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INFLUENCE OF TEMPERATURE AND HOST SPECIES ON THE DEVELOPMENT OF *CRYPTOCARYON IRRITANS*

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ABSTRACT: The course of infection of the parasitic ciliate *Cryptocaryon irritans* was followed on *Lates calcarifer* and *Macquaria novemaculeata* at 20 and 25 C. The parasite was originally isolated from locally caught *Acanthopagrus australis*. At 20 C trophonts stayed on the fish longer, tomonts took longer to excyst, and the resulting theronts were larger than at 25 C. On *L. calcarifer* at 20 C, trophonts grew slowly at first but eventually became significantly larger (mean tomont diameter 466 × 400 μm) than at 25 C (mean diameter 373 × 320 μm). On *M. novemaculeata*, trophonts never grew as large as on *L. calcarifer* and at 20 C they grew poorly. The number of theronts produced per tomont was directly related to the size of the tomont but was not influenced by incubation temperature. The tomont incubation period was not related to the diameter of the tomont but was significantly influenced by the host origin of the tomont. Theront size was also significantly affected by the host origin of the tomont but not the diameter of the tomont. These results show that *C. irritans* exhibits variability in morphometrics on different hosts and under different temperature conditions. This variability needs to be taken into account if utilizing morphometric data for separating strains of *C. irritans*.

The parasitic ciliate *Cryptocaryon irritans* Brown, 1951 causes 'white spot' disease of marine fishes. It is an important pathogen of captive fishes, with many reports of epizootics of *C. irritans* in marine aquaria (Sikama, 1937; Brown, 1951; Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969) and, more recently, in mariculture facilities worldwide (Huff and Burns, 1981; Colorni, 1985; Kaige and Miyazaki, 1985; Rasheed, 1989; Diamant et al., 1991; Leong, 1992). *Cryptocaryon irritans* exhibits low host specificity when fish are confined and is considered a danger to virtually all mariculture where water temperatures are over 19 C. The life cycle of *C. irritans* involves 4 developmental stages (Sikama, 1961; Colorni, 1987) and is similar to that of *Ichthyophthirius multifiliis* Fouquet, 1876 ('Ich' in freshwater fishes). The terminology used here follows that of Lom and Dykova (1992). The histophagous trophont feeds in the epidermis of the skin and gills of the fish. After a period of time the trophont leaves the fish and sinks to the substrate, where it forms a cyst or tomont. The tomont undergoes a series of palintomic divisions producing infective stages or theronts. Upon excystment the theronts are liberated to infect new hosts.

Sikama (1961) regarded *C. irritans* as having a worldwide distribution in tropical waters, but other authors (Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969) considered outbreaks of *C. irritans* in their aquaria to be due primarily to importation of the disease with fishes from other aquaria and not from local wild fish. Indeed, there is very little known about *C. irritans* in wild fish populations and equally little information regarding the existence of different strains or species of *Cryptocaryon*. The possible existence of strains or species of *I. multifiliis* has been suggested (Nigrelli et al., 1976) and recent evidence seems to support this. Clayton and Price (1992) found significant differences in the infectivity of different isolates of *I. multifiliis*, and Dickerson et al. (1993) have shown differences in immobilization antigens of isolates of *I. multifiliis* which have the potential for use as biochemical markers to define strain differences. There also appears to be some evidence for strains of *C. irritans*, as a *Cryptocaryon*-like ciliate from the relatively cool waters of the Mediterranean Sea has been described as different from a tropical (Red Sea) *C. irritans* isolate in some characteristics (Diamant et al., 1991).

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A *C. irritans*-like ciliate is found on estuarine fish around Brisbane, Australia, in waters that seasonally range from 15 to 28 C. Ribosomal RNA studies being conducted in this laboratory will indicate whether there are genetic differences between this and overseas isolates of *C. irritans*, but it is also important to examine characteristics of the isolates in other ways (Thompson and Lymbery, 1990), such as morphometrics (size of trophonts, tomonts, number of theronts/tomont) and morphology (live observation, silver staining, scanning electron microscopy, transmission electron microscopy), to gain a better understanding of differences between isolates. Morphometric comparisons of isolates may be 1 method of identifying strains of *C. irritans*, as there are some variations in the measurements published previously by various authors (Table I). However, it is not clear how factors such as water temperature and host fish species affect morphometric parameters. Hence, we examined the effects of these factors using a local isolate of *C. irritans* to determine whether morphometric data are taxonomically useful.

We examined the infection dynamics of a local isolate of *C. irritans* at 20 C and 25 C. Infection experiments were performed on barramundi *Lates calcarifer* (Bloch, 1790) and Australian bass *Macquaria novemaculeata* (Steindachner, 1866). Both are catadromous fish that are produced by local fish hatcheries. The availability of these fish reared in freshwater from local fish hatcheries allowed infections to be performed with the assumption that all fish were naive to *C. irritans* infections.

MATERIALS AND METHODS

Experimental infections

Juvenile *L. calcarifer* (mean length 1.5 cm) were obtained from The Southern Fisheries Centre, Brisbane, Australia, and maintained at 25 ± 1 C and 10 ppt salinity. Juvenile *M. novemaculeata* (mean length 2.0 cm) were obtained from Abington Fish Hatchery, Childers, Australia, and maintained at 20 C and 7 ppt. A few days prior to experiments all fish were adapted to 30 ppt salinity, which was then maintained throughout. A local isolate of *C. irritans* was obtained from specimens of yellow fin bream *Acanthopagrus australis* (Günther, 1859) captured from Moreton Bay, Brisbane, Australia, and maintained by serial passage through naive *L. calcarifer* held at 26 C and 35 ppt. Newly encysted tomonts were collected with a fine paint brush from the bottom of tanks that had held infected *L. calcarifer*. These were rinsed with several washes of 35 ppt seawater filtered to 0.22 μm and incubated at room temperature (25 ± 3 C). Within 10 hr of the excystment of tomonts, 2–3 ml of the infective theront suspension (approximately 100–200 theronts/ml) was

TABLE I. Comparison of some of the previously published measurements of the various stages of the life cycle of *Cryptocaryon irritans*.

Authority	Brown (1951)	Sikama (1961)	Nigrelli and Ruggieri (1966)	Colorni (1985)	Burgess (1992)
Host species	Various	Various	Various	*Sparus aurata*	*Chelon labrosus*
Temperature	—	20–23 C	22–25 C	24 ± 1 C	25 ± 1 C
Duration of trophont on host (days)	—	—	—	3–7 Peak 4–5	2.9–4.7 Mean 3.5
Incubation period of tomont (days)	—	—	6–9 Peak 8	3–28 Peak 6 ± 2	3.5–35 Peak 5–7
Mean trophont size L × W (μm)	*r*: 70–400 μm	*r*: 66 × 34 to 452 × 360	*r*: 48 × 27 to 450 × 350	—	353 × 205 (*r*: 203 × 129 to 452–332)
Mean tomont size L × W (μm)	—	200–300 (*r*: 90–400)	*r*: 170 × 95 to 441 × 252	241 × 214 (*r*: 160 × 150 to 370 × 310)	326 × 306 (*r*: 160 × 154 to 406 × 369)
Number of theronts/tomont	—	Up to 100 or more	Up to 200 or more	No more than 200	119–292 Mean 198
Mean theront size L × W (μm)	40–56 long	65 × 35	56.5 × 35	57 × 25 (*r*: 50 × 20 to 70 × 30)	56 × 32 (*r*: 40 × 22 to 69 × 44)

applied with a pipette to fish previously anesthetized in a benzocaine bath (100 mg/L). Infections were performed under a dissecting microscope in petri dishes held at a slight angle from the horizontal (head of fish up) so that theronts would not contact the gills. Portions of the rear of the fish body, caudal, second dorsal, and ventral fins were exposed to theronts during this process. Fish were exposed for 3–5 min before being allowed to recover individually in 3-L aquaria containing 30 ppt seawater. Salinity was measured with a refractometer.

Growth of trophonts

Infected fish were maintained individually in 3-L aquaria at either 20 ± 1 C or 25 ± 1 C in temperature-controlled rooms under an approximate 12L:12D photoperiod. The mean length of *L. calcarifer* used in experimental infections was 6.5 cm (*r*: 3.7–9.5, n = 22) at 20 C and 6.7 cm (*r*: 3.7–12.0, n = 16) at 25 C. The mean length of the *M. novemaculeata* used was 6.1 cm (*r*: 4.7–8.6, n = 12) at 20 C and 7 cm (*r*: 4.4–9.0, n = 13) at 25 C. There was no significant difference between species (ANOVA, $P = 0.47$) or temperatures (ANOVA, $P = 0.46$) in the lengths of the fish used. The mean weight of both species of fish was around 5–7 g. All fish maintained at 25 C were examined every day postinfection (PI), whereas fish maintained at 20 C were examined daily from day 2 PI. During daily examinations, fish were anesthetized with benzocaine (100 mg/L), transferred to a petri dish, and examined externally under a dissecting microscope. The number and diameter of the trophonts visible on the body and fins were recorded for each fish, with trophont diameters being measured in situ using a calibrated ocular micrometer. The fish were then placed in a new (scrubbed and dried out) 3-L aquarium to recover and were incubated individually as before. All fish were examined as such for 2–3 days after they showed no further signs of infection.

Collection of tomonts from experimentally infected fish

Aquaria that had previously contained fish from the experiments described above were carefully decanted to leave approximately 1–2 cm of seawater covering the bottom. The aquaria were then placed upon a background of black plastic where the tomonts encysted on the glass bottom were counted, collected with a fine paintbrush, and pipetted into a petri dish where their diameters were measured. The entire bottom of each aquarium was then methodically brushed with a fine paintbrush to dislodge any remaining unseen tomonts and trophonts. These, along with the tank sediment, were then poured into a petri dish and examined under a dissecting microscope where their numbers and diameters were recorded. This process was repeated for 2–3 days after each fish ceased dropping tomonts.

Incubation of tomonts and theront production

Tomonts collected from aquaria containing wild-caught *A. australis* held at 22 C and experimentally infected *L. calcarifer* and *M. novemaculeata* held at 25 C were placed individually in 25-well tissue culture plates and incubated under a 12L:12D photoperiod at either 20 C or 25 C in 3 ml of 30-ppt seawater filtered to 0.22 μm. A total of 460 tomonts was collected from *L. calcarifer* (n = 16, mean length 6.7 cm; *r*: 3.7–12.0 cm), 100 from *M. novemaculeata* (n = 13, mean length 7 cm; *r*: 4.4–9.0 cm), and 328 from *A. australis* (n = 35, mean length 14.3 cm; *r*: 7–26 cm). Tomonts were observed daily and the water was changed every 5 days over an observation period of 15 days. Tomont diameter (measured under a dissecting microscope), number of days to excystment (incubation period), and the number of theronts produced per tomont were recorded. Theronts from freshly excysted tomonts were killed with 2–3 drops of concentrated formalin before counting with a hand counter. The mean length of the theronts produced by each tomont was estimated by measuring a sample of 10–15 formalin-fixed theronts from each tomont under a compound microscope. Tomont and theront diameters were measured with a calibrated ocular micrometer.

Statistics

Statistical analysis of the data was done on SAS software (SAS version 6.02, SAS Institute Inc., Cary, North Carolina). The significance of differences in fish lengths, trophont, tomont, and theront diameters or numbers, and incubation periods amongst species and temperatures was tested by analysis of variance (ANOVA) or general linear modelling (GLM). Values for the probability level (P) are given in the text. Tukey's test was used to distinguish between the significantly different mean values. Probability values of less than 0.01 were considered significant for ANOVA and GLM and less than 0.05 was considered significant for Tukey's test procedures. Regressions were generated by Lotus Freelance Plus version 3.01 software (Lotus Development Corp. Cambridge, U.K.).

RESULTS

Experimental infections

Most of the tomonts incubated to produce theronts for these infections excysted between 0200 and 0600 hr. Consequently, most infections of fish were performed before noon. Theronts applied to the caudal fins of both *L. calcarifer* and *M. novemaculeata* swam with a spiralling motion until they contacted the fish. Some theronts immediately attached to the fish surface with their anterior portion and burrowed into the epidermis in a spiral motion. This process took 30–60 sec in most cases at 22–24 C. Other theronts came in contact with the epidermis but did not penetrate during the 5-min exposure period.

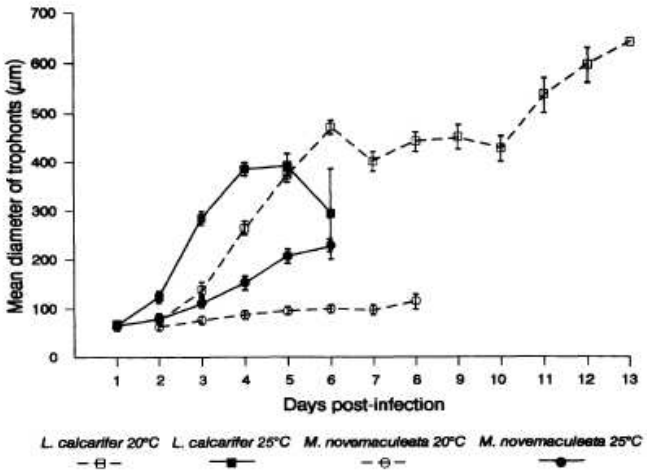

FIGURE 1. Mean diameter of trophonts measured on *Lates calcarifer* and *Macquaria novemaculeata* in experimental infections at 20 and 25 C Bars denote standard errors of means.

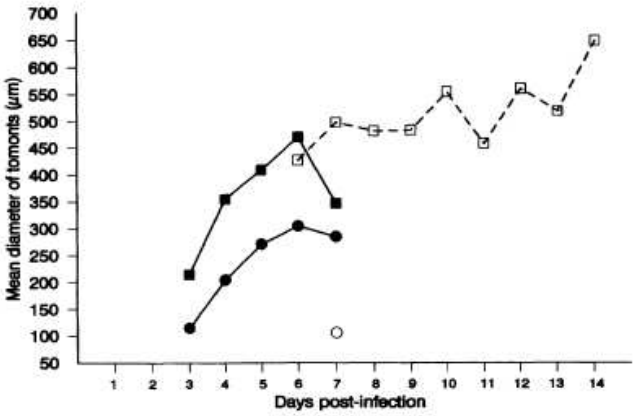

FIGURE 3. Mean diameters of tomonts collected daily from aquaria containing experimentally infected *Lates calcarifer* and *Macquaria novemaculeata* at 20 and 25 C. From *L. calcarifer* at 20 C (—□—), mean 466 μm (*r:* 210–700 μm, n = 756), and at 25 C (—■—), mean 373 μm (*r:* 130–620 μm, n = 827); from *M. novemaculeata* at 20 C (—○—), mean = 106 μm (*r:* 76–136 μm, n = 2), and at 25 C (—●—), mean = 288 μm (*r:* 115–460 μm, n = 270).

Growth of trophonts

Trophont diameter increased with increasing residence time on both species of fish at a rate that varied significantly with water temperature (GLM, $P = 0.0001$) and fish species (GLM, $P = 0.0001$) (Fig. 1).

The diameter of trophonts on *L. calcarifer* at 25 C was significantly larger (GLM, $P = 0.0001$) than at 20 C for days 2, 3, and 4 PI, but by days 5 and 6 PI there was no significant difference in trophont diameter between temperatures (GLM, $P = 0.33$ and 0.07, respectively). The largest trophonts recorded on *L. calcarifer* (770 μm) were measured on day 4 PI at 25 C and on days 6, 8, and 9 PI at 20 C. The diameters of trophonts on *M. novemaculeata* at 25 C were also significantly larger (GLM, $P = 0.0001$) than at 20 C for all days 2–6 PI. The largest trophont recorded on *M. novemaculeata* was 490 μm at 25 C on day 6 PI, whereas at 20 C the largest trophonts were 180 μm, these being measured on days 6 and 7 PI. The diameter of trophonts recorded on *M. novemaculeata* was significantly smaller (GLM, $P = 0.0001$) than on *L. calcarifer* for all days 2–8 PI at 20 C and for days 1–5 PI at 25 C. At day 6 PI at 25 C there was no significant difference (GLM, $P = 0.27$) in trophont diameter between the 2 host species.

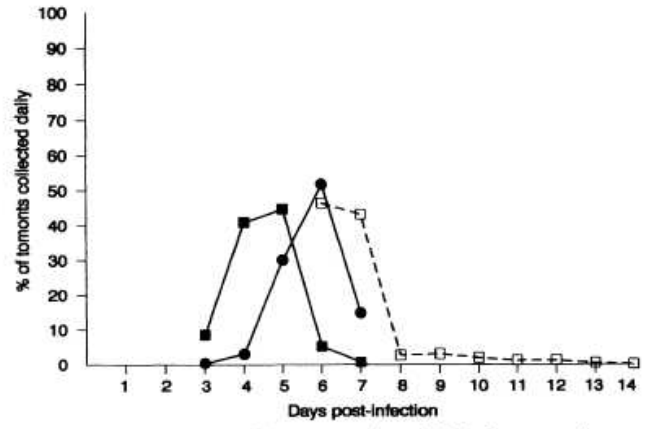

FIGURE 2. Percentage of tomonts collected daily from aquaria containing experimentally infected *Lates calcarifer* and *Macquaria novemaculeata* at 20 and 25 C. Total number of tomonts collected were: from *L. calcarifer* at 20 C (—□—), n = 756, and at 25 C (—■—), n = 827; from *M. novemaculeata* at 20 C (—○—), n = 2, and at 25 C (—●—), n = 270.

Collection of tomonts

Tomonts were collected from *L. calcarifer* at 25 C from day 3 PI, with peak numbers being collected on days 4 and 5 PI, whereas those at 20 C began dropping tomonts on day 6 PI with most being collected by day 7 PI (Fig. 2). The mean residence time of trophonts on *L. calcarifer* was 6.8 days at 20 C and 4.5 days at 25 C. The diameter of tomonts collected from *L. calcarifer* at both 20 and 25 C increased with days PI (Fig. 3) and corresponded to the trophont data in Figure 1. The diameter of tomonts from *L. calcarifer* at 20 C (mean 466 × 400 μm) was significantly larger (GLM, $P = 0.0003$) than at 25 C (mean 373 × 320 μm).

Tomonts from *M. novemaculeata* at 25 C were collected from day 3 PI, with peak numbers being collected on days 5 and 6 PI. Only two tomonts were collected from *M. novemaculeata* at 20 C, these being collected on day 7 PI; however, small trophonts (mean diameter 96 μm, n = 37) were collected between days 3 and 7 PI at this temperature. The mean residence time of trophonts on *M. novemaculeata* was 5.4 days at 20 C and 5.2 days at 25 C. The diameter of tomonts collected from *M. novemaculeata* at 25 C (mean 288 × 250 μm) was much larger than at 20 C (mean 106 × 100 μm).

Tomonts collected from *M. novemaculeata* at 25 C (mean 288 μm) were significantly smaller (GLM, $P = 0.0001$) than those from *L. calcarifer* at this temperature (mean 373 μm). The tomonts collected from *M. novemaculeata* at 20 C (mean 106 μm) were much smaller than those from *L. calcarifer* at this temperature (mean 466 μm).

TABLE II. Data collected from excystment of tomonts collected from experimentally infected *Lates calcarifer* and *Macquaria novemaculeata* at 25 C and from wild *Acanthopagrus australis* at 22 C. Tomonts were incubated at either 20 or 25 C for 15 days.

Incubation temperature	Host origin of tomont											
	L. calcarifer				*M. novemaculeata*				*A. australis*			
	Minima	Maxima	Mean	n	Minima	Maxima	Mean	n	Minima	Maxima	Mean	n
20 C												
Number incubated	—	—	—	225	—	—	—	50	—	—	—	161
Number excysted	—	—	—	108	—	—	—	16	—	—	—	69
% Excysted	—	—	—	48	—	—	—	32	—	—	—	43
Incubation period (days)	6	11	6.9	94	8	10	8.7	16	7	10	7.6	16
Tomont diameter (μm)	230	540	384	82	155	435	322	16	360	720	472	57
Number of theronts/tomont	90	605	248	82	20	330	141	13	100	870	307	57
Mean theront length (μm)	48	86	64	36	61	72	68	12	49	71	59	40
25 C												
Number incubated	—	—	—	235	—	—	—	50	—	—	—	167
Number excysted	—	—	—	87	—	—	—	28	—	—	—	99
% Excysted	—	—	—	37	—	—	—	56	—	—	—	59
Incubation period (days)	3	12	5.2	70	4	6	4.3	28	4	7	5.4	17
Tomont diameter (μm)	155	590	404	82	205	410	317	28	335	640	490	48
Number of theronts/tomont	25	689	258	82	40	240	135	22	85	1,030	363	48
Mean theront length (μm)	38	74	58	36	49	66	61	21	45	63	53	32

Tomont incubation and theront production

The diameter of the tomonts that excysted from *L. calcarifer*, *M. novemaculeata*, and *A. australis* differed significantly between host species (GLM, $P = 0.0001$). Tukey's test showed that tomonts from *A. australis* were significantly larger than those from *L. calcarifer*, which were again significantly larger than those from *M. novemaculeata* (Table II).

There was no significant relationship between the diameter of tomonts and either the length of the tomont incubation period (GLM, $P = 0.81$) or the mean length of the theronts produced by that tomont (GLM, $P = 0.39$) after variation due to temperature was taken into account. However, larger tomonts from all 3 host species produced significantly more theronts (GLM, $P = 0.0001$) (Fig. 4). Temperature did not significantly affect the number of theronts produced per tomont after variation due to tomont diameter was taken into account (GLM, $P = 0.49$).

Both temperature and host origin of the tomont significantly influenced the tomont incubation period (GLM, $P = 0.0001$ and $P = 0.008$, respectively). There was also a significant interaction (GLM, $P = 0.0001$) between the effects of temperature and host species on tomont incubation period. Tukey's test showed this was because at 20 C tomonts from *M. novemaculeata* took significantly longer to excyst (mean 8.7 days) than those from either *A. australis* (7.6 days) or *L. calcarifer* (6.9 days), but at 25 C the situation was reversed, with tomonts from *M. novemaculeata* having significantly shorter incubation periods (mean 4.3 days) than either *A. australis* (mean 5.4 days) or *L. calcarifer* (mean 5.2 days). Overall the tomont incubation period at 25 C was significantly shorter than at 20 C (GLM, $P = 0.0001$) after taking into account variation due to the host origin of the tomont.

Temperature and host origin of the tomont also significantly influenced theront length. Theronts were significantly larger at 20 C than at 25 C (GLM, $P = 0.0001$), and after variation due to incubation temperature was taken into account the theronts from tomonts from *A. australis* were found to be significantly smaller (GLM, $P = 0.0001$) than those from either *L. calcarifer* or *M. novemaculeata*.

DISCUSSION

Water temperature and host species both had significant effects on numerous aspects of the development of *C. irritans* in these experiments. These factors hence must be taken into account if morphometrics are to be used in conjunction with other methods in studies to identify strains of *C. irritans*.

Development of *C. irritans* on these hosts was modified greatly by temperature. At 25 C the residence time of trophonts on both hosts in these experiments were similar to that found by Colorni (1985) for infections of *Sparus aurata* and by Burgess and Matthews (1994b) on *Chelon labrosus* at similar temperatures. At 20 C, trophonts remained on *L. calcarifer* for longer periods of time similar to *Ichthyophthirius multifiliis* at these temperatures (MacLennan, 1942; Ewing and Kocan, 1987). Although initial growth of trophonts on *L. calcarifer* at 20 C was slower than at 25 C, the mean diameter of the trophonts (and consequently tomonts) measured from *L. calcarifer* was larger at 20 C than at 25 C. Smaller trophonts at higher temperatures have also been found for *I. multifiliis*. Trophonts of *I. multifiliis* from carp averaged around 1,000 μm diameter at 2–5 C, 600 μm at 15 C, and 500 μm at 25 C (Wagner, 1960). MacLennan (1942) attributed similar findings of smaller trophonts of *I. multifiliis* at higher temperatures to increased activity of trophonts at higher temperatures causing rupture of the host epidermis sooner than at lower temperatures. It seems from the present study that the increased residence time of trophonts on *L. calcarifer* at 20 C may also contribute to their larger mean diameters, as the few trophonts that remained on *L. calcarifer* for greater than 10 days at 20 C were much larger than those that left the host and encysted earlier.

Throughout these experiments on hosts of both species, there

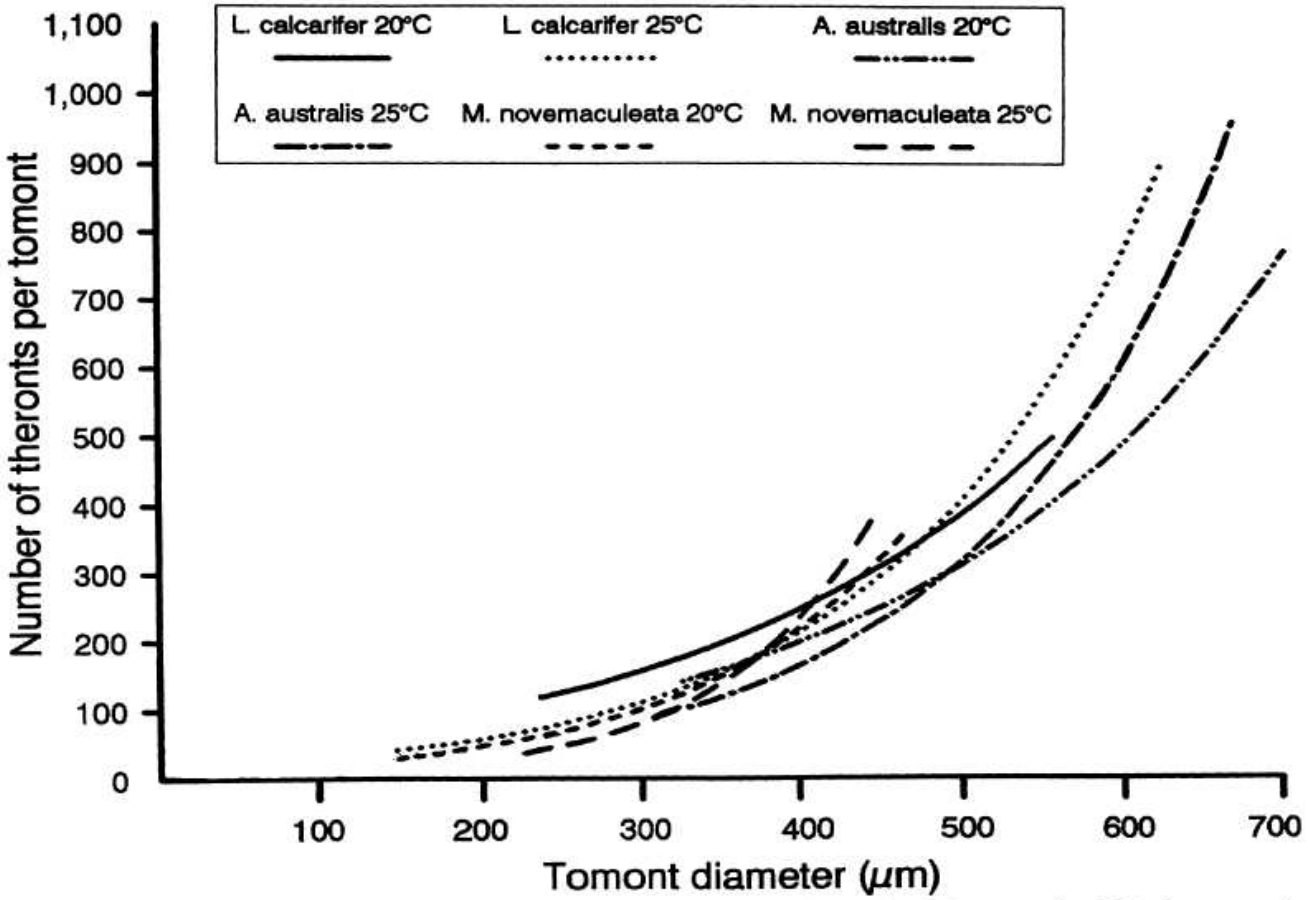

FIGURE 4. Graphic representation of the relationship between tomont diameter and the number of theronts produced. Fitted curves are for exponential regressions (formula $y = ae^{bx}$). The values for each curve are: for tomonts from *Acanthopagrus australis* incubated at 20 C, a = 33.3, b = 0.004, $r^2 = 0.532$ (n = 51); incubated at 25 C, a = 11.7, b = 0.006, $r^2 = 0.669$ (n = 44). Tomonts from *Lates calcarifer* incubated at 20 C, a = 40.9, b = 0.004, $r^2 = 0.465$ (n = 82); incubated at 25 C, a = 15.8, b = 0.006, $r^2 = 0.767$ (n = 82). Tomonts from *Macquaria novemaculeata* incubated at 20 C, a = 10.2, b = 0.007, $r^2 = 0.681$ (n = 22); incubated at 25 C, a = 3.38, b = 0.01, $r^2 = 0.915$ (n = 13).

was a large range of trophont diameters at any given day PI, even though all fish were exposed to theronts for only 5 min. Large variations in the size of trophonts of similar age was also observed for *I. multifiliis* (by MacLennan, 1942), but it has since been shown that some trophonts of *I. multifiliis* reproduce in the host epidermis (Ewing et al., 1988). Division of *C. irritans* trophonts in the host epidermis was not observed in these experiments. Often the number of trophonts observed on a fish and the number of tomonts subsequently collected from that fish were identical, indicating that, in these cases at least, division of trophonts does not occur in *C. irritans* infections.

The mean incubation period of tomonts in these experiments decreased as temperature increased from 20 C to 25 C, as previously found for *C. irritans* by Cheung et al. (1979). These results contrast to the findings of Xu et al. (1991) who in their observations of a Chinese isolate of *C. irritans* recorded the mean incubation period of tomonts from *Pagrosomus major* at 20 C and 25 C to be 4.0 and 7.2 days, respectively. Variations in the tomont incubation period between these aforementioned studies may be due to these authors passaging *C. irritans* on different fish species, as here the host origin of tomonts significantly influenced their mean incubation period. Our findings that the tomont diameter does not significantly affect the tomont incubation period confirms the observations of Nigrelli and Ruggieri (1966) and Colorni (1985).

The temperature at which tomonts were incubated was shown to influence theront size. Those tomonts incubated at 20 C produced significantly larger theronts than those at 25 C. This indicates a trend where at low temperatures large tomonts are produced, which liberate large theronts. It is not known whether large theronts are more effective at finding hosts at lower temperatures, but it could be hypothesized that larger theronts have greater energy reserves and may be infective for longer periods than smaller theronts. In these experiments, when theronts were placed onto fish fins, initial infection time was rapid, with most successful invasions taking less than 60 sec. However, it appears that invasion of fish is a process requiring considerable energy, and the use of theronts greater than 6–8 hr old resulted in low numbers of trophonts becoming established. This agrees with the observations of Yoshinaga and Dickerson (1994), who recorded only 4% recovery from infections using theronts greater than 6 hr old, and Burgess and Matthews (1994a), who calculated maximum theront viability at the moment of excystment. It seems that the timing of excystment of tomonts is a critical

stage in the life cycle of *C. irritans*, and it would be selectively advantageous for strategies to develop that increase both the timespan of theront infectivity and the chances of theronts infecting hosts soon after excystment, when infectivity is highest. One of the latter strategies is known to include excystment being entrained to circadian rhythms (Burgess and Matthews, 1994b; Yoshinaga and Dickerson, 1994), and there may be others, yet unknown.

The relatively poor growth of *C. irritans* on *M. novemaculeata* in these experiments illustrates how the growth of *C. irritans* can vary greatly between hosts of different species. One possible explanation is that *M. novemaculeata* demonstrated innate or acquired resistance to *C. irritans*. Clayton and Price (1992) found significant interspecific differences in the susceptibility of freshwater fish to *I. multifiliis*. They attributed this to the fact that the goodeid fish species *Ameca splendens* and *Ilyodon xantusi* were recently derived from wild populations, and were more susceptible to *I. multifiliis* infections than the poecilid species *Xiphophorus maculatus* and *Iiphophorus variatus*, which had a long history of domestication and presumably were selected for resistance to *I. multifiliis*. A more recent study confirmed the existence of genetic factors in determining the resistance of these fish to *I. multifiliis* infections (Clayton and Price, 1994). The 2 fish species used here, though known to be previously unexposed to *C. irritans*, were obtained from different hatcheries and thus most likely had different histories of exposure to other ciliates. This may explain their differences in susceptibility to *C. irritans* infections.

Another possible explanation for the poor growth of *C. irritans* on *M. novemaculeata* could be variation between fish species in their susceptibility to stress associated with abnormal temperatures. At 20 C, *L. calcarifer* is below its preferred temperature range in the wild, i.e., 23–36 C (Russel and Garrett, 1985). In comparison, *M. novemaculeata* prefers waters between 12 and 24 C (Battaglene et al., 1989), hence the substantial growth of *C. irritans* on *L. calcarifer* at 20 C could be a result of the stress that occurs in fish when temperatures are not optimal (Elliot, 1981). Furthermore, the isolate of *C. irritans* used here, though collected from wild *A. australis*, was maintained by serial passage using naive *L. calcarifer* as the host species. It may be possible that prolonged exposure of *C. irritans* to a particular host may lead to a predisposition for infecting that host, in this case *L. calcarifer*.

The trophonts and tomonts of *C. irritans* collected from *L. calcarifer* and *A. australis* in the present study were on average substantially larger than previously recorded. However, the residence time of trophonts on the host and the tomont incubation periods were similar at given temperatures to those recorded previously and are not influenced by tomont diameter. It seems, therefore, that infections of *L. calcarifer* and *A. australis* will increase at greater rates at a given temperature than infections recorded in previous studies. This is because the reproductive capacity of tomonts is directly related to their diameter (Sikama, 1961; Nigrelli and Ruggieri, 1966; Colorni, 1985; this paper). Tomonts that average around 250 μm diameter, like those recorded in previous studies, only produce around 200 theronts (Sikama, 1961; Nigrelli and Ruggeri, 1966; Colorni, 1985; Burgess and Matthews, 1994a). In the present study, the mean diameters of tomonts collected from *L. calcarifer* and *A. australis* were substantially larger and produced many more theronts, so infections of both *L. calcarifer* and *A. australis* under these conditions would seem to have the potential for being even more devastating than previously recorded for *C. irritans* on fish of other species.

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LITERATURE CITED

BATTAGLENE, S. C., P. J. BEEVERS, AND R. B. TALBOT. 1989. A review of research into the artificial propagation of Australian Bass (*Macquaria novemaculeata*) at the Brackish Water Fish Culture Research Station, Salamander Bay, 1979 to 1986. Fisheries Bulletin 3, NSW Agriculture and Fisheries, 11 p.

BROWN, E. M. 1951. A new parasitic protozoan, the causal organism of a white spot disease in marine fish *Cryptocaryon irritans* gen. & sp. n. Agenda of Scientific Meetings of the Zoological Society London **11:** 1–2.

BURGESS, P. J. 1992. *Cryptocaryon irritans* Brown, 1951 (Ciliophora): Transmission and immune response in the mullet *Chelon labrosus* (Risso, 1826). Ph.D. Thesis. University of Plymouth, Plymouth, U.K., 328 p.

———, AND R. A. MATTHEWS. 1994a. A standardized method for the in vivo maintenance of *Cryptocaryon irritans* (Ciliophora) using the grey mullet *Chelon labrosus* as an experimental host. Journal of Parasitology **80:** 288–292.

———, AND ———. 1994b. *Cryptocaryon irritans* (Ciliophora): Photoperiod and transmission in marine fish. Journal of the Marine Biological Association of the United Kingdom **74:** 535–542.

CHEUNG, P. F., R. F. NIGRELLI, AND G. D. RUGGERI. 1979. Studies on cryptocaryoniasis in marine fish: Effect of temperature and salinity on the reproductive cycle of *Cryptocaryon irritans* Brown, 1951. Journal of Fish Diseases **2:** 93–97.

CLAYTON, G. M., AND D. J. PRICE. 1992. Interspecific and intraspecific variation in resistance to ichthyophthiriasis among poeciliid and goodeid fishes. Journal of Fish Biology **40:** 445–453.

———, AND ———. 1994. Heterosis in resistance to *Ichthyophthirius multifiliis* infections in poeciliid fish. Journal of Fish Biology **44:** 59–66.

COLORNI, A. 1985. Aspects of the biology of *Cryptocaryon irritans*, and hyposalinity as a control measure in cultured gilt-head sea bream *Sparus aurata*. Diseases of Aquatic Organisms **1:** 19–22.

———. 1987. Biology of *Cryptocaryon irritans* and strategies for its control. Aquaculture **67:** 236–237.

DIAMANT, A., G. ISSAR, A. COLORNI, AND I. PAPERNA. 1991. A pathogenic *Cryptocaryon*-like ciliate from the Mediterranean Sea. Bulletin of the European Association of Fish Pathologists **11:** 122–124.

DICKERSON, H. W., T. G. CLARK, AND A. A. LEFF. 1993. Serotypic variation among isolates of *Ichthyophthirius multifiliis* based on immobilisation. Journal of Eukaryotic Microbiology **40:** 816–820.

ELLIOT, J. M. 1981. Some aspects of thermal stress on freshwater teleosts. *In* Stress and fish, A. D. Pickering (ed.). Academic Press, London, U.K., p. 209–245.

EWING, M. S., S. A. EWING, AND K. M. KOCAN. 1988. *Ichthyophthirius* (Ciliophora): Population studies suggest reproduction in host epithelium. Journal of Protozoology **35:** 549–552.

———, AND K. M. KOCAN. 1987. *Ichthyophthirius multifiliis* (Ciliophora) exit from gill epithelium. Journal of Protozoology **34:** 309–312.

HUFF, J. A., AND C. D. BURNS. 1981. Hypersaline and chemical control of *Cryptocaryon irritans* in red snapper, *Lutjanus campechanus* monoculture. Aquaculture **22:** 181–184.

KAIGE, N., AND T. MIYAZAKI. 1985. A histopathological study of white spot disease in Japanese flounder. Fish Pathology **20:** 61–64. [In Japanese, English summary.]

LEONG, T. S. 1992. Diseases of brackish water and marine fish cultured in some Asian countries. *In* Diseases in Asian aquaculture, 1. M. Shariff, R. P. Subasinghe, and J. R. Arthur (eds.). Fish Health Section, Asian Fisheries Society, Manila, Philippines, p. 223–236.

LOM, J., AND I. DYKOVA. 1992. Protozoan parasites of fishes. Elsevier, London, U.K., 315 p.

MACLENNAN, R. F. 1942. Growth in the ciliate *Ichthyophthirius* II. Volume. Journal of Experimental Zoology **91:** 1–13.

NIGRELLI, R. F., K. S. POKORNY, AND G. D. RUGGIERI. 1976. Notes on *Ichthyophthirius multifiliis,* a ciliate parasitic on fresh-water fishes, with some remarks on possible physiological races and species. Transactions of the American Microscopical Society **95:** 607–613.

———, AND G. D. RUGGIERI. 1966. Enzootics in the New York Aquarium caused by *Cryptocaryon irritans* Brown, 1951 (=*Ichthyophthirius marinus* Sikama, 1961), a histophagous ciliate in the skin, eyes and gills of marine fishes. Zoologica **51:** 97–102.

RASHEED, V. M. 1989. Diseases of cultured Brown-Spotted Grouper *Epinephelus tauvina* and Silvery Black Porgy *Acanthopagrus cuvieri* in Kuwait. Journal of Aquatic Animal Health **1:** 102–107.

RUSSELL, D. J., AND R. N. GARRETT. 1985. Early life history of Barramundi *Lates calcarifer* (Bloch), in North-eastern Queensland. Australian Journal of Marine and Freshwater Research **36:** 191–201.

SIKAMA, Y. 1937. Preliminary report on the whitespot disease in marine fish. Suisan-Gakukai **7:** 149–160. [In Japanese.]

———. 1961. On a new species of *Ichthyophthirius* found in marine fishes. Science Report of the Yokosuka City Museum **6:** 66–70.

THOMPSON, R. C. A., AND A. J. LYMBERY. 1990. Intraspecific variation in parasites—What is a strain? Parasitology Today **6:** 345–348.

WAGNER, G. 1960. Der Entwicklungszyklus von *Ichthyophthirius multifiliis* Fouquet und der einfluß physikalischer und chemischer außenfaktoren. Zeitschrift fuer Fisherei und deren Hilfswissenschaften **9:** 425–443. [In German.]

WILKIE, D. W., AND H. GORDIN. 1969. Outbreak of cryptocaryoniasis in marine aquaria at Scripps Institution of Oceanography. California Fish and Game **55:** 227–236.

XU, R. L. et al. 1991. Influence of temperature on the development of *Cryptocaryon irritans.* Annual Bulletin of the Society of Parasitology, Guandong Province **12:** 34–36. [In Chinese.]

YOSHINAGA, T., AND H. W. DICKERSON. 1994. Laboratory propagation of *Cryptocaryon irritans* on a saltwater-adapted *Poecilia* hybrid, the black molly. Journal of Aquatic Animal Health **6:** 197–201.