The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Effects of Early Weaning on Growth and Digestive Enzyme Activity in Larvae of Sea Bass (*Dicentrarchus labrax* L.)

Cuneyt Suzer[1]*, Kursat Firat[1], Sahin Saka[1] and Arif Karacaoglan[2]

[1] Ege University, Faculty of Fisheries, Aquaculture Department, 35100 Bornova, Izmir, Turkey

[2] Hatko Sea Products Inc., Oren, Milas, Mugla, Turkey

(Received 5.8.06, Accepted 30.10.06)

Key words: sea bass, digestive enzymes, early weaning, growth, survival

Abstract

The influence of early weaning on digestive enzymes, growth, and survival in sea bass larvae were investigated. Larvae were reared in a closed sea water system and first fed *Artemia* nauplii eight days after hatching. Three weaning periods were compared by introducing a microparticulate diet on day 15, 20, or 25. The control continued to be fed only *Artemia.* The lowest growth and survival rates were obtained in the 15-day group while the highest were in the 25-day group. After weaning, protease activities (trypsin, chymotrypsin, elastase, pepsin) dropped in all groups, indicating malnutrition. Amylase activity slightly increased in all experimental groups due to the higher starch content in the microparticulate diet than in *Artemia* and the absolute and relative lipid contents were lower in groups fed the microparticulate diet. Lipase activity suddenly dropped after weaning but slightly rose from day 35 until the end of the experiment (day 40). Due to decreased larvae development, survival, and digestive enzyme activity, weaning at 20 days after hatching, synchronous to formation of the stomach and enzymatic activity, is strongly recommended.

Introduction

Larvae rearing of marine fish mainly depends on live feeds such as the rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* and *Artemia* sp. However, the large amounts of *Artemia* cysts needed to maintain an uninterrupted food chain and nutritional quality by enrichment require large investments. This is especially relevant in sea bass, where live food accounts for 79% of the production costs for juveniles up to 45 days old. In the first three months of life, live food represents 50% of the feed costs even though it constitutes only 1.6% of the total dry weight of the required food (Person-Le Ruyet et al., 1993). More and more investigations are focusing on early weaning and its effects on digestive physiolo-

* Corresponding author. E-mail: cuneyt.suzer@ege.edu.tr

gy in marine fish larvae. In addition, intensive research is being conducted to find full or partial replacements for live food organisms (Kolkovski et al, 1996; Fernandez-Diaz and Yufera, 1997; Rosenlund et al., 1997; Cahu et al., 1998; Southgate and Partridge, 1998). Improvements in extruder feed technology and new food formulae have increased larvae survival and quality (Cahu and Zambonino Infante, 2001; Kolkovski, 2001), while higher water temperatures may enhance larval metabolism, potentially improving their readiness to accept inert diets (Applebaum, 1989).

The digestive functions of marine fish larvae, including sea bass, undergo major developmental changes during the first month of life and until acquisition of an adult mode of digestion (Cahu and Zambonino Infante, 1995). Recent studies focused on the functional changes in the digestive tract during larval development by studying the onset and variation of pancreatic and intestinal digestive enzymes and the response of these enzymes to diet concentration and composition. In this respect, early weaning of marine fish larvae onto microparticulate diets has been remarkable and is accepted as a substitute for live foods (Baskerville-Bridges and Kling, 2000; Hamlin and Kling, 2001; Alves et al., 2005; Curnow et al., 2006).

There is literature on the early weaning of sea bass larvae onto microparticulate diet (Cahu and Zambonino Infante, 1994; Cahu et al., 1999), but no earlier studies have measured digestive enzymes (pepsin, chymotrypsin, elastase, lipase) during weaning. The aims of this study were to investigate the effects of early weaning on digestive proteolytic and lipolytic enzyme activity and to measure the above digestive enzymes.

Materials and Methods

Broodstock and egg incubation. Twelve female (mean 2.4 kg) and 12 male (mean 2.1 kg) European sea bass (*Dicentrarchus labrax* L.) were selected from wild breeders and stocked in a 16-m^3 tank with a seawater supply of 35 l/min, a natural photoperiod (8 h light:16 h dark), and a water temperature of 14-15°C. The broodstock were fed frozen cuttlefish (*Sepia officinalis*) and *Leander squilla* (*Palaemon elegans*) daily as the primary food source. Spawned eggs were immediately collected in 500-µm mesh collectors. Following fertilization, viable buoyant eggs were separated from dead ones and incubated in 300-l incubators at an initial density of 4000 eggs/l with a gentle flow of 14.5±0.5°C sea water. Oxygen saturation was over 85%, salinity 38‰, pH around 7.6, and ammonia and nitrite below 0.011 mg/1.

Larvae rearing. Larvae were reared in three closed seawater systems that included three dark-gray cylinder-conical 3-m^3 tanks per system. Larvae were stocked at 100 individuals/l. Water temperature, dissolved oxygen, salinity, pH, ammonia, and nitrite were monitored daily. Water temperature was maintained at 15-20°C (temperature increased from 15 to 16°C between days 1 and 7, from 16 to 19°C between days 8 and 21, and from 19 to 20°C between days 22 and 40). Oxygen, salinity, and pH were maintained at >85%, 38‰, and 7.8, respectively. Ammonia and nitrite were kept constant and below 0.01 mg/1. The water exchange rate was gradually increased with the age of the larvae. Light, supplied by fluorescent tubes, was 50-100 lux at the water surface. The photoperiod was set at 16 h light:8 h dark.

Newly hatched larvae were fed *Artemia* nauplii (AF 430, INVE Aquaculture, Ghent, Belgium) at 1-2 individuals/ml from day 8, *Artemia* nauplii (AF 480, INVE Aquaculture, Ghent, Belgium) at 1-2 individuals/ml from day 12, and *Artemia* metanauplii (EG, Artemia Systems SA) at 2-4 individuals/ml from days 15 until weaning (Fig. 1). Nauplii and metanauplii were enriched with Protein Selco (Artemia Systems SA, Ghent, Belgium). A microparticulate diet (Proton, INVE Aquaculture, Ghent, Belgium) was introduced on day 15 (group A), 20 (group B), or 25 (group C). The control group was fed only *Artemia* metanauplii until end of the experiment.

Sampling and analytical procedure. The growth rate was monitored by sampling 30 larvae from each tank at 5-day intervals. Specific growth rate (SGR) was calculated as SGR = 100 (ln final wt - ln initial wt)/days reared.

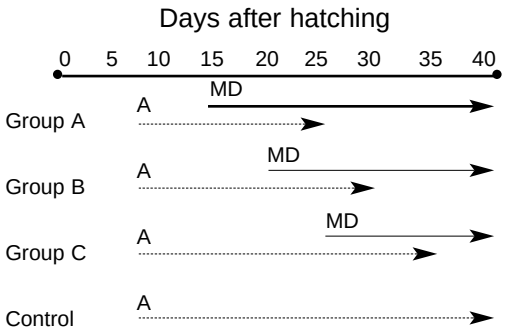

Fig. 1. Weaning of European sea bass (*Dicentrarchus labrax* L.) larvae from *Artemia* (A) to microparticulate diet (MD).

Survival was determined at the end of the experiment.

Pooled samples of larvae (50-250 individuals, depending on age and size) were collected for enzyme analysis at the same hour, before food distribution, on days 8, 11, 15, 17, 20, 22, 25, 27, 30, 35, and 40. Whole body homogenates were used for enzymatic assay. Samples were homogenized in five volumes (v/w) of ice-cold distilled water. Extracts for enzyme assay were obtained after homogenization of larvae (35 mg/ml) in cold 50 mM Tris-HCl buffer, pH 8.0, followed by centrifugation at 13.500 x *g* for 30 min at 4°C.

Trypsin activity was measured using Nα-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as the substrate (Tseng et al., 1982). Chymotrypsin activity was assayed using benzoyl-L-tyrosine ethyl ester (BTEE) as the substrate according to the method of Worthington (1982). Elastase activity was measured using succinyl-$(L\text{-alanine})_3$-p-nitroanilide as the substrate (Bieth et al., 1974). Pepsin was assayed by a modification of the method of Worthington (1982) whereby, in test tubes, 100 ml of the homogenate was incubated at 37°C with 500 ml of hemoglobin substrate 2% w/v hemoglobin in 0.06 HCl. The reaction was stopped after 10 min using 1 ml of trichloroacetic acid (TCA 5%). In the blank tubes, the homogenate was added only after TCA. The precipitates were centrifuged for 6 min at 4000 x *g* and the optical density of the test tube supernatant was read at 280 nm against the blank. Amylase activity was measured using starch as the substrate (Metais and Bieth, 1968). Lipase activity was measured using the method of McKellar and Cholette (1986) as modified by Versaw et al. (1989), using β-naphtyl caprylate as the substrate. One unit of lipase activity was defined as 1 mg of β-naphtol released per minute. Enzyme activities were expressed as specific activities, i.e., U/mg soluble protein. Protein was determined by the Bradford procedure (Bradford, 1976). All spectrophotometric analyses were performed with a Jenway 6300 UV-visible spectrophotometer.

Statistical analysis. All measurements were carried out in triplicate. Results are given as means±SD. The variance homogeneity of the data was analyzed using the Levene test. Survival data were compared by Fischer's chi-square test and enzymatic activity data were compared by one-way ANOVA, followed by Newman-Keul's multiple range test, when significant differences at the 0.05 level were found. Statistical analyses were performed by SPSS 11.0 software.

Results

Larvae development. Larvae hatched between 89 and 91 h. The hatching rate was calculated as 92%. Egg and oil globule diameters were 1119.64±39.24 µm and 328.21± 28.52 µm, respectively. The average total length and weight of the newly hatched larvae were 3.85±0.2 mm and 0.21±0.1 mg. Total lengths and weights at the end of the experiment (day 40) are given in Table 1 while growth is shown in Fig. 2.

Enzymatic activity. In all groups, trypsin activity increased from day 8 until weaning and then decreased (Table 2). The lowest activity was measured in group A. In groups B and C, tryptic activity decreased 75% from day 20 to day 30 (Fig. 3). At the end of the experiment, there were no significant differences between groups A, B, and C but there was a significant difference between the control and the experimental groups.

As with trypsin, chymotrypsin activity

Table 1. Length, weight, growth, and survival at the end of the experiment (40 days).

	Group A	*Group B*	*Group C*	*Control*
Total length (mm)	18.45±1.9	20.86±2.1	22.12±3.6	20.76±4.3
Weight (mg)	34.21±4.1	45.56±3.7	48.78±5.3	44.34±3.6
SGR (%/day)	4.92	9.02	9.17	8.95
Survival (%)	18.2	37.5	41.4	44.3

There were no significant differences in total length, weight, or survival (p>0.05) between groups B, C, and the control. Group A significantly differed (p<0.05) from the other groups.

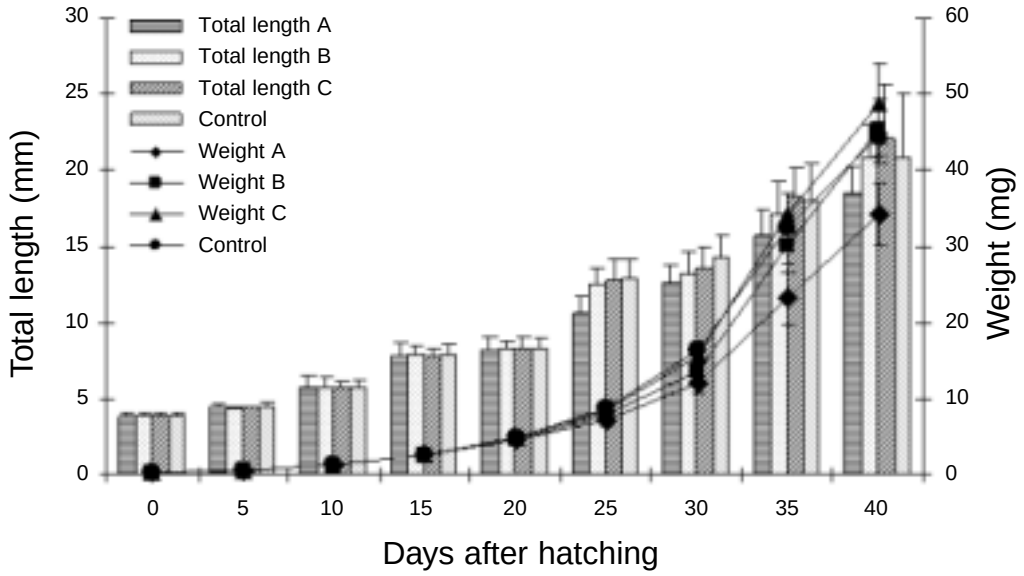

Fig. 2. Growth of European sea bass larvae weaned from *Artemia* to a microparticulate diet at 15 (Group A), 20 (Group B), or 25 (Group C) days after hatching. Each mean±SD represents the average of 30 larvae.

increased until weaning and then slowly dropped in all experimental groups. In contrast, chymotrypsin increased in the control until day 30 and then slightly dropped. The highest specific activity of chymotrypsin was in control group. There was no significant difference between group C and the control but these groups significantly differed from groups A and B.

Elastase activity followed a similar pattern as chymotrypsin in all experimental groups, increasing over 4.55, 3.82, and 4.71 times in groups A, B, and C, respectively, until weaning, then decreasing 80% for groups A and B and 30% in group C. At the end of the experiment, there were no differences between group C and the control, but these groups significantly differed from groups A and B.

Table 2. Digestive enzymes in whole body homogenates of European sea bass larvae weaned from *Artemia* to a microparticulate diet 15 (Group A), 20 (Group B), or 25 (Group C) days after hatching (DAH). The control was fed *Artemia* only.

DAH	*Trypsin (mU/mg)*	*Chymotrypsin (mU/mg)*	*Elastase (mU/mg)*	*Pepsin (U/mg)*	*Amylase (U/mg)*	*Lipase (mU/mg)*
Group A						
8	25.3 ± 4.5	108.3 ± 13.2	0.27 ± 0.11	0	0.34 ± 0.02	67.5 ± 23.7
11	46.6 ± 5.2	369.6 ± 23.6	0.56 ± 0.11	0	0.89 ± 0.07	128.9 ± 11.5
15	78.8 ± 13.8	668.8 ± 45.3	1.23 ± 0.09	0	2.20 ± 0.32	168.7 ± 21.6
17	21.1 ± 8.5	347.1 ± 122.5	0.17 ± 0.13	0	11.82 ± 2.12	97.7 ± 11.6
20	13.7 ± 6.3	321.7 ± 84.8	0.15 ± 0.11	0	8.82 ± 1.58	76.5 ± 31.6
22	15.4 ± 2.6	305.4 ± 93.7	0.11 ± 0.09	0	6.82 ± 1.24	71.1 ± 24.9
25	17.4 ± 7.5	278.5 ± 69.3	0.1 ± 0.09	2.27 ± 0.26	7.73 ± 0.78	67.5 ± 13.5
27	24.7 ± 4.9	264.7 ± 53.7	0.09 ± 0.06	6.16 ± 1.49	7.36 ± 1.49	73.6 ± 16.3
30	19.1 ± 3.3	196.1 ± 88.7	0.17 ± 0.08	4.99 ± 0.84	5.90 ± 0.84	66.2 ± 16.1
35	22.4 ± 3.6	227.4 ± 142.1	0.12 ± 0.09	5.34 ± 1.23	6.23 ± 1.23	81.5 ± 16.8
40	18.7 ± 7.2	257.7 ± 112.4	0.13 ± 0.06	5.98 ± 0.92	6.64 ± 1.63	98.8 ± 27.4
Group B						
8	24.5 ± 3.1	114.5 ± 11.6	0.35 ± 0.12	0	0.41 ± 0.06	74.8 ± 13.3
11	43.7 ± 7.3	372.7 ± 35.2	0.76 ± 0.09	0	0.71 ± 0.03	133.2 ± 16.8
15	84.3 ± 14.2	684.3 ± 67.3	1.14 ± 0.11	0	2.31 ± 0.21	178.1 ± 18.5
17	74.8 ± 9.4	831.8 ± 123.6	1.67 ± 0.14	0	1.89 ± 0.22	187.7 ± 16.8
20	86.6 ± 19.4	1167.6 ± 214.8	1.34 ± 0.12	0	0.92 ± 0.34	203.1 ± 13.8
22	16.2 ± 6.3	894.2 ± 143.7	0.14 ± 0.11	0	10.46 ± 2.89	93.5 ± 28.5
25	17.9 ± 4.6	843.9 ± 167.3	0.12 ± 0.11	2.68 ± 0.36	9.47 ± 0.57	74.8 ± 14.8
27	18.7 ± 4.9	748.7 ± 252.7	0.12 ± 0.09	7.25 ± 1.57	7.25 ± 2.57	71.2 ± 17.3
30	14.2 ± 4.2	654.2 ± 98.3	0.11 ± 0.11	5.84 ± 0.52	5.84 ± 2.52	65.3 ± 12.7
35	19.3 ± 7.3	679.3 ± 187.2	0.13 ± 0.11	4.52 ± 0.78	6.89 ± 1.18	89.4 ± 21.2
40	17.6 ± 7.4	529.6 ± 127.2	0.17 ± 0.07	6.22 ± 1.27	7.26 ± 1.24	97.5 ± 31.5
Group C						
8	21.2 ± 5.5	111.2 ± 16.2	0.32 ± 0.13	0	0.59 ± 0.08	69.3 ± 17.6
11	37.5 ± 6.9	366.5 ± 16.8	0.65 ± 0.26	0	0.64 ± 0.08	144.5 ± 17.3
15	81.3 ± 11.5	674.4 ± 77.2	1.06 ± 0.21	0	2.75 ± 0.14	181.3 ± 27.4
17	67.8 ± 13.1	837.8 ± 153.7	1.34 ± 0.26	0	1.72 ± 0.27	198.3 ± 17.4
20	79.4 ± 9.4	1262.4 ± 326.4	1.43 ± 0.21	0	1.42 ± 0.24	216.6 ± 22.6
22	88.9 ± 10.3	1814.9 ± 342.9	1.56 ± 0.19	0	1.29 ± 0.32	229.6 ± 35.1
25	91.2 ± 8.4	1783.8 ± 278.3	1.51 ± 0.37	5.26 ± 0.68	2.72 ± 0.73	247.3 ± 19.7
27	57.3 ± 4.1	1673.3 ± 263.9	1.15 ± 0.33	7.68 ± 1.65	12.53 ± 3.24	154.5 ± 38.5
30	25.8 ± 3.5	1785.8 ± 273.2	1.13 ± 0.22	4.82 ± 1.43	4.82 ± 3.43	126.3 ± 15.3
35	34.5 ± 5.3	1578.5 ± 341.4	1.17 ± 0.33	5.65 ± 1.25	7.27 ± 0.96	141.8 ± 34.1
40	22.4 ± 9.3	1624.4 ± 337.8	1.13 ± 0.42	6.70 ± 1.33	7.53 ± 1.17	157.4 ± 29.3
Control						
8	22.6 ± 4.2	112.6 ± 15.2	0.28 ± 0.11	0	0.49 ± 0.03	81.3 ± 21.5
11	45.4 ± 3.4	375.4 ± 33.7	0.62 ± 0.16	0	0.88 ± 0.12	133.8 ± 25.4
15	88.2 ± 5.7	692.7 ± 49.3	1.14 ± 0.13	0	2.89 ± 0.43	174.3 ± 33.2
17	82.7 ± 9.6	839.7 ± 162.8	1.72 ± 0.21	0	1.86 ± 0.21	189.2 ± 21.6
20	66.1 ± 6.8	1264.6 ± 329.2	1.63 ± 0.23	0	0.62 ± 0.17	211.4 ± 23.3
22	83.8 ± 11.2	1833.8 ± 426.3	1.74 ± 0.16	0	0.25 ± 0.23	242.9 ± 39.5
25	87.2 ± 9.9	1879.5 ± 462.5	1.82 ± 0.28	1.26 ± 0.33	1.28 ± 0.56	258.3 ± 24.8
27	77.5 ± 12.6	1967.5 ± 242.5	1.85 ± 0.25	1.73 ± 0.58	1.73 ± 0.78	273.8 ± 44.7
30	89.5 ± 15.2	2538.4 ± 189.4	1.93 ± 0.17	1.67 ± 1.29	1.67 ± 0.87	304.3 ± 22.7
35	114.3 ± 23.2	2146.3 ± 442.7	1.91 ± 0.23	2.12 ± 0.89	1.95 ± 0.52	291.6 ± 38.5
40	104.3 ± 31.6	1949.3 ± 328.4	1.82 ± 0.33	2.96 ± 1.18	1.78 ± 0.89	298.5 ± 25.6

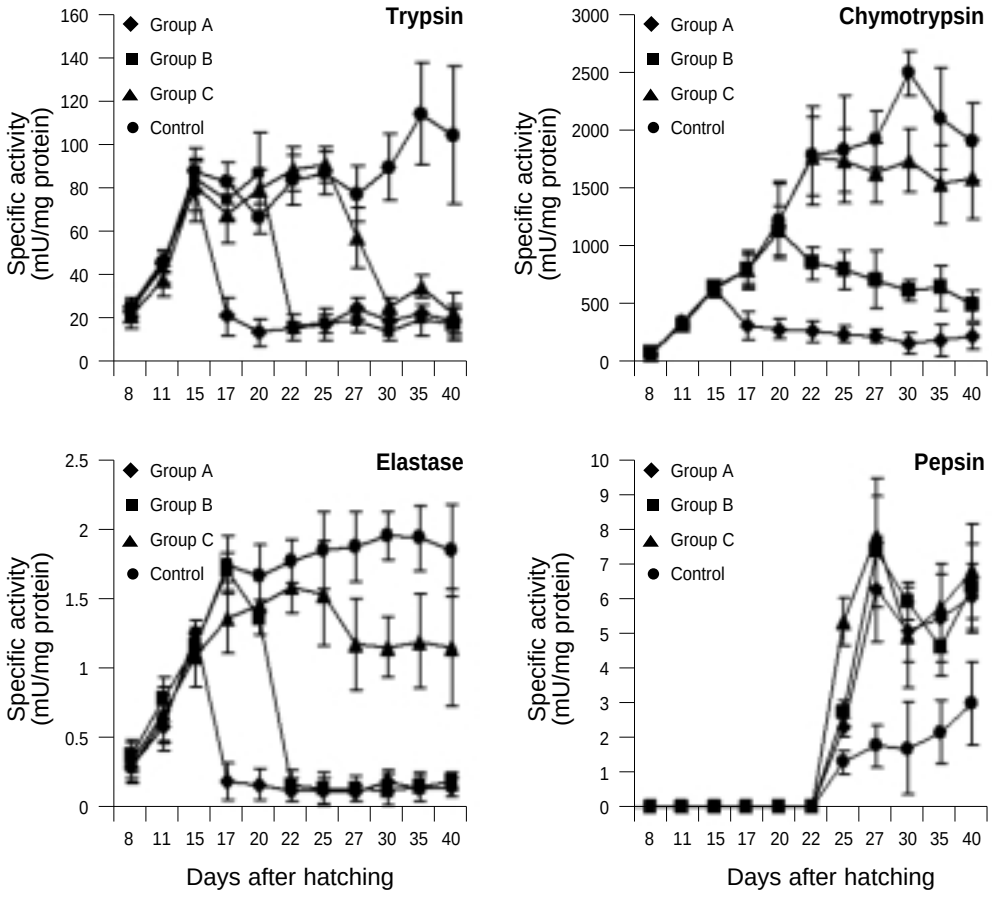

Fig. 3. Specific activity of proteases assayed in whole larvae homogenates from sea bass larvae weaned from *Artemia* at 15 (group A), 20 (group B), or 25 (group C) days. The control was fed *Artemia* throughout. Results are expressed as means±SD (n = 5).

In all groups, pepsin was first detected on day 25 (concurrent with stomach formation), increased to day 27, decreased by 30%, and rose until the end of experiment. The highest pepsin activity was in group C. There were no significant differences between the experimental groups but the control group was significantly lower.

Amylase activity sharply increased after weaning in the experimental groups and then decreased, while it was nearly constant in the control group (Fig. 4). There were no significant differences between experimental groups but they significantly differed from the control. In contrast, lipase sharply declined by 50% after weaning in the experimental groups but increased in the control. There were no differences among the experimental groups but they significantly differed from the control.

Discussion

As expected, early weaning strongly affected growth and survival. Growth and development in group A (weaned at 15 days) were inferior to the other groups, similar to results reported by Cahu and Zambonino Infante (1994) and

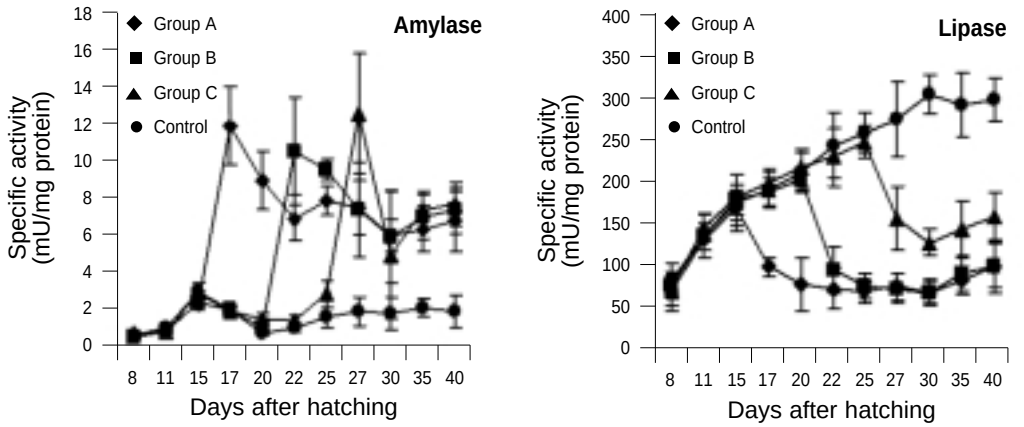

Fig. 4. Specific activity of lipase and amylase assayed in whole larvae homogenates from sea bass larvae weaned from *Artemia* at 15 (group A), 20 (group B), or 25 (group C) days. The control was fed *Artemia* throughout. Results are expressed as means±SD (n = 5).

Person-Le Ruyet et al. (1993). It is not easy to formulate a compound diet adequate for fish larvae because the nutritional requirements of fish larvae cannot be estimated by traditional nutritional approaches (Cahu and Zambonino Infante, 2001). As of now, commercially formulated diets do not support larvae growth. Curnow et al. (2006) used three weaning protocols and two commercial microparticulate diets, Gemma and Proton, in *Lates calcarifer* larvae. The Gemma diet produced better growth, higher survival, and reduced cannibalism. Nevertheless, as pointed out by the authors, it could be that both the biochemical composition and the amino acid and lipid profiles affected these parameters. In our study, although only the Proton microparticulate diet was used, we obtained relatively better results than earlier similar studies.

The substitution of live food by formulated diets and co-feeding of sea bass larvae are closely related to digestive enzyme activity. Therefore, although larvae can be weaned at metamorphosis or when a functional stomach is formed, early introduction of a microparticulate diet can result in lower growth performance and enzymatic activity, as found for sea bass *Lates calcarifer* (Walford and Lam, 1993), Japanese flounder *Paralichthys olivaceus* (Kanazawa et al., 1989), Atlantic cod *Gadus morhua* (Baskerville-Bridges and Kling, 2000), and haddock *Melanogrammus aeglefinus* (Hamlin and Kling, 2001).

Digestive enzyme activity can be an indicator of food acceptance and digestive capacity of the offered feed. Trypsin and chymotrypsin, for example, are specific to pancreatic protein hydrolysis (Nolting et al., 1999; Zambonino Infante and Cahu, 2001). Main factors affecting trypsin and chymotrypsin activity in fish larvae are rearing techniques (clear or green water), age, feeding protocol, and food characteristics (Cahu and Zambonino Infante, 2001; Zambonino Infante and Cahu, 2001). In our study, trypsin activity was higher before introduction of the microparticulate diet and significantly decreased in all groups after start of the diet, similar to results reported by Nolting et al. (1999). These researchers also found that the protein content of larvae fed *Artemia* nauplii was higher and the growth of larvae fed microparticulate diet was lower. The trypsin activity contribution of *Artemia* can be as much as 5% of the total assayed activity in 20-day-old sea bass larvae (Cahu and Zambonino Infante, 1995).

Pepsin activity usually appears together with the formation of a functional stomach in fish larvae (Cahu and Zambonino Infante, 2001; Kolkovski, 2001). Pepsin was first detected on day 24 in sea bass larvae by Zambonino Infante and Cahu (1994). Similar to their findings, this activity was measured for the first time on day 25 in our study. The lower survival in groups A and B, weaned on days 15 and 20, respectively, was related to the absence of pepsin and a functional stomach. Possibly the microparticulate diet was not digested and the *Artemia* metanauplii were insufficient for the larvae. However, it could be that the lower survival resulted from starting the microparticulate diet five days before the detection of pepsin. A functional stomach and pepsin were reported on day 17 for sea bass *Lates calcarifer* (Walford and Lam, 1993), day 40 for gilthead sea bream *Sparus aurata* (Moyano et al., 1996), day 25 for common pandora *Pagellus erythrinus.* (Suzer et al., 2006), and day 30 for red porgy *Pagrus pagrus* (Darias et al., 2005).

Elastases are serine proteases that hydrolyze amides and esters produced in the pancreas as inactive zymogen and proelastase, and activated in the duodenum by trypsin. Usually, they break down elastin, the specific protein of elastic fibers, and digest other proteins such as fibrin, collagen, hemoglobin, albumin, and proteoglycan. These are used in association with other enzymes such as trypsin, chymotrypsin, and collagenase. In our study, pancreatic elastase activity had a similar pattern as tryptic activity. Caruso and Genovese (2000) recorded that elastase activity had a similar enzymatic profile as total protease and aminopeptidase activity in the Sparids, *Pagellus erythrinus*, *P. acarne*, and *P. bogaraveo*. A similar pattern between elastase and protease (trypsin and chymotrypsin) activity was found in the Atlantic salmon, *Salmo salar* (Nordrum et al., 2003).

Generally, amylase activity is first detected before mouth opening and is higher during the lecitotrophic stage. It is stimulated by glycolytic chains, glycogen, and starch. Feeding characteristics and regime are affected by amylase activity in fish larvae, especially during weaning, probably due to the starch content of the microparticulate diet (Cahu and Zambonino Infante, 2001). Similarly, it seems that sharp increases of amylase activity in early weaned sea bass larvae are related to the starch content in the microparticulate diet (Cahu and Zambonino Infante, 1994).

The lipase plateau in the control suggests that the maximum capacity of lipolytic enzyme synthesis was reached. This activity gradually decreased with age in all experimental groups, perhaps due to the composition of the microparticulate diet but perhaps caused by ineffective utilization of the diet by the larvae. This phenomenon was acceptable in whole larval homogenates; it suggests lower enzymatic activities and reduced pancreatic secretion of enzymes in larvae fed the microparticulate diet. Similar results were reported for turbot *Scophthalmus maximus* (Hoehne-Reitan et al., 2003) and sea bass larvae (Zambonino Infante and Cahu, 1999) where lipolytic activities (neutral lipase and phospholipase A_2) in larvae fed microparticulate diet were relatively lower than in larvae fed live prey.

In conclusion, the present study demonstrates that, whatever the development stage, larvae were able to modulate their digestive enzyme activities in response to a change in diet (Cahu and Zambonino Infante, 1994). Growth and survival were lower in group A than in the other groups due to retarded formation of the stomach and gastric glands, indicating that gastric activity and pepsin secretion need to occur for digestion of a microparticulate diet. Applebaum (1989) suggested that raising the water temperature increases larvae metabolism and activity, potentially improving their readiness to accept inert diets. Introduction of a microparticulate diet and digestion of all its ingredients could result in enhanced growth and survival of sea bass larvae after formation of the stomach and activity of gastric glands approximately on day 25. Further studies should focus on early weaning and digestive enzyme expression after introduction of a microparticulate diet in other cultured species.

Acknowledgements

We would like to express our sincere gratitude to the staff of Hatko Sea Products Inc., Milas, Mugla, Turkey, where the experiments were conducted, for their most efficient technical assistance.

References

Alves Jr. T.T., Vinicius R., Cerqueira V.R. and J.A. Brown, 2005. Early weaning of fat snook (*Centropomus parallelus* Poey 1864) larvae. *Aquaculture*, 253:334-342.

Applebaum S., 1989. Can inert diets be used more successfully for feeding larval fish? (Thoughts based on indoor feeding behavior observations). *Pol. Arch. Hydrobiol.*, 36:437.

Baskerville-Bridges B. and L.J. Kling, 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. *Aquaculture*, 189:109-117.

Bieth J., Spiess B. and C. Wermuth, 1974. The synthesis and analytical use of a highly sensitive and convenient substrate of elastase. *Biochem. Med.*, 11:350.

Bradford M.M., 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72:248-254.

Cahu C.L. and J.L. Zambonino Infante, 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. *Comp. Biochem. Physiol.*, 109A:213-222.

Cahu C.L. and J.L. Zambonino Infante, 1995. Maturation of the pancreatic and intestinal digestive function in sea bass (*Dicentrarchus labrax*): effect of weaning with different protein sources. *Fish Physiol. Biochem.*, 14:431-437.

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

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

Cahu C.L., Zambonino Infante J.L., Quazuguel P. and M.M. Le Gall, 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. *Aquaculture*, 171:109-119.

Caruso G. and L. Genovese, 2000. Osservazioni sulla distribuzone di enzimi digestivi in differenti specie di Sparidi. *Biol. Mar. Medit.*, 7: 621-623.

Curnow J., King J., Partridge G. and S. Kolkovski, 2006. Effects of two commercial microdiets on growth and survival of barramundi (*Lates calcarifer* Bloch) larvae within various early weaning protocols. *Aquac. Nutr.*, 12:247-255.

Darias M.J., Murray H.M., Martinez-Rodriguez G., Cardenas S. and M. Yufera, 2005. Gene expression of pepsinogen during the larval development of red porgy *(Pagrus pagrus)*. *Aquaculture*, 248:245-252.

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

Hamlin H. J. and L.J. Kling, 2001. The culture and early weaning of larval haddock *Melanogrammus aeglefinus* using a microparticulate diet. *Aquaculture*, 201:61-72.

Hoehne-Reitan K., Kjorsvik E. and K.I. Reitan, 2003. Lipolytic activities in developing turbot larvae as influenced by diet. *Aquac. Int.*, 11:477-489.

Kanazawa A., Koshio S. and S. Teshima, 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* fed microbound diets. *J. World Aquac. Soc.*, 20:31-37.

Kolkovski S., 2001. Digestive enzymes in fish larvae and juveniles-implications and application to formulated diets. *Aquaculture*, 200:181-201.

Kolkovski S., Tandler A. and M.S. Izquierdo, 1996. The effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass *Dicentrachus labrax* larvae. *Aquaculture*, 148:313-322.

McKellar R.C. and H. Cholette, 1986.

Determination of the extracellular lipases of *Pseudomonas fluorescens* spp. in skim milk with the beta-naphthyl caprylate assay. *J. Dairy Res.*, 53:301.

Metais P. and J. Bieth, 1968. Détermination de l'α-amylase par une microtechnique. *Ann. Biol. Clin.*, 26:133-142.

Moyano F.J., Diaz M., Alarcon F.J. and M.C. Sarasquete, 1996. Characterisation of digestive enzyme activity during larval development of gilthead seabream (*Sparus aurata*). *Fish Physiol. Biochem.*, 15:121-130.

Nolting M., Ueberschar B. and H. Rosenthal, 1999. Trypsin activity and physiological aspects in larval rearing of European sea bass (*Dicentrarchus labrax*) using live prey and compound diets. *J. Appl. Ichthyol.*, 15:138-142.

Nordrum S., Olli J.J., Rosjo C., Holm H. and A. Krogdahl, 2003. Effects of graded levels of medium chain triglycerides and cysteine on growth, digestive processes and nutrient utilization in sea water reared Atlantic salmon (*Salmo salar,* L.) under ad libitum feeding regime. *Aquac. Nutr.*, 9:263-274.

Person-Le Ruyet J., Alexandre J.C., Thebaud L. and C. Mugnier, 1993. Marine fish larvae feeding: Formulated diets or live prey? *J. World Aquac. Soc.,* 24(2):211-224.

Rosenlund G., Stoss J. and C. Talbot, 1997. Co-feeding marine fish larvae with inert and live diets. *Aquaculture*, 155:183-191.

Southgate P.C. and G.J. Partridge, 1998. Development of artificial diets for marine finfish larvae: problems and prospects. pp. 151-171. In: S.S.Z. De Silva (ed.). *Tropical Mariculture*. Academic Press, San Diego, CA, USA.

Suzer C., Saka S. and K. Fırat, 2006. Effects of illumination on early life development and digestive enzyme activities in common pandora *Pagellus erythrinus* L. larvae. *Aquaculture*, 260:86-93.

Tseng H.C., Grendell J.H. and S.S. Rothman, 1982. Food, deodenal extracts, and enzyme secretion by the pancreas. *Am. J. Physiol.*, 243:G304-G312.

Versaw W.K., Cuppett S.L., Winters D.D. and L.E. Williams, 1989. An improved colorimetric assay for bacterial lipase in nonfat dry milk. *J. Food Sci.*, 54:1557-1558.

Walford J. and T.J. Lam, 1993. Development of the digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. *Aquaculture*, 109:187-205.

Worthington T.M., 1982. *Enzymes and Related Biochemicals.* Biochemical Products Division, Worthington Diagnostic System, Freehold, NJ, USA.

Zambonino Infante J.L. and C.L. Cahu, 1994. Influence of diet on pepsin and some pancreatic enzymes in sea bass *Dicentrarchus labrax* larvae. *Comp. Biochem. Physiol.*, 109:209-212.

Zambonino Infante J.L. and C.L. Cahu, 1999. High dietary lipid levels enhance digestive tract maturation and improve *Dicentrarchus labrax* larval development. *J. Nutr.*, 129: 1195-1200.

Zambonino Infante J.L. and C.L. Cahu, 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. *Comp. Biochem. Physiol.*, 130C:477-487.