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Assessment of Two Microbound Artificial Diets for Weaning Asian Sea Bass *(Lates calcarifer,* Bloch)

P. S. LEE[1, 2], P. C. SOUTHGATE[1] and D. S. FIELDER[3,4]

[1]*Zoology Department, James Cook University of North Queensland, Townsville, Queensland 4811, Australia*
[2]*Present address: Aquatas Pty. Ltd., P.O. Box 156, Kingston, Tasmania 7051, Australia*
[3]*Sea Harvest Pty. Ltd., Mourilyan, Queensland 4858, Australia*
[4]*Present address: NSW Fisheries, Port Stephens Research Centre, Taylors Beach, New South Wales 2301, Australia*

Abstract

Two microbound diets (MBD) containing marine fish roes were assessed as weaning diets for Asian sea bass/barramundi *(Lates calcarifer)* over a 5-d growth trial. The larvae were successfully weaned on the two MBD tested which supported greater growth than a commercially available weaning diet developed for European bass *(Dicentrarchus labrax)* which is currently used as a weaning diet in commercial culture of *L. calcarifer* in Australia. The results indicate that marine fish roes are a suitable basis for weaning diets for *L. calcarifer,* and that MBD are a suitable way of presenting these diets. The inexpensive and easily prepared MBD used in this study may therefore represent a viable alternative to the commercial diets currently available to marine finfish hatcheries.

Introduction

The inclusion of artificial food in the diet of marine fish larvae (weaning) is a critical stage in intensive larval rearing. Weaning reduces the dependence on live feeds, and therefore reduces hatchery running costs. Person-Le Ruyet (1991) described three weaning strategies which have been applied to different species of marine fish larvae, with differing levels of success; 1) weaning at first feeding has been achieved with plaice *(Pleuronectes platessa)* and sole (Solea solea), although with lower survival than achieved with live feeds (Person-Le Ruyet 1991); 2) larvae may be reared for some time on live feed, which is then replaced abruptly with artificial feed; this strategy is used for European sea bass *(Dicentrarchus labrax)* (Person-Le Ruyet 1991), or; 3) the replacement may be gradual, occurring over several days, as occurs in the culture of red sea bream *(Pagrus major)* and Japanese flounder *(Paralichthys olivaceus)* (Kanazawa et al. 1989).

Asian sea bass or barramundi (*Lates calcarifer*, Bloch) are widely cultured throughout Southeast Asia and Australia (Copland and Grey 1987). Studies to date on the use of artificial feeds for first feeding *L. calcarifer* larvae have been unsuccessful (Walford et al. 1991; Southgate and Lee 1993) because early *L. calcarifer* larvae are unable to digest artificial food particles. Walford and Lam (1993) showed that digestive enzyme activity is low in early *L. calcarifer* larvae but increases as the larvae approach metamorphosis. As such, larval rearing of *L. calcarifer* is still dependent on the provision of live food organisms such as rotifers (*Brachionus* sp.) and *Artemia*.

Weaning of *L. calcarifer* typically occurs close to metamorphosis, approximately 20-25 d after hatching (Mackinnon 1987; Fuchs and Nedelec 1991). In Asian hatcheries, *L. calcarifer* larvae are commonly weaned onto finely minced fish flesh (Maneewong 1987); however, dry formulated feeds are used for weaning intensively reared larvae in Australia (MacKinnon 1987). At Sea Harvest, a commercial producer of *L. calcarifer* in north Queensland, Australia, a commercially available microparticulate diet (Sevbar 1°Age and 2°Age, Frippak Feeds, Sanofi Aquaculture, France) is used routinely for weaning. Although developed for weaning European sea bass and sea bream (*Sparus auratus*), these diets have been used very successfully for weaning *L. calcarifer*. At Sea Harvest, weaning commences at the completion of the larval phase and is routinely conducted over a 3-7 d period.

This study assesses the potential of two microbound diets (MBD) containing marine fish roes as a weaning diet for *L. calcarifer*, and compares growth with that obtained with Sevbar. MBD are composed of nutrients trapped with the particle matrix. They can be manufactured in a range of sizes and have been used successfully as artificial feeds for the larvae of a number of species of fish (Adron et al. 1974; Teshima et al. 1982; Kanazawa et al. 1989). Marine fish roes have shown promise as a basis for starter diets for fish in previous studies (Garatun-Tjeldsto et al. 1987, 1989) and, in general, they have a biochemical composition suitable for use in marine fish diets (Fernandez-Reiriz et al. 1988). In particular, they contain high levels of n-3 highly unsaturated fatty acids (HUFA) (Tocher and Sargent 1984; Fernandez-Reiriz et al. 1988; Vlieg and Body 1988), which are essential for dietary nutrients for marine fish larvae including *L. calcarifer* (Rimmer et al. 1994).

Materials and Methods

MBD were made containing either mackerel (*Scomberomorus commerson*) or mullet (*Mugil* sp.) roe. Frozen roes were thawed, skinned and homogenized using a Silverson L4R homogenizer. The homogenates were then freeze-dried and powedered in a ball-mill. MBD containing the powdered roe were manufactured as described by Teshima et al. (1982). Gelatin (swine skin, 300 bloom) was used to bind the mackerel roe (diet MBD/G) and carrageenan to bind the mullet roe (diet MBD/C). The roe/binder mixtures were freeze-dried, milled and sieved to an appropriate particle size (Table 1). Due to the adhesive properties of diet MBD/C, it could only be sieved to a size range of

Table 1. Proximate composition and particle size of the weaning diets (% dry weight).

Component	Diet		
	Sevbar[1]	MBD/G	MBD/C
Protein[2]	55.0	51.5	58.7
Lipid[3]	10.0	17.3	21.9
Ash[4]	14.0	4.8	3.9
Moisture[5]	6.0	3.0	2.9
Particle size (μm)	100-300	118-200	300-500

[1]Manufacturer's information.
[2]Determined as Kjeldhal nitrogen x 6.25.
[3]Determined gravimetrically after chloroform:methanol extraction (Folch et al. 1957).
[4]Mass remaining after combustion at 500°C for 15 h.
[5]Determined after drying for 24 h at 100°C.

300-500 μm. All MBDs were vacuum-packed in plastic sachets and stored in a refrigerator until use (approximately 2 weeks). The final level of gelatin and carrageenan in the complete dried diets was 10% and 5% (w/w), respectively. The proximate composition of each MBD is shown in Table 1.

After hatching, larvae were reared intensively according to a standard protocol similar to that described by Rimmer and Reed (1991). Larvae were initially fed a diet of rotifers (*Brachionus* sp.) and later *Artemia* nauplii followed by adult *Artemia* and advanced nauplii. At 23 d post-hatch, at a total length (TL) of 7-10 mm, fish were stocked into aerated 10-l plastic aquaria at a density of 20 l-1. Three replicate aquaria were used to assess each of four dietary treatments; MBD/C, MBD/G, Sevbar 1° Age and controls.

During the first 2 d of the growth trial, larvae receiving artificial diets were fed these at a rate of 10% body wet weight in each of two morning feeds, approximately 3 h apart, followed by a single afternoon feed of adult *Artemia* at a rate of 3 ml^{-1}. For the final 3 d of the trial, artificial diets were fed to larvae at a rate of 10% body wet weight in each of four feeds, approximately 3 h apart. Larvae in the control group received no artificial diets. Artificial diets were resuspended in 1 μm filtered seawater by vigorous shaking, and introduced dropwise to the aquaria. Daily body wet weight estimates were obtained from tabulated values of age, length and wet weight of cultured *L. calcarifer* (D.S. Fielder, unpubl. data).

Water changes were carried out prior to feeding on each day, and 2 h after final feeding on the final 3 d. At each water change, approximately 80% of the water was removed by siphoning through a 390-μm nylon mesh screen. The trial was conducted over 5 d, at the end of which fish were collected in a 390-μm mesh screen, washed with filtered seawater followed by 3% (w/v) ammonium formate, and oven dried at 50°C. Thirty randomly chosen individuals from each replicate aquarium were weighed using a Cahn 21 Electrobalance. Ash was determined as mass remaining after heating at 500°C for 4 h. Data were analyzed using nested ANOVA and significantly different means were determined using the Least Significant Difference test (Zar 1984).

Results

Survival, standard length, dry weight and ash content of larvae from each dietary treatment are shown in Table 2. Highest mean survival was shown by control larvae; however, survival did not differ significantly ($P>0.05$) between treatments.

Larvae in the control group decreased in dry weight during the experiment. In contrast, larvae receiving all three of the weaning diets increased in dry weight during the trial, although, final dry weight did not differ significantly between treatments ($P>0.05$); however, in contrast to larvae receiving Sevbar, those receiving the MBDs had a significantly greater dry weight at the completion of the trial than control group larvae ($P<0.05$) (Table 2).

Table 2. Survival, length, dry weight and ash-free dry weight (mean ± SEM) of *L. calcarifer* larvae at the completion of the weaning trial.

Diet	Survival (%)	Length (mm)[1]	Dry weight (mg)[2]	Ash (%)[3]
Control	90.7±6.06[a]	7.87±0.37[a]	2.42±0.11[a]	17.57±0.24
Sevbar	76.3±8.57[a]	8.30±0.30[a]	3.17±0.14[a,b]	16.81±0.31
MBD/C	85.3±2.85[a]	8.66±0.25[a]	3.49±0.17[b]	16.56±0.04
MBD/G	63.0±6.43[a]	8.21±0.31[a]	3.45±0.12[b]	17.92±1.06

[1]Initial length 8.49 (±1.13) mm.
[2]Initial dry weight 3.03 (±0.14) mg.
[3]Initial ash content 16.65 (±0.20) %.
[a,b]Figures in columns sharing a common superscript are not significantly different ($P>0.05$).

Discussion

Barramundi larvae were successfully weaned on the two MBD tested, under the experimental weaning protocol. Both MBD had suitable physical characteristics to allow ingestion, and growth of larvae fed these diets indicates that the larvae could digest ingested particles. The results show that mackerel and mullet roe are a suitable basis for a weaning diet for *L. calcarifer*. Both diets supported greater growth of *L. calcarifer* larvae than achieved with the commercial weaning diet.

Previous studies have shown that first-feeding *L. calcarifer* larvae readily ingest the MBD described here, but are unable to digest them and ultimately starve to death (Southgate and Lee 1993). Similarly, *L. calcarifer* larvae are unable to digest protein-walled microcapsules although digestion is enhanced if fed in combination with rotifers (Walford et al. 1991). The results of this study indicate that older *L. calcarifer* larvae are able to digest both gelatin and carrageenan MBD and assimilate their nutrients. Walford and Lam (1993) showed that older *L. calcarifer* larvae are better equipped for digesting artificial food particles; the development of the stomach and pepsin-type enzyme activity in the gut of *L. calcarifer* larvae is not well established until they approach metamorphosis.

The major potential problem with using artificial diets for larval fish is that of maintaining water quality. Dry artificial food particles are usually negatively buoyant and may settle from suspension and decompose; particles may be subject to direct bacterial attack (Muir and Sutton 1994) and also allow nutrients to leach into culture water. All three artificial feeds used in this study resulted in a certain degree of cloudiness to the culture water indicating that water quality was adversely affected. While more efficient particle suspension may be obtained through appropriate tank design and aeration (Backhurst and Harker 1988), reduced nutrient leaching from MBD may be possible through microencapsulation of the food particles (Langdon et al. 1985).

MBD composed of various binders have now been used with a certain degree of success with the larvae of a number of marine fish (Adron et al. 1974; Teshima et al. 1982, 1984; Kanazawa et al. 1989). The nutritional value of MBD has, however, been shown to vary with the type of binder used. For example, Fuchs and Nedelec (1991) found alginate-bound MBD to be nutritionally inferior to carrageenan-bound diets; they proposed that this was a result of the inability of larvae to digest alginate-bound diets. The growth of larvae in this study indicates that *L. calcarifer* larvae are capable of digesting MBD bound with gelatin and carrageenan. The MBD used in this study had noticably different physical characteristics; the gelatin-bound diet was drier and less sticky than the carrageenan-bound diet and the stickiness of the latter prevented it from being seived to less than the 300 mm. This may be related to differences in the dietary ingredients (e.g., binders, roe) or it may reflect differences in chemical composition of the MBD. For example, carrageenan-bound MBD had a higher lipid content (21.9%) than gelatin-bound MBD (17.3%) and this may have affected the adhesive properties of the dried diet.

The MBD used in this trial are simple and relatively cheap to prepare, and they supported good rates of growth when compared to a commercial weaning diet for marine fish larvae. As such, they may offer a viable alternative to commercial diets currently available to marine finfish hatcheries. The simplicity of their preparation may also allow hatcheries to prepare weaning diets on-site ensuring fresh artificial weaning diets are available when required.

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