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Effects of temperature and host species on the life cycle of *Cryptocaryon irritans*

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ABSTRACT

Cryptocaryon irritans, an important protozoan ciliate, infects almost all species of marine teleosts and causes heavy economic losses in aquaculture. In industrial aquaculture, culture container rotation is an effective method to control cryptocaryoniasis. However, the accurate frequency and times of container rotation for different temperatures or fish species has not been reported but is very important information for the application of the method. In the present study, we investigated the duration of *C. irritans* trophont residence and theront production in three fish species at temperatures from 18 °C to 32 °C with 2 °C intervals. The results indicate that duration of trophont residence and theront excystment was shorter with rises in temperature, especially from 18 °C to 22 °C. In different fish species, the length of trophont residence varied whereas the length of theront excystment was very similar at the same temperature. In line with these data, we determined the frequency and times that tomonts were removed, and results demonstrated that all the tomonts can be completely removed with 2 or 3 days interval for 2 or 3 times (from 22 °C to 32 °C), and the removal of tomonts was identical in three fish species at the same temperature. At 18 °C and 20 °C, however, the interval of tomont removal was > 4 days. Lastly, we verified the removal of tomonts with *Trachinotus ovatus* as host at 22 °C and 30 °C by a placemat removal method. In conclusion, these data provide an important basis for defense against *C. irritans* infection by the interruption of its life cycle in an intensive aquaculture system.

1. Introduction

Cryptocaryon irritans (Brown, 1951) is an obligate protozoan ciliate, which infects almost all marine teleosts species in tropical and subtropical regions, causing “white spot” disease and considerable mortalities mainly in aquarium and cultured fish (Colorni and Burgess, 1997). Because of its widespread distribution, indiscriminate host specificity, and high level of virulence, *C. irritans* has become an important parasite in aquaculture, and one which leads to heavy economic losses worldwide, including South China (Chen et al., 2008; Luo et al., 2008; Mai et al., 2015). The life cycle of *C. irritans* has four stages, namely parasitic trophont, off-host protomont, reproductive tomont, and infective theront (Colorni and Burgess, 1997). The trophont is the only parasitic stage that lives in the epidermis of the skin, gills, and eyes, where it impairs the physiological function of these organs. The living characteristics of trophonts make them difficult to be removed by chemical immersion, while oral drugs against trophonts are very limited (Hirazawa et al., 2001; Kawano et al., 2012; Rigos et al., 2013; Yoshinaga et al., 2011).

Diverse methods, such as physical, traditional herbs and immune prophylaxis, were reported to prevent or control cryptocaryoniasis (Luo et al., 2007; Yanong, 2009). These methods include freshwater immersion, heat treatment, drying treatment, ozone treatment, ultraviolet radiation, culture container rotation, and vaccination by exposure to living theronts or intraperitoneal injection with inactivated theronts. Among them, culture container rotation is an effective measure to control *C. irritans* infection by interrupting the life cycle and preventing the proliferation of this parasite. Previously, Colorni (1987) has reported that replacement of sand on the tank bottom could control *C. irritans* infection. Our laboratory has established a method of removing diseased fish from one tank to a new tank to cure cryptocaryoniasis (unpublished data). Goto et al. (2015) have reported that cryptocaryoniasis could be prevented by moving diseased fish from shallow closed bay water to further offshore in a sea cage-culture. Recently, Jiang et al. (2016) have optimized this method by using a placemat placed on the bottom of a tank and then replacing this placemat instead of removing sand or fish. This method is very easy to perform with little stress to cultured fish and can be used in an industrial aquaculture. In fact, we are now designing a device to make the replacement of the placemat easier.

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In this method, however, knowing when and how many times the placemat should be replaced is very important, and which determines whether fish are reinfected by new excysted theronts and whether all the tomonts are transferred from the culture system. To obtain these data, in the present study, we studied the duration of trophont residence and theronts excystment with different temperatures and host species. Based on these data, we confirmed the frequency and times that tomonts are removed dependent on the temperature or host species, which improves the effectiveness of the placemat removal method in the defense against *C. irritans* infection in aquaculture.

2. Materials and methods

2.1. Parasites and fish

C. irritans used in this study were originally isolated from infected pompano *Trachinotus ovatus*, and then identified according to the morphological features (Dan et al., 2006). They were propagated and preserved in our laboratory using *T. ovatus* as the host following the method described by Dan et al. (2006, 2009).

Pompano *T. ovatus* (156.2 ± 12.4 g), orange-spotted grouper *Epinephelus coioides* (176.5 ± 13.3 g), and three-banded sweetlip *Plectorhinchus cinctus* (168.4 ± 14.2 g) were purchased from the Marine Fisheries Development Center of Guangdong Province in China, and where no disease outbreak was reported during the course of the culture. The gills and mucus from ten fish were observed using a light microscope to ensure there was no parasite infection, and their serum was tested for not immobilization of *C. irritans* theronts. Fish were then reared in a flow-through water system at 28 °C for two weeks and fed twice daily with commercial pellet feed. During the trial, the salinity of seawater was 25–29‰, DO was ≥ 5 mg/L, pH was 7.5–8.1, and inorganic nitrogen was < 0.1 mg N/L.

2.2. Challenge and tomont collection

Before the challenge, each species of fish were randomly divided into eight groups, with the water temperature at 18 °C, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C, and 32 °C (± 0.2 °C), respectively, and acclimatized for 3 weeks at 1000-L round plastic tanks. Each group contained three parallel subgroups and ten fish in each subgroup. For the challenge, fish were exposed to *C. irritans* at a dose of 10,000 theronts/fish for 2 h, after which the seawater was continuously circulated. A tarpaulin selected by Jiang et al. (2016) with the size equal to the bottom of the plastic tanks was placed at the bottom of each tank 24 h after infection. The tarpaulin was removed every 6 h to collect tomont, and the time the last tomonts were collected was recorded.

2.3. Tomonts incubation and theronts excystment

The tomonts, collected from each group in Section 2.2 were incubated in 100 mL glass beakers with 50 mL sterilized seawater at the same water temperature of the corresponding host culture, the seawater was changed every 6 h. The exchanged seawater was observed under the microscope to check for theronts, and the time that theronts began to excyst was recorded.

2.4. Control test

Based on the data obtained in Section 2.2 and 2.3, we used pompanos *T. ovatus* as host, and 22 °C and 30 °C as rearing temperature to elevate the control of *C. irritans* infection by the placemat removal method as described previously by Jiang et al. (2016). In brief, fish were divided into three groups at each temperature (22 °C and 30 °C), placemat removal group (I), infection control group (II) and blank control group (III), with 30 fish in each group. Before the challenge, a tarpaulin was placed in the tank bottom of group I. Then, fish in group I and II were infected with *C. irritans* at a dose of 10,000 theronts/fish, while fish in group III were not treated. The tarpaulin in group I was renewed every 3 days three times (22 °C) or 2 days 2 times (30 °C). The fish surviving infection by *C. irritans* in all groups were cultured until one month, and then the skin and gills were sampled to observe trophonts of *C. irritans* under a microscope.

2.5. Statistical analysis

Data are expressed as mean ± standard error. The significance of differences between samples was determined by One-Way ANOVA with Duncan's test, and the level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of temperature and host species on the residence time of trophonts

To determine the removal times of tomont to control *C. irritans* infection, the residence duration of trophonts at different temperatures and in different host species were studied (Fig. 1 and SFig. 1). In total, the residence time of trophonts in all fish species was reduced with an increase of water temperature (18–30 °C), especially from 18 °C to 24 °C. From 30 °C to 32 °C, however, no significant change in the residence time was observed in all fish species. On the other hand, the residence time of trophonts was not completely consistent between each fish species at the same temperature (Fig. 1 and SFig. 1). For example, from 18 °C to 22 °C, the residence time of trophonts in *P. cinctus* was longer than in *T. ovatus* and *E. coioides*, while the residence time of trophonts was shorter in *P. cinctus* than in *T. ovatus* from 24 °C to 28 °C. The longest residence of trophonts was 272 ± 9.2, 254 ± 3.5, 248 ± 12.5 h for *P. cinctus*, *T. ovatus* and *E. coioides* at 18 °C, respectively, and the shortest resident time was about 83 h at 30 °C and 32 °C for all fish species (Fig. 1).

3.2. Effects of temperature and host species on the initiation of theront excystment

To determine the removal frequency of tomonts in order to control *C. irritans* infection, the time of theronts excystment initiation was recorded at different temperature using tomonts collected from different host species (Fig. 2 and SFig. 2). Similar to the effect of temperature on the residence time of trophonts, the time of theront excystment initiation was reduced following an increase in temperature, but significant change was observed from 18 °C to 22 °C, while there was only slight fluctuation when the temperature was > 24 °C. For tomonts collected

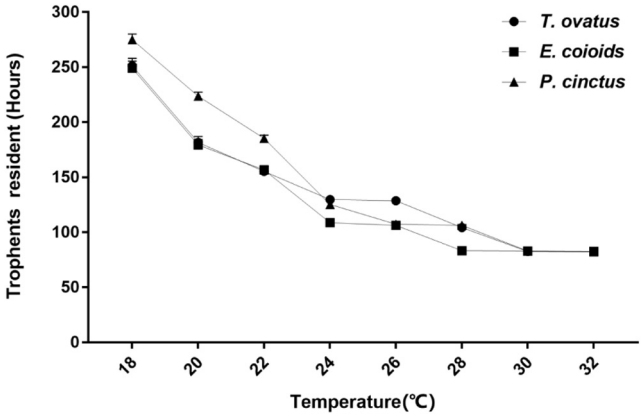

Fig. 1. The residence time of trophonts in *T. ovatus*, *E. coioides* and *P. cinctus* at different temperatures (18 °C–32 °C). Data are expressed as mean ± standard error.