Journal of Marine Biology and Aquaculture

OMMEGA Publishers
Answering Intelligence

Research Article Open Access

The Tolerance of Rabbitfish *Siganus oramin* to the Ichthyotoxic Alga *Chattonella marina*

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Abstract

Raphidophycean flagellates, *Chattonella marina* is an ichthyotoxic algal species. Its blooms induced massive mortality of wild and cultivated fishes, which caused economic loss all over the world. Previous studies suggested respiratory disorder was the major reason of the fish death upon *C. marina* exposure and fishes with high respiratory rates might be sensitive to *C. marina*. In this study, rabbitfish (*Siganus oramin*) which has high respiratory rate was studied. In 48h exposure to *C. marina*, only 30% mortality was found. Besides, the respiratory rate of *C. marina* exposed rabbitfish was significantly higher than the control groups only after 24h. Therefore, the rabbitfish showed relatively high resistance to *C. marina* as compared to other studied fish species with high respiratory rate. Besides, the survival fish also had significantly lower ratio of gill damage, and anaerobic respiration was not dominant. Although it suffered respiratory disorder in exposure of *C. marina*, its blood glucose did not decrease to lethal level. The gill protection ability of rabbitfish might be the key to resist to toxic effect of *C. marina*. Further investigation on the mechanism of gill protection may help to make better management strategies in fish farming when facing the red tide of *C. marina*.

Received date: July 19, 2017
Accepted date: October 06, 2017
Published date: October 07, 2017

Citation: Yiu, S.K.F., et al. The Tolerance of Rabbitfish *Siganus oramin* to The Ichthyotoxic Alga *Chattonella marina*. (2017) J Marine Biol Aquacult 3(2): 1- 4.

DOI: 10.15436/2381-0750.17.1612

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Keywords: *Chattonella marina*; *Siganus oramin*; Harmful algae; Hypoxia; Rabbitfish

Introduction

Raphidophycean flagellates *Chattonella marina*, first reported in India in 1954, is a highly toxic species causing harmful algal blooms (HABs) associated with massive mortality of wild and cultivated fish in Japan, China and Australia[1-3]. Several bioactive compounds have been found in cultures of raphidophytes: reactive oxygen species[4], brevetoxin[5], free fatty acid[6], nitric oxide[7], hemagglutinins and hemolysins[8]. However, some studies have rebutted that the ROS and brevetoxin were not the ichthyotoxins of *C. marina*[9,10]. Besides, there are no direct evidences to indicate that the hemolysis and oxidation of hemoglobin do occur in *C. marina* exposed fish[11]. Therefore the ichthyotoxic mechanism of *C. marina* is still highly controversial and the reason why some fish can survive in exposure of *C. marina* is also unclear.

The susceptibilities of different fish species exposed to *C. marina* had been investigated, including goldlined seabream (*Rhabdosargus sarba*), Russell's snapper (*Lutjanus russellii*), marine medaka (*Oryzias melastigma*), green grouper (*Epinephelus coioides*)[12], red seabream (*Pagrus major*)[13], yellowtails (*Seriola quinqueradiata*)[14] and damselfish (*Acanthochromis polycanthus*)[6]. Toxic effect of *C. marina* on fish was mainly inducing gill damage, the subsequent fermentable fuel exhaustion was the major cause of fish death[11].

Teleost *Siganus* spp. are tolerant to various environmental stresses such as high concentration of aqueous ammonia[15], aqueous nitrite[16], a wide range of temperature[17], salinity[18], and marine white spot disease[19-20]. Moreover, it is an excellent food which has been widely cultured in the Mediterranean and

Indo-Pacific Regions[21]. The rabbitfish, *Siganus oramin*, with a wide geographic distribution, can be utilized as a good bio-indicator for quick monitoring on the local environmental condition[22].

Rabbitfish were adopted in this study to explore their responses to the exposure of toxic *C. marina*. Parameters in physiology (respiratory rate), histopathology (gill histology) and biochemistry (hematology) were investigated. The findings can hopefully contribute to develop better HABs management strategies in fish farming.

Methods and Materials

The rabbitfish (length: 10.2 ± 1.1 cm, weight: 12.0 ± 4.3 g) were captured from Ma Liu Shui Public Pier, Hong Kong. The fish were raised in the tank with fully aerated local sea water (salinity 33 ± 2‰). They were fed with common fish food every other day and the sea water was renewed once a week to keep good water quality. Feeding was ceased 2 days prior to the expo-sure experiments.

The raphidophyte *Chattonella marina* (Subrahmanyan) Hara et Chihara (NIES-3) was provided by the National Institute of Environmental Studies (NIES), Japan and maintained in modified K medium[23] with a salinity of 33 ± 2‰, and kept at 25 ± 1°C at a photon flux density of 100 µm photons $m^{-2}s^{-1}$ in a 12:12h light:dark cycle. The illumination was provided by cool white fluorescent lamps. To ensure the observed effect was caused by *C. marina* toxicity but not the physical disturbance of the algal cells, a non-toxic alga, *Dunaliella tertiolecta* was used as nega-tive control. The maintenance condition of *D. tertiolecta* was the same as *C. marina*.

The exposure experiment was divided into three groups, *C. marina* (CM) as treatment, while seawater (SW) and *D.tertiolecta* (DT) as controls. For the algal culture, *C. marina* was cultured in 2 L plastic tank until reached the mid-log growth phase (around 10,000 cells mL^{-1}) which was in the most toxic stage[24]. *D.tertiolecta* was also cultured in the same condition as *C. marina* until the cell density reached around 10,000 cells mL^{-1}. For each group, ten fishes were used (one fish in a tank, n = 10). Fish mortality, frequency of operculum movement (indicator of respiratory rate), gill histology and blood glucose level of all groups were also monitored. The whole exposure took 48 hours.

The frequency of operculum movement was measured for 1 min at: the very beginning, 6h, 24h,and 48h of the exposure. During the 48h exposure, the moribund fish (showing intoxication symptom, e.g. imbalanced swimming or lying on the bottom of the tank) in CM exposure was removed immediately for blood collection and gill histological examination. The survival fish from the CM exposure were all sampled after 48h exposure together with the ones from SW and DT controls. All sampled fish were put in the ice bath to lower their activity, and the blood was collected from caudal vein by syringe. The blood samples were immediately centrifuged 10,000 rpm at 4°C for 1min to obtain the serum. The blood glucose concentration in serum was analyzed by glucose assay kit (Biovision, K606). The fish gill arches were cut and the gill filament were then separated carefully and examined under light microscope.

To determine the lethal level of blood glucose caused by hypoxia, a hypoxia experiment was conducted. The fishes were put into the tank and a plastic bag was covered on the sea water surface to prevent gaseous exchange. The blood was collected until the fish showing hypoxia symptom (e.g. lying on the bottom), and then blood glucose level was determined by using glucose assay kit (Biovision, K606).

One-way ANOVA and S-N-K post-hoc test were used to test the null hypothesis that there was no significant difference between the means of each parameter in three experimental groups. Statistical analysis was performed using SPSS 17.0. The level of statistical significance was set at $p < 0.05$.

Results and Discussion

The respiratory rates (indicated as operculum movement rate) of rabbitfish in all groups were decreasing with time (Figure 1). No significant differences were observed among three groups at 6 h and 24 h, respectively. At 48 h, the frequency of operculum movement of rabbitfish exposed to *C. marina* was significantly higher than those in DT and SW controls, but no sig-nificant difference was observed between DT and SW controls. The significant higher respiratory rate of rabbitfish exposed to *C. marina* indicated that rabbitfish suffered stress caused by the toxic effect of *C. marina*. In previous studies, the fishes had dis-tress and their LT50 were within few hours[6,12-14]. In this study, the mortality of rabbitfish exposed to *C. marina* was only 30%. The present study shows rabbitfish was tolerant to toxic *C. marina* to certain extent. It reveals rabbitfish might partially avoid the harmful effect of *C. marina* in the wild.

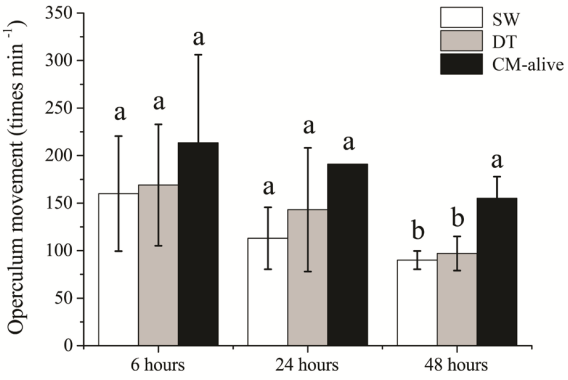

Figure 1: Respiratory rate of *Siganus oramin* during the exposure to seawater (SW), *Dunaliella tertiolecta* (DT), and *Chattonella marina* (CM) at 6h, 24h, 48h (Mean ± Standard Deviation, one way ANOVA, S-N-K test, $p < 0.05$).

Examining the status of rabbitfish gill filaments under light microscope (Figure 2), no irregular filament was observed in Sword groups while part of the filament in CM treatment showed irregularity. There was significantly higher ratio of irregular filament in moribund fish as compared to the fish a live in *C. marina* treatment (Figure 3). The filament (and lamella) was designed to maximize gill surface area for oxygen loading to the blood and to minimize water-blood diffusion distance[25]. Gill damages observed in this study indicates gill surface area for gaseous exchange in rabbitfish was reduced after exposed to *C. marina*. It showed that rabbitfish had difficulty in respiration due to gill damage, so that it increased operculum movement rate to

obtain more oxygen. This should be the reason why rabbitfish had higher respiratory rate in *C. marina* exposure as compared to the control groups at 48h. However, the mechanism of gill damage by *C. marina* is not clear. It is necessary to further investigate how gill damage is induced by *C. marina*.

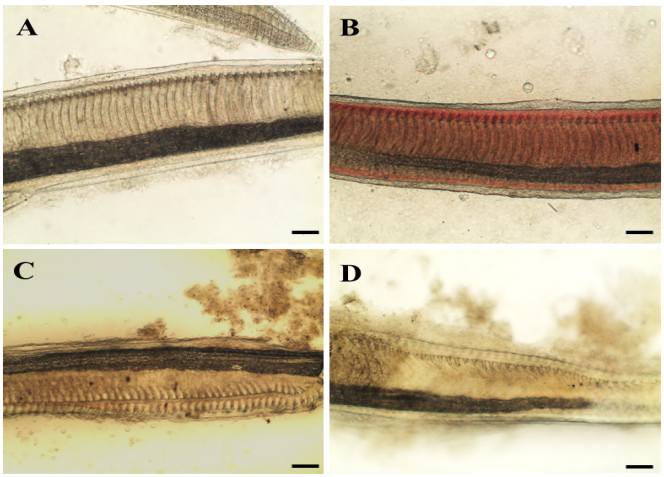

Figure 2: Gill of *Siganus oramin* exposed to seawater (SW), *Dunaliella tertiolecta* (DT) and *Chattonella marina* (CM) under light microscope. Gill filaments in SW (A), DT (B), CM-alive (C) and CM-mr-b(D). Alive fish in CM (CM-alive); Moribund Fish in CM (CM-mrb). Scale bars = 100 μm.

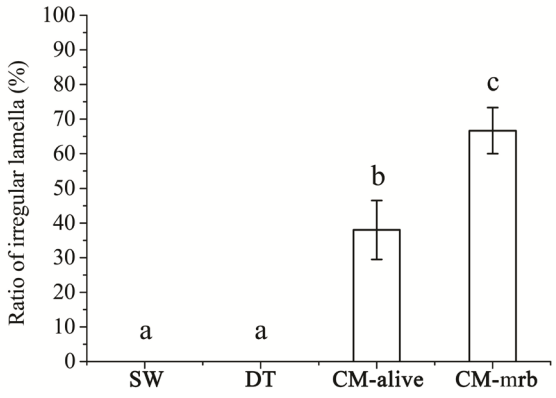

Figure 3: Ratio of irregular lamella in *Siganus oramin* exposed to seawater (SW), *Dunaliella tertiolecta* (DT), and *Chattonella marina* (CM). Alive fish in CM (CM-alive); Moribund Fish in CM (CM-mrb) (Mean ± Standard Deviation, one-way ANOVA, S-N-K test, $p < 0.05$).

Significantly lower blood glucose concentration was found in *C. marina* exposed moribund fish (CM-mrb) as compared to the fish alive in CM, DT and SW groups (Figure 4). It revealed the moribund fish from *C. marina* exposure suffered more stress. Based on the respiratory rate and gill histological findings, higher frequency of operculum movement and gill damage of rabbitfish only occurred in *C. marina* exposed fish. These two factors indicated that *C. marina* exposed rabbitfish was under respiratory distress. Besides, severer gill damage was observed in the moribund fish as compared to fish alive in *C. marina* exposure (CM-alive), the subsequent declined oxygen uptake put more stress on the moribund fish. On the other hand, the lethal blood glucose level was determined by the hypoxia(HYP) experiment, no significant difference was found between blood glucose levels of CM-mrb and HYP (Figure 4). Hence, the reason for fish death caused by *C. marina* should be that fish had much severe respiratory distress which leads to more glucose consumption till reaching lethal level eventually, as agreed by another study[11].

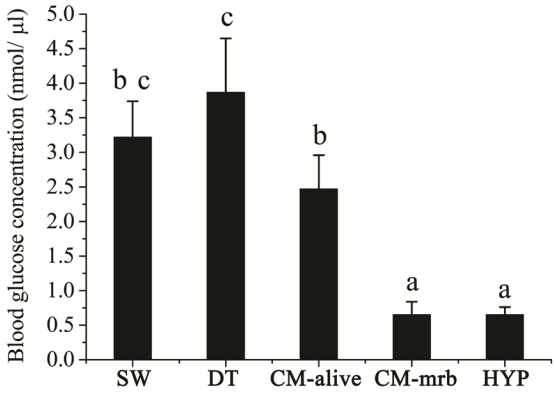

Figure 4: Blood glucose concentration of *Siganus oramin* exposed to seawater (SW), *Dunaliella tertiolecta* (DT), *Chattonella marina* (CM) and hypoxia (HYP). Alive fish in CM (CM-alive), Moribund Fish in CM (CM-mrb) (Mean ± Standard Deviation, one-way ANOVA, S-N-K test, $p < 0.05$).

Previous study suggested the fish with high respiratory rate (101 ± 12 times min^{-1}) might be sensitive to *C. marina*, because the rapid gill disturbance led to the anaerobic respiration and the subsequent fermentable fuel exhaustion cause quick death[24]. In this study, rabbitfish also had a relative high respiratory rate (90 ± 10 time's min^{-1}, in SW control) but its mortality in CM treatment was low. Consequently, rabbitfish was believed to have unique characteristic to resist the toxicity of *C. marina*, in which its gill protection ability might be the key.

In summary, this study demonstrated that the toxicological response of rabbitfish to the exposure of *C. marina*. Gill damage was the crucial cause of fish death. The results revealed that only a small portion of *C. marina* exposed rabbitfish had a significant gill damage and most of the rest had much less gill damage and eventually survived. Nevertheless, the gill protection ability of rabbitfish as a mechanism against the toxic effects of *C. marina* is still unknown. It is essentially necessary to further investigate the mechanism of gill protection mechanism, which may help develop better HAB management strategies in fish farming when facing the threat from harmful algal blooms of *Chattonella marina*.

Acknowledgements: Appreciations go to Dr. Meng YAN for helping the revision of manuscript from City University of Hong Kong.

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