Comparative ontogenetic development of two marine teleosts, gilthead seabream and European sea bass: new insights into nutrition and immunity

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Abstract

Gilthead seabream and European sea bass are two of the most commonly farmed fish species. Larval development is critical to ensure high survival rates and thus avoid unacceptable economic losses, while nutrition and immunity are also important factors. For this reason this paper evaluates the ontogenetic development of seabream and sea bass digestive and immune systems from eggs to 73 days post-fertilisation (dpf) by assessing the expression levels of some nutrition-relevant (*tryp, amya, alp and pept1*) and immune-relevant (*il1b, il6, il8, tnfa, cox2, casp1, tf, nccrp1, ighm* and *ight*) genes. The results point to similar ontogenetic development trends for both species as regard nutrition and differences in some immunity related genes.

Keywords: ontogeny; teleost; gilthead seabream; European sea bass; immunity; nutrition.

1. Introduction

Gilthead seabream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) are the most commonly farmed fish species in Europe (FAO, 2014), where a very successful productive industry depends on combining improved knowledge of their biology and improved farm management. However, obtaining larvae from eggs and subsequent larval development is one of the key steps in the productive cycle, as it is for the culture of other fish species of potential interest for the aquaculture industry. Successful marine larviculture requires not only an effective feeding schedule based on the nutritional requirements and digestive capabilities of developing larvae, in which an effective and mature immune system is of prime importance (Conceição et al., 2010; Parker et al., 2012). Suitable production of larva requires large facilities, high maintenance costs and intensive labour to produce the desired amount of life foods constantly (Kanazawa, 2003). Hence, new advances in our knowledge of the larval digestive system and its interaction with live food in each larvae period constitute a major challenge.

In teleost fish, the emergence of primary and secondary lymphomyeloid organs, head-kidney, spleen and thymus, takes place at different larval stages. The wide variability between species in this respect can be explained by the differing lengths of the embryonic period and larval development, but also by the fact that the development of larvae is strongly affected by culture conditions such as temperature and/or salinity (Falk-Petersen, 2005). In both gilthead seabream and European sea bass, the histological development of the lymphoid organs has been studied previously (Abelli et al., 1996; Josefsson and Tatner, 1993). However, little is known about the timing of the appearance of different immune response gene markers. Furthermore, autologous adaptive immunocompetence acquisition in fish larvae usually follows the first

appearance of lymphoid organs (Lam et al., 2004; Nakanishi, 1986) but is not always correlated with the same (Mulero et al., 2008). In addition, as occurs in other vertebrates, some proteins in fish are maternally transferred from the female to an immunologically naïve offspring (Grindstaff et al., 2003; Zhang et al., 2013). Moreover, the maternal transfer of mRNAs to the oocytes after spawning should not be ruled out as gene expression in larvae will determine the timing of immune system development in offspring and the subsequent effective immune response of these offspring (Huttenhuis et al., 2006; Magnadottir et al., 2005).

Improved knowledge of the development of the digestive capability and immunity in fish larvae could help reduce the high mortality rates in hatcheries and overcome a recurring production bottleneck in the aquaculture industry. Thus, the aim of this work was to know the pattern of expression of some genes that code relevant molecules for (i) nutrition, such as the enzyme responsible for hydrolysing proteins (*tryp*), alpha bonds for polysaccharides (*amya*) and phosphate groups (*alp*), and for carrying oligopeptides in the digestive tube (*pept1*); for (ii) different innate immune responses, such as inflammation (interleukin (il) 1 beta (*il1b*), *il6, il8*, tumor necrosis factor alpha (*tnfa*) and ciclooxygenase-2: *cox2*), apoptosis (caspase 1: *casp1*), antimicrobial peptides (transferring: *tf*) and cytotoxicity (non-specific cytotoxic cell receptor protein 1: *nccrp1)* and for (iii) adptative immune responses such as different populations of B lymphocyte markers (immunoglobulin M, *ighm*, and immunoglobulin T, *ight*) during the ontogenetic development of two of the most relevant commercial species in the Mediterranean area, the gilthead seabream and the European sea bass.

2. Material and methods

2.1. Experimental design

Broodstocks of gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) were bred and kept at the *Instituto Español de Oceanografia* (*IEO*, Mazarrón, Murcia), where the fry was bred and maintained. Natural seawater (38 ‰ salinity) was heated to 17 ± 1 ºC and filtered through mechanical and biological substrates. The temperature increased naturally and reached 26 ºC by the end of the experiment. Gilthead seabream larvae were bred using the "green water" technique in a 5,000-l round tank with an initial density of about 60 eggs/l. During the experiment, the light intensity was 1,000 lux at the water surface, and the photoperiod was 16:8 (L:D). Water renewal was limited to 2 % daily during the first 20 days of culture and was achieved by the addition of 70 ml/m^3 of a microalgae concentrated solution (Phytobloom, Necton) containing 80 % *Nanochloropsis oculata*. Subsequently, continuous water renewal (30 %/h) and light aeration were provided in the tank. Larvae were successively fed with enriched (Selco, Inve Animal Health) rotifers from 6 to 24 days post-fertilisation (dpf), *Artemia nauplii* (Inve Animal Health) from 20 to 35 dpf, enriched Instar II *Artemia* from 31 to 58 dpf and a commercial dry pellet diet (Skretting) from 54 dpf onward. The European sea bass larvae were kept in dark during the first 40 days after hatching. The light intensity of 1,000 lux at the water surface and the 16:8 (L:D) photoperiod were maintained. The specimens were subsequently fed with enriched Instar II *Artemia* and a commercial dry pellet diet (Skretting) from 54 dpf onwards. Three pools of eggs and larvae at different time points post fertilization (0, 3, 6, 10, 13, 17, 24, 31, 45, 59 and 73 dpf) were sampled and stored at -80ºC in TRIzol® reagent (Life Technologies) for latter RNA isolation. The experiments described comply with the Guidelines of the European Union Council (2010/63/EU), the Bioethical

Committee of the University of Murcia (Spain) and the *Instituto Español de Oceanografía* (Spain) for the use of laboratory animals.

2.2. Analysis of gene expression by real-time PCR (qPCR)

Total RNA was extracted from pooled eggs or larvae using TRIzol® reagent according to the manufacturer's instructions. Total RNA (1 µg) was treated with DNase I to remove genomic DNA and the first strand of was cDNA synthesized by reverse transcription using SuperScript III Reverse Transcriptase (Life Technologies) with an oligo-dT_{18} primer (Life Technologies). To check integrity, 1µl of each RNA sample was run in 2% agarose gel.

Real-time PCR was performed as described elsewhere (Cordero et al., 2015). Four putative endogen genes were evaluated following the method described by Pfaffl et al. (2004). The stability of the Ct-values for each candidate in both species is represented in Supplementary Figure S1. Based on another specific study for European sea bass larval stages (Mitter et al., 2009) and an analysis of the four endogen gene transcriptions by the BestKeeper® software, the gene expression of each target gene was corrected by the most suitable reference gene (*ef1a*) according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Gene names follow the guidelines of the Zebrafish Nomenclature Committee (ZNC). The primers used were designed using the OligoPerfect™ Designer Tool (Thermo Fisher Scientific) and are shown in Table 1. Before the experiments, the specificity of each primer pair was studied using positive and negative samples. Amplified products from positive samples were run in 2% agarose gels and sequenced. After these verifications, all amplifications were performed in duplicate cDNAs and repeated once to confirm the results. Negative controls with no template were always included in the reactions.

2.3. Statistical analysis

The results are expressed as mean ± standard error mean (SEM). Data were statistically analysed by one-way analysis of variance (ANOVA) to determine differences between groups followed by a post hoc Tukey test. Normality of the data was previously assessed using a Shapiro-Wilk test and homogeneity of variance was also verified using the Levene test. Statistical analyses were conducted using SPSS for Windows V19. Differences were considered statistically significant when $P<0.05$ and represented with different letters for each stage.

3. Results

3.1. Digestive and transport component transcription increased during the first days

The transcript profile of the genes that code for several digestive enzymes (*tryp*, *amya* and *alp*) and a transport protein located in the digestive tube (*pept1*) (Fig. 1) was analysed. The expression of *tryp*, was expressed in eggs of both species, and higher values were found from 6 dpf onward, with comparable levels of expression in both species. On the other hand, *amya* was undetected in eggs, and later gene expression levels increased from 3 to 17 dpf in both species. The expression of *tryp*, *alp* and *pept1* showed very similar profiles in both species, the only exception being the undetectable *pept1* in European sea bass eggs.

3.2. Innate immune-related genes were expressed differently in both species during development

The following transcription of genes involved in inflammation, phagocytosis apoptosis and cytotoxicity innate immune responses were studied (Fig. 2): interleukin (il) 1 beta (*il1b*), *il6*, *il8*, tumor necrosis factor alpha (*tnfa*), ciclooxygenase-2 (*cox2*), caspase 1 (*casp1*), transferrin (*tf*) and non-specific cytotoxic cell receptor protein 1 (*nccrp1*). The

168 mRNA transcripts of *il1b*, *tnfa* were not detected in eggs in either of the species
169 analyzed, while *il6* transcripts were not detected in gilthead seabream eggs (Fig. 2). Two
170 of the pro-inflammatory cytokines (*il1b* and *tnfa*) were undetected in eggs, but
171 expressed in all the larvae stages of both species (Fig. 2). Transcripts of the rest of the
172 genes analyzed were observed from eggs onwards. Interestingly, *il6*, *tnfa*, *casp1* and
173 *nccrp1* showed higher levels of expression during European sea bass development than
174 in gilthead seabream, while *tf* showed higher expression levels in gilthead seabream
175 than in European sea bass larvae.

176 **3.3 Adaptive immune genes are detected at all larval stages**

177 We have analysed the gene expression profile of B lymphocyte (immunoglobulin M,
178 *ighm*, and immunoglobulin T, *ight*) cell markers (Fig. 3). In the case of *ighm*, gene
179 expression was detected at 3 dpf in gilthead seabream, however, *ighm* was expressed
180 also in European sea bass eggs. In both cases, the expression had significantly increased
181 by the end of the larval stages. On the other hand, *ight* transcription was found in eggs
182 from both species. The gene expression of *ight* remained constant in the case of
183 European sea bass, and decreased in larval stages until 31 dpf and showed no alteration
184 at the end of seabream larval stages.

185

186 **4. Discussion**

187 In the development of the digestive system, the expression levels of the genes that code
188 for two key digestive enzymes, *tryp* and *amya*, have been described in several teleosts
189 (Galaviz et al., 2012; García-Gasca et al., 2006; Murashita et al., 2014, 2013; Pérez et
190 al., 1998; Ribeiro et al., 1999), although most studies have only focused on dietary
191 changes. Our aim was to show the pattern of gene expression in naïve gilthead seabream
192 and European sea bass during the complete ontogeny process. In both species the

193 maximum expression levels of both enzyme transcripts were reached around 17-20 dpf ,
194 when a mature digestive system has already developed (Sahlmann et al., 2015).
195 Previous ontogenetic studies have described both *tryp* and *amya* gene expression peaks
196 in the European sea bass (Zambonino-Infante and Cahu, 2001). Furthermore, our data
197 are supported by previous observations in Bluefin tuna, in which *amya* gene expression
198 was undetected in eggs (Murashita et al., 2014). Another digestive enzyme, *alp*, which
199 carried out the dephosphorylation and is also responsible for the development of the
200 brush border enterocytes (Henning et al., 1994), greatly increased from eggs to 3 dpf. It
201 has been reported that mouth and anus open at 3-6 dph in gilthead seabream (Elbal et
202 al., 2004), thus, our data point to a rapid increase in *alp* gene expression before larval
203 feeding starts. Finally, the importance of *pept1* in the first stages lies in the fact that it is
204 a strong candidate for major nutrient carriers because of its non-selective and high-
205 capacity transporting function for di- and tri-peptides (Daniel, 2004), and it is only
206 expressed in the intestine (Ahn et al., 2013). In agreement with our data, in Japanese eel
207 larvae, the expression of pept1 had significantly increased by 6 dpf, after which it
208 remained constant (Ahn et al., 2013). All these data together suggest that first stages
209 (from hatching to 6-10 dpf) are critical in the development of a suitable digestive system
210 in teleost, and that highest gene expression is achieved around 17-20 dpf, coinciding
211 with the appearance of a mature digestive system.
212 The role of nutrition and its impact on immunity and disease resistance in fish have also
213 been established (Blazer, 1992). Inadequate nutrition and/ or the improper balance of
214 nutrients within the diet can have a major impact on the proper development of
215 lymphoid organs and several immune functions. Bearing this in mind, we next analysed
216 several-related innate and adaptive immune response genes, focusing on the
217 immunocompetence acquisition process from eggs to 73 dpf. Several studies in gilthead

seabream and European sea bass have described the development of lymphomyeloid organs using histological techniques (Abelli et al., 1996; Dos Santos et al., 2000; Josefsson and Tatner, 1993; Schrøder et al., 1998) and the appearance, during ontogeny, of immune molecules through PCR sequencing or microarrays, although most of these studies do not refer to eggs (Darias et al., 2008; Mulero et al., 2008; Yúfera et al., 2012). In vertebrates, inflammatory events will result in a cytokine cascade, whereby *tnfa* is released, followed by *il1b* and then *il6* and *il8* (Secombes et al., 2001). In addition, prostaglandins (especially the inducible *cox2*) induced by these cytokines and, in turn, by nitric oxide, also play a critical role in inflammation (Hirata and Narumiya, 2012; Salvemini et al., 1993; Secombes et al., 2001). Finally, the inflammatory *casp1* is the gene encodes the protein responsible for activating *il1b* (Franchi et al., 2009; López-Castejón et al., 2008; Reis et al., 2012). Our results show that *il8, cox 2* and *casp1* are expressed in eggs and early stages of larva, but that *il1b, tnfa* and *il6* are not expressed in eggs, as supported to some extend by previous studied on gilthead seabream using conventional PCR (Mulero et al., 2008). There are very few previous reports about the expression of these molecules in fish eggs, although the mRNA of other important innate immune components have been reported at the time of hatching in carp (Huttenhuis et al., 2006). Furthermore, our data suggest for the first time a conserved development of these pro-inflammatory molecules, which show similar patterns in the ontogeny of both fish species. Some peaks in the gene expression levels have previously been reported around 17-20 dph (Varela et al., 2012), in agreement with our data in the case of *il1b* in both species and *casp1* in gilthead seabream. As regards the gene expression of other innate immune factors, it is known that transferrin is able to create a bacteriostatic environment by limiting the availability of iron to replicating pathogens (Stafford and Miodrag, 2003) as well as to act as primary activator of fish macrophages

(Stafford et al., 2001). It has been found that *tf* is constitutively expressed in adult sea bass specimens (Neves et al., 2009). In larval stages, a previous short report on Indian carp detected *tf* mRNA expression 6 dph and onwards by PCR (Nayak et al., 2011). Transferrin plays vital roles in iron homeostasis, which is essential for cellular growth, while it also has a clear role in immunity as antimicrobial peptide (Grayfer et al., 2014; Gómez and Balcázar, 2008). In the present study, gene expression was observed in eggs, later increasing, as expected, due to cellular growth and because hatching larvae are exposed to many bacteria in the aquatic environment. In agreement with our data, changes in *tf* transcripts have also been reported during early development in *Megalobrama amblycephala* (Ding et al., 2015). Lastly, to our knowledge, this is the first time that the expression of *nccrp1* has been evaluated in eggs and larval stages of teleost. The *nccrp1* decays in transcription from eggs (especially significant in sea bass) and peaks around 17 dpf, after which it remains almost constant during the rest of the developmental process. As regards adaptive immunity, we observed the expression of *ighm* and *ight* genes from eggs and from 3 dpf onwards in gilthead seabream and European sea bass, respectively. Although, several studies using conventional PCR did not detect mRNA levels of immunoglobulins in eggs (Mulero et al., 2008; Parker et al., 2012), others, including the present one, using rtPCR did (Seppola et al., 2009). Interestingly, *ighm* and *ight* were detected in eggs of gilthead seabream and Atlantic cod (Seppola et al., 2009) long before the onset of the lymphomyeloid organ development. However, our data also showed that both gene levels subsequently increased from 45 dpf onward in both species, matching the development of a mature spleen in fish (Falk-Petersen, 2005). The differences observed between the two species studied might be due to evolutionary divergence although further studies including more fish species will be needed to clarify this issue.

In fish, maternal immunity refers to the immunity transferred via the eggs from mother to offspring, a process in which both innate and adaptive immune-relevant factors are transferred at protein level (Swain and Nayak, 2009; Zhang et al., 2013). However, transcript levels of innate and adaptive factors, such as C3, α2-macrogloblulin, serum amyloid A, C1/MASP2, the antimicrobial peptides, cathelecidin and lysozyme, and the immunoglobulins, IgM and IgD (Huttenhuis et al., 2006; Seppola et al., 2009) have also been reported in previous studies. In the present study, we report the presence of several cytokines, including *il6* and *il8*, but not *il1b* and *tnfa*, as well as the presence of *cox2*, *tf*, *casp1*, *nccrp1* and *ighm* and *ight* in eggs, suggesting maternal transfer of innate and adaptive factors at transcript level since these are present in the larvae earlier than the onset of the development of lymphomyeloid organs. This issue highlights the need to revise the immune system ontogeny of fish at transcriptional level, using the highly sensitive techniques that are available nowadays in order to improve our knowledge of such an important biological process as passive maternal immunity. In summary, we have studied the expression profile of nutrition and immune genes in gilthead seabream and European sea bass from eggs to 73 dpf larvae, finding that they are essential for the suitable development of the larvae of both species.

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442 **Table 1.** Information of primers used for qPCR.

Gene	Symbol	GenBank ID	Organism	Primer sequence (5´→3´)	GO: Biological process
elongation factor 1-alpha	*ef1a*	AF184170	*S. aurata*	F: CTGTCAAGGAAATCCGTCGT R: TGACCTGAGCGTTGAAGTTG	Translational elongation GO:0006414
		FM019753	*D. labrax*	F: CGTTGGCTTCAACATCAAGA R: GAAGTTGTCTGCTCCCTTGG	
beta-actin	*actb*	X89920	*S. aurata*	F: GGCACCACACCTTCTACAATG R: GTGGTGGTGAAGCTGTAGCC	Protein folding GO:0006457
		AJ537421	*D. labrax*	F: TCCCTGGAGAAGAGCTACGA R: AGGAAGGAAGGCTGGAAAAG	
alpha-tubulin	*tuba*	AY326430	*S. aurata*	F: AAGATGTGAACTCCGCCATC R: CTGGTAGTTGATGCCCACCT	Microtubule-based process GO:0007017
		AY326429	*D. labrax*	F: ACGAGGCCATCTACGACATC R: GGCCGTTATGGACGAGACTA	
vimentin	*vim*	FM155527	*S. aurata*	F: CGCTTACCTGTGAGGTGGAT R: GTGTCTTGGTAACCGCCTGT	Intermediate filament-based process GO:0045103
		FM018579	*D. labrax*	F: AGCGCCAGATTAGAGAGCTG R: GCCATCTCGTCCTTCATGTT	
trypsin	*tryp*	AF316852	*S. aurata*	F: GGTCTGCATCTTCACCGACT R: AAAGGCAGCAGAGTGATGGT	Digestion GO:0007586
		AJ006882	*D. labrax*	F: GCACCATGTGCACAGTCTCT R: ACAGGATGGGGATGTTCAAG	
alpha-amylase	*amya*	AF316854	*S. aurata*	F: TGGTGGGACAATCAGAGTCA R: GTCCAGGTTCCAGTCGTCAT	Digestion GO:0007586
		AJ310653	*D. labrax*	F: GATCACCAGATGCAACAACG R: CTGAACCAGCTTCCACATGA	
alkaline phosphatase	*alp*	AY266359	*S. aurata*	F: TTACTGGGCCTGTTTGAACC R: ATCCTTGATGGCCACTTCCAC	Metabolic process GO:0008152
		FJ860000	*D. labrax*	F: TTACCTCTGTGGGGTCAAGG R: TAGCCCATTTGAGGATGGAG	
peptide transporter 1	*pept1*	GU733710	*S. aurata*	F: TTGAACATAACGTCGGGTGA R: AATTTTGCATTTCCCTGTGG	Transport GO:0006810
		FJ237043	*D. labrax*	F: TGAGATCGACACACACAGCA R: AAACGCTCGCAGAACTCATT	
interleukin 1-beta	*il1b*	AJ277166	*S. aurata*	F: GGGCTGAACAACAGCACTCTC R: TTAACACTCTCCACCCTCCA	Inflammatory response GO:0006954
		AJ269472	*D. labrax*	F: CAGGACTCCGGTTTGAACAT R: TTGTCCCCTTTTGAATGGAC	
interleukin 6	*il6*	AM749958	*S. aurata*	F: AGGCAGGAGTTTGAAGCTGA R: ATGCTGAAGTTGGTGGAAGG	Inflammatory response GO:0006954
		AM490062	*D. labrax*	F: ACTTCCAAAACATGCCCTGA R: CTCCTTAGACTGACCAGCGG	
interleukin 8	*il8*	AM765841	*S. aurata*	F: GCCACTCTGAAGAGGACAGG R: TTTGGTTGTCTTTGGTCGAA	Inflammatory response GO:0006954
		AM490063	*D. labrax*	F: GTCTGAGAAGCCTGGGAGTG R: GCAATGGGAGTTAGCAGGAA	

tumor necrosis factor alpha	*tnfa*	AJ413189	*S. aurata*	F: CTGTGGAGGGAAGAATCGAG R: TCCACTCCACCTGGTCTTTC	Inflammatory response GO:0006954
		DQ200910	*D. labrax*	F: AGCCACAGGATCTGGAGCTA R: GGACAGCTACAGAAGCGGAC	
ciclooxigenase 2	*cox2*	AM296029	*S. aurata*	F: GAGTACTGGAAGCCGAGCAC R: GATATCACTGCCGCCTGAGT	Inflammatory response GO:0006954
		AJ630649	*D. labrax*	F: AGCACTTCACCCACCAGTTC R: AAGCTTGCCATCCTTGAAGA	
caspase 1	*casp1*	AM490060	*S. aurata*	F: ACGAGGTGGTGAAACACACA R: GTCCGTCTCTTCGAGTTTGC	Apoptotic process GO:0006915
		DQ198376	*D. labrax*	F: CCAGATCGTGGGTGTTTTCT R: TCTTCAAAGCGTTGCATGAC	
transferrin	*tf*	JF309047	*S. aurata*	F: CAGGACCAGCAGACCAAGTT R: TGGTGGAGTCCTTGAAGAGG	Response to bacterium GO:0009617
		FJ197144	*D. labrax*	F: TGCCAAAAACCTGATGTTCA R: TAAGGGAACGGACAATGCTC	
non-specific cytotoxic cell receptor protein 1	*nccrp1*	AY651258	*S. aurata*	F: ACTTCCTGCACCGACTCAAG R: ACTTCCTGCACCGACTCAAG	Positive regulation of cell proliferation GO:0008284
		FM022070	*D. labrax*	F: TGGGGTGAGATACGTCCACT R: TGGTTTTGGTTGCTCTGACA	
immunoglobulin mu heavy chain	*ighm*	AM493677	*S. aurata*	F: CAGCCTCGAGAAGTGGAAAC R: GAGGTTGACCAGGTTGGTGT	Adaptive immune response GO:0002250
		FN908858	*D. labrax*	F: AGGACAGGACTGCTGCTGTT R: ACAACAGCAGACAGCAGGTG	
immunoglobulin tau heavy chain	*ight*	FM145138	*S. aurata*	F: TGGCAAATTGATGGACAAAA R: CCATCTCCCTTGTGGACAGT	Adaptive immune response GO:0002250
		FM010886	*D. labrax*	F: TCACTTGGCAAATTGATGGA R: AGAACAGCGCACTTTGTTGA	

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Figure legends:

Figure 1. Expression of some nutrition-related genes in gilthead seabream (black) and European sea bass (grey) larvae. Results are expressed as mean ± SEM (n=3 pools). Differences are expressed with different capital and lower case letters for gilthead seabream and European sea bass, respectively, and were considered significant when $p<0.05$.

Figure 2. Expression of genes involved in innate immunity in gilthead seabream (black) and European sea bass (grey) larvae. Results are expressed as mean ± SEM (n=3 pools). Differences are expressed with different capital and lower case letters for gilthead seabream and European sea bass, respectively, and were considered significant when $p<0.05$.

Figure 3. Expression of genes involved in adaptive immunity in both gilthead seabream (black) and European sea bass (grey) larvae. Results are expressed as mean ± SEM (n=3 pools). Differences were expressed with different capital and lower case letters for gilthead seabream and European sea bass, respectively, and were considered significant when $p<0.05$.

Figure 1

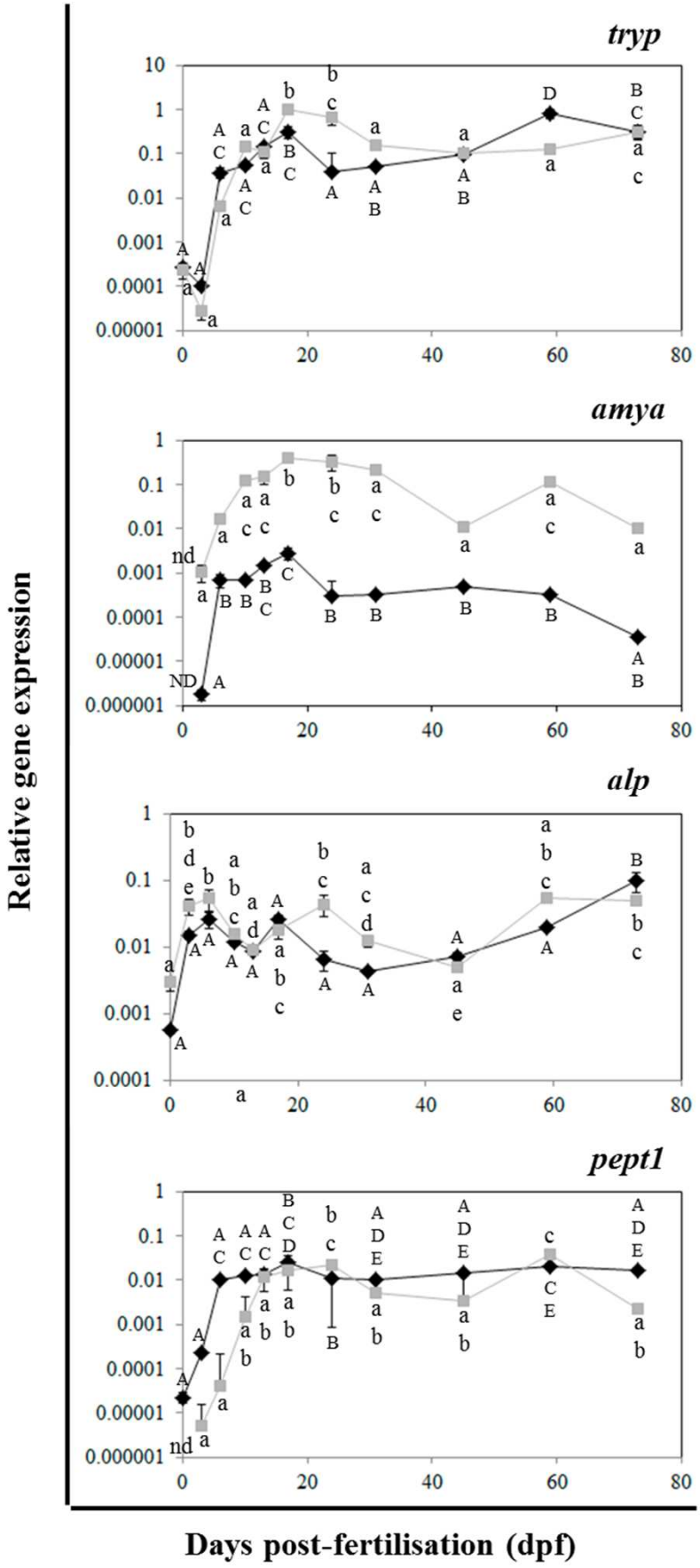

tryp
amya
alp
pept1
Relative gene expression
Days post-fertilisation (dpf)

Figure 2

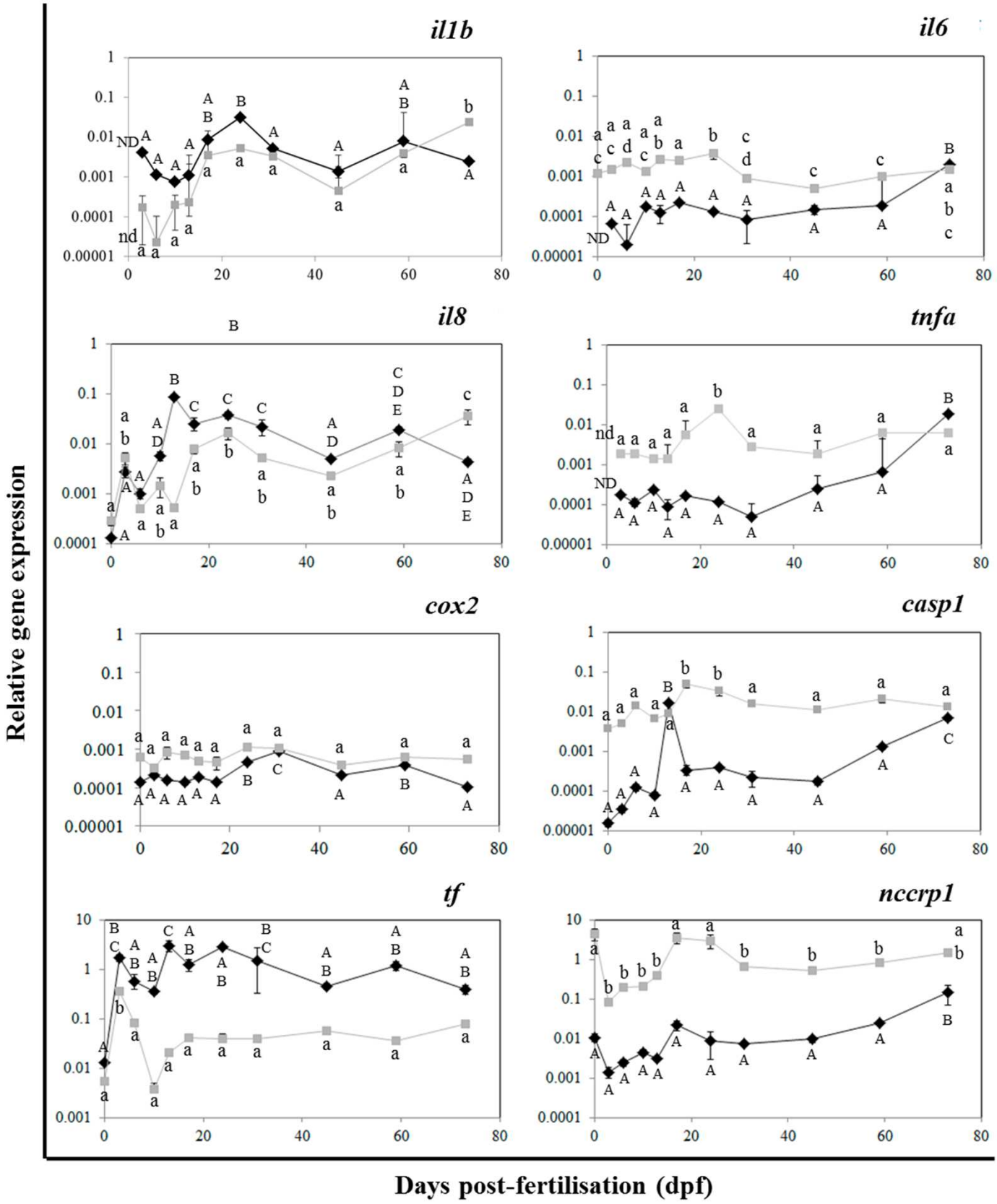
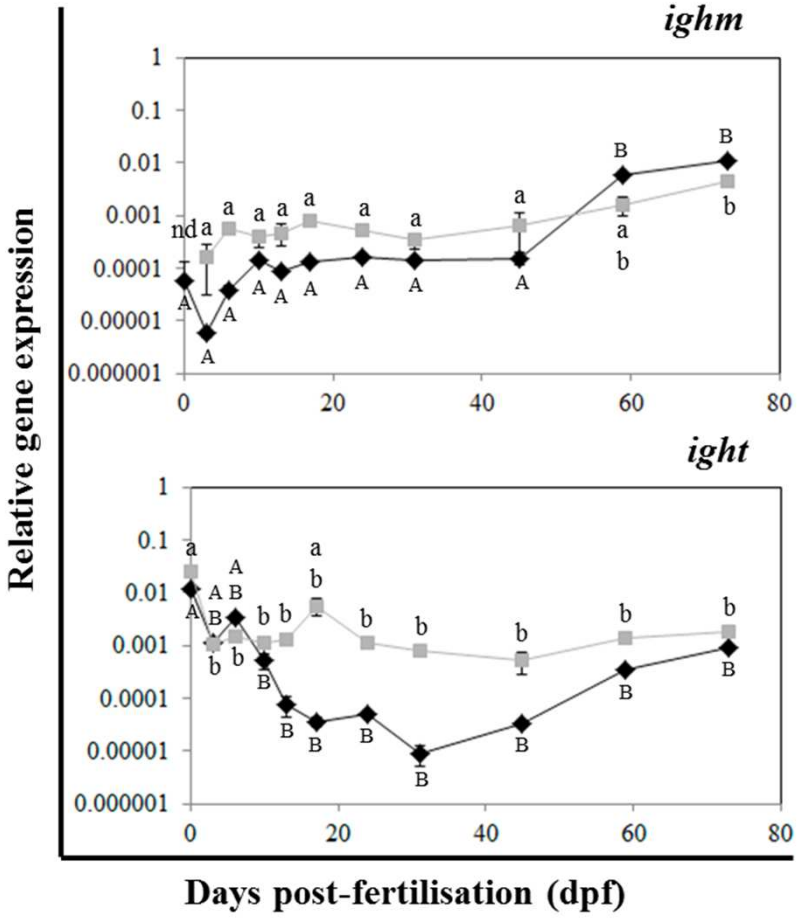

il1b
il6
il8
tnfa
cox2
casp1
tf
nccrp1
Relative gene expression
Days post-fertilisation (dpf)

Figure 3


ighm
ight
Relative gene expression
Days post-fertilisation (dpf)
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0
20
40
60
80

Highlights

-The knowledge of nutrition and immunity is essential to the survival of fish larvae.

-Comparative ontogenetic profile was studied in two farmed fish species by qPCR.

-The digestive enzymes encode by *tryp*, *amya*, *alp*, *pept1* showed similar patterns.

-The immune-related genes were the most variable between both stages and species.