

# The Prevalence of *Cryptocaryon irritans* in wild marine ornamental fish from Vietnam

**K V Van and D T Ninh**

Department of Aquatic Environment and Fish Pathology, Faculty of Fisheries, Vietnam National University of Agriculture. Hanoi, Vietnam

E-mail: kvvan@vnua.edu.vn

**Abstract.** Wild-caught ornamental marine fish from NhaTrang (Khanh Hoa) and Ha Long (Quang Ninh) in Vietnam were examined over the three sampling batches corresponding to the spring, summer and autumn times for the prevalence of *Cryptocaryon irritans*. Out of a total of 211 fish (15 species), 143 (67.7 %) were found to be infected with the mean intensity of 7.67 parasites per field of view (x4 magnification). The prevalence of *C. irritans* in fish caught during the spring (91.0 3% on average) was significantly higher than that of the fish caught during the summer (39.29 %). A wide variation in the prevalence of the parasite was shown among the fish species. The highest prevalence and intensity of the infection occurred in *Platax teira*, *Diodon holocanthus*, *Paracanthurus hepatus* at 100 % of infection and density of 12 parasites/field of view (x4 magnification) while and the lowest prevalence of *C. irritans* appeared on *Rhinocanthus aculeatus*, *Zanclus cornutus*, and *Zebrasoma veliferum* with less than 50% of fish infected. Clinical signs of fish infected of *C. irritans* showed such as tiny white spots on skin, gills, and fins; ragged fins, changes in skin colour, cloudy eyes and increase mucus production.

## 1. Introduction

Ornamental fishes are receiving an increased attention due to the growth of local and global demand and the consequent bloom of small and medium commercial aquariums and marine museums for leisure activities in Vietnam. A vast number of aquatic species have been successfully adapted to live in the aquarium. Marine ornamental fishes are more colorful and shapely. These fish groups are, therefore, more attractive to aquarists. However, ornamental fishes kept in aquariums are susceptible to numerous diseases particularly parasitic diseases. *Cryptocaryon irritans* infections in fish are a significant disease problem for marine aquarists and commercial marine culture worldwide.

*Cryptocaryon irritans*, a ciliated protozoan parasite, is one of the most devastating parasites of marine fish cultured in temperate and tropical areas. This parasite is an obligate ectoparasite that causes Cryptocaryonosis, also known as the white spot disease in marine fish [1]. Although *C. irritans* is commonly found in tropical, subtropical and warm temperate waters at low infection intensity [2], infection by this parasite has emerged as a major problem in confined environments, such as in marine culture and aquariums due to the buildup of the parasite and high population density of fish in these systems [3].

*Cryptocaryon irritans* has a direct life cycle [4]. This means infection can spread within a group of



fish without the need for another animal host for development. However, the life cycle is complex. It includes 4 stages that develop on and off the fish, which is briefly described by Yanong [5] as follows: The trophont is the “feeding stage,” during which the parasite is found embedded within the tissues of the fish. After the trophont leaves the fish, it becomes a protomont before encysting and transforming into a to mont, or “reproductive stage.” The tomont develops and divides into numerous tomites, which eventually leave the cyst as the ronts, the free-swimming infective stages. Theronts actively seek fish hosts for a new life cycle.

The length of the entire life cycle varies, depending upon a number of factors, including the strain of *Cryptocaryon*, temperature, and fish host [4, 6-9]. Even for a specific strain of the parasite and fish host, the life cycle may vary by weeks or months [4]. An average life cycle appears to be 1 to 2 weeks. However, life cycle durations may range from 6 days to 11 weeks, primarily because of the unpredictability of tomont development [4, 9, 10].

The most commonly observed stage is the trophont or “feeding” stage. The trophont is found on the fish, usually underneath the outer skin layers. Spherical or pear-shaped, with cilia all over its body, the trophont will normally be seen “rolling” or rotating slowly under the epithelium. Because the trophont is embedded within the skin, it is relatively protected from any potential treatments [5]. Trophonts can range in size from about 48 x 27 µm to 452 x 360 µm. The trophont feeds on the body fluids and cells of the fish for about 3–7 days before leaving the host. Trophonts will also actively leave fish that have died, but are not immediately infective. They require additional time to develop from protomonts to tomonts, just as they would if leaving a live host [5].

Although numerous studies have been conducted on various aspects of *C. irritans*, information on infections related to this parasite on marine ornamental fish in Vietnam is very limited. The report aims to present the current results and updated information on the infection of *C. irritans* on wild-caught marine ornamental fish in Vietnam. The study was conducted at Vinpearl Land Aquarium Times City, which is one of the largest commercial marine aquaria in Vietnam.

## 2. Methodology

The study was conducted from 2/2016 to 12/2016 through three sampling batches corresponding to the times of the spring, summer and autumn of the year. In total 211 fish of 15 marine ornamental fish species captured from Ha Long and Nha Trang were examined (table 1). The fish were transported by closed system oxygen packed polythene bags [11]. After transportation, the fish were held in separated recovery tanks to examine the infection of *Cryptocaryon irritans* according to the method described by Dogiel (1929) cited by Ha Ky and Te [12]. During this acclimation period, infected fish were also visually observed in terms of external signs and behaviour changes. To inspect the presence of the parasites, mucus samples from gills and skin of the fish were taken and then smeared onto clean microscope slides to observe under the microscope with magnification powers of x4, x10, x40 and x100.

Based on the collected data, the prevalence and intensity of infection by seasons and fish species were calculated as follows:

Prevalence of infection = number of infected fish/total number of fish inspected x 100 %

Infection intensity = total number of parasites on fields (4x10)/total number of fields with the presence of parasite.

The data were collected and analysed on Excel 2010.

**Table 1.** Summary information on fish species, sample batches, sample size and sources of fish used in the study. The first, second and the third sample batches were in the spring, summer and autumn respectively. The fish used in the study were captured from coastal areas of Nha Trang (Khanh Hoa) and Ha Long (Quang Ninh).

No	English name	Scientific name	Sampling batch (number of sampled fish)			Sample size	Sampling sites
			1	2	3		
1	Picasso Triggerfish	<i>Rhinecanthus aculeatus</i>	5	5	5	15	NhaTrang- KhanhHoa
2	Spotted Parrotfish	<i>Cetoscarus ocellatus</i>	5	4	5	14	
3	Philippine Butterflyfish	<i>Chaetodon adiergastos</i>	6	6	6	18	
4	Blue Surgeonfish	<i>Paracanthurus hepatus</i>	4	0	4	8	
5	Tomato Clownfish	<i>Amphiprion frenatus</i>	5	5	6	16	
6	Long-spine porcupinefish	<i>Diodon holocanthus</i>	3	0	4	7	
7	Teira batfish	<i>Plataxteira</i>	6	5	5	16	
8	Domino damselfish	<i>Dascyllus trimaculatus</i>	6	5	6	17	
9	Lionfish	<i>Pterois volitans</i>	4	0	3	7	
10	Oriental Sweetlips	<i>Plectorhinchus vittatus</i>	5	5	5	15	
11	Golden Rabbitfish	<i>Siganus guttatus</i>	6	6	6	18	QuangNinh NhaTrang KhanhHoa
12	Sailfin tang	<i>Zebrasoma veliferum</i>	4	3	4	11	
13	Moorish Idol	<i>Zanclus cornutus</i>	5	4	5	14	
14	Royal angelfish	<i>Pygoplites diacanthus</i>	4	0	3	7	
15	Common Clownfish	<i>Amphiprion ocellaris</i>	10	8	10	28	
<b>Total</b>			<b>78</b>	<b>56</b>	<b>77</b>	<b>211</b>	

### 3. Results and discussion

In the study, a total of 211 marine ornamental fish of 15 species were sampled at 2 – 3 sampling batches corresponding to the spring, summer and autumn time. Three to ten fish individuals from each fish species were sampled for each sampling batch. Most of the fish used in the study were captured from NhaTrang – KhanhHoa (almost 90 %); other small numbers (10 %) were sourced from Ha Long – QuangNinh (table 1).

The prevalence of *Cryptocaryon irritans* infections ranged between 33.3 and 100 % for the 15 fish species examined with the overall prevalence of 66.8 % (table 2). Previous research by Diggles and Lester [7] has reported the similar prevalence (66.7%) of *C. irritans* infection on marine wild caught fish in Australia. However, a much lower prevalence (22.44 %) of freshwater “Ich” infecting on juvenile grass carp has been found by Van and Tho *et al.* [11].

**Table 2.** Summary data from 211 fish of 15 species sampled at three sampling batches from Nha Trang and Ha Long to examine for the infection of *C. irritans*. The data of fish length and mean prevalence are present as mean  $\pm$  SE.

STT	Fish species	No of Fish sampled	Mean Fish length (cm)	No of Fish infected <i>C. irritans</i>	Prevalence (%)
1	<i>Rhinecanthus aculeatus</i>	15	15.2 $\pm$ 0.63	5	33.33
2	<i>Cetoscarus ocellatus</i>	14	13.1 $\pm$ 0.54	8	57.14
3	<i>Chaetodon adiergastos</i>	18	7.9 $\pm$ 0.34	15	83.33
4	<i>Paracanthurus hepatus</i>	8	8.4 $\pm$ 0.49	6	75.0
5	<i>Amphiprion frenatus</i>	16	8.9 $\pm$ 1.03	12	75.0
6	<i>Diodon holocanthus</i>	7	13.5 $\pm$ 2.57	6	85.71
7	<i>Platax teira</i>	16	12.3 $\pm$ 1.35	13	81.25
8	<i>Dascyllus trimaculatus</i>	17	8.4 $\pm$ 0.68	10	58.82
9	<i>Pterois volitans</i>	7	11.2 $\pm$ 1.14	7	100
10	<i>Plectorhinchus vittatus</i>	15	12.0 $\pm$ 0.59	12	80.0
11	<i>Siganus guttatus</i>	18	7.8 $\pm$ 0.35	15	83.33
12	<i>Zebrasoma veliferum</i>	11	8.8 $\pm$ 0.53	5	45.45
13	<i>Zanclus cornutus</i>	14	7.8 $\pm$ 0.42	6	42.86
14	<i>Pygoplites diacanthus</i>	7	9.8 $\pm$ 0.63	5	71.43
15	<i>Amphiprion ocellaris</i>	28	5.8 $\pm$ 0.23	18	64.29
Total/mean		211		143	67.77 $\pm$ 7.23

Wide variations of the prevalence of *C. irritans* infections in fish were shown among the sampling batches for each fish species (Table 3). Fish caught in the spring infected by *C. irritans* are at the highest prevalence of 91.03% on average with various fish species infected at 100% of prevalence such as *C. adiergastos*, *P. hepatus*, *A. frenatus*, *P. volitans*. The lower prevalence of *C. irritans* infections was observed in the fish captured during the autumn (64.94 %), while the lowest value occurred on the fish captured during the summer (39.29 %). According to Yoshinaga [14], who conducted a study on the impacts of temperature and dissolved oxygen levels on the development of *C. irritans*, temperatures for the optimal growth of most strains of *Cryptocaryon* are between 23 and 30 °C [10, 14]. The development of the parasite was significantly damaged at the temperature above 31°C and no parasite was recovered from any fish reared at 34°C. These could be the reasons related to the variation of the higher prevalence of parasite infections on fish captured in the spring compared to the summer. A similar trend of growth was also reported on *I. multifiliis*, a freshwater parasite by Van Duijn [15], and on *Centrocestus formosanus*, a trematode parasite infecting common carp [11].

There was a significant difference in the prevalence of *C. irritans* infection among the fish species from the same sampling batch. The extremely high prevalences of infection on the fish groups of lionfish and long-spine porcupinefish were observed at 85.56 – 100% while the other groups of fish such as *Rhinecanthus aculeatus*, *Zebrasoma veliferum* and *Zanclus cornutus* were infected with *C. irritans* at less than 50 % of prevalence. The variation of the prevalence of infections among species could be due to the difference in susceptibility of the fish to the parasites [4,6,16,]. The same results were also reported by Diggles and Lester [7] when these authors examined the infection of this parasite on wild-caught fish from Australia. Differences in the susceptibility of wild hosts may be due to unequal exposure of hosts to infective stages because of differences in host behaviour or ecological niche [7].

Regarding infection intensity, the present results show the mean intensity of *C. irritans* on ornamental fish, which is 7.64 parasites per field of view (4x10 magnification) (table 3). The highest mean intensity was at 2.20  $\pm$  2.27 parasites per field of view on *P. teira* and the lowest intensity at 5.67

$\pm 1.09$  parasites per field of view on *D. trimaculatus*. A previous study done by Diggles and Lester [7] reported a much lower intensity of *C. irritans* at 12.9 parasites per infected fish on wild fish. Another study conducted by Van et al. According to Van *et al.* [13], on infection of *Ichthyophthirius multifiliis* on juvenile of grass carp informed an infection intensity of 6.68 parasites per field of view.

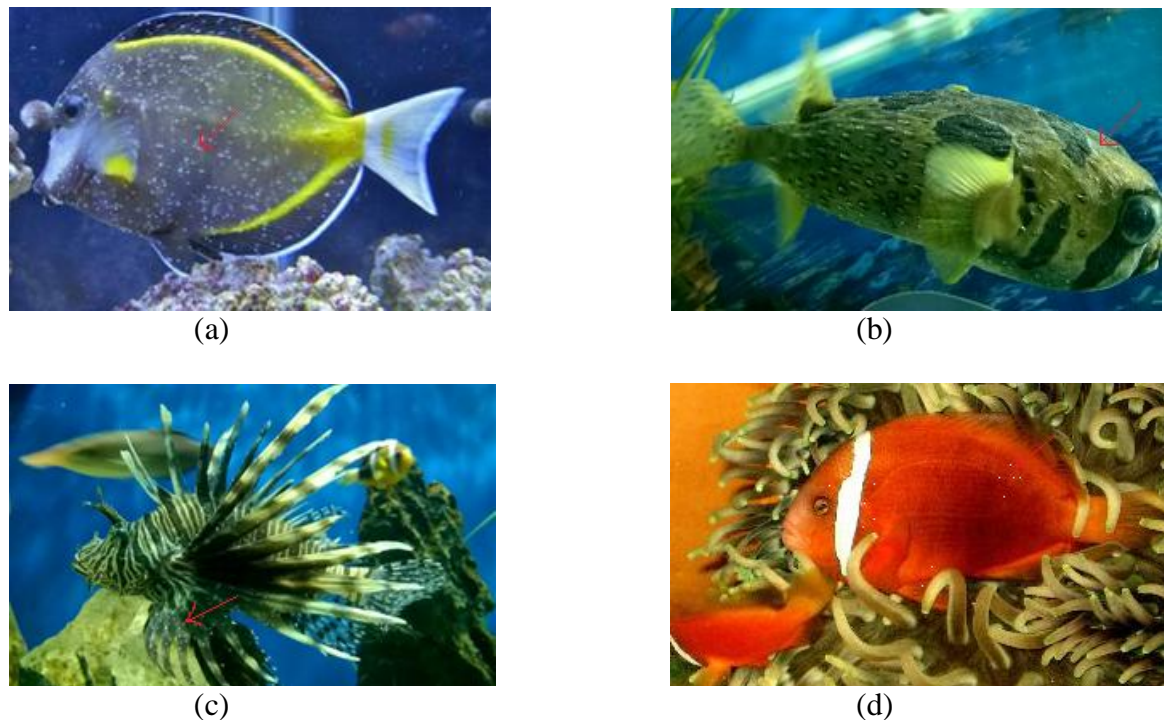
**Table 3.** Prevalence of *C. irritans* infection by seasons and fish species and mean intensity of infection calculated for each species. The symbol “-” means no sample. The data on mean intensity and prevalence are presented as mean  $\pm$  SE. The column with different letters indicates a significant difference.

No	Fish species	Prevalence (%)			Mean Intensity
		Spring	Summer	Winter	
1	<i>R. aculeatus</i>	60	0	40	6.80 $\pm$ 2.39
2	<i>C. ocellatus</i>	80	25	60	7.71 $\pm$ 1.92
3	<i>C. adiergastos</i>	100	66.7	83.3	8.90 $\pm$ 2.30
4	<i>P. hepatus</i>	100	-	50	10.86 $\pm$ 2.03
5	<i>A. frenatus</i>	100	40	83.3	6.13 $\pm$ 1.51
6	<i>D. holocanthus</i>	100	-	75	10.22 $\pm$ 2.29
7	<i>P. teira</i>	100	60	80	12.20 $\pm$ 2.27
8	<i>D. trimaculatus</i>	83.3	40	50	5.67 $\pm$ 1.09
9	<i>P. volitans</i>	100	-	100	7.10 $\pm$ 1.03
10	<i>P. vittatus</i>	100	60	80	8.72 $\pm$ 0.34
11	<i>S. guttatus</i>	100	66.7	83.3	8.22 $\pm$ 1.29
12	<i>Z. veliferum</i>	75	0	50	6.20 $\pm$ 1.27
13	<i>Z. cornutus</i>	60	25	40	7.67 $\pm$ 1.09
14	<i>P. diacanthus</i>	100	-	33.3	6.10 $\pm$ 1.03
15	<i>A. ocellaris</i>	100	25	60	6.78 $\pm$ 1.34
Mean		91.0 <sup>a</sup> $\pm$ 8.08	39.3 <sup>c</sup> $\pm$ 12.08	64.9 <sup>b</sup> $\pm$ 10.72	7.64 $\pm$ 1.34

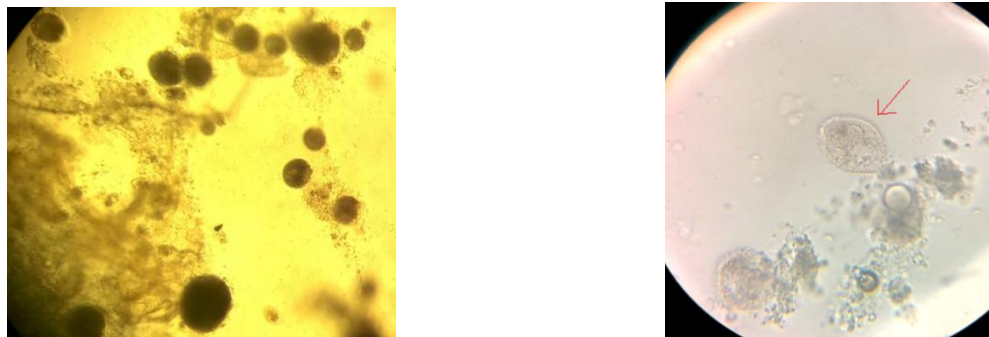
The infected fish with a mean intensity higher than 10 parasites per field of view showed clear white spots on the skin, gills and eyes (figure 1). At the advanced stages of infection, these white spots were visible to naked eyes. Other typical signs were pale fills, ragged fins, changes in skin colour, cloudy eyes and increased mucus production. The behaviours of the infected fish also changed including hanging at the surface or at the sites of incoming water, acting lethargic, scratching (flashing) or swimming abnormally, and rapidly breathing due to the presence of the parasites on gills leading to damages of gill tissue. At this stage, sporadic to mass mortality of infected fish occurred. Similar typical signs of infected fish were described in various studies on infection of *C. irritans* [4,17].

Under the microscopes, the parasites appeared in a round, spherical or oval shapes, measuring from 180 to 700  $\mu$ m, depending upon the age of infection and stage of maturity (figure 2). Throughout the body of the infected fish, the parasite emerged within the epidermis of skin and also in the epithelia of gills. Invasions of subepidermal tissues have previously been attributed to advanced stages of the disease when underlying tissues are exposed by erosion and peeling of the epithelium.





**Figure 1.** White spots appeared on skin and fins of infected fish (a) *P. hepatus*; (b) *D. holocanthus*; (c) *P. volitans* (d) *A. frenatus*



**Figure 2.** *Cryptocaryon irritans* identified by examining the fresh skin and gill tissues of the infected fish under the microscope (x4; x10). The parasites appeared in a round to oval shape and greyish structures due to various life stages of the parasite.

#### 4. Conclusion

It is clear that *Cryptocaryon irritans* is common all yearround in ornamental marine fish sampled in Vietnam. However, the prevalence and intensity of the infection vary widely with the fish host and captured seasons. Some species are very susceptible to the parasite infection (up to 100 % infected) while others are infected at a much lower rate (less than 50 % infected). A seasonal variation in the prevalence and intensity of *C. irritans* infection was also clearly presented. The highest prevalence of infection occurred during the spring and the lower values were shown in the summer. These findings provide important information which can be employed by aquarists in keeping and treating this parasite for ornamental fish in a confined condition.

## 5. References

- [1] Colorni A and Burgess P 1977 *Aquar. Sci. Conserv.* **1** 217-238
- [2] Wrighta, AG and Colornib, A 2002 *Eur. Jof. Protistology.* **37** 375-378
- [3] Yoshinaga T, Dickerson HW 1994 *J. Aquat. Anim. Health.* **6** 197-201
- [4] Colorni A and Burgess P 1997 *Aquar. Sci. Conserv.* **1** 217-238
- [5] Yanong R P E 2009 *Cryptocaryon irritans* infections (marine white spot disease) in fish. In: UF/IFAS Extension FA p 164
- [6] Colorni A 1985 *Dis Aquatic Organism.* **1** 19–22
- [7] Diggles B K and Lester R J G 1996 *Dis Aquatic Organism.* **25** 159–167
- [8] Diggles B K and Lester R J G 1996 *Jof Parasitol.* **82** 45–51
- [9] Yambot A V, Song Y L and Sung H H 2003 *Dis Aquatic Organisms* **54** 147–156
- [10] Dickerson H W 2006 *Int. Cambridge MA* **1** 116–153
- [11] Van K V and Tho N V 2013 Van K V and Tho N V 2013 *J Veterinary Sci Tech* **20** 74-81
- [12] Ha Ky and Te B Q 2007 Vietnam Freshwater Fish. *J. Sci Technol.* 212-213
- [13] Vn K V, Tuyen N V, Hoai T D and An N T 2015 *J.Sc. Devel.* **13** 200-205
- [14] Yoshinaga T 2001 *Fish. Pathol.* **36** 231–235
- [15] Van Duijn C Jr 1967 *Diseases of Fishes* 2 (London: Illiffe Books)
- [16] Wilkie D W and Gordin H 1969 *California Fish and Game* **55** 227–236
- [17] Noga E J 1996 *Fish disease: diagnosis and treatment* Mosby-Year Book Inc St Louis MO 367