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Formulation of Semi-Purified Diets for Striped Bass, *Morone saxatilis*, Larvae

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Abstract: Recent years have witnessed a dramatic rise in the intensive culture of striped bass, *Morone saxatilis* due to its reduced supply from capture fisheries and high market price. One of the major problems that limit its mass culture is non-availability of seed in sufficient quantities due to unreliability of live feeds and their high cost, *Artemia* for example. The main objective of these studies was, complete replacement of live food with artificial diets or at least reduce its quantity in larval food without compromising growth and survival of fish. Based on the chemical composition of natural zooplankton, the presumed ideal diet for striped bass larvae. various iso-caloric and iso-nitrogenous semi-purified diets were formulated. The chemically analyzed ingredients were homogenously blended, microencapsulated and fed to larval fish. Commercial starter diets were also used for comparison. There were two feeding trials. Eight and 12-day-old striped bass was used in trial 1 and 2 respectively. Former had 9 dietary treatments and was continued for two weeks. Instar I live *Artemia* nauplii and zooplankton served as control while their encapsulated forms were secondary controls. Fish fed on live *Artemia* and zooplankton exhibited the highest growth and survival comparatively lower in zooplankton fed group. Encapsulated live food ranked second. Fish in the rest of the treatments could gain only 0.1-5.8 mg and survival ranged just from 25-43%. Later trial, on the other hand, had 11 treatments and lasted four weeks. Same age and weight larval fish, were fed on live *Artemia* (control) nauplii, *Artemia* capsules and casein based semi-purified diet. All the diets were processed following the same procedures as implied in former trial. But unlike previous trial tannic acid was incorporated in encapsulation material of some of the diets to enhance capsule stability and dropped in others. Similarly betain was included in selected inert diets to boost diet acceptability. Some of the inert diets were also periodically supplemented with live *Artemia* nauplii. Fish reared on live *Artemia* maintained its superiority in growth and survival over its counterparts. Tannic acid containing diets with periodic supplementation of live *Artemia* gave 40-50% survival and reasonable growth while performance in rest of the treatments remained very poor. Fish grew only up to 11-25 mg and survival could not exceed 25%. It appears that striped bass larvae can not digest microcapsules due to poorly developed digestive system.

Key words: *Artemia*, striped bass, semipurified diets, microencapsulation

INTRODUCTION

Like other fish species production of striped bass, *Morone saxitilis* in commercial hatcheries still depends on supply of live food such as rotifers, *Brachionus plicatilis* and *Artemia* (Cahu and Infante, 2001). Pro-larvae and larvae, soon after yolk sac absorption, demands an exogenous and continuous source of food due to its rudimentary digestive system (Blaxter, 1969). Feeding fish larvae from hatching to metamorphosis on live organisms such as algae and micro crustaceans is technically difficult and may be impractical for large scale operations (Gabaudan, 1984). Persistent availability of sufficient food of sustainable quality around the larval fish is and has been a major constraint (Sorgeloos, 1980; Watanabe *et al.*, 1983). Collection of *Daphnia*, *Artemia* and *Brachionus* from natural sources can import pathogens and parasites to the hatchery (Uys and Hecht, 1985). Pathogen free and cost effective compound diet substitution for live prey is therefore crucial for lowering production cost and ensuring

sustainable supply of quality fish seed (Uys and Hecht, 1985; Bautista *et al.*, 1989).

Formulation of an effective compound diet for fish larvae is not easy to achieve. Nutritional requirements of larval stages of most of the species are not known and are hard to know by traditional nutritional approaches and further they change with the development of larvae (Dabrowski, 1984). Further the diet should be palatable, nutritionally adequate and particle size should be compatible to the oral cavity of the target animal and must be the true representative of the whole. These particles should resemble their natural counterparts in elasticity and collapsibility so that fish chemically and visually can recognize them as food (Appelbaum, 1980). Moreover, the individual particles must be fully protected from nutrient leaching. Poor diet stability leads to nutrient leaching, particle dissolution and subsequently bacterial fouling (Langdon, 2003). Feed should have a low moisture level to extend the shelf life and inhibit the growth of microorganisms.

Person Le Ruyet *et al.* (1993) formulated a diet adequate to sustain good growth and survival in European seabass, *Dicentrarchus labrax*. Similarly Zmbonino Infante *et al.* (1997) obtained significant growth and good survival in sea bass when fed only compound feed. Cahu *et al.* (1998) has achieved up to 35% survival when fed exclusively on compound feed from mouth opening with no cannibalism. The survival rate can be attributed to the efficiency of the compound diet, since unfed sea bass larvae do not survive after day fifteen. Concurrently some survival has been obtained in sea bream, *Sparus aurata* (Fernanandez-Diaz and Yufera, 1997) and in *Pagrus major* (Takeuchi *et al.*, 1998) but survival remained very poor in larval *Chitala* when fed on boiled egg-yolk (Sarkar *et al.*, 2006).

Microencapsulated diets appear to be a good option to over come these limitations. They can combine the texture of moist diets and the high stability of the best dry pellets. Their high stability may protect the nutrients from leaching and make them available to fish (Leibovitz *et al.*, 1987). They are consumed by estuarine fish species e.g., *Menidia beryllina* and crustaceans (Leibovitz *et al.*, 1987; Yufera *et al.*, 2002). The objective of present studies was to formulate nutritionally balanced and well protected water stable diets for striped bass. Diet formulae were based on the composition of natural zooplankton and particles were carefully encapsulated. The prepared diets were evaluated against live *Artemia*, live zooplankton and some commercially known starter diets.

MATERIALS AND METHODS

Experimental protocol and setup: Eight and 12-day old striped bass larvae were transported in oxygen permeated plastic bags, from the University of Maryland's Crane Aquaculture Facility (Trial 1) and the Verplanck Hatchery Hudson River, New York (Trial 2) respectively. They were transferred to 190-L aquaria and acclimated to the laboratory conditions in 5‰ saline water for three days. The experimental system consisted of acrylic cylindrical fish egg hatching jars (150 x 46H cm; 6L capacity), modified after the design of Buss (1959). On the 4th day, 11 and 15 day post hatching, fish were randomly placed into 28 (Trial 1) and 31 (Trial 2) jars. A random sample of five fish was taken from the original stock for preliminary weight and length measurements (Table 5). Fish were raised in 5‰ saline water at 24-26°C, under ambient photoperiod and low level constant aeration.

Every morning before the first feeding, the jars were cleaned, uneaten feed and extraneous material was removed from the jars. Dead fish was removed and counted and one third of the total water replaced. The fry were fed 100% of their wet weight throughout the study period at 10.00, 14.00 and 18.00 h with 3% daily increase. At the end of each experiment, the water was

completely drained off and the fish were harvested on a 150 µm net suspended in 2-phenoxy ethanol. The euthanized animals were blotted dry, weighed by an electronic balance to the nearest 0.1 mg and measured with a caliper to the nearest 0.1 mm.

Preparation of diets:

Diet 1 (LA): *Artesia* nauplii were hatched from a single batch of Reference *Artemia* cysts II (RAC II) (Bengtson *et al.*, 1985) in 500 ml separatory funnels containing 30% saline water with pH range of 7.75-8.3 and at 26°C temperature under constant light and aeration. After 36 h of incubation, aeration was stopped and the instar I nauplii, were allowed to settle at the bottom of the hatching flask and then collected on a 150 µm sieve. They were rinsed with deionized water and blotted dry. The nauplii were weighed and homogenously dispersed in the tank once every day early in the morning.

Diet 2 (LZ): Live zooplanktons, predominantly *Brachionus plicatilis*, collected daily from Pettaquamscutt River Narragansett, R.I., were concentrated at a plankton net of 110 µm mesh. Up to day three of the experiment, they were treated exactly like *Atremia* nauplii and fed to fish but due to mortality of zooplankton during the long handling time, the strategy was changed. They were transferred to a graded beaker that contained 50 ml sea water and examined for their total biomass. Repeated weighing and dissolution gave qualitative and quantitative estimation of zooplankton in a given volume of water. This practice was repeated every 3rd day to make sure that fish were not under fed.

Diet 3 (EAN): RAC II *Artemia* were hatched as in diet 1. The instar I nauplii were harvested on a 150 µm sieve, rinsed with de-ionized water and blotted dry on a paper towel. They were weighed and encapsulated according to the procedure explained for diet # 5 in this series except that the albumin-alginate ratio was 1:1.

Diet 4 (EZ): Zooplanktons were collected as in treatment 2 and were sieved through several meshes to remove extraneous material and undesirable organisms present in sample water. They were thoroughly rinsed with deionized water, blotted dry, weighed and encapsulated following the procedure as explained for diet # 5.

Diet 5 (ECD): Casein (Erie Foods International, Inc., IL) contained 94% protein, 0% fats 1% ash and 5% moisture (Table 1). Casein, vitamix, mineral mix and carboxymethyl cellulose (Table 1), were individually powdered in a coffee grinder and sieved through 106 µm mesh. The diet was formulated based on the chemical composition of zooplanktons (Table 2). The pre-sieved ingredients were then thoroughly mixed in Sorvall

Table 1: Ingredient composition of casein and yeast based artificial diets for striped bass, *Morone Saxatilis*, larvae

| | % in diet | |
Ingredient	Casein based	Yeast based
Casein	60.0	-
Yeast	-	78.7
Vitamix	5.0	2.6
Mineral mix	4.0	0.6
Menhaden fish oil	13.0	10.9
Fatty acyl methyl esters	4.2	5.05
Stay-C (Ascorbyl-PP)	0.1	0.05
Carboxymethylcellulose	13.7	2.1

Omani-Mixer. Intermixing of dry ingredients was followed by the addition of menhaden fish oil (Zapata Haynie Corporation, Virginia), stay-C (VTI Vitamin Technologies International, ID) and fatty acid methylesters (Ocean Organics, RI). The mixture obtained was then encapsulated (Leibovitz, 1990).

Diet 6 (EYD): Autolysed yeast extract (Rhone-Poulenc Food Ingredients Division, PA) was finely ground (106 μm) and mixed with measured amount of fish oil, fatty acid methylesters, vitamins and minerals and then encapsulated (Leibovitz, 1990). Protein, fat, ash and moisture contents of yeast were 77, 1, 13 and 9% respectively.

Diet 7 (AP200): Closed formula commercial starter diet (AP200) (Ziegler Brothers Inc., PA), was analyzed for its nutritional information and fed to fish without any further modification.

Diet 8 (EAP200): Encapsulated diet # 7.

Diet 9 (ABD): *Artemia* meal-based commercial starter diet (PDM Associates, CT), was analyzed and sieved through 150 and 250 μm mesh and fed to fish without any modification.

Preparation of diets for trial 2: Live, encapsulated and formulated diets were processed following the same procedures as described for different diets in trial 1. The differences were only addition of tannic acid and attractant in some diets (Table 5).

Chemical analysis: Crude protein, ash and moisture was determined by the AOAC (1984) methods (Table 2). The amino acids were analyzed (Table 4) by the Pico-Tag method (Bennet and Solomon, 1986) that involves the protein hydrolysis (Ng *et al.*, 1987) and pre-column derivatization of the amino acids followed by Reverse Phase High Performance Liquid Chromatography (HPLC) (O' Hare *et al.*, 1987).
Crude lipids were estimated (Table 2) by the Bligh and Dyer (1959) Method as modified by Kates (1986). Fatty acid profile (Table 3) was determined using the NOAA

protocol (1988) of National Marine Fisheries for the analysis of marine fish oil.

Experimental design: Both feeding trials were designed as "Completely Randomized Design" CRD). The treatment group fed on live *Artemia* nauplii served as control in both experiments while "unfed" group was negative control. There were three replicates in each treatment with 25 individuals in each replica except unfed that had single replica. All the jars were numbered and randomly allotted to treatment groups.

Statistical analysis: One Way Analysis of Variance followed by Duncan's Multiple Range Test, was used to evaluate the statistical significance of the treatment differences. Differences between the treatment means were considered significant at $p < 0.5$ level.

RESULTS

Feeding activity and behavior: Both live diets (*Artemia* nauplii and zooplankton) were eagerly taken up by fish and they consumed all the available live organisms within a short span of time. Response of larvae was slow towards inert diets. On the 3rd day of the experiment, fish larvae present in casein-based diet, Ap200 powder and encapsulated *Artemia* group, hesitantly started to swallow the capsules. The response was comparatively better towards AP200 powder. Sometimes fish spit out the particles after devouring it. The food particles were visible through transparent larval gut by day five. During the next one and a half week fish appeared healthy and active in those diet groups where they had showed some positive response towards feed. The fish fed on encapsulated yeast-based diet, casein-based diet without tannic acid and unfed control, were totally dead at the end of the first week.
Cannibalism was visually observed and was more prominent in encapsulated casein-based diet, yeast-based diet, encapsulated AP200 and encapsulated *Artemia* without tannic acid. One fish running after the other and devouring it as a whole when convenient, was seen many times during feeding trials. At the end of the trial mortality was calculated by difference. Number of live fish recovered, was subtracted from the initial stock. The missing fish were considered the victims of cannibalism. Data showed that 17-18% of the total stock was lost due to this problem.

Survival and growth

Trial 1 survival: Survival was significantly higher in *Artemia* fed group than rest of the treatment groups (Table 5). Consequential mortality started in treatment 6 (yeast based diet) and treatment 10 (unfed) on the second day of experiment and all the individuals were dead on or before day nine (Fig. 1). Although mortality

Table 2: Proximate analysis of live, live encapsulated, formulated and commercial diets on dry weight basis

Nutrients	Diet #								
	1	2	3	4	5	6	7	8	9
Protein	61	57	61	57	57	57	53	53	44
Fats	11	17	11	17	17	17	10	10	11
Ash	11	25	11	25	4	19	13	13	12
Moisture	-	-	5	5	5	5	5	5	13

Note: Diet # follows the pattern as in feeding trial 1(Material and Method section)

Table 3: Fatty acid composition (mg/gm dry weight) of live and inert diets

FAMES	Diet #						
	1 and 3	2	4	5	6	7 and 8	9
14:0	1.1	1.9	1.9	1.5	1.6	2.6	1.4
14:1	0.9	-	-	0.1	0.1	-	0.8
15:0	0.6	-	-	0.1	0.1	0.2	0.4
15:1	0.5	-	-	-	-	-	-
16:0	12.5	7.5	7.5	4.3	4.5	12.9	10.9
16:1	12.4	4.2	4.2	2.4	2.2	3.4	7.9
17:0	3.0	-	-	0.4	0.4	0.5	0.8
16:2w4	4.0	-	-	0.4	0.4	0.6	1.3
18:0	4.0	1.0	1.0	1.4	1.4	3.4	2.9
18:1w9	33.5	3.3	3.3	4.4	4.4	10.7	18.4
16:3w4	-	-	-	0.2	0.2	-	-
18:2w9	10.0	-	-	0.2	0.2	-	-
18:2w6	-	0.7	0.7	0.2	0.4	14.8	8.6
20:0	-	-	-	-	-	0.2	-
18:3w3	0.5	0.4	0.4	5	4.7	1.7	5
20:1w9	4.5	-	-	-	1.2	1.5	-
18:4w3	-	1.9	1.9	0.7	0.6	0.7	1.0
21:5	-	-	-	-	-	0.2	-
20:2w6	-	-	-	-	-	-	-
20:3w6	-	-	-	0.1	0.1	1.1	-
22:0	-	-	-	2.2	2.2	1.0	-
20:4w6	-	-	-	0.2	0.2	-	-
20:5w3	4.5	4.3	4.3	7.1	7.1	4.1	4.1
22:5w3	5.5	-	-	0.1	0.1	-	-
22:6w6	-	-	-	0.3	0.3	-	-
22:5w3	-	-	-	0.9	0.8	0.5	-
22:6w3	-	5	5	4.6	4.6	5.1	-

had started in the other treatment groups, the rate of mortality, however, was comparatively low. Fish fed on live zooplankton was the second highest in survival. Survival (%) was similar in encapsulated *Artemia* nauplii, encapsulated zooplankton, encapsulated casein-based diet and encapsulated AP200. Survival, however, was comparatively lower in *Artemia* meal based diet (19%) but not statistically different from aforementioned inert diets (Table 5).

Growth: The fish fed on live food (*Artemia* and zooplankton) showed a substantial increase in weight. The final weight was 16 mg for fish fed on *Artemia* nauplii and 12 mg for live zooplankton. The respective weight gain was 486% and 314% over the initial body weight in both treatment groups. The weight gain in the rest of the treatment groups ranged from 0-139%. The fish fed on encapsulated zooplankton, AP200, casein and *Artemia* meal based diet did not grow at all. The larvae fed on AP200 capsules and *Artemia* meal based diet showed 14% reduction in their initial weight. Similar variations were noted when their specific growth rates were compared (Table 5).

Trial 2 survival: Significantly higher survival (78.7%) was observed in live *Artemia* fed group than rest of the treatments, even after 30 days of rearing (Table 5). Like the previous experiment, mortality had started on the second day but the rate was low compared to the first experiment. Mortality rate in live *Artemia* group leveled off after 11 days. A sharp jump in mortality was observed in casein-based diet without tannic acid and unfed control and all the fish were dead in the first half of the 2nd week of experiment (Fig. 2). Fish fed on casein-based diet with tannic acid and tannic acid +attractant survived up to the beginning of the third week. Mortality then started and all the fish were dead within a short period of time (Fig. 2). Among the surviving treatments, casein-based diet
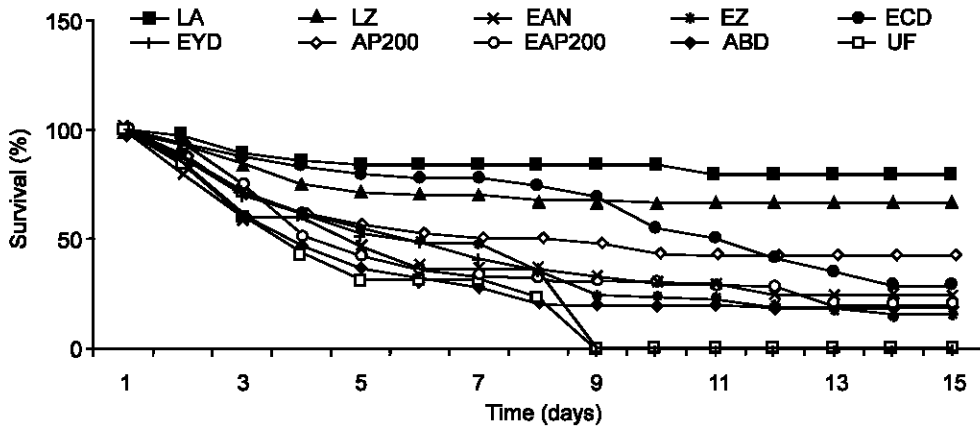
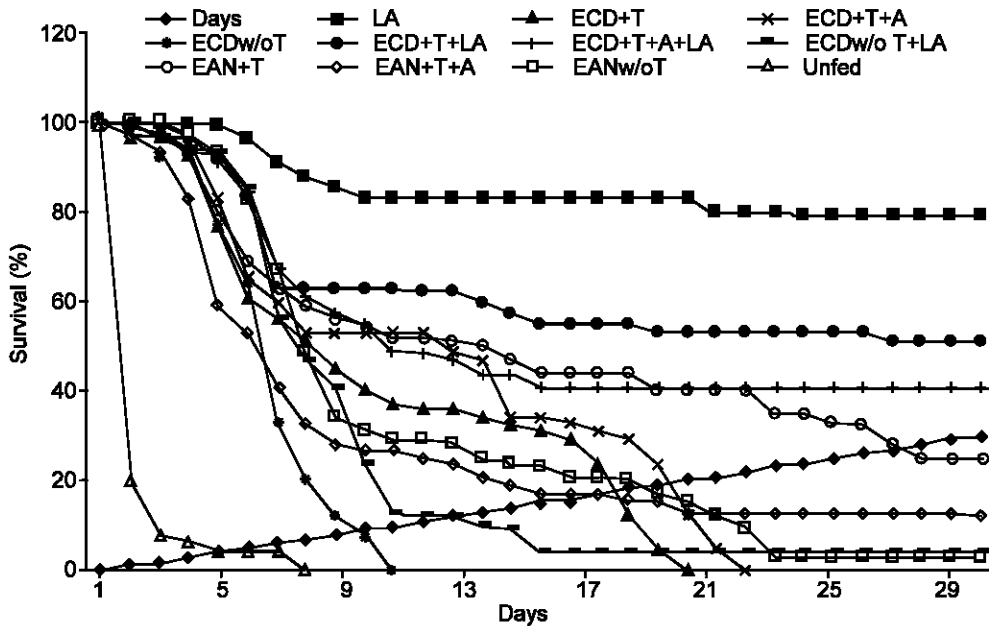Fig. 1: Survival (%) of striped bass larvae in trial 1

Fig. 2: Survival (%) of striped bass larvae in trial 2

containing tannic acid with live *Artemia* supplementation every 4th day, exhibited significantly higher survival (51%) than those where live *Artemia* was omitted and capsules were prepared without tannic acid. Survival in these groups ranged from 6% (casein without Tannic Acid + live *Artemia*; *Artemia* capsules without tannic acid) to 40% (casein + Tannic Acid + Attractant + live *Artemia*). Fish fed on diets with tannic acid-containing microcapsules showed overall better survival. Periodic supplementation of live *Artemia* amplified this response (Fig. 2).

Growth: The fish fed on live *Artemia* nauplii yielded the highest weight (Table 5) while those fed on combined diets (live *Artemia* + artificial diets) were second in growth. *Artemia* capsules prepared with and without tannic acid, showed significant growth differences, more

in tannic acid containing capsules and very poor where tannic acid was excluded (Table 5).

DISCUSSION

During these studies larvae fed on experimental diets, ingested some diets but not others. The reasons for their total refusal to encapsulated yeast-based diet (Trial 1) and casein-based diet without tannic acid (Trial 2) might be small mouth size or poor diet quality that failed to stimulate gustatory responses. Larval fish responded slowly towards casein-based diet with tannic acid in capsule material and or where attractants were also added. Unlike the former, in the later, processing modifications, supported survival of larval fish up to 3rd week of experiment but sudden mortality then ensued and all the individuals perished within a very short period of time. There can be multiple factors governing the

Table 4: Amino acid composition (mgs/gm of dry weight) of diets

AA	mgs in diet						
	1 and 3	2	4	5	6	7 and 8	9
ASP	31.2	40.4	40.4	29.4	60.9	44.6	30
GLU	153	89.2	89.2	139	70.2	83.6	79.3
SER	30.5	20	20	30.3	30.4	26.3	16.5
GLY	22.7	39.7	39.7	19.6	29.1	38.5	22.4
HIS	24.1	12.1	12.1	21.6	8.4	12.6	8.5
ARG	31.8	54.7	54.7	31.2	49.7	50.7	29.9
THR	20.8	21.6	21.6	18.2	33.8	25.2	13.9
ALA	16.9	42.8	42.8	17	54.5	30.1	22.9
PRO	85.7	39.6	39.6	78.6	30.5	35.2	28.8
TYR	35.7	27.6	27.6	34.3	2.3	11.9	10.3
VAL	28.6	32.1	32.1	27	34.6	2.1	23.7
MET	12.3	12.6	12.6	10.8	5.3	11.9	7.8
CYS	-	0.8	0.8	0.2	0.4	2.1	-
ISO	18.2	22.8	22.8	19.9	25.3	21.5	20.2
LEU	29.2	38.1	38.1	30	33.7	33.2	31.1
PHE	20.8	24.3	24.3	20.1	21.5	23.8	19
LYS	46.7	37	37	41.8	47.2	34.5	27

Table 5: Survival (%), final wet body weight (mg) and Specific Growth Rate (SGR) of striped bass in feeding trials 1 and 2. Data are presented as mean±SE. Initial mean wet weight of the larvae was 2.8±0.3 and 2.6±0.3 mg respectively. Values in a column followed by the same superscript letters are not significantly different from each other (p<0.05)

Treatment[1]	Survival	Weight	SGR[2]
(LA)[1]	80±4[a]	16±5[a]	11.7
(LA)[2]	79±6[a]	64±17	10.7
(LZ)[1]	67±14[b]	12±5[b]	9.5
(EAN)[1]	25±10[c]	7±2[c]	5.8
(EAN+T)[2]	25±0[b]	16±7[b]	6
(EAN+T+A)[2]	12±6[c]	15±5[b]	6
(EAN W/O T)[2]	6±3[d]	11±3[c]	4.7
(EZ)[1]	27±8[c]	3±1[d]	0.2
(EYD)[1]	0		
(ECD)[1]	29±7[c]	3±1[d]	-0.1
(ECD+T)[2]	0		
(ECD+T+A)[2]	0		
(ECD W/O T)[2]	0		
(ECD+T+LA)[2]	51±13[e]	26±9[d]	7.7
(ECD+T+A+LA)[2]	40±24[f]	26±7[d]	7.7
(ECD W/O T+LA)[2]	6±1[d]	26±4[d]	7.7
(AP200)[1]	43±9[d]	6±2[c]	4.7
(EAP200)[1]	28±17[c]	2±0.1[d]	-0.6
(ABD)[1]	19±8[e]	3±1[d]	0.2

A: Superscript 1, 2 represents trials, T stands for tannic acid and A for attractant. Detail of other abbreviations can be seen under heading, "Diet Preparation" in "Material and Method" section.

B: SGR (Average daily growth as percentage of the initial weight) = ln (final weight) - ln (initial weight) x 100 ÷ No. of days

rearing process of this tiny creature and will be tried to explain in the following text.

Nutrient level in the artificial diets or their chemical composition is the first step to consider in diet formulation. Since the nutrient requirements of the larval striped bass like most of the other marine fish species (Park *et al.*, 2006) are not known, hence it is impossible to formulate a balanced diet and/or even balanced diet can be nutrient-deficient due to nutrient leaching (Medgyesy and Wieser, 1982; Langdon, 2003; Yufera *et al.*, 2002) if diet particles are not fully protected. These problems were very carefully addressed in the present studies. The diets were based on the nutrient composition of natural zooplankton, the presumed ideal diet for larval striped bass. The diet particles were well protected in albumen-alginate capsules, whose ability to prevent leaching has been documented by (Leibovitz, 1990; Ozkizilick and Chu, 1996; Yufera *et al.*, 2002).

The encapsulated diet particles were though non-motile, yet their retention time in the water column was much longer than anticipated and they were available to larvae providing ample opportunity for sufficient intake. Larvae were observed schooling both at the top and bottom striking the capsules and they were visible inside the transparent gut of the larvae soon after feeding. It means that like other species such as blue spotted goby (Clack, 2006) and *Chitala chitala* larvae (Sarkar *et al.*, 2006), larval striped bass was also capable of capturing and ingesting non-living and non-motile prey.

Stout outer albumen-alginate tannic acid bonded coating can be a barrier to the proper dissolution of capsules and ultimate release of nutrients. Walford *et al.* (1991) found that protein membrane capsules did not break down even after 2 h retention in the gut of sea bass larvae and the capsules were expelled intact. Bengtson *et al.* (1993) in his detailed histological studies discovered that digestion of albumen-alginate capsules in striped bass larvae differed from that of live *Artemia*. Although the capsules were broken down in the passage through the digestive tract, very little of capsule contents were actually digested and absorbed. This indicates that there was no proper mechanical or physiological mechanism in larval gut which could breakdown the outer coating of the capsule and let the nutrients go in the gut. It was obvious from the

superiority in growth and survival of fish fed on AP200 powder (Trial 1) to that of its counterpart. Might be the nutrients in mash were comparatively more available (Tonheim *et al.*, 2005) to digestive set-up and they supported growth and survival of fish.

Dabrowski (1979), Dabrowski *et al.*(2000), Yufera *et al.* (2000) and Cahu and Infante (2001) demonstrated the importance of exogenous enzymes for larvae, available in live foods. Live foods transferred 70% of total protease activity in whitefish larvae (Hofer, 1985) and 89-94% of the total esterase, 79-88% exonuclease, 43-60% protease and 15-27% amylase in turbot larvae (Munilla-Moran *et al.*, 1990). Kurokawa *et al.* (1998) on the other hand observed that only 0.6% protease of the total enzyme, present in 2-day-old Japanese sardine larval gut, was due to rotifers. Our observations support some but contradicts with others and affirm that some enzymes were present in larval gut at the onset of first feeding but might not be in sufficient quantities to support on going digestive processes. Sirvas-Cornejo *et al.* (2007) has recently observed better growth in post larval *Fenneropenaeus indicus* when he supplemented microencapsulated diet with genetically modified enzyme (protease) producing bacteria. Similarly Keysami *et al.* (2007) have reported higher survival and faster rate of metamorphosis in those *Macrobrachium rosenbergii* larvae which were fed on *Artemia* treated with *Bacillus subtilis* bacterium than those which were hatched in the absence of bacteria. Hence it can be deduced from recent and previous studies that addition of an appropriate exogenous enzyme source is must for successful weaning of larvae on artificial diets.

When larvae were fed on combined diets (artificial diets + live *Artemia* nauplii on every 4th day), growth and survival remained in between to those fed on sole artificial diets or live *Artemia* with no cannibalism at all. Battaglene and Cobcroft (2007) observed reduced rate of malformations and better growth in striped bass trumpeter larvae when fed on mixed (artificial + live food) feeding schedule. Investigations of these workers strongly support and confirm our findings. It seems that inert diets offered, maintained proper nutrient balance and motility, collapsibility and characteristic colour/odor of live food, not only instigated larvae to take more feeds but also transferred some digestive factors into the larval gut. The combined effect of two dietary sources helped the larvae to nearly approach control group in growth and survival. Tonheim *et al.* (2007) and Conceicao *et al.* (2007) have similar findings in their investigative work. Therefore, total elimination of live food for successful larval rearing (Fletcher *et al.*, 2007), at least at this time, without reducing growth potential, needs further extensive research work.

Addition of attractants did not make any difference to the performance of larval fish though quite encouraging results have been obtained previously in larval yellow perch (Dabrowski *et al.*, 2000). Fatty acid and amino acid composition was almost uniform in all the diets implied because the formulation was based on the composition of the natural food. Concentration of fatty and amino acids was even higher in some inert diets. Unlike previous studies (Turner and Rooker, 2005), this slight variation in present situation apparently did not play any differential role in larval growth and survival, hence do not warrant further explanation.

From these studies it can be concluded that there is not a single factor contributing in rearing of larval fish but a series of factors. Digestive capabilities and dietary requirements of the larvae need to be determined through the combination of biochemical, physiological and morphological studies. Feed formulation then need to be based on the integration of information obtained from these studies which is attractive to larvae, can stimulate the required enzymes at and when required and are digestible eliminating the dependence on costly and unreliable natural foods. Specific sites for digestion and absorption of nutrients in the larval gut need to be thoroughly studied by the use of radioisotopes and fluorescence dyes. Not less important is to learn the inherent attractant in natural food that motivates the larvae and stimulates the ingestion of live foods. Further refinement in encapsulation technology for the safe delivery of nutrients and their release at suitable sites and at appropriate time is of great significance in preparation of effective weaning diets which will be a major breakthrough in larval rearing technology.

REFERENCES

AOAC (Association of Official Analytical Chemists), 1984. Official Methods of Analysis, 14th Edn. AOAC, Arlington Virginia, USA.

Appelbaum, S., 1980. Versuche zur geschmacksperzeption einiger susswasserfische in larvalen und adulten stadium. Archives Fischereiwiss, 31: 105-114.

Battaglene, S.C. and J.M. Cobcroft, 2007. Advances in the culture of striped trumpeter larvae: A review. Aquaculture, 268: 195-208.

Bautista, N.M., O.M. Millamena and A. Kanazawa, 1989. Use of Kappa-carragenan microbound diet (C-MBD) as feed for *Penaeus monodon* larvae. J. Marine Biol., 103: 169-173.

Bengtson, D.A., A.D. Beck and K.L. Simpson, 1985. Standardization of the nutrition of fish in aquatic toxicological testing. Pages 431-446 in C.B. Cowey, A.M. Mackie and J.G. Bell, Eds. Nutrition and feeding in fish. Acadmic Press, London.

Bengtson, D.A., D. Borrus, H.E. Leibovitz and K.L. Simpson, 1993. Studies on digestive structure and function in larvae of inland silversides, *Menidia beryllina*. In Physiological and Biological Aspects of Fish Development (B. Walther and H.J. Fyhn, Eds.), pp: 199. Uni. Bergen, Bergen, Norway.

Bennet, H.P.J. and S. Solomon, 1986. Use of Pico-Tag methodology in the chemical analysis of peptides with carboxyl terminal amides. J. Chromatography, 359: 221-230.

Blaxter, J.H.S., 1969. Development: eggs and larvae. Pages 178-152 In H.S. Hoar and D.J. Randall Eds. Fish physiology, volume 3. Acadmic press, New York, New York, USA.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. and physiol., 37: 911-937.

Buss, K., 1959. Jar culture of trout eggs. The progressive Fish Culturist, 21: 26-29.

Cahu, C.L., J.L. Zambonino-Infante, A.M. Escaffre, P. Bergot and S. Kaushik, 1998. Preliminary results on sea bass, *Dicentrarchus labrax* larvae rearing with compound diet from first feeding. Comparison with carp Cyprinus carpiuo larvae. Aquaculture, 169: 1-7.

Cahu, C. and J.Z. Infante, 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture, 200: 161-180.

Clack, B.W., 2006. Development of microparticulate feeds and methods to improve acceptability of artificial diets by blue spotted goby (*Asterropteryx semipunctata*). Oregon State University (HMSC) Publications, 121 the Valley Libraray, Corvallis, USA.

Conceicao, L.E.C., L. Reibero, S. Engrola, C. Aragao, S. Morais, M. Lacuisse, F. Soares and M.T. Dinis, 2007. Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). Aquaculture, 268: 64-81.

Dabrowski, K., 1979. Role of proteolytic enzymes in fish digestion. Pages 107-126 In E. Styczynskjurewicz, E. Backiel, T. Jaspers and E. Persoone Eds. Cultivation of fish fry and its live food. European Mariculture Society Special Publication 4, Bredene, Belgium.

Dabrowski, K., 1984. The feeding of fish larvae: present state of the art and perspectives. Reprod. Nutr. Dev., 24: 807-833.

Dabrowski, K., S. kolkovski and C. Theis, 2000. Application of New Biotechnology of Micro-encapsulation. Department of Natural Resources, Project Number: R/A-12, The Ohio State University, USA, pp: 1-3.

Fernanandez-Diaz, C. and M. Yufera, 1997. Detecting growth in gilthead sea bream *Sparus aurata* L. larvae fed microcapsules. Aquaculture, 153: 93-102.

Fletcher, Jr. R.C., W. Roy, A. Davie, J. Taylor, D. Robertson and H. Migaud, 2007. Evaluation of new particulate diets for early weaning of Atlantic cod (*Gadus morhua*): Implications on larval performance and tank hygiene. Aquaculture, 263: 35-51.

Gabaudan, J., 1984. Posthatching morphogenesis of the digestive system of striped bass. Doctoral dissertation. Auburn University, Alabama, USA.

Hofer, R., 1985. Effects of artificial diets on the digestive process of larvae. Pages 213-216. In Nutrition and Feeding in Fish, C.B. Cowey, A.M. Mackie and J.B. Bell Eds. Institute of Marine Biochemistry Aberdeem, Scotland.

Kates, M., 1986. Lipid extraction procedures. Pages 347 In Techniques of Lipidology: Isolation, Analysis and Identification of lipids. Elsevier Press, Amesterdam.

Keysami, M.A., C.R. Saad, K. Sijam, H.M. Daud and A.R. Alimon, 2007. Effect of *Bacillus subtilis* on growth and survival of larvae *Macrobrachium rosenbergii* (de Man). Aquaculture Nutr., 13: 131-136.

Kurokawa, T., M. Shirashi and T. Suzuki, 1998. Quantification of exogenous protease derived from zooplankton in the intestine of Japanese sardine (*Sardinops melnoticus*) larvae. Aquaculture, 161: 491-499.

Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae. Aquaculture, 227: 259-275.

Leibovitz, H.E., D.A. Bengtson, P.D. Maugle and K.L. Simpson, 1987. Effects of dietary *Artemia* lipid fractions on growth of larval inland silversides, *Menidia beryllina*. Pages 469-478 In W. Decleir, I. Moens, P. Sorgeloos and E. Jaspers Eds. *Artemia* Research and its Applications, volume 3. Universa Press Wettern, Belgium.

Leibovitz, H.E., 1990. Abumen-alginate microcapsules for delivering food to larval inland. silversides, *Menidia beryllina*. Doctrol dissertation. University of Rhode Island, USA.

Medgyesy, N. and W. Wieser, 1982. Rearing of white fish, *Coregonus lavaretus*, with frozen zooplankton by means of new feeding apparatus. Aquaculture, 28: 327-337.

Munilla-Moran, R., J.R. Stark and A. Barbour, 1990. The role of exogenous enzymes in digestion, of cultured turbot larvae, *Scophthalmus maximus*. Aquaculture, 88: 337-350.

Ng, L.T., A. Pascaud and M. Pasvaud, 1987. Hydrochloric acid hydrolysis of protein and determination of tryptophan by Reverse Phase High Performance Liquid Chromatography. Analytical Biochem., 167: 47-52.

NOAA, 1988. Fatty acid composition. Pages 6-11 In VanDolah, F.M. and Galloway, S.B. Eds. Biomedical Test Materials program: Analytical Methods for the Quality Assurance of Fish oil. Charleston, SC: U.D. Department of Commerce, USA.

O' Hare, M.M.T., O. Tortora, U. Gether, H.V. Nielson and T.W. Schwartz, 1987. High Performance Liquid Chromatography of phenylthiocarbamyl derivatives of amino acids and side chain derivatized amino acids. J. Chromatography, 389: 379-388.

Ozkizilick, S. and F.L. Chu, 1996. Preparation and Characterization of a complex microencapsulated diet for striped bass, *Morone Saxatilis*, larvae. J. Microencapsul., 13: 331-43.

Park, G.H., V. Puvanendran, A. Kellett, C.P. Christopher and A.J. Brown, 2006. Effect of enriched rotifers on growth, survival and composition of larval Atlantic cod (*Gadus morhua*). J. Marine Sci., 63: 285-295.

Person Le Ruyet, J., J.C. Alexandre, L. Thebaud and C. Mugnier, 1993. Marine fish larvae feeding: formulated diets or live preys? J. World Aquac. Soc., 24: 211-224.

Sarkar, U.K., W.S. Lakra, P.K. Deepak, R.S. Nagi, S.K. Paul and A. Srivastava, 2006. Performance of different types of diets on experimental larval rearing of endangered *Chitala chitala* (Hamilton) in re-circulatory system. Aquaculture, 261: 141-150.

Sirvas-Cornejo, S., J.W. Latchford and D.A. Jones, 2007. Effect of microencapsulated diets supplemented with genetically modified bacteria on the growth and survival of *Fenneropenaeus indicus* postlarvae. Aquaculture Nutr., 13: 10-16.

Sorgeloos, P., 1980. The use of brine shrimp in aquaculture. Pages 25-26. In G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers Eds. The brine shrimp *Artemia*, Volume 3: Ecology, culturing and use in aquaculture. Universa press, Wettern Belgium.

Takeuchi, T., N. Ohkuma, S. Ishida, W. Ishizuka, M. Tomota, H. Hayasawa and H. Miyakawa, 1998. Development of micro-particle diet for marine fish larvae. VIII Int. Symp. Nutrition and Feeding of Fish. Las Palmas, Spain, June 1-4. pp: 193.

Tonheim, K.S., M. Espe, K. Hamre and I. Ronnestad, 2005. Pre-hydrolysis improves utilization of dietary protein in the larval teleost Atlantic halibut (*Hippoglossus hippoglossus* L.) J. Experimental Marine Biol. and Ecol., 321: 19-34.

Tonheim, K.S., A. Nordgreen, I. Hogoy, K. Hamre and I. Ronnestad, 2007. *In vitro* digestibility of water-soluble and water-insoluble protein fractions of some common fish larval feeds and feed ingredients. Aquaculture, 262: 426-435.

Turner, J.P. and J.R. Rooker, 2005. Effect of dietary fatty acids on the body tissues of larval and juvenile cobia and their prey. J. Experimental Marine Biol. and Ecol., 322: 13-27.

Uys, W. and T. Hecht, 1985. Evaluation and preparation of an optimal dry feed for the primary nursing of *Clarias gariepinus* larvae. Aquaculture, 47: 173-183.

Walford, J., T.M. Lim and T.J. Lam, 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., C. Kitajima and S. Fujita, 1983. Nutritional value of live organisms used in Japan for mass propagation of fish: A review. Aquaculture, 34: 115-143.

Yufera, F-D., S. Pasuial, D. Moyano, G. Alercon and G-G. Parra, 2000. Towards an inert diet for first feeding gilthead seabream, Sparus aurata l. larvae. Aquaculture Nutr., 6: 143.

Yufera, M., S. Kolkovski, C. Fernandez Diaz and K. Dabrowski, 2002. Free amino acid leaching from protein walled microencapsulated diet for fish larvae. Aquaculture, 214: 273-287.

Zmbonino Infante, J.L., C.L. Cahu and A. Peres, 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. J. Nutr., 127: 608-614.