Aquaculture 424–425 (2014) 120–130

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of different weaning strategies on survival, growth and digestive system development in butter catfish *Ompok bimaculatus* (Bloch) larvae

Pravata Kumar Pradhan [a,*], Joykrushna Jena [a], Gopa Mitra [b], Neeraj Sood [a], Enric Gisbert [c]

[a] National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkusha, Lucknow 226002, Uttar Pradesh, India
[b] Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751002, Odisha, India
[c] IRTA, Centre de Sant Carles de la Ràpita, Unitat de Cultius Aqüícoles, Crta. de Poblenou km 5.5, 43450 Sant Carles de la Ràpita, Tarragona, Spain

ARTICLE INFO

Article history:
Received 16 August 2013
Received in revised form 20 December 2013
Accepted 23 December 2013
Available online 4 January 2014

Keywords:
Butter catfish
Weaning
Live feed
Microdiet
Feeding strategy
Histology

ABSTRACT

The effects of different feeding regimes on survival, growth and morphogenesis of the digestive system in butter catfish, *Ompok bimaculatus* (Bloch) larvae were investigated in the present study. Eight different feeding regimes varying on the type of food (*Artemia* nauplii, zooplankton or microdiet) and the time after hatching at which those different food items were offered to larvae were evaluated in order to find the most convenient weaning strategy for butter catfish larvae. The results indicated that the larvae weaned after 7 days post hatch (dph) showed similar survival rates with those fed just live prey (*Artemia* nauplii or zooplankton), whereas early weaning (before 7 dph) resulted in poor survival. In terms of growth performance, larvae fed solely on *Artemia* nauplii, showed the best results followed by those fed with zooplankton and those fed on a combination of *Artemia* nauplii and zooplankton. In contrast, the larvae fed under the other feeding regimes including the microdiet showed similar final growth values. With regard to the effect of different weaning strategies on the morphogenesis of the digestive system, the results indicated that early weaning delayed the development of the stomach, intestine and pancreas. However, at the end of the trial at 17 dph, no differences in the level of development were observed among treatments regardless of the weaning protocol tested, which indicated the high plasticity of butter catfish to different nutritional conditions once their digestive system was completely developed and larvae were adapted to the microdiet. Hence, it was concluded that it is feasible to rear butter catfish larvae with zooplankton without dependence upon *Artemia* nauplii, and also that larvae may be weaned onto microdiets after a short period of co-feeding when weaning takes place after 7 dph. These findings would be very useful to improve the actual larval rearing techniques for this promising catfish species from the Indian sub-continent.

1. Introduction

Butter catfish (*Ompok bimaculatus*), a fish species belonging to the family of Siluridae, has a very high consumer preference in the North Eastern and Eastern states and also in the mainlands of India. The species is a delicacy in those areas and it is considered as one of the highly priced food fish (US$ 8–12/kg). In addition, this species is categorized as endangered by the IUCN and it faces a high risk of extinction (CAMP, 1997), which raises the issue of the sustainable exploitation of this biological resource. Consequently, the aquaculture of this catfish species has a double interest. Firstly, the culture of butter catfish may be of interest for diversifying the freshwater aquaculture in India and other neighboring countries; secondly, its culture may also be used for conservational purposes and restocking programs (NBFGR, 2011). Independently of the final purpose of this activity, there is an urgent need for reliable, effective and efficient larval rearing techniques to ensure consistent production of good quality fry.

Larviculture of butter catfish is an extremely seasonal activity, since it mainly relies on the wild available zooplankton usually growing in ponds nearby indoor larval rearing facilities. These ponds are regularly harvested and the live preys transferred into the indoor larval rearing facilities. In addition to limitations in the regular supply of live prey produced in ponds, their biochemical composition is quite variable (Mitra et al., 2007), depending on pond's primary production (van der Meeren and Jørstad, 2001). These limitations may be overcome with the use of *Artemia* as a food source, since the supply and regular production of this type of live prey is assured year-round and its biochemical profile can be manipulated by means of the use of commercial or tailor-made emulsions (Øie et al., 2011). However, the large demand for *Artemia* cysts and their shortage has resulted in an important increase in price of this type of live prey, which is really a problem for their massive use in developing third-world countries. Thus, the use of microdiets could partially alleviate such problems by substituting live food; but their use soon after hatching often leads to low survival and poor growth performance due to improper larval digestive capabilities, unbalanced composition, palatability or physical characteristics of the dry feed or to the low attractiveness of the non-mobile particle for the

* Corresponding author. Tel.: +91 522 2442441; fax: +91 522 2442403.
E-mail address: pradhanpk1@gmail.com (P.K. Pradhan).

0044-8486/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.12.041

fish larva (Cahu and Zambonino-Infante, 2001; Liu et al., 2012; Person-Le Ruyet et al., 1993). Combined feeding of fish larvae with live prey and manufactured microdiets (referred to as co-feeding) has been reported to alleviate problems related with the low acceptance and digestibility of microdiets in marine and freshwater fish larvae and also increases the success of early weaning to these type of diets (Alves et al., 2006; Curnow et al., 2006a,b; Holt et al., 2011; Liu et al., 2012, among others). Although live food has been successfully substituted by microdiets in a reduced number of marine and freshwater species; milkfish *Chanos chanos* (Santiago et al., 1983); African catfish *Clarias gariepinus* (Appelbaum and Van Damme, 1988); lake whitefish *Coregonus clupeaformis* (Harris and Hulsman, 1991); Atlantic cod *Gadus morhua* (Baskerville-Bridges and Kling, 2000); European sea bass *Dicentrarchus labrax* (Cahu and Zambonino-Infante, 2001), Siberian sturgeon *Acipenser baerii* (Gisbert and Williot, 2002); pike-perch *Sander lucioperca* (Ostaszewska et al., 2005) and/or yellowtail kingfish (Kolkovski et al., 2010), there is not a universal weaning strategy for fish larvae, since this may be considered as a species-specific process that is primarily related to the development and maturation of the digestive function (Cahu and Zambonino-Infante, 2001; Lazo et al., 2011). Consequently, the timing of organ development and its associated physiological functions are affected by the general life history and reproductive guild of each species, as well as by a variety of abiotic and biotic factors. Among them, temperature, water quality, food availability and composition have been generally considered as some of the most important (Zambonino Infante and Cahu, 2007). This study aims to contribute to reduce the massive mortality in the culture of this catfish species, associated with the dietary transition from live food (*Artemia* nauplii and zooplankton) to a compound feed. In particular, we have investigated under controlled laboratory conditions how different feeding regimes, using mono-specific diet or combinations of live food and microdiets through various weaning regimes, influenced the survival, growth performance and development of digestive system in butter catfish larvae. This new information is expected to be very useful for improving actual larval rearing practices for butter catfish, one of the most interesting and promising fish species for diversification in freshwater aquaculture in the Indian sub-continent.

2. Materials and methods

2.1. Source of larvae

This trial took place at the College of Fisheries, Central Agricultural University in Agartala, Tripura (India) under natural photoperiod conditions according to the rainy season of the year (25°53′ N, 91°55′ E). Eggs were obtained by artificial breeding. Thus, butter catfish females (44–49 g; n = 3) were injected with Ovaprim® at 1.0 ml kg^{-1} body weight (BW) and male butter catfish (44–46 g; n = 3) with Ovaprim® at 0.5 ml kg^{-1} BW. After 9–10 h of hormonal injection, females were stripped individually into dry enamel trays, whereas milt was obtained from the males individually by surgically removing the testes, which were macerated to produce a suspension to be mixed with the eggs for fertilization. Eggs were subsequently washed thoroughly with clean water and total volume of eggs were determined and hatched in $3 \times 1 \times 0.60$ m^3 cement incubators at 27.0 ± 1.1 °C with mild aeration as described by Pradhan et al. (2012). Newly hatched larvae were left in the incubators until the age of 1 day post hatch (dph) when 1600 larvae were randomly selected for the trial.

2.2. Experimental procedures and feeding regimes

One hundred larvae (1 dph, 0.4 mg in wet weight) were stocked in sixteen glass aquaria ($60 \times 30 \times 30$ cm^3) containing 25-l water each at a density of 4 larva l^{-1} and the experiment started at 2 dph. Two air stones were used in each aquarium to provide aeration and promote a homogeneous distribution of feed in the water column. During the rearing period, water temperature, dissolved oxygen and pH values were maintained at 27.0–28.1 °C, 6–8 mg l^{-1} (87% saturation) and 6.8–7.6, respectively. Water quality was maintained in all aquaria by daily renewal of 30% of their total volume.

Eight different dietary treatments or feeding regimes (two replicates each) varying on the type of food (*Artemia* nauplii, zooplankton or microdiet) and the age at which those different food items were offered to larvae, were designed in order to set up the most convenient feeding regime and weaning strategy for butter catfish larvae (Fig. 1). Thus, larvae were reared under the following feeding regimes (FR) from the onset of the exogenous feeding at 2 dph until 17 dph: FR-A, larvae fed with non-enriched *Artemia* nauplii (OSI PRO 80™, Ocean Star International, Inc., USA) from 2 to 17 dph; FR-B, larvae fed with zooplankton (Cyclopoidea) collected from a nearby pond from 2 to 17 dph; FR-C, larvae fed with a commercial microdiet (Frippak Fresh CAR #1, INVE®, Belgium) from 2 to 17 dph; FR-D, larvae fed with non-enriched *Artemia* nauplii from 2 to 8 dph, zooplankton from 6 to12 dph and the microdiet from 10 to 17 dph; FR-E, larvae fed with zooplankton from 2 to 7 dph and the microdiet from 5 to 17 dph; FR-F, larvae fed with zooplankton from 2 to 9 dph and the microdiet from 7 to 17 dph; FR-G, larvae fed with zooplankton from 2 to 11 dph and the microdiet between 9 and 17 dph; FR-H, larvae fed with zooplankton from 2 to 13 dph and the microdiet from 11 to 17 dph. In all cases, larvae were fed with different diets to apparent satiation four times per day (08:00, 12:00, 16:00 and 20:00 h) as described by Mollah and Tan (1982). They were considered satiated when their foraging behavior ceased and larvae assembled in the corners and/or at the bottom of the aquaria. The weaning procedure in feeding regimes D-H consisted of decreasing the frequency of live prey (*Artemia* nauplii and zooplankton) and increasing the frequency of microdiet feeding (4/0, 3/1, 2/2, 1/3, 0/4) within 2 consecutive days. All the uneaten food and fecal residues were siphoned out every morning before first feed distribution at 08:00 h.

2.3. Larval sampling, growth measurements and histological procedures

The effect of different feeding regimes on butter catfish larval performance was assessed at 7, 12 and 17 dph. At these sampling dates, 10 larvae from each experimental aquarium were randomly selected, sacrificed with an overdose of anesthetic (tricaine methanesulphonate, MS-222; Sigma), rinsed in distilled water, and individually measured in total length (TL) and body weight (BW) as described in Pradhan et al. (2012). The specific growth rate (SGR) in BW and TL of larvae for different experimental periods (2–7, 7–12, 12–17 and 2–17 dph) was determined

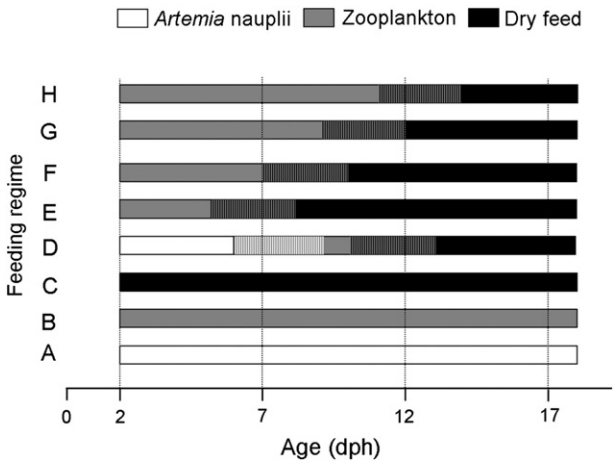

Fig. 1. Eight different feeding regimes varying on the type of food (*Artemia* nauplii, zooplankton or microdiet) and the age at which those different food items were offered to butter catfish larvae.

by using the following formula: SGR (% day^{-1}) = [(ln $F_{BW,\ TL}$ – ln $I_{BW,\ TL}$) t^{-1}] × 100; where F is the final BW or TL value at a given time, I is the initial BW or TL value, and t is the duration in days of the considered period. Final survival was evaluated by counting the animals surviving at the end of the experiment and calculated according to Buckley et al. (1984), which considers the number of sampled individuals during the experiment.

In order to evaluate the effects of different FR on the morpho-anatomical development of the digestive system, the same larvae that were used for body size measurements, were fixed in 4% buffered formalin (pH = 7.0) at 7, 12 and 17 dph. Then, larvae were dehydrated in a graded series of ethanol, embedded in paraffin, cut into serial sagittal sections (2–3 μm thick) and mounted with Eukit® in gelatinized slides . Sections were stained by Harris' Hematoxylin and Eosin (HE) procedure for general histomorphological observations, while periodic acid-Schiff (PAS) was used to detect neutral glycoconjugates in intestinal mucous cells and glycogen deposits in the liver (Pearse, 1985). The development of the digestive system in butter catfish larvae was compared among different FR according to Pradhan et al. (2012). The level of fat deposits (spherical unstained vacuoles corresponding to lipids dissolved during the embedding process of the larva in paraffin) was evaluated in the liver and intestine. These organs are considered reliable nutritional and physiological biomarkers because their histological organization is very sensitive to dietary changes (Gisbert et al., 2008). Histological images were obtained by light microscopy (Nikon SMZ800 coupled to a digital camera Olympus DP70) and analyzed by using a digital image analysis software package (ANALYSIS™; Soft Imaging Systems GmbH, Münster, Germany). The areas covered by fat deposits (ACFD) in the tissue sections of liver and posterior intestine were measured according to Boglino et al. (2012) and Treviño et al. (2011), respectively. In addition, the percentage of hepatocytes with fat deposits was quantified by counting the proportion of cells with fat inclusions with regard to the total of hepatocytes within the image field. For each histological parameter, we analyzed between three and five image fields from each of the five specimens examined by experimental replicate.

2.4. Statistical analyses

Data from growth performance and survival from each experimental group were compared by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) method for *post hoc* multiple comparisons, as differences that are significant according to HSD are judged real significant. The values of ACFD and percent of hepatocytes with fat deposits were also subjected to two-way ANOVA analysis to compare the interactive effects of larval age and different feeding regimes. In all cases, the assumptions of normality adjustment and homogeneity of variance were checked by using Kolmogorov–Smirnov's and Levene's tests, respectively (Zar, 1999). Variables that were expressed as percentage were arcsin square root transformed. The accepted level for statistical significance was $P < 0.05$. The mean values of SGR and survival are expressed as mean ± standard error of the mean (SEM), since they could not be calculated for individual larva, whereas the rest of the data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted with SPSS (version 17.0, USA).

3. Results

3.1. Larval survival

Data on final survival and growth performance of butter catfish larvae reared under different feeding regimes are summarized in Table 1. Diets and weaning strategy significantly affected larval survival at 17 dph ($P < 0.05$). Larval survival in groups weaned after 7 dph (FR-D, F, G and H) was similar to those groups fed solely on live prey, *Artemia* nauplii (FR-A) or zooplankton (FR-B) with values ranging from 65.0 to 78.7% ($P > 0.05$). In contrast, larvae reared under the FR-C (microdiet offered from first feeding) and FR-E (early weaning at 5 dph) showed the lowest survival rates (48.8 ± 6.2% and 58.7 ± 3.8%, respectively; $P < 0.001$).

3.2. Larval growth

At 7 dph, the maximum growth in terms of both TL and BW (10.8 ± 0.1 mm; 8.6 ± 1.0 mg) was found in larvae fed with FR-D, while those fed with FR-C showed the poorest growth performance (6.7 ± 0.04 mm; 2.4 ± 0.3 mg; $P < 0.05$; Fig. 2). In this sense, larvae fed FR-C were a 38.0% shorter and 72.0% lighter than those recorded in larvae from the FR-D. The rest of dietary treatments (A, B, E–H) showed intermediate values with regard to FR-C and D. Data on SGR in terms of TL and BW followed the same pattern (Table 1). At 12 dph, the maximum growth in terms of both TL and BW was recorded in larvae FR-A (21.2 ± 0.1 mm; 59.5 ± 4.9 mg). Similarly to 7 dph, larvae fed FR-C showed the minimum growth in terms of TL (9.4 ± 0.02 mm) and BW (7.8 ± 0.5 mg; $P < 0.05$; Fig. 2), which were 56% and 87% lower than those recorded in larvae fed FR-A. The rest of dietary treatments (B and D–H) showed intermediate values with regard to FR-A and C. SGR values in terms of BW and TL recorded between 7 and 12 dph followed the similar trend than those of BW and TL.

At the end of the trial (17 dph), final larval mean growth in terms of BW was the highest in larvae FR-A (339.8 ± 0.6 mg), followed by those larvae fed FR-B (229.4 ± 3.1 mg; $P < 0.05$). Although, larvae fed FR-D had the third highest growth (151.2 ± 35.4 mg), it was not significantly different from the rest of the treatment groups with the exception from that recorded in larvae fed FR-A. Butter catfish larvae from FR-C showed the minimum growth in BW (81.3 ± 22.76 mg), which was a 76%, 64.5% and 46.2% lower than those recorded in larvae from the FR-A, B and D, respectively. No significant differences were observed in the mean BW values between larvae from FR-C, E–H ($P > 0.05$). Between 12 and 17 dph, SGR values in terms of BW of larvae from FR-C and B treatments were significantly higher than the larvae fed FR-D, E, F and G ($P < 0.05$). Between 2 and 17 dph, larvae from FR-A, B and D showed the maximum SGR values in terms of BW with values ranging from 45.0 to 39.5% day^{-1} ($P > 0.05$). SGR values from larvae fed FR-C, E–H were similar ($P > 0.05$), but significantly lower than those recorded in larvae from FR-A ($P < 0.05$). SGR in terms of TL followed a similar trend with the SGR values based on BW.

3.3. Effects of weaning strategy on digestive system morphogenesis

The histological description of the development of the digestive system in butter catfish larvae under standard rearing conditions was already described in Pradhan et al. (2012); thus, this kind of information is not presented in this study. In particular, feeding regimes and weaning strategies tested in this study affected the histological differentiation of different regions of the digestive system in butter catfish larvae. In most feeding regimes, the stomach, composed by its glandular and non-glandular regions (Fig. 3A, E), was already differentiated at 7 dph, with the exception of larvae fed FR-C (Fig. 3B), which did not show a developed stomach at this point. Although stomach morphogenesis was more advanced at 12 dph with regard to the previous sampling interval, larvae fed FR-C still showed retardation in the level of development of this digestive organ, as larvae showed a reduced number of gastric glands and thinner gastric mucosa layer (95.8 ± 15.5 μm in depth; Fig. 3C, F), with regard to the other treatments (370.2 ± 155.0 μm in depth). At the end of the study, no differences in stomach development could be detected among all feeding regimes (Fig. 3D, G, H).

At 7 dph, the pattern of lipid accumulation in the intestine of butter catfish larvae along larval development followed that already described by Pradhan et al. (2012), regardless of the feeding regime considered. At 7 dph, the intestine in butter catfish was coiled and the pre-valvular

Table 1
Survival, body weight, specific growth rate (SGR) of body weight, total length and SGR of total length of butter catfish larvae at 7, 12 and 17 days post hatch (dph)reared under different feeding regimes.

Feeding regime	Survival (%)	Body weight (mg)			SGR (body weight)			Total length (cm)			SGR (total length)		
		7 dph	12 dph	17 dph	2–7 dph	7–12 dph	12–17 dph	2–17 dph	7 dph	12 dph	17 dph	2–7 dph	7–12 dph	12–17 dph	2–17 dph
A	65.0 ± 5.0 ^{b}	5.2 ± 0.8 ^{b}	59.5 ± 22.9 ^{d}	339.8 ± 53.0 ^{c}	50.5 ± 0.04 ^{b}	48.5 ± 2.1 ^{b}	34.9 ± 1.2 ^{ab}	44.9 ± 0.0 ^{c}	0.9 ± 0.1 ^{b}	2.12 ± 0.3 ^{e}	3.8 ± 0.1 ^{b}	22.2 ± 0.2 ^{b}	16.9 ± 0.9 ^{b}	11.5 ± 0.8 ^{a}	16.9 ± 0.0 ^{b}
B	66.0 ± 6.0 ^{b}	6.0 ± 1.8 ^{b}	28.8 ± 9.7 ^{bc}	229.4 ± 81.2 ^{b}	54.4 ± 0.4 ^{b,c}	30.9 ± 2.6^{a}	41.6 ± 2.4 ^{b}	42.3 ± 0.0 ^{b,c}	0.9 ± 0.1 ^{bc}	1.6 ± 0.2 ^{bcd}	3.3 ± 0.4 ^{ab}	22.6 ± 0.2 ^{b}	11.2 ± 0.1 ^{ab}	13.9 ± 0.2 ^{a,b}	15.9 ± 0.0 ^{a,b}
C	48.7 ± 6.3 ^{a}	2.5 ± 0.6 ^{a}	7.8 ± 2.0 ^{a}	81.3 ± 45.8 ^{a}	36.4 ± 1.9 ^{a}	23.0 ± 1.0 ^{a}	46.5 ± 3.1 ^{b}	35.3 ± 1.3 ^{a}	0.7 ± 0.1 ^{a}	0.9 ± 0.1 ^{a}	2.6 ± 0.6 ^{a}	16.0 ± 0.9 ^{a}	6.8 ± 0.5 ^{a}	22.2 ± 1.6 ^{b}	14.4 ± 0.7 ^{a}
D	66.0 ± 5.0 ^{b}	8.6 ± 1.9 ^{c}	47.4 ± 7.5 ^{cd}	151.2 ± 43.1 ^{ab}	61.2 ± 1.6 ^{d}	34.3 ± 1.1 ^{a}	22.9 ± 3.8 ^{a}	39.5 ± 1.1 ^{a,b,c}	1.0 ± 0.1 ^{c}	1.9 ± 0.2 ^{de}	3.2 ± 0.2 ^{ab}	25.6 ± 1.1 ^{b}	11.6 ± 0.2 ^{ab}	10.2 ± 1.9 ^{a}	15.8 ± 0.0 ^{a,b}
E	58.7 ± 3.8 ^{a}	7.0 ± 1.8 ^{bc}	35.9 ± 9.6 ^{bc}	125.3 ± 64.9 ^{a}	57.3 ± 0.9 ^{c,d}	32.2 ± 4.7 ^{a}	25.0 ± 0.3^{a}	38.2 ± 1.1 ^{a,b}	1.0 ± 0.1 ^{bc}	1.8 ± 0.2 ^{cde}	2.9 ± 0.5 ^{a}	23.7 ± 0.4 ^{b}	12.0 ± 1.2 ^{ab}	9.6 ± 0.1 ^{a}	15.1 ± 0.3 ^{a,b}
F	66.2 ± 6.3 ^{b}	7.1 ± 2.1 ^{bc}	35.4 ± 14.5 ^{bc}	107.5 ± 38.4 ^{a}	57.6 ± 0.7 ^{c,d}	31.8 ± 1.9 ^{a}	21.6 ± 2.9 ^{a}	37.0 ± 1.8 ^{a,b}	0.9 ± 1.4 ^{bc}	1.7 ± 0.2 ^{bcd}	2.8 ± 0.3 ^{a}	22.6 ± 0.6 ^{b}	12.0 ± 1.1 ^{ab}	10.1 ± 1.3 ^{a}	14.9 ± 0.6 ^{a,b}
G	77.5 ± 2.5 ^{b}	7.4 ± 2.1 ^{c}	24.3 ± 12.1 ^{ab}	85.4 ± 23.3 ^{a}	58.4 ± 0.6 ^{c,d}	23.7 ± 0.8^{a}	24.7 ± 3.8^{a}	35.2 ± 1.3^{a}	1.0 ± 0.1 ^{bc}	1.5 ± 0.3 ^{bc}	2.7 ± 0.2 ^{a}	23.5 ± 0.2 ^{b}	8.1 ± 0.7^{a}	12.0 ± 1.8 ^{a}	14.6 ± 0.4 ^{a}
H	78.7 ± 1.2^{b}	7.0 ± 1.9 ^{bc}	22.2 ± 13.1 ^{ab}	113.2 ± 27.1 ^{a}	57.2 ± 1.1 ^{c,d}	23.0 ± 1.6^{a}	32.5 ± 0.1 ^{a,b}	37.5 ± 0.9 ^{a,b}	0.9 ± 0.1 ^{bc}	1.4 ± 0.3 ^{b}	3.0 ± 0.2 ^{ab}	22.6 ± 0.6^{b}	8.1 ± 2.4 ^{a}	15.0 ± 0.9^{a,b}	15.2 ± 0.2 ^{a,b}

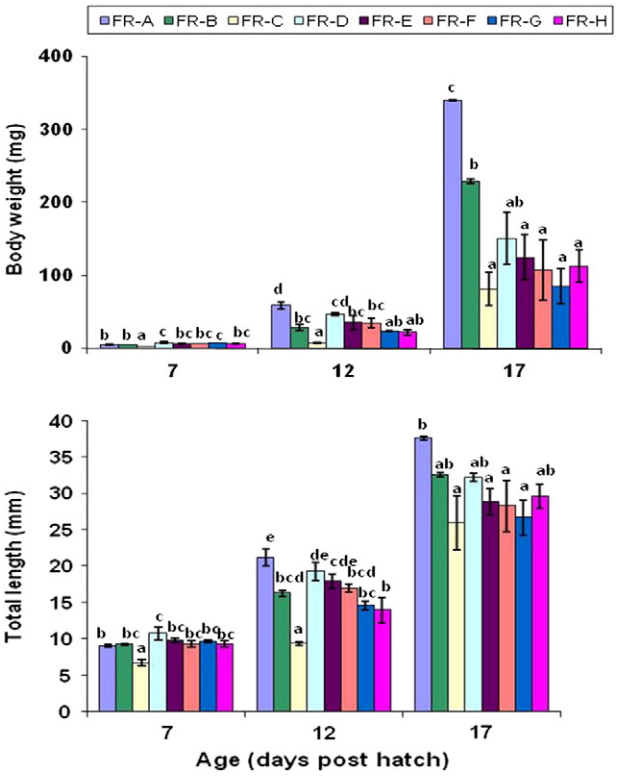

Fig. 2. Growth performance (body weight and total length) of butter catfish larvae at 7, 12 and 17 days post hatch reared under different feeding regimes. Different letters indicate the existence of statistical differences among groups (ANOVA, $P < 0.05$).

(anterior) and post-valvular (posterior) intestine were clearly distinguishable. In all feeding regimens with the exception of larvae fed FR-C, the intestinal mucosa was deeply convoluted, and the anterior and posterior intestine differed in the size of transversal mucosal folds that were larger in the anterior than in the posterior intestine (Fig. 4A, B, D). Larvae fed FR-A and D contained large number of intra- and intercellular lipid vacuoles (502.7 ± 43.9 μm^2) between the enterocytes lining the intestinal epithelium whereas intra- and intercellular lipid deposition in the intestinal mucosa of larvae fed the rest of feeding regimes were significantly ($P < 0.05$) lower (80.2–100.5 μm^2). At 12 dph, larvae from FR-A and D showed a considerable decrease in the number of large lipid vacuoles, whereas some few small lipid inclusions were present in intercellular spaces (Fig. 4C). However, the intestinal mucosa in larvae fed FR-C showed a large deposition of lipids, showing symptoms of steatosis (Fig. 4E). At the end of the study, the level of intestinal fat deposits decreased in all groups, including larvae from FR-C that showed the highest fat accumulation at earlier ages (Fig. 4F).

At 7 dph, the exocrine pancreas of larvae from all feeding regimes contained eosinophilic zymogen granules inside polyhedral basophilic pancreocytes, although the size of the pancreocytes were larger in butter cattish larvae fed FR-A and D (65.3 ± 12.9 μm in diameter). The exocrine pancreas in larvae fed FR-C had smaller pancreocytes in comparison to the rest of dietary treatments (27.1 ± 5.6 μm) (Fig. 5A, B), and these differences were also observed at 12 dph. At 17 dph, larvae from all tested feeding regimes, including FR-C, had abundant zymogen granules within pancreocytes and no differences were observed between larvae of different feeding regimes (Fig. 5C, D).

The liver was already well differentiated at 7 dph in larvae from all feeding regimes, although there were differences in the level of fat accumulation among them measured in terms of ACFD values. The effects of different feeding regimes on the level of fat deposition in the liver of butter catfish larvae are presented in Figs. 6 and 7. The two-way ANOVA result showed that the percentage of hepatocytes with fat deposits, as well as the ACFD values in the butter catfish larvae were

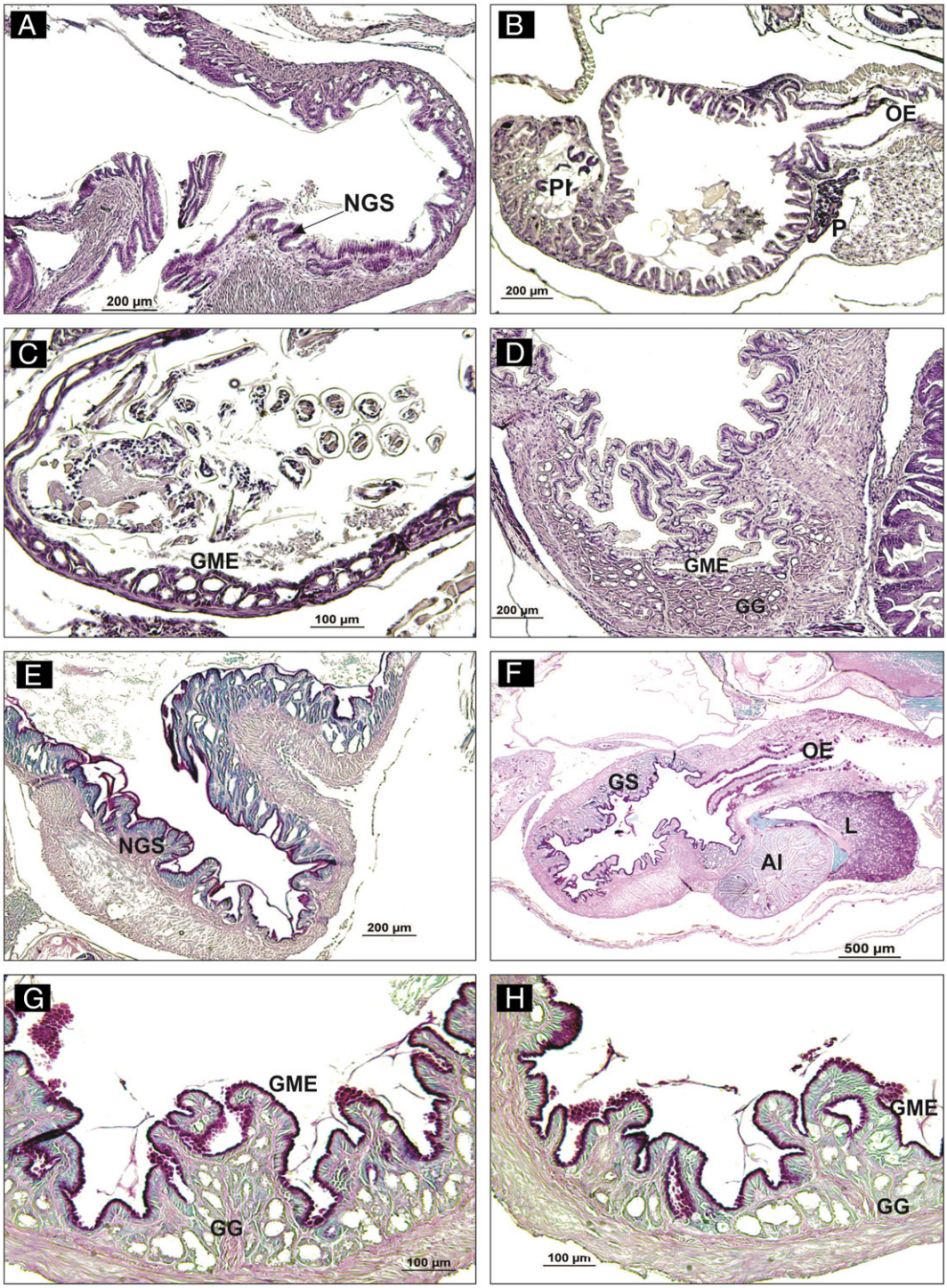

Fig. 3. Histological sections of the stomach of butter catfish larvae at different ages (days post hatch, dph). A, general view of the glandular and non-glandular regions of stomach of a larva from the feeding regime (FR) E at 7 dph. B, general view of the gut of a larva from FR-C; note development of the intestine, liver and pancreas, but the absence of the stomach. C, longitudinal section of the stomach of a larva from FR-C at 12 dph showing a thinner gastric mucosal layer. D, stomach of a larva from FR-C at 17 dph showing a well developed stomach with a thick mucosal layer. E, detail of the glandular and non-glandular regions of the stomach of a larva from FR-B at 7 dph. F, detail of the gut of a larva from FR-C at 12 dph showing the development of the glandular and non-glandular stomach regions of the stomach. G, H, detail of the gastric mucosal layer from FR-C and FR-D at 17 dph, respectively. Note the absence of major histological differences among both digestive organs. Abbreviations: *AI*, anterior intestine; *GG*, gastric gland; *GS*, glandular stomach; *GME*, gastric mucosal epithelium; *I*, intestine; *L*, liver; *NGS*, non-glandular stomach; *OE*, esophageal epithelium; *P*, pancreas; *PI*, posterior intestine. Staining: hematoxylin–eosin (A–D), Periodic acid-Schiff (E–H).

significantly ($P < 0.001$) influenced by the interactive effects of both age and feeding regimes (Fig. 7). From an ontogenic point of view, the comparison of the level of fat deposition in the liver sampled between different ages indicated that larvae from FR-A and D showed a decrease, in areas covered by fat deposits values at 17 dph compared to their respective lower age group of larvae ($P < 0.05$) (Fig. 6A, B). In contrast, larvae from FR-C showed an increase in the level of hepatic fat deposits in terms of areas covered by fat deposits in the liver between 7 and 12 dph ($91.3 \pm 10.0\ \mu m^2$), whereas they remained stable until the end of the study ($98.2 \pm 7.2\ \mu m^2$) (Fig. 6C, D). No significant differences

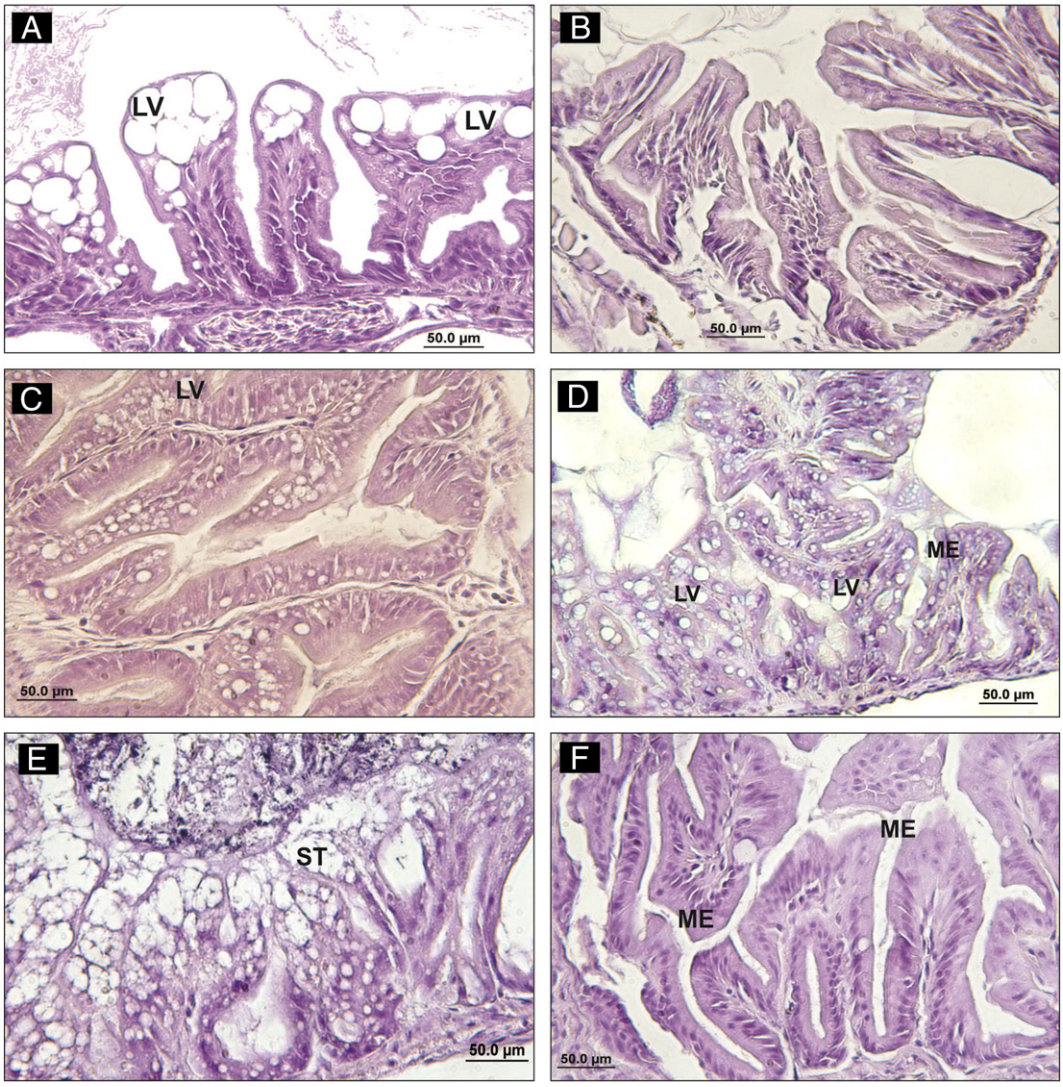

Fig. 4. Histological sections of the intestine of butter catfish larvae at different ages (days post hatch, dph). A, enterocytes with large lipid vacuoles of a larva from FR-A at 7 dph. B, enterocytes of a larva from FR-B showing no lipid inclusions. C, intestinal mucosa of a larva from FR-A at 12 dph showing smaller sized lipid vacuoles compared to larvae of 7 dph. D, detail of anterior intestine of a larva from FR-C with lipid vacuoles and smaller mucosal folds. E, intestinal mucosa of a larva from FR-C at 12 dph showing symptoms of steatosis. F, intestinal mucosa of a larva from FR-C at 17 dph showing well developed mucosal folds and no lipid inclusions. Abbreviations: *LV*, lipid vacuoles; *ME*, mucosal epithelium; *ST*, steatosis Staining: hematoxylin–eosin.

($P > 0.05$) were observed in the level of fat accumulation in the hepatic tissue along larval age in the rest of feeding regimes (Fig. 6E, F). The percentage of hepatocytes containing fat deposits in their cytoplasm increased with larval age in all dietary groups ($P < 0.05$), with the exception of larvae from FR-E where remained constant along larval development ($P > 0.05$).

The comparison of hepatic fat deposits (Fig. 7), between different feeding regimens at particular ages revealed that at 7 dph larvae from FR-A showed the maximum level of ACFD values (171.0 ± 31.8 µm^2) (Fig. 6G) followed by FR-D (112.5 ± 7.6 µm^2) with regard to the rest of experimental groups ($P < 0.05$). No hepatic fat deposits were detected in butter catfish larvae from FR-C, whereas fat accumulation (ACFD) in larvae from the other feeding regimens ranged between 32.8 and 56.0 µm^2. The percentage of hepatocytes containing fat deposits in their cytoplasm was similar in larvae from FR-A, D and E with values ranging between 30.9 ± 2.1% and 47.4 ± 3.1% ($P > 0.05$), but they were significantly higher than the rest of dietary treatments ($P < 0.05$). At 12 dph, the level of hepatic fat accumulation in larvae fed FR-A was higher (203.4 ± 36.43 µm^2) with regard to the other feeding regimes with ACFD values that ranged between 25.4 to 91.3 µm^2 ($P < 0.05$). At this age, the percentage of hepatocytes with fat deposits was highest in the larvae of FR-G (52.8 ± 3.3%), whereas it was lowest in the FR-D (28.2 ± 11.22%). In the rest of the feeding regimes the values were similar ($P > 0.05$). At 17 dph, larvae fed FR-C showed the maximum level of fat deposits in the hepatic tissue (ACFD = 98.2 ± 7.3 µm^2) (Fig. 6H) in comparison to the rest of dietary treatments ($P < 0.05$), which showed ACFD values ranging between 41.7 and 72.3 µm^2. The percentage of hepatocytes with fat deposits in the larvae from FR-E was the lowest (21.5 ± 4.1%) followed by larvae from FR-C (32.7 ± 7.0%) and FR-A (42.0 ± 3.8%; $P < 0.05$), whereas larvae from FR-B, C and F-H had the values ranging from 51.1 ± 7.9% to 66.0 ± 8.9% ($P > 0.05$).

4. Discussion

Larval survival is one of the most important parameters to consider when evaluating the success of any weaning process. In this sense, it is generally accepted that the adaptation of fish larvae to microdiets requires protocols to adapt them to a period of drastic morphological, physiological, and behavioral changes and to their nutritional and

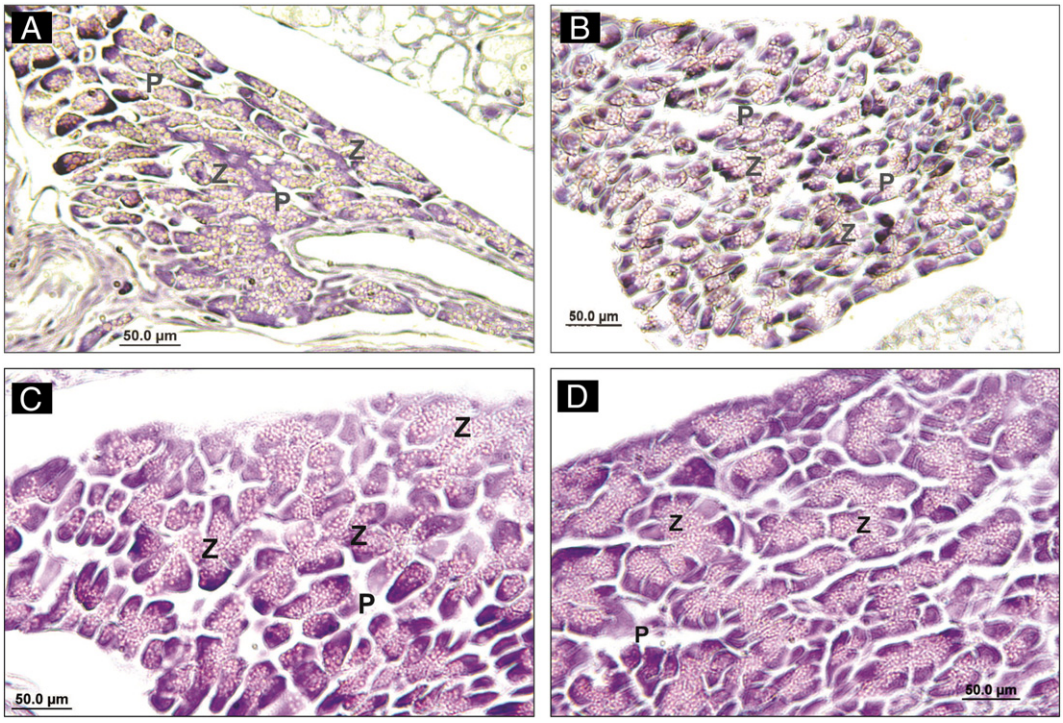

Fig. 5. Histological sections of the pancreas of butter catfish larvae at different times (days post hatch, dph). A, general view of the exocrine pancreas of a larva from FR-A at 7 dph showing pancreocytes arranged in acini containing zymogen granules. B, exocrine pancreas of a larva from FR-C; note smaller size of pancreocytes compared to FR-A. C, D, the exocrine pancreas of larvae from FR-C and E, respectively showing no difference in size of pancreocytes at 17 dph. Abbreviations: *P*, pancreocytes; *Z*, zymogen granules. Staining: hematoxylin–eosin.

environmental requirements (Rao, 2003), whereas the stage of development is the most important independent variable affecting growth performance and nutritional condition of larvae (Andrade et al., 2012). Under the present experimental conditions, butter catfish larvae weaned after 7 dph in the feeding regimes F–H showed similar survival rates with larvae that only on live prey (*Artemia* nauplii or zooplankton) with values ranging from 65.0 to 78.7%. However, early weaning of butter catfish larvae resulted in poor survival with values ranging from 48.8 to 58.7%, as it was previously reported in other catfish species such as *C. gariepinus* (Hogendoorn, 1980) and the sampa *Heterobranchus longifilis* (Kerdchuen and Legendre, 1994), although results are not directly comparable in terms of the effects of different microdiets on weaning success due to different dietary composition of feeds among studies and recent advances in microdiet formulations and manufacturing processes (Holt et al., 2011; Langdon and Barrows, 2011).

The effect of the weaning strategy on larval growth is also an important parameter to consider when hatchery managers evaluate different larval rearing protocols, although this parameter may not be as critical as survival, since differences in larval size may be compensated at further stages of the rearing process (Ali et al., 2003; Valente et al., 2013). In the present trial, butter catfish larvae fed *Artemia* nauplii (solely, FR-A) showed the best results in terms of growth performance (339.8 ± 53.0 mg and 3.8 ± 0.1 cm) followed by larvae fed with zooplankton (FR-B; 229.4 ± 81.2 mg and 3.3 ± 0 .4 cm) and combination of both (FR-D; 151.2 ± 43.1 mg and 3.2 ± 0.2 cm). In contrast, all larvae offered the microdiet showed similar final growth values in terms of BW and TL with values ranging from 81 to 125 mg and 2.6 to 2.9 cm, respectively. Similar observations were made on other catfish species such as African catfish *C. gariepinus* (van Damme et al., 1990), Japanese catfish *Silurus asotus* (Hirakawa et al., 1997), redtail catfish *Mystus nemurus* (Kamarudin et al., 2011) fed *Artemia* nauplii in comparison to microdiets, as well as in many other freshwater and marine fish species (Holt et al., 2011). Under present experimental conditions, the lower performance of butter catfish larvae weaned early in comparison to those fed *Artemia* nauplii may be attributed to the fact that gastric glands were not completely functional regardless of their level of development identified by means of histological procedures. A progressive shift in activity from alkaline to acid proteases has been reported for this species between 15 and 21 dph when animals exhibited fully functional digestive system (Pradhan et al., 2013). Thus, the absence of a functional stomach and its associated acid-pepsin-mediated digestion could be a limiting influence on the digestibility of dietary compounds in early weaned butter catfish larvae. Live prey is a highly digestible protein source for fish larvae, while other protein sources such as fish meal have a low digestibility (Rønnestad et al., 2013). In this sense, several authors have reported that African catfish fed microdiets showed growth rates comparable to those fed live food only after the complete development of the stomach (Verreth and Tongeren, 1989; Verreth et al., 1992). In addition, other studies have suggested that other than providing some essential micronutrients, *Artemia* might contribute to the activation of zymogens or digestive hormones (Petkam and Moodie, 2001) or might trigger the secretion of endogenous enzymes with regard to microdiets (Pedersen and Hjelmeland, 1988). Additionally, higher tryptic activities were observed in larvae fed *Artemia* compared with the weaned larvae in pikeperch *Sander lucioperca* (Hamza et al., 2007). These observations might be in agreement with our results, which indicated that the pancreocytes' size at 7 dph was comparatively larger (65.3 ± 12.9 µm) in larvae from FR-A and FR-D in which *Artemia* nauplii were offered to larvae in comparison to the rest of dietary treatments (27.1 ± 5.6 µm).

One unexpected result from our experiment was that larvae fed *Artemia* nauplii grew better (339.8 ± 53.0 mg and 3.8 ± 0.1 cm) than those fed wild zooplankton (cyclopoid copepods) (229.4 ± 81.2 mg and 3.3 ± 0.4 cm), which generally is the contrary when dealing with marine fish larvae fed these two types of live preys (Øie et al., 2011). Copepods have generally lower total lipid and neutral lipid contents as compared with *Artemia*, but have a higher proportion of phospholipids and n-3/n-6, as well as higher total and free amino acid contents (see reviews in Ahlgren et al., 2009; Øie et al., 2011). Thus, differences in growth performance between both groups of butter catfish larvae may

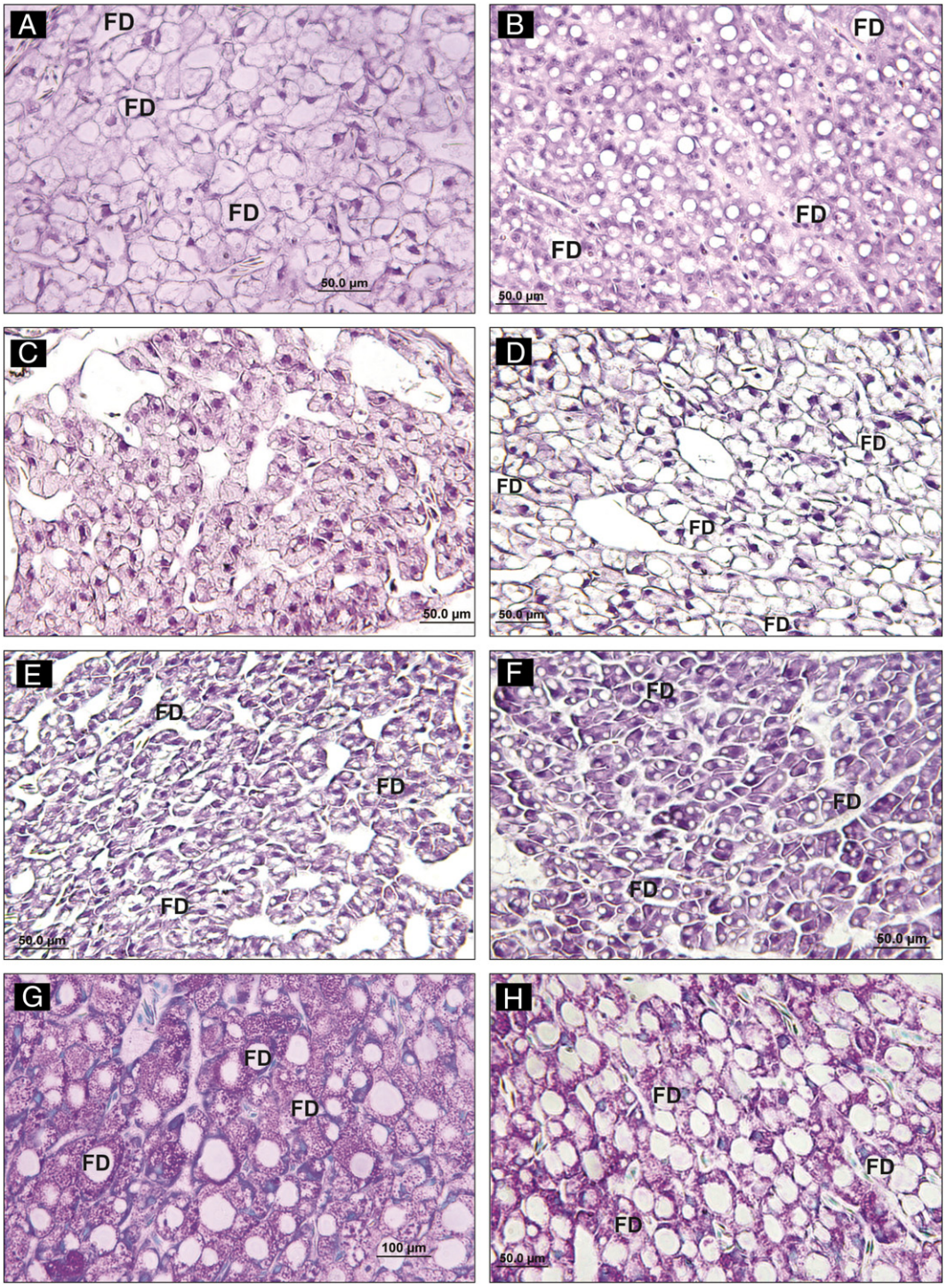

Fig. 6. Histological sections of the liver of butter catfish larvae at different times (days post hatch, dph). A, detail of the liver of a larva from FR-A at 7 dph showing large numbers of fat deposits. B, liver of a larva from FR-A at 17 dph showing decrease in size and increase in numbers of fat deposits. C, liver of a larva from FR-C at 7 dph showing no fat deposits. D, liver of a larva from FR-C at 17 dph showing large numbers of fat deposits. E, F, liver of larvae of FR-E at 7 and 17 dph, respectively, showing no change size and number of fat deposits. G, H, liver of larvae from FR-A and FR-C at 7 and 17 dph, respectively stained with Periodic acid-Schiff (PAS) showing large numbers of fat deposits. Abbreviations: *FD*, fat deposits. Staining: hematoxylin–eosin (A–F), Periodic acid-Schiff (PAS) (G–H).

be linked to different nutritional value of both live preys. In this sense, freshwater copepods have a lower specific P content in comparison to their marine counterparts, whereas the N:P ratio of marine zooplankton is typically higher than that of freshwater zooplankton (Sterner and Hessen, 1994). In addition, the lower growth performance of larvae fed copepods may be hypothesized due to the presence of numerous diapause eggs in this type of prey, since diapause eggs are resistant to digestion in many fish species and they might represent a considerable indigestible female copepod biomass (Bartholmé et al., 2005; Conway et al., 1994; Flinkman et al., 1995), although this hypothesis could not be validated under the present experimental conditions. The above-mentioned differences as well as other nutritional variations between both types of live preys might explain the differences in growth performance observed between larvae fed *Artemia* nauplii and cyclopoid copepods (FR-A and FR-B, respectively) or different requirements by this species which are covered better by *Artemia*. However,

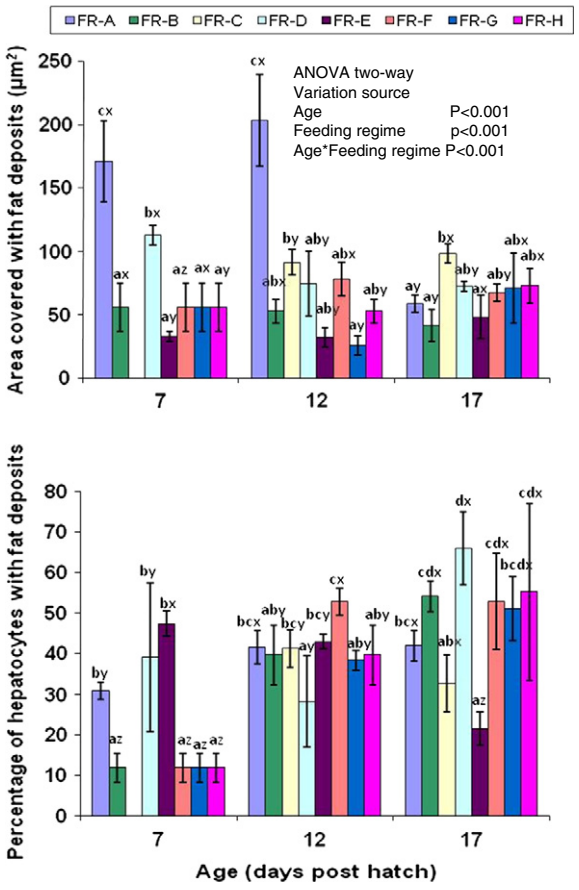

Fig. 7. Effects of different feeding regimes and ages on the level of fat deposition (ACFD) in the liver of butter catfish larvae. Different letters a, b, c, d correspond to significant differences ($P < 0.05$) in the level of fat deposition among feeding regimes (one-way ANOVA) and the letters x, y, z denote age wise significant differences ($P < 0.001$) in the level of fat deposition in the same feeding regime. *P* value for their interaction effect ($P < 0.001$) (two-way ANOVA).

further research on the nutritional value of wild freshwater zooplankton is needed in order to characterize and optimize its nutritional properties in terms of its suitability for fish larval feeding purposes.

How lipid content and composition in the diet is utilized by the various organs and tissues of fish is reflected in their structure (Gisbert et al., 2008). As the different digestive organs employed different cellular mechanisms in response to qualitative and quantitative changes of the diet, their use as nutritional and physiological biomarkers is well established in fish larvae. In this sense, changes in the histological organization of the liver, the exocrine pancreas, the intestine and muscular fibers have been used on a regular basis as histological targets to analyze the nutritional condition of fish larvae and elucidate the effects of different dietary regimes or nutrients on larval physiology, nutrition and early development. These tissues and organs are especially sensitive to non-optimal feeding conditions or nutritional stress during larval development, because they are under progressive and intensive morphogenesis, and consequently, they respond rapidly and sensitively to nutritional disorders (see reviews in Gisbert et al., 2008; Cahu et al., 2009). In the present study, butter catfish larvae fed *Artemia* nauplii at 7 and 12 dph had higher values of ACFD in the liver and intestine with regard to the rest of dietary treatments, whereas it decreased by 17 dph. The same trend with regard to the positive correlation between the levels of intestinal and liver fat accumulation was reported by several authors (Boglino et al., 2012; Papadakis et al., 2009), confirming that this organ responds sensitively to changes in the diet in butter catfish larvae. This trend is consistent with existing data on the process of maturation of the digestive tract in butter catfish (Pradhan et al., 2012) and the acquisition of an adult mode of digestion at the end of the larval period (Pradhan et al., 2013). In contrast, larvae fed wild zooplankton showed a lower degree of hepatic and intestinal fat deposits, which might be due to the lower total lipid content a higher proportion of phospholipids and n-3/n-6 in comparison to *Artemia* nauplii (Ahlgren et al., 2009; Øie et al., 2011). Dietary phospholipids have been found to greatly affect lipid digestion, absorption and transport in cultured fish, as it is generally accepted that they have a beneficial effect on lipid emulsification in the intestinal lumen (Tso, 1994), whereas their absence in the diet may lead to morphological alterations in the epithelial gut (Liu et al., 2012). Thus, the higher accumulation of fat deposits in the intestine of *Artemia*-fed butter catfish larvae with regard to larvae fed zooplankton may indicate that although larvae were able to satisfactorily digest and absorb dietary lipids, they displayed a reduced lipid transport capacity (Cahu et al., 2009; Gisbert et al., 2005). In this sense, this accumulation pattern has led to the suggestion that intestinal lipid inclusions may be considered as a temporary storage form of re-esterified fatty acids when the rate of lipid absorption exceeds the rate of lipoprotein synthesis, or because of an inability to metabolize lipids (Kjørsvik et al., 1991; Segner et al., 1993). At the end of the trial, larvae fed microdiet showed a higher degree of fat accumulation that both groups fed exclusively live prey which might be attributed to the different lipid total content and profile of diets. In any case, the accumulation of lipids in the intestine or liver resulted in a potential pathological situation that might have affected cell functionality and ultimately the larval performance, since no signs of epithelial abrasion, cellular necrosis, and/or inflammatory reactions along the intestinal mucosa were detected as a consequence of large lipid deposits (Gisbert et al., 2008).

The physiology and morphogenesis of larval digestive tract might be stimulated or impaired, depending on how co-feeding is performed (Cahu and Zambonino-Infante, 2001). In fact, depending on the type of microdiet and feeding regime the development and maturation of the digestive system might be delayed or promoted (Vega-Orellana et al., 2006; Engrola et al., 2007, 2010; Kamarudin et al., 2011; Liu et al., 2012 among others). Under the present experimental conditions, the exocrine pancreas of butter catfish larvae fed exclusively with live preys (*Artemia* nauplii and zooplankton) or co-fed with live prey and a microdiet for different periods of time had a normal histological organization with a large pancreocytes (65.3 ± 12.9 μm) arranged in acini and a high amount of zymogen granules, whereas that of larvae fed only the microdiet was characterized by smaller pancreocytes (27.1 ± 5.6 μm) and a lesser quantity of zymogen granules between 7 and 12 dph. These results indicated impairment in the degree of maturation and functionality of the exocrine pancreas, as well as that of the glandular stomach (Gisbert et al., 2008). In this sense, some authors have reported that an improper or abrupt weaning schedule might delay the development of the stomach (Hamza et al., 2007; Liu et al., 2012). In butter catfish larvae, early weaning did not only affect the morphogenesis of digestive glands, but also the development of the intestine, as larvae fed exclusively a microdiet showed a reduction in number and size of intestinal folds, which might also have affected larval performance since the intestine is the major site for nutrient digestion and absorption (Gisbert et al., 2008). Regardless of the weaning schedule tested, no differences in the level of cellular organization and development was observed among treatments at the end of the study, which indicated high plasticity of butter catfish larvae to different nutritional conditions once their digestive system was completely developed and larvae were adapted to the microdiet (Pittman et al., 2013; Rønnestad et al., 2013).

5. Conclusions

Early larval feeding has been one of the main limiting factors that have compromised the development of catfish farming in Indian rural areas, since *Artemia* is a really expensive food item and its production as live feed requires specialized facilities to produce (Chepkirui-Boit

et al., 2011). Therefore, feeding protocols or diets that reduce dependence on this type of live prey are of technical and economic interest (González et al., 2008). Feeding regimes where butter catfish larvae were fed exclusively with a microdiet from the onset of exogenous feeding or fed during a short period of time (3 days) with live feed and then switched to microdiet lead to growth arrest and poor survival. The results from this study indicated that it is feasible to rear butter catfish larvae with zooplankton without dependence upon *Artemia* nauplii, and also that larvae may be weaned onto microdiets after a short period of co-feeding when weaning takes place after 7 dph. However, present results were obtained under small experimental conditions and need to be further verified at a commercial scale.

Acknowledgments

This work was funded by the International Foundation for Science, Sweden (IFS grant A/4422-1) and the histological studies were conducted at the IRTA (Sant Carles de la Ràpita, Spain) under an IFS travel grant provided to the first author. The authors also gratefully acknowledge the College of Fisheries, Agartala, Tripura for providing facilities to carry out this work and the State Fisheries Department, Government of Tripura for providing the larvae. The first author would like to sincerely thank Dr. K.B. Andree and O. Bellot (IRTA-SCR), Dr. A.V. Suresh (Integrated Aquaculture International), Dr. H. Priyadarsi and Dr. A.B. Patel, College of Fisheries, Tripura for their help and encouragement.

References

Ahlgren, G., Vrede, T., Goedkoop, W., 2009. Fatty acid ratios in freshwater fish, zooplankton and zoobenthos — Are there specific optima? In: Arts, M.T., Brett, M.T., Kainz, M. (Eds.), Lipids in Aquatic Ecosystems. Springer Science + Business Media, pp. 147–178.

Ali, M., Nicieza, A., Wotton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4, 147–190.

Alves, T.T., Cerqueira, V.R., Brown, J.A., 2006. Early weaning of fat snook (*Centropomus parallelus* Poey 1864) larvae. Aquaculture 253, 334–342.

Andrade, C.A.P., Nascimento, F., Conceicao, L.E.C., Linares, F., Lacuisse, M., Dinis, M.T., 2012. Red porgy, *Pagrus pagrus*, larvae performance and nutritional condition in response to different weaning regimes. J. World Aquacult. Soc. 43, 321–334.

Appelbaum, S., Van Damme, P., 1988. The feasibility of using exclusively dry diet for rearing of Israeli *Clarias gariepinus* (Burchell) larvae and fry. J. Appl. Ichthyol. 4, 105-l10.

Bartholmé, S., Samchyshyna, L., Santer, B., Lampert, W., 2005. Subitaneous eggs of freshwater copepods pass through fish guts: survival, hatchability, and potential ecological implications. Limnol. Oceanogr. 50, 923–929.

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

Boglino, A., Gisbert, E., Darias, M.J., Estévez, A., Andree, K.B., Sarasquete, C., Ortiz-Delgado, J.B., 2012. Isolipidic diets differing in their essential fatty acid profiles affect the deposition of unsaturated neutral lipids in the intestine, liver and vascular system of Senegalese sole larvae and early juveniles. Comp. Biochem. Physiol. 162A, 59–70.

Buckley, L.J., Turner, S.I., Halavik, T.A., Smigielski, A.S., Drew, S.M., Laurence, G.C., 1984. Effects of temperature and food availability on growth, survival, and RNA–DNA ratio of larval sand lance (*Ammodytes mericanus*). Mar. Ecol. Prog. Ser. 15, 91–97.

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

Cahu, C., Gisbert, E., Villeneuve, L.A.N., Morais, S., Hamza, S., Wold, P.A., Zambonino-Infante, J.L., 2009. Influence of dietary phospholipids on early ontogenesis of fish. Aquacult. Res. 40, 989–999.

CAMP, 1997. Conservation assessment and management plan (CAMP) workshop for freshwater fishes of India. NBFGR, Lucknow 327.

Chepkirui-Boit, V., Ngugi, C.C., Bowman, J., Oyoo-Okoth, E., Rasowo, J., Mugo-Bundi, J., Cherop, L., 2011. Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed *Artemia* and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. Aquacult. Nutr. 17, 82–89.

Conway, D.V.D., Mc Fadzen, I.R.D., Tranter, P.R.G., 1994. Digestion of copepod eggs by larval turbot *Scophthalmus maximus* and egg viability following gut passage. Mar. Ecol. Prog. Ser. 106, 303–309.

Curnow, J., King, J., Bosmans, J., Kolkovski, S., 2006a. The effect of reduced artemia and rotifer use facilitated by a new microdiet in the rearing of barramundi *Lates calcarifer* (Bloch) larvae. Aquaculture 257, 204–213.

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

Engrola, S., Conceição, L.E.C., Dias, L., Pereira, R., Ribeiro, L., Dinis, M.T., 2007. Improving weaning strategies for Senegalese sole: effects of body weight and digestive capacity. Aquacult. Res. 38, 696–707.

Engrola, S., Dinis, M.T., Conceicao, L.E.C., 2010. Senegalese sole larvae growth and protein utilization is depressed when co-fed high levels of inert diet and *Artemia* since first feeding. Aquacult. Nutr. 16, 457–465.

Flinkman, J., Vuorinen, I., Christiansen, M., 1995. Calanoid copepod eggs survive passage through fish digestive tracts. ICES J. Mar. Sci. 51, 127–129.

Gisbert, E., Williot, P., 2002. Advances in the larval rearing of Siberian sturgeon. J. Fish Biol. 60, 1071–1092.

Gisbert, E., Villeneuve, L., Zambonino-Infante, J.L., Quazuguel, P., Cahu, C.L., 2005. Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. Lipids 40, 609–618.

Gisbert, E., Ortiz-Delgado, J.B., Sarasquete, C., 2008. Nutritional cellular biomarkers in early life stages of fish. Histol. Histopathol. 23, 1525–1539.

González, A., Celada, J.D., González, R., García, V., Carral, J.M., Sáez-Royuela, M., 2008. *Artemia* nauplii and two commercial replacements as dietary supplement for juvenile signal crayfish, *Pacifastacus leniusculus* (Astacidae), from the onset of exogenous feeding under controlled conditions. Aquaculture 281, 83–86.

Hamza, N., Mhetli, M., Kestemont, P., 2007. Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae. Fish Physiol. Biochem. 33, 121–133.

Harris, K.C., Hulsman, P.F., 1991. Intensive culture of lake whitefish (*Coregonus clupeaformis*) from larvae to yearling size using dry feeds. Aquaculture 96, 255–268.

Hirakawa, N., Takeuchi, T., Sakai, K., Watanabe, T., 1997. Feeding trial of larval Japanese catfish using live food or formulated micro-diets. J. Tokyo Univ. Fish. 83, 43–52.

Hogendoorn, H., 1980. Controlled breeding of the African catfish, *Clarias lazera* (C. and V.) III. Feeding and growth of fry. Aquaculture 21, 233–241.

Holt, C.J., Webb, K.A., Rust, M.B., 2011. Microparticulate diets: testing and evaluating success. In: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, UK, pp. 353–372.

Kamarudin, M.S., Otoi, S., Saad, C.R., 2011. Changes in growth, survival and digestive enzyme activities of Asian redtail catfish, *Mystus nemurus*, larvae fed on different diets. Afr. J. Biotechnol. 10, 4484–4493.

Kerdchuen, N., Legendre, M., 1994. Larval rearing of an African catfish, *Heterobranchus longifilis*, (Teleostei, Clariidae): a comparison between natural and artificial diet. Aquat. Living Resour. 7, 247–253.

Kjørsvik, E., van der Meeren, T., Kryvi, H., Arnønnson, J., Kvenseth, P.G., 1991. Early development of the digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. J. Fish Biol. 38, 1–15.

Kolkovski, S., Curnow, J., King, J., 2010. Development towards commercialization of marine fish larvae feeds — Microdiets. Project No. 2004/258. Fisheries Research Report No. 198. Department of Fisheries, Western Australia (180 pp.).

Langdon, C., Barrows, R., 2011. Microparticulate diets: technology. In: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, UK, pp. 335–351.

Lazo, J.P., Darias, M.J., Gisbert, E., 2011. Ontogeny of the digestive tract. In: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, UK, pp. 5–46.

Liu, B., Zhu, X., Lei, W., Yang, Y., Han, D., Jin, J., Xie, S., 2012. Effects of different weaning strategies on survival and growth in Chinese longsnout catfish (*Leiocassis longirostris* Gunther) larvae. Aquaculture 364–365, 13–18.

Mitra, G., Mukhopadhyay, P.K., Ayyappan, S., 2007. Biochemical composition of zooplankton community grown in freshwater earthen ponds: nutritional implication in the culture of fish larvae and early juveniles. Aquaculture 272, 346–360.

Mollah, M.F.A., Tan, E.S.P., 1982. Effects of feeding frequency on the growth and survival of catfish (*Clarias macrocephalus*) larvae. Indian J. Fish. 29, 1–9.

NBFGR, 2011. Proceedings of National Consultation on Species Prioritization for Ex situ Conservation and Freshwater Aquaculture, 17–18 September 2011. NBFGR, Lucknow 22.

Øie, G., Reinta, K.I., Evjemo, J.O., Støttrup, J., Olsen, Y., 2011. Live feeds. In: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, UK, pp. 307–334.

Ostaszewska, T., Dabrowski, K., Czumińska, K., Olech, W., Olejniczak, M., 2005. Rearing of pike-perch larvae using formulated diets — first success with starter feeds. Aquacult. Res. 36, 1167–1176.

Papadakis, I.E., Zaiss, M.M., Kyriakou, Y., Georgiou, G., Divanach, P., Mylonas, C.C., 2009. Histological evaluation of the elimination of *Artemia* nauplii from larval rearing protocols on the digestive system ontogeny of shi drum (*Umbrina cirrosa* L.). Aquaculture 253, 334–342.

Pearse, A.G.E., 1985. Histochemistry. Theoretical and applied. Vol. 2: Analytical Technology. Churchill Livingstone, New York 1055.

Pedersen, B.H., Hjelmeland, K., 1988. Fate of trypsin and assimilation efficiency in larval herring *Clupea harengus* following digestion of copepods. Mar. Biol. 97, 467–476.

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

Petkam, R., Moodie, G.E.E., 2001. Food particle size, feeding frequency, and the use of prepared food to culture larval walking catfish, *Clarias macrocephalus*. Aquaculture 194, 349–362.

Pittman, K., Yúfera, M., Pavlidis, M., Geffen, A.J., Koven, W., Ribeiro, L., Zambonino-Infante, J.L., Tandler, A., 2013. Fantastically plastic: fish larvae equipped for a new world. Rev. Aquacult. 5, S224–S267.

Pradhan, P.K., Jena, J.K., Mitra, G., Sood, N., Gisbert, E., 2012. Ontogeny of the digestive tract in butter catfish *Ompok bimaculatus* (Bloch) larvae. Fish Physiol. Biochem. 38, 1601–1617.

Pradhan, P.K., Jena, J.K., Mitra, G., Sood, N., Gisbert, E., 2013. Ontogeny of the digestive enzymes in butter catfish *Ompok bimaculatus* (Bloch) larvae. Aquaculture 372–375, 62–69.

Rao, T.R., 2003. Ecological and ethological perspectives in larval fish feeding. J. Appl. Aquac. 13, 145–178.

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Rev. Aquacult. 5, S59–S98.

Santiago, C.B., Bañes-Aldaba, M., Songalia, E.T., 1983. Effect of artificial diets on growth and survival of milkfish fry in freshwater. Aquaculture 34, 247–252.

Segner, H., Rösch, R., Verreth, J., Witt, U., 1993. Larval nutritional physiology: studies with *Clarias gariepinus*, *Coregonus lavaretus* and *Scophthalmus maximus*. J. World Aquacult. Soc. 24, 121–134.

Sterner, R.W., Hessen, D.O., 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annu. Rev. Ecol. Syst. 25, 1–29.

Treviño, L., Alvarez-González, C.A., Perales-García, N., Arevalo-Galan, L., Uscanga-Martínez, A., Marquez-Couturier, G., Fernández, I., Gisbert, E., 2011. A histological study of the organogenesis of the digestive system in bay snook *Petenia splendida* Gunther, 1862 from hatching to the juvenile stage. J. Appl. Ichthyol. 27, 73–82.

Tso, P., 1994. Intestinal lipid absorption. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract. Raven Press, New York, pp. 1867–1907.

Valente, L.M.P., Moutou, K.A., Conceição, L.E.C., Engrola, S., Fernandes, J.M.O., Johnston, I.A., 2013. What determines growth potential and juvenile quality of farmed fish species? Rev. Aquacult. S168–S193.

Van Damme, P., Hilde, R., Frans, O., 1990. The adaptation of *Clarias gariepinus* larvae to dry feed. In: Hirano, R., Hanyu, I. (Eds.), Proceedings of the Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 303–305.

van der Meeren, T., Jørstad, K.E., 2001. Growth and survival of Arcto-Norwegian and Norwegian coastal cod larvae (*Gadus morhua* L.) reared together in mesocosms under different light regimes. Aquacult. Res. 32, 549–563.

Vega-Orellana, O.M., Fracalossi, D.M., Sugai, J.K., 2006. Dourado (*Salminus brasiliensis*) larviculture: weaning and ontogenetic development of digestive proteinases. Aquaculture 252, 484–493.

Verreth, J., Tongeren, M.V., 1989. Weaning time in *Clarias gariepinus* (Burchell) larvae. Aquaculture 83, 81–88.

Verreth, J., Toreele, E., Spazier, E., Sluiszen, A.V.D., Rombout, J., Booms, R., Segner, H., 1992. Development of a functional digestive system in the African catfish *Clarias gariepinus* (Burchell). J. World Aquacult. Soc. 23, 286–298.

Zambonino Infante, J.L., Cahu, C.L., 2007. Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: applications to diet formulation. Aquaculture 268, 98–105.

Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall International, NJ, USA.