Comparative Biochemistry and Physiology, Part A 197 (2016) 43–51

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream (*Sparus aurata*) during ontogeny

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ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 2 February 2016
Accepted 10 March 2016
Available online 14 March 2016

Keywords:
Digestive enzymes
Daily rhythms
Fish larvae
Digestive physiology
Sparus aurata

ABSTRACT

In order to identify daily changes in digestive physiology in developing gilthead seabream larvae, the enzyme activity (trypsin, lipases and α-amylase) and gene expression (*trypsinogen-try*, *chymotrypsinogen-ctrb*, *bile salt-activated lipase-cel1b*, *phospholipase A_2-pla2* and *α-amylase-amy2a*) were measured during a 24 h cycle in larvae reared under a 12 h light/12 h dark photoperiod. Larvae were sampled at 10, 18, 30 and 60 days post-hatch. In each sampling day, larvae were sampled every 3 h during a complete 24 h cycle. The enzyme activity and gene expression exhibited a marked dependent behavior to the light/darkness cycle in all tested ages. The patterns of activity and expression of all tested enzymes were compared to the feeding pattern found in the same larvae, which showed a rhythmic feeding pattern with a strong light synchronization. In the four tested ages, the activities of trypsin, and to a lesser extent lipases and amylase, were related to feeding activity. Molecular expression of the pancreatic enzymes tended to increase during the night, probably as an anticipation of the forthcoming ingestion of food that will take place during the next light period. It follows that the enzymatic activities are being regulated at translational and/or post-translational level. The potential variability of enzyme secretion along the whole day is an important factor to take into account in future studies. A particularly striking consequence of the present results is the reliability of studies based in only one daily sample taken at the same hour of the day, as those focused to assess ontogeny of digestive enzymes.

1. Introduction

The impressive growth rates recorded in larval fish are supported by both their high ingestion rates and adequate processing of food. The sequential acquisition of functional digestive capacities in developing larvae has been biochemically assessed in many fish species (e.g. Cahu and Zambonino-Infante, 1994; Martínez et al., 1999; Ribeiro et al., 1999; Gawlicka et al., 2000; Lazo et al., 2000; Zambonino-Infante et al., 2008; Gisbert et al., 2009; Suzer et al., 2013). Most of these studies reported the activities of the main pancreatic and/or intestinal digestive enzymes involved in the hydrolysis of proteins, lipids and carbohydrates. In spite of the variety of experimental conditions and species, the available information indicates that teleosts display a quite similar ontogenetic pattern (Rønnestad et al., 2013), though some differences exist between species with herbivorous–omnivorous and carnivorous feeding habits once the juvenile stage has been attained. Recently, the molecular expression profiles of the main digestive enzyme precursors have been analyzed, this giving a more complete understanding of the ontogeny of the digestive capacity in several species (Darias et al., 2007; Kortner et al., 2011; Srichanun et al., 2013; Murashita et al., 2014; Mazurais et al., 2015). Nevertheless, most of these studies were based on a single sampling per day schedule, which may offer a wrong picture taking into account the great variations in feeding behavior of fish larvae within a one day period. In fact, several studies have evidenced a rhythmic feeding pattern during the larval stage, primarily driven by the light/dark daily cycles (Østergaard et al., 2005; Ma et al., 2006; Wang et al., 2008; Navarro-Guillén et al., 2015). In larvae of visual feeders, food ingestion occurs during the illumination period and ceases in the dark period, with an hourly profile characteristics for each species (Kotani and Fushimi, 2011; Rønnestad et al., 2013). It is therefore expected that food processing within the gut will be related to this feeding pattern.

Gilthead seabream (*Sparus aurata*) is a marine species of high commercial importance in the Mediterranean aquaculture. Different aspects of its larval rearing, ontogeny and physiology have been studied for years (Yúfera et al., 2011). A first study on the ontogeny of digestive enzyme activities during the first month of life was reported by Moyano et al. (1996). In a recent study, we have determined the ontogenetic changes in the molecular expression of these digestive enzymes during the first two months of life considering a single sampling per day schedule (Mata-Sotres et al., 2016). These studies revealed that the enzymatic equipment and ontogenetic pattern of this species resemble those described in other carnivorous fish during their juvenile stage.

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http://dx.doi.org/10.1016/j.cbpa.2016.03.010
1095-6433/

Table 1
Primer pairs used for q-PCR primer sequences, amplicon sizes (bp), reaction efficiencies (E) and Pearson's coefficients of determination (R^2) are indicated.

Gene	Fwd sequence (5′–3′)	Rev sequence (5′–3′)	Size (bp)	E	R^2
try	TGAACATCCCCATCCTGTCT	GTAGCCCCAGGACACAACAC	172	1.00	1.00
ctrb	ATCCAACGGCTTTCATTTCTG	GCCATAGCCCTTATTGTGCTC	124	1.00	0.99
cel1b	TGGACAATGCCTACTCCACA	GCAGCCTGAGTAGGAACCAG	121	0.97	0.99
pla2	CCAGACCATCTTCACCATCC	CACCCAATCCACAGGAGTTC	114	0.97	0.99
amy2a	AACCACGACAACCAGAGAGG	GCCCATCCAGTCATTCTGAT	186	1.00	0.99
actb	TCCTGCGGAATCCATGAGA	GACGTCGCACTTCATGATGCT	108	1.00	0.99

Furthermore, we have described that the gilthead seabream larvae exhibit a rhythmic feeding behavior both under light/dark or permanent illumination regimes (Mata-Sotres et al., 2015). Studies on early juveniles of this species show a clear postprandial pattern in the digestion modulated by the feeding frequency and protocol when fed on commercial feeds (Yúfera et al., 2004, 2014; Montoya et al., 2010). During the larval rearing, the individuals exhibit a clear rhythm in their feeding activity in spite of the permanent availability of live prey in the water column. The question is to what extent this feeding rhythm influences the digestive function in general and the activity of each digestive enzyme in particular.

The aim of this study was to assess changes in the molecular expression and activities of the digestive enzymes during the whole daily cycle at different moments of the larval life of gilthead seabream, and to provide a better understanding on the regulation of the digestive function in developing fish larvae.

2. Materials and Methods

2.1. Rearing Conditions

Gilthead seabream fertilized eggs were supplied by the Laboratory of Marine Cultures at the Faculty of Marine and Environmental Sciences (Puerto Real, Cádiz, Spain) and transferred to ICMAN animal experimentation facilities (REGA number ES110280000311). After hatching, the larvae were reared in 250 L circular tanks at constant

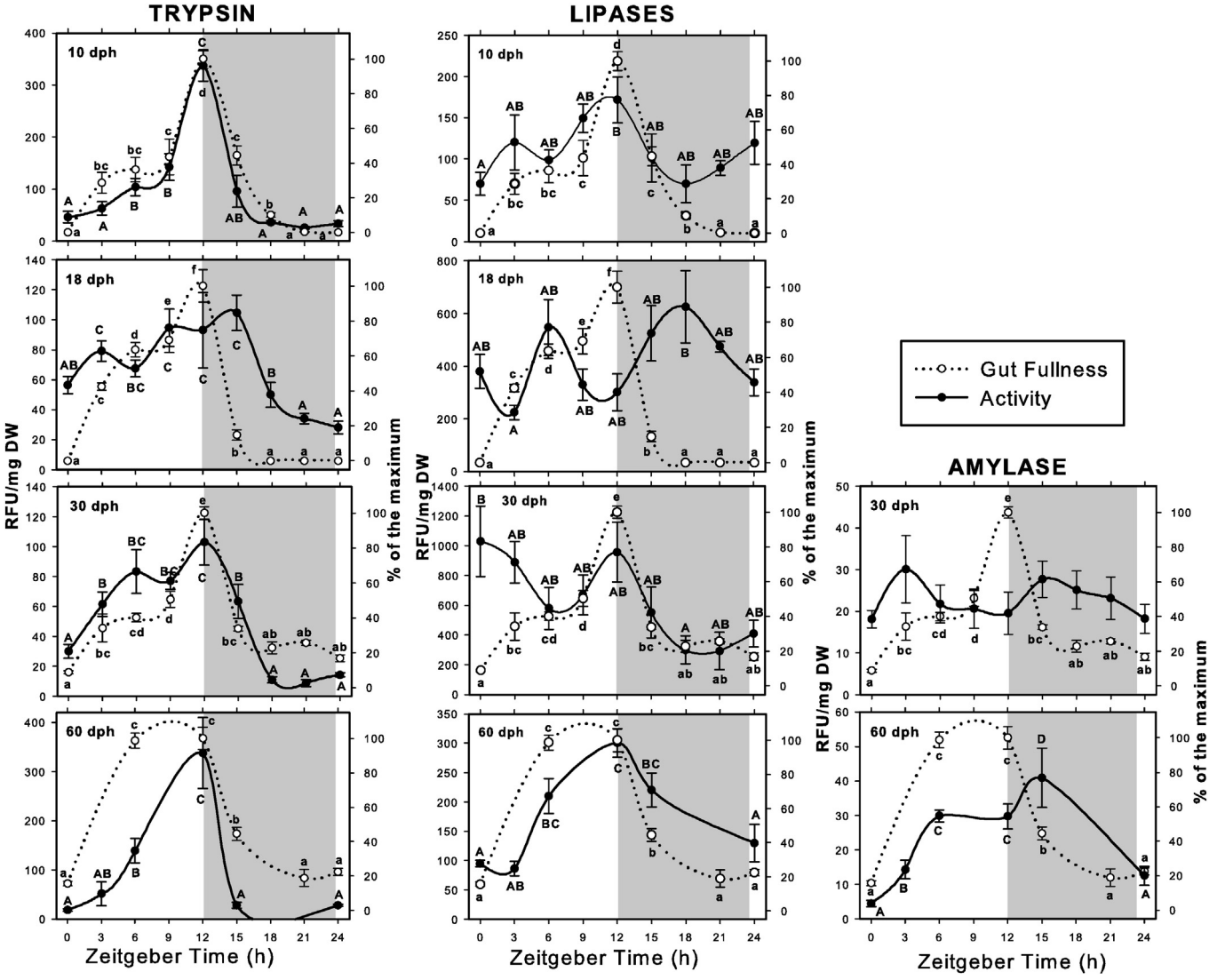

Fig. 1. Trypsin, lipase and amylase activities (solid line) compared with the gut fullness (dashed line; Mata-Sotres et al., 2015) of *S. aurata* larvae at 10, 18, 30 and 60 dph (n = 9). Different letters represent significantly different values ($P < 0.05$) within the same age (mean ± SEM). The white and gray parts of the graph symbolize the light and dark periods of the cycle.