Aquaculture Reports 10 (2018) 1–7

Contents lists available at ScienceDirect

Aquaculture Reports

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

Development of immune functionality in larval and juvenile crimson snapper *Lutjanus erythropterus* (Bloch 1790)

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ARTICLE INFO

Keywords:
Immune system
Enzyme activity
Ontogenetic development
Crimson snapper *Lutjanus erythropterus*

ABSTRACT

Ontogenetic development of the immune system in crimson snapper (*Lutjanus erythropterus* Bloch 1790) larvae was histologically and enzymatically studied from hatch to 36 days post-hatch (DPH). Primitive hepatopancreas appeared on 2 DPH and renal tubules started hematopoiesis on 4 DPH. The spleen anlage appeared on 6 DPH and the thymus formed on 14 DPH. Total activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and sodium-potassium adenosine triphosphatase (Na^+ K^+-ATPase) gradually increased after hatch, and showed a sharp increase after 29 DPH during the transitional feeding period from *Artemia* to inert feed. The specific activities of SOD, CAT, and GPX showed a trend of sharp increase and reached the maximum level on 4 DPH when exogenous feeding started, except for Na^+ K^+-ATPase where the peak occurred on10 DPH. The specific activities of these five enzymes reached the peak during the food transition from rotifers to *Artemia*, but the total activity of enzymes showed an increasing trend as fish grew. The present study provides new knowledge of the development of functional enzymes relevant to fish larvae immunity, sheds light on the understanding of the change of larval health, and improves hatchery management of crimson snapper.

1. Introduction

The immune system is functionally important to defend the invasion of alien pathogens, reduce the chance of infection, and maintain body homeostasis. During the course of ontogenetic development, there exists a trade-off between energy demand for immunity and other physiological functions. In marine fish, the early larval stage represents a critical period for fish growth and survival in aquaculture (Watanabe and Kiron 1994), because larvae are vulnerable and prone to disease infection during this stage. Studies on the development of the immune system of fish larvae have been conducted on a few number of fish species (Padrós and Crespo, 1996; Romano et al., 1997; Watts et al., 2003; Liu et al., 2004; Santamaría et al., 2004; Xiao et al., 2008; Xiao et al., 2013) where the kidney, spleen, and thymus are regarded as the major immune organs. In organ ontogeny, the kidney usually develops earlier than the spleen and thymus. However, the developmental order of these organs is highly variable in fish (Zapata et al., 2006). The timing of lymphoid production is crucial during early development, and potentially determines the survival of fish larvae.

In aerobic organisms, an antioxidant defense system has evolved to protect cells from damage by reactive oxidant species (ROS). The prevention of oxidation is an essential process in all aerobic organisms, as a decrease of antioxidant protection may lead to cytotoxicity, mutagenicity and carcinogenicity (Mates, 2000). Concomitantly, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR) have been found in most fish species (Rudneva, 1997). Together with these enzymes, some lower-molecular-weight antioxidants such as carotenoids, vitamins E, K and C, amino acids, and peptides (glutathione) are also detected in fish tissues (Martinez-Alvarez et al., 2005). Knowledge of antioxidant enzyme expression at the early stage of fish ontogeny is important in understanding the origin and formation of protective mechanisms during early life history of organisms. The activity of antioxidant enzymes such as SOD, CAT, and GPX has been proposed as indicators of immune responses in many species (Fitzgerald 1992; Aceto et al., 1994; Peters and Livingstone 1996; Arun and Subramanian 1998; Mourente et al., 1999; Rudneva 1999; Zielinski and Portner, 2000; Peters et al., 2001; Campa-Córdova et al., 2002; Dandapat et al., 2003).

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https://doi.org/10.1016/j.aqrep.2018.02.001
Received 21 July 2017; Received in revised form 17 February 2018; Accepted 24 February 2018
Available online 07 March 2018
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Thus, histogenesis of immune organs along with ontogeny of antioxidant enzymes should be useful indication for understanding the development of immune system.

The Na^+, K^+ −ATPase is an transporting enzyme which uses adenosine triphosphate (ATP) as energy to transfer K^+ into the cell and Na^+ out of the cell membrane. It plays a key role in maintaining the ionic and osmotic balance in a cell. The Na^+ K^+-ATPase has been widely investigated in euryhaline and marine teleost (Foskett and Scheffey 1982; de Renzis and Bornancin 1984; Zadunaisky 1984). The Na^+ K^+-ATPase is expressed at high levels in the mitochondria-rich cells. These cells are involved in salt secretion in seawater and ion uptake in freshwater organisms (Foskett and Scheffey 1982; Zadunaisky 1984; Avella and Bornancin 1990; Marshall 1995; Uchida and Kaneko 1996; Hiroi et al., 1998).

The crimson snapper *Lutjanus erythropterus* (Bloch 1790) is an economically important species in South China Sea, but its population has declined due to overfishing (Guo et al., 2007). Because of its high market price and flesh quality, it has become one of the most economically important species for aquaculture in China. However, the poor rearing technology of crimson snapper larvae is still a major bottleneck hindering this aquaculture because of high mortality during the early life stage. The reason for low survival is unknown as there is a lack of information on the ontogenetic development of crimson snapper larvae. Therefore, studies of the immune system in larval and juvenile crimson snapper would provide new knowledge in fish physiology and benefit aquaculture of crimson snapper. In this study, the ontogenetic development of some enzymes relevant to fish immunity and the developmental changes in the histological structure in crimson snapper larvae were examined, aiming to understand the immune system ontogeny in crimson snapper larvae.

2. Materials and methods

The experimental materials in this study was obtained from an early study on the ontogenetic development of the digestive enzymes of this species (Cui et al., 2017).

Fertilized eggs of crimson snapper were obtained from Shenzhen Longqizhuang Aquaculture Hatchery (Guangdong Province, P.R. China), and were transported to Shenzhen R&D Station of South China Sea Fisheries Research Institute and hatched in 500-L fiberglass incubators at 27.5 °C with a hatching rate of 93.5 ± 1.9%. On 2 days post hatching (DPH), larvae were stocked into three 2500-L larval rearing tanks at a density of 60 fish L^{-1}. Rearing tanks were supplied with filtered seawater through 5-μm pores from the bottom with a daily exchange rate of 200% tank volume. The experimental condition, rearing protocol, fish sampling, and growth measurements were described by Cui et al. (2017). During the experimental period, water temperature was controlled at 27.5 ± 1 °C. The water quality was maintained at ammonia and nitrite < 0.06 mg L^{-1}, salinity 34‰, and pH 8.0.

2.1. Histological analysis procedures

On each sampling day, 20 fish larvae from each rearing tanks were fixed in 10% neutrally buffered formalin after anesthesia in 5% AQUI-S (AQUI-S New Zealand, Lower Hutt, New Zealand). Each fixed fish was individually embedded in a paraffin block and sectioned in serial sagittal cuts (5 μm thick) using a Leica RM 2135 rotary microtome (Nussloch, Germany). The hematoxylin–eosin (HE) stain was used for general histological observations. The slide with sections was mounted permanently using DePex (Shanghai, China). The sections of five fish were randomly examined under a light microscope. Photographs were taken with the Olympus digital camera attached to the microscope (Shanghai, China).

2.2. Enzymatic assay

On each sampling day, the fish specimens were thoroughly rinsed in distilled water to remove external salt and then immediately stored in liquid nitrogen. The whole fish was homogenized for enzymatic assays. For each assay, a pooled sample of 5–100 frozen fish was thawed, weighed and homogenized using a glass homogenizer on ice in 0.2 M NaCl (w/v). The suspensions were centrifuged at 13 300*g* for 10 min at 2 °C. Then, the supernatant was incubated in the enzyme substrate under 25 °C and read on a spectrophotometer (SpectraMax M5, USA) at the target wavelength. All the measurements were carried out in triplicate.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured using a SOD activity colorimetric assay kit (Catalogue number: A001-1, Nanjing Jiancheng Bioengineering Institute, P.R. China). In the assay, SOD combined with free radicals to form nitrite. The formed nitrite reacted with the chromogenic agent to generate purple color which was read at 550 nm on a spectrophotometer.

Catalase (CAT, EC 1.11.1.6) activity was measured using a CAT activity colorimetric assay kit (Catalogue number: A007-1, Nanjing Jiancheng Bioengineering Institute, P.R. China). In the assay, the reaction of CAT cleaving with substrates could be stopped with ammonium molybdate, and the remaining substrates would react with ammonium molybdate by releasing chromophore, and the released chromophore was measured at 405 nm.

The activity of sodium-potassium adenosine triphosphatase (Na^+K^+-ATPase, EC 3.6.3.9) was measured using a Na^+K^+-ATPase activity colorimetric assay kit (Catalogue number: A070-2, Nanjing Jiancheng Bioengineering Institute, P.R. China). In the assay, Na^+K^+-ATPase was cleaved into ADP and inorganic phosphorus, and the amount of inorganic phosphorus was measured at 636 nm.

Glutathione peroxidase (GPX) activity was qualified using a GPX colorimetric assay kit (Catalogue No. A005, Nanjing Jiancheng Bioengineering Institute, P.R. China).

The total enzyme activity was expressed as milli-units per larval fish or units per larval fish (mU $larva^{-1}$ or U $larva^{-1}$). The specific activity was expressed as units per milligram of protein (U mg^{-1} protein). Soluble protein of crude enzyme extracts was quantified using the bicinchoninic acid protein assay kit (Catalogue No. BCA1 & B9643; Sigma-Aldrich, St. Louis, MO, USA).

2.3. Statistical analysis

The measurement of each variable was presented as the mean of pooled fish larvae ranging from 5 to 200 individuals on a sampling day with three replicates. Mean values of both total activities and specific activities of each digestive enzyme between sampling dates were compared with one-way ANOVA (PASW Statistics 18.0; Chicago, IL, USA). When a significant difference was found, Tukey's test was performed for multiple range comparisons with the level of significance set at $P < 0.05$. All the data were tested for normality, homogeneity and independence before ANOVA. When the homogeneity of variances was violated, a nonparametric test (the Welch test) was performed.

3. Results

3.1. Morphometric changes and fish growth

The standard length of crimson snapper larvae and juveniles during the experimental period is shown in Fig. 1. The growth of larvae was continuously recorded from the hatch to the end of the experiment at 36 DPH.

3.2. Development of the immune system

On 2 DPH, crimson snapper larvae carried a large yolk sac that

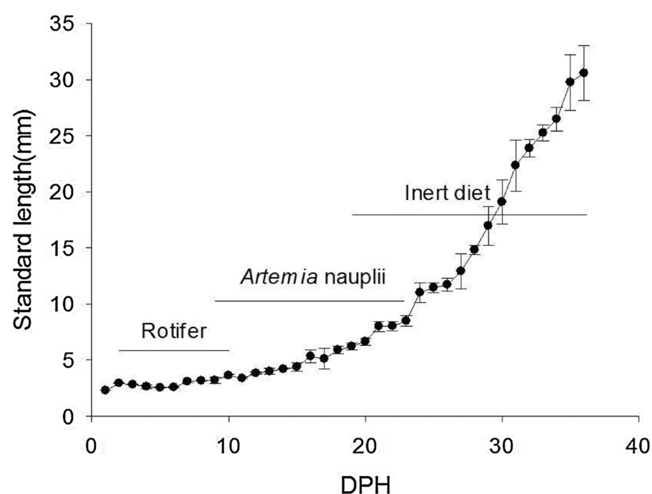

Fig. 1. Standard length (Mean ± SD) of crimson snapper *Lutjanus erythropterus* larvae fed different diets over time.

occupied most of the abdominal cavity, and the digestive tract was a straight and undifferentiated tube attached dorsally to the yolk sac (Fig. 2a). From 2–3 DPH, the incipient liver and pancreas appeared. The renal tubules were clearly visible on 4 DPH (Fig. 2b), and the inner wall of the tube possessed eosinophilic secretory cells. At the same time, the development of anterior kidney was primitive, containing undifferentiated stem cells whose cytoplasm was lightly basophilic. The spleen anlage was first seen at 6 DPH, and was surrounded by the pancreas. The interior of spleen anlage had many tiny capillaries (Fig. 2c). On 8 DPH, as the development of the anterior kidney proceeded, the number of lymphocytes between renal tubules increased significantly (Fig. 2d). On 10 DPH, the spleen continually increased in size and the margin of the spleen became obvious (Fig. 2e). On 12 DPH, the lightly stained hematopoietic stem cells were aggregated on the outside of renal tubular, and the number of renal tubules began to decrease (Fig. 2f). On 14 DPH, the basophilic kidney cells began to increase, and the number of renal tubules further decreased (Fig. 2g). Meanwhile, the thymus appeared in the position of the third and fourth gill arch, and located on the back of the gill cavity. The thymus was in a long rod shape, and uniformly stained with blue color. On 16 DPH, the cross-section of spleen became heart-shaped, and the size of spleen further increased (Fig. 2h). The morphological development of lymphoid organs of the anterior kidney was complete between 19 and 22 DPH (Fig. 2i and j), but the migration of lymphocytes between anterior kidney and thymus was not observed. The large vessels appeared on the outside of the spleen, and reticular cells and fibrous tissues distributed inside the spleen.

3.3. SOD activity

The total activity of SOD was detected on one DPH at 3.34 ± 0.59 U larva^{-1} and remained low until 10 DPH at 24.15 ± 8.81 U larva^{-1} (Fig. 3, $P > 0.05$). Then the total activity of SOD began to show a slight increase on 23 DPH at 166.62 ± 2.27 U larva^{-1}, and remained at a similar level until 29 DPH ($P > 0.05$). Thereafter, the total activity of SOD sharply increased from 140.09 ± 13.41 U larva^{-1} on 29 DPH to 588.92 ± 45.52 U larva^{-1} on 33DPH ($P < 0.05$) and remained at a similar level until the end of the experiment.

The specific activity of SOD was 1.51 ± 0.11 U mg^{-1} protein at hatch, and then increased rapidly and reached the maximum (3.64 ± 0.19 U mg^{-1} protein) on 4 DPH ($P < 0.05$). Subsequently, the specific activity of SOD decreased rapidly to 1.25 ± 0.10 U mg^{-1} protein on 6 DPH ($P < 0.05$), and remained at a relatively stable level until 16 DPH ($P > 0.05$). Then the specific activity of SOD slightly decreased to 0.25 ± 0.05 U mg^{-1} protein on 36 DPH (Fig. 3).

3.4. CAT activity

The total activity of CAT was 3.34 ± 0.57 U larva^{-1} on one DPH and remained at a low level until 10 DPH (15.51 ± 2.39 U larva^{-1}, Fig. 4). Then it slightly increased to 112.29 ± 8.90 U larva on 23 DPH ($P < 0.05$) and remained at the same level until 29 DPH (84.44 ± 7.21 U larva^{-1}) ($P > 0.05$). Thereafter, the total activity of CAT rapidly increased to 371.63 ± 65.72 U larva^{-1} on 36 DPH ($P < 0.05$).

The specific activity of CAT in the larvae and juveniles was 1.02 ± 0.17 U mg^{-1} protein at hatch, and increased rapidly to 4.44 ± 1.19 U mg^{-1} protein on 2 DPH ($P < 0.05$). Then, it decreased to 0.91 ± 0.22 U mg^{-1} protein on 6 DPH ($P < 0.05$). Subsequently, the specific activity of CAT gradually decreased to 0.41 ± 0.06 U mg^{-1} protein on 26 DPH ($P < 0.05$). On 29 DPH, the specific activity of CAT decreased to the minimum (0.12 ± 0.10 U mg^{-1} protein, $P < 0.05$), and remained at a low level until the end of the experiment.

3.5. GPX activity

The development pattern of the total activity of GPX was similar to that of SOD (Fig. 5). The total activity of GPX was 3.57 ± 0.24 U larva^{-1} on one DPH, and remained at a relative low level before 10 DPH (16.04 ± 1.04 U larva^{-1}, $P > 0.05$). On 12 DPH, the total activity of GPX began to slightly increase from 34.01 ± 2.90 U larva^{-1} to 163.29 ± 3.24 U larva^{-1} on 26 DPH. From 29 DPH, the total activity of GPX sharply increased from 144.50 ± 2.73 U larva^{-1} to 345.62 ± 13.93 U larva^{-1} on 34 DPH ($P < 0.05$).

The specific activity of GPX was 1.08 ± 0.08 U mg^{-1} protein at hatch, and rapidly increased to the maximum level of 3.13 ± 0.36 U mg^{-1} protein on 4 DPH ($P < 0.05$). Then it sharply decreased to 1.78 ± 0.41 U mg^{-1} protein on 5 DPH, which was similar to the initial level ($P > 0.05$). After 5 DPH, the specific activity of GPX gradually decreased, but remained at a similar level with the initial specific activity of GPX until 23 DPH ($P > 0.05$). Finally, it reached the minimum of 0.26 ± 0.01 U mg^{-1} protein on 36 DPH.

3.6. Na^+K^+-ATPase activity

The total activity of Na^+K^+-ATPase was relative low from one DPH (2.03 ± 0.51 U larva^{-1}) to 8 DPH (3.93 ± 0.28 U larva^{-1}) (Fig. 6, $P > 0.05$). Then it began to increase, and the total activity of Na^+K^+-ATPase reached 77.44 ± 18.92 U larva^{-1} on 10 DPH. After 10 DPH, the total activity of Na^+K^+-ATPase slightly fell back. Then, it recovered to 82.21 ± 27.22 U larva^{-1} on 26 DPH, which was similar to the level on 10 DPH ($P > 0.05$). From 29 DPH, the total activity of Na^+K^+-ATPase sharply increased from 47.37 ± 11.24 U larva^{-1} to 550.30 ± 38.30 U larva^{-1}.

The specific activity of Na^+K^+-ATPase in larvae and juveniles was 0.45 ± 0.33 U mg^{-1} protein on one DPH. From 2 DPH to 3 DPH, it rapidly increased to 2.63 ± 0.43 DPH ($P < 0.05$), and then sharply decreased to 0.54 ± 0.04 U mg^{-1} protein on 8 DPH, which was similar to the initial hatching level ($P > 0.05$). The specific activity of Na^+K^+-ATPase reached the peak level (5.58 ± 1.48 U mg^{-1} protein) on 10 DPH, and then sharply decreased to the initial hatching level. From 12 DPH to the end of the experiment, the specific activity of Na^+K^+-ATPase remained at a stable level similar to the level of newly hatched larvae ($P > 0.05$).

4. Discussion

Before the immune system is fully developed, fish mainly rely on non-specific immunity including cellular immunity and humoral immunity (Uribe et al., 2011). In this study, the immune organs of crimson

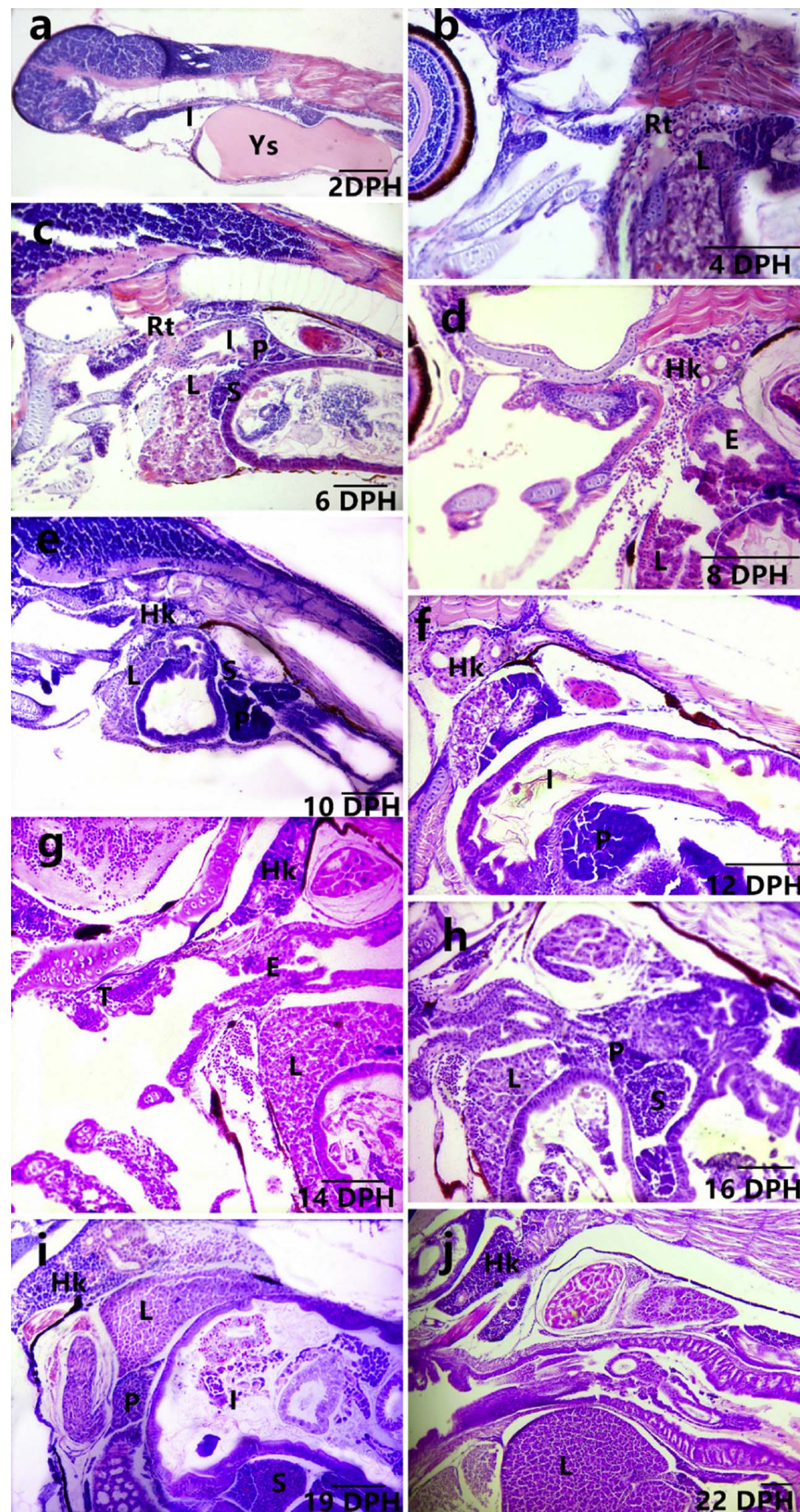

Fig. 2. Morphological observations and sagittal sections of the crimson snapper immune organs (E, esophagus; Hk, anterior kidney; I, intestine; L, liver; P, pancrease; Rt, renal tubule; S, spleen; T, thymus; Ys, yolk sac).

snapper matured after rapid development in the larvae and juvenile stages, and the developmental order in the early stage was anterior kidney, spleen, and thymus.

On 4 DPH, the anterior kidney contained some undifferentiated cells which could be the hematopoietic stem cells indicating that the anterior kidney began to perform hematopoietic function at this time. This is similar to the orange-spotted grouper *Epinephelus coioides* (Wu and Lin 2003). Then on 12 DPH, the renal tubular began to gradually decrease, indicating that the anterior kidney part is no longer the main organ of urination, which is similar to the golden pompano *Trachinotus ovatus* (Cai et al., 2012). The spleen primordia formed on 6 DPH, and significantly increased in size at 10 DPH, indicating that the spleen has

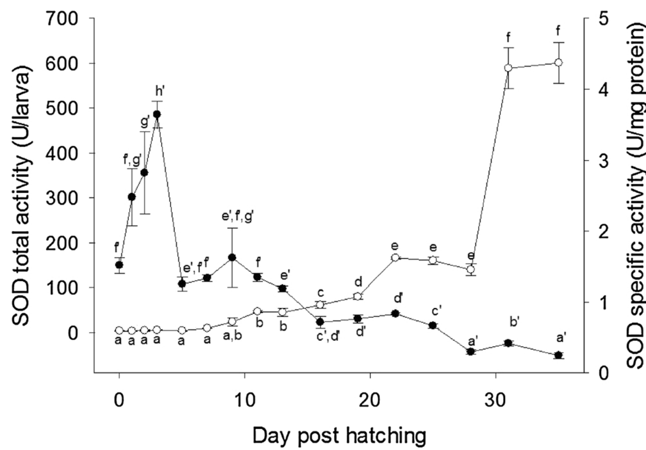

Fig. 3. The total activity of SOD activity in U larva^{-1} (dark circle) and the specific activity of amylase in U mg^{-1} protein (blank circle) from 1 to 36 days post-hatch (DPH). Mean ± SD (n = 3) with the same superscript letter is not significantly different between time point comparisons ($P < 0.05$).

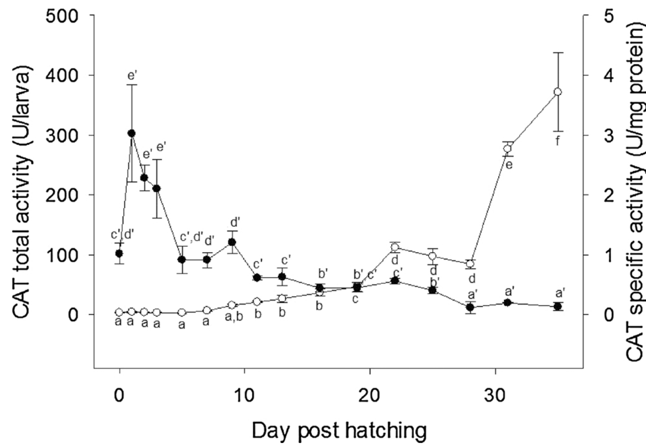

Fig. 4. The total activity of CAT activity in U larva^{-1} (dark circle) and the specific activity of amylase in U mg^{-1} protein (blank circle) from 1 to 36 days post-hatch (DPH). Mean ± SD (n = 3) with the same superscript letter is not significantly different between time points comparisons ($P < 0.05$).

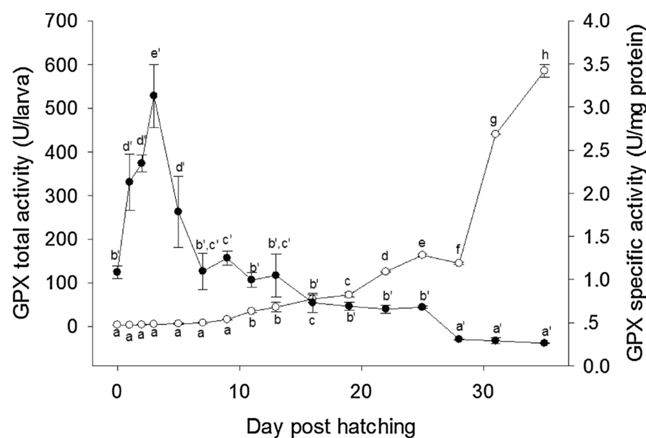

Fig. 5. The total activity of GPX activity in U larva^{-1} (dark circle) and the specific activity of amylase in U mg^{-1} protein (blank circle) from 1 to 36 days post-hatch (DPH). Mean ± SD (n = 3) with the same superscript letter is not significantly different between time point comparisons ($P < 0.05$).

performed hematopoietic function. On 22 DPH, large blood vessels appeared near the spleen and a large number of cells entered the spleen, indicating that the spleen began to participate in blood circulation.

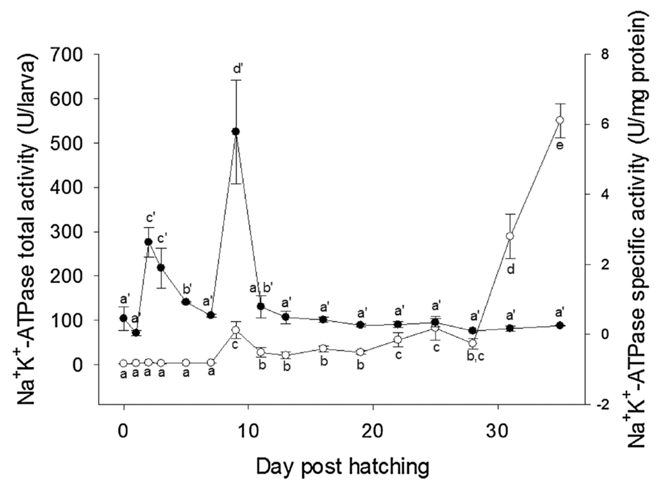

Fig. 6. The total activity of Na^+ K^+-ATPase activity in U larva^{-1} (black circle) and the specific activity of amylase in U mg^{-1} protein (clear circle) from 1 to 36 days post-hatch (DPH). Mean ± SD (n = 3) with the same superscript letter is not significantly different in multiple comparisons ($P < 0.05$).

Hematopoietic stem cells were found in the anterior kidney of newly hatched turbot *Scophthalmus maximus* larvae, suggesting that the same stem cells migrate to the immature thymus during the early development of anterior kidney (Padros and Crespo 1996). A lymphocytic “bridge” was found between the anterior kidney and thymus at the early developmental stage of teleost fishes such as yellow croaker *Pseudosciaena croce* and golden snapper *Sparus aurata*, suggesting that there is cell migration between the anterior kidney and thymus (Xu et al., 2007; Josefsson and Mary 1993). Tatner (1985) labeled 6-month-old thymocytes with isotope tritium, and found that the thymocytes migrated into peripheral lymphoid organs such as spleen and kidney, but it is not clear whether cell migration and development of immune organs occurred at this stage. In this study, the sections of early development of crimson snapper were continuously observed. The results showed that there was a separate reticular tissue and muscle tissue between thymus and anterior kidney. The migration of lymphocyte cells between thymus and anterior kidney was not found, which is similar to the orange-spotted grouper *Epinephelus coioides* (Wu and Lin 2003). The continual section observations of crimson snapper did not show disappearance of the thymus at the early developmental stages, which has been found in the flounder *Paralichthys olivaceus* (Liu et al., 2004)

Oxygen is necessary for aerobic organisms, but the peroxidation products such as superoxide radical, hydrogen peroxide and hydroxyl radical are highly destructive to aerobic organisms (Ahmad 1995). These can oxidize proteins, DNA and lipids and destroy cell structures, resulting in more vicious cycles of free radicals (Sies, 1986). To counteract the toxicity caused by peroxide, all living aerobic organisms have evolved with an antioxidant system. Previous research has shown that the antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR) exist in the body of most fishes (Rudneva 1997). Some molecules with antioxidant effect such as carotenoids, vitamin C, vitamin E, vitamin K, amino acids and peptides have been also found in most fish together with antioxidant enzymes (Martinez-Alvarez et al., 2005; Bragadottir 2001).

According to the free radical theory, with the attack of free radicals against antioxidant defense system, the resilience of antioxidant defense system decreases with age (Harman 1956). However, little is known about the link between the aging of fish and degradation of antioxidant systems. In the present study, the SOD and CAT activities in erythrocytes in the liver of larvae and juvenile fish were much higher than those in older fish (Wdzieczak et al., 1982). Antioxidant enzyme

activities are also related to feeding patterns and nutrient supply. GPX and CAT activities in herbivorous fish are lower than those in carnivorous fish at the same age (Radi et al., 1987). Some studies have shown that the increase of lipids and vitamins in the diet has impacted the antioxidant defense system (Mourente 2002; Rueda-Jasso 2004). In these reports, the addition of lipids and vitamins has a positive effect on CAT and SOD activities in the liver of fish. The environment also has a significant effect on antioxidant enzyme activities. For instance, strong light can increase the SOD activity in the blood to prevent the damage from ultraviolet rays (Fitzgerald 1992). Temperature has an impact on survival, fecundity and growth of fish (Dippner 1997; Klyashtorin 1997; O'Brien and Fox 2000). Similarly, changes in temperature can affect the dissolved oxygen in the environment, which affects the formation of free radicals and antioxidant enzyme activities (Portner 2002). It has been reported that the level of SOD can regulate the internal environment disorders caused by temperature changes (Filho and Giulivi 1993). Environmental pollution can force fish to enhance immunity (Rodriguez-Ariza et al., 1993). For example, the CAT activity significantly increased in channel catfish and trout when the water was polluted by suspended matter (Ahmad and Hamid 2000; Santos et al., 2004). The SOD, CAT and GPX activities were significantly increased in freshwater catfish and eel in the water polluted by paper mills. Parasitic infections also affect the response of the antioxidant immune system (Dautremepuits et al., 2003). In the present study, SOD, CAT and GPX activities reached the maximum on 4 DPH, indicating that the water environmental stress may be most serious at this time. On 4 DPH, the yolk sac was exhausted, and the larvae required exogenous nutrition. The total enzyme activity from 1 to 36 DPH showed an increasing trend, indicating that the immune system of larvae and juvenile crimson snapper is gradually improved. The significant increase of Na^+K^+-ATPase activity on 10 DPH was probably due to the intake of *Artemia* nauplii and the removal of *Chlorella vulgaris* from the tanks, but the specific reasons still need further study.

Although the crimson snapper has characteristics of strong resistance to disease, the deformity and mortality of larvae are still critical issues in aquaculture. In this study, the histological changes were related to the development of immune function over time as evidenced by the concomitant dynamics of enzyme activities. This study provides baseline information to study the development of immune function during the early life of crimson snapper, which sheds light on future research to improve hatchery technology and seedling production for larval fish rearing.

Acknowledgments

This project was funded by Nature Science Foundation of Hainan Province (317289), and Special Scientific Research Funds for Central Non-profit Institutes, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (2014YJ01).

References

Aceto, A., Amicarelli, F., Sacchetta, P., Dragani, B., Bucciarelli, T., Masciocco, L., Miranda, M., Di llio, C., 1994. Developmental aspects of detoxifying enzymes in fish (*Salmo iridaeus*). Free Radic. Res. 21, 285–294.
Ahmad, I., Hamid, T., 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochim. Biophys. Acta 1523, 37–48.
Ahmad, S., 1995. Preface. Oxidative Stress and Antioxidant Defenses in Biology. Chapman & Hall, NY xi–xvii.
Arun, S., Subramanian, P., 1998. Antioxidant enzymes in freshwater prawn Macrobrachium malcolmsonii during embryonic and larval development. Comp. Biochem. Physiol. B 121, 273–277.
Avella, M., Bornancin, M., 1990. Ion fluxes in the gills of freshwater and seawater salmonid fish. Anim. Nutr. Trans. Proces. 1–13.
Bragadottir, M., 2001. Endogenous Antioxidants in Fish. Master Degree Thesis, Department of Food Science. University of Iceland.
Cai, W., Ou, Y., Jia'er, L.I., Sun, P., 2012. Development of immune organs at early stages of *Trachinotus ovatus*. South China Fish Sci. 8 (5), 39–45.
Campa-Córdova, A.I., Hernández-Saavedra, N.Y., Ascencio, F., 2002. Superoxide dismutase as modulator of immune function in American white shrimp (*Litopenaeus vannamei*). Comp. Biochem. Physiol. C 133 (4), 557–565.
Cui, K., Cheng, D., Ma, Z., Qin, J.G., Jiang, S., Sun, D., Ma, S., 2017. Ontogenetic development of digestive enzymes in larval and juvenile crimson snapper *Lutjanus erythopterus* (Bloch 1790). Aquacult. Res. http://dx.doi.org/10.1111/are13278. online first stage.
Dandapat, J., Chainy, G.B.N., Rao, K.J., 2003. Lipid peroxidation and antioxidant defence status during larval development and metamorphosis of giant prawn, *Macrobrachium rosenbergii*. Comp. Biochem. Physiol. C 135, 221–233.
Dautremepuits, C., Betoulle, S., Vernet, G., 2003. Stimulation of antioxidant enzymes levels in carp (*Cyprinus carpio*) infected by *Ptychobothryum* sp. (Cestoda). Fish Shellfish Immunol. 15, 467–471.
de Renzis, G., Bornancin, M., 1984. Ion transport and gill ATPase. In: In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology Vol. XB. Academic Press, New York, pp. 65–104.
Dippner, J.W., 1997. Recruitment success in different fish stocks in the North Sea in relation to climate variability. Deutsche Hydrogr. Zeitschr. 49, 277–293.
Filho, D.W., Giulivi, C., 1993. Antioxidant defences in marine fish-I. Teleosts. Comp. Biochem. Physiol. C 106, 409–413.
Fitzgerald, J.P., 1992. Comparative analysis of superoxide dismutase activitiew in a range of temperate and tropical teleost fish. Comp. Biochem. Physiol. B 101 (1–2), 111–114.
Foskett, J.K., Scheffey, C., 1982. The chloride cell: definitive identification as the salt-secretory cell in teleosts. Science 215, 164–166.
Guo, Y.S., Wang, Z.D., Liu, C.W., Liu, L., Liu, Y., 2007. Phylogenetic relationships of south China sea snappers (Genus *Lutjanus*; family lutjanidae) based on mitochondrial DNA sequences. Mar. Biotechnol. 9 (6), 682–688.
Harman, D.A., 1956. Theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.
Hiroi, J., Kaneko, T., Seikai, T., 1998. Developmental sequence of chloride cells in the body skin and gills of Japanese flounder (*Paralichthys olivaceous*) larvae. Zool. Sci. 15, 455–460.
Josefsson, S., Mary, F.T., 1993. Histogenesis of the lymphoid organs in sea bream (*Sparus aurata* L.). Fish Shellfish Immunol. 3 (1), 35–49.
Klyashtorin, L., 1997. Pacific Salmon: Climate-linked Long-term Stocks Fluctuations, vol. 5. PICES Press, pp. 2–34.
Liu, Y., Zhang, S.C., Jiang, G.L., Yang, D., Lian, J., Yang, Y., 2004. The development of the lymphoid organs of flounder, *Paralichthys olivaceus*, from hatching to 13 months. Fish Shellfish Immunol. 16 (5), 621–632.
Marshall, W.S., 1995. Transport processes in isolated teleost epithelia: opercular epithelium and urinary bladder. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Cellular and Molecular Approaches to Fish Ionic Regulation. Academic Press, New York, pp. 1–23.
Martinez-Alvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish. 15 (1), 75–88.
Mate, J.M., 2000. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153, 83–104.
Mourente, G., Tocher, D.R., Diaz, E., et al., 1999. Relationship between antioxidants, antioxidant enzyme activities and lipid peroxidation products during early development in *Dentex dentex* eggs and larvae. Aquaculture 179, 309–324.
Mourente, G., 2002. Increased activities of hepatic antioxidant defense enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidized oil: attenuation by dietary vitamin E. Aquaculture 214, 343–361.
O'Brien, C.M., Fox, C.J., 2000. Climate variability and north sea cod. Nature 404, 142.
Padrós, F., Crespo, S., 1996. Ontogeny of the lymphoid organs in the turbot *Scophthalmus maximus*: a light and electron microscope study. Aquaculture 144, 1–16.
Peters, L.D., Livingstone, D.R., 1996. Antioxidant enzyme activities in embryologic and early larval stages of turbot. J. Fish Biol. 49, 986–997.
Peters, L.D., Porte, C., Livingstone, D.R., 2001. Variation of antioxidant enzyme activities in sprat (*Sprattus sprattus*) larvae in mixed zooplankton from the southern North Sea. Mar. Pollut. Bull. 42, 1087–1095.
Portner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A 132, 739–761.
Radi, A.A.R., Matkovics, B., Csengeri, I., 1987. Comparative studies of the phospholipid fatty acids and the antioxidant enzyme activities in fish with different feeding habits. Comp. Biochem. Physiol. B 87, 49–54.
Rodriguez-Ariza, A., Peinado, J., Pueyo, C., 1993. Biochemical indicators of oxidative stress in fish from polluted littoral areas. Can. J. Fish. Aquat. Sci. 50, 2568–2573.
Romano, N., Baldassini, M.R., Terribili, F.R., et al., 1997. Histological observations on lymphomyeloid organs of the Antarctic fish *Trematomus bernacchii* (Teleostei: notothеniidae). Polar Biol. 18 (5), 358–362.
Rudneva, I.I., 1997. Blood antioxidant system of Black Sea elasmobranch and teleost. Comp. Biochem. Physiol. C 118, 255–260.
Rudneva, I.I., 1999. Antioxidant system of Black Sea animals in early development. Comp. Biochem. Physiol. C 122, 265–271.
Rueda-Jasso, R., 2004. Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles. Aquaculture 231, 417–433.
Santamaría, C.A., Marín de Mateo, M., Traveset, R., Sala, R., Grau, A., Pastor, E., Sarasquete, C., Crespo, S., 2004. Larval organogenesis in common dentex: *Dentex dentex* L. (Sparidae): histological and histochemical aspects. Aquaculture 237, 207–228.
Santos, M.A., Pacheco, M., Ahmad, I., 2004. *Anguilla anguilla* L: antioxidants responses to in situ bleached kraft pulp mill effluent outlet exposure. Environ. Int. 30, 301–308.
Sies, H., 1986. Biochemistry of oxidative stress. Ang Chem-Int. Ed. 25, 1058–1071.
Tatner, M.F., 1985. The migration of labeled thymocytes to the peripheral lymphoid organs in the rainbow trout (*Sparus aurata* L.). Fish Shellfish Immunol. 3 (1), 35–49.

Uchida, K., Kaneko, T., 1996. Enhanced chloride cell turn over in the gills of chum salmon fry in seawater. Zool. Sci. 13, 655–660.

Uribe, C., Folch, H., Enriquez, R., Moran, G., 2011. Innate and adaptive immunity in teleost fish: a review. Vet. Med. 56 (10), 486–503.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124 (S1–4), 223–251.

Watts, M., Kato, K., Munday, B.L., Burke, C.M., 2003. Ontogeny of immune system organs in northern bluefin tuna (*Thunnus orientalis*, Temmick and Schlegel 1844). Aquac. Res. 34, 13–21.

Wdzieczak, J., Zalesna, G., Wujec, E., 1982. Comparative studies on superoxide dismutase, catalase, and peroxidase levels in erythrocytes and livers of different freshwater and marine fish species. Comp. Biochem. Physiol. B 73, 361–365.

Wu, J.Y., Lin, H.R., 2003. Ontogeny of lymphoid organs in the orange-spotted grouper (*Epinephelus coioides*). Acta Zool. Sin. 49 (6), 819–828.

Xiao, Z.Z., Yu, D.D., Sun, Z.Z., Xu, S.H., et al., 2008. Ontogeny of immune organs during early development stage of barfin flounder (*Verasper moseri*). Mar. Sci. 32 (7), 88–92.

Xiao, Z.Z., He, T., Li, J., Gao, T.X., 2013. Ontogeny of the immune system in rock bream *Oplegnathus fasciatus*. Chin. J. Oceanol. Limnol. 31 (5), 1028–1035.

Xu, X.J., Weng, C.H., Wang, J., Sun, Y., 2007. Ontogeny of immune organs during early development stage of *Pseudosciaena crocea*. Acta Oceanol. Sin. 29 (3), 105–113.

Zadunaisky, J.A., 1984. The chloride cell: the active transport of chloride and the paracellular pathways. In: In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology Vol. XB. Academic Press, New York, pp. 129–176.

Zapata, A., Diez, B., Cejalvo, T., Gutierrez-de Frias, C., Cortes, A., 2006. Ontogeny of the immune system of fish. Fish Shellfish Immunol. 18, 126–136.

Zielinski, S., Portner, H.O., 2000. Oxidative stress and antioxidative defense in cephalopods: a function of metabolic rate or age. Comp. Biochem. Physiol. B 125, 147–160.