Advances in the Nutrition and Feeding of the Bullseye Puffer *Sphoeroides annulatus*

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ABSTRACT

The present report describes the research done to date on the nutritional requirements and feeding of the bullseye puffer *Sphoeroides annulatus*. After hatching, the larvae of the species have been successfully reared with the microalgae *Isochysis* sp. and *Nannochloropsis oculata*, the rotifer *Brachionus rotundiformis* and the brine shrimp *Artemia*. The live food feeding schedule starts at day 1 post-hatchand last until day 29 post-hatch, time when the larvae are weaned onto artificial microdiets prepared with protein sources adequated to the capacities for digestion of marine fish larvae. Weaning is done over a five days period with a post-weaning survival of 49%. The highest growth in juveniles fed dry diets with different protein levels was achieved with a 55% protein content. As for the lipids, a preliminary experiment indicates that 5.6% lipid in the diet produces better growth than a diet with 9.5%. Practical dry diets with different protein sources for the grow-out of *S. annulatus* have been tested, the best results were obtained with a diet prepared with fresh ingredients. Further testing of different meals of marine origin is currently done to formulate dry diets for the grow-out of the species. Broodstock feeding is currently done with a mixed ration of a dry diet formulated with 42% protein and 14% lipid with a trout diet with 42% protein and 10% lipid.

INTRODUCTION

Several marine fish species are subject of study in Mexico to evaluate their potential for fish farming (Avilés-Quevedo 2000). The bullseye puffer (*Sphoeroides annulatus* Jenyns, 1842) is a marine fish with high potential for aquaculture in the Pacific coast. This species is found throughout the Gulf of California and along the eastern Pacific from San Diego, USA to Peru (Thomson, Findley & Kerstitch 1987). This fish has economic importance in northwest Mexico where it can reach a market price of US$ 5/kg gutted and headed fish or

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US$ 9/kg fillet. *S. annulatus* is a similar species to the tiger puffer *Takifugu rubripes*, a high value species cultured in Japan and Korea (Kanazawa 1980; Yang, Cho & Kim 1993; Takii *et al.,* 1995). The bullseye puffer might become one of the first marine fish species in the Mexico to be cultivated at commercial scale from hatchery-produced larvae to grow-out fish. Recent advances in the fish maturation and reproduction (Duncan *et al*., in press), studies on the embrionary development (Martínez-Palacios *et al*., in press) and the controlled production of larvae and juveniles (Abdo de la Parra *et al*., 2001; García-Ortega, Abdo & Hernández in press) made possible the production of fish for the study of its nutrient requirements and feeding. Consequently, it is feasible the evaluation of the species for its culture at commercial scale. At present, there are not commercial feeds available for the grow-out of this species. Thus, in this report are presented the advances obtained to date on the nutritional research for the development of feeds and feeding strategies for the culture of *S. annulatus*.

NUTRITION AND FEEDING OF LARVAE

Live food

After hatching, marine fish larvae rely on their own nutrient resources in the yolk sac for growth. The duration of the yolk reserves depends on the egg and fish size and the environmental conditions. Once the yolk sac reserves are depleted, the acquisition of nutrients in first feeding fish will depend on the ingestion of plankton organisms found in their natural habitat. Under culture conditions, the most used live food organisms to fed marine fish larvae are different species of microalgae and copepods, the rotifer *Brachionus* sp. and the brine shrimp *Artemia*. They provide the best results in terms of fish larvae growth and survival in comparison to artificial diets (Jones, Kamarudin & Le Vay 1993; Watanabe & Kiron 1994). In the larviculture of *S. annulatus*, a protocol of live food feeding for hatchery-produced larvae has been developed for the first days to above 30 days after hatching (Abdo *et al*., 2001) (Fig. 1). At 26-29°C, *S. annulatus* larvae start the exogenous feeding 4-5 days after hatching, therefore, at day 1 after hatching a mix of microalgae (*Isochrysis* sp. and *Nannochloropsis oculata*) is added to the larval tanks at a concentration of 100,000 cells per ml to pre-condition the water in the tanks. The microalgae are added continuously until day 11 post-hatch. Mixed microalgal diets provide essential nutrients for animal growth and development and are more nutritionally balanced than single microalgal diets (Brown, Jeffrey & Garland 1989). Moreover, microalgae stimulate the digestive processes in marine fish larvae and contribute to the stablishment of the early gut flora (Reitan *et al*., 1997). The microalgae also served as food for the rotifer *Brachionus rotundiformis*, which is used to fed the larvae from day 4 to 11 post-hatch at a density of 5 rotifers per ml, and from day 11 to 20 post-hatch at a density of 10 rotifers per ml. The rotifers are grown in a mix of *Isocrhysis* sp. and *N. oculata*. During the microalgae and rotifer feeding period, the food density is monitored four times a day and adjusted to maintain the required density. The amount of rotifers is reduced to 5 per ml from day 20 until day 26, which is the last days of rotifer feeding. Approximately at day 20 the fish mouth is sufficiently big to start feeding on newly hatched *Artemia* nauplii, which are offered to the fish larvae four times daily in increasing amounts from 0.1 to 3 nauplii per ml until day 29 post-hatch when the weaning period is started. Hatching, harvesting and storing of *Artemia* are done following the procedures described for its use in aquaculture (Sorgeloos *et al*., 1996). The growth of *S. annulatus* larvae fed according to the described live food protocol is shown in Fig. 1. During the rotifer phase the growth rate of bullseye puffer does not present an exponential increase as in the larvae of other fish species (Conceição, 1997). The growth rate improves during the feeding phase with *Artemia* nauplii from day 21 post-hatch onwards. Throughout the larval stages of marine fishes, growth and survival are affected mostly by the nutritional factors. The content of essential fatty acids in rotifers and *Artemia* is an important factor affecting their nutritional value as food for marine fish larvae. Because both live good organisms are deficient in two essential fatty acids, i.e. ecoisapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3), different techniques have been developed for their nutritional improvement with oil emulsions rich in highly unsaturated fatty acids (HUFA) (Sorgeloos, Léger & Lavens 1988) or by the use of selected microalgae (Brown *et al*., 1977). For larvae of *S. annulatus* it is expected that growth and survival during the live food phase can be improved by enrichment of rotifers and *Artemia* with HUFA, as it has been demonstrated in the tiger puffer (Han, Yoshimatsu & Kitajima, 1995) and the larvae of other marine fish species (Rainuzzo, Reitan & Olsen, 1997; Sargent *et al*., 1999). Thus, further research is necessary in larvae of bullseye puffer to improve the larval growth and survival by the use of enriched rotifers and *Artemia*, and by the use of selected microalgae and copepods with adequate HUFA profiles.

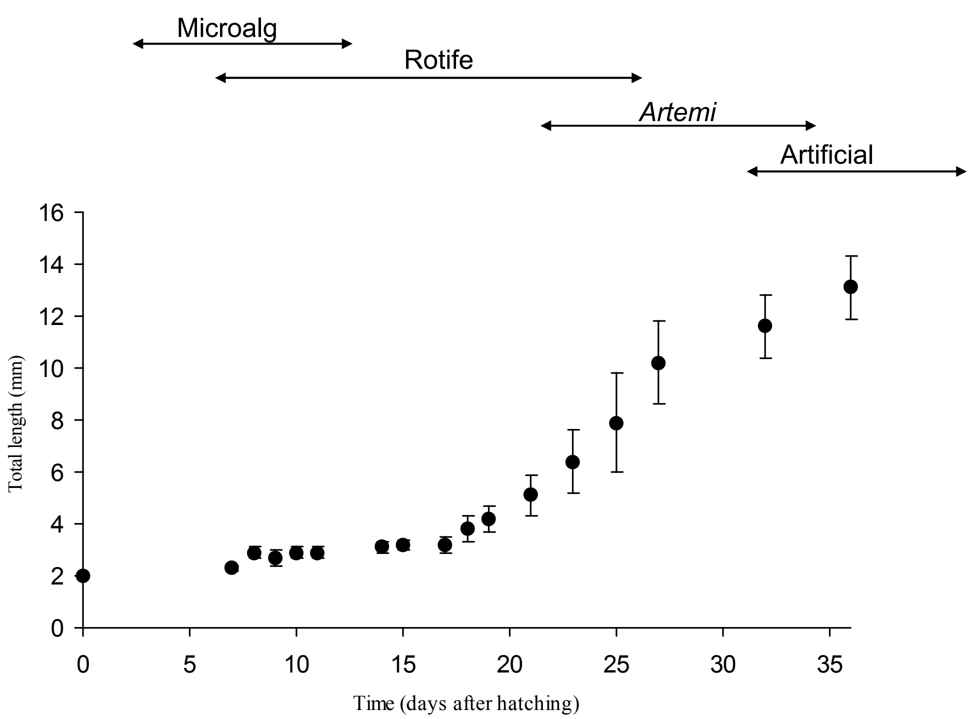

Fig. 1. Mean total length of bullseye puffer larvae during the live food feeding phase.

Weaning diets

Weaning experiments were carried out to test the suitability of experimental microparticulated diets for feeding hatchery-produced *S. annulatus* larvae, to test a weaning protocol for the species and to determine earlier weaning times (García-Ortega *et al*., in press). In a first experiment two experimental diets were prepared with different protein sources: diet 1 with decapsulated cysts of *Artemia* and fishmeal, and diet 2 with a combination of fishmeal, squid, tuna gonad and shrimp meal. The ingredients were mixed with water and a binder, milled, dried and ground to a particle size of 420-700 µm. The protein and lipid contents in the diets were respectively: 48.4 and 12.3% in diet 1 and 54.1 and 13.5% in diet 2. Both diets were tested with 60-days-old fish, which were fed with live food until this age. Weaning was done in five days by gradually reducing the numbers of *Artemia* nauplii until their complete substitution by the artificial microdiet . Fish survival after 11 weeks of feeding was 92% for diet 1, 85% for diet 2, and 95% for the control fish fed *Artemia* nauplii. Among the artificial microdiets, higher final weight and length were obtained with diet 1. This experiment demonstrated that bullseye puffer can be adequately reared with artificial dry diets. Therefore, diet 1 was further ground to a particle size of 190-420 µm and was used for testing earlier weaning times to reduce the period of live food feeding. For this experiment, three different times for initiation of weaning were tested in sibling fish larvae, i.e. at 29, 34, and 39 days post-hatch. After 23 days of feeding, small differences in weight, length and survival were found among weaning treatments. When weaning started at day 29 post-hatch, the fish larvae grew from an initial weight of 38.4 mg and length of 11.1 mm to a final weight and length of 405.7 mg and 25.1 mm respectively. Small differences in growth were found when compared to the other two weaning treatments (Fig. 2). Final survival when weaning at day 29 post-hatch was 49.3%. These studies demonstrated that microparticulated diets formulated with a combination of decapsulated cysts of *Artemia* and fishmeal as protein source are suitable weaning diets for bullseye puffer larvae. In previous nutritional studies with fish larvae, the combination of decapsulated cysts of *Artemia* with fishmeal provided a diet with higher protein digestibility than a diet with fishmeal as the sole protein source (García-Ortega *et al*., 2001). Thus, feeding studies with *S. annulatus* indicate that the larvae can be weaned at a weight of 38.4 mg or at day 29 post-hatch and possibly earlier without considerable reduction of growth or survival compared to fish weaned at day 39 post-hatch. This represents an attractive aspect of the species to take into consideration for its culture at commercial scale.

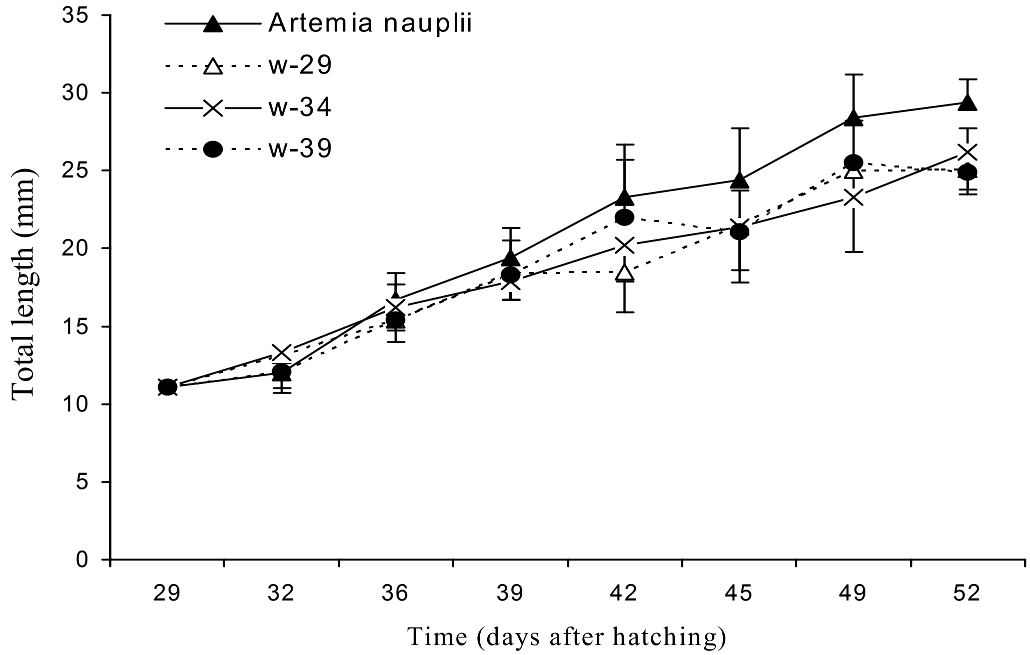

Fig. 2. Mean total length of bullseye puffer larvae weaned at 29, 34 and 39 days post-hatch (w-29, w-34 and 2-39 respectively). One control treatment was fed exclusively *Artemia* nauplii.

NUTRIENT REQUIREMENTS OF JUVENILES

Protein

A feeding experiment was conducted to study the effect of different protein levels on growth of juvenile bullseye puffer. Seven isocaloric diets were formulated with different protein levels (30, 35, 40, 45, 50, 55 and 60% diet dry weight). Fish meal (80% of total dietary protein) and casein (20% total dietary protein) were used as protein sources with fish oil as the lipid source with a formulation of 10% in each diet (Table 1). Each dietary treatment was tested in triplicate with 40 fish in each 300 liter tank with an initial fish weight of 2.5 (±0.1) g. Fish were fed *ad libitum* five times daily during eight weeks. Temperature, salinity and fish survival were monitored daily and fish weight was measured every two weeks. The mean temperature during the experiment was 29.6 (±1.5) °C, the salinity was constant at 34 ppt and survival was above 90% in all diet treatments. At the end of the experiment, the highest growth was obtained in fish fed the diet with 55% protein followed by the fish fed the diets with 50 and 60% protein (Fig.3). A similar protein requirement was estimated for the tiger puffer *T. rubripes* for which a level of 50% protein in the diet provided the highest growth, followed by the diets with 45 and 60% (Kanazawa *et al*., 1980). Similar results have been obtained for other marine fish species (Tucker 1992; Kaushik, 2002; Koven, 2002).

Table 1. Formulation (% dw) and proximate composition of semi-purified diets for the determination of protein requirement in *S. annulatus* juveniles.

	Diets (% protein)						
Ingredients	30	35	40	45	50	55	60
Fish meal	35.3	41.2	47.1	53.0	58.8	64.7	70.6
Casein	7.1	8.3	9.5	10.7	11.9	13.1	14.3
Fish oil	8.5	8.2	7.9	7.6	7.4	7.1	6.9
Dextrin	44.1	35.3	28.5	21.7	14.9	8.1	1.2
Vitamin premix	2	2	2	2	2	2	2
Mineral premix	2	2	2	2	2	2	2
Vitamin C	1	1	1	1	1	1	1
Alginate	2	2	2	2	2	2	2
Proximate composition (% dw)							
Protein	32.6	37.2	43.4	48.5	51.3	57.2	63.4
Lipid	8.1	6.7	7.5	6.9	7.5	7.8	7.0
Ash	9.0	10.0	11.4	12.4	13.1	15.1	15.7
Estimated nutrients							
Protein	30	35	40	45	50	55	60
Lipid	10	10	10	10	10	10	10
Energy kJ/g diet	18.3	18.3	18.4	18.4	18.4	18.5	18.5

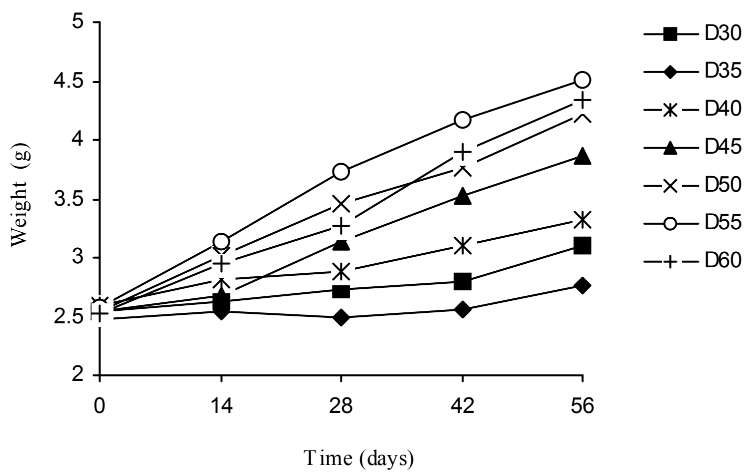

Fig. 3. Mean growth in weight of bullseye puffer juveniles fed seven isocaloric diets with different levels of protein: 30, 35, 40, 45, 50, 55,and 60% (dw of the diet).

Lipids

A preliminary study with 19 g bullseye puffer showed that this species probably has low lipid requirements. Two isonitrogenous diets with fish oil (cod liver oil) as lipid source were tested. The diet containing 5.6% lipid yielded higher growth rate than a diet with 9.5% lipid. The optimum lipid level, as determined with pollack liver oil in diets for *T. rubripes* was determined at 6% or less (Kanazawa *et al.*, 1980). The lipid requirement for puffer fish is lower than other marine fish such as yellowtail (15% with pollack liver oil and 45% dietary protein) (Deshimaru, Kuroki & Yone, 1982) and red sea bream (15% fish oil with 52% dietary protein) (Takeuchi, Shiina & Watanabe, 1991). Further research is necessary to determine the optimum level of lipid for *S. annulatus*. Likewise, it is necessary to determine the requirements of essential fatty acids, specifically HUFA's, in the different live stages of the species for the elaboration of balanced feeds for every culture phase.

Other nutrients

There is no information available on the requirements of *S. annulatus* for carbohydrates, amino acids, vitamins and energy. As with other marine fish species, these studies are necessary for the development of suitable growth-out diets. To date, the feed used for *S. annulatus* has been formulated following to the recently acquired knowledge on its specific nutritional requirements combined with the known requirements of *T. rubripes*.

PRACTICAL DIETS AND FEEDING PRACTICES

Juvenile and grow-out

Several materials and feeds were used in the first trials for maintenance and grow-out of bullseye puffer. Raw minced fish and squid were supplied to the fish in combination with trout pellets. Although the fish could be maintained in this way, their growth was generally low. In order to start a programme for the development of practical diets for the grow-out of the species, a feeding experiment was conducted to test dry diets formulated with different protein sources. Fish meal and shrimp head meal were used as the basal protein in all three diets in the experiment with complementary protein from squid liver meal in diet 1, crab meal in diet 2, and a mix of fresh tuna gonad and fresh squid in diet 3 (Table 2). The dry diets were tested in triplicate with 5.3 g fish fed during nine weeks and feeding was done until satiation five times a day. At the end of the experiment, the growth rate and the feed convertion ratio (FCR) were significantly higher with diet 3 (Table 2). The best results were obtained with the diet prepared with fresh ingredients, probably for their higher amino acid conetent and the enhaced palatibility by use of fresh squid (Tacon & Barg, 1998). However, for the development of practical diets for this species we aim to use dry meals that are commercially available to avoid the use of fresh ingredients in the diets preparation. In this sense, diets 1 and 2 provided good results that can be used as starting point for the development of a grow-out diet.

Table 2. Protein source (% dw) and proximate composition of dry practical diets for *S. annulatus*, and its specific growth rate and feed convertion ratio after a feeding period of nine weeks.

	Diet		
Protein source	1	2	3
Fish meal	41	52.6	33.2
Squid liver meal	30		
Crab meal		22.6	
Fresh tuna gonad			16
Fresh squid			15
Shrimp head meal	15	15	15
Proximate composition (% dw)			
Protein	50.6	53.3	49.6
Lipid	12.5	8.1	12.4
Ash	15.6	23.4	16.1
Dry matter	94.8	94.8	93.8
Specific growth rate*	1.17^{b}	1.4^{b}	2.07^{a}
Feed conversion ratio*	3.0^{b}	2.5^{b}	1.2^{a}

*Values in the same row with the same superscript are not significantly different (P>0.05).

A new dry diet was prepared considering the diet formulations in the previous experiment and this is currently tested with *S. annulatus* in grow-out trials in cages. The grow-out diet were formulated at 50% protein and 6% lipid contents.

Broodstock

Wild broodstock of *S. annulatus* are captured and acclimatized to hatchery conditions for egg production. During the first days after they arrival to the hatchery, the fish are fed with minced fish fillet and squid. Subsequently the fish are fed with a diet based on formulations for *T. rubripes* (Kanazawa, 1991). A dry diet with 42% protein and 14% lipid and a trout diet with 45% protein and 10% lipid are provided to the fish twice a day at a ration equivalent to 2% of the mean body weight as maintenance ration.

FUTURE RESEARCH

Important advances have been made with regard to larval feeding. However, the requirements for HUFA are not known yet and it is expected that enrichment of rotifers and *Artemia* with HUFA can improve larval growth in a similar way as in other marine fish species. Live food enrichment might also allow testing early weaning protocols with the larvae. Improvements on broodstock nutrition are also expected to produce higher quality larvae. With this regard, the study of the effect of dietary HUFA and vitamins on broodstock production of higher quality eggs and larvae, might help to improve the larval survival. As for the juveniles and grow-out fish nutrition, a next step is to the study of amino acid and vitamin requirements, and to determine the optimal feeding frequencies and rations for the improvement of their FCR. The development of grow-out diets and feeding protocols that yield maximum growth rates will help in reducing the time to reach the market size. Although the nutritional research with *S . annulatus* is still in its early stages, results obtained so far are encouraging and further work is being planned to help in the development of production techniques for its culture at commercial scale.

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