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Immunological, ionic and biochemical responses in blood serum of the marine fish *Trachinotus ovatus* to poly-infection by *Cryptocaryon irritans*

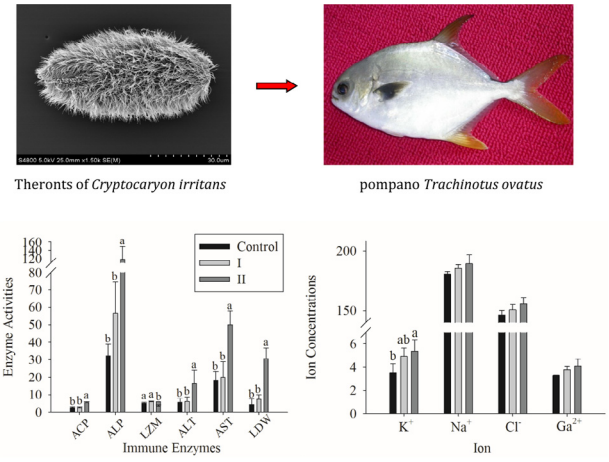
Fei Yin [a,*], Peng Sun [a], Baojun Tang [a], Xueming Dan [b], Anxing Li [c,**]

[a] *Key Laboratory of East China Sea and Oceanic Fishery Resources Exploitation, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China*
[b] *College of Animal Science, South China Agricultural University, Guangzhou, Guangdong Province 510642, China*
[c] *Key Laboratory for Aquatic Products Safety of Ministry of Education, State Key Laboratory of Biocontrol, The School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong Province 510275, China*

HIGHLIGHTS

- Serum ion and biochemical parameters were influenced by infection with *C. irritans*.
- High level of protective immunity following poly-infection by *C. irritans*.
- ACP and AKP may play crucial roles in the immune response against this disease.
- Multiple infections enhance pompano's immunity while causing limited harm.

GRAPHICAL ABSTRACT



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ABSTRACT

To investigate the response of pompano fish (*Trachinotus ovatus*) to white spot disease, we used the protozoan *Cryptocaryon irritans* to infect live 450-g specimens at concentrations of 40,000 theronts/fish. We assessed the relative infection intensity (RII), serum immobilizing titer, and immunity-related enzyme activities (ACP, AKP, LZM), and assessed feeding, serum ion concentrations (Na^+, Cl^-, Ca^{2+} and K^+) and blood biochemistry (ALT, AST, LDH) of pompano. The fish were then treated with a lethal dose of *C. irritans* (70,000 theronts/fish) and the number of deaths was recorded. We found that the relative infection intensities of the control group, group I, and group II were 0, 0.630 ± 0.179, and 0.014 ± 0.006. Poly-infection induced a significant increase in the serum immobilizing titer (853.33 ± 295.60) of group II. In terms of the

* Corresponding author. Fax: +86 21 6581 2067.
E-mail address: yinf@ecsf.ac.cn (F. Yin).
** Corresponding author. Fax: +86 020 84115113.
E-mail address: lianxing@mail.sysu.edu.cn (A. Li).

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biochemical assessment, group II had significantly higher alkaline phosphatase and acid phosphatase activities than the other groups, and the lowest lysozyme activity ($P < 0.05$), compared to higher activity in the control group and the highest level in group I. Only the fishes of group I had stopped feeding after treatment. The concentrations of Na^+, Cl^-, and Ca^{2+} in blood serum did not differ significantly among the three groups, but K^+ concentration increased with the increasing infection frequency. Alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase activities in fish of group II were significantly higher than those of the other groups. Survival of the fish subjected to the lethal dose of *C. irritans* was 0, 0, and 100 in groups control, I, and II, respectively. In conclusions, based on the food intake of group II, along with the results of relative infection intensity, serum immobilizing titer, and survival, we speculate that the fish in that group acquired high protective immunity following poly-infection by *C. irritans*, experiencing limited harm for pompano.

The protozoan *Cryptocaryon irritans* (originally classified as *Ichthyophthirius marinus*) is a parasitic ciliate of marine teleost fish (Matthews and Burgess, 1995). The condition it causes is cryptocaryonasis, commonly known as "ich", first reported in aquarium fish (Sikama, 1937) but later found to also cause severe damage in mariculture systems. After a fish is infected with *C. irritans*, white spots with clear edges are formed in the lesions, thus this is also known as the "white spot" disease of marine fish (Cheung et al., 1979; Colorni and Burgess, 1997). Over the course of years of development of marine fish aquaculture in many countries, increase in the density of aquaculture and general mismanagement has led to a greater frequency of white spot disease in marine fish culture zones, causing huge economic losses to farmers and related institutions (Colorni, 1985).

To explore the mechanism of pathogenesis and find effective prevention methods, researchers have used approaches such as changing fish between ponds, and physical/chemical pest control (Huff and Burns, 1981; Kawano et al., 2012). However these results are usually not applicable to large water bodies. Other challenges stem from the fact that the chemicals themselves are often toxic, their residues are polluting, and their doses are not easy to determine. Acquired protection to *C. irritans* was demonstrated nearly 20 years ago, using the grey mullet (*Chelon labrosus*) as an experimental host (Burgess and Matthews, 1995). Since then, protective immunity has been confirmed on the immunized grouper (*Epinephelus coioides*) (Bai et al., 2008; Luo et al., 2007; Yambot and Song, 2006), Mozambique tilapia (*Oreochromis mossambicus*) (Misumi et al., 2011) and the pompano (*Trachinotus ovatus*) (Dan et al., 2008), as indicated by high antibody titers in blood serum and mucus of exposed fish, and higher survival and fewer parasites in vaccine-immunized fish compared to control groups. Our previous study indicated that epithelial infection with *C. irritans* can, to some extent, protect fish from later infection, but it also causes decreased food consumption and retarded growth (Dan et al., 2008; Yin et al., 2014b). So, we speculated that low-dose poly-infection may be useful for improving immunity while not causing too much harm to fish.

Many factors including environmental conditions (e.g., photoperiod, fish stocking density, temperature, salinity), host physiology (e.g., reproductive cycle, age, gender, and nutritional status of fish), and pathogen biology (e.g., of viruses, bacteria, ectoparasites) have been reported to impact on blood parameters of fish. The evaluation of blood chemistry parameters in fish is a routine and important tool in clinical veterinary medical practice. This simple technique can provide essential information on the physiological status of fish and therefore help clinicians to make appropriate treatment decisions (Chen et al., 2004).

T. ovatus is an important farmed fish in China, where it is highly valued for its taste and highly nutritious content. During the commercial development and expansion of artificial culturing of this species, the white spot disease is a serious threat to pompano aquaculture. Numerous studies have demonstrated that this pathogen can be efficiently propagated in the animal model *T. ovatus*. A standardized procedure for doing so was established by means of infecting fish with a sub-lethal dose of theronts (Dan et al., 2006). This approach provided a foundation for further studies of *C. irritans*, such as pathogenicity and disease control (Li et al., 2013; Yin et al., 2014a). Further, protective immunity has also been confirmed in immunized *T. ovatus* (Dan et al., 2008), which is positively correlated with the duration of *C. irritans* infection (Burgess and Matthews, 1995; Dan et al., 2008). The humoral immune responses and host protection of pompanos against *C. irritans* were determined. When fish immunized with live theronts were challenged with a sub-lethal dose of theronts of *C. irritans*, the reduction rate of relative infection intensity (RII) of *C. irritans* was 50.6%. When fish were challenged with a lethal dose of theronts of *C. irritans*, the survival rate of immunized fish was 60% (Dan et al., 2008).

In this study, we infected *T. ovatus* up to 10 repeats at 40,000 theronts/fish. By comparing changes in pompanos' relative infection intensity (RII), serum immobilizing titer (SIT), immunity-related enzyme activities, feeding, survival, serum ion concentrations and biochemical parameters after infection, we investigated whether a high level of protection is conferred by poly-immunization of *T. ovatus* against *C. irritans*.

1. Materials and methods

1.1. Host fish

Healthy pompano with an average body mass of 450 g were purchased from fish farms in Aotou Town, Huizhou City, Guangdong Province, China for use in this study. A sample of 10 randomly selected pompano was examined; no parasites were detected on the skin or gills of these fish, and no immobilization occurred when theronts were incubated in fish blood serum (Bai et al., 2008). Pompano were acclimatized in 1000-L aquaria for 2 weeks prior to the experiment and fed twice a day with commercially produced feed equal to 3% the fish's body weight in total. Feces were sucked away each time before feeding. The conditions for aquaculture were salinity 29–31‰, water temperature 27 ± 1 °C, Non-ionic ammonia concentrations <0.02 mg/L, inorganic nitrogen <0.2 mg/L, water flow rate 200 L/h, light intensity 1000 lx, and photoperiod 14 L:10 D.

1.2. Parasites

The *C. irritans* were derived from a naturally infected pompano, and pompano was then used as the animal model to establish the passage system. When passed to the 5th generation, a sufficient amount of theronts was collected for the experiments.

1.3. Experimental methods

Active theronts that hatched within 1 h were collected and the concentration of parasites was calculated (Dan et al., 2006). Fish were divided randomly and equally into three groups. Each infection group contained 3 parallel subgroups, each subgroup containing 10 fish: fish in group I were infected only once, at the tenth week from the