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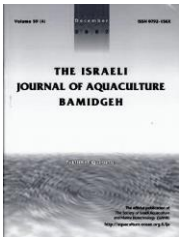
ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

The Israeli Journal of Aquaculture - Bamidgeh, IJA_71.2019.1614, 9 pages



Comparative Analysis of Immune Function of Three Wild Mandarin Fish Populations in Yuanjiang River Basin

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Keywords: *Siniperca chuatsi*; IgM; immune function; lysozyme activity

Abstract

Mandarin fish *Siniperca chuatsi* (Basilewsky) is a unique species in East Asia and has very important ecological and economic value. There are significant differences in immune function between different populations of Mandarin fish. However, up to now, the comparative study of immune indices of different populations of wild mandarin fish has been scarce. In the present study, the transcription level of Immunoglobulin M (IgM) and blood serum immunologically related enzyme activity of three wild Mandarin fish populations from Wuqiangxi reservoir, Lingjintan reservoir, and Taoyuan reservoir of Yuanjiang river basin were detected. In head kidney, spleen and in the liver of Mandarin fish, the transcription level of IgM was highest in Taoyuan reservoir followed by Lingjintan reservoir, and was lowest in Wuqiangxi reservoir. The catalase activity of Mandarin fish was highest in Taoyuan reservoir followed by Wuqiangxi reservoir, and was lowest in Lingjintan reservoir. Total number of white blood cells, lysozyme activity, acid phosphatase activity, superoxide dismutase (SOD) activity, and alkaline phosphatase activity of Mandarin fish were in line with immunoglobulin M gene expression, which were highest in Taoyuan reservoir followed by Lingjintan reservoir, and lowest in Wuqiangxi reservoir. This indicated that the immune function of Mandarin fish from Taoyuan reservoir was strongest, and these fish had the greatest resistance to harmful invaders such as parasite, bacteria, and virus compared to Lingjintan reservoir group and Wuqiangxi reservoir group. The results of this study provide a theoretical basis for disease-resistant breeding and germplasm optimization in mandarin fish breeding production, and may be valuable in future research.

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Introduction

Mandarin fish *Siniperca chuatsi* (Basilewsky), a world-famous freshwater fish, is a unique species in East Asia and has very important ecological and economic value. It is mainly distributed in China, Korean Peninsula, Japan and other places (Hu et al., 2018). Since the success of artificial reproduction, fish farms often pay attention only to reproductive performance during reproduction, ignoring other traits of mandarin fish which could be of economic importance. Long-term artificial breeding has led to serious degeneration of *Siniperca chuatsi* germplasm, especially the decline of disease resistance and the increase of morbidity and mortality.

As in higher vertebrates, fish rely on both specific and non-specific mechanisms to protect themselves against invading pathogens (Xia et al., 2017). In fish, the primary lines of non-specific defense are the skin and mucus (Kumar et al., 2013). As a first line of defense, various peptides/proteins such as lysozymes, antibodies, complement factors and other lytic factors are present in serum where they prevent colonization of microorganisms, leading to prevention of infection and disease (Misra et al., 2006; Alexander and Ingram, 1992; Das et al., 2009). Immunoglobulin M (IgM) is the first antibody made by the body, and plays a crucial role in the first line of defense in the specific immune system (Wang et al., 2006; Ellis, 2001). IgM is mainly produced by B cells in the spleen and lymph nodes and is widely distributed in the blood. In teleost fish, IgM is the main immunoglobulin molecule, which is a tetramer composed of covalently linked heavy and light chains (Basha et al., 2013). IgM plays an important role in humoral immunity, especially in resisting bacterial antigen invasion.

Neutrophils are considered the source of lysozyme and the enzyme appears to be much more bactericidal than lysozyme of higher vertebrates (Ellis, 2001). Lysozyme activity functions as a primary defence factor of non-specific humoral immunity in preference to cellular defence mechanisms (Basha et al., 2013). Fish serum lysozyme is believed to be of leukocyte origin (Kumar et al., 2013). Lysozyme plays an important role in innate immunity by lysis of the bacterial cell wall and thus stimulates the phagocytosis of bacteria (Ellis, 1990). Its ability to disrupt the cell walls of certain pathogens makes lysozyme a natural antagonist to harmful invaders like parasite, bacteria and virus. Lysozyme occurs prominently in fish serum and mucus (Wang et al., 2006). Lysozyme activity may be enhanced at relatively low concentrations of pollutants, and it has been proved that lysozyme activity was induced by a relatively low dosage of mercury exposure (Low and Sin, 1996). Serum lysozyme activity significantly increased in low dose of MC group (Qiao et al., 2013).

Serum lysozyme is used as an indicator of innate immune response in fish (Tort et al., 2003). An increased level has been considered to be a natural protective mechanism in fish (Ingram, 1980).

Superoxide dismutase (SOD) is one of the main anti-oxidant defense enzymes generated in response to oxidative stress that converts the highly toxic superoxide anions into hydrogen peroxide (Xia et al., 2017). Chang et al. had found that SOD decreases in *P. monodon* against WSSV-infected (Chang et al., 2003). The activity of SOD was significantly lowered in WSSV-infected *F. indicus* (Sarathi et al., 2008). The activity of superoxide dismutase responsible for the scavenging of reactive oxygen species (ROS) decreased leading to the increases of superoxide anion (Xia et al., 2017). An increase in the superoxide anion production against pathogens is considered to be beneficial after exposing shrimp to immunostimulants (Downs et al., 2001).

Catalase activity (CAT) is one of the primary antioxidant enzymes involved in ROS removal (Xia et al., 2017). Superoxide anion (O^{-}_{2}) was reduced to H_2O_2 by SOD, and H_2O_2 was converted to water and oxygen by CAT (Xia et al., 2017). The production of O_2- has been reported as an accurate method to measure the effectiveness of potential immunostimulants (Song and Hsieh, 1994). An increase in H_2O_2 and superoxide anion is considered to be beneficially protecting disease with respect to increased immunity (Song and Hsieh, 1994).

Increased phosphatase activity indicates higher breakdown of the energy reserve, which is utilized for the growth and survival of fish (Xia et al., 2017). Alkaline phosphatase played an important role in metabolic regulation, which directly involved in the transfer of phosphate group and calcium phosphorus metabolism. Alkaline

phosphatase also could change the surface structure of the pathogen to strengthen the recognition and phagocytosis of pathogens (Xia et al., 2017). Activity of alkaline phosphatase was increased in the group of fish fed with turmeric over different days (Xia et al., 2017). An increase in the alkaline phosphatase activity is considered to be beneficial for fish disease resistance (Xia et al., 2017). Acid phosphatase activity was found to increase post-challenge with *Aeromonas hydrophila* (Das et al., 2009).

In China's aquaculture, the mandarin fish *Siniperca chuatsi* (Basilewsky) has a relatively high market value and is important in stocking fisheries in lakes and reservoirs (Zhang et al., 2003). There are significant differences in immune function between different populations of fish. Yuan river is the third largest tributary of the Yangtze river. It is also the river with the largest amount of water and the most abundant fish resources in the waters of Xiang, Zi, Yuan, and Li in Dongting Lake (Yin et al., 2015). However, up to now, the comparative study of immune indices of different populations of wild mandarin fish in Yuanjiang river basin has rarely been studied. Therefore, the present study aims to compare the immune indices of three wild mandarin fish populations from Wuqiangxi reservoir, Lingjintan reservoir, and Taoyuan reservoir of Yuanjiang river basin. The results of this study will provide theoretical basis for disease-resistant breeding and germplasm optimization in mandarin fish breeding production, which has a high value of research and application.

Materials and Methods

Experimental fish

Mandarin fish (body weight: 100 ± 5 g) were collected from Wuqiangxi reservoir (WQX), Lingjintan reservoir (LJT), and Taoyuan reservoir (TY) of Yuanjiang river. Before the beginning of the experiment, the fish were acclimatized in quarantine plastic tanks filled with aerated freshwater at 24±2°C for two weeks. The experiment was approved by the guidelines of Institutional Animal Care and Use Committees (IACUC) of Hunan University of Arts and Science, Changde, China.

Total number of white blood cells

Ten fish were collected respectively from Wuqiangxi reservoir, Lingjintan reservoir, and Taoyuan reservoir, and anesthetized by immersion in water containing 0.1 ppm tricaine methane sulfonate (MS-222). Whole blood was collected from the caudal vein using syringes and needles that were rinsed with heparin. The total number of white blood cells was determined using the Diagnostic Reagent Kits purchased from Nanjing Jian Cheng Bioengineering Institute (China). 1 mL blood sample per individual was centrifuged at 1000 g for 5 min in order to separate the blood serum. Then the blood serum was stored at -20°C for catalase activity (CAT), alkaline phosphatase activity (AKP), acid phosphatase activity (ACP), T-SOD and lysozyme activity test.

Lysozyme activity

Lysozyme activity was measured using the turbidity assay. Lysozyme standard product powder (80,000 U/mg) was used as a standard, and 1 mg lyophilized *Micrococcus lysodeikticus* in sodium phosphate buffer (pH 5.75) was used as substrate. 20 µL plasma sample was added to 2 mL of substrate, and the reduction in the transmittance at 530 nm was determined after 20 s and 8 min incubation. One unit of lysozyme activity was defined as increase in transmittance of 0.001 per min.

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical-dependent reactions using the Ransod Kit (Randox, Crumlin, UK). Briefly, the reaction mixture (1.7ml) contained xanthine (0.05 mM) and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT, 0.025 mM) dissolved in CAPS 50 mM (pH 10.2) and EDTA (0.94 mM). In the presence of xanthine oxidase (80 U l1, 250 µl), superoxide and uric acid were produced from xanthine. Then, the superoxide radical reacted with INT to produce a red formazan dye. The optical density was measured at 505 nm, and the rate of reaction was estimated from the absorbance readings 30 s and 3 min after adding xanthine oxidase. A reference standard SOD was supplied with the Ransod Kit. One unit of SOD was defined as the amount required to

inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units ml-1.

Catalase, Alkaline phosphatase, and Acid phosphatase activity

In the experiments, we measured catalase activity (CAT), alkaline phosphatase activity (AKP), and acid phosphatase activity (ACP) of blood serum. CAT, AKP, and ACP activity was determined using the Diagnostic Reagent Kits purchased from Nanjing Jian Cheng Bioengineering Institute (China).

Immunoglobulin M gene expression assay

For the analyses of the effects of hydropower cascade development on the immunoglobulin M gene expression, head kidney, spleen, and liver were taken from three groups (WQX, LJT, and TY), respectively. Total RNA was extracted from the samples.

To quantify the expression of IgM in Mandarin fish, quantitative real-time PCR (qRT-PCR) was performed using specific primers (Table 1), and the product of these primers was sequenced to conform specificity. The [reverse transcription] (qRT-PCR) was carried out in triplicate on a Rotor-Gene Q real-time PCR Detection System (QIAGEN, Dusseldorf, Germany) using the SYBR ® Premix Ex Taq™ II (Takara, Japan) according to the manufacturer's instructions using specific primers (Table 1). Quantitative Real-time PCR reaction was carried out in 20 mL volume mixtures containing 10 mL SYBR qPCR Mix, 1 mL of each primer (10 mM), 2 mL cDNA and 6 mL ddH_2O. PCR amplification reaction was completed with the following profile: initial denaturation at 94℃ for 30 s followed by 40 cycles of 10 s at 94℃, 30 s at 58℃ and 30 s at 72℃. The PCR reaction carried out without cDNA sample was used as control. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that a single PCR product was detected. According to the equation: fold change = E - ΔΔ CT, where ΔΔ CT = (CT target gene - CT β-actin), gene expression values could be calculated as fold change in the target gene relative to the reference gene β-actin standard.

Table 1. The primer sequences for quantitative real-time PCR.

Primer name	*Primer sequence (5'–3')*
IgM-F	GACGAGAAAGGAAACGAA
IgM-R	TGGAGGCAGCATAAACAC
β-actin-F	GGAGAAGCTGTGGTACGTCG
β-actin-R	GTTGTAGGTGGTCTCGTGGA

Statistical analysis

Data are presented as mean value ± standard error (SE); mean values of immune indices of mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir were compared using the one-way analysis of variance by Duncan's test of STATISTICA software package (Version 6.0, Statsoft, Inc.). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Results

Total number of white blood cells

The total number of white blood cells of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY) is shown in Fig. 1. The total number of white blood cells was 2.62×10^{10} per mL blood in Takara, Japan group, 3.11×10^{10} per mL blood in LJT group and 9.93×10^{10} per mL blood in TY group. The total number of white blood cells was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 1).

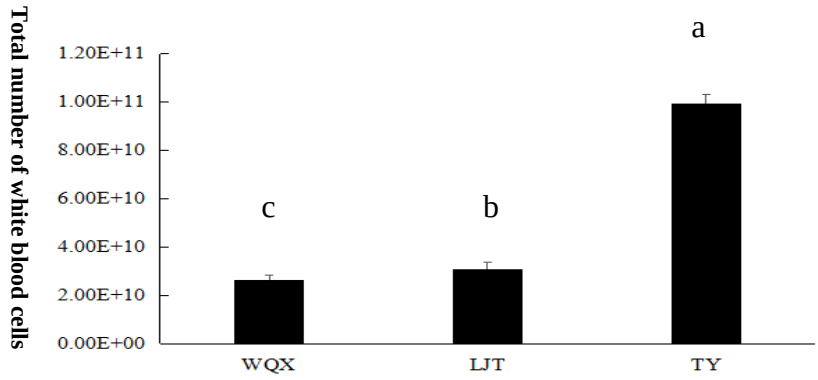

Fig. 1. Total number of white blood cells of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Lysozyme activity

The lysozyme activity of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir is shown in Fig. 2. Lysozyme activity was 2.41 U/ml in WQX group, 6.25 U/ml in LJT group and 6.78 U/ml in TY group. Lysozyme activity was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 2).

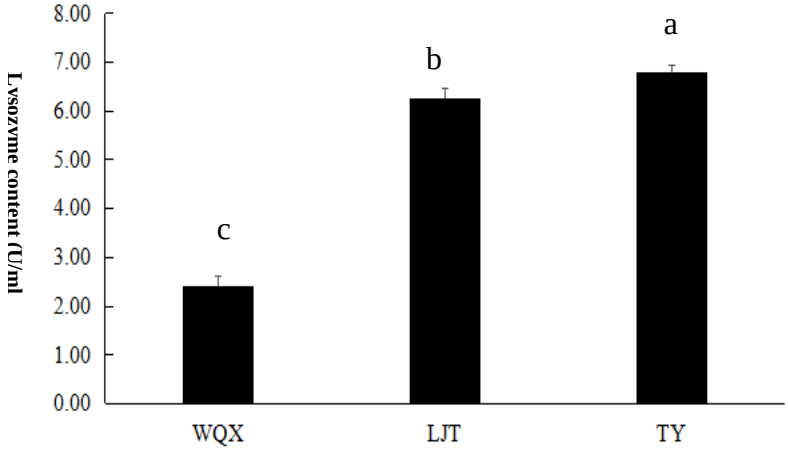

Fig. 2. Lysozyme activity of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) activity of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir was shown in Fig. 3. Superoxide dismutase activity was 104.28 U/ml in WQX group, 104.89 U/ml in LJT group and 143.17 U/ml in TY group. Superoxide dismutase activity was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 3).

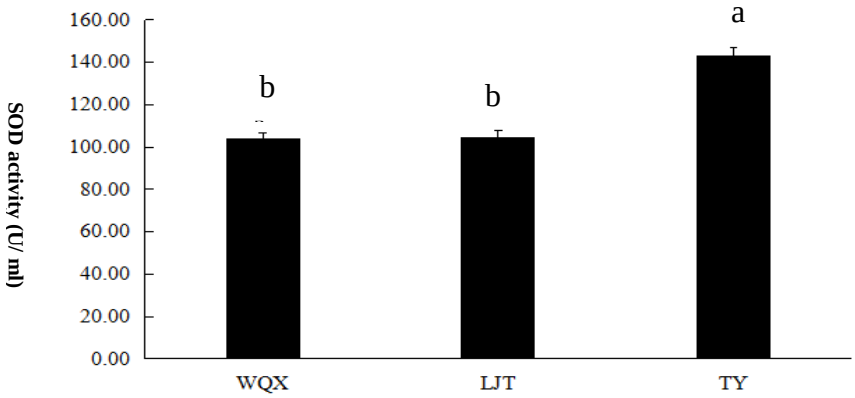

Fig. 3. Superoxide dismutase (SOD) activity of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Catalase activity (CAT)

The catalase activity (CAT) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir is shown in Fig. 4. Catalase activity was 5.04 U/ml in WQX group, 1.94 U/ml in LJT group and 21.06 U/ml in TY group. Catalase activity was highest in TY group followed by WQX group, and was lowest in LJT group (Fig. 4).

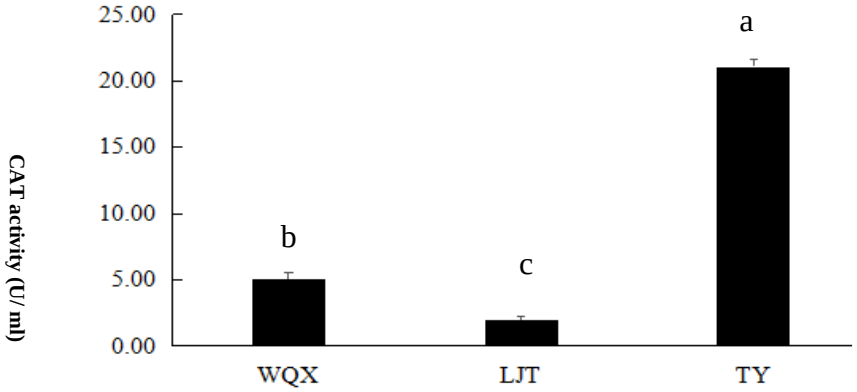

Fig. 4. Catalase activity (CAT) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Alkaline phosphatase activity (AKP)

The alkaline phosphatase activity (AKP) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir is shown in Fig. 5. Alkaline phosphatase activity was 2.6 U/ml in WQX group, 3.31 U/ml in LJT group and 4.47 U/ml in TY group. Alkaline phosphatase activity was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 5).

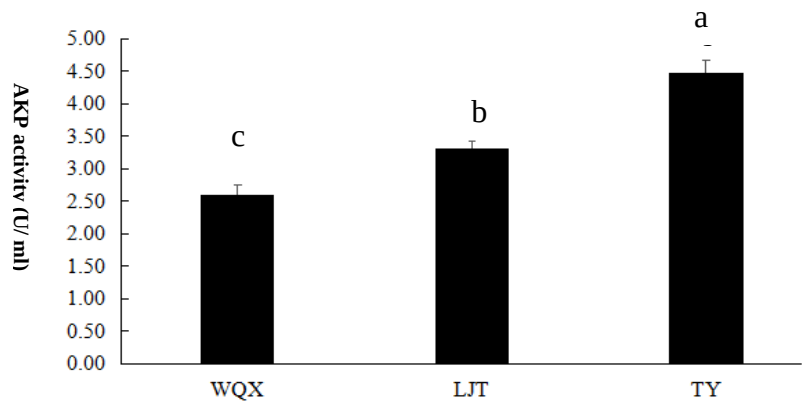

Fig. 5. Alkaline phosphatase activity (AKP) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Acid phosphatase activity (ACP)

The Acid phosphatase activity (ACP) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir was shown in Fig. 6. Acid phosphatase activity was 3.69 U/ml in WQX group, 4.58 U/ml in LJT group and 13.08 U/ml in TY group. Acid phosphatase activity was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 6).

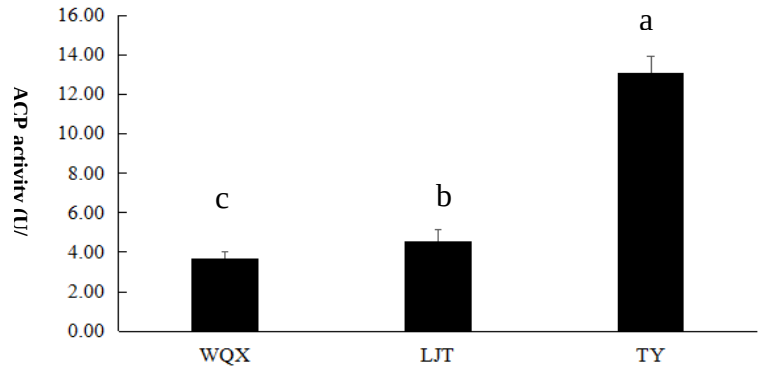

Fig. 6. Acid phosphatase activity (ACP) of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir (WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Immunoglobulin M gene expression

The relative expression level of IgM gene of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir was shown in Fig. 7. In WQX, LJT and TY

groups, the transcription level of IgM was highest in head kidney compared to spleen and liver. In head kidney, spleen and liver of Mandarin fish, the transcription level of IgM was highest in TY group followed by LJT group, and was lowest in WQX group (Fig. 7).

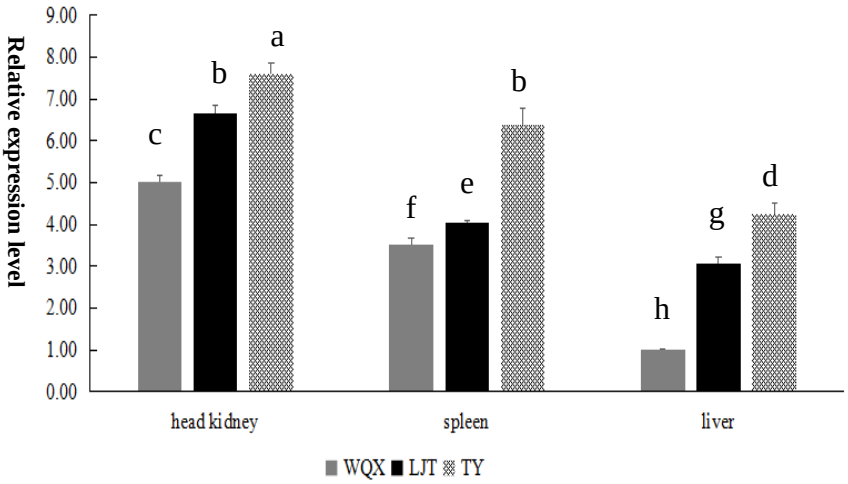

Fig. 7. The relative expression level of IgM gene of Mandarin fish from Wuqiangxi reservoir, Lingjintan reservoir and Taoyuan reservoir(WQX, LJT and TY). Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Discussion

The present preliminary study compared, for the first time, the immune indices of Mandarin fish from Wuqiangxi reservoir (WQX), Lingjintan reservoir (LJT) and Taoyuan reservoir (TY) of Yuanjiang river basin. The results of this study provided a theoretical basis for disease-resistant breeding and germplasm optimization in mandarin fish breeding production, which had a high value of research and application.

Immunoglobulin M (IgM) is the first antibody made by the body, and provides a crucial first line defense for the immune system (Xia et al., 2014). Immune system organs (such as bone marrow, lymphatic system and others) maintain normal immune functions (Xia et al., 2018). Head kidney comprising cytokine-producing lymphoid cells from the immune system and endocrine cells secreting cortisol, catecholamines, and thyroid hormones is a major immune organ in fish (a specific bone marrow equivalent). It plays an important role in immune response of teleostei while the spleen is the last genesia-organ in the development of the lymphatic system in teleost (Xia et al., 2015). The liver's lymphocyte population is selectively enriched in natural killer and natural killer T cells, play critical roles in first-line immune defense against invading pathogens (Racanelli and Rehermann, 2006). In the present study, the relative expression level of IgM in WQX, LJT and TY groups was highest in head kidney compared to the spleen and liver, and as such is a predominant immune organ. IgM is the predominant immunoglobulin molecule in teleosts. In head kidney kidney, spleen and liver of Mandarin fish, the transcription level of IgM was highest in TY group followed by LJT group, and was lowest in WQX group. It indicated that the degree of lymphocyte proliferation and immune function of Mandarin fish from Taoyuan reservoir was strongest compared to Mandarin fish from Wuqiangxi reservoir and Lingjintan reservoir.

In the present study, blood serum lysozyme activity of Mandarin fish was highest in TY group followed by LJT group, and was lowest in WQX group. This indicated that the blood serum of Mandarin fish from Taoyuan reservoir had strongest resistance to harmful invaders like parasite, bacteria and virus compared to Mandarin fish from Wuqiangxi reservoir and Lingjintan reservoir. Also in our study, SOD activity was highest in TY group, which is in line with the blood serum lysozyme activity. There was no significant difference between LJT group and WQX group. The highest level of SOD activity was considered to be beneficial after exposing Mandarin fish from Taoyuan reservoir to immunostimulants compared to LJT and WQX groups. CAT activity was highest in the TY group, which is in line with the blood serum lysozyme activity and SOD activity, which

indicated that Mandarin fish from Taoyuan reservoir had strongest resistance to harmful invaders

A notable increase of hemolymph ammonia accumulation and a serious depression of total hemocyte count were observed in *Eriocheir sinensis* following 1 day exposure to 20 mg/L ammonia-N (Hong et al., 2007). In comparison the blue shrimp *L. stylirostris* exposed to 1.5 and 3 mg/L ammonia decreased its total hemocyte count by 15% and 51%, respectively compared to the control (Moullac and Haffner, 2000). The total hemocyte count of *H. diversicolor supertexta* exposed to 3.16, 5.37 and 10.34 mg/L ammonia-N decreased significantly by 19%, 29%, and 34%, respectively after 72 h (Cheng et al., 2004). Total number of white blood cells of *Megalobrama amblycephala* decreased significantly in relation to NH_3-N exposure (Xia et al., 2018). In the present study, the total number of white blood cells of Mandarin fish was highest in TY group followed by LJT group, and was lowest in WQX group, which was in line with immunoglobulin M gene expression.

In the present study, the blood serum alkaline phosphatase activity and acid phosphatase activity were highest in TY group followed by LJT group, and were lowest in WQX group, which were in line with lysozyme activity, superoxide dismutase (SOD) activity, total number of white blood cell and IgM gene expression in head kidney, spleen and liver. It indicated that the immune function of Mandarin fish from Taoyuan reservoir was strongest, and it had strongest resistance to harmful invaders like parasite, bacteria and virus compared to LJT group and WQX group.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (Grant No. 31572619), Hunan Natural Science Foundation (Grant No. 2018JJ3373), China Postdoctoral Science Foundation(2019M652748) and Co-construction of Provinces and Ministries State Key Experiment on Developmental Biology of Freshwater Fishes Open-end Fund project (Grant No.2017KF007)

The authors declare no conflict of interests.

References

Alexander J.B., Ingram G.A., 1992. Noncellular non-specific defence mechanism of fish. *Annu Rev Fish Dis,* 2: 249–279.

Basha K.A., Raman R.P., Prasad K.P., Kumar K., Nilavan E., Kumar S., 2013. Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton). *Fish Shellfish Immunol,* 35(5):1433-1441.

Chang C.F., Su M.S., Chen H.Y., 2003. Dietary-1,3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish Shellfish Immunol*, 15:297-310.

Cheng W., Hsiao I.S., Chen J.C., 2004. Effect of ammonia on the immune response of Taiwan abalone Haliotis diversicolor supertexta and its susceptibility to Vibrio parahemolyticus. *Fish Shellfish Immunol, 17: 193-202.*

Das B.K., Debnath C., Patnaik P., Swain D.K., Kumar K., Misrhra B.K., 2009. Effect of β-glucan on immunity and survival of early stage of *Anabas testudineus* (Bloch). *Fish Shellfish Immunol*, 27(6): 678-683.

Downs C., Fauth J.E., Woodley C.M., 2001. Assessing the health of grass shrimp (*Palaemonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. *Mar Biotechnol*, 3: 380-397.

Ellis A.E., 1990. Immunity to bacteria in fish. *Fish Shellfish Immunol*, 9: 291-308.

Ellis A.E., 2001. Innate host defense mechanisms of fish against viruses and bacteria. *Dev Comp Immunol*, 25: 827-839.

Hong M.L., Chen L.Q., Sun X.J., Gu S.Z., Zhang L., Chen Y., 2007. Metabolic and immune responses in Chinese mitten-handed crab (*Eriocheir sinensis*) juveniles exposed to elevated ambient ammonia. *Comp Biochem Physiol C Toxicol Pharmacol*, 145: 363-369.

Hu Y.T., Duan G.Q., Ling J., He J., Hou G.J., 2018. Genetic variation analysis of breeding population and wild population of *Siniperca chuatsi*. *Jiangsu agri sci,* 40(26): 197-199

Ingram G.A., 1980. Substances involved in the natural resistance of fish to infection - a review. *J Fish Biol,* 16: 23-60.

Kumar S., Raman R.P., Pandey P.K., Mohanty S., Kumar A., Kumar K., 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. *Fish Shellfish Immunol*, 34(2): 564-573.

Low K.W., Sin Y.M., 1996. In vivo and in vitro effects of mercuric chloride and sodium selenite on some non-specific immune responses of blue gourami, *Trichogaster trichopterus* (Pallus). *Fish Shellfish Immunol*, 6: 351–362.

Misra CK, Das BK, Mukherjee SC, Pattaink P., 2006. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. *Aquaculture* 255: 82–94.

Moullac G.L, Haffner P., 2000. Environmental factors affecting immune response in Crustacea. *Aquaculture*, 191: 121-131.

Qiao Q., Liang H., Zhang X., 2013. Effect of cyanobacteria on immune function of crucian carp (*Carassius auratus*) via chronic exposure in diet. *Chemosphere*, 90(3): 1167-1176.

Racanelli V., Rehermann B., 2006. The liver as an immunological organ. *Hepatology*, 43: 54-62.

Sarathi M., Nazeer Basha A., Ravi M., Venkatesan C., Senthil Kumar B., Sahul Hameed A.S., 2008. Clearance of white spot syndrome virus (WSSV) and immunological changes in experimentally WSSV-injected *Macrobrachium rosenbergii*. *Fish Shellfish Immunol*, 25:222-230.

Song Y.L., Hsieh Y.T., 1994. Immunostimulation of tiger shrimp Penaeus monodon haemocytes for generation of microbicidal substances: analysis of reactive oxygen species. *Dev Comp Immunol*, 18: 201-209.

Tort L., Balasch J.C., Mackenzie S., 2003. Fish immune system. A cross roads between innate and adaptive responses. *Imunología*, 3: 277-286.

Wang W.B., Wang Y.P., Hu W., 2006. Effects of the "all-fish" growth hormone transgene expression on non-specific immune functions of common carp, *Cyprinus carpio* L. *Aquaculture*, 259: 81–87.

Xia H., Liu W.J., Wu K., Wang W.M., Zhang X.Z., 2015. Spatio-temporal expression of blunt snout bream (*Megalobrama amblycephala*) mIgD and its immune response to Aeromonas hydrophila. *Cent Eur J Immunol*, 40 (2): 132-141.

Xia H., Song T., Wang L., Jiang L.S., Zhou Q.T., Wang W.M., Liu L.G., Yang P.H., Zhang X.Z., 2018. Effects of dietary toxic cyanobacteria and ammonia exposure on immune function of blunt snout bream (*Megalabrama amblycephala*). *Fish Shellfish Immunol*, 78:383-391.

Xia H., Tang Y., Lu F.H., Luo Y.S., Yang P.H, Wang W.B., Jiang J.G., Li N., Han Q., Liu F., Liu L.G., 2017. The effect of *Aeromonas hydrophila* infection on the non-specific immunity of blunt snout bream (*Megalobrama amblycephala*). *Cent Eur J Immunol*, 42(3): 239-243.

Xia H., Wu K., Liu W.J., Wang W.M., Zhnag X.Z., 2014. Molecular cloning and expression analysis of immunoglobulin M heavy chain gene of blunt snout bream (*Megalobrama amblycephala*). *Fish Shellfish Immuno*l, 40(1):129-35.

Yin G.S., Liu H.P., Wang Y.Z., 2015. Environmental impact assessment of downstream section of cascade development in Yuan River basin. *Water Res Plan Design*, 5: 43-46.

Zhang Y.A, Nie P, Wang Y.P., Zhu Z.Y., 2003. cDNA sequence encoding immunoglobulin M heavy chain of the mandarin fish *Siniperca chuatsi*. *Fish Shellfish Immunol,* 14 : 477-480.