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Infection and Propagation of *Cryptocaryon irritans* on Golden Pompano (*Trachinotus* spp.) in Vietnam

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Abstract

Cryptocaryon irritans is an ectoparasitic protozoan responsible for white spot disease, posing a significant threat to mariculture systems in Vietnam. This study aimed to isolate and propagate *C. irritans*, and to establish an experimental infection model in *Trachinotus* spp. (5-7cm in length) under laboratory conditions. Between February and June 2025, a total of 106 fish samples were sourced from aquaculture operations in Quang Ninh and Khanh Hoa, revealing an average *C. irritans* infection rate of 59.43%, with the highest prevalence observed during March and April. Infected fish exhibited clinical signs including skin darkening, mucus loss, white spots on the skin and gills, fin erosion, ulcerations, and tissue damage. The parasite was successfully cultured at the trophont stage and was propagated to the tomont and theront stages at 28-30°C, achieving a conversion efficiency of 61.7% after 48 hours and yielding an average of 150-300 theronts per tomont. These theronts were introduced to *Trachinotus* spp. at a density of 5,000 theronts per 250L tank (50 fish) at a temperature of 28°C. The infection progressed rapidly, with trophonts observed within 2-3 days and cumulative mortality reaching 100% by day seven. The established infection model offers a robust platform for further research in epidemiology, immunology, and therapeutic interventions against *C. irritans*.

Keywords

Cryptocaryon irritans, mariculture system, parasite infection, *Trachinotus* spp.

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Introduction

The ciliated protozoan parasite *Cryptocaryon irritans* Brown, 1951 (Ciliophora: Holophryidae) is a significant pathogen in marine aquaculture and is known as the causative agent of white spot disease in fish (Li *et al.*, 2022). It can infect nearly all marine fish species

(Dan *et al.*, 2006; Yin *et al.*, 2019), particularly high-value cultured species such as the orange-spotted grouper, *Epinephelus coioides* (Zeng *et al.*, 2023), barramundi, *Lates calcarifer* (Gibson-Kueh, 2012; Pattipeiluhu *et al.*, 2024), and golden pompano, *Trachinotus blochii* (Sun *et al.*, 2022), as well as ornamental fish kept in indoor-aquarium systems (Van & Nhinh, 2018). *C. irritans* parasitizes the skin, gills, and fins, damaging epithelial cells and impairing respiratory function (Zheng *et al.*, 2020; Li *et al.*, 2022). Infected fish typically exhibit lethargy, reduced growth rates, and mortality ranging from sporadic cases to massive losses (Zhou *et al.*, 2023; Zhou *et al.*, 2024).

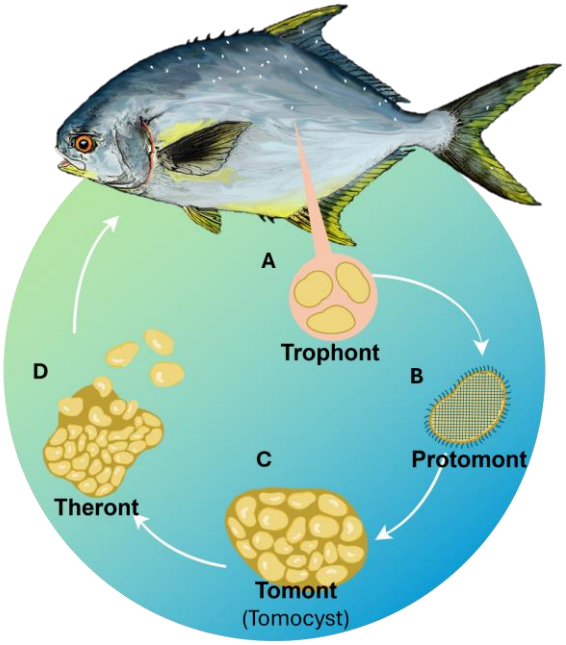
The parasite develops through three main stages (**Figure 1**): trophont (on the host), tomont (encysted stage), and theront (infective stage). Theronts are sensitive to chemical treatments due to the absence of a cyst wall, whereas trophonts and tomonts are resistant, requiring precise timing and targeted strategies for effective treatment (Colorni & Burgess, 1997; Li *et al.*, 2022). In Vietnam, outbreaks of white spot disease have been reported during the spring-summer season (Van, 2018; Van & Nhinh, 2018), affecting high-value cultured species (Hanh *et al.*, 2018). *Trachinotus* spp. (including *T. blochii* and *T. ovatus*) are widely promoted for commercial aquaculture in Vietnam due to their rapid growth, desirable flesh quality, and adaptability to industrial farming conditions. However, *Trachinotus* spp. are highly susceptible to *C. irritans* infection, particularly at the juvenile stage, when their immune defenses are still underdeveloped.

Although numerous studies have addressed the biology, life cycle, prevention, and treatment of *C. irritans* (Colorni & Burgess, 1997; Li *et al.*, 2022; Zhong *et al.*, 2023), reports on the development of a controlled infection model for golden pompano under laboratory conditions in Vietnam remain limited. The aims of this research were to establish a standardised protocol for parasite infection and propagation, understand the disease's progression, evaluate therapeutic agents and vaccines, and implement effective control strategies.

Materials and Methods

Ethics statement

The study was conducted in accordance with the regulations of the Vietnam National University



Note: (A) Adult trophont ("white spots") visible on the host; (B) Trophonts leave the fish body, become tomonts, and form tomocysts; (C) Tomites reproduce within the tomocyst; (D) Free-swimming theronts emerge from the tomocyst and seek a new host.

Figure 1. Life cycle of C. irritans

of Agriculture and were approved to be carried out through research project code NV2025-09-07TĐ. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Study duration and location

Sampling focused on fish populations exhibiting clinical signs of infection with the parasite *C. irritans* such as lethargy, schooling avoidance, reduced feeding, skin darkening, mucus loss, ulcers, white spots, rubbing behavior, or disorientation, particularly during the spring-summer transitional period, when post-stocking outbreaks are frequently reported. The research was conducted between February and June 2025. *Trachinotus* spp. specimens exhibiting clinical signs were selected for sampling. Fish were collected from a marine fish farm in the coastal areas of Quang Ninh and Khanh Hoa provinces. Samples were transported live in oxygenated bags to the laboratory at the Department of Aquatic Environment and Fish Pathology, Faculty of Fisheries, Vietnam National University of Agriculture, for parasite isolation and propagation.

Cryptocaryon irritans isolation

Cryptocaryon irritans was isolated following the method described by Watanabe *et al*. (2020), with modifications that were suitable for practical conditions in Vietnam. Infected fish were kept in a separate tank containing filtered and sterilized seawater (30ppt). Trophonts were allowed to detach from the fish body over a period of four hours and settled to the bottom of the tank. They were then collected using a bottom filter mesh (30-50µm), rinsed, centrifuged, and transferred into 50mL Falcon tubes containing sterile seawater (30ppt, UV-sterilized, autoclaved if needed). No nutrient medium was used, as tomonts develop autonomously. Trophonts were incubated at a temperature between 28°C and 30°C to develop into tomonts. After 24 hours, tomonts were transferred into Falcon 24-well plates (10 per well) with sterile seawater (1mL sterile seawater/well) and observed daily under a light microscope (40× magnification).

Identification was based on morphological characteristics and motility according to standard descriptions (Diggles & Lester, 1996; Colorni & Burgess, 1997; Li *et al.*, 2022). The distinguishing features of each stage were as follows: **Trophont** – Actively motile, oval to spherical in shape, with granular cytoplasm; **Protomont** – A transitional stage after leaving the host; cells were still motile but showed reduced activity and eventually attached to the bottom of the culture well; **Tomont** – Fully encysted stage characterized by a transparent cyst wall. Tomonts were non-motile and gradually underwent binary and multiple fission; **Tomite** – Multiple small daughter cells visible inside the tomont cyst, clearly separated by membranes; and **Theront** – Free-swimming, ciliated stage, approximately 20-30µm in size, actively moving in the culture medium.

Theronts released from the tomonts were collected from the culture wells at 24-48 hours post-encystment. For each sample, 100µL of the supernatant was gently pipetted and loaded into a Neubauer hemocytometer (0.1mm × 0.0025mm^2). The number of motile theronts was observed and recorded. Each sample was counted in five randomly selected squares of the chamber, and the average was used for calculation.

Cryptocaryon irritans infection procedure

Disease-free golden pompano *Trachinotus* spp. fingerlings, measuring 5-7cm in length (1.32-1.59g per fish), were imported from reputable hatcheries in Quang Ninh Province. Three to five specimens were randomly clinically checked to ensure that fish were not infected with pathogens. The fish were reared in 250L plastic tanks at a density of 50 fish per tank, with continuous aeration and periodic supplementation with sterilized seawater (**Figure 2**).

The infection experiment followed the method described by Yin *et al.* (2014), in which 5,000 theronts (collected from section 2.3) were added to each tank for three hours, after which the fish were transferred to new tanks. Three fish specimens were sampled daily to assess infection intensity and prevalence. These fish were not included in the mortality count of the experiments after sampling. The survival rate and clinical signs were monitored

daily for seven days. No mortality or abnormal behaviors were observed in the control group during the experiment.

The causative parasite was re-identified based on clinical observations and microscopic examination based on the characteristic signs of *Cryptocaryon irritans* infection using the morphological features described in previous studies (Colorni & Burgess, 1997; Li *et al.*, 2022).

To maintain clean and sustainable *C. irritans* lines, the trophonts that detached from fish between days 5-7 were collected using mesh filters. The infection procedure was then repeated to maintain a stable and continuous *C. irritans* strain for long-term research.

Results

Infection status and associated clinical signs of C. irritans on Trachinotus spp.

A total of 106 specimens of golden pompano *Trachinotus* spp., including 49 specimens from Quang Ninh Province, and 57 specimens from Khanh Hoa Province, were examined. Among them, 63 specimens tested positive for *C. irritans*, accounting for 59.43% of all sampled fish. The infection prevalence varied by month, with initial outbreaks observed in February and March, showing rates ranging from 33.33% to 50.00%. Higher infection rates were recorded in April and May, with prevalences of 76.25% and

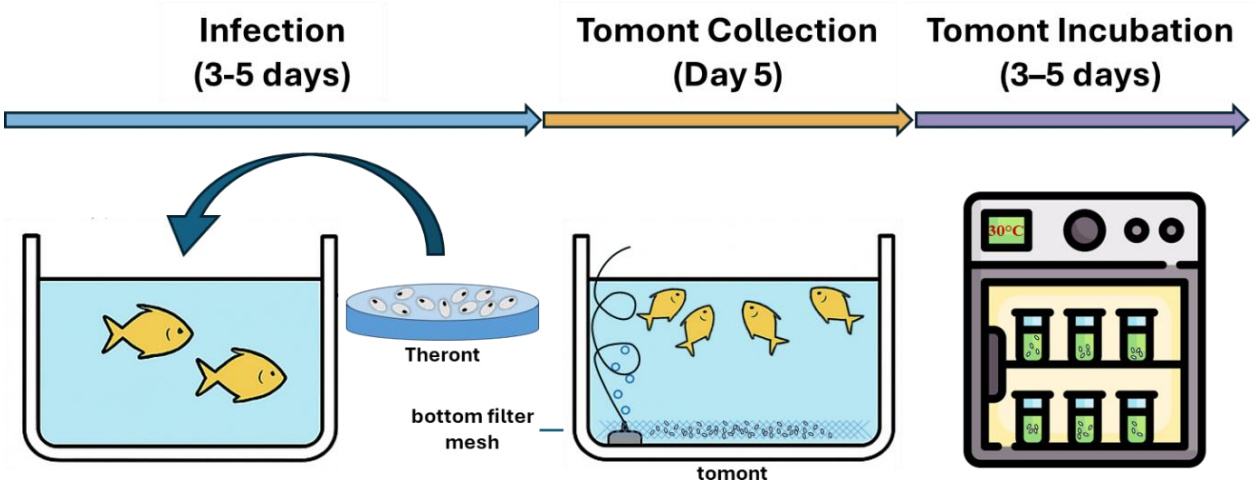
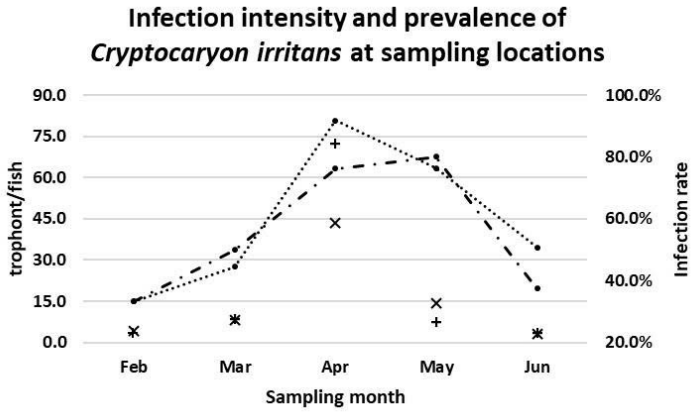


Figure 2. Schematic of *C. irritans* isolation, propagation, and infection protocol



Note: Mean infection intensity (trophonts/fish) in Quang Ninh (+; n = 49) and Khanh Hoa (×; n = 57); and Infection prevalence in Trachinotus spp. from Quang Ninh (– •–) and Khanh Hoa (····•····)

Figure 3. The prevalence and intensity of *Cryptocaryon irritans* infection on *Trachinotus* spp. in Quang Ninh and Khanh Hoa provinces

80.00% in Quang Ninh, and 91.67% and 76.39% in Khanh Hoa, respectively. In June, infection rates declined to 37.50% and 50.79% in Quang Ninh and Khanh Hoa, respectively. Infection intensity was assessed based on trophont counts. The peak intensity was observed in April, with averages of 72.2 trophonts per fish in Quang Ninh and 43.7 trophonts per fish in Khanh Hoa. In other months, the intensity was lower, ranging from 3.3 to 14.2 trophonts per fish (**Figure 3**).

The primary clinical signs observed in infected *Trachinotus* spp. included darkened or uneven body coloration (80.60%) and the presence of white spots on the skin and/or gills (67.16%). Additionally, mucus loss and fin erosion were observed in 46.27% of the fish, while haemorrhagic ulcers were recorded in 34.33%. Gill lesions, including necrosis and excessive mucus secretion, were found in 22.39% of specimens. Mild exophthalmia (pop-eye) was observed in 17.91% of the infected fish (**Figure 4**).

Isolation and propagation

Trophonts of *C. irritans* were sampled promptly, immediately after detachment from the fish host. In the first experimental batch, the transformation rate was 43.3% after 24 hours and 61.7% after 48 hours.

During the first 24 hours post-isolation, trophonts were observed in the protomont stage (**Figure 5A**) – a transitional phase in which they detach from the host and attach to the bottom surface of the well. At this stage, the organisms exhibited an oval shape, a clearly defined cyst wall, and homogenous cytoplasm.

Within the following 24-48 hours, these protomonts developed into tomonts. Initial binary fission was observed (**Figure 5B**), followed by multiple divisions resulting in clusters of 4, 8, or more daughter cells (**Figure 5C-E**) and the boundaries between daughter cells became increasingly distinct as the tomonts matured (**Figure 5F-G**), indicating readiness for excystment. Eventually, the tomocysts ruptured (**Figure 5H**), releasing infective theronts into the surrounding medium. Theronts appeared as highly motile, ellipsoidal organisms propelled by cilia (**Figure 5I**), capable of surviving and actively searching for a host for 12-24 hours post-release. Over the seven-day incubation period at 28-30°C, each well produced 1,500-3,000 theronts, corresponding to approximately 150-300 theronts per trophont.

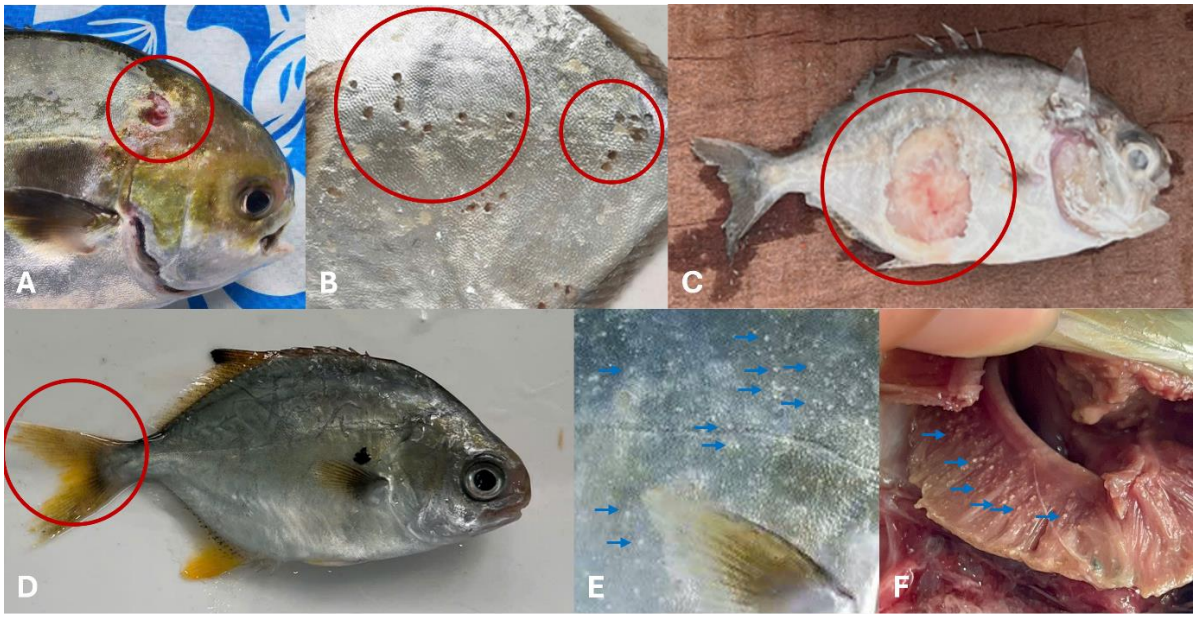
Cryptocaryon irritans infection

Theronts obtained from *in vitro* propagation were used to infect juvenile *Trachinotus* spp. at a density of 5,000 theronts per tank (50 fish) for a three-hour exposure period. Then, the fish were transferred to new tanks and monitored conservatively for seven days (**Figure 6**).

Following experimental infection of *Trachinotus* spp. with theronts, disease progression and clinical signs were monitored daily over the seven-day post-infection period. On the initial day, no fish mortality or detectable infection were observed. However, theronts were observed attaching to and penetrating the epithelium of the skin and gills. Within the first 24 hours, small-sized trophonts (15-20µm) began to appear in infected fish, with one out of three fish testing positive and an average infection intensity of three trophonts per fish. These findings confirmed the parasite's rapid attachment, invasion, and parasitism capacity. By day four, all examined fish (3/3) tested positive for *C. irritans*, with a peak infection intensity of 166 trophonts per fish, while the cumulative mortality rate reached 21%.

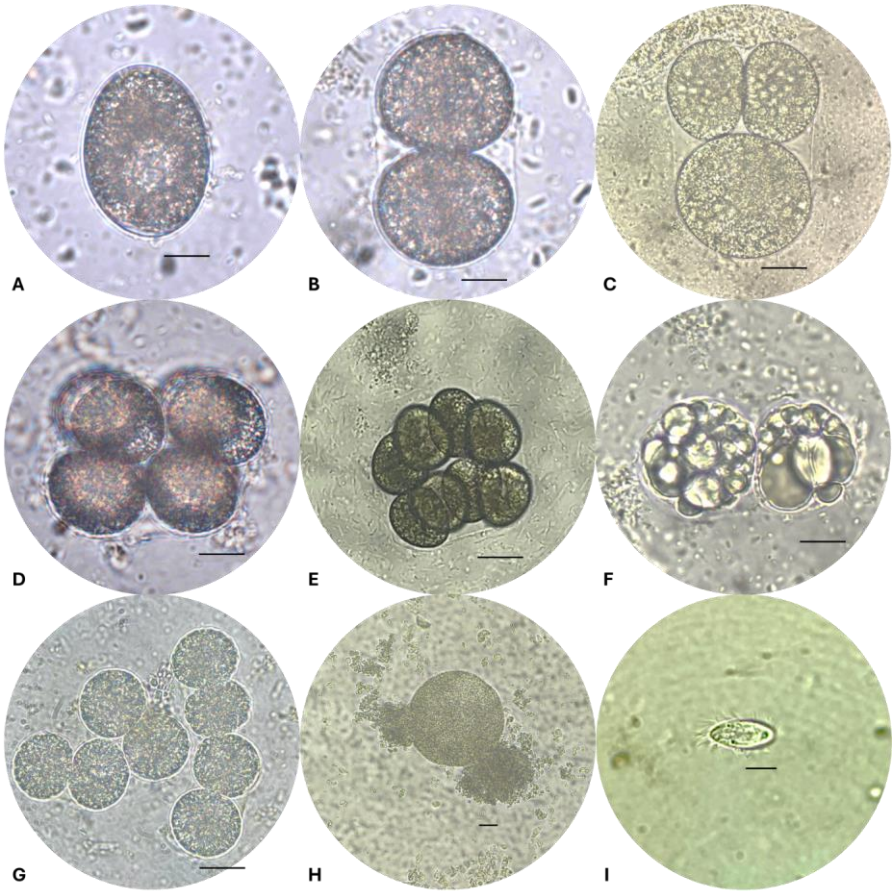
Discussion

Cryptocaryon irritans outbreaks tend to increase during seasonal transitions, particularly when temperature and salinity fluctuate or when juvenile fish are stressed due to recent stocking. For instance, in a study conducted in a public aquarium setting, it was found that most ornamental marine fish experienced higher infection rates during the spring-summer transition, with an overall prevalence of 67.7% (Van & Nhinh, 2018). Similarly, Diggles & Lester (1996) reported high infection intensities between March and June in wild marine fish sampled off the coast of Queensland, Australia. These findings are in line with the current study, in which elevated infection rates were also



Note: (A-C) Hemorrhagic ulceration on the skin and gills (indicated by red circles); (D) Skin darkening and fin erosion; (E-F) White spots on skin and gills (indicated by blue arrows).

Figure 4. Clinical signs observed in *Trachinotus* spp. infected with *C. irritans*



Note: (A) Isolated trophont transitioning into the protomont stage (18-24h post-isolation); (B-G) Tomont division into multiple daughter cells (tomites); (H) Mature cyst rupture releasing theronts after 3-5 days; (I) Free-swimming theront with elliptical shape and active movement via cilia, seeking a host. (40×magnification; Scale bar = 10µm)

Figure 5. The developmental stages of *Cryptocaryon irritans*

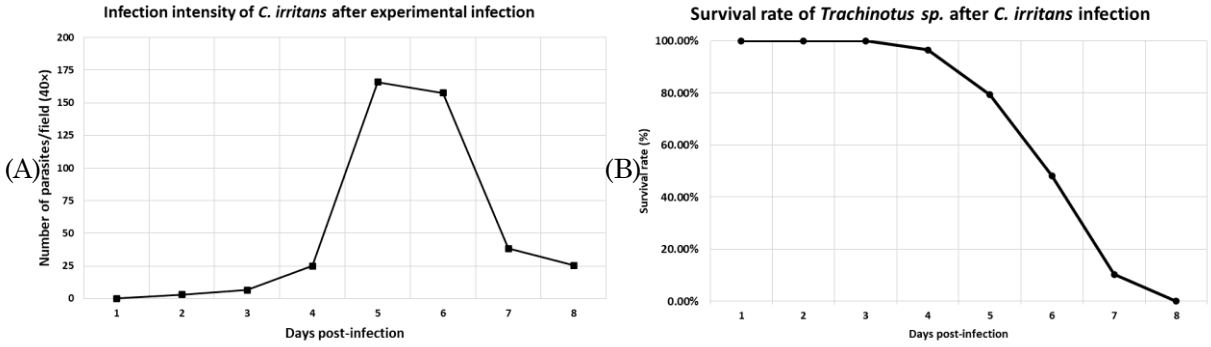


Figure 6. The experimental infection of *C. irritans* to *Trachinotus* spp. (A) Infection intensity; (B) Survival rate of *Trachinotus* spp. after infection

observed during the spring–early summer period in cultured *Trachinotus* spp.

Clinically infected fish in this study typically exhibited lethargy, separation from the school, surface swimming, and reduced responsiveness to external stimuli. In cases where symptoms were more severe, mortality frequently occurred within 24-48 hours, particularly when a clearly visible “white spot” was present. The clinical signs and gross lesions recorded here are consistent with those reported in previous findings, such as Zeng *et al.* (2023), further confirming the typical pathology of *C. irritans* infection in marine fish.

The accurate timing of trophont collection and the application standardized isolation techniques enabled the acquisition of clean trophont samples for propagation. The tomont transformation rates observed in this study, 43.3% at 24 hours and 61.7% at 48 hours, fall within the ranges reported in previous studies (Yambot & Song, 2004; Dan *et al.*, 2006; Li *et al.*, 2022), which documented conversion rates between 40% and 70% under optimal conditions. However, continued monitoring across successive propagation cycles is necessary to better understand generational variability in developmental transitions.

The experimental infection of *Trachinotus* spp. under laboratory conditions revealed a characteristic pattern of disease progression, aligning with the established life cycle and pathogenic mechanism of *C. irritans* (Colorni & Burgess, 1997; Li *et al.*, 2022). Both infection prevalence and intensity increased substantially from day one to day four, accompanied by the onset of clinical signs such as surfacing behavior, lethargy, and the appearance of "white spots" on the skin and gills. From day five onward, although the survival rate declined markedly (52% on day five, 10% on day six, and 0% by day seven), the infection intensity began to decrease due to the transference stages of *C. irritans* from trophont to tomont (detached from fish). Comparable findings were reported by Yin *et al.* (2014), who reported that marbled rockfish (*Sebastiscus marmoratus*) exposed to a similar theront density exhibited comparable mortality patterns. However, the researchers only monitored fish for 96 hours (four days), whereas the current investigation extended observation to seven days. This longer monitoring period provides a more comprehensive understanding of disease progression and highlights critical time points for early diagnosis and intervention in managing *C. irritans* outbreaks under culture conditions.

Over the seven-day incubation period at 28-30°C, each well produced 1,500-3,000 theronts, equivalent to approximately 150-300 theronts per trophont. These results are comparable to previous findings, which also claimed that theront yield is closely associated with the tomont size and maturation status (Colorni, 1985; Watanabe *et al.*, 2018; Li *et al.*, 2022). This high reproductive output under controlled conditions underscores the parasite’s potential for rapid spread in aquaculture systems if not properly managed.

Conclusions

The method for infection and propagation of *C. irritans* on golden pompano (*Trachinotus* spp.) juveniles (5-7cm in length) was

successfully established, demonstrating the ability to maintain a viable parasite line under laboratory conditions in Vietnam. From 106 fish samples collected from hatcheries and marine farms in Quang Ninh and Khanh Hoa provinces between February and June 2025, 63 samples (59.43%) were positive for *C. irritans*, with the highest prevalence recorded during the early stocking months (March-April). Infected fish exhibited typical clinical signs including darkened skin, white spots on the skin and gills, excessive mucus loss, fin erosion, hemorrhage, ulceration, necrosis, and tissue damage.

Acknowledgements

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