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# Effect of Dietary Regimes on Development of Digestive System of Stinging Catfish, *Heteropneustes fossilis* (Bloch) Larvae

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**A B S T R A C T**

In the present study, effects of different dietary regimes on development of digestive system in stinging catfish, *Heteropneustes fossilis* (Bloch) larvae were investigated. Seven different dietary regimes (DR), varying in food type (Artemia nauplii, zooplankton or microdiet) and their combinations, were evaluated in triplicate. With regard to effect of different dietary regimes on the morphogenesis of the digestive system, it was observed that weaning had no effect on the timing of development of the digestive organs. However, at the end of study (22 dph), no differences were observed in the development of digestive system among different dietary regime regardless of the weaning strategies investigated. Therefore, it can be concluded that it is feasible to rear stinging catfish larvae without dependence upon artemia nauplii, and the larvae may be weaned to microdiets after 7 dph. This information would be very helpful to improve the larval rearing techniques for this important candidate catfish species in the Asian countries.

## Introduction

*Heteropneustes fossilis* (Bloch), also known as stinging catfish is one of the economically and therapeutically important fish species in many Asian countries particularly, in India, Thailand, Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar, Indonesia, and Cambodia (Burgess, 1989; Akand *et al.*, 1991). The species is highly preferred because of its delicious taste, nutritional and medicinal properties and high market price (Chakraborty and Nur, 2012). Moreover, this catfish can be cultured at high stocking density in wastewater aquaculture system. Because of these characteristics, the species has high market demand and economic value (US$ 6-8/kg) in these countries. Importantlly, *H. fossilis* has been prioritized as one of the fish species for aquaculture and considered as a candidate species for the diversification of freshwater aquaculture in India (NBFGR, 2011). However, in the country, the aquaculture of this species is not commercially successful due to unavailability of quality seed. High mortality rates during larval rearing and scant knowledge of their feeding strategies are major constraints of farming

success. Further, wild seed collection is almost negligible due to habitat alterations (Vijayakumar *et al.,* 1998). Therefore, it is the need of time to develop effective, reliable and efficient larval rearing techniques and dietary regimes to ensure consistent production of quality seeds to meet the demand.

In fish larviculture, zooplankton and artemia are usually provided as feed during first feeding. However, low production and high price of live feed are major bottlenecks for their production on commercial level (Person-Le Ruyet *et al.,* 1993). Moreover, mass culture of live feed requires high costs and is labour intensive along with the highly variable nutritional value of live feeds (Person-le Ruyet *et al.,* 1993; Cahu & Zambonino Infante, 2001; Langdon, 2003). Therefore, formulated microdiets could be used for the replacement of live food. However, the use of formulated microdiets immediately after hatching, can cause poor survival and growth of larva due to inappropriate development of digestive system (Jones *et al.,* 1993; Person-Le Ruyet *et al.,* 1993; Watanabe and Kiron, 1994). Furthermore, the early introduction of formulated di*et al.,*one has found limited success in freshwater species (Koven *et al.,* 2001). There are no universal weaning strategies for fish larvae because of difference in timing of development, maturation and functionality of digestive system (Cahu and Zambonino Infante, 200; Lazo *et al.,* 2011). Consequently, variability in timing of organ development and associated physiological functions affected due to difference in reproductive guilds. It is also affected because of environmental rearing conditions, especially water temperature. In this paper, authors have investigated how different dietary treatments affected the digestive tract development in *H. fossilis* under controlled laboratory conditions. The new information generated in the present study would be helpful in improving the understanding of feeding strategies and actual larval rearing techniques for *H. fossilis*, one of the most popular and promising catfish for diversification of freshwater aquaculture in Asia.

## Materials and Methods

### Source of larvae

The experimental trials were conducted at indoor hatchery of fish genetic resource centre of ICAR-NBFGR, Lucknow, India. Larvae were produced by induced spawning of sexually mature fish (male & female) of *H. fossilis*. Briefly, both female (145–169 g) and male (87–95 g) stinging catfish were injected intramuscularly with synthetic hormone Ovarim®, (Congruent Pharmachem Pvt. Ltd. Mumbai, India) at the rate of 1.0 ml and 0.5 ml kg $^{-1}$ body weight, BW respectively. Stripping of females, sperm collection and fertilization were done following the protocol described by Puvaneswari *et al.,* (2009). The fertilized eggs were subsequently incubated in Fiber Reinforced Plastic (FRP) tray incubators at 28.0 ± 1.1 $^{\circ}$C with continuous water flow. Newly hatched larvae were kept in the incubators until the age of 1 dph.

### Experimental procedures and feeding regimes

A total of 1260 larvae were randomly selected for the experiment. Sixty larvae (1 dph, 0.2 mg) were stocked in 21 circular FRP tanks containing 30 L water each (stocked at a density of 2 larva $l^{-1}$), and the feeding experiment started at 2 dph. Aeration was given in each tank to provide dissolved oxygen and help in a homogeneous distribution of feed throughout the water column. Water quality parameters such as water temperature, dissolved oxygen and pH values were maintained at 28.0–29.1 °C, 6–8 mg $l^{-1}$ and 6.8–7.6, respectively during the experiment period.

Seven different dietary regimes varying in food type (Artemia nauplii, zooplankton or microdiet) and duration were evaluated in triplicate. The age at which different food items were given to larvae, were intended to find out the most convenient dietary regime and weaning strategy for stinging catfish larvae (Fig. 1). Therefore, larvae were reared under the following dietary regimes (DR) from the onset of the exogenous feeding at 2 dph until 22 dph: DR-A, larvae fed with non-enriched Artemia nauplii (OSI PRO 80™, Ocean Star International, Inc., USA) from 2 to 22 dph; DR-B, larvae fed with mixed zooplankton (Copepods, Cyclops and cladocerans) collected from a fish pond from 2 to 22 dph; DR-C, larvae fed with a commercial microdiet (Micro Elite 50, Lucky Star®, Taiwan) from 2 to 22 dph; DR-D, larvae fed with non-enriched Artemia nauplii from 2 to 8 dph, zooplankton from 6 to12 dph and the microdiet from 10 to 22 dph; DR-E, larvae fed with zooplankton from 2 to 7 dph and the microdiet from 5 to 22 dph; DR-F, larvae fed with zooplankton from 2 to 10 dph and the microdiet from 7 to 22 dph; DR-G, larvae fed with zooplankton from 2 to 12 dph and the microdiet between 9 and 22 dph. In all the treatments, larvae were fed with different diets to satiation three times per day (08:00, 12:00 and 16:00 h) for 20 days (Mollah and Tan, 1982).

The feeding protocol in dietary regimes D-G composed of decreasing the frequency of live feed (Artemia nauplii and zooplankton) and increasing the frequency of formulated diet feeding (4/0, 3/1, 2/2, 1/3, 0/4) within 2 consecutive days. The feed waste and faecal residues were siphoned out daily before first feeding provided at 08:00 h and 30% water exchange was done in all the tanks.

## Larval sampling for histological procedures

At each sampling day, 5 larvae from each experimental tanks (n = 15 per dietary treatment) were randomly sampled and larvae were fixed in 10% buffered formalin (pH = 7.0) at 7, 12, 17 and 22 dph. Then, larvae were dehydrated in a graded series of ethanol, cleaned in chloroform, embedded in paraffin, cut into serial sagittal sections (3–4 μm thick), mounted on glass slide and air dried. Sections were deparaffinized with graded series of xylene and stained by Harris' Hematoxylin and Eosin (HE) procedure for general histomorphological observations. The development of the digestive system in stinging catfish larvae was compared among different dietary regimes according to Pradhan *et al.*, (2014). Histological images were obtained by an Olympus (BX 53, Japan) light microscope.

## Results and Discussion

Histological assessment of condition of larvae in early development stages is one of the most accurate indicator of nutritional status (Ferron and Leggett, 1994). Further, histological observations can be made from several tissues from a single specimen, which respond at different rates to food deprivation and diets, enabling a more precise description of the nutritional state of an individual (Gisbert *et al.*, 2008). The development of the digestive tract in stinging catfish larvae have been already studied through histological description under standard rearing condition (unpublished data). However, in this paper authors have tried to understand the effect of different dietary regimes and weaning strategies on the histological differentiation of different parts of the digestive tract in stinging catfish larvae.

In the present study, in all the dietary regimes, the stomach was differentiated at 7 dph (Fig. 1A, C), however, in the case of dietary regimes DR-A and DR-D, stomach had more number of gastric glands and thicker gastric mucosal layer compared to other dietary regimes. On the other hand, in the rest of the

dietary regimes, stomach had lesser number of gastric glands and thinner gastric mucosal layer and stomach of larval group fed with DR-C had lowest growth (Fig. 1B).

At 12 dph and 17 dph, although the development was more advanced as compared to the previous sampling, the pattern of development in different dietary regimes was similar to 7 dph. At 22 dph, stomach development was almost similar among all dietary regimes (Fig. 1D). Similar to the present findings, Pradhan *et al.,* (2014) reported that *O. bimaculatus* fed with microdiet showed retardation in the development of stomach in terms of number of gastric glands and gastric mucosal layer. However, authors have reported that at the end of the experimental period of 15 days, no difference in stomach development was observed between different dietary regimes.

Development of a functional stomach associated with the production of HCl and pepsin by gastric glands is considered as a crucial event for enabling young fish to digest compound diets (Zambonino-Infante *et al.,* 2008; Rønnestad *et al.,* 2013). In *C. gariepinus,* it has been reported that although larvae were able to be reared on microdiets from the onset of exogenous feeding, their growth rates were not comparable to those fed live food until their stomach became functional (Verreth and van Tongeren 1989; Verreth *et al.,* 1992).

Therefore, the larval period in terms of nutrition ends with the completion of a functional stomach (Segner *et al.,* 1993), and consequently, the stomach differentiation is considered as a decisive event in the nutritional physiology of larvae and could be used as an external marker for the start of weaning (Verreth *et al.,* 1992; Senger *et al.,* 1993).

At 7 dph, the intestine of stinging catfish larvae showed the similar pattern in all the dietary regime and no lipid accumulation were observed along larval development regardless of the dietary regime (Fig. 1E, F). The complexity of the intestinal morphology in fish varies with age and is also influenced by the quantity and quality of the feed (Gisbert *et al.,* 2004, 2008; Rønnestad *et al.,* 2013). It is generally accepted that the intestine is the major site for lipid absorption and larvae fed high levels of neutral lipids show intracellular and intercellular accumulation of fat in the intestine, while fish fed with low/moderate levels of lipids shows normal appearance and organization. Studies on the intestinal morphology of larvae have been conducted in various species of catfish, such as *O. bimaculatus* (Pradhan *et al.,* 2014), *P. punctifer* (Gisbert *et al.,* 2014), *R. quelen* (Silveira *et al.,* 2013) and *C. gariepinus* (Verreth *et al.,* 1992), showing that the intestinal morphology affects the digestibility and absorption of nutrients (Khojasteh, 2012). In European sea bass larvae, it has been reported that differences in lipid absorption and accumulation in the intestinal mucosa are influenced by dietary lipid levels (Gisbert *et al.,* 2005). In the present study, in all the dietary regimes, intestinal mucosa had normal appearance and organization and no differences were observed in the deposition of lipids in the intestine between different dietary regimes. However, in contrast to the present findings in *O. bimaculatus,* larvae fed with artemia had led to higher accumulation of fat deposits in the intestine. In fish larvae, lipid droplets are not considered as part of the endoplasmic reticulum of Golgi apparatus of enterocytes and in many cases, they do not appear to be enclosed by any membrane.

This has led to the suggestion that intestinal lipid inclusions are a temporary storage form of re-esterified fatty acids in cases when the rate of lipid absorption exceeds the rate of lipoprotein synthesis (Sheridan, 1988), or because an inability to metabolise lipids

(Kjørsvik *et al.*, 1991). Therefore, it may be assumed that the stinging catfish larvae have better ability to metabolize lipids. The liver is considered reliable nutritional and physiological biomarker because its histological organization is very sensitive to dietary changes.

**Fig.1** Histological sections of stomach and intestine of stinging cat fish larvae at different ages (days post hatch, dph). A, general view of stomach of larva from dietary regime, DR- A at 7 dph. B, general view of glandular stomach of larva from dietary regime, DR- C at 7 dph. C, Histological sections of stomach of larvae from DR D at 7 dph. D, Histological sections of stomach of larvae from DR B at 22 dph. E, intestinal sections of mucosa of larva from DR C at 7 dph. F, intestinal sections of mucosa of larva from DR G at 7 dph. Abbreviations: GG, gastric gland; ME, Mucosal epithelium; NGS, non-glandular stomach. Staining: hematoxylin-eosin

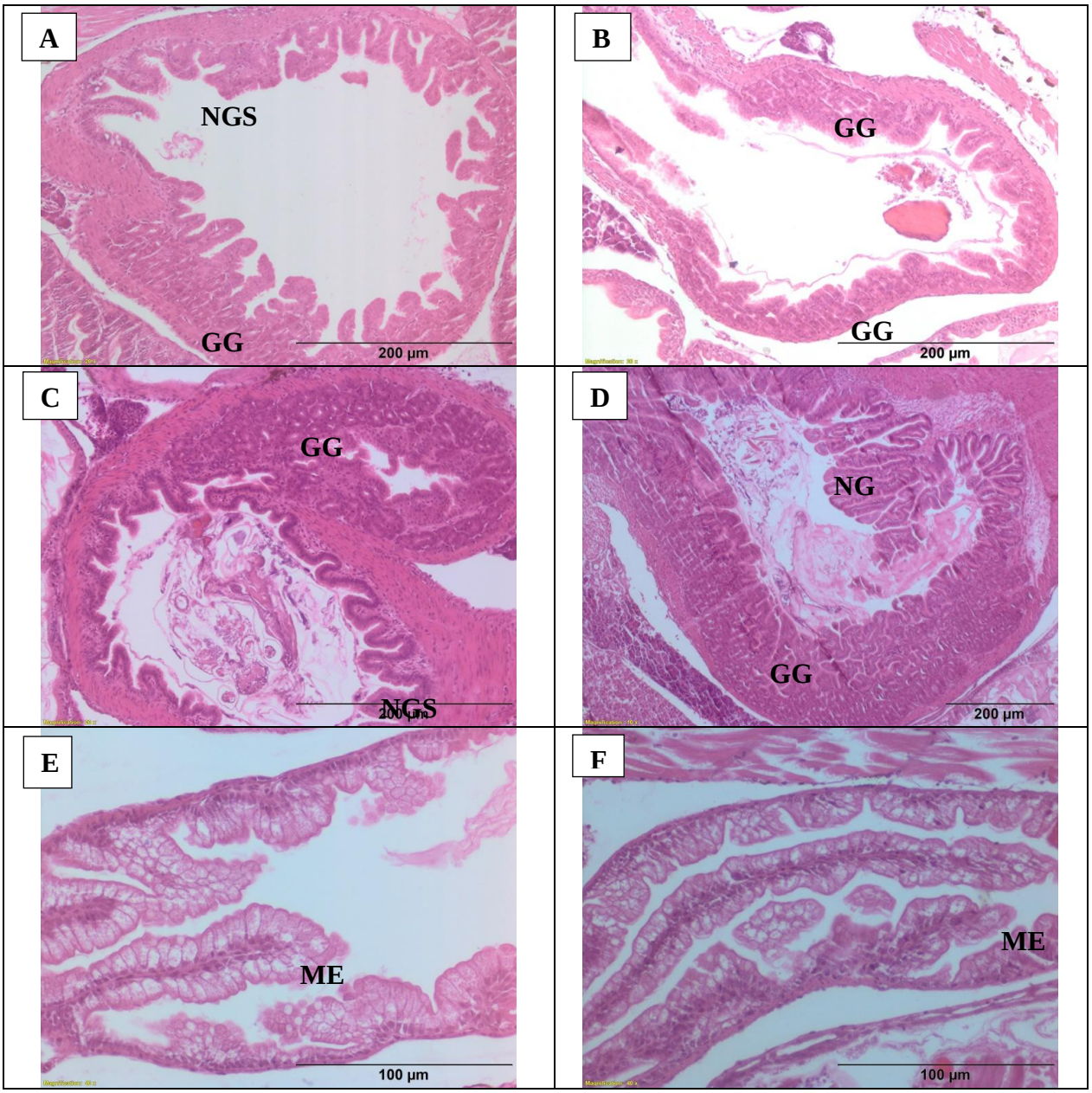
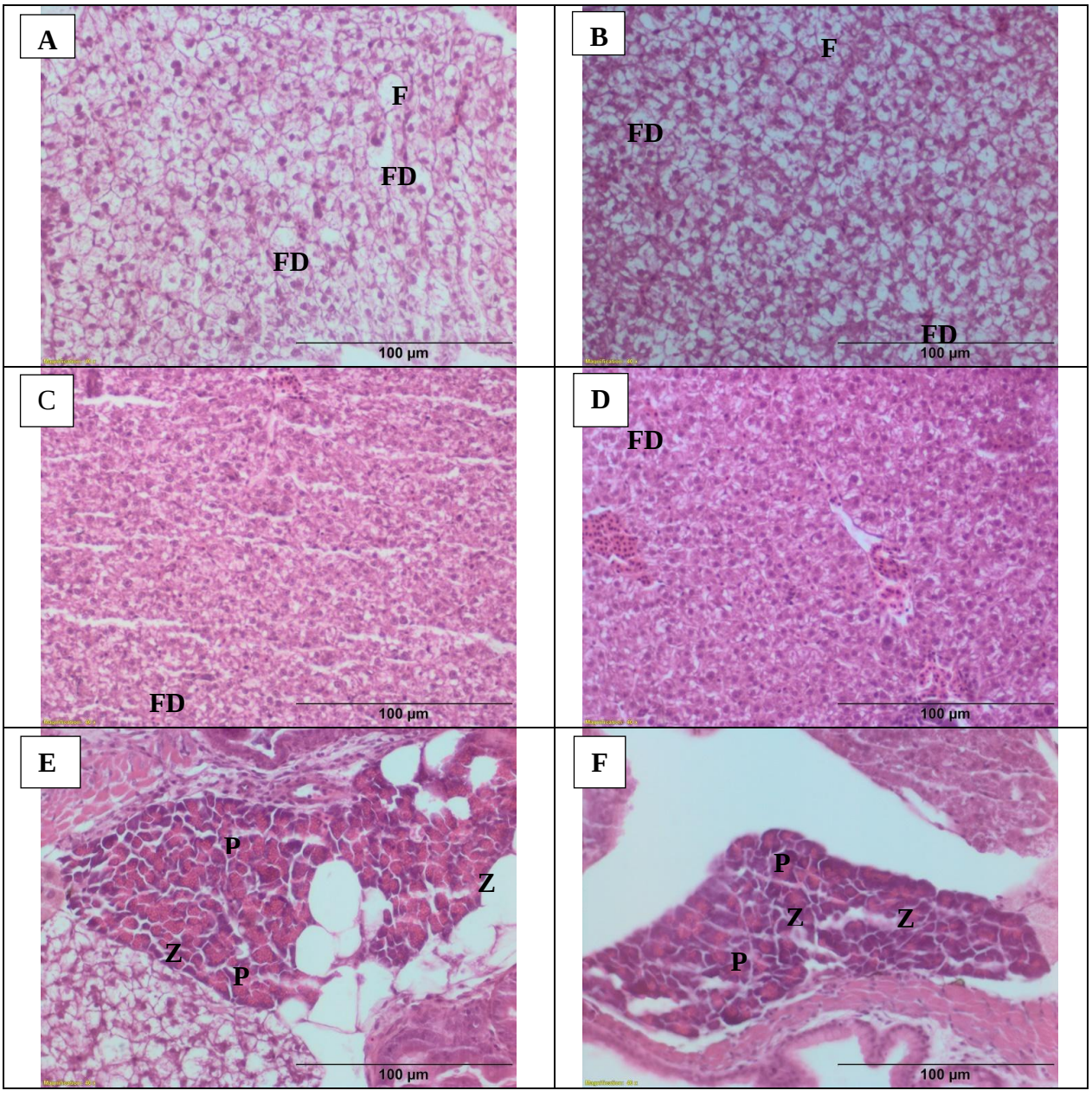


**Fig.2** Histological sections of liver and pancreas of stinging catfish larvae at different age. A, section of liver of larvae from DR A at 7 dph showing fat deposits; B, section of liver of larvae from DR D at 7 dph showing fat deposits; C, liver of larvae from DR A at 22 dph showing reduced fat deposits. D, liver of larvae from DR D at 22 dph showing reduced fat deposits; E, Pancreas of larvae from DR A showing zymogen granules in pancreatocyte at 7 dph; F, Pancreas of larvae from DR C showing zymogen granules in pancreatocyte at 7 dph. Abbreviations: P; pancreatocytes, Z; zymogen granules. Staining: hematoxylin-eosin




The same trend with regard to the positive correlation between the levels of liver lipid accumulation has been reported by several authors (Boglino *et al.*, 2012; Papadakis *et al.*, 2009, Pradhan *et al.*, 2014), confirming that this organ responds sensitively to changes in the diet in stinging catfish larvae. In the present study, in other dietary regimes (except DR-A and DR-D) in which larvae were fed with mixed zooplankton, microdiet and

combination of both, showed no/very less level of lipid accumulation in liver, which may 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).

A functional exocrine pancreas is characterized by differentiated organ morphology (acinar gland), including developed excretory ducts and the presence of acidophilic zymogen granules (pancreatic enzyme precursors) (Hoehne-Reitan and Kjørsvik, 2004). This early differentiation and morphogenesis of the exocrine pancreas facilitates its use at a cytological and biochemical level as a good biomarker of the nutritional condition of the larvae. Imbalanced diet induces degeneration of the exocrine pancreas (Yúfera *et al.,* 1993; Crespo *et al.,* 2001). In the present study, exocrine pancreas of stinging catfish larvae fed exclusively with DR-A (Artemia nauplii) and co-fed with Artemia, zooplankton and microdiet (DR-D) had larger pancreocytes arranged in acini and a high amount of zymogen granules, whereas that of larvae fed with other dietary regimes had comparatively smaller pancreocytes and a lesser quantity of zymogen granules. Further, other studies have suggested that in addition to providing some essential micronutrients, Artemia might be contributing to the activation of zymogens or digestive hormones (Petkam and Moodie, 2001) or might be triggering the secretion of endogenous enzymes in comparison to artificial diets and zooplanktons (Pedersen and Hjelmeland, 1988). In addition, it has also been observed that larvae fed with Artemia had higher tryptic activities compared with the weaned larvae in pikeperch *Sander lucioperca* (Hamza *et al.,* 2007). All these observations support our results, which indicated that the pancreocytes size at 7 dph were comparatively larger in larvae from DR-A and DR-D in which Artemia nauplii were offered to larvae in comparison to the rest of dietary treatments (Fig.2 C). The exocrine pancreas in larvae fed DR-C had smaller pancreocytes in comparison to the rest of dietary treatments (Fig. 2D). These differences were also observed at 12 dph and at 17 dph. At the end of the study (22 dph) in all the dietary treatment larvae had abundant zymogen granules and no differences were observed between larvae of different dietary regimes.

Similar to our present findings, Cahu and Zambonino-Infante, (2001) reported that the physiology and morphogenesis of larval digestive tract might be stimulated or impaired, depending on how co-feeding is performed. In addition, the type of microdiet and feeding regime also have been reported to play an important role in the development and maturation of the digestive system (Vega-Orellana *et al.,* 2006; Engrola *et al.,* 2007, 2010; Kamarudin *et al.,* 2011; Liu *et al.,* 2012). 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 stinging catfish, regardless of the weaning schedule tested, no differences in the level of cellular organization and development were observed among treatments at the end of the study, which indicated high plasticity of this 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). Similar to the present study, in shi drum *Umbrina cirrosa*, Papadakis *et al.,* (2009) also observed lower growth in early weaned larvae without any influence on the timing of the appearance of the various components of the digestive system. Therefore, it can be concluded that it is feasible to rear stinging catfish larvae without dependence upon artemia nauplii, and the larvae may be weaned to microdiets after 7 dph.

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