Tocher et al., 1985) is most likely extended into adult life. Neutral lipid fatty acids, on the other hand, which tend to reflect the fishes' diet have higher levels of 20:1n-3 and 22:1n-11 in wild compared with cultured fish (Owen & Middleton, 1977; Lambertsen & Braekkan, 1965; Gatten et al., 1983). This

results from a transition to a wax ester-rich copepod diet that larvae of these fishes undergo in the wild at around day 60 post-hatch (Gatten et al., 1983). From hatching and up to day 60 the dominant neutral lipid fatty acids of larvae reared in large ecosystems are the n-3 C:20 and C:22 HUFA (Gatten et al., 1984). This would suggest a similar HUFA profile in the phospholipid fraction although this has not yet been evaluated. The present experiments clearly show that C:20 or C:22n-3 HUFA are equally suitable for growth of herring larvae during its first month post-hatch. Owing to the relatively slow SGR of this species, however, if an EFA requirement for 22:6n-3 does exist it might have been met (in larvae reared in the presence of Nannochloropsis) by the fatty acid carried through from the egg. On the other hand it is possible that this species has a capacity for bio-converting the 20:5n-3 to 22:6n-3. If a dietary requirement for 22:6n-3 exists in herring it will most likely manifest itself at the transition stages which in clupeoids are noted for the proliferation of pyloric caeca, coiling of the gut and thickening of body musculature (O'Connell, 1981; Blaxter & Hunter, 1982). Supporting this hypothesis is the fact that at their later growth stages larval herring will not grow on a diet of brine shrimp alone (Blaxter, 1966) and that the mortalities are preceded (at about day 30 post-hatch) by a decline in assimilation efficiency (Klumpp & von Westerenhagen, 1986) which, as pointed out earlier, is one of the symptoms of dietary fatty acid deficiencies.

The dietary requirements of marine fish larvae, with the exception of turbot, have so far not been assessed in such a way that will distinguish between the effectiveness of 22:6n-3 or 20:5n-3 as EFA. Growth and survival of red sea bream larvae was found to be

substantially improved when yeast mass-cultured rotifers were enriched with Chlorella minutissima (Kitajima et al., 1979), which was due to the input of 20:5n-3 by the algae (Watanabe et al., 1983b). Larval red sea bream were also compared for growth and survival over a 27 d rearing trial on diets of rotifers containing either 20:5n-3 (grown on Chlorella) or both 20:5n-3 and 22:6n-3 (grown on w-yeast), showing minor improvements in growth and survival when both HUFAs were available (Kitajima et al., 1980). Tentatively, one could therefore suggest that for red sea bream either C:20 or C:22 HUFA are suitable EFA, which would put it in the same category as the plaice. Other larvae for whom EFA requirements were assessed using the rotifer plus enrichments method are black sea bream Mylio macrocephalus and the ayu Plecoglosus altivelis (Watanabe et al., 1983b). In the former both C:20 and C:22 were found to be essential and no distinction was drawn between them. While for the ayu, a species that spends its early life history in fresh waters (similar to salmonids), 18:3n-3 was found to be an essential fatty acid. However a further enhancement of ayu growth was achieved through use of C:20 and C:22 HUFA in the diets (Oka et al., 1980).

Evaluation of larval fatty acid requirements has also been carried out using Artemia enriched with various marine lipids, algae or yeasts grown in the presence of marine lipids (Watanabe et al., 1982c; Dendrinos & Thorpe, 1987). Yet again these experiments were not designed to differentiate between requirements for C:20 or C:22 HUFA, both of which were found to be essential for growth of larval flounder Paralichthys olivaceus, rock sea bream Oplegnathus fasciatus, later stages of the red sea bream and Dover sole.

As a result of the facts presented here either through the experimental work, or those found in the literature, one might conclude that with respect fo dietary HUFA, fish larval requirements resemble those of their adult counterparts. In practical terms this implies that when formulating diets for larvae its FA profile should qualitatively resemble that of the adult fish. However, due to their higher growth rates, the ration of essential fatty acids required by the larvae might possibly be higher than that of the adults as suggested by the 1.3% compared with 0.8-1% (HUFA as percent of dry diet) needed in larvae versus adult turbot (Le-Milinaire et al., 1983; Castell et al., 1986). On the other hand Watanabe et al. (1983b) suggest that 0.3-0.4% HUFA (percent dry weight of food particle) are the necessary levels of these fatty acids for good growth and survival of juvenile marine fishes.

C. The effects of the diets on pigmentation in plaice

The occurrence of albinism in hatchery reared flatfishes is well documented. It has often been observed in plaice and has been associated with rearing conditions such as food quantity (Riley, 1966) or larval population density (Shelbourne, 1974). Apart from a probable reduced viability for released albino juveniles in the wild, the lack of pigmentation could expose the fish, at the ongrowing stage, to the harmful effects of UV radiation. Abnormally pigmented fish also have a reduced market value as in the case of Japanese flounder Paralychthys olivaceus (Seikai, 1985a). Recent publications tend to relate albinism in flatfishes to dietary factors (Seikai, 1985a,b; Seikai et al., 1987).

The present work on plaice showed a pronounced effect of the diet on the occurrence of albinism. Rotifers which had been

enriched with Isochrysis consistently conferred higher ratios of normal to abnormal pigmented juveniles compared with rotifers enriched with Nannochloropsis. It should be noted, however, that this ratio differed between the two experiments and that in both years it was never an all-or-nothing response. In the present experiments pigmentation of juvenile fish could not be associated with any growth or development responses of larvae to the diets as these parameters were unaffected by it. Dietary effects on pigmentation have also been noted in the Japanese flounder. These fish show a high percent of normal pigmentation when raised on natural zooplankton (Seikai, 1985b), or if Artemia of the San-Francisco rather than the Tien tsin or Brazil strains are employed (Seikai et al., 1987). The dietary factor(s) linked with the pigmentation are not yet known. Mineral, heavy metals, pesticides as well as lipid and fatty acid composition of the Artemia strains mentioned above, did not reveal any differences which could explain pigment abnormalities (Seikai et al., 1987). On the other hand chloroplast pigments, such as chlorophylls and carotenoids, are typical to algae from diverse taxonomical classes (e.g. Norgard et al., 1974). The present results suggest that one should look at these differences for clues as to the dietary factor(s) required for normal flatfish pigmentation.

In the present experiments, larvae reared on rotifers enriched with either of the algae were grown on to metamorphosis on Artemia. The differences in pigmentation could, therefore, only be attributed to the rotifer feeding phase which extended from first-feeding and up to transition, stages 1c-3c inclusive (Ryland, 1966). A similar finding has been reported for the Japanese flounder. Seikai (1985a) has shown that the extent of the unpigmented area on

the fishes' ocular side, and the ratio of albino to normal fish in a population, can vary with the feeding regime. Larvae fed on rotifers (enriched with Chlorella and w-yeast) for progressively longer intervals, before being transferred to a diet of Artemia nauplii (Brazilian strain), showed fewer pigmentation abnormalities at metamorphosis. The turning point seemed to occur when larvae were about 12.9-13.2 mm (TL), which corresponds with the final stages of notochord flexure and initiation of eye migration (Fukuhara, 1986). It therefore seems most likely that pigmentation in flatfishes is related to developmental events taking place during the period immediately preceeding metamorphosis. If so, the dietary component(s) necessary for normal pigmentation should be present in the diet up to this time.

D. Relations of growth to survival

Fish larvae have to grow in order to survive (Jones, 1973; Laurence, 1977), their growth rates are high and furthermore, seem to be genetically predetermined (Houde & Scheckter, 1981). The present herring and turbot nutritional experiments show, in addition, that at increasing growth rates survival is directly and proportionally increased (see Figs 14 for herring and 25 for turbot). Furthermore, in the yeast-enriched rotifer treatments where growth was curtailed, though not through any starvation effects, mortalities were highest. Growth and survival rates of marine fish larvae can also be enhanced through elevating the density of prey organisms in their environment. This effect has often been reported, amongst others, for winter flounder Pseudopleuronectes americanus (Laurence, 1977), sea bream Archosargus rhomboidalis, lined sole Archirus lineatus, bay anchovy Anchoa mitchilli (Houde, 1978;

Houde & Schekter, 1981) and herring (Werner & Blaxter, 1981; Chekley, 1984).

The parameter chosen for presenting the mortality component differed between the two species. Turbot, which were followed throughout most of their early life history (up to metamorphosis), were presented by the daily mortality rates (the coefficients of the mortality regressions). On the other hand, mortality models could not be fitted to the data on herring as on day 28 these larvae had only completed about one third of their larval cycle. Any model employed for the herring would have, therefore, been predictive rather than factual. For this reason data for these larvae were presented by their survival values at the termination of the experiment.

In the turbot experiment the larvae feeding on rotifers enriched with Isochrysis had lower mortality and final survival rates compared with those larvae feeding on Nannochloropsis-enriched rotifers. This is contrary to the expected higher survival at lower mortality rates. Using the mortality regressions for turbot larvae it was possible to back-calculate from day 8, the first day that mortalities in the tanks could be counted, to the initial stocking numbers on day 1. These calculated values were then compared with the 1,250 larvae initially stocked in each tank. Doing so revealed that 800, 500 and 700 larvae were unaccounted for in Isochrysis tanks a, b and c respectively, and that from the Nannochloropsis treatments tank b was missing 600 larvae. Although a mistake in the stocking procedures could be used to explain the disparity in these numbers, it is most unlikely to have been centered mainly on the Isochrysis treatment tanks. Likewise, suggestions that mortalities were taking place, but were unobserved,

would be subject to the same criticism. This differential mortality, or unaccounted larvae, is disturbing as the lower larval densities in the tanks where growth rates were fastest could in itself be invoked to explain the differential growth rates, rather than the differences between the diets. However, it is plausible that the reduction in the numbers of survivors in these tanks was the result of cannibalism and that cannibalism behaviour commences at development stages 4b-4c, which distinguishes the stage distributions (Fig. 23) of larvae in the fastest growing tanks from those of larvae in tanks where growth rates were slower. Cannibalism is, in fact, very common among turbot larvae at the stages of metamorphosis, when one can easily observe aggressive behavioural patterns such as fin biting. This problem is sometimes alleviated in hatcheries by removing the larger larvae to separate tanks.

One of the characteristic growth patterns of larvae reared in hatcheries is the increase in the range of sizes as the larvae get older. This has been termed the "size hierarchy" effect (see Blaxter, 1975). In the present turbot feeding experiment the distribution of developmental stages for 15 day old larvae (Fig. 23) was from the initiation of hypural element formation (stage 2c) to the initial stages of eye migration (stage 4c), the respective range of lengths was 5.1 to 7.9 mm and that of dry weights was 95 to 1050 µg. With such large size differences predation of faster on slower growing individuals is a distinct possibility. Furthermore, the skewed stage distributions, for all Isochrysis-enriched rotifer treatments, suggests that there was a differential loss of larvae from the lower developmental stages.

E. Ingestion rates and conversion efficiencies

A steady increase in the number of organisms, their size and total weight in the guts of fish larvae is to be expected in relation to the increasing prey capture efficiency (e.g. Hunter, 1980), as well as increases in mouth size and gut volume related to larval size. This trend has been observed in species such as striped knifejaw Oplegnathus fasciatus (Fukusho, 1979), bream Archosargus rhomboidalis (Stepiens, 1976), as well as in clupeoids (Blaxter & Hunter, 1982). In the larval flatfishes winter flounder (Laurence, 1977), and turbot (Kuhlman et al., 1981 and this study) the relation of gut contents to larval weight has been found to be linear, so that the gut contents are a constant percent of body weight. The proportionality of gut volume to fish weight is maintained in adult flatfishes, such as dab Limanda limanda (Jobling et al., 1977), and turbot (Flowerdew and Grove, 1979). The use of rotifers in the present experiments produced a proportionality factor for turbot larvae of 13%. This is larger than the 4.7% evaluated for the same species by Kuhlman et al. (1981), who fed their larvae on copepods. Such differences could have arisen from the different sampling methodologies or the diverse food particles employed. In this respect, the soft, spherical rotifers used in the present study might well pack better than the irregular shaped, hard copepods used by the latter authors. The proportionality of gut volume to larval weight suggests that, throughout the larval period, the handling capacity of the digestive system for a given species is constant per body mass. Therefore, unless a larvae will increase its digestive enzyme levels or complement, its assimilation efficiency should be expected to remain constant.

The precise relation of gut contents to weight of herring larvae has, up to the present study, not been evaluated. However, herring larvae grown in large ecosystems, and therefore feeding on a mixture of wild zooplankton, do show an increase in both the size-range and quantity of organisms in their guts with age (Gamble et al., 1981). It was therefore most intriguing to observe that the logical linear relationship did not hold in the present herring feeding trials and that the gut contents relative to body dry weight dropped considerably from 8% to 2.5% between days 12 and 18 post-hatch. This, however, did not reflect a loss of appetite since no reduction in the daily intake of food was recorded during this period. The decrease in the gut contents would only have occurred if the residence time of the food relative to the rate of ingestion was decreasing.

In adult fish the passage of food down the alimentary canal has been studied in relation to the rate at which nutrients are transferred from the stomach to the intestine. It transpires that the stomach contracts at a regular frequency and that the volume secreted at each contraction through the pylorus (the stroke volume) is probably regulated via receptors in the upper intestine. These receptors are sensitive to certain nutrients (amino acids) as well as acidity levels (Jobling, 1986). According to Govoni et al. (1986) passage of food through the larval alimentary canal is mediated, in the lack of the longitudinally orientated smooth muscle of the muscularis, via peristaltic contractions of a single layer of smooth muscle. In some species this is also assisted by ciliated cells (Iwai & Rosenthal, 1981; Watanabe, 1984b). However physiological regulation of peristalsis and evacuation rates has not yet been elucidated for larvae.

Gut evacuation time, for herring larvae continuously feeding on Artemia nauplii, has been estimated at 4-10 h (Rosenthal & Hempel, 1970) and at 3-7 h, or over 7 h (Werner & Blaxter, 1980, 1981). Herring larvae feeding on copepods have slower evacuation rates of 0.7-3 h (Pedersen, 1984). Apart from being related to ambient temperatures (Blaxter, 1965; Laurence, 1971) and prey species (Fossum, 1983) evacuation rates in herring larvae are strongly influenced by prey density. The increased ingestion rates at high prey densities can physically displace food in the gut, in particular when a large food particle such as Artemia is employed (Werner & Blaxter, 1981). Increase in ingestion rate itself has also been shown to result directly in higher evacuation rate (Pedersen, 1984). This could have an overriding effect on gut evacuation rate when prey organisms do not fill the gut to its maximum capacity. Indeed, unlike the larger Artemia, the rotifers used in the present experiments were never observed to fill the gut completely. Thus, it is quite possible that the decrease in gut contents, observed in the present herring experiments, was a direct result of the increased evacuation rate as the ingestion rate increased with age.

Flatfish larvae differ from the clupeoids in being able to complete all or most of their early life history on a small food particle, such as rotifers (Howell, 1973, and this study). In other species food size has often been implicated as one of the factors limiting the growth of fish larvae. In E. mordax, growth stops at about 6 mm when feeding on the dinoflagellate Gymnodinium splendens, but continues to 20 mm when feeding on Brachionus (Hunter, 1976a, 1980). This has been associated with the lower calorific content of the algae 5×10^{-5}cal compared with 17.9×10^{-5}cal for the rotifer. Apart from the relation of calories

gained per bite, the degree to which a food particle is digested will relate to the surface/volume ratio available for enzyme activity and its residence time in the gut. If, as the present results for herring suggest, small prey are rapidly defaecated, then some increase in their size might improve their residence time and consequently, the efficiency with which they are assimilated. Furthermore, the inflection points in curves of gut contents on larval size, such as that found for herring larvae in the present experiments, could be a way for deciding when to increase the particle size offered to the larvae.

Weight-specific ration, expressed as percent body weight consumed per day, was larger for turbot (80%/d) than the herring (30%/d). This is to be expected when one considers that the former species was raised at a higher temperature and exhibited a much faster growth rate. Houde & Schekter (1981) found that at equal temperatures and prey densities, and for a given larval dry weight, eel like larvae with a straight gut (Anchoa mitchilli) will consume more food compared to larvae with a coiled gut (Achirus lineatus), namely 140 compared with 90%/d respectively. The authors associated this with the shorter retention time of food in larvae with straight guts, necessitating higher feeding rates.

Daily ration estimates for Atlantic herring have mostly been obtained for larvae feeding on Artemia. Checkley (1984) showed that herring larvae consumed prey in relation to its availability, with weight-specific rations ranging from 1-11% of body nitrogen/d. The highest ration was found for larvae with a specific growth rate of 4%/d. On a dry-weight basis this equals a daily ration of about 17%/d assuming that nitrogen in terms of dry weight equals 12.2% for the larvae (same author) and 8.1% for Artemia (Leger et al.,

1986). Klumpp & von Westernhagen (1986) estimated the weight-specific ration during the first month of larval life to be 16-28%/d, with an average value of 21%/d specific growth rate between days 8-33 was 4.5%/d. These values correspond well with the present results even though the food used here was Brachionus. Herring larvae grown under natural conditions have been estimated to consume prey at a rate of 16%/d at a specific growth rate of 7.7%/d, with larvae feeding on wild zooplankton at a concentration of 12 items/l (Gamble et al., 1981).

As yet there are no estimates of daily ration in turbot. Weight-specific ration estimates for other flatfishes are 90%/d for lined sole (Houde & Schekter, 1981), 30-300%/d for winter flounder (Laurence, 1977), 48-160%/d for summer flounder, Paralichthys dentatus (Buckley & Dillmann, 1982) and 15-39%/d for plaice (Klumpp & von Westernhagen, 1986).

Gross growth efficiencies, K1, for herring in the present study ranged between 20-40% and for turbot, ranged between 20-60%. Estimated K1 values for marine fish larvae, other than the above two species, range from 5 to 40% (Houde & Schekter, 1983) or 40-80% (Klumpp & von Westernhagen, 1986). The latter authors estimated K1 values for herring larvae in the range 20-27%. Checkley (1984) estimated that the K1 for herring larvae could attain maximal values of 40-50%.

K1, being the ratio in percentage of the ingested food to growth, is functionally related to ingestion. The value of K1 is 0 when ingestion only meets the demands of basic metabolism, is maximal at some intermediate ingestion value (the optimal ration), and declines at high ingestion rates (Brett & Grove, 1979).

In the present study, fluctuations in K1 values could not be related to any particular developmental events noted in the larvae. However, they did tend to fluctuate in accordance with the daily weight-specific ration estimates. Reduction of K1 with increasing rations in marine fish larvae have also been noted by Stepiens (1976); Laurence (1977); Houde & Schekter (1981); Checkley (1984); Boehlert & Yoklavich (1984) and Klumpp & von Westernhagen (1986). This is associated with the lowering of assimilation efficiencies at increased ingestion rates, due to the rapid passage of food particles through the gut (Ryer & Boehlert, 1983; Boehlert & Yoklavich, 1984; Govoni et al., 1982). In herring larvae offered Artemia at high densities it has been noted that digestion of the food is incomplete (Werner & Blaxter, 1981). In the Pacific herring, carbon assimilation decreases at the increasing ingestion rates that result from high rotifer densities (Boehlert & Yoklavich, 1984). Lowering of the gross growth efficiency will also take place when food is consumed in excess of the intake relating to the maximal growth rate a species is capable of. Furthermore, rates of ammonia excretion increase asymptotically with the amount of food ingested (Klumpp & von Westernhagen, 1986). This implies that the high metabolic expenditure for digestion and assimilation at high food intakes will detract from the gross growth efficiency.

Estimates of food consumed daily or hourly by fish larvae reveal an exponential increase as a function of larval age (Stepiens, 1976; Buckley & Dillman, 1982), or length (Okauchi et al., 1980; Barahona-Fernandez & Conan, 1981). Since weight of larvae is an exponential function of time or an allometric function of length, the ingestion relations can be linearised if weights are employed as

estimates of both larval age and its daily ration (Barahona-Fernandez & Conan, 1981; Buckley & Dillman, 1982). Table 31 and Fig. 32a,b show the relation of daily ingestion to body weight in a number of fish larvae from diverse taxonomical orders such as Clupeiformes, Perciformes and Pleuronectiformes. It also includes data for herring and turbot obtained in this study. Excluded from the Table are those studies in which daily ingestion was calculated on the basis of gut contents multiplied by evacuation rates and hours of feeding (Hunter & Kimbrell, 1980; Laurence, 1977) or hourly feeding rates multiplied by feeding duration (Werner & Blaxter, 1981). This is because: a, larvae do not necessarily feed at a constant rate (Hofer & Burkle, 1986), b, gut evacuation rates are related to feeding rates (Werner & Blaxter, 1981) and c, food levels both in nature and in a larval rearing tank are not necessarily constant.

The weight-specific ingestion obtained from this model is the coefficient of proportionality relating weights of ingested prey to larvae and is expressed in units of d^{-1}. It differs from weight-specific ration (e.g. Houde & Schekter, 1981) which is the ratio, in percentage, of ingestion to body weight on a single day. It also differs from the estimate used by Checkley (1984) which was the average nitrogen consumption (µg) by larvae over a period of time, t, divided by the average bodily nitrogen content of larvae during the same period.

The regression constant (b) becomes a proportionally smaller fraction of the daily weight-specific ration with increasing larval age. In other words, the tendency to consume food in excess of the weight-specific ingestion decreases with increasing age. This is probably the basis of the progressive reduction in the weight-

specific ration often observed as larvae grow older (Klumpp & von Westernhagen, 1986; Stepiens, 1976; Houde & Schekter, 1981). The reduced rations in older larvae have often led to the suggestion that due to age related improvements in the digestive systems, the efficiency of food utilisation increases allowing for reductions in weight-specific rations. Indeed, blenny larvae, which are more developed at hatching compared with most other planktonic fish larvae, having a fully functional digestive system, (Klumpp & von Westernhagen, 1986) do not exhibit any reduction of their weight-specific ingestion throughout their larval period.

Larval flatfish have often been reported to exhibit improved gross growth efficiencies near metamorphosis (Klumpp & von Westernhagen, 1986; Buckley & Dillman, 1982; Cetta & Capuzzo, 1982; Laurence, 1977), perhaps resulting from an improvement in both the efficiency of prey capture and of the developing digestive system. In turbot, grown at 20°C, the stomach and pyloric caeca evolve from about day 15 post-hatch when larval TL is 6.9-7.7 mm, an event that also delineates the onset of peptic digestion (Cousin et al., 1985). This event relates to morphological stages 4a-4c (Al-Maghazachi and Gibson, 1986) which are characterised by the flexure of the notochord. In Japanese flounder the stomach is formed at day 29 post-hatch and peptic digestion is apparent from day 30 (O'Connell, 1981 after Yasunaga, 1972). This event also relates to the period characterised by notochord flexure, the morphological stages E-F of Fukuhara (1986). Peptic digestion is probably more efficient than the assimilation via pinocytosis of macromolecules in the hind-gut prevalent in the larval forms (Govoni et al., 1986). One can therefore assume that the development of the gastric glands and peptic digestion is the

probable cause for the improved assimilation noted in larval flatfishes prior to transformation. In the present study turbot did not display an improvement in gross growth efficiency since the stomach was probably not yet functional throughout the period in which these larvae were tested.

It is well established that in fish, after metamorphosis, the growth efficiency decreases with age due to rising metabolic costs associated with weight (Gerking, 1971; Brett, 1979). In larval fishes, however, there are conflicting opinions regarding the possibility of an enhancement or a reduction in assimilation efficiencies associated with larval development (Govoni et al., 1986). Resolving this conflict might be possible if the definition of the larval stage was to be made on a physiological basis. Thus marine fish could be considered as larvae up to the event of gastric gland formation and initiation of peptic digestion. Following this event the fish would enter their juvenile stage. This type of definition has been employed by Cousin et al. (1985) and O'Connell (1981). Accordingly the larvae of the flatfishes previously mentioned will exhibit an improved assimilation only during the juvenile stage. However, their larval stage would be characterised by a constant assimilation efficiency which is in accordance with what is known for other larvae such as spot, Leiostomus xanthurus (Govoni et al., 1982) and the fresh water fish Abramis abrama (Sorokin & Panov, 1966). As pointed out by Govoni et al. (1986) there is so far no histological or biochemical evidence supporting the suggestions of increased assimilation efficiencies within the larval period.

Examination of the ingestion data, in the linearised form in which it has been presented in Table 31, suggests that some other

Table 31. Relations of weight specific ingestion (I = dry weight food consumed/larval dry weight/d) and specific growth rates (SGR = (Δ ln W/Δ t) x 100), for fish larvae.

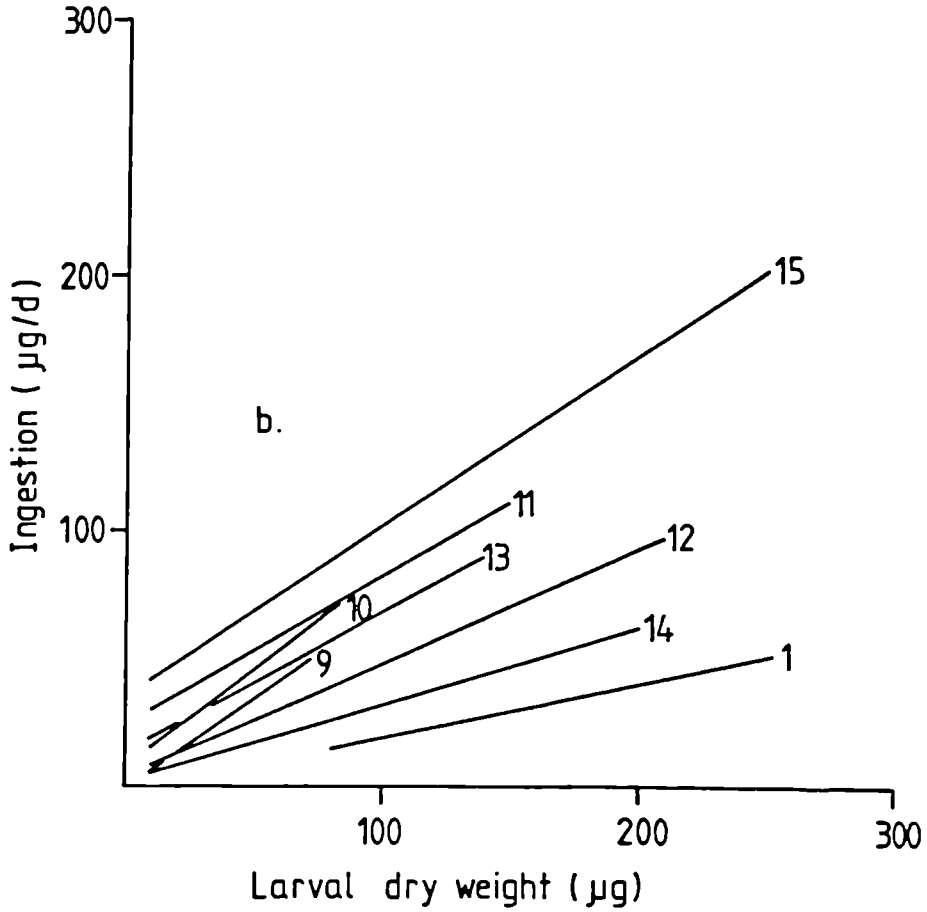
Species	Prey	Temp.	Ingestion			SGR	Authors
		°C	(I=a W+b;µg/µg)			%/d	
			a	b	r		
Clupea harengus	Rotifer	11	0.13	49.7	0.79	9.3	Minkoff, this study
	Wild zooplankton	9-12	0.16*			7.7	Gamble et al., 1981(1)
	Artemia nauplii	7-9	0.11*			4.0	Checkly Jr., 1984
	Artemia nauplii	9	0.19	3.52	0.96	5.5	Klumpp & von Westernhagen, 1986(2)
Pleuronectes platessa	Artemia nauplii	9	0.18	20.1	0.99	11.0	Klumpp & von Westernhagen, 1986(2)
	Rotifer	7-12	0.09	90.7	0.88	6.5	Howell, 1973(3)
Blennius pavo	Rotifer + Artemia	24	0.31	0.03	1.0	18.9	Klumpp & von Westernhagen, 1986(2)
Scophthalmus maximus	Rotifer	19	0.43	38.5	0.83	19.9	Minkoff, this study
Oplegnathus fasciatus	Rotifer	21-23	0.67	-41.5	0.99	22.1	Fukusho, 1979(4)
Acanthopagrus schlegeli	Rotifer	17-23	0.57	71.6	0.99	26.1	Okauchi et al., 1980(4)
Archosargus rhomboidalis	Wild zooplankton	23	0.69	-1.2	0.95	21.7	Stepien Jr., 1976(2)
		26	0.75	7.9	0.94	27.2	
		29	0.57	24.3	0.91	31.9	
Anchoa mitchili	Wild zooplankton	26	.21	7.09	.97	23.4	Houde & Schekter, 1981(2)
			.24	22.5	.94	24.5	
			.55	78.7	.91	34.4	

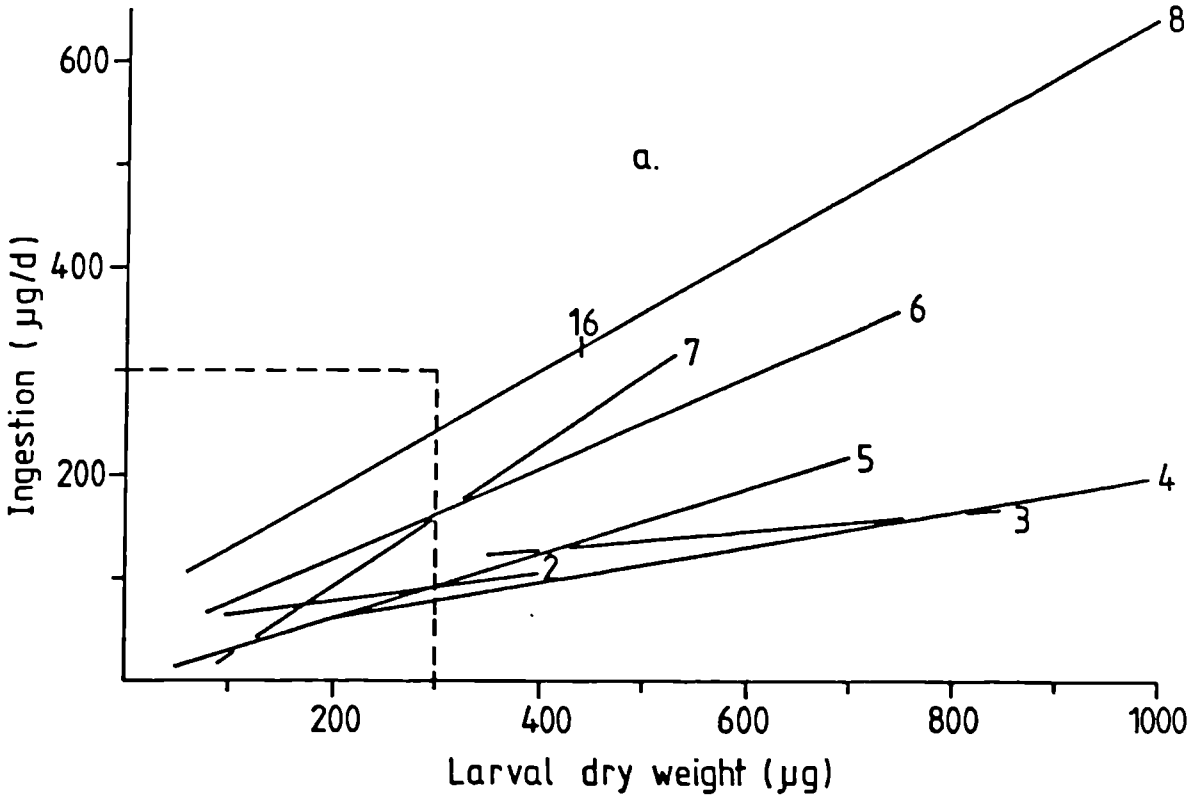
Table 31 (continued)

Species	Prey	Temp.	Ingestion (I=a W+b;µg/µg)			SGR	Authors
		°C	a	b	r	%/d	
Archosargus rhomboidalis	Wild zooplankton	26	.17	2.02	.94	18	Houde & Schekter, 1981(2)
			.45	2.32	.98	22.4	
			.55	13.6	.92	33.6	
Achirus lineatus	Wild zooplankton	28	.18	2.1	.99	17	Houde & Schekter, 1981(2)
			.30	2.2	.98	21	
			.67	35.4	.94	29	
Coregonus sp.	Moina sp.	10	.16*			5.9	Hofer & Burkle, 1986(5)

(1): Data presents assessments on larvae grown in large (310 m^3) enclosures.
(2): Weight specific ingestion and SGR were evaluated directly from published data.
(3): Data on preserved larval lengths were converted to dry weights using a 7% shrinkage factor for length and the relation logW=5.51logTL-2.68 (Minkoff, this study). Rotifer numbers were converted to dry weight 1 rotifer = 0.3µg.
(4) Larval wet weights converted to dry weights using an 0.2 conversion factor. Rotifer dry weight estimated as in (3).
(5): Wet weights converted to dry weights using an 0.2 factor for the larvae and an 0.09 factor for Moina. Estimates for larval ages 8-31 days only.
*: Weight specific ingestion as estimated by the author.

Fig. 31. Relations for daily ingestion estimates of fish larvae in dry weight food consumed/larval dry weight. The area delimited by the broken line of Fig. 31a is presented in Fig. 31b. The figure relates to the regressions calculated in Table 31: 1, herring (Klumpp & von-Westernhagen, 1986); 2, herring (Minkoff, this study); 3, plaice (Howell, 1973); 4, plaice (Klumpp & von-Westernhagen, 1986); 5, blenny (Klumpp & von-Westernhagen, 1986); 6, turbot (Minkoff, this study); 7, Japanese striped knifejaw (Fukusho, 1979); 8, porgy (Okauchi et al., 1980); 9, 10, 11 sea bream (Stepiens, 1976); 12, 13 sea bream (Houde & Schekter, 1981); 14, 15, lined sole (Houde & Schekter, 1981); 16, bay anchovy (Houde & Schekter, 1981).


300
200
100
Ingestion (µg/d)
b.
15
11
12
13
10
14
9
1
100
200
300
Larval dry weight (µg)


600
400
200
Ingestion (µg/d)
a.
8
16
6
7
5
4
3
2
200
400
600
800
1000
Larval dry weight (µg)

factors could be involved in the reduced weight-specific rations commonly observed as larvae get older. As noted earlier the regression constant (b) assumes a higher proportion of the weight specific ingestion in younger compared with older larvae. One might regard the regression constant (b) as a product of the experimental methodology. Accordingly the magnitude of (b) could be indicative of: food supplied in excess of an optimal requirement, and in particular to the yonger larvae; some adaptive responses of the growing larvae to their artificial surroundings which is expressed in the lowering of the required daily ration, or some general estimate relating to the deviation of the culture conditions from those experienced by larvae in the wild. With regard to the supply of excess food, the results of Houde & Schekter (1981) show that at reduced prey concentration the magnitude of (b) diminishes and the decline in weight-specific ingestion exhibited in older larvae is less apparent. However, it should be noted that lowering prey availability also reduced the specific growth rate of these larvae.

In the present experiments the weight-specific ingestion of herring was 13%/d and the average weight-specific ration was 30%/d (Table 23). The weight-specific ingestion compares better to the daily ration of 16%/d estimated for ecosystem populations by Gamble et al. (1981). This might suggest that the further 17% of body weight consumed daily by larvae in the present study, seemingly a "superfluous feeding", was a necessity imposed by the confinements of the rearing containers. Such confinement probably results in higher intraspecific competition for food, and probably more avoidance manoeuvres relating to encounters with the walls and other larvae. Interactions of this kind would tend to increase the metabolic demands of the larvae, so leading to increased feeding

levels. Optimization of the rearing, through evaluation of parameters such as tank volumes, might therefore reduce the weight-specific ration required by larvae at a given specific growth rate or conversely, increase the specific growth rate for a given weight-specific ration. Theilacker (1980b) showed increased growth rates and nutritional condition of jack mackerel Trachurus symmetricus reared in 100 l compared with 10 l tanks. However, although it is generally recognised that rearing larvae is preferable in volumes of 10^2-10^3 l the field remains unexplored.

Weight-specific ingestion is lower for species living in temperate (8-12°C) than sub-tropical (20-25°C) waters. Table 31 also lists the SGR values calculated for these larvae from the presented data, showing that larvae from sub tropical waters also exhibit higher specific growth rates. The relation of weight-specific ingestion to SGR in units of %/d, for data where food was not a limiting factor, is plotted in Fig. 32. The figure also includes data for herring from Gamble et al., 1981 (I=16%/d, SGR=7.7%), Checkley, 1981 (I=11%/d, SGR=4%/d) and data for a fresh water coregonid from Hofer & Burkle, 1986 (I=16%/d, SGR=5.9%/d). The regression fitted to these points is described by the equation:

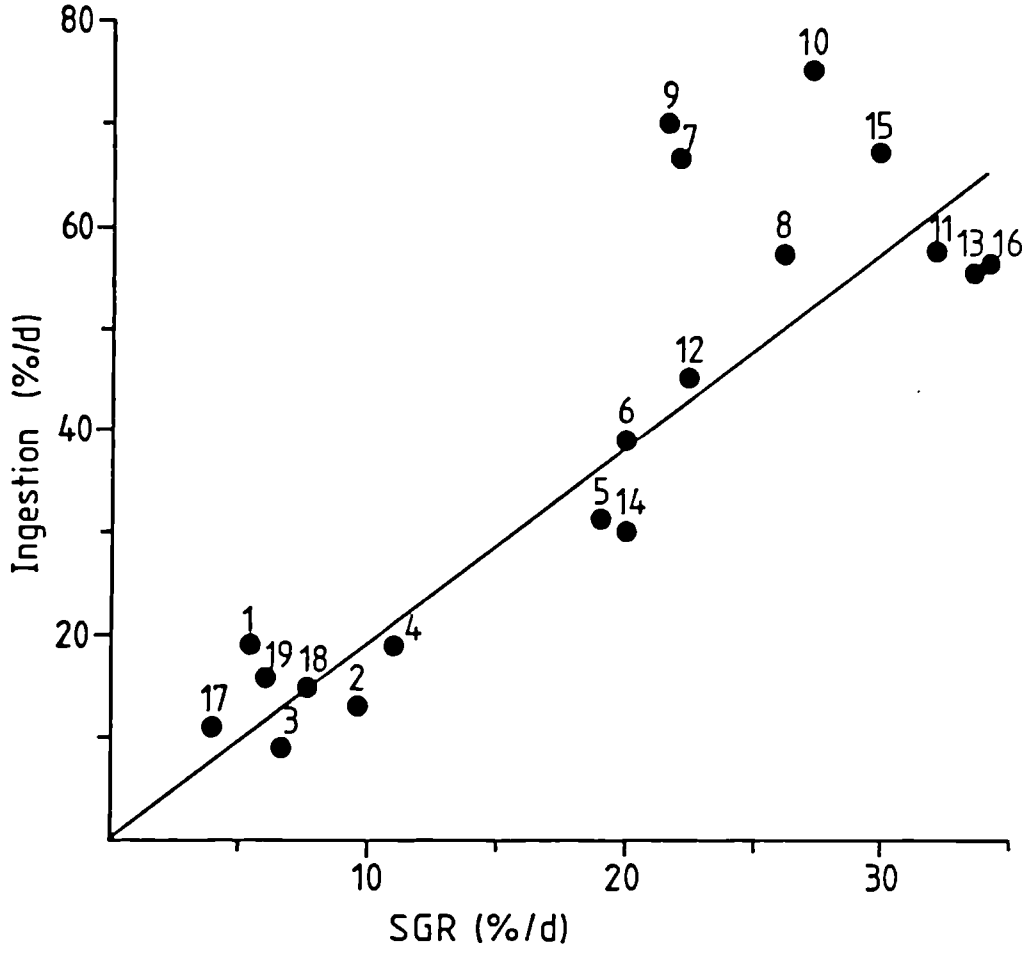
$$I=1.91 \times SGR+3.46; \quad (r=0.82; \; p < 0.001)$$

I and SGR in units of %/d.

From this it can be assumed that marine fish larvae have a minimal daily food requirement which is roughly twice their daily weight increment.

In the equation presented here the SGR was chosen as the independent variable. The value of the specific growth rate, whenever food was not limiting, was assumed to be characteristic for any one species within its physiological range. This is not to say

Fig. 32. The relation of weight specific ingestion to the specific growth rate, both in percent/d for a number of fish larvae. The figure relates to the data presented in Table 31. 1, herring (Klumpp & von-Westernhagen, 1986); 2, herring (Minkoff, this study); 3, plaice (Howell, 1973); 4, plaice (Klumpp & von-Westernhagen, 1986); 5, blenny (Klumpp & von-Westernhagen, 1986); 6, turbot (Minkoff, this study); 7, Japanese striped knifejaw (Fukusho, 1979); 8, porgy (Okauchi et al., 1980); 9, 10, 11 sea bream (Stepiens, 1976); 12, 13 sea bream (Houde & Schekter, 1981); 14, 15 lined sole (Houde & Schekter, 1981); 16, bay anchovy (Houde & Schekter, 1981); 17, herring (Checkley, 1984); 18, herring (Gamble et al., 1981); 19, white fish (Hofer & Burkle, 1986).


80
60
40
20
Ingestion (%/d)
10
20
30
SGR (%/d)
1
19
18
17
3
2
4
5
14
6
12
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that SGR will not vary with fluctuations in temperature as shown by Stepiens (1976), or ration as shown by Houde & Schekter (1981), and Checkley (1984). However, it is most unlikely that a subtropical species will function in the environment of a temperate species and vice versa.

Checkley (1984) also noted the relation of SGR to weight-specific ingestion and showed that in herring larvae the SGR increased linearly with increasing ingestion. In this case ingestion was used as the independent variable. Furthermore by normalizing data available from the literature to 8°C using a Q10 value of 2.3 (on the assumption that Q10 is constant throughout) he established a universal exponential relation:

$$g=0.13+0.088\log i \quad (r=0.85;\ p < 0.001)$$

g and i in units of d^{-1}

Thus, although he does not claim that all larvae can exist and function at any temperature his equation implies that rates of growth and ingestion will vary proportionally with temperature. This is what the linear model developed in the present study shows. In an attempt to fit the data in Fig. 31 to an exponential model, similar to the one used by Checkley, it was found that the residual sum of square was larger than that obtained for the linear model presented here. The exponential model was therefore rejected.

So far it has generally been assumed that one should approach with caution direct comparisons of ingestion parameters obtained by independent workers on different larval species. This is due to the fact that measurements related to feeding rates of larvae could be affected by the number of fish and the type and concentration of prey in the experimental container as well as by variables such as illumination, the volume of the holding facilities and the duration of

feeding (Theilacker & Dorsey, 1980). However relations such as the one developed here, or that of Checkley (1984) overcome these difficulties and suggest that the relationship between ingestion and specific growth rate is similar in a wide range of species.

The linear relation shown above could be used to evaluate daily rations and optimal feeding strategies for larvae reared in hatcheries. This is of particular importance when inert diets are used due to the fact that zooplankton are mostly fed on an individuals/volume basis but inert diets have to be introduced on a weight-specific basis.

CONCLUSIONS

The present study is the first of its kind in which a few diet combinations derived from a single food organism were tested on a spectrum of marine fish larvae. The two main contributions to be drawn from this study are:

a. The n-3 highly unsaturated fatty acid requirements of larvae are similar to those of the adult forms. Incorporating both 20:5n-3 and 22:6n-3 into the diet will guarantee its suitability for most larvae, though in some cases, such as herring and plaice, 22:6n-3 is not a necessity.

b. The quantity of food a larva consumes daily approximates twice its daily weight increment on a dry weight basis. This figure can help construct feeding schedules for larvae when employing inert diets, thus avoiding excess feeding that could lead to tank pollution.

Concerning the algal species employed there is a strong indication that pigmentation of juvenile flatfish will benefit from

the incorporation of I. galbana into their diet prior to metamorphosis. The nutrients in the algae responsible for this phenomenon might possibly be certain carotenoids. Otherwise, N. oculata proved to be an excellent alga for maricultural purposes in having rapid growth rates, achieving high densities and by being able to withstand culture conditions which are not aseptic.

The use of rotifers as nutritional probes seem to be limited to investigating either daily ration, or fatty acid requirements of their consumers. This technique does not enable us to take the quantum leap to the formation of the elusive inert diet required for hatchery purposes. Nevertheless, the results presented here suggest that the composition of the diet should ideally resemble that of the rotifer with respect to proximate as well as amino acid composition. Future research should centre on the types and sources of proteins that the larval digestive system, prior to the evolution of the stomach and gastric glands, is able to digest.

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Appendix I - Fatty acid analysis

Ia. Total lipid extraction:

Samples were thawed on ice then transferred to tubes where volumes of $CHCl_3$ and seawater were estimated. The volumes were adjusted to give $CHCl_3$:Methanol:sea water ratio of 8:4:3. This was followed by vortex mixing and teflon/glass homogenizing for 2 min on ice. Biphasic layer formation was speeded by centrifugation at 1,500 rpm (MSE Super-Miror) for 30 s. The aqueous upper layer was discarded and the lower organic layer filtered through Whatman No. 1 paper, followed by evaporation to dryness under oxygen free nitrogen (OFN) overnight in a desiccator in vacuo.

Ib. Fatty Acid Methyl Ester Preparation (FAME Prep)

Total lipid extracts were redisolved in 1% H_2SO_4 in MeOH plus toluene in 2:1 V/V ratios. The mixture was left at 50°C overnight in loosely stopped tubes after flushing with OFN. Following this 5 ml distilled H_2O and 5 ml Hexane + 0.05% butylated hydroxy toluene (BHT = anti oxidant) were added, the mixture was vortex mixed and centrifuged as described previously. The upper layer was removed to a second tube whilst the remaining mixture underwent a repeat of the H_2O and Hexane treatment and the upper layer was added to the first lot. Into this second tube 3.5 ml of 2% $KHCO_3$ (aq) was added followed by vortex mixing and centrifugation. The upper layer was removed, a spatula full of anhydrous Na_2SO_4 was added, and it was vortex mixed and centrifuged. The liquid layer was removed to another tube where it was

evaporated to dryness under OFN. It was then redissolved in hexane and BHT, streaked on TLC plate and co-chromatographed with FAME standards, using solvent mix Hexane/Diethyl Ether/Glacial Acetic Acid (85:15:1) by vol. The TLC purified FAME were scraped into tubes and eluted from silica using 10 ml Hexane + BHT, vortex mixed, centrifuged and the liquld layer removed and evaporated to dryness under OFN. This was then redissolved in Hexane + BHT and analyzed by GLC.

Ic. GLC - Analysis

Carried out on a Packard 436 capillary gas chromatograph using a Chranpack CP-WAX-52 CB 0.32 x 0.45 mm column (50 m). Flame ionisation detector operated at 250°C. Sample injected via an on-column injector. The column was held at 50°C for 0.1 min after which the oven temperature was programmed to increase to 190°C at a rate of 39°C/min and then to rise at 1.5°C/min to 225°C maximum which was maintained for 15 mins. Peaks were identified by reference to known standards and quantitated by a Shinadzu CR3A recording integrator operating on a time lock program - lock being on from 0.1 min to 10.5 min of the 50 min analysis. (This is to miss Hezane + BHT solvent from quantitative analysis of methyl esters).