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Effects of nutritional enhancement of live food organisms on growth and survival of Barramundi/Seabass *Lates calcarifer* (Bloch) larvae

M.A. RIMMER (1) and A. REED (2)

Queensland Department of Primary Industries,

(1) Fisheries Research Branch, Northern Fisheries Research Centre, Box 5396, CAIRNS Mail Centre, Queensland 4871, Australia.

(2) Food Research and Technology Branch, Malanda Research Laboratory, P.O. Box 1, MALANDA, Queensland 4885, Australia.

Abstract — Larvae of barramundi (*Lates calcarifer* Bloch) reared intensively in some Australian hatcheries have suffered periodic high mortalities which have been ascribed to nutritional deficiencies in the live food organisms used, particularly deficiencies of polyunsaturated fatty acids. Barramundi larvae were reared in an experimental system and fed on four diets, representing combinations of supplemented and unsupplemented rotifers (*Brachionus plicatilis*) and supplemented and unsupplemented brine shrimp (*Artemia salina*). Supplementation of rotifers and brine shrimp with a commercially available microencapsulated diet increased the levels of several polyunsaturated fatty acids in the food organisms. Two test diets (both using freshly hatched brine shrimp) produced near total mortality by day 30, while the other two (using supplemented brine shrimp) produced negligible mortality over the same period. Supplementation of rotifers and brine shrimp resulted in significantly larger larvae at day 22 compared with larvae fed on unsupplemented rotifers and newly hatched brine shrimp. The enhancement of the fatty acid composition of live food organisms used in intensive rearing is discussed with respect to the observed effects of different diets on growth and survival of barramundi larvae.

INTRODUCTION

Barramundi (*Lates calcarifer*) is the premier sportfish of northern Australia. It supports an important commercial wildstock fishery and is a major component of the recreational fishery of Queensland, the Northern Territory and Western Australia. Because barramundi are catadromous, this species cannot support self-maintaining populations in freshwater

impoundments, which are important recreational fishing areas throughout eastern Australia. For this reason, there is considerable public demand for barramundi to be stocked into impoundments, as well as into rivers and streams where existing barramundi populations are believed to be in decline. In addition, there is increasing interest in this species for aquaculture, due to the consistently high prices demanded by barramundi on the Australian market.

The Northern Fisheries Research Centre (N.F.R.C.) in Cairns has designed a small-scale hatchery to develop techniques for rearing barramundi. Many of the hatchery techniques developed at N.F.R.C. are now in use at commercial hatcheries in Queensland. These commercial hatcheries are primarily involved in aquaculture of « plate size » barramundi (c. 500 g) for the restaurant trade, but most also produce barramundi fry for stocking.

Barramundi reared at N.F.R.C. have periodically suffered severe mortalities (up to 90 % in some batches) at around 12 to 14 days after hatching. The symptoms involved a stress response where the larvae swam erratically following any disturbance, followed by « fainting », then death or recovery. Histological examination of the larvae showed extensive vacuolation of the brain and spinal cord and accumulation of excessive fat deposits in the liver (Rodgers and Barlow 1987).

Commercial barramundi hatcheries in northern Queensland have reported frequent occurrences of a similar mortality syndrome. Larval mortality problems are adversely affecting production from Australian barramundi hatcheries and are a significant threat to the establishment of a viable barramundi aquaculture industry in Australia.

From a survey of available literature on intensive rearing of marine fish larvae, Rodgers and Barlow (1987) tentatively ascribed the observed symptoms to nutritional deficiencies (particularly of polyunsaturated fatty acids) in the live food organisms used to feed the barramundi larvae (rotifers *Brachionus plicatilis* and brine shrimp *Artemia salina*).

Following the adoption of supplementary feeding techniques for rotifers and brine shrimp using a microencapsulated diet known to be high in polyunsaturated fatty acids (Frippak « CAR 1 » and « Booster ») during the 1987-88 season, this syndrome has not recurred at N.F.R.C. (Rimmer et al., 1988).

In order to investigate the nutritional basis of the mortality syndrome, a series of experiments was conducted at N.F.R.C. to test the effects of nutritional enhancement of live food organisms on growth and survival of barramundi larvae reared under intensive conditions. This paper presents the results of the initial experiments which compared four diets, based on the use of supplemented and unsupplemented rotifers and brine shrimp in combination.

MATERIALS AND METHODS

Barramundi larvae used in these experiments were reared from fertilised eggs obtained from wild spawning barramundi at Weipa in the north-eastern Gulf of Carpentaria in northern Queensland. Larvae were

air-freighted to Cairns, and arrived at N.F.R.C. around the time of hatching (12-17 hours after fertilization at 28-30°C). Although the term « larva » is used throughout this paper to describe the early life history stages of barramundi, the larger fish used in these experiments are properly termed « juvenile » (Kendall et al., 1984). Barramundi larvae metamorphose at 8-12 mm in length (MacKinnon, 1987; Russell, 1987). For convenience, the term « larva » has been used to describe the fish used in these experiments.

LARVAL FEEDING SCHEDULE

Barramundi larvae reared intensively at N.F.R.C. were fed rotifers at 10-20/ml from day 2 (where hatching is designated as day 1) to day 14, and brine shrimp at 2/ml from day 8, increasing to 5/ml by day 12 and continuing at 5/ml until day 18.

Rotifers were reared outdoors on algae (usually *Chlorella* species) together with small quantities of yeast. Rotifers were harvested daily in the morning and fed to the barramundi larvae that afternoon and the following morning. Rotifers to be fed in the afternoon were supplemented with Frippak « Booster » in aerated 3 litre plastic soft drink bottles for 4 hours at 1.0 g dry weight of microcapsules per litre of water with a rotifer concentration of 4 million rotifers per litre. Rotifers to be fed the next morning were supplemented for 20 to 22 hours at 0.2 g of microcapsules per litre with a rotifer concentration of 2 million rotifers per litre.

Brine shrimp nauplii (« Aquarium Products » brand) were harvested daily and starved for 24 hours to ensure that the yolk was absorbed before they were offered to the barramundi larvae or were supplemented. Brine shrimp starved for 24 hours were fed to barramundi larvae from day 8 to day 12; from day 13 to day 18 the larvae were fed on supplemented brine shrimp. Supplementary feeding of brine shrimp took place in a 50 litre glass aquarium. Microcapsules were added at 0.3 g/l and brine shrimp were maintained at densities of 0.5-1.0 million per litre.

EXPERIMENTAL LARVAL REARING UNIT

The experimental larval rearing unit used in these experiments comprised 22 individual chambers each of 2 litres capacity. The design of the unit ensured that fish in each chamber were subjected to identical conditions of water quality, temperature and light. Water from a header tank gravity-fed to the rearing chambers, from which it drained via small nylon screens (63, 120 or 200 microns aperture) which could be changed to permit the retention of various sized organisms. The water from the rearing chambers collected in a sump and was pumped back to the header tank via a biological filter. Temperature was maintained at $29 \pm 1^\circ\text{C}$ by air-conditioning the room and heating water in the header tank. Lighting was provided by fluorescent lamps at an intensity of 400 lux at the water surface. Water quality parameters (temperature, pH, salinity, ammonia, nitrite and nitrate) were monitored daily.

EXPERIMENTAL DESIGN

For the experiments described here, 16 chambers were used to test 4 replicates of 4 diets in a block design. The diets tested were :

- Diet 1* : Rotifers reared on *Chlorella* only; newly hatched brine shrimp.
- Diet 2* : Rotifers reared on *Chlorella* then supplemented with Frippak « Booster »; newly hatched brine shrimp.
- Diet 3* : Rotifers reared on *Chlorella* only; brine shrimp starved for 24 h (day 8 to day 12), then brine shrimp starved for 24 hours before supplementation with Frippak « Booster » (from day 13 on).
- Diet 4* : Rotifers reared on *Chlorella* then supplemented with Frippak « Booster »; brine shrimp starved for 24 hours (day 8 to day 12), then brine shrimp starved for 24 h before supplementation with Frippak « Booster » (from day 13 on).

These diets were designed to represent the original (unsupplemented) diet in use when the mortality syndrome was first encountered at N.F.R.C. and enhanced (supplemented) diets during both the rotifer and brine shrimp feeding phases of the larval rearing period. The larvae were fed twice daily to ensure that they had constant access to freshly supplemented food organisms. Before each feed, approximately 90 % of the water from each chamber was siphoned through a large surface area screen (200 or 400 microns) to remove food organisms while retaining barramundi larvae.

The experiment was run twice, once to determine daily mortality patterns (N1) and again to investigate the effects of the different diets on growth (N2). The same procedures and experimental design were used in both experiments. The density of hatched larvae was estimated volumetrically; 545 larvae were introduced to each chamber for experiment N1 and 133 for N2. Mortalities were monitored daily by counting dead larvae, although in practice, mortalities could only be accurately estimated after about day 10, when larvae were large enough to leave visible corpses. Experiment N1 was terminated at day 30 when cumulative mortalities in two treatments were at or near 100 %. Experiment N2 was terminated at day 22, when all survivors were preserved in 10 % formalin for later measurement of total length (TL); this measurement was used to compare growth between treatments.

HISTOLOGY

Samples of dead and live moribund larvae were taken for histological examination at irregular intervals. Most of the larvae sampled live at day 22 in experiment N2 (a total of 389 larvae) were used for histological examination. Specimens for histology were preserved in 10 % formalin, processed using conventional wax embedding techniques, sectioned and stained with haematoxylin and eosin.

FATTY ACID ANALYSES

Samples for fatty acid analysis were sieved to remove small particles (particularly algal cells and microcapsules) and then extracted with chloroform/methanol (2:1 v/v) using the modified methods of Folch *et al.* (1957) and Bligh and Dyer (1959) and stored under nitrogen at -25°C until analysed.

The excess solvent was removed using a rotary evaporator and the lipid residue taken up in a minimum of hexane. The base-catalysed transesterification procedure of Christopherson and Glass (1969) was used to prepare the fatty acid methyl esters from the lipid solution. The esters were separated by gas-liquid chromatography on a Shimadzu R1-A with a $2.1\text{ m} \times 3\text{ mm i.d.}$ glass column packed with 15% OV-275 on 100/120 Chromosorb PAW-DMCS. The column oven was temperature programmed from 190° to 220°C increasing at $2^{\circ}\text{C}/\text{min}$ and the carrier gas (nitrogen) flow rate was $65\text{ mL}/\text{min}$.

The peaks were identified and quantified on a Shimadzu RPR-G1 GC processor calibrated using the methyl esters of authentic triacylglycerol standards supplied by Sigma (Sigma Chemical Co., St Louis, MO, USA). A comparison was also made with a standard methylated cod liver oil sample supplied by R. Johns of the University of Melbourne.

RESULTS

Nutritional Enhancement of Live Food Organisms

The effects of supplementary feeding with Frippak « Booster » microencapsulated diet on the fatty acid composition of rotifers and brine shrimp are shown in Figures 1 and 2. The fatty acid composition of barramundi eggs (which Dendrinis and Thorpe (1987) suggested reflects the optimal fatty acid composition of the larval diet) is also shown for comparison.

The fatty acid composition of the rotifers fed to barramundi larvae did not closely match the composition of the egg yolk (Fig. 1). Rotifers were found to contain lower levels of 16:0 than egg yolk, but higher levels of 16:1. Unsupplemented rotifers were deficient in four fatty acids which were found in barramundi egg yolk: 18:2, 22:4n-6, 22:5n-6 and 22:6n-3. Supplementation increased the levels of 22:6n-3 to about 3% which is still well below the level found in barramundi egg yolk (17%). Supplemented rotifers were still lacking 22:4n-6 and 22:5n-6 which were present in barramundi egg yolk. Levels of 20:5n-3 in unsupplemented rotifers (11-12%) were much greater than those found in barramundi egg yolk (4%) and supplementation of rotifers only provided a slight increase in 20:5n-3 to 13%.

The fatty acid composition of brine shrimp did not closely match the fatty acid composition of barramundi egg yolk. Brine shrimp had lower levels of all the saturated fatty acids than was found in the egg yolk, but higher levels of 18:1, 18:2 and 18:3 (Fig. 2). The fatty acids 20:0, 20:4n-3

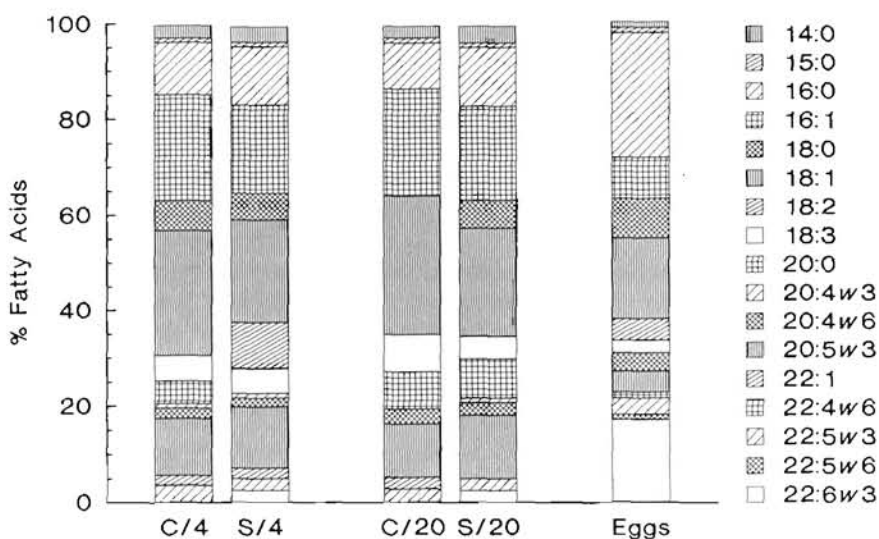


Fig. 1. — Fatty acid composition of rotifers used in diets 1-4. C/4 : not supplemented, used 4 hours after harvest (p.m. feed, diets 1 and 3); S/4 : supplemented at 1.0 g microcapsules/litre for 4 hours after harvest (p.m. feed, diets 2 and 4); C/20 : not supplemented, used 20 hours after harvest (a.m. feed, diets 1 and 3); S/20 : supplemented at 0.2 g microcapsules/litre for 20 h after harvest (a.m. feed, diets 2 and 4). Eggs : egg yolk from eggs stripped from spawning barramundi at Weipa, Queensland.

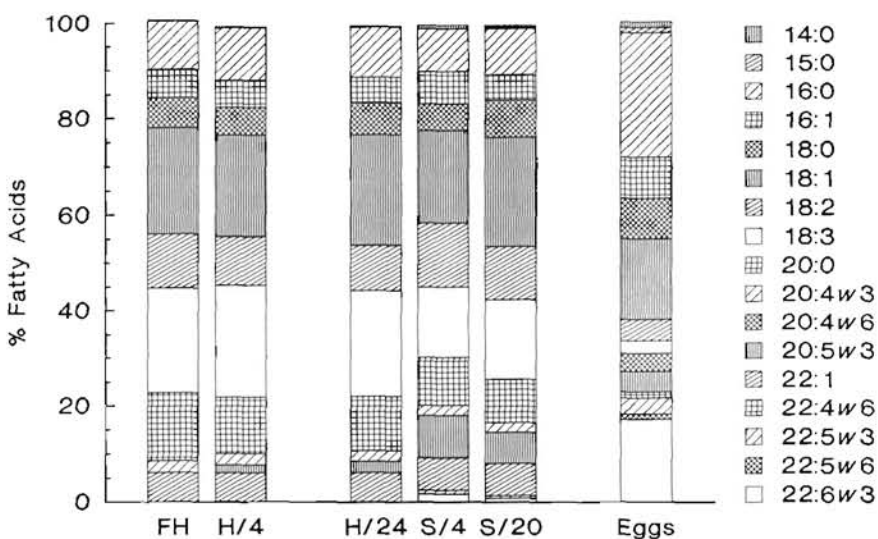


Fig. 2. — Fatty acid composition of brine shrimp used in diets 1-4. FH : freshly hatched brine shrimp (a.m. feed, diets 1 and 2); H/4 : brine shrimp harvested about 4 hours after hatching (p.m. feed, diets 1 and 2); H/24 : brine shrimp harvested about 24 hours after hatching (a.m. and p.m. feeds, diets 3 and 4, day 8 to day 12); S/4 : brine shrimp harvested about 24 hours after hatching, then supplemented at 0.3 g microcapsules/litre for 4 hours (p.m. feed, diets 3 and 4, day 13+); S/20 : brine shrimp harvested about 24 hours after hatching, then supplemented at 0.3 g microcapsules/litre for 20 hours (a.m. feed, diets 3 and 4, day 13+). Eggs : egg yolk from eggs stripped from spawning barramundi from Weipa, Queensland.

and 22:1 were present in brine shrimp but not in barramundi egg yolk. Freshly hatched brine shrimp were deficient in 5 fatty acids found in barramundi egg yolk: 20:4n-6, 22:4n-6, 22:5n-3, 22:5n-6 and 22:6n-3. Brine shrimp supplemented with microcapsules showed increased levels of 20:5n-3 (7.9%), 22:5n-3 (1%) and 22:6n-3 (1.2%) (Fig. 2), but still lacked 20:4n-6, 22:4n-6 and 22:5n-6 which were present in barramundi egg yolk.

Water quality

Water temperature ranged from 28-30°C; pH from 7.8-8.0; salinity from 27-32 g/l; ammonia from 0-0.2 mg/l total NH₃; nitrite from 0-0.2 mg/l; nitrate was constant at about 20 mg/l.

Survival

The four test diets showed dramatically different effects on survival of barramundi larvae. Larvae fed on diets 1 and 2 began showing stress symptoms (pale colouration, erratic swimming followed by « fainting ») on day 18. Large-scale mortalities began on day 20 (N1) or day 21 (N2), with mortality tapering off after 5 or 6 days (Fig. 3 and 4). In comparison, larvae fed on diets 3 and 4 had negligible mortalities over the same period.

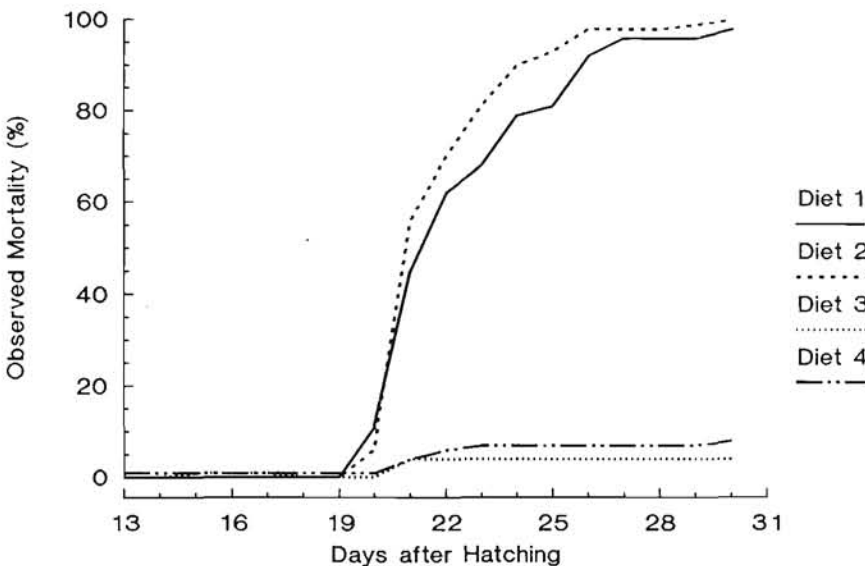


Fig. 3. — Cumulative mortality of barramundi larvae in experiment N1 from day 13.

Growth

The four test diets produced significantly different growth rates in barramundi larvae. Larvae fed on diets 3 and 4 were significantly larger

at day 22 than those fed on diets 1 and 2 (ANOVA, $P < 0.01$). Larvae fed on diets 1 and 2 averaged 8.29 mm TL and 8.69 mm TL respectively at day 22, while larvae fed on diets 3 and 4 averaged 9.98 mm TL and 10.48 mm TL respectively at day 22 (Fig. 5).

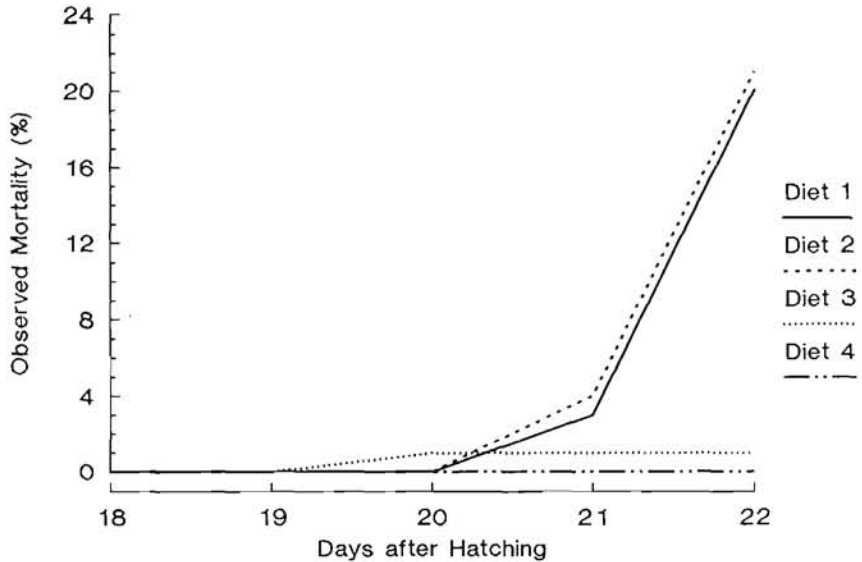


Fig. 4. — Cumulative mortality of barramundi larvae in experiment N2 (terminated at day 22).

When the effects of unsupplemented rotifers (used in diets 1 and 3) and supplemented rotifers (used in diets 2 and 4) were analysed, the results indicate that barramundi larvae fed on supplemented rotifers were significantly larger at day 22 than those fed on unsupplemented rotifers (ANOVA, $P < 0.01$). Larvae fed on unsupplemented rotifers averaged 9.11 mm TL while those fed on supplemented rotifers averaged 9.54 mm TL at day 22 (Fig. 6).

Similarly, barramundi larvae fed on starved and supplemented brine shrimp (used in diets 3 and 4) were significantly larger at day 22 than those fed on newly hatched brine shrimp (used in diets 1 and 2) (ANOVA, $P < 0.01$). Larvae fed on newly hatched brine shrimp averaged 8.47 mm TL while those fed on starved and supplemented brine shrimp averaged 10.20 mm TL at day 22 (Fig. 7).

Histology

Larvae fed on diets 3 and 4 showed no abnormal pathology. Several larvae fed on diets 1 and 2 showed some minor vacuolation of the spinal cord at day 22.

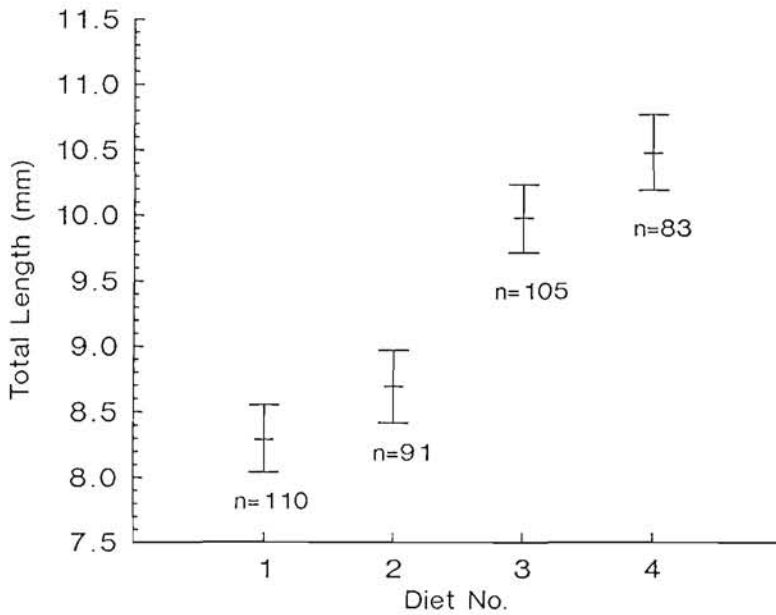


Fig. 5. — Total length of barramundi larvae at day 22, fed on four test diets (see text for details). Means and 95 % confidence limits shown; numbers below bars represent sample sizes.

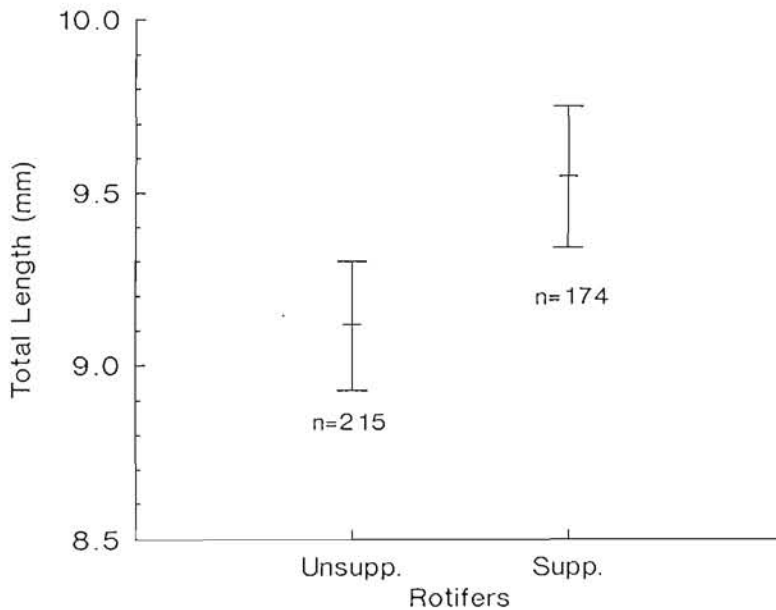


Fig. 6. — Total length of barramundi at day 22, fed on unsupplemented rotifers (diets 1 and 3) and supplemented rotifers (diets 2 and 4). Means and 95 % confidence limits shown; numbers below bars represent sample sizes. freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4). Means and 95 % confidence limits shown; numbers below bars represent sample sizes.

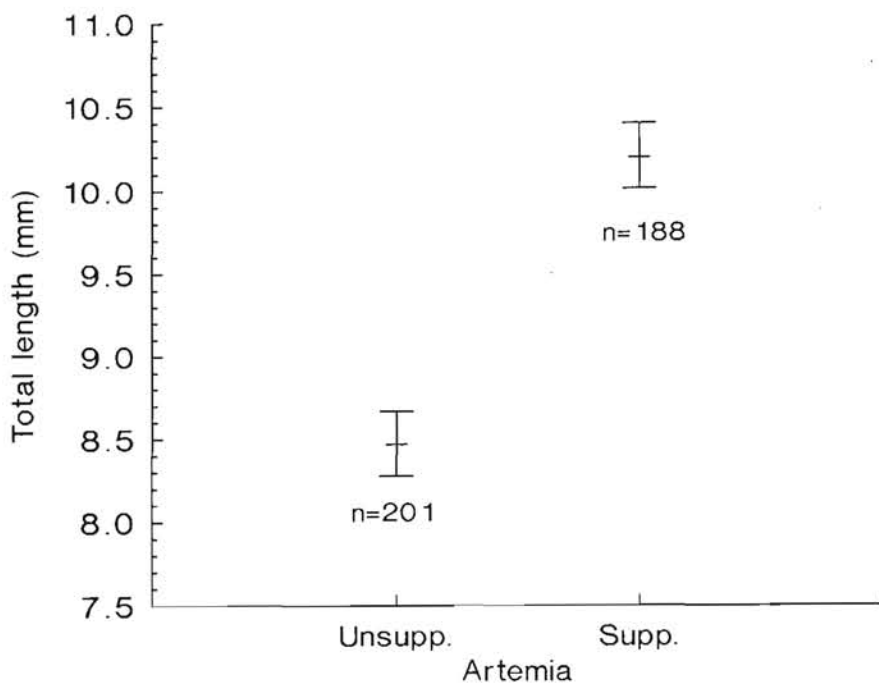


Fig. 7. — Total length of barramundi larvae at day 22, fed on freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4). Means and 95% confidence limits shown; numbers below bars represent sample sizes.

Tab. 1. — Presence (+) or absence (–) of polyunsaturated fatty acids found in barramundi egg yolk in the four dietary treatments used in experiments N1 and N2

Fatty Acid	Rotifers		Brine Shrimp	
	Unsupp.	Supp.	Newly hatched	Supp.
18 :2	–	+	+	+
20 :4n-6	+	+	–	–
20 :5n-3	+	+	+	+
22 :4n-6	–	–	–	–
22 :5n-3	+	+	–	+
22 :5n-6	–	–	–	–
22 :6n-3	–	+	–	+

DISCUSSION

Numerous studies have shown the importance of fatty acids, particularly long chain fatty acids of the n-3 series, in the diet of marine fish larvae. The essential fatty acids for marine fish are generally considered to be the C20 and C22 unsaturated fatty acids (Watanabe *et al.*, 1983; New, 1986), although survival in some species may also be limited by the levels of C18 fatty acids in the diet (Dendrinis and Thorpe, 1987).

Microencapsulated diets provide a convenient method of enhancing the fatty acid composition of live food organisms without the disadvantages inherent with other techniques, such as the degradation of water quality that may accompany the use of oil-based supplements (Rodgers and Barlow, 1987; Rimmer *et al.*, 1988). Several studies (Walford and Lam, 1987; Ahmed and Jones, 1988; Budd, 1988) have investigated the relationship between microcapsule concentration and supplementation duration for enhancing the polyunsaturated fatty acid composition of rotifers and brine shrimp using a microencapsulated diet. Generally, short periods of supplementation (4-8 hours) have been found to be most effective in increasing the levels of polyunsaturated fatty acids (Walford and Lam, 1987; Ahmed and Jones, 1988; Budd, 1988).

In the present study, levels of 22 :6n-3 were maintained for up to 20 hours supplementation at a level comparable with that found after 4 hours supplementation (Fig. 1 and 2).

Dendrinis and Thorpe (1987) suggested that the requirements of Dover sole (*Solea solea*) larvae for 20 :5n-3 and 22 :6n-3 fatty acids during early larval development could be met by the small quantities in the food used, and that after this period these requirements could be met by elongation and desaturation of shorter-chain fatty acids such as 18 :3n-3. If this hypothesis is adopted for barramundi larvae, the presence or absence of a particular fatty acid may be more important than the precise proportions of each fatty acid in the organism.

The C14, C16 and C18 saturated and monounsaturated fatty acids found in barramundi egg yolk were also found in the live food organisms fed to the larvae (Fig. 1 and 2). However, many of the polyunsaturated fatty acids found in barramundi egg yolk were absent from the live food organisms used in the test diets; these are summarised in Table 1.

The major difference in the fatty acid composition of supplemented and unsupplemented rotifers was the presence of 18 :2 and 22 :6n-3 in supplemented rotifers (Fig. 1). Barramundi larvae fed rotifers deficient in these two fatty acids showed similar survival to those fed rotifers with enhanced levels of 18 :2 and 22 :6n-3, suggesting that these fatty acids are not essential for survival of barramundi larvae. In addition, the absence of 18 :2 and 22 :6n-3 from the rotifer diet did not predispose the larvae to the effects of the mortality syndrome seen at day 20+ (Fig. 3).

The main differences between the fatty acid composition of diets 1 and 2 (freshly hatched brine shrimp) and diets 3 and 4 (starved and

supplemented brine shrimp) were the presence of 22 :5n-3 and 22 :6n-3 and the increased levels of 20 :5n-3 in the supplemented brine shrimp used in diets 3 and 4 (Fig. 2). Unsupplemented brine shrimp were found to lack 22 :5n-3 and 22 :6n-3; newly hatched brine shrimp lacked 20 :5n-3 but brine shrimp used about 4 hours after hatching had a low concentration of 20 :5,n-3 (2%). However, since a deficiency of 22 :6n-3 did not adversely affect survival of barramundi during the rotifer feeding phase, it is unlikely that such a deficiency would cause extensive mortalities during the brine shrimp feeding phase, when larval organogenesis is well advanced and the metabolic functions of the larvae are presumably more competent (Kendall *et al.*, 1984; Dendrinis and Thorpe, 1987). The deficiency of 22 :5n-3 and the low level of 20 :5n-3 in newly hatched brine shrimp may have caused, or contributed to, the mortalities seen in fish reared on diets 1 and 2. Alternatively, the overall deficiency of C20 and C22 polyunsaturated fatty acids in newly hatched brine shrimp may have influenced survival of barramundi larvae fed on these diets.

Supplementation of both rotifers and brine shrimp also improved growth rates. The difference in the mean size at day 22 of fish fed on diet 1 (8.29 mm TL) and those fed on diet 4 (10.48 mm TL) is substantial and would offer real advantages to hatcheries using intensive culture techniques by reducing the length of time the fish are in the hatchery. Faster growth (presumably associated with better nutrition) may also contribute to better « quality » of larvae and fry.

Future research into the nutritional requirements of barramundi larvae will involve experimental investigation of the effects of varying proximate composition and amino acid composition of the live food organisms used during intensive rearing.

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