Weaning of bullseye puffer (Sphoeroides annulatus) from live food to microparticulate diets made with decapsulated cysts of Artemia and fishmeal

Article *in* Aquaculture International · December 2003

DOI: 10.1023/A:1024100618838

CITATIONS
36

READS
202

3 authors, including:

Armando García-Ortega
University of Hawai'i at Hilo
39 PUBLICATIONS **1,046** CITATIONS

SEE PROFILE

Crisantema Hernández
Research Center for Food and Development A.C.
102 PUBLICATIONS **1,284** CITATIONS

SEE PROFILE

Aquaculture International **11:** 183–194, 2003.

Weaning of bullseye puffer (*Sphoeroides annulatus*) from live food to microparticulate diets made with decapsulated cysts of *Artemia* and fishmeal

A. GARCÍA-ORTEGA*, I. ABDO and C. HERNÁNDEZ
*Laboratory of Nutrition and Larviculture, Centro de Investigación en Alimentación y Desarrollo (CIAD), Unidad Mazatlán, Apdo. Postal 711, C.P. 82010, Mazatlán, Sinaloa, México; *Author for correspondence (e-mail: agarcia@victoria.ciad.mx)*

Abstract. Two experiments were carried out to test microparticulate diets for weaning hatchery-produced larvae and juveniles of bullseye puffer *Sphoeroides annulatus*. The diets were formulated with different protein sources: diet 1 with a combination of decapsulated cysts of *Artemia* and fishmeal, and diet 2 with a combination of fishmeal, squid, tuna gonad and shrimp meal. In the first experiment 60-days-old fish were weaned with the microdiets over five days. 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. Once it was determined that bullseye puffer can be adequately reared with artificial dry diets, diet 1 was used to test earlier times for weaning to reduce the period of *Artemia* feeding. In the second experiment, three different times were tested for initiation of weaning in sibling fish larvae, i.e., at 29, 34, and 39 days post-hatch. Small differences in weight, length and survival were found among weaning treatments after 23 days of feeding. When weaned at day 29 post-hatch, 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. Final survival in this treatment was 49.3%. The reduced period of *Artemia* feeding would provide an economical alternative for the species to take into consideration for its culture at commercial scale.

Key words: *Artemia* cysts, Bullseye puffer, Larviculture, Microparticulate diets, Protein source, Weaning

Introduction

The aquaculture production of marine species in Mexico and other Latin American countries has been mainly restricted to penaeid shrimp. Nevertheless, the importance for diversification of marine aquaculture in the region has been recently recognized. Thus, research on the larval rearing of new candidate fish species for aquaculture has been carried out including the flounders *Paralichthys orbignyanus* (Cerqueira 1995) and *P. woolmani* (Bennetti 1997), the Pacific yellowtail *Seriola mazatlana* (Bennetti 1997) and

the spotted sand bass *Paralabrax maculatofasciatus* (Avilés-Quevedo et al. 1995; Alvarez-González et al. 2001).

The bullseye puffer (*Sphoeroides annulatus*) is a potential marine species for aquaculture on the Pacific coast of Mexico. This species is found throughout the Gulf of California and along the eastern Pacific from San Diego, USA to Peru (Thomson et al. 1987). Recently, significant advances have been made on its reproduction in captivity (Duncan and Rodriguez 2001) and larval rearing with live food organisms (Abdo et al. 2001). However, there is no published information on weaning times and artificial diets for this fish. When evaluating a species for commercial culture, it is necessary to formulate and test suitable artificial diets for weaning and on-growing. During development, the fish larvae pass through important anatomical and physiological changes, which affect their nutritional requirements (Govoni et al. 1986). Thus, the larval development of cultured fish can be delayed if inappropriate artificial diets are supplied (Cahu and Zambonino Infante 1994). Moreover, high mortalities can occur during weaning if fish larvae present low ingestion rates or poor food digestion (Watanabe and Kiron 1994). The biological value of artificial microdiets in marine fish larvae can be improved if an easy-to-digest protein source is provided. The digestibility of artificial microdiets for fish larvae can be improved by the use of decapsulated cysts of *Artemia*, which are a highly digestible protein source (García-Ortega et al. 2000). The decapsulated cysts in combination with fish meal and other protein sources were tested in weaning experiments with larvae and juveniles of bullseye puffer. In this study, two experiments were carried out to test the suitability of experimental microparticulate diets for feeding hatchery-produced bullseye puffer larvae, to test a weaning protocol for the species, and to determine earlier weaning times.

Materials and methods

Two experimental diets were formulated with different protein sources: diet 1 was prepared with a mix of decapsulated cysts of *Artemia* and fishmeal, and diet 2 was made with a mix of fishmeal, squid, tuna gonad and shrimp meal (Table 1). The diets were prepared by mixing the ingredients with water and a cold binder to avoid using high temperatures in their preparation. After thorough mixing of the ingredients, the paste was milled and dried at 40 °C, then ground manually in a mortar and sieved to a particle size of 420–700 μm. The diets were analyzed for N content by Kjeldahl analysis with a Kjeltec Autoanalyzer (1030, Foss Tecator AB, Sweden) and crude protein content was calculated as crude nitrogen content multiplied by 6.25. The total lipid content was determined by soxhlet extraction with petroleum ether for 6 h

Table 1. Formulation and proximate composition of artificial diets made of different protein sources for weaning bullseye puffer (*Sphoeroides annulatus*)

	Diet	
	Diet 1	Diet 2
Formulation (% dw)		
Artemia decapsulated cysts[a]	74	NA
Sardine fishmeal[b]	15.7	27.5
Squid	NA	20.1
Tuna gonad[c]	NA	19.7
Shrimp meal	NA	18.8
Cod liver oil[d]	2.6	2.1
Vitamin premix[e]	1.7	3.2
Mineral premix[e]	1	2
Lecithin[d]	NA	1
Dextrin[d]	NA	2.1
BHT[d]	NA	0.5
CMC[f]	5	NA
Alginate[d]	NA	3
Proximate composition (% dw)		
Protein	48.4	54.1
Lipid	12.3	13.5
Ash	9.4	19.5
Dry matter	91.5	92.2

[a]*Artemia* shell free, INVE Aquaculture Inc. Utah, USA.
[b]Harinas y Aceites de Occidente, S.A., Mazatlan, Mexico.
[c]PINSA S.A. de C.V., Mazatlan, Mexico.
[d]Drogueria Cosmopolita, S.A. de C.V., Mexico.
[e]Malta Cleyton, Malta Texo de Mexico S. A. de C. V.
[f]Carboxymethylcellulose Sodium salt, Fluka Chemie, Buchs, Switzerland.
NA = Not Applicable.

following the procedure of AOAC (1984). The ash was determined by incinerating the samples in a muffle furnace at 550° during 12 h, and the dry matter was determined by drying the sample in an oven at 105 °C for 16 h and weighing to the nearest 0.001 g. Each determination in the proximate composition analyses was carried out with three replicates.

Larvae of bullseye puffer were obtained by induced spawning of wild broodstock using LHRHa (Duncan and Rodriguez 2001). A sample was taken

from mature fish and oocyte diameter determined. Fish with oocytes greater than 0.5 mm were selected for treatment with LHRHa. Two injections of LHRHa were given, with the first 20 μg per kilo of fish and two days later the second at a concentration of 40 μg per kilo. Three days after the first injection the fish begin to spawn. Eggs initiated hatching 72 h after fertilization at 27.5 °C and the larvae started exogenous feeding four days after hatching. The mean wet weight and length of newly hatched larvae was 0.32 mg and 2.0 mm respectively. The larvae were reared by gradually introducing microalgae (*Nannochloropsis oculata* and *Isochrysis* sp.) from day 1 to 11 after hatching, rotifers (*Brachionus rotundiformis*) from day 4 to 26, and live *Artemia* nauplii from day 21 until the initiation of weaning following the live food feeding scheme described for this species (Abdo et al. 2001). The weaning experiments were carried out in a seawater flow-through system with nine 600 L black fiberglass tanks with constant illumination and aeration. In the first experiment the two microparticulate diets were tested in 60-days-old fish with a mean initial wet weight = 0.69 g and length = 3.0 cm at a density of 50 fish per tank. Three replicate tanks were used for each diet treatment and the control with live *Artemia* nauplii. The fish were weaned over a period of five days, in which the nauplii were completely replaced by the diets from a density of one nauplii per ml to zero at a daily rate of 20%. During weaning, the first feeding of the day was done with the microparticulate diet to allow maximal acceptance of the diet, and the first of two daily nauplii feedings was gradually postponed 30 minutes per day. During the live nauplii feeding, the water flow in the fish tanks was interrupted for one hour to avoid the loss of *Artemia*. The microparticulate diets were given *ad libitum* five times daily at 09.00, 12.00, 15.00, 18.00 and 20.00 for 11 weeks. The wet weight and total length were measured in 10 fish per tank every week, with the exception of week 10.

In a second weaning experiment, 29-days-old sibling larvae with a mean initial wet weight = 38.4 mg and length = 11.1 mm were stocked in four 600 L tanks with flow-through at a density of 150 fish per tank. Diet 1 was used to test three different times for the initiation of weaning, i.e., at 29, 34, and 39 days after hatching. One tank was exclusively fed live *Artemia* nauplii as a control for the entire experiment whereas the fish in the weaning treatments were fed with *Artemia* nauplii until the day for the initiation of weaning. Weaning and feeding were carried out as indicated for the first experiment, except that a smaller particle size of diet 1 was used (190–420 μm) and the density of live *Artemia* nauplii at the initiation of weaning was 0.5 nauplii per ml. The wet weight and length of 10 larvae per tank were measured every three days, and the experimental period lasted 23 days. At the start of the experiment the mouth size of 24 larvae was

measured under a stereoscopic microscope with a digital vernier caliper (CD-6, Mitutoyo Corporation, Japan). In both experiments, the solid waste in each tank was siphoned out every day, the water temperature and salinity were recorded daily and survival was determined at the end of the experimental period.

The data on weight, length and survival in fish larvae fed the artificial microdiets from the first experiment were submitted to a one-way ANOVA (Sokal and Rohlf 1995) to find if a significant difference ($p < 0.05$) existed between the diet treatments.

Results

The ingredient and proximate composition of the two diets is presented in Table 1. The protein and lipid contents were slightly higher in diet 2. This was probably due to the inclusion of several protein sources in the formulation. These ingredients might also contain lipid, which contributed to the total lipid content in the diet. The high ash value in diet 2 is due to the high ash content in two protein sources included in this diet, i.e., sardine fishmeal (16.6%) and shrimp meal (19.9%).

During the first weaning experiment, the water temperature fluctuated between 29.4–30.6 °C and salinity from 28–32 ppt. With regard to the effect of the diets on growth, no significant differences ($p > 0.05$) were found in weight between the diet treatments (Figure 1). However, higher final weight (Figure 1), length and survival (Table 2) were achieved with diet 1. At this fish age, feeding with live *Artemia* nauplii did not produce higher growth than the artificial diets. Survival in the artificial diet treatments was high (> 85%) and close to the survival achieved with the live food control (Table 2). Once it was determined that larvae of bullseye puffer can be adequately reared with artificial diets, earlier times for weaning were tested to reduce the period of feeding with live *Artemia* nauplii.

In this second experiment, the water temperature and salinity fluctuated between 29.7–30.8 °C and 32–34 ppt respectively. Small differences in weight (Figure 2), length and survival (Table 3) were found among weaning treatments. The control larvae fed with *Artemia* nauplii yielded the highest final weight and survival. The mouth size in the larvae at the start of the experiment was 0.9 mm (± 0.1) and corresponded to 8.5% of the total length of the larvae at day 29 post-hatch.

In both experiments the larvae presented a positive response to the progressive substitution of live food by microparticulate diets during the five day weaning period. A very active swimming and feeding behavior was observed in every occasion the diets were supplied to the fish. Visual veri-

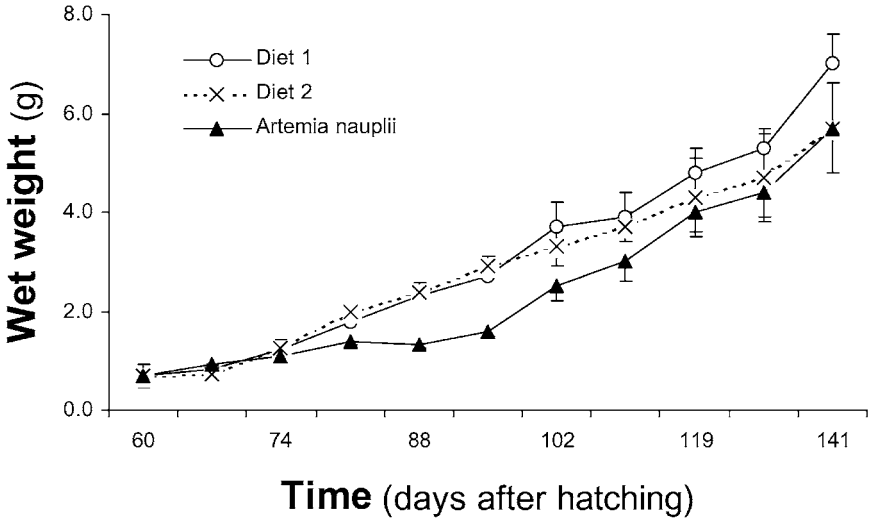

Figure 1. Weight of bullseye puffer during and after weaning with microparticulate diets made with different protein sources and one control treatment fed live *Artemia* nauplii. No significant differences ($p > 0.05$) were found among the artificial diet treatments. Weaning was done over a period of five days and the fish were fed for 11 weeks.

Table 2. Initial and final length and survival of bullseye puffer weaned with two microparticulate diets made of different protein sources and one control treatment with live *Artemia* nauplii. Weaning was done over a period of five days and the fish were fed for 11 weeks

Fish age	Length (cm)		
(days after hatching)	Diet 1	Diet 2	*Artemia* nauplii
60	$3.0 \pm 0.3^*$	$3.0 \pm 0.3^*$	3.0 ± 0.3
141	$6.2 \pm 0.0^*$	$5.9 \pm 0.3^*$	6.0 ± 0.0
Final survival (%)	$92.0 \pm 2.0^*$	$85.3 \pm 3.1^*$	94.7 ± 4.2

For the two artificial diet treatments the means in the same row with an asterisk are not significantly different ($p > 0.05$).

fication of larval feeding and food presence in the gut confirmed that the microparticulate diets were well accepted by the fish. The feeding behavior in this species during the first days of weaning presented a tendency to first ingest the inert feed particle, then expel it and almost immediately ingest it again. Apparently, the fish larvae pass through an adaptation period during the change from a soft and wet food item, like *Artemia* nauplii, to a hard and dry feed particle, like the microparticulate diet. After weaning, when most of the larvae were habituated to the new diet, the fish at once ingested every particle of the artificial diet. A remarkable aspect of feeding this species with

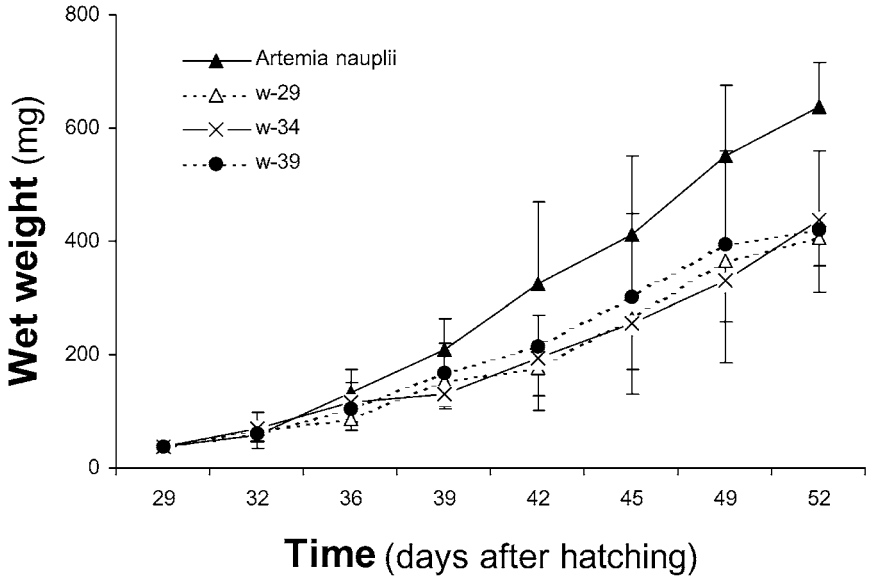

Figure 2. Weight of bullseye puffer larvae weaned with diet 1 at different times, i.e., at 29, 34, 39 days post-hatch (w-29, w-34, w-39 respectively). One control treatment was fed only live *Artemia* nauplii. Weaning was done over a period of five days and the fish were fed for a 23 days.

Table 3. Initial and final length and survival of bullseye puffer larvae weaned with diet 1 at different fish ages, i.e., at 29, 34, 39 days post-hatch (w-29, w-34, w-39 respectively). One control treatment was fed exclusively with live *Artemia* nauplii. Weaning was done over a period of five days and the fish were fed for a total of 23 days

Fish age	Length (mm)			
(days after hatching)	w-29	w-34	w-39	*Artemia* nauplii
29	11.1 ± 0.5	11.1 ± 0.5	11.1 ± 0.5	11.1 ± 0.5
52	25.1 ± 1.3	26.2 ± 2.7	24.9 ± 2.8	29.4 ± 1.5
Final survival (%)	49.3	46.0	44.7	62.7

artificial diets was the observation that fish larvae were able to detect and ingest the feed particles on the water surface, as well as the feed particles which were slowly sinking in the water column, and the particles resting in the tank bottom. Moreover, there was no cannibalism observed during or after weaning in both experiments. Similarly, no apparent problems with diseases were observed.

Discussion

A critical period in the larviculture of marine fish is the weaning phase. Weaning diets that are well accepted, digested and that fulfill the nutritional requirements of the fish are essential for a successful production of larval and juvenile fish. Diets formulated with a mixture of different protein sources have been successfully used in the larval culture of red seabream *Pagrus major* and ayu *Plecoglossus altivelis* (Kanazawa et al. 1982), Japanese flounder *Paralichthys olivaceus* (Kanazawa et al. 1989) and European sea bass *Dicentrarchus labrax* (Person Le Ruyet et al. 1993). In our study, the diets formulated with different protein sources were well accepted by bullseye puffer larvae and produced satisfactory fish growth and survival. The diets had similar protein content to the artificial diets tested successfully in the larvae of other marine fish species, e.g., 52% in diets for gilthead seabream *Sparus aurata* (Fernández-Díaz and Yúfera 1997) and milkfish *Chanos chanos* (Duray and Bagarinao 1984), and were close to the optimum protein and lipid requirements for juveniles of the tiger puffer Takifugu rubripes cultured in Japan (Kanazawa et al. 1980; Takii et al. 1995). In previous nutritional studies with fish larvae, the incorporation of decapsulated cysts of *Artemia* as the major protein source in combination with fishmeal provided a diet with higher protein digestibility than a diet with fishmeal as the only protein source (García-Ortega et al. 2001). An advantage of feeding decapsulated cysts of *Artemia* over freshly hatched nauplii is that the energy content in the cysts is higher than in nauplii (Vanhaecke et al. 1983). Moreover, the proximate composition and the amino acid and fatty acid profiles in decapsulated cysts are similar to those in live nauplii (García-Ortega et al. 1998). With regard to the application of *Artemia* in the formulation of artificial diets, it was demonstrated that the supplementation of various extracts from *Artemia* into microdiets for gilthead seabream *Sparus aurata* larvae significantly increased the artificial diet intake (Kolkovski et al. 1997). These results support the use of extracts or decapsulated cysts of *Artemia* in microdiets for larvae of marine fish to improve their intake compared with microdiets made exclusively of fishmeal or other ingredients commonly used in the design of diets for juveniles or adult fish. Another important aspect of the use of microparticulate diets as weaning diet is the advantage that cold binders can be used during their preparation and high processing temperatures can be avoided. High temperatures might have detrimental effects on the protein quality, causing protein denaturation and decreasing their solubility, which are events that might lead to changes in the structural properties of the protein molecules (Boye et al. 1997). These changes have been associated with a decline in the protein and amino acid digestibility in fish feeds (Opstvedt et al. 1984).

Growth results in experiment 1 were similar as those obtained for tiger puffer *Takifugu rubripes* of the same age (Han and Yoshimatsu 1997). Survival in the microparticulate diets treatments was high, which is an indication of the suitability of rearing *S. annulatus* with artificial diets at this fish age. Alternatively, when live *Artemia* nauplii are offered from day 21 post-hatch until day 60 (i.e., 40 days of *Artemia* feeding) the commercial culture of the species is compromised due to the high cost of *Artemia* production. In any case, the growth of bullseye puffer fed live nauplii was not higher than with the artificial diet treatments, which indicates that fish at this age no longer require *Artemia*.

A weaning period of five days provided sufficient time for the larvae of bullseye puffer to adapt to the dry artificial diets and avoided high fish mortalities. This represents an advantage for *S. annulatus* over other marine fish species like the Dover sole *Solea solea*, in which the longer the period of live *Artemia* feeding, the more difficult it is to wean the larvae onto a formulated diet (Appelbaum 1985). In the first experiment, bullseye puffer did not present problems in adapting to a dry artificial diet despite being fed 40 days with live *Artemia* nauplii. Feeding five times a day permitted the fish to have sufficient food available to avoid problems with cannibalism, which can be a serious constraint in applied fish larviculture (Hecht and Pienaar 1993).

The results in the present study indicate that bullseye puffer 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 on a commercial scale.

Conclusions

1. Microparticulate diets formulated with a combination of decapsulated cysts of *Artemia* and fishmeal as protein source are suitable weaning diets for bullseye puffer larvae.
2. Weaning during a five days period provided sufficient time for the fish larvae to adapt from live *Artemia* nauplii to the dry inert diets. A feeding frequency of five times a day (09.00, 12.00, 15.00, 18.00 and 20.00) was adequate and avoided problems with fish cannibalism.

3. Larvae of bullseye puffer can be weaned onto artificial diets at a weight of 38.4 mg or day 29 post-hatch with an acceptable survival (49%) after 23 days of feeding with microparticulate diets.

Acknowledgements

The authors thank B. González and I. Martínez for technical assistance, INVE Aquaculture Inc. for providing shell free *Artemia* cysts and Malta Cleyton S.A. for providing the vitamin and mineral premixes. This work was supported by CONACyT, Mexico, ref. 211083-1, exp. 235 and project nr. J28342B.

References

Abdo de la Parra I., García-Ortega A., Martínez-Bodríguez I., González-Rodríguez B., Velasco G., Hernández C. and Duncan N. 2001. Larval rearing of the Mexican bullseye puffer *Sphoeroides annulatus* under hatchery conditions. In: C.I. Hendry, G. van Stappen, M. Wille and Sorgeloos, P. (eds), Larvi 2001 – Fish and Shellfish Larviculture Symposium. European Aquaculture Society Special Publication No. 30. September 3–6, 2001, Ghent, Belgium, pp. 4–7.

Alvarez-Gonzalez C.A., Ortiz-Galindo J.L., Dumas S., Martínez-Díaz S.F., Hernández-Ceballos D.E., Grayeb-del Alamo T., Moreno-Legorreta M., Peña-Martínez R. and Civera-Cerecedo R. 2001. Effect of stocking density on the growth and survival of spotted sand bass *Paralabrax maculatofasciatus* larvae in a closed recirculation system. Journal of the World Aquaculture Society 32: 130–137.

AOAC 1984. Official methods for analysis of the Association of Official Analytical Chemists, 14th edition. Arlington, VA, 1141 pp.

Appelbaum S. 1985. Rearing of the Dover sole, *Solea solea* (L.), through its larval stages using artificial diets. Aquaculture 49: 209–221.

Avilés-Quevedo A., McGregor-Pardo U., Rodríguez-Ramos R., Hirales-Cosío O., Huerta-Bello M. and Iizawa M. 1995. Biología y cultivo de la cabrilla arenera *Paralabrax maculatofasciatus* (Steindachner, 1868) SEPESCA-JICA, Mexico, p. 85.

Bennetti D.D. 1997. Spawning and larval husbandry of flounder (*Paralichthys woolmani*) and Pacific yellowtail (*Seriola mazatlana*), new candidate species for Aquaculture. Aquaculture 155: 307–318.

Boye J.I., Ma C.Y. and Hardwalkar V.R. 1997. Thermal denaturation and coagulation of proteins. In: S. Damodaran and A. Paraf (eds), Food Proteins and Their Applications. Marcel Dekker Inc., New York, pp. 25–56.

Cahu C.L. and Zambonino Infante J.L. 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comparative Biochemistry and Physiology 109A: 213–222.

Cerqueira V.R. 1995. Marine finfish larviculture in Brazil: current status and perspectives. In: J. Calderón and P. Sorgeloos (eds), Memorias II Congreso Ecuatoriano de Acuicultura. October 20–25, 1993, Guayaquil, Ecuador, pp. 125–130.

Duncan N. and Rodríguez G. 2001. Induced spawning of the bullseye puffer *Sphoeroides annulatus* using LHRHa. In: Book of Abstracts Aquaculture 2001. World Aquaculture Society, Orlando, USA, January 21–25, 2001, p. 196.

Duray M. and Bagarinao T. 1984. Weaning of hatchery-bred milkfish larvae from live food to artificial diets. Aquaculture 41: 325–332.

Fernández-Díaz C. and Yúfera M. 1995. Capacity of gilthead seabream, *Sparus aurata* L., larvae to break down dietary microcapsules. Aquaculture 134: 269–278.

García-Ortega A., Verreth J.A.J., Segner H., Coutteau P., Huisman E.A. and Sorgeloos P. 1998. Biochemical and enzymatic characterization of decapsulated cysts and nauplii of the brine shrimp *Artemia* at different developmental stages. Aquaculture 161: 501–514.

García-Ortega A., Koussoulaki A., Boer H. and Verreth J. 2000. *In vitro* protein digestibility of *Artemia* decapsulated cysts and nauplii, and of microbound diets for larval fish. Aquaculture Research 31: 475–477.

García-Ortega A., Huisman E.A., Sorgeloos P. and Verreth J. 2001. Evaluation of protein quality in microbound starter diets made with decapsulated cysts of *Artemia* and fishmeal for fish larvae. Journal of the World Aquaculture Society 32: 317–329.

Govoni J.L., Boehlert G.W. and Watanabe Y. 1986. The physiology of digestion in fish larvae. Environmental Biology of Fishes 16: 59–77.

Han K. and Yoshimatsu T. 1997. Early developmental stages in laboratory-reared puffer fish, *Takifugu rubripes*. Scientific Bulletin of the Faculty of Agriculture, Kyushu University 51: 157–165.

Hecht T. and Pienaar A.G. 1993. A review of cannibalism and its implications in fish larviculture. Journal of the World Aquaculture Society 24: 246–261.

Kanazawa A., Teshima S., Sakamoto M. and Shinomiya A. 1980. Nutritional requirements of the puffer fish: purified test diet and the optimum protein level. Bulletin of the Japanese Society of Scientific Fisheries 46: 1357–1361.

Kanazawa A., Teshima S.I., Inamori S., Sumida S. and Iwashita T. 1982. Rearing of larval red sea bream and ayu with artificial diets. Memoirs of the Faculty of Fisheries, Kagoshima University 31: 185–192.

Kanazawa A., Koshio S. and Teshima S.I. 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* fed microbound diets. Journal of the World Aquaculture Society 20: 31–37.

Kolkovski S., Arieli A. and Tandler A. 1997. Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. Aquaculture International 5: 527–536.

Opstvedt J., Miller R., Hardy R.W. and Spinelli J. 1984. Heat-induced changes in sulfhydril groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gaidneri*). Journal of Agricultural and Food Chemistry 32: 929–935.

Person Le Ruyet J., Alexandre J.C., Thébaud L. and Mugnier C. 1993. Marine fish larvae feeding: formulated diets or live prey? Journal of the World Aquaculture Society 24: 211–224.

Sokal R.R. and Rohlf F.J. 1995. Biometry. The Principles and Practice of Statistics in Biological Research, third edition. W.H. Freeman and Company, New York, pp. 207–271.

Takii K., Ukawa M., Nakamura M. and Kumai H. 1995. Suitable lipid level in brown fish meal diet for tiger puffer. Fisheries Science 61: 841–844.

Thomson D.A., Findley L.T. and Kerstitch A.N. 1987. Reef Fishes of the Sea of Cortez. The Rocky-shore Fishes of the Gulf of California. The Arizona University Press, Tucson, pp. 243–244.

Vanhaecke P., Lavens P. and Sorgeloos P. 1983. International study on *Artemia*. XVII. Energy consumption in cysts and early larval stages of various geographical strains of Artemia. Annales de la Societe Royale Zoologique de Belgie 113: 155–164.

Watanabe T. and Kiron V. 1994. Prospects in larval fish dietetics. Aquaculture 124: 223–251.