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Cryptocaryon irritans Brown 1951, the cause of 'white spot disease' in marine fish: an update

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Cryptocaryon irritans Brown 1951, a holotrichous ciliate parasite of marine fishes, causes 'marine white spot disease'. In aquaria, *C. irritans* can cause acute damage and heavy mortalities to marine teleosts. Although first described 60 years ago, only within the last decade has detailed information emerged concerning its life cycle, transmission and pathogenesis. An update of our knowledge of this important aquarium fish parasite is presented here.

KEYWORDS: *Cryptocaryon irritans*, white spot disease, ciliate, Colpodida, parasitic protozoa

INTRODUCTION

In the confines of an aquarium, many protozoan parasites encounter ideal conditions for proliferation. Among the most important and potentially devastating are the protozoan ectoparasites. By feeding on the skin and gills they cause irritation, epithelial hyperplasia, respiratory difficulty and lesions that often become secondarily infected with bacteria or fungi.

Cryptocaryon irritans Brown 1951 is a holotrichous ciliate that parasitizes marine fishes in temperate and tropical seas. In the wild, heavy infections are presumably rare because of the generally low density of its hosts and its relatively short parasitic stage. However, under aquarium conditions *C. irritans* can overwhelm an entire fish population in a few days (de Graaf, 1962; Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969; Hignette, 1981; Ayroud, 1982; Giavenni, 1982).

Cryptocaryon irritans invades the skin, eyes and gills, impairing the physiological function of these organs. The clinical signs of cryptocaryonosis include pinhead-sized whitish nodules (Fig. 1), mucus hyperproduction, skin discoloration, anorexia and respiratory distress. Infected fishes can exhibit behavioural changes, presumably due to pruritus, such as fin tremors, hyperactivity and sudden darting movements. In severe cases, fish become lethargic and hover just beneath the water surface and have corneal cloudiness and ragged, opaque fins due to sloughing skin. The gills are often pale with lamellae clumped together by excess mucus.

Fig. 1. Grey mullet (*C. labrosus*) heavily infected with *C. irritans*. The fish is 70 mm total length.

HISTORY OF *C. IRRITANS*

Cryptocaryon irritans may have first been observed over a century ago when Kerbert (1884) reported an infection similar to ichthyophthiriosis in *Mustelus* and *Acanthias*. However, these are elasmobranchs, which are considered to be naturally resistant to *C. irritans* (Lom, 1984). Sikama (1937) first described 'a ciliate parasite' affecting over 45 species of marine fishes in aquaria of the Tokyo Imperial University Institute for Fisheries. This disease was remarkably similar to the ichthyophthirosis of freshwater fishes caused by *Ichthyophthirius multifiliis* Fouquet (Sikama, 1937). When he later published the work in a more extensive form in German, Sikama (1938) referred to it as 'Weisspünktchenkrankheit' or 'white spot disease' of marine fishes. Later still, Sikama (1960) again discussed the disease in a Japanese article. In the same year, in a Chinese publication, Nie and Lee (1960) called Sikama's ciliate *Ichthyophthirius marinus* sp. nov. pro *Ichthyophthirius* sp. Sikama. It was only in 1961, however, that Sikama (1961, 1962) himself finally named the parasite *I. marinus* sp. nov. Brown (1951), unaware of Sikama's (1937, 1938) reports, named the same organism *C. irritans* based on a preliminary description of the parasite obtained from marine fish of the Aquarium of the Zoological Society of London. Although Brown's (1951) ignorance of Sikama's (1937, 1938) publications was questioned by Canella (1972), who also found the name crypto-caryon (= hidden nucleus) inappropriate, the name given

by Brown has been accepted by the scientific community and *C. irritans* is today the universally accepted name for this parasite.

C. IRRITANS AND ITS ALLEGED 'FRESHWATER COUNTERPART' *I. MULTIFILIIS*

In the popular aquarium literature, cryptocaryonosis is often referred to as 'marine ich' or 'marine white spot disease', implying a close taxonomic relationship between *C. irritans* and *I. multifiliis*. Although living in different aquatic habitats, these two ciliates have several features in common: they are both histophagous ectoparasites, undergo a polymorphic life cycle that includes encystment and share a similar dispersal mechanism represented by actively swimming infective forms. However, recent studies on *C. irritans*' ultrastructure (Colorni and Diamant, 1993; Matthews *et al*., 1993) and molecular biology (Diggles and Adlard, 1995, 1997) show that *C. irritans* and *I. multifiliis* are taxonomically more distant than their extraordinary similarities had led researchers to believe. After comparing partial DNA sequences, Diggles and Adlard (1995) found grounds to justify the present position of *I. multifiliis* within the Oligohymenophorea (order Hymenostomatida) but proposed that *C. irritans* be assigned to the Colpodea (order Colpodida), further widening the taxonomic divergence of these two ciliates by placing them in separate classes. Thus, it appears that the remarkable similarity in survival strategies of these two ciliates results from convergent evolution rather than phylogenetic proximity (Colorni and Diamant, 1993; Diggles and Adlard, 1995, 1997).

Cryptocaryon is a monotypic genus. Studies by Diamant *et al*. (1991) and Colorni and Diamant (1993) suggested that more than one species of *Cryptocaryon* may exist. A Red Sea isolate (Colorni and Diamant, 1993) was similar to the isolate described by Nigrelli and Ruggieri (1966) except for several minor differences, including the presence of a possible protrusible apparatus and more numerous kinetid rows in the Red Sea isolate. These two isolates were considerably smaller than two Australian isolates (Diggles and Lester, 1996a,b), even considering that *C. irritans* varied in size on different hosts and at different temperatures (Diggles and Lester, 1996a). More recently, intraspecific variants of *Cryptocaryon* were confirmed in isolates from geographically distant sites (Diggles and Adlard, 1995, 1997; Diggles and Lester, 1996b). Nevertheless, the diversities reported thus far are not substantial enough to justify the separation of *Cryptocaryon* into different species.

LIFE CYCLE

Crytocaryon irritans has a direct (i.e. does not require an intermediate host), quadriphasic life cycle with parasitic and off-host stages (Fig. 2).

The terminology used for the developmental stages of the Ophryoglenina (Canella and Rocchi-Canella, 1976) is generally accepted despite the fact that recent evidence excluded *C. irritans* from this suborder. Thus, 'phoront' describes the stage after contact with the host is established and attachment ensues, 'trophont' is the parasitic stage feeding on the host, upon leaving the host the mature trophont sheds its cilia and becomes a 'protomont' which

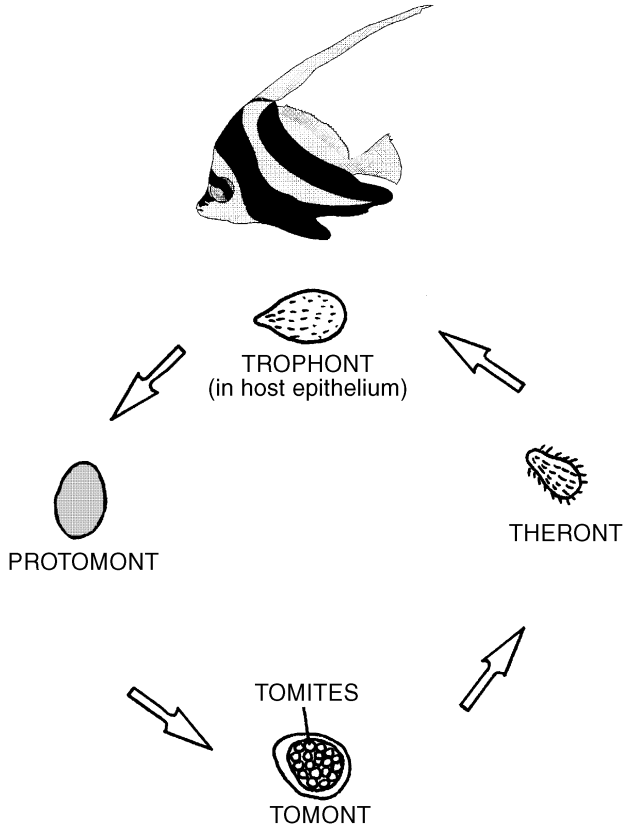


Fig. 2. Life cycle of *C. irritans*. Not to scale.

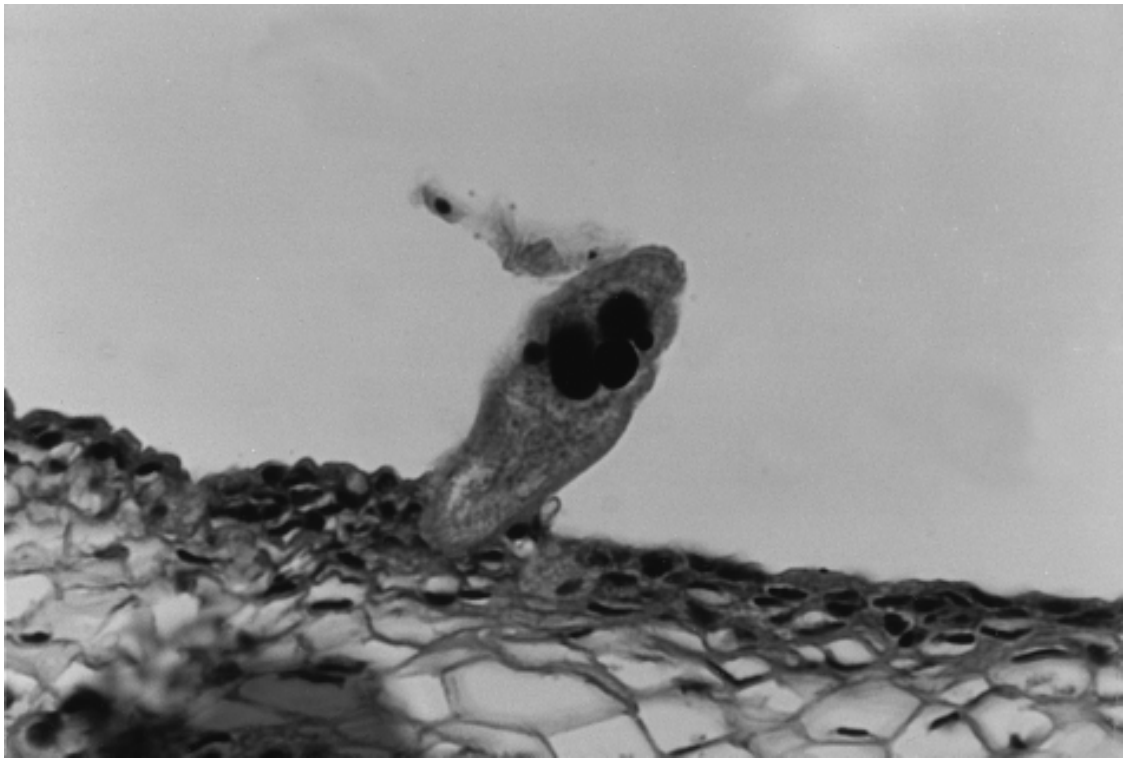
adheres to the substratum and encysts, 'tomont' is the encysted, benthic, dividing stage, 'tomites' are the daughter cells and 'theront' is the excysted, free-swimming, non-feeding, infective stage. The mean duration of *C. irritans*' life cycle is 1–2 weeks at 24–27 °C (Colorni, 1992). However, some tomonts can prolong their cycle to as long as 10 weeks.

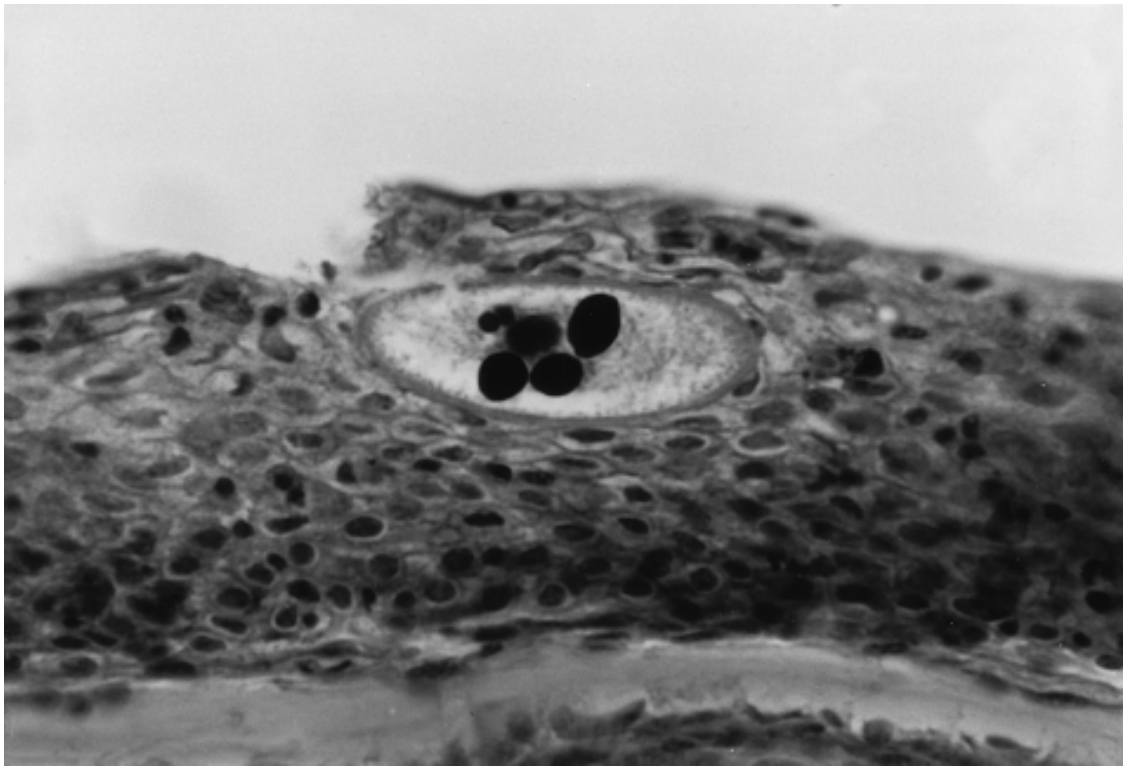
Trophonts

The phoront rapidly becomes a trophont (Figs 3–5). Trophonts are up to 452 μm (Table 1), spheroidal to pear-shaped and revolve continuously within the host epithelium (Fig. 6). The trophonts of a Red Sea isolate fed on body fluids, tissue debris and whole cells of gilthead seabream *Sparus aurata*. Their growth phase culminated when the trophonts spontaneously exited the host after 3–7 days, with a peak at 4–5 days (Colorni, 1985; Colorni and Diamant, 1993) and fell to the bottom as protomonts.

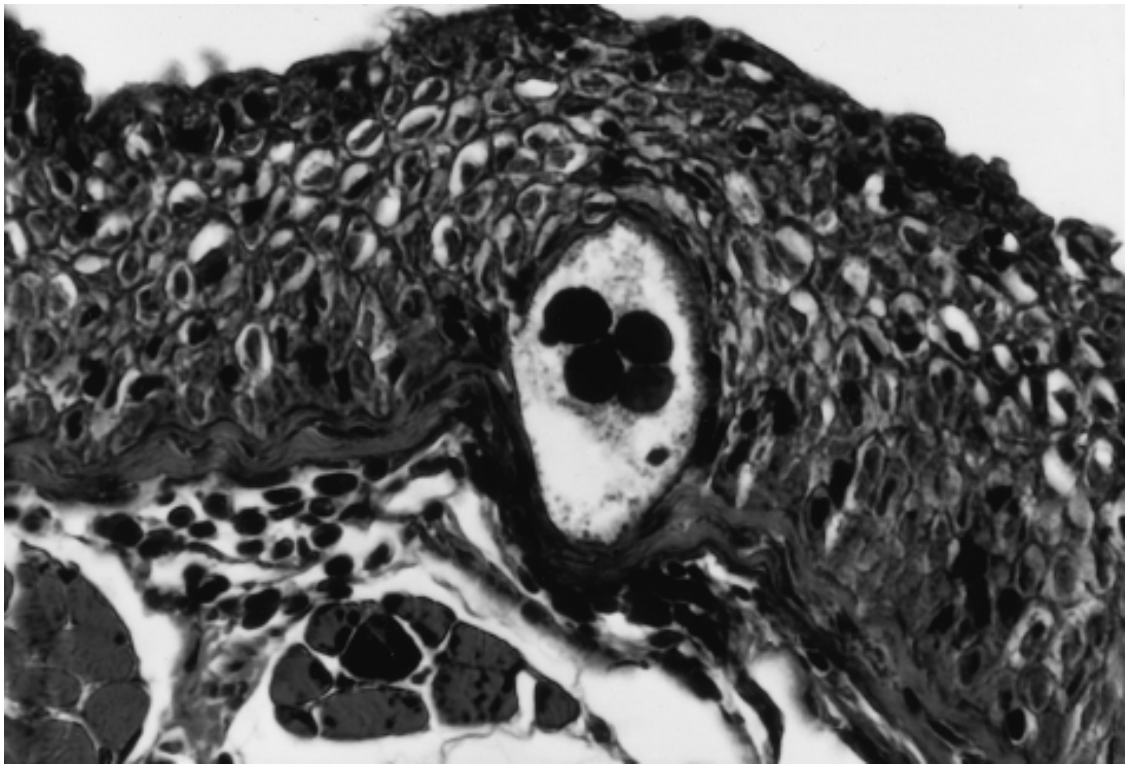
Protomonts

The exit of protomonts is influenced by circadian rhythms, as the great majority exit in early morning darkness (Burgess and Matthews, 1994b). After shedding their cilia and flattening the surface ridges (Matthews *et al*., 1993), the parasites 'crawl' on the bottom usually for 2–8 h (Burgess and Matthews, 1994b), but





Figs 3–5. Sequence of theront penetration into host epithelium and settlement on the germinative layer.



Figs. 3–5. (*continued*)

sometimes for up to 18 h (Colorni, 1992) with active 'probing' movements of the apical pole. After their movements slow to immobility, adhesion to the substratum and encystment ensue, with the cyst hardening in 8–12 h (Colorni, 1985). Trophonts also leave upon death of the host, indicating that the parasites do not exit because of their increased size and consequent rupture of the surrounding host epithelium (Brown, 1963), but rather because of some active process.

Tomonts

Tomonts (w × l) range from 94.5 × 170 to 252 × 441 μm (Nigrelli and Ruggieri, 1966) (Table 1 and Figs 7 and 8). The Red Sea isolate measured 150 × 160 to 310 × 370 μm (Colorni, 1985). The Australian isolates varied from 197 × 684 μm to 210 × 763 μm, according to the water temperature and the species of fish infected (Diggles and Lester, 1996a,b,c) (Table 1). Host death accelerates the transition from trophont to protomont. Trophonts that exit the fish prematurely when the fish dies cannot infect a new host and all encyst within 18 h (Colorni, 1985), including those <24 h old. However, tomonts must be at least 50 × 50 μm to produce viable tomites (Colorni, 1985). Tomonts undergo a sequence of often asymmetric binary fissions, culminating in numerous daughter tomites. Although mature trophonts on the same fish often spontaneously exit within a narrow time period (16–18 h), the tomont development and subsequent

theront release are very asynchronous, even when the tomonts are incubated under identical conditions (Nigrelli and Ruggieri, 1966; Colorni, 1985; Burgess and Matthews, 1994a) (Fig. 8).

Theronts

Theronts are oval to claviform (Fig. 9). Freshly excysted Red Sea theronts are $20-30 \times 50-70\ \mu$m (Colorni, 1985) (Table 1). However, the theront size varies with geographic location, host species and temperature. The Australian trophonts stayed on the fish longer, tomonts took longer to excyst and the theronts were larger when fish were infected at 20 °C compared to 25 °C (Diggles and Lester, 1996a). In the Red Sea isolate, no more than 200 theronts were ever produced by a tomont and their number was positively correlated with the tomont volume. In contrast, an isolate from the Caribbean pomacanthid *Holacanthus tricolor* produced up to 292 theronts per tomont, with a mean of 198 theronts per tomont (Burgess, 1992).

Theronts spiral actively and are attracted to fish. In large numbers, they can kill post-larvae within minutes. The theront's lifespan is less than 24 h for most isolates (Nigrelli and Ruggieri, 1966; Burgess and Matthews, 1994a; Yoshinaga and Dickerson, 1994). Red Sea isolates may commonly survive 36 h and, in a few cases, weak ciliary movements may be seen at up to 48 h (Colorni, 1985). However, the theront infectivity decreases considerably after the first 6–8 h post-excystment (Burgess, 1992; Yoshinaga and Dickerson, 1994; Diggles and Lester, 1996a). A low percentage of infectivity is present at 10–12 h post-excystment, but drops to zero at 18 h (Burgess, 1992). The theront emergence from the tomonts is influenced by circadian rhythms (at least under a laboratory-controlled photoperiod), usually taking place during the dark phase (Burgess and Matthews, 1994b).

ASYNCHRONY OF TOMONT DEVELOPMENT

Even under identical incubation conditions tomonts vary considerably in the time required to form theronts (Nigrelli and Ruggieri, 1966; Colorni, 1992; Burgess and Matthews, 1994a; Diggles and Lester, 1996b). Thus, theront excystment is very asynchronous, occurring between 3 and 72 days and peaking at 6 ± 2 days (Colorni, 1992). This differs significantly from *I. multifiliis*, where the theront excystment takes only 18–24 h at 23 °C (Dickerson and Dawe, 1995).

The reason for asynchronous excystment is unclear. There is no relationship between the tomont size and excystment time (Nigrelli and Ruggieri, 1966; Colorni, 1992; Diggles and Lester, 1996a,b). In fact, a large and a small tomont may produce theronts at the same time, even though the smaller tomont undergoes fewer divisions. When tomites do not form until at least 2 weeks, a mass of endoplasm remains undifferentiated and fewer live theronts are produced (Colorni, 1992). Whatever the cause, asynchronous excystment prevents simultaneous exhaustion of all tomonts, facilitates theront dispersal in time and appears so advantageous to *C. irritans* that the phenomenon should be interpreted as a strategy for survival (Colorni, 1985).

Table 1. Distribution of *C. irritans* in either feral fishes or captive fishes of local or known origin

Bibliographic source	Isolation	Facilities	Geographic source
Sikama (1937, 1938, 1961)	Japan	AQM	Pacific Ocean
Brown (1951)	London	AQM	Unknown
Laird (1956)	Fiji	WLD	Fiji, Pacific Ocean
de Graaf (1962)	The Netherlands	AQM	Ceylon (Sri Lanka), Indian Ocean (?)
Nigrelli and Ruggieri (1966)	New York	AQM	Hawaii and Indo-Pacific Ocean (?)
Wilkie and Gordin (1969)	California	AQM	Hawaii and Indo-Pacific Ocean (?)
Tareen (1980)	Kuwait	MCL	Persian Gulf
Huff and Burns (1981)	Florida	MCL	Northwest Florida and Southeast Alabama
Colorni (1985)	Israel	MCL	Red Sea
Kaige and Miyazaki (1985)	Japan	MCL	Pacific Ocean
Leong and Wong (1988)	Malaysia	MCL	South China Sea
Rasheed (1989)	Kuwait	MCL	Persian Gulf
Gallet de Saint Aurin *et al.* (1990)	Martinique	MCL	Caribbean Sea, Atlantic Ocean
Diamant *et al.* (1991)	Israel	MCL	Mediterranean Sea
Burgess (1992)	London	AQM	Unknown
Xu *et al.* (1992)	China	MCL	South China Sea
Hua *et al.* (1994)	China	MCL	South China Sea
Bunkley-Williams and Williams (1994)	Puerto Rico	WLD	Caribbean Sea, Atlantic Ocean
Diggles and Lester (1996a,c)	Australia	WLD	Southeast Queensland, Pacific Ocean

AQM, public aquariums; MCL, mariculture facilities; WLO, wild fish.

GEOGRAPHICAL DISTRIBUTION

Most reports of *C. irritans* infections involve marine aquaria and closed mariculture systems. There are few published reports of *C. irritans* in wild fishes; however, such data are important for determining the origin of various isolates and their natural distribution (Table 1). Knowing the distribution of *C. irritans* is important in assessing the disease risk associated with the use of local seawater sources for closed culture systems.

Cryptocaryon irritans has been recorded from wild fishes from the coastal waters of Japan (Sikama, 1961; Kaige and Miyazaki, 1985); Heron Island, the Great Barrier Reef, Australia (Diggles and Adlard, 1995); the Red Sea, Israel (Colorni, 1985) and the Caribbean, Puerto Rico (Bunkley-Williams and Williams, 1994). In Fiji, of 36 fish species from a coral reef only one (*Epinephelus merra*) harboured a light *C. irritans* infection (Laird, 1956). In mariculture, *C. irritans* has been a problem in Australia and New Zealand (Hine, 1982; P.M. Hine personal communication), China (Xu *et al*., 1992; Hua *et al*., 1994), Thailand (Tookwinas, 1990a,b), Malaysia (Leong and Wong, 1988), other parts of South East Asia (Cheong, 1990), Kuwait (Tareen, 1980; Rasheed, 1989), the French West Indies (Gallet de Saint Aurin *et al*., 1990) and the southern USA (Huff and Burns, 1981).

Temperature (°C)	Trophont size (μm)	Tomont size (μm)	Theront size (μm)	Maximum number of theronts
20–23	66 × 34–452 × 360	452 × 360	65 × 35	100 or more
	70–400		40–56	
24–26				
22–25	48 × 27–450 × 350	94.5 × 170–441 × 252	56.5 × 35	200 or more
20–26				
23–25		60 × 60–370 × 310	20 × 50–30 × 70	Up to 200
		50–450 × 30–50		
27–30	220–250	220–250	35–55	
24–26	353–205	326–306	40 × 22–69 × 44	292
20		250–450	50	337
15–27		210–763 197–684 221–505		1030

Wilkie and Gordin (1969) identified *C. irritans* on a single opaleye (*Girella nigricans*) collected from a tidal pool near the Scripps Institution of Oceanography, California; they believed that the parasite may have originated in effluent water from the Scripps Aquarium, which had a history of *C. irritans* outbreaks.

Cryptocaryon irritans was considered to be restricted to warmwater marine environments. However Diamant *et al.* (1991) found that *C. irritans* has a counterpart in the cooler waters of the eastern Mediterranean and Diggles and Lester (1996c) collected *Cryptocaryon*-infected fishes from Moreton Bay, Queensland, Australia, where the water temperature can fall to 15 °C. By comparing the rDNA sequences of isolates from Australia, Israel and the USA, Diggles and Adlard (1997) confirmed the existence of warm water and cold water intraspecific variants of *C. irritans*.

TRANSMISSION PATTERNS

To complete its life cycle, *C. irritans* must successfully infect a suitable host. The mechanism of host location is unknown. Although *C. irritans* exhibits weak phototaxis (Nigrelli and Ruggieri, 1966; Burgess and Matthews, 1994b), theronts can infect fish in complete darkness, suggesting that light does not play an

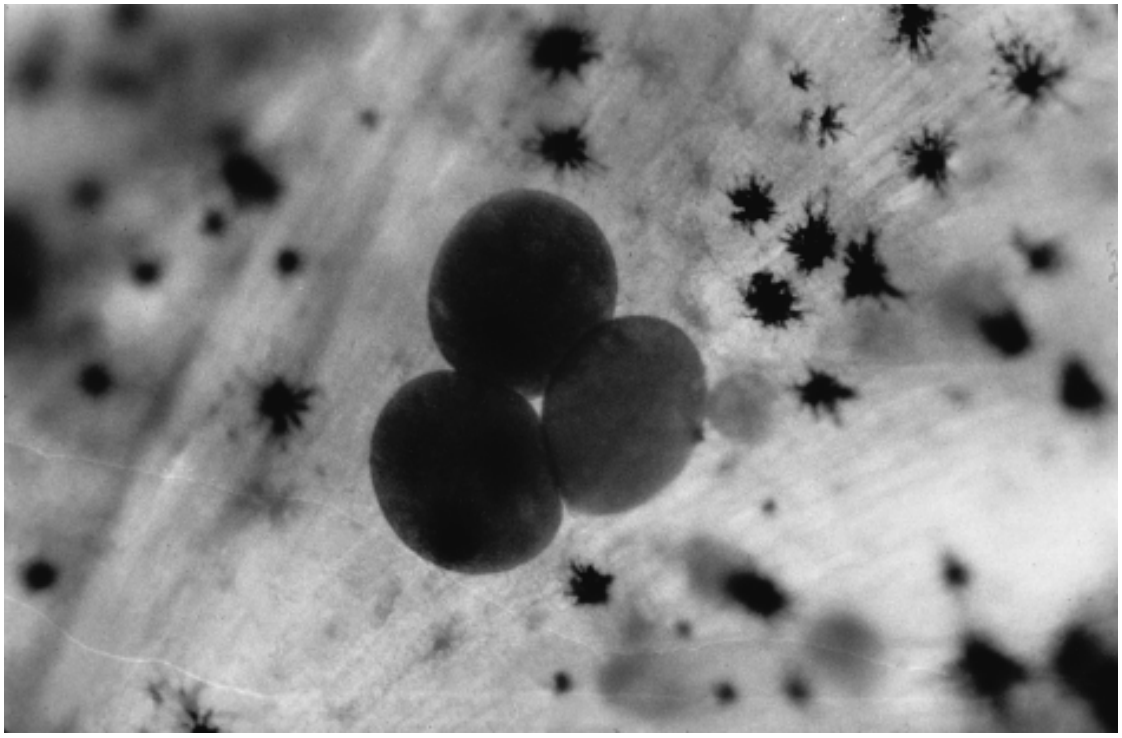


Fig. 6. Cluster of three mature trophonts (approximately 200 μm each). The smaller, dark, stellate bodies are host chromatophores.

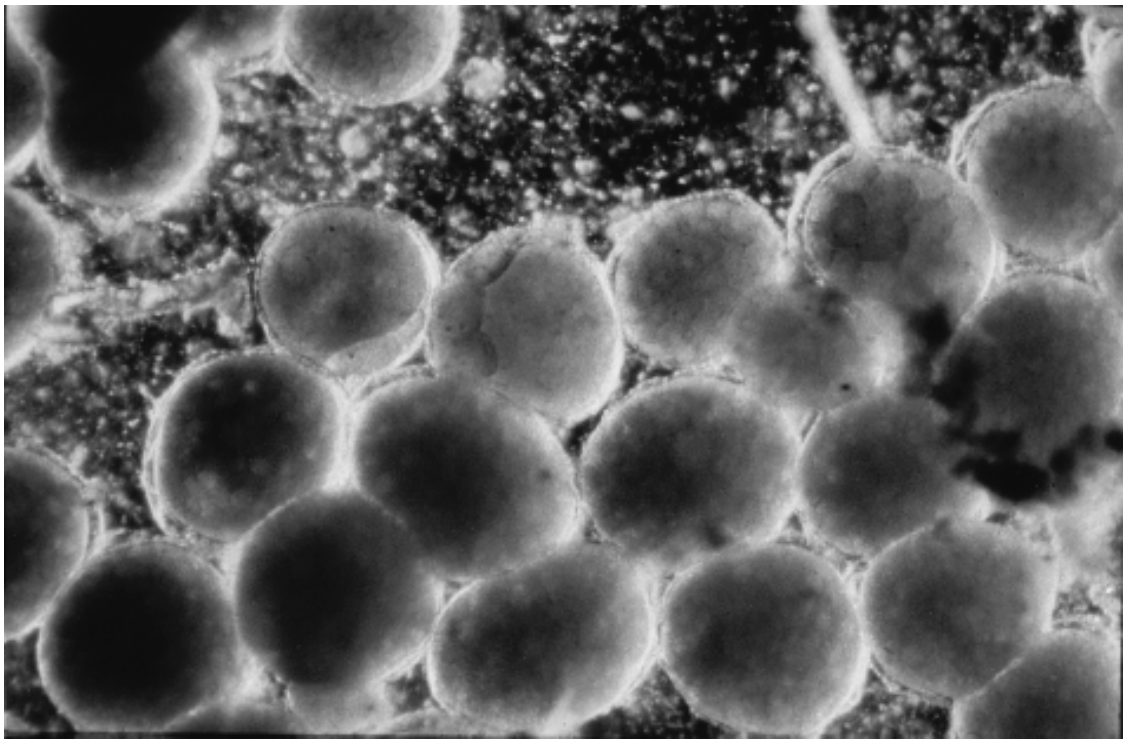


Fig. 7. *In vitro* monolayer aggregation of tomonts.

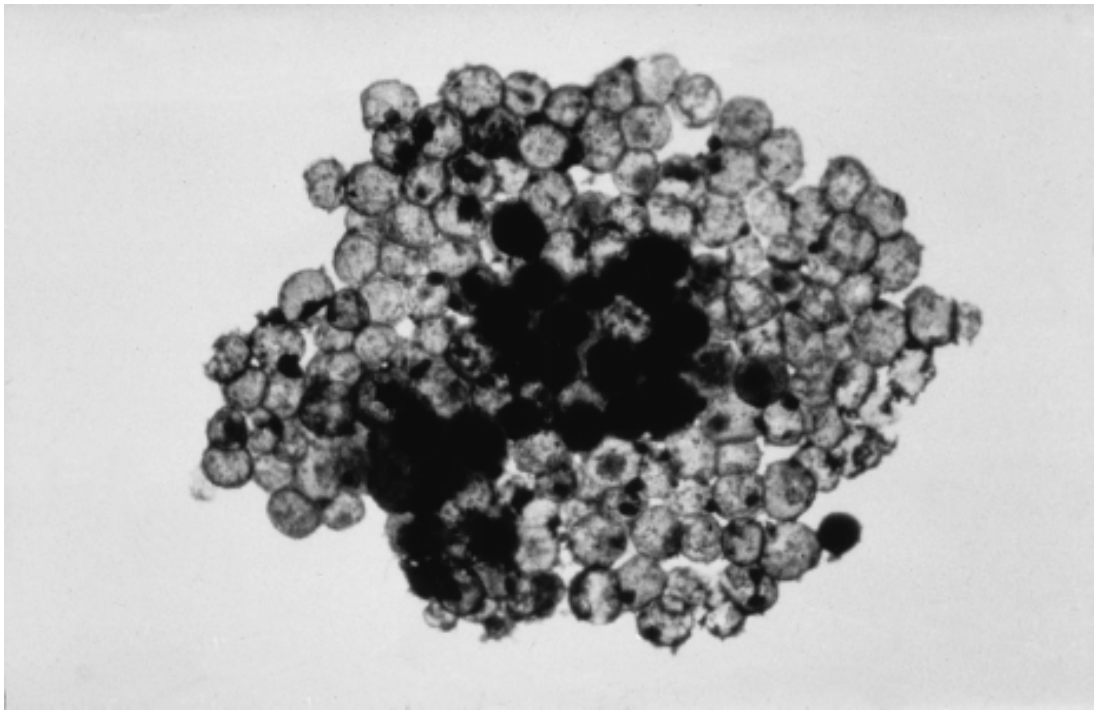


Fig. 8. *In vitro* asynchronous development of tomonts. Dark tomonts contain tomites, whereas the lighter ones are empty 'shells' from which the theronts have excysted.

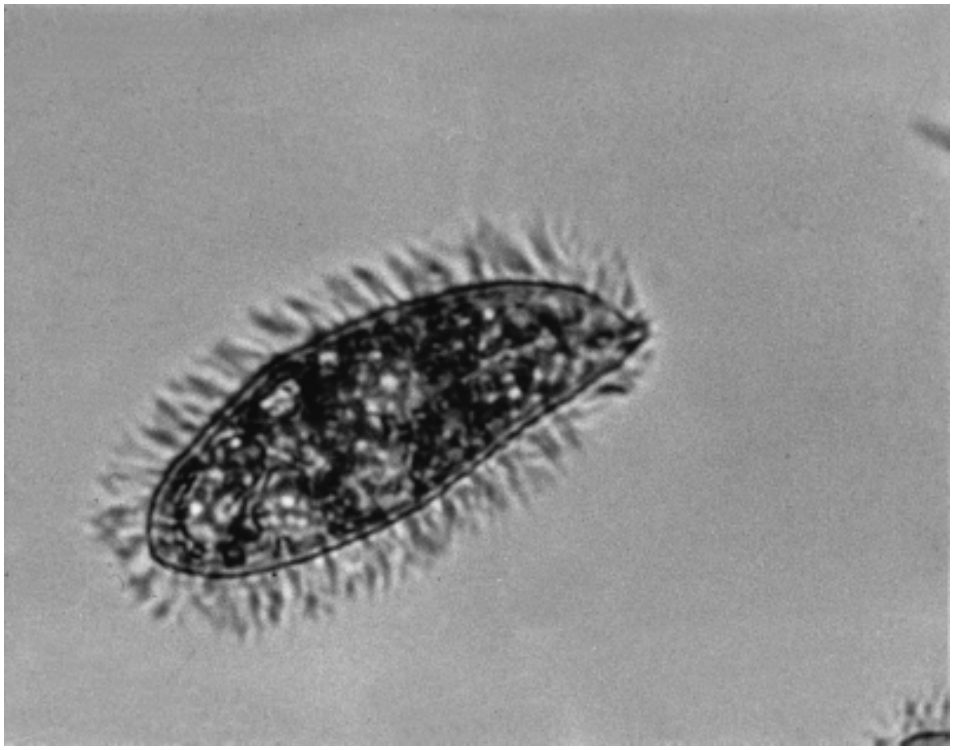


Fig. 9. Free-swimming theront (approximately 60 μm).

important part in transmission (Burgess and Matthews, 1994b). Furthermore, photoreceptors have been identified in other ciliates (Pill-Soon and Walker, 1981) but not in *C. irritans*. Other factors such as chemoattraction require investigation.

The possibility that *C. irritans* randomly contacts a moving fish seems unlikely, especially considering the low density of potential hosts in the natural environment. Although theronts are highly motile (speeds of 5.4 $m\,h^{-1}$ having been recorded under static water conditions; Burgess, 1992), they are much slower than fish. Another constraint on transmission is the limited time (<18 h) during which a theront remains infective.

Laboratory studies (Burgess and Matthews, 1994b) have revealed that mature trophonts exit the host and theronts excyst during darkness. Most reef fishes are inactive at night (Starck and Davis, 1966; Lowe-McConnell, 1987), some being in a state of torpor (Randall, 1968). Burgess and Matthews (1994b) speculated that nocturnal excystation would increase the chances of theronts infecting a host by providing them with a static target. Similarly, the distance to be covered by the protomonts from the host to substratum would be shorter at night if the host is resting near the seabed. The nocturnal exit of the protomonts might also reduce predation by planktonivorous fishes, most of which are diurnal feeders (Burgess and Matthews, 1994b).

Not all theronts successfully locate and infect a host; even under seemingly ideal laboratory conditions, only approximately 5–20% succeed (Matthews and Burgess, 1995). Nevertheless, within an aquarium, the parasite burden can increase approximately 10-fold every 6–8 days (Burgess, 1992). This enormous reproductive potential explains the often rapid build-up of infection levels in aquaria.

HOST SUSCEPTIBILITY

In nature, different fish species appear to vary in their susceptibility to *C. irritans* infections (Diggles and Lester, 1996c). However, in captivity, this phenomenon may be due to a difference in their adaptability to confinement, handling and the environmental conditions. With the exception of elasmobranchs, which are considered resistant (Lom, 1984), *C. irritans* is virtually non-specific in its host selection (Sikama, 1937, 1938; Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969; Burgess and Matthews, 1995a). Consequently, *C. irritans* can cause widespread mortalities in marine aquaria housing a mixed community of fish species. In this respect, it is similar to *I. multifiliis*, which also has a low host specificity (Dickerson and Dawe, 1995). Under experimental aquarium conditions, *C. irritans* can also infect temperate marine fishes held in water of >18 °C, as well as freshwater or brackish water fishes adapted to seawater (Burgess and Matthews, 1995a). There is no evidence that *C. irritans* can infect marine invertebrates or other non-piscine species.

HOST IMMUNITY

The immunological consequences of the intimate and relatively long contact between *C. irritans* and its host were studied by Burgess (1992) and Burgess and

Matthews (1995b) who used the grey mullet (*Chelon labrosus*) as an experimental host. They demonstrated the development of acquired immunity following challenge with theronts, which lasted for up to 6 months. However, many fish were not completely protected and produced a few trophonts upon challenge. This might explain renewed outbreaks of *C. irritans* at very long intervals of time: fish which survive the initial outbreak will develop a certain degree of protection and sustain small numbers of trophonts, thereby enabling the parasite to cycle at low levels in the aquarium. Any event which subsequently diminishes the fish's immunity, such as physiological stress due to adverse environmental conditions or the introduction of non-immune fish to the aquarium, could enable the parasite population to increase rapidly, causing a renewed outbreak of cryptocaryonosis. A similar association between acquired immunity and epidemics has been described for *I. multifiliis* (Dickerson and Dawe, 1995).

HISTOPATHOLOGY OF INFECTIONS

Cryptocaryon irritans trophonts live completely embedded within the host's epithelium, often completing their growth without producing any noticeable harm to the fish. This is in contrast to opportunistic ciliates (e.g. *Tetrahymena* spp., *Uronema marinum*, and *Philasterides dicentrarchi*) that can invade the body deeply and rapidly inflict lethal injuries (see Cheung *et al*., 1980; Dragesco *et al*., 1995). The failure of *C. irritans* to penetrate into the dermis is an intriguing aspect of the relationship between this parasite and its host, perhaps indicating a high degree of host–parasite adaptation (Colorni, 1992).

After contact is made with the host integument, the theront forces its way into the epithelium, disrupting the epithelial cells and settling adjacent to the germinative layer. The invasion process is very rapid and is often completed within 5 min. The tissue damage caused by theront penetration heals rapidly and there is no sign of penetration damage in fish with 1 day old trophonts. The growing trophont gradually displaces the overlying epithelial layers, giving rise to nodules appearing macroscopically as 'white spots'. In general, a moderate primary infection and secondary reinfection do not induce substantial histopathological changes in the surrounding tissues (Colorni, 1992).

Usually only one trophont occupies a site in the host's integument. However, in heavy infections two to four trophonts can occupy the same 'burrow'. Clusters of up to 40 trophonts are often observed between the fin rays. In heavily infected areas, the epithelium shows progressive degeneration and the outer layers may slough. Apoptosis in host cells was recently demonstrated by Yoshinaga *et al*. (1997).

In branchial tissue, the parasites settle on the basement membrane of the gill filament and rapidly (within 20–30 min) become enclosed by a thin layer of epithelial cells. Fusion of the secondary lamellae and irreversible obliteration of the interlamellar spaces occurs only after frequent, heavy reinfections. When infecting the eyes, *C. irritans* settles on the corneal basement membrane. In severe cases, corneal hyperplasia gives the eyes a cloudy appearance.

After the trophont drops from the host, the tissues heal within 1 week, so long as no secondary (e.g. bacterial) infection occurs. Mortalities occur only after successive, severe reinfections (Colorni, 1992).

DIFFERENTIAL DIAGNOSIS

By the second day of infection, the trophonts will normally be barely visible to the naked eye and appear as discrete, small, white nodules. The trophonts are usually most obvious on the fins. On dark or brightly coloured fishes, the white foci are easily visible, but are often hardly noticeable on silvery, whitish or pale-coloured fishes.

Cryptocaryonosis can only be definitively diagnosed by the microscopic observation of the continuously revolving, pear-shaped ciliates in fresh fin or gill clips or skin scrapings. The aquarium hobbyist literature contains reports of diagnostic confusion between *C. irritans* and other parasitic marine ciliates such as *Uronema marinum* (family Philasteridae Kahl, 1971) and *Brooklynella hostilis* (family Hartmannulidae Poche, 1913). The distinguishing features and life cycles of parasitic ciliates of marine fishes have been reviewed comprehensively by Lom and Dyková (1992).

CONTROL MEASURES

The difficulty in eradicating *C. irritans* from marine aquaria and mariculture systems arises from the complexity of its life cycle, in particular the prolonged development of some tomonts and the consequently asynchronous excystment of the infective theronts. In aquarium systems, the control of *C. irritans* currently relies mainly on the use of chemicals.

Chemical methods

A broad spectrum of chemical agents has been used to control *C. irritans* and all are applied as either short- or long-duration baths. Formalin is variably successful under aquarium and mariculture conditions (Herwig, 1978; Moe, 1982; Rasheed, 1989). Dyes such as acriflavine, malachite green and methylene blue have been used either alone or in conjunction with other chemicals, notably formalin (Van Duijn, 1973; Herwig, 1978, 1979; Tookwinas, 1990b). Several antiprotozoal agents originally developed for medical and veterinary applications, notably quinine derivatives (de Graaf, 1973; Kingsford, 1975; Herwig, 1978, 1979; Huff and Burns, 1981), metronidazole (Herwig, 1978) and pyrimethamine (Kingsford, 1975), have also been tried. Other chemicals used against *C. irritans* include potassium permanganate, sodium chlorite, sulphathiazole, nitrofurazone and penicillin (Wilkie and Gordin, 1969; Herwig, 1978, 1979). A number of commercially formulated treatments are also available to the aquarist. Many of these treatments were originally used to control *I. multifiliis* and have not been tested for their effectiveness in controlling *C. irritans*.

Copper, usually as copper sulphate, has been widely used, either alone or in combination with biological dyes and other chemicals such as formalin (Wilkie and Gordin, 1969; Herwig, 1978; Andrews *et al.*, 1988; Untergasser, 1989). However, copper-based remedies are ichthyotoxic and also highly toxic to certain marine invertebrates (de Graaf, 1973; Andrews *et al.*, 1988) which largely precludes their use in systems housing both fish and invertebrates. Furthermore, copper is inactivated in seawater due to interaction with carbonates and other

compounds (Blasiola, 1976; Keith, 1981; Colorni, 1987). Although its activity can be prolonged by using citric acid or acetic acid as a chelating agent (Herwig, 1978), there may be a problem in trying to measure chelated copper with commercial kits. The regular monitoring and adjustment of the copper concentration is necessary in order to maintain a therapeutic level (Wilkie and Gordin, 1969); this problem is compounded in open or semi-closed aquarium systems where the chemotherapeutant is continually diluted. Despite their disadvantages, copper compounds currently rank among the chemical treatments of choice for *C. irritans*.

Brief details of some of the more commonly applied chemicals for treating *C. irritans* are given in Table 2. A critical evaluation of these various remedies is not possible for several reasons. First is the lack of data on the efficacy of different treatments under similar environmental conditions, host species and parasite burdens. Second, most studies on chemical control were undertaken more than 15 years ago, when the biology and life cycle of *C. irritans* were less well known. For example, recent immunological studies on *C. irritans* (Burgess, 1992) suggest that the reduction in infection levels following chemical treatment might, in some cases, be partly due to acquired immunity rather than the treatment alone. Clearly, host immunity to *C. irritans* should be taken into account when assessing the efficacy of chemotherapeutants; *in vitro* studies of the chemical action against the non-parasitic stages of *C. irritans* would therefore seem warranted.

Various stages of *C. irritans* differ in their vulnerability to chemical action. A commercial remedy, based on quinine, *para*-rosaniline and aminoacridine, was shown to be most effective in destroying theronts, less effective against protomonts and ineffective against tomonts when applied at the manufacturer's recommended dosages (Burgess, 1992). Chemotherapeutants added to the water are rarely absorbed through the skin of the fish in a quantity sufficient to affect the trophonts (Herwig, 1978, 1979). As tomonts, the cyst wall of *C. irritans* is similarly impervious to medication (the prolonged period of tomont development in *C. irritans* makes this parasite more difficult to eradicate than *I. multifiliis*). Consequently, chemotherapeutics that destroy *C. irritans* during its parasitic or encysted phase would probably also kill the fish (Herwig, 1978); some of the early literature on chemical treatments should therefore be interpreted with caution. Clearly, an effective chemical treatment that exhibits low ichthyotoxicity is still awaited.

One further consideration when applying chemical treatments is their possible effect on biological filters. The chemicals used to combat *C. irritans* and which are considered toxic to nitrifying bacteria include acriflavin, formaldehyde, methylene blue and malachite green (Cassidy, 1995). Copper sulphate is considered to be non-toxic to nitrifying bacteria when applied at therapeutic levels (0.15–0.25 p.p.m.) (Cassidy, 1995).

As the effectiveness of chemotherapeutants is most likely based on the vulnerability of the protomont and theront stages to chemical action, the timing of the application can be critical, in particular for chemicals which have a limited period of activity. Thus, a chemical treatment may be made more effective if administered after dark, when the trophonts leave the protection of their hosts and the theronts excyst (Burgess and Matthews, 1994b; Yoshinaga and Dickerson, 1994).

Table 2. Chemicals used to control *C. irritans* in aquaria

Chemical	Formulation and dose	Method of application	Bibliographic source
Copper sulphate	0.15–0.25 p.p.m.	Immersion for 3–10 days	Herwig (1978)
Copper sulphate	0.15 p.p.m. (as free Cu) Solubilized with citric acid (1 : 5 citric acid : copper sulphate)	Continuous immersion	Wilkie and Gordin (1969)
Copper sulphate and methylene blue	Copper sulphate 1 g, methylene blue 2 g, citric acid 0.25 g and H_2O 1 l. Dose: 1.25 $cm^3\,l^{-1}$	Immersion. Repeat at half dose on days 4 and 8. Recommended maintainance of copper level between 0.15 and 0.2 $mg\,l^{-1}$	Untergasser (1989)
Formalin	0.2 cm^3 $gallon^{-1}$ [a]	Immersion	Herwig (1978)
Formalin, cupric acetate and Tris buffer	Formalin 100 cm^3, cupric acetate 8 g and Tris 92 g. Dose: 1 cc 25 $gallons^{-1}$ [a]	Immersion. Single treatment usually sufficient, but repeat if necessary	Nigrelli and Ruggieri (1966)
Quinacrine hydrochloride	4–6 mg $gallon^{-1}$ [a]	Immersion. Applied as two doses, given 1 day apart. Strong light inactivates chemical action	Herwig (1978)

[a]US gallon.

Physical methods

Various physical control strategies (Colorni, 1987), each with their own limitations, have been devised in Israel. Contrary to both Herwig (1978) and Cheung *et al.*'s (1979) hypotheses, hyposalinity does not upset the osmotic balance of the trophonts, which are not adversely affected when a prolonged (18 h) freshwater treatment is administered to the host (Colorni, 1992). However, the tomonts are very sensitive to rapid salinity decreases (Colorni, 1985) and four consecutive hyposalinity (8–10 p.p.t.) treatments for 3 h at 3 day intervals can eradicate the disease within 7–10 days. Some tropical fishes though are stenohaline and do not tolerate this treatment well. A second method consists of moving the infected fishes between two different tanks at the same frequency as in the hyposalinity treatment, the tanks being dried and cleaned between uses. These two methods are practical only when limited volumes of water and a limited number of fishes need to be treated. In a third method, a 1–2 cm layer of fine sand is spread uniformly over the bottom of the treatment aquarium. Three days later, the sand is removed by suction and a new layer is deposited. This operation must be repeated four times at 3 day intervals. This treatment offers the protomont a substratum suitable for encystment, yet which is easily removable. The method is practical in aquaria in which fishes co-exist with sponges, corals and other delicate sessile invertebrates that cannot survive any drastic handling. The efficacy of all three methods is based on the fact that the tomonts are damaged, destroyed or removed before they can complete the reproductive process.

Two other methods for the control of *C. irritans* have been reported in the literature: ultraviolet (UV) irradiation and ozonation. UV irradiation at approximately 2537 Å destroys some fish pathogens, including protozoa (Hoffman, 1974; Gratzek *et al.*, 1983). Spotte (1979) estimated from the size of the theront stage that the minimal lethal dose of UV required to destroy *C. irritans* would be 800 000 $\mu W\,s^{-2}$. UV could prevent the spread of *I. multifiliis* between multi-aquarium closed systems, but it is not effective in controlling infections within an individual aquarium (Gratzek *et al.*, 1983); the same is likely to apply to *C. irritans*. Regarding the use of ozone, Wilkie and Gordin (1969) reported that the administration of 8 mg $O_3\,h^{-1}$ in a 15 gallon aquarium prevented infection in opaleye held for 21 days in an infected aquarium.

Immunological methods

The demonstration by Burgess (1992) and Burgess and Matthews (1995b) that fish can develop acquired immunity to *C. irritans* suggests the potential for an immunological approach to controlling cryptocaryonosis. An effective vaccine against *C. irritans* would offer the advantage of a prophylactic rather than a curative approach to control, thereby eliminating the need to administer potentially ichthyotoxic chemical treatments. Unfortunately, no vaccine currently exists for use against *C. irritans*. Furthermore, Burgess (1992) showed that mullet which developed acquired protection to *C. irritans* remained fully vulnerable to infection with *I. multifiliis*. Therefore, the candidate vaccines currently being evaluated against *I. multifiliis* (reviewed by Dickerson and Dawe (1995)) probably will not confer protection against *C. irritans*.

PREVENTIVE MEASURES

In view of the inherent difficulties in eradicating *C. irritans* from aquarium systems, a preventative approach is preferred. Several procedures have been applied to reduce the risks of accidentally transmitting *C. irritans* to uninfected aquaria.

Colorni (1987) described a method for preventing the accidental transmission of *C. irritans* via newly acquired fish. This involved four consecutive movements of the new fish at 3 day intervals between two different isolation tanks (the tanks being dried and cleaned between uses). This method interrupts the life cycle of the parasite and ensures that the fishes can be introduced safely into uninfected aquaria.

Studies on tomont settlement (Burgess, 1992) have indicated that *C. irritans* can encyst on a variety of surfaces, including dead coral, rocks and the moulted exoskeletons of crustacea. The parasite will also encyst on aquarium equipment such as undergravel filters. These studies indicate that aquarium decor and equipment are potential sources of *C. irritans* if transferred directly from one aquarium to another without first being dried or disinfected. Tomonts can be destroyed by immersion for 3 h in freshwater (P.J. Burgess, personal observation).

Seawater can also be a potential source of theronts if obtained from an area where *C. irritans* is endemic. Since theronts are infective for less than 18 h (Burgess, 1992), the storage of seawater for at least 24 h renders it free of *C. irritans*, provided no tomonts or infected fishes are present.

CONCLUDING REMARKS

Recent studies have greatly enhanced our understanding of *C. irritans*, its taxonomic position, geographical distribution, life cycle and transmission. Intraspecific variants of this ciliate have been detected, some existing outside the tropics, in temperate waters. In terms of control strategies, several 'vulnerable' features of *C. irritans* have been uncovered, including the limited lifespan of the theronts and the circadian influence on transmission which can be exploited to render chemical treatments more effective. Although various degrees of success have been recorded in managing cryptocaryonosis, all existing control methods have one or more inherent limitations, being either ichthyotoxic to varying degrees, incompletely effective or unsuitable for use with large volume systems or those housing invertebrates. Although classified as an ectoparasite, by embedding completely in the host's epithelium, *C. irritans* maintains a more intimate relationship with its host than most other ectoparasites. This fact suggests that immunological control may be possible in the future; however the development of a vaccine has been hindered by the requirements for long-lasting effectiveness, the practicality of the delivery route, cost-effectiveness and, as *C. irritans* also affects food fishes, human safety aspects. At present, strict prophylaxis remains the best strategy for maintaining aquaria free of *C. irritans*.

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