



Acceptability of complex microencapsulated diets by striped bass (*Morone saxatilis*) larvae

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Abstract

The acceptability of two microencapsulated diets (casein-walled complex microcapsules, CWC, and CWC containing water and lipid soluble fractions of *Artemia*, MCWC) by striped bass larvae, from 7 to 21 days posthatching (DPH), was tested. These two microencapsulated diets were fed to striped bass larvae exclusively (CWC or MCWC alone, 1.1 mg/larva/day) or as partial replacement of live food, *Artemia* nauplii (two nauplii/ml + MCWC, 1.1 mg/larva/day). Controls were unfed larvae and larvae fed with a full ration of *Artemia* (five nauplii/ml) or 40% ration of *Artemia* (two nauplii/ml). The ingestion {(number of larvae with food items in the gut ÷ total number of larvae examined) × 100%} of CWC and MCWC by larvae were 42% and 75%, respectively at 7 DPH, while the ingestion in groups fed with full, or 40% ration *Artemia*, and fed 40% *Artemia* plus MCWC were nearly 100% (96%–98%). On 14 DPH, the ingestion by the larvae fed CWC and MCWC increased markedly, reaching 100%. The survival of the larvae fed solely CWC and MCWC were 44% and 54% respectively, lower than the two controls fed full or 40% ration of *Artemia* and the group fed MCWC + 40% *Artemia*. The survival of the later three groups ranged from 72 to 78%. All the unfed larvae died by 19 DPH. The larvae fed a full ration of *Artemia* had the highest mean wet weight and total length. Supplementing the group fed 40% ration *Artemia* with MCWC slightly improved the growth of the larvae compared to the nonsupplemented group. At the end of the experiment, larvae in that group had significantly greater wet weight gain and total length than the nonsupplemented group. The results of this study revealed that a complex-protein walled capsule is readily ingested by striped bass larvae at first feeding. Potentially, the complex-protein microencapsulated diet containing *Artemia* extracts can be used as partial replacement of live food. However, to achieve such a goal, study is needed to improve its nutritional quality and digestibility, in particular. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Culture of marine fish larvae relies heavily on the use of live food organisms, such as rotifers and *Artemia* nauplii. Although live food used in larval culture is superior to any artificial diet to date, it has certain disadvantages when used extensively. The nutritional quality of live food varies significantly from batch to batch as a result of changes in biochemical composition (Watanabe et al., 1983). The production of live food is costly and time consuming (Ehrlich et al., 1989; Jones et al., 1987) and the ability to adjust the size and composition of live food to meet the specific requirements of the larvae is limited (Jones et al., 1987; Jones et al., 1993).

Acceptability of artificial diets by first feeding marine fish larvae varies enormously and restricts the development of a nutritionally complete diet. Promising but limited success has been reported for marine fish larvae fed microparticulate or microencapsulated diets as partial or full replacements for live food (Adron et al., 1973; Kanazawa et al., 1982; Applebaum, 1985; Leibovitz et al., 1987; Kanazawa et al., 1989). Kanazawa et al. (1982) showed that the growth and survival of red sea bream (*Chrysophrys major*) and Ayu (*Plecoglossus altivelis*) improved markedly when fed either zein microcoated or nylon–protein microcapsules in addition to live rotifers. In a separate study, inland silverside, *Menidia beryllina*, larvae fed microencapsulated diets containing freeze-dried *Artemia* survived relatively well, however, growth was severely retarded (Leibovitz et al., 1987). Studies indicate that striped bass larvae do not accept artificial diets as first food and feeding the larvae solely on these diets results in total mortality (Tuncer et al., 1990; Webster and Lovell, 1990). However, it is unclear whether poor survival and growth of striped bass larvae are due to low diet acceptability and/or nutritional incompleteness of the diet.

We recently developed a complex crosslinked protein-walled microcapsule by encapsulating lipid-wall capsules containing water soluble nutrients along with other dietary nutrients into a crosslinked protein membrane (Ozkizilcik and Chu, 1996). The advantage of this diet is its ability to release low molecular weight attractants (i.e., free amino acids, nucleotides) from the protein wall while other essential nutrients, such as water soluble vitamins are retained in the lipid-wall capsules encapsulated in the diet. The objective of this study was to determine if striped bass larvae would accept and grow on the complex protein-walled capsules as partial or full replacement of *Artemia*, 7 through 21 days posthatching (DPH).

2. Materials and methods

2.1. Larval fish culture

Six DPH striped bass larvae were obtained from Hudson River Utilities' Hatchery

(Verplanck, NY, USA). Approximately 1800 larvae were transferred to each individual conical tank (60 l) in a recirculating system. Culture water (21°C, 3 ppt) was passed through a 5 μm filter and crushed oyster shells to eliminate uneaten food items, fecal particles, and ammonia. Ammonia levels were <200 μM in all tanks throughout the experiments. The culture tanks were continuously aerated. Feeding was initiated at 7 DPH and the experiments were terminated at 21 DPH.

2.2. Diet preparation

Complex crosslinked protein-walled capsules were prepared according to the method described by Ozkizilcik and Chu (1996). Briefly, 20 g of the dietary ingredient mixture (Table 1), which consisted of casein, menhaden meal, starch, amino acid mixture resembling the amino acid composition of *Artemia* (Seidel et al., 1980; Dabrowski and Rusiecki, 1983), cholesterol, lecithin, and attractants, was added to 100 ml of 0.02 N NaOH containing 0.2% (w/v) urea and stirred until the casein dissolved. Lipid-walled capsules containing vitamin and mineral mixtures (Teshima et al., 1982) were prepared as described by Langdon and Siegfried (1984) and Chu et al., (1987) and then added to the above dietary mixture. Fat soluble vitamins were dissolved in menhaden oil before the lipid-walled capsules were prepared. The dietary mixture containing lipid-walled capsules was then atomized into swirling 1% (v/v) adipoyl chloride–cyclohexane solution containing 2% (w/v) crude lecithin. The microparticles were allowed to crosslink at the water–cyclohexane interface for 15 min. The capsule slurry was poured into chilled cyclohexane to quench the reaction. The capsules were washed with cyclohexane to remove excess adipoyl chloride and freeze dried for storage. This process produced crosslinked protein-walled microcapsules of a mean diameter of 153 μm (130

Table 1
Dietary ingredients (% by weight) of crosslinked complex protein-walled microcapsules prepared as described in the Materials and methods section

| | Crosslinked protein-walled capsules CWC ^a (%) |
|------------------------------------|---|
| Casein | 20 |
| Menhaden meal | 35 |
| Starch | 5 |
| Amino acid mix ^b | 5 |
| Cholesterol | 1 |
| Lecithin | 1 |
| Lipid-walled capsules ^c | 25 |
| Attractants ^d | 8 |

^a CWC = Casein-walled complex capsule

^b Seidel et al., 1980; Dabrowski and Rusiecki, 1983.

^c Lipid-walled capsules were prepared as described in Materials and methods. Aqueous core of lipid-walled capsules contained 5 g vitamin mix and 5 g mineral mix (Teshima et al., 1982) dissolved in 20 ml of distilled water. Fat soluble vitamins were dissolved in menhaden oil before the lipid-walled capsules were prepared. Quantities were adjusted according to the lipid composition of the diet.

^d Attractants included glycine (2 g), betaine (2 g), inosine (1 g), inosine 5'-monophosphate (1 g), guanosine (1 g) and guanosine 5'-monophosphate (1 g).

μm to 200 μm) and consisted of lipid-walled capsules containing essential vitamins and minerals, and other dietary compounds (i.e., menhaden meals, starch) encapsulated within a crosslinked protein wall. The capsules consisted of 53% protein and 21% lipids as determined by analyses (Lowry et al., 1951; Bligh and Dyer, 1959; Holland and Gabbot, 1971). Feeding the casein-walled capsules (CWC) to 7 DPH larvae in a preliminary experiment revealed that only 46% of the examined larvae ($n = 25$) ingested the CWC. In an attempt to increase ingestion, CWC were modified to contain water and lipid soluble extracts (MCWC) of newly hatched *Artemia* nauplii. To prepare the extract, 30 g wet weight of *Artemia* was homogenized in 80% (v/v) ethanol. The homogenate was centrifuged and the upper phase was removed and dried in a rotary evaporator. The ethanol soluble residue was redissolved in distilled water and added to the dietary mixture. The residue was dried and lipids were extracted with chloroform–methanol (2:1 v/v). *Artemia* lipids were then added in the lipid mixture used in the preparation of lipid-walled capsules.

2.3. Feeding experiment

Treatments consisted of CWC, MCWC, full ration *Artemia* (five nauplii/ml), 40% ration *Artemia* (two nauplii/ml), and MCWC + 40% *Artemia*. A total of 1800 larvae were placed in each larval tank. All experimental treatments were carried out in duplicate. Our previous trials showed that the growth of larvae was determined by the number of *Artemia* nauplii present in the culture water (unpublished observations). Therefore, 100% and 40% ration were determined to be five and two nauplii/ml. Microencapsulated diets were fed to the larvae at 1.1mg/larva/day, based on the dry weight of *Artemia*, four separate times/day (0.275mg/larva/time). The number of *Artemia* nauplii in the culture tanks that received full or 40% live food was checked twice daily and necessary amounts were added to maintain the concentrations (i.e., five nauplii/ml for full ration and two nauplii/ml for 40% ration). The *Artemia* cysts were hatched daily in 60 l transparent tanks in 1 μm filtered estuarine water (24 ppt) at 28°C. Freshly hatched nauplii were collected on 150 μm filters, rinsed well and stored live at 10°C until feeding.

2.4. Ingestion and breakdown of microcapsules by the larvae

Ingestion of *Artemia* and microencapsulated diets by 7 and 14 DPH larvae was examined microscopically. Thirty minutes after feeding, 25 larvae were sampled and examined for the presence of food items in the digestive tract. Percent of ingestion was calculated as: number of larvae with food items in the gut \div total number of larvae examined \times 100.

To determine the extent of breakdown of complex microencapsulated diets, five larvae (13 DPH) were removed from the culture tanks approximately 30 min after feeding. Individual larvae ($n = 5$) were placed in a 24-well tissue culture plate containing 2 ml of culture water. Individual larvae were transferred to a microscope slide with a Pasteur pipet at 0 min, 30 min, 1.5 and 3.5 h and examined under a microscope. Photomicrographs were taken at $5\times$ (Olympus BH2 microscope equipped with an SC-35

Olympus camera) to reveal the state of gut contents through the transparent body of the larva. No mortality was observed during the examination.

2.5. Larval fish measurement

Larvae were sampled from each tank at 14 and 21 DPH to measure wet weights and fish length (25 larvae/tank, totally 50 larvae/treatment). Wet weights (mg) of individual larvae were determined to the nearest 0.1 mg after blotting dry on a paper towel. Total fish length (mm) was measured to nearest 0.1 mm. using an optical micrometer (Mnostat). At the end of the experiment (21 DPH), survival was determined and is expressed as percentages ($\% = \text{number of fish that survived at the end of the experiment} \div \text{the number of larvae at the beginning of the experiment} \times 100$).

2.6. Statistical analyses

Results are presented as means \pm SD. Tank and treatment effects were evaluated by two-way analysis of variance (ANOVA) using the statistical package (SYSTAT) followed by the Newman–Keuls multiple comparison test. Differences were considered statistically significant if $P \leq 0.05$.

3. Results

Microscopic observation showed that the complex protein-walled microcapsules were readily ingested by the first feeding striped bass larvae (Table 2). The inclusion of *Artemia* extracts in the capsules appeared to improve the ingestion of CWC by first feeding larvae. On 7 DPH, 75% of the larvae accepted the MCWC as first food, while only 42% of the larvae ingested CWC. On 14 DPH, the ingestion rate of these two diets increased markedly, reaching 100%, as other treatments.

Microscopic examination of the digestive tract of the larvae at 30 min, 1, 2 and 4 h after feeding is illustrated in Fig. 1. The digestive tract was full of capsules at 30 min (Fig. 1A). Nearly half the ingested capsules dissipated after 1 h (Fig. 1B). The capsule aggregate shrank in size during the following 3 h, almost completely residing in the anterior section of the gut at 4 h postfeeding (Fig. 1D).

All unfed larvae had died by 19 DPH. The survival of other treatments ranged from 44 to 78%. Surprisingly the larvae fed 40% *Artemia* had the highest survival at 21 DPH (Table 2). Survival of the larvae fed solely on CWC or MCWC were 54 and 44% respectively, lower than that of the other treatment groups. They did not show any apparent growth. The larvae fed full ration of *Artemia* had the highest mean wet weight and total length at both 14 and 21 DPH. Supplementing the group fed 40% ration *Artemia* with MCWC slightly improved the growth of the larvae (21 DPH) compared to the nonsupplemented group, although a lower growth was observed in the 14 DPH larvae of the supplemented group than the nonsupplemented group. At the end of the experiment, larvae in that group had significantly greater wet weight gain and total length than the nonsupplemented group (Table 2). Although including the MCWC

Table 2

Ingestion rate (% = number of larvae with food items in the gut ÷ total number of larvae examined × 100) of striped bass larvae at first feeding (7 DPH) and 14 DPH, total length (mm), wet weight (mg), and survival (%) in feeding experiment^a

| Treatments ^b | Ingestion rate | | Total length (mm) | | Wet weight (mg) | | Survival |
|---|----------------|--------|------------------------|-------------------------|------------------------|-------------------------|----------|
| | 7 DPH | 14 DPH | 14 DPH | 21 DPH | 14 DPH | 21 DPH | |
| Unfed | – | – | 5.33±0.04 ^A | – | 0.68±0.01 ^A | – | – |
| <i>Artemia</i> (5 nauplii/ml) | 98 | 100 | 8.29±0.30 ^D | 12.15±0.50 ^E | 3.55±0.50 ^E | 11.80±1.67 ^D | 72 |
| <i>Artemia</i> (2 nauplii/ml) | 96 | 100 | 7.66±0.30 ^C | 10.75±0.71 ^D | 2.92±0.33 ^D | 7.56±0.50 ^B | 78 |
| MCWC ^c + <i>Artemia</i> (2 nauplii/ml) | 96 | 100 | 7.60±0.10 ^C | 11.45±0.07 ^C | 2.72±0.35 ^C | 8.92±0.05 ^C | 72 |
| MCWC | 75 | 100 | 5.66±0.06 ^B | 5.73±0.14 ^A | 1.12±0.01 ^B | 0.86±0.01 ^A | 44 |
| CWC ^d | 42 | 100 | 5.76±0.16 ^B | 6.00±0.06 ^B | 1.11±0.02 ^B | 1.09±0.03 ^A | 54 |

^a The initial (7 DPH) total length = 5.55±0.11 mm; the initial wet weight = 0.90±0.09 mg.

^b Treatments designated similar letters (A, B, C, D, E) are statistically insignificant ($P > 0.05$, ANOVA, Student–Newman–Kuels test).

^c MCWC = modified casein-walled complex capsules.

^d CWC = casein-walled complex capsules.

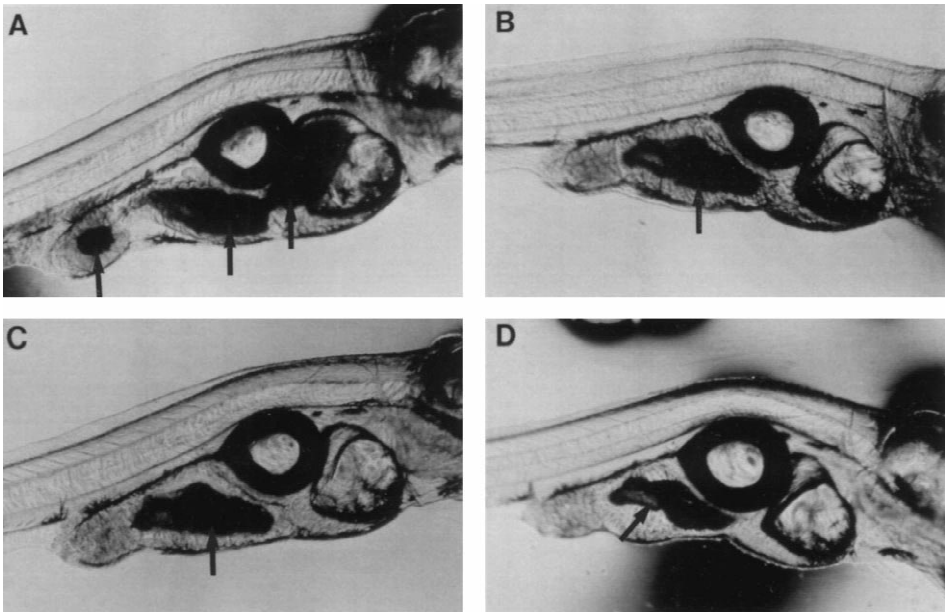


Fig. 1. Capsule aggregates (arrows) in the digestive tract (lateral views) of 13 days posthatching striped bass larvae fed complex casein-walled microcapsules; (a) 30 min, (b) 60 min, (c) 120 min and (d) 240 min after feeding.

containing *Artemia* extract improved the ingestion of the complex capsules by first feeding larvae, the CWC containing no *Artemia* extract appeared to achieve a better growth and survival at the end of the experiment.

4. Discussion

Protein-walled microcapsules have found extensive use in the culture of marine organisms since their modification for aquatic filter feeders (Jones et al., 1974). Numerous studies have investigated the acceptability and digestibility of protein-walled microcapsules by adult (Langdon, 1989) and larval oysters (Chu et al., 1987), shrimp larvae (Jones et al., 1987) and fish larvae (Applebaum, 1985; Kanazawa et al., 1982). The use of microencapsulated diets has expanded to commercial scale only in penaeid shrimp culture (Jones et al., 1987).

In the present study, we demonstrated that MCWC containing *Artemia* extracts were ingested by 75% of the first feeding striped bass larvae. This result was promising. Earlier studies reported that striped bass larvae did not accept artificial diets as first food (Tuncer et al., 1990; Webster and Lovell, 1990). Although capsule aggregate in the gut dissipated within 1 h of feeding (Fig. 1B), microscopic observation indicated the remaining aggregate shrank in size in the following 3 h, suggesting slow digestion. Langdon (1989) reported a 40% absorption efficiency of ^{14}C crosslinked protein-walled capsules by *Crassostrea gigas*, which was lower than live food. In the present study, an exclusive diet of complex protein-walled capsules did not support growth of the larvae, although a relatively high survival rate (44% for MCWC, 54% for CWC) was achieved. Similar results were reported for *M. beryllina* (Leibovitz et al., 1987), *P. altivelis* and *Plecoglossus major* (Kanazawa et al., 1982).

Increased growth did not occur in larvae supplemented with MCWC until 21 DPH suggesting that it takes time for the larvae to adapt to the diet. It is speculated that a better growth could be achieved, if the feeding was extended through metamorphosis to juvenile stage. The lack of growth of larvae fed solely CWC capsules was probably contributed to by two factors: (1) imbalance of amino acids (the amino composition of *Artemia* is different from casein; Seidel et al., 1980; Teshima et al., 1986); and (2) low digestibility of crosslinked protein wall.

Digestibility of microencapsulated diets increased when live food was offered as a dietary supplement (Kanazawa et al., 1982; Walford et al., 1991). Therefore, poor growth of fish larvae fed artificial diets may also be the result of an inadequate level of digestive enzymes (Dabrowski, 1979). Dabrowski (1979) suggested that the initial digestive processes in the rudimentary digestive tract of the fish larvae are facilitated by the enzymes present in live food. Although striped bass larvae possess tryptic (Baragi and Lovell, 1986) and lipolytic enzymes (Ozkizilcik et al., 1996) at first feeding, enzymes derived from live food may help in digestion and stimulate enzyme secretion in fish larvae. If such a condition does exist, then the complex microencapsulated diets offer a potential method of providing dietary enzymes to larval fish.

The semipermeable nature of the crosslinked protein-wall allows the release of low molecular weight attractants, such as free amino acids, to stimulate ingestion by larvae,

while retaining the highly water soluble nutrients in the lipid-walled capsules. Improved ingestion in first feeding 7 DPH larvae fed MCWC suggests that phagostimulants are important. Striped bass larvae may feed primarily by chemical stimulus, rather than vision. In a preliminary experiment, it was found that when larvae were placed in a container in complete darkness, 97% of the larvae were able to capture *Artemia* nauplii. However, further study is required to verify this observation.

In summary, this study revealed that complex protein-walled capsules are readily ingested by striped bass larvae as first food. Potentially, complex-protein microencapsulated diet containing *Artemia* extracts can be used as partial replacement of live food and the vehicle for dietary enzyme and drug delivery. However, to achieve these goals, study is needed to improve its nutritional quality and digestibility particularly.

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References

- Adron, J.W., Blair, A., Cowey, C.B., 1973. Rearing of plaice (*Pleuronectes platessa*) larvae to metamorphosis using an artificial diet. Fish. Bull. 72, 353–357.
- Applebaum, S., 1985. Rearing the Dover Sole, *Solea* (L), through its larval stages using artificial diets. Aquaculture 49, 209–221.
- Baragi, V., Lovell, R.T., 1986. Digestive enzyme activities in striped bass from first feeding through larva development. Trans. Am. Fish. Soc. 115, 478–484.
- Bligh, E. G., Dyer, W. J., 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
- Chu, F.-L.E., Webb, K.L., Hepworth, D., Casey, B.D., 1987. Metamorphosis of larvae of *Crassostrea virginica* fed microencapsulated diets. Aquaculture 64, 185–197.
- Dabrowski, K., 1979. Role of proteolytic enzymes in fish digestion. in: Styczynska-Jurewicz, E., Backiel, E., Jaspers, T., Persoone, E. (Eds.), Cultivation of Fish Fry and Its Live Food, European Mariculture Society Special Publication, Vol. 4, Belgium, pp. 107–126.
- Dabrowski, K., Rusiecki, M., 1983. Content of total and free amino acids in zooplanktonic food of fish larvae. Aquaculture 30, 31–42.
- Ehrlich, K.F., Cantin, M.C., Rust, M.B., 1989. Growth and survival of larval and post-larval smallmouth bass fed a commercially prepared dry feed and/of *Artemia* nauplii. J. World Aquacult. Soc. 20, 1–6.
- Holland, D. L., Gabbott, P. A., 1971. A microanalytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. Mar. Biol. Assoc. UK 51, 659–668.

- Jones, D.A., Kamarudin, M.S., Le Vay, L., 1993. The potential for replacement of live feeds in larval culture. J. World Aquacult. Soc. 24, 199–210.
- Jones, D.A., Kurmaly, K., Arshard, A., 1987. Penaeid shrimp hatchery trials using microencapsulated diets. Aquaculture 64, 133–146.
- Jones, D.A., Munford, J.G., Gabbott, P.A., 1974. Microcapsules as artificial food particles for aquatic filter feeders. Nature 247, 233–235.
- Kanazawa, A., Teshima, S.-I., Inamori, S., Sumida, S., Iwashita, T., 1982. Rearing of larval Red Sea bream and Ayu with artificial diets. Mem. Fac. Fish., Kagoshima Univ. 3, 185–192.
- Kanazawa, A., Koshio, S., Teshima, S.-I., 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese Flounder, *Paralichthys olivaceus* fed microbound diets. J. World Aquacult. Soc. 20, 31–37.
- Langdon, C.J., 1989. Preparation and evaluation of protein microcapsules for a marine suspension-feeder, the Pacific oyster *Crassostrea gigas*. Mar. Biol. 102, 217–224.
- Langdon, C.J., Siegfried, C.A., 1984. Progress in the development of artificial diets for bivalve filter feeders. Aquaculture 39, 135–153.
- Leibovitz, H.E., Bengtson D.A Maugle, P.D., 1987. Effects of dietary *Artemia* lipid fractions on growth and survival of larval inland silversides, *Menidia beryllina*. in: Sorgeloos, P., Bengtson, D.A., Declair, W., Jaspers, E. (Eds.), *Artemia* Research and Its Application. Ecology, Culturing, Use in Aquaculture, Universa Press, Vetteren, Belgium, p. 556.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265–275.
- Ozkizilcik, S., Chu, F.-L.E., 1996. Preparation and characterization of a complex microencapsulated diet for striped bass *Morone saxatilis* larvae. J. Microencap. 13, 331–343.
- Ozkizilcik, S., Chu, F.-L.E., Place, A.R., 1996. Ontogenetic changes of lipolytic enzymes in striped bass (*Morone saxatilis*). Comp. Biochem. Physiol. 113B, 631–637.
- Seidel, C.R., Kryznowek, J., Simpson, K.L., 1980. International study on *Artemia* XI. Amino acid composition and electrophoretic protein patterns of *Artemia* from five geographical locations. in: Sorgeloos, P., Bengtson, D.A., Declair, W., Jaspers, E. (Eds.), *Artemia* Research and Its Application. Ecology, Culturing, Use in Aquaculture, Vol. 3, Universa Press, Vetteren, Belgium, p. 456.
- Teshima, S.-I., Kanazawa, A., Sakamoto, M., 1982. Microparticulate diets for the larvae of aquatic animals. Min. Rev. Daa File Fish. Res. 2, 67–86.
- Teshima, S.-I., Kanazawa, A., Yamashita, M., 1986. Dietary value of several proteins and supplemental amino acids for larvae of the prawn *Penaeus japonicus*. Aquaculture 51, 225–235.
- Tuncer, H., Harrell, R.M., Houde, E.D., 1990. Acceptance and consumption of food by striped bass and hybrid larvae. J. World Aquacult. Soc. 21, 22–234.
- Walford, J., Lim, T.M., Lam, T.J., 1991. Replacing live foods with microencapsulated diets in the rearing of seabass (*Lates calcarifer*) larvae: do the larvae ingest and digest protein-membrane microcapsules? Aquaculture 92, 225–235.
- Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for the mass propagation of fish: a review. Aquaculture 34, 115–143.
- Webster, C.D., Lovell, R.T., 1990. Comparison of live brine shrimp nauplii and nonliving diets as first food for striped bass larvae. Prog. Fish Cult. 52, 171–175.