# Effects of *Cryptocaryon irritans* infection on the survival, feeding, respiratory rate and ionic regulation of the marbled rockfish *Sebastiscus marmoratus*

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SUMMARY

To clarify the effects of a *Cryptocaryon irritans* infection on the physiological functions of the marbled rockfish *Sebastiscus marmoratus*, this study utilized *C. irritans* at concentrations of 2500; 5000; 7500; 10000; 20000; and 30000 theronts/fish to infect marbled rockfish weighing 45 ± 3 g. The survival rate, food intake, respiratory rate, serum ion concentrations and gill $Na^+/K^+$-ATPase activity were determined. With the increase of the infection concentration and the passage of time, the survival rate of the rockfish gradually decreased. The groups infected with more than 5000 theronts/fish had stopped feeding within 4 days. The respiratory rates of the fish in the groups infected with 2500 and 5000 theronts/fish initially increased and then decreased. In contrast, the respiratory rate of the fish in the groups infected with more than 7500 theronts/fish was elevated to levels significantly higher than the control group after 12 h. The $Na^+/K^+$-ATPase activity and serum $Na^+$ and $Cl^-$ concentrations increased with increasing infection concentration. In conclusion, the physiological functions of the fish infected with low concentrations of *C. irritans* can be effectively restored, whereas a high concentration infection induced severe stress. The declined food intake and accelerated respiratory rate could be useful for an early warning system as important indicators.

Key words: *Cryptocaryon irritans*, *Sebastiscus marmoratus*, survival, food intake, respiratory rate, ion content, $Na^+/K^+$-ATPase activity.

## INTRODUCTION

*Cryptocaryon irritans*, originally classified as *Ichthyophthirius marinus*, is a parasitic ciliate of marine teleost fish (Matthews and Burgess, 1995). It has a direct (i.e. does not require an intermediate host), quadriphasic life cycle, involving four developmental stages (trophont, protomont, tomont and theront). Trophont is the parasitic stage, which feeds on the host cells and tissues debris. Upon leaving the host the mature trophont sheds its cilia and becomes a protomont which adheres to the substratum and encysts. Tomont is the encysted, benthic, dividing stage and theront is the excysted, free swimming, non-feeding, infective stage (Colorni and Burgess, 1997). After a fish is infected with *C. irritans*, white spots with clear edges are formed in the lesions, thus earning the name 'white spot' disease (Cheung *et al*. 1979; Colorni and Burgess, 1997). Over the years, due to the development of marine fish culture in many countries, the increase in the density of aquaculture, and aquaculture mismanagement, the 'white spot' disease occurs frequently in marine fish culture zones and causes huge economic losses to farmers and related institutions (Colorni, 1985).

After being infected with *C. irritans*, the fish mainly present with reduced feeding behaviour (Kawano *et al*. 2012) and swimming and breathing abnormalities. Severe infection causes tissue damage, osmotic imbalance, decreased resistance to disease (Colorni and Burgess, 1997; Liu *et al*. 2012), loss of appetite, breathing difficulties, and, eventually, the death of the host (Matthews and Burgess, 1995). To find effective prevention methods and to explore the pathogenic mechanism, researchers have used approaches such as immune prevention (Luo *et al*. 2007; Bai *et al*. 2008), changing ponds, physical and chemical pest control (Huff and Burns, 1981;

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Yan *et al*. 2008), and gene quantification (Li *et al*. 2011*a*, *b*). Because the infection cycle of *C*. *irritans* is relatively short, the mean duration of *C*. *irritans*' life cycle is only one week at 27 ± 0·5 °C (Dan *et al*. 2006), and the initial low concentration infection and the second 'enhanced' infection have less than a 1-week interval during the disease seasons. One tomont (trophont) can divided into 200–300 tomites (theronts) (Colorni, 1985; Xu *et al*. 1992). So the 'enhanced' infection often causes an acute death of the fish. In actual production, it is often too late to start taking action when farmers discover the infection. Therefore, an early warning of the 'white spot' disease of marine fish and a rapid diagnosis of the infection level and the disease extent will help to effectively treat the disease. Research has shown that a low concentration infection dose not causes mass death in the fish but triggers an immune response in the fish to a certain extent (Luo *et al*. 2007). Effective methods can be utilized to save the lives of a large quantity of fish when facing moderate levels of infection; however, there is no effective approach for combating high concentrations of infection. In aquaculture, it is difficult to determine the extent of infection in the fish, and a valid judgement can only be made based on various behaviours and physiological responses of the fish.

The marbled rockfish, *Sebastiscus marmoratus*, is a traditional type of game fish. It is mainly distributed in the western North Pacific but is also found in the South China Sea, East China Sea, the Yellow Sea and the Bohai Sea. In recent years, marbled rockfish culture in China has been on the rise. Sun *et al*. (2011) successfully established a *C*. *irritans* passage system by using marbled rockfish as the host (Sun *et al*. 2011). However, changes in the physiological functions of this fish caused by the 'white spot' disease have not yet been reported. In this study, the marbled rockfish was used as the subject and was infected with different concentrations of *C*. *irritans* theronts. By comparing the survival, food intake, respiratory rate, serum $Na^+$ and $Cl^-$ concentrations, and the $Na^+/K^+$-ATPase activity of the marbled rockfish at different time-points post-infection, we explored the pathogenic pattern and mechanism and developed a system to evaluate the disease extent of the fish, thus providing the basis to take appropriate measures and to determine a favourable timing.

## MATERIALS AND METHODS

### Cryptocaryon irritans *strain*

The *C*. *irritans* GD1 strain, which was established by the State Key Laboratory of Biocontrol at Sun Yat-sen University, was used in the experiments. This strain was derived from a naturally infected *Trachinotus ovatus* (500 ± 50 g), and *T*. *ovatus* was then used as the animal model to establish the GD1 strain passage system. When passed to the 5th generation, a sufficient amount of theronts was collected for the experiments.

### *Experimental fish*

The marbled rockfish (45 ± 3 g) was purchased in Aotou Town, Huizhou City, Guangdong Province. Ten fish were randomly chosen and an immobilization assay was used to measure the immobilization effects of the serum on the *C*. *irritans* theronts to ensure that this batch of fish had not been previously infected with *C*. *irritans* (Bai *et al*. 2008). Healthy fish were selected and raised in 252-L aquariums (L × W × H: 60 cm × 60 cm × 70 cm), with each aquarium containing 20 fish. The aquaculture water was seawater that was filtered twice through a sand filter, and the flow rate was 60 L $h^{-1}$. The fish were fed twice daily (8:00 and 15:00) with mixed wild fish meat (purchased and stored at −20 °C until feeding), and the amount of meat provided each day was approximately 3% of the fish wet weight. Before each feeding, the residual feed and feces were removed by suction, and the residual feed was weighed. The salinity, water temperature, light intensity and photoperiod for aquaculture were 29–31‰, 27 ± 1 °C, 1000 L ×, and 14 L: 10 D, respectively.

### *Experimental methods*

Based on the method described by Dan *et al*. (2006), active theronts that hatched within 1 h were collected, and the infection concentration was calculated (Dan *et al*. 2006; Misumi *et al*. 2012). Six infection concentrations were used to infect the experimental fish: I: 2500; II: 5000; III: 7500; IV: 10 000; V: 20 000; and VI: 30 000 theronts/fish. The uninfected group (0 theronts/fish) was used as the control group. Each infection group contained 6 parallel subgroups, 3 for measurement of survival rate, food intake and respiratory rate, and the other 3 for measurement of serum ion concentrations and the $Na^+/K^+$-ATPase activity. The infection was performed in 252 L aquariums with 5 L of water for each fish. The infection lasted for 2 h in the dark. The fish were then transferred to clean seawater and raised under normal culture conditions. Throughout the course of the entire experiment, the physicochemical conditions of the water were the same as those of the relaying period.

### *Measurement of survival rate, food intake and respiratory rate*

Every 12 h, we recorded the number of dead fish in each group and calculated the survival rate, as follows: survival rate (%) = 100 * number of surviving fish/initial number of fish.

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Every day (d), we recorded the amount of feed for each group of fish and the residual feed weight and calculated the food intake, as follows: food intake (g) = the total amount of feed in each experimental aquarium – residual feed weight.

Every 12 h, 6 fish were randomly selected from each experimental group. We recorded the number of breaths and calculated the respiratory rate, as follows: respiratory rate (beats/min) = the number of breaths/the length of time for the counting.

### *Sample collection and testing*

Samples were collected from groups I, II, III and IV as well as the control group at 24, 48, 72 and 96 h post-infection. At each time-point, 3 fish were randomly removed from each aquarium. After the fish were anaesthetized with 0·15 mL $L^{-1}$ clove oil, blood samples were collected from the tail vein with 1-mL sterile syringes, placed in a sterile centrifuge tube, and allowed to stand at room temperature for 1 h, which was followed by standing still at 4 °C overnight. The blood samples were then centrifuged at 1330 *g* for 10 min, and the serum from the top layer was taken and stored in a −20 °C freezer. Subsequently, the fish were dissected on an ice tray, and the gills were removed, rinsed quickly in cooled normal saline and the excess of water was removed with a paper tissue. Samples from tissue were minced and mixed with nine volumes of chilled homogenization solution (100 mM imidazole-HC1 buffer, pH 7·6, 5 mM $Na_2EDTA$, 200 mM sucrose, 0·1% sodium deoxycholate, 4 °C). The homogenate was centrifuged at 1330 *g* for 10 min at 4 °C. According to the requirements, the supernatant was diluted and used for enzyme activity and total protein measurements.

The serum ion concentrations ($Na^+$ and $Cl^-$) and the branchial $Na^+/K^+$-ATPase activity were measured with assay kits (developed by the Nanjing Jiancheng Bioengineering Institute) according to the protocol supplied with the kits. The $Na^+$ and $Cl^-$ concentrations were measured by turbidimetry. The $Na^+/K^+$-ATPase activity was determined using a colorimetric method (Na/K-ATPase kit, product code: A070-2). The activity unit was defined such that 1 enzyme activity unit was equal to the amount of ATPase required to produce 1 $\mu$mol of inorganic phosphorus from the decomposition of ATP in tissues containing 1 mg protein within 1 h, namely micromolar phosphorus/mg of protein hour [$\mu$mol Pi·(mg prot·h)$^{-1}$]. The total branchial protein contents of the marbled rockfish under different concentrations of theronts were determined using the Bradford method (Bradford, 1976) with bovine serum albumin as standard.

### *Data analysis and statistical methods*

One-way analysis of variance (ANOVA) was used to compare the survival, feeding, respiratory rate, branchial Na+/K+-ATPase activity and content of ions in the marbled rockfish between the infection treatments. All of the variables were tested for normality and homogeneity before the one-way ANOVA was applied. If the infection treatments differed significantly, a Duncan multiple comparison procedure was applied to compare the different means of the infection treatments. Statistical analyses were performed using Statistical Package for the Social Sciences 11.5.

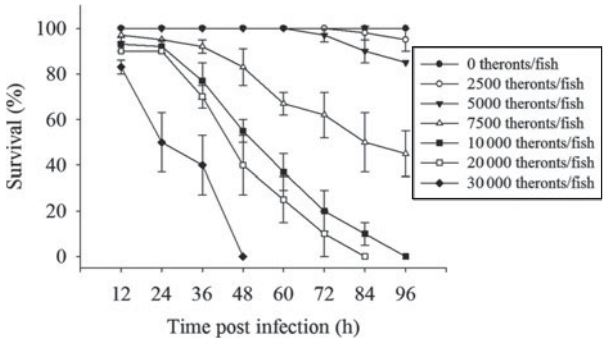


Fig. 1. Survival of marbled rockfish (*S. marmoratus*) during acute *C. irritans* infection.

## RESULTS

### *Mortality*

Analysis of variance showed that infections with different concentrations of *C. irritans* theronts had significant effects on the survival rate of the marbled rockfish ($P<0·05$, Fig. 1). Multiple comparisons showed that at each time-point after 12 h, the survival rate of the marbled rockfish showed a significant downward trend with the increase of infection concentration ($P<0·05$). At 12, 24 and 36 h, the survival rates of the marbled rockfish in four groups, group I (2500 theronts/fish), group II (5000 theronts/fish), group III (7500 theronts/fish), and the control group (0 theronts/fish), were significantly higher than those of the other three groups, group IV (10 000 theronts/fish), group V (20 000 theronts/fish) and group VI (30 000 theronts/fish) ($P<0·05$); the differences between the three low infection concentration groups and the control group were not significant ($P>0·05$). From 48 to 84 h, the survival rate of group III was significantly decreased, and it was lower than the survival rates of group I, group II and the control group ($P<0·05$). The marbled rockfish in group VI and group V had all died within 48 and 84 h, respectively. At 96 h, the survival rate of group II was significantly decreased, and it was lower than that of group I and the control group ($P<0·05$); however, the difference between group I and the control group was not significant ($P>0·05$). By 96 h, the marbled rockfish in group IV had all died.

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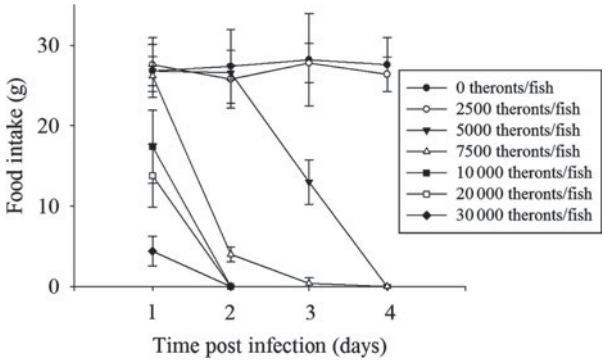


Fig. 2. Food intake of marbled rockfish (*S. marmoratus*) during acute *C. irritans* infection.

### *Effects of* C. irritans *on the food intake*

Infections with different concentrations of *C. irritans* theronts had significant effects on the average daily food intake of the marbled rockfish ($P<0{\cdot}05$, Fig. 2). Multiple comparisons showed that from the first day to the fourth day, the food intake of the marbled rockfish showed a significant downward trend with the increase of infection concentration ($P<0{\cdot}05$). On the first day, the average daily food intake of the marbled rockfish in three groups, group IV, group V and group VI, was significantly lower than the other four groups, groups I, II and III and the control group ($P<0{\cdot}05$), while the differences among the four low concentration groups were not significant ($P>0{\cdot}05$). On the second day, the average daily food intake of group III was significantly decreased, and it was lower than the food intake of group I, group II and the control group ($P<0{\cdot}05$); the differences among group I, group II and the control group were not significant ($P>0{\cdot}05$). Fish in groups IV, V and VI had stopped feeding by the second day. On the third day, the average daily food intake of group II was significantly decreased, and it was lower than the food intake of group I and the control group ($P<0{\cdot}05$); the difference between group I and the control group was not significant ($P>0{\cdot}05$). On the fourth day, the fish in group II and group III had stopped feeding; however, fish in the control group and group I did not show a significant decline in food intake ($P>0{\cdot}05$).

### *Effects of* C. irritans *on the respiratory rate*

Infections with different concentrations of *C. irritans* theronts had significant effects on the respiratory rate of the marbled rockfish ($P<0{\cdot}05$, Fig. 3). Multiple comparisons showed that from 12 to 96 h, the respiratory rates of the marbled rockfish in group I and group II first showed an upward trend, followed by a downward trend ($P<0{\cdot}05$). The respiratory rates of the marbled rockfish in group I at 48 and 72 h and those of the marbled rockfish in group II at 12, 24, 48, 60 and 84 h were significantly higher than the control group ($P<0{\cdot}05$). In contrast, there were no significant differences between the respiratory rates at 12 and 96 h for group I or group II ($P>0{\cdot}05$). From 12 to 96 h, the respiratory rates of the marbled rockfish in group III, IV, V and VI showed a fluctuant upward trend ($P<0{\cdot}05$). The respiratory rates of the marbled rockfish at each time-point between 24 and 96 h were significantly higher than that at 12 h ($P<0{\cdot}05$). In addition, except for the 12-h time-points of group III and IV, the other time-points of the 4 concentration groups all showed a respiratory rate that was significantly higher than the control group ($P<0{\cdot}05$).

### *Effects of* C. irritans *on the branchial enzyme activity and serum ion concentrations*

Infections with different concentrations of *C. irritans* theronts had significant effects on the $Na^+/K^+$-ATPase activity and the serum $Na^+$ and $Cl^-$ concentrations of the marbled rockfish ($P<0{\cdot}05$, Fig. 4). Multiple comparisons showed that as the infection concentration increased, the $Na^+/K^+$-ATPase activity showed an overall upward trend. The enzyme activities of group IV at various time-points were significantly higher than those of the other 4 groups at the corresponding time-points ($P<0{\cdot}05$). The enzyme activities of group II and group III were significantly higher than those of the control group and group I at 48, 72 and 96 h ($P<0{\cdot}05$). With the passage of time post-infection, the $Na^+/K^+$-ATPase activities of the control group, group I and group IV did not change ($P>0{\cdot}05$); however, the $Na^+/K^+$-ATPase activity of group II increased gradually ($P<0{\cdot}05$) and that of group III first showed an upward trend and then a downward trend ($P<0{\cdot}05$). The $Na^+$ contents of group IV at the 48 and 72 h time-points were significantly higher than those of the other 4 groups ($P<0{\cdot}05$). At 24, 48, 72 and 96 h, there were no significant differences in the $Na^+$ content among the control groups and groups I, II and III ($P>0{\cdot}05$). With the passage of time after infection, the $Na^+$ contents of the control group and group I did not change significantly ($P>0{\cdot}05$), and those of group II and group III first increased and then decreased ($P<0{\cdot}05$). The $Na^+$ contents of group IV at 48 and 72 h were significantly higher than that at 24 h ($P<0{\cdot}05$). At 24 h, the $Cl^-$ content of group IV was significantly higher than those of the control group, group I and group II ($P<0{\cdot}05$). At 48 and 72 h, the $Cl^-$ contents of group IV were significantly higher than those of the other 4 groups ($P<0{\cdot}05$). At 96 h, there were no significant differences in the $Cl^-$ contents among the control group and groups I, II and III ($P>0{\cdot}05$). With the passage of time after infection, the $Cl^-$ contents of the control group and group I did not change significantly ($P>0{\cdot}05$), whereas those of groups II,

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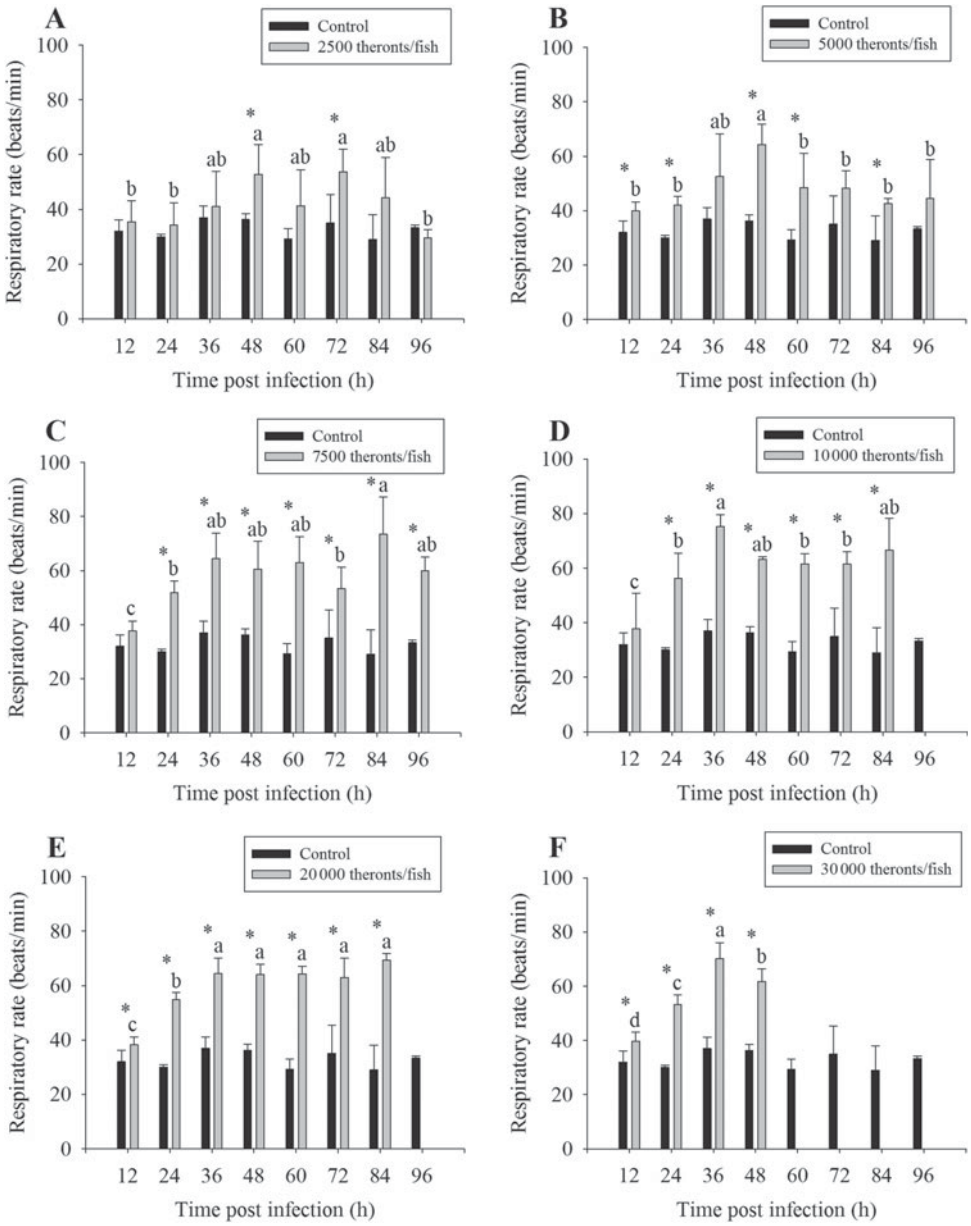


Fig. 3. Respiratory rate of marbled rockfish (*S. marmoratus*) during acute *C. irritans* infection. Different superscripts indicate significant difference among the different sampling time points; asterisks indicate significant difference between infected groups and controls at the same sampling time point ($P < 0.05$, Duncan's multiple comparison).

III and IV first increased and then decreased ($P < 0{\cdot}05$).

## DISCUSSION

*Cryptocaryon irritans* can infect most marine teleost fish (Burgess and Matthews, 1995). In the case of aquaculture with a high density, the fish are susceptible to *C. irritans* disease and die in large numbers (Colorni, 1985; Colorni and Burgess, 1997). Studies suggest that one of the main reasons leading to the death of the host fish in stress might be that *C. irritans* invades the gills. The invasion stimulates the gill filaments to secrete large amounts of mucus and causes inflammation and erosion of the gill filaments, thereby inducing tissue necrosis and resulting in an ion concentration imbalance and respiratory disorders. Finally, the host shows breathing difficulty or respiratory failure (Diggles and Adlard, 1997; Li *et al*. 2011*a*; Yoshinaga *et al*. 2011).

Reports have shown that the mortality rates of *Epinephelus* sp., *Pseudosciaena crocea* and *T. ovatus* after a *C. irritans* infection were up to 50, 75 and 100%, respectively (Chen *et al*. 2011). In this study, the *C. irritans* infection had a significant effect on the survival rate of the marbled rockfish. The survival rate of the marbled rockfish was gradually reduced with the increased passage of time post-infection. In addition, the infection concentration also significantly affected the survival rate of the fish. Luo *et al*. (2007) showed that infection concentrations less than 60000 theronts/fish did not cause death in

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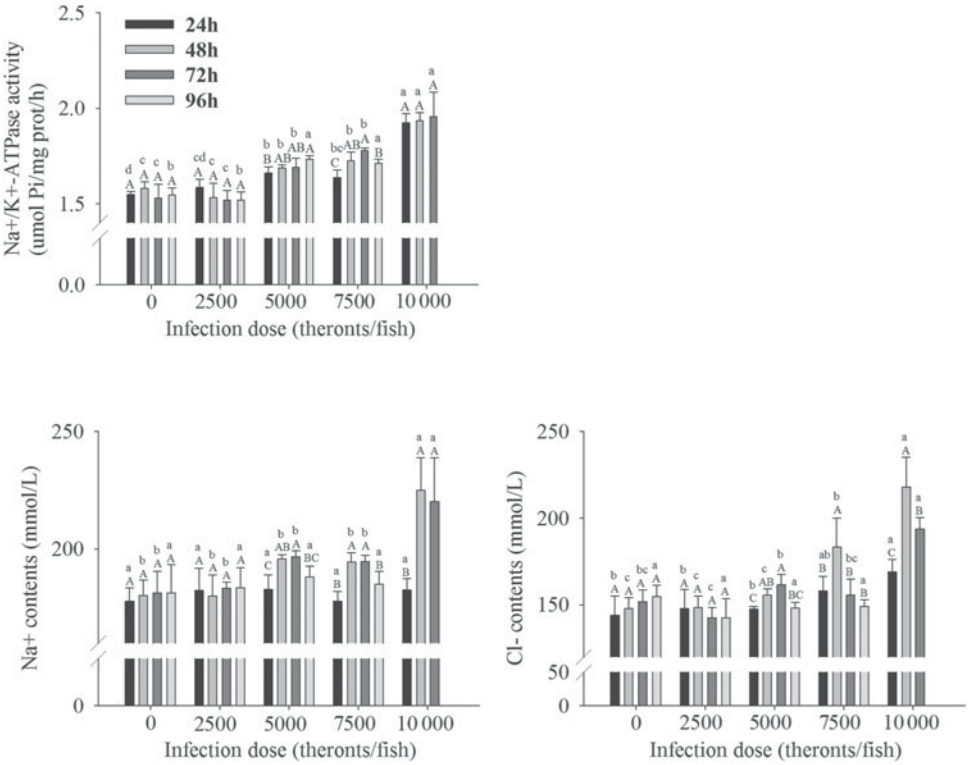


Fig. 4. Branchial $Na^+/K^+$-ATPase activities and serum $Na^+$, $Cl^-$ contents of marbled rockfish (*S. marmoratus*) during acute *C. irritans* infection.

orange-spotted grouper (*Epinephelus coioides*) that weighed 20 g, whereas an infection concentration of 120 000 theronts/fish killed all experimental fish within a week. Dan *et al.* (2006) showed that when infecting *T. ovatus* weighing 167·8 g with *C. irritans*, only a portion of the fish were killed if the concentration was less than 12 000 theronts/fish, and all fish were killed if the concentration was 18 000 theronts/fish. An infection concentration of 56 667 theronts/fish could cause a small number of tilapia to die on the fourth day after infection (Misumi *et al.* 2012). This study showed that the survival rates of groups infected with 0, 2500, 5000 and 7500 theronts/fish at 96 h post-infection were 100, 95, 85 and 45%, respectively. In contrast, all fish in the groups infected with 10 000, 20 000 and 30 000 theronts/fish had died within 96, 84 and 48 h, respectively. In summary, the lethal concentration of *C. irritans* for the grouper is far higher than those for tilapia, *T. ovatus*, and the marbled rockfish. This result suggests that there are great differences in the resistance to the *C. irritans* infection between different species of fish (Colorni, 1985). In addition, researchers have found that certain types of bony fish have a natural anti-*C. irritans* feature. For example, when experimentally infecting the rabbitfish (*Siganus oramin*) with a concentration of 10 000 theronts/fish, it was found that the resulting intensity of infection was far less than that of other varieties of fish such as grouper. An in-depth analysis found that the serum of rabbitfish could lead to cilia loss and cell rupture (Wang *et al.* 2010) because the blood of this fish contains L-amino acid oxidase, which is a protein with an antimicrobial activity to some pathogenic organisms (Wang *et al.* 2011). Currently, the development and utilization of this protein has also become a research focus.

Stress caused by external factors will result in reduced food intake by fish. A prolonged time of stress and aggravated stress level can even lead to stopped feeding (Aguado Giménez and García García, 2002; Peng *et al.* 2011). Therefore, a lowered appetite is often an indicator of sick or dying fish. Reports have indicated that fish infected with *C. irritans* present a significantly decreased appetite (Chen *et al.* 2011). Red sea bream and puffer fish die in large numbers on the second day after a loss of appetite (Kawano *et al.* 2012). The relationship between the degree of loss of appetite and the infection concentration or time has not been reported. This study showed that with an increase in the infection concentration, the food intake of the marbled rockfish was significantly reduced. On the second day of infection, fish in the groups infected with 10 000, 20 000 and 30 000 theronts/fish had already stopped feeding. On the fourth day, fish in the groups infected with 5000 and 7500 theronts/fish had also stopped feeding. In contrast, the food intakes of the fish in the control group and the group infected with 2500 theronts/fish did not show significant changes. This result suggests that fish in the low concentration groups suffered relatively milder levels

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of stress. The relatively adequate food intake of fish in the 2500-theronts/fish group also enabled the physical recovery of the marbled rockfish after the parasites detached.

The temperature, salinity, dissolved oxygen, chemical substances and other factors all affect the breathing condition of the animal (Affonso and Rantin, 2005; Cerezo Valverde *et al*. 2006). When suffering from a *C*. *irritans* infection, the fish will also show symptoms of breathing difficulty by opening the mouth and by an elevated breathing frequency (Chen *et al*. 2011). A histological observation has shown that gill invasion by *C*. *irritans* can cause tissue swelling, which results in a reduced gill lamella gap and reduced gill surface area available for oxygen exchange (Misumi, 2009; Li *et al*. 2011*a*). It has been shown that during hypoxia, animals tend to increase the respiratory rate and ventilation volume (Cerezo Valverde *et al*. 2006). This study used different concentrations of theronts to infect the marbled rockfish and found that the respiratory rate of each group of fish was increased by varying degrees. The respiratory rate of the control group of fish did not change significantly over time. From the beginning of the experiment to 96 h, the respiratory rates of the groups infected with 2500 and 5000 theronts/fish first showed an upward trend and then a downward trend. Taken together with the observation that the two groups of fish still showed a survival rate above 85% and had a relatively adequate food intake at 96 h (the food intake of fish in the 5000 theronts/fish group gradually rebounded after 96 h), it is reasonable to conclude that after most of the trophozoites detached from the host at 60 h, i.e. after the stress level was reduced, the physiological functions of the above groups of fish could be restored to different degrees. This recovery ability is also an important prerequisite so that after the primary *C*. *irritans* infection of a low concentration, most fish can still survive and effectively activate their own immune defence (Matthews and Burgess, 1995; Li *et al*. 2011*a*). Twelve h after exposure to the parasite, the respiratory rates of the other groups of fish rose to higher levels, and the levels were significantly higher than that of the control group. Especially for fish infected with concentrations over 10 000 theronts/fish, the respiratory rate did not show obvious signs of decline from the initial infection to the point where all fish had died. This result might be because the gill tissue of the fish in the high concentration infection groups had been severely damaged (Li *et al*. 2011*a*) and had lost its function, and even the increased respiratory rate could not provide enough oxygen for survival.

ATPase is an important enzyme for the regulation of ions in fish. The gill is the key organ for the regulation of ions in fish, and it is also the site directly affected by the parasitic infection of the trophonts of *C*. *irritans* (Cheung *et al*. 1979). When the fish increases the frequency of opening and closing the gill cover and mouth, the fish also continuously ingests large amounts of seawater. To maintain the relative stability of the ion concentrations in the intracellular environment and the osmotic balance between the intracellular and extracellular environment, the fish will discharge most monovalent ions through the gill chloride cells and certain amounts of divalent ions through the kidneys. The ion discharge is mediated by the functions of the ATPase (Lin *et al*. 2004). When the fish is severely infected with *C*. *irritans*, the gill chloride cells are damaged, which greatly interferes with the discharge of ions and causes a serum osmotic imbalance (Misumi, 2009). In this study, the serum $Na^+$ and $Cl^-$ concentrations of the 2500; 5000; and 7500 theronts/fish groups did not show significant changes at any time-point compared with the control group, except that the serum $Cl^-$ concentration of the 7500 theronts/fish group was significantly increased at 48 h. In contrast, at 48 h post-infection, the serum $Na^+$ and $Cl^-$ concentrations of the 10 000 theronts/fish group were significantly higher than those of the other groups. This result might be because the gill damage is relatively mild for low infection concentration groups such as the 2500 theronts/fish group, the fish have a strong ability of self-adjustment, and the respiratory rate drops after some of the trophonts detach from the fish, thereby avoiding the accumulation of large quantities of ions. When stress is severe, stress hormones (cortisol and catecholamines) can regulate the ATPase activity and, therefore, compensate for the deteriorated ion pump function due to the damaged chloride cells (Misumi, 2009). In the present study, with the passage of time post-infection, the serum $Na^+$ and $Cl^-$ concentrations of the 7500 theronts/fish group initially showed an upward trend, followed by a downward trend. We speculate that the elevated $Na^+/K^+$-ATPase activity can, to some extent, promote an ion efflux. The results of the 5000 theronts/fish infection group was between those of the 2500-theronts/fish group and the 7500 theronts/fish group, and in addition to the strong self-recovery ability, the elevated $Na^+/K^+$-ATPase activity also played a certain role. However, although the $Na^+/K^+$-ATPase activity of the 10 000 theronts/fish group was significantly higher than those of the control group and other infected groups, excess ions could not be discharged due to the severe damage to the gill (Misumi, 2009; Li *et al*. 2011*a*).

In summary, a *C*. *irritans* infection of the marbled rockfish can lead to a reduced survival rate and food consumption, increased respiratory rate, and changes in the ion concentrations and $Na^+/K^+$-ATPase activity. The physiological functions of fish infected with low concentrations of *C*. *irritans* theronts can be effectively restored, whereas a high concentration infection induced severe stress, which leads to acute pathology and death. The experimental results in this study suggest that the declined food intake

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and accelerated respiratory rate could be useful for an early warning system as important indicators. When the above symptoms appeared, the 'Cryptocaryoniasis' could be determined by observing the 'white dots' in gills or on skin or combined with the microscope detection.

## FINANCIAL SUPPORT

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