Kuroshio Biosphere
Vol. 5, Mar. 2009, pp. 1-7 + 5 pls.

EFFECT OF CILIATES ON THE BRANCHING CORAL *ACROPORA FORMOSA*: LABORATORY EXPERIMENT AND MICROSCOPIC OBSERVATIONS

By

Shashank KESHAVMURTHY*[1], Kimio FUKAMI[1], and James Davis REIMER[2, 3]

Abstract

In this work, we investigate the mechanisms of ciliate infection in the coral *Acropora formosa*. The results of laboratory experiments showed that healthy corals kept at 24 °C in aquaria and inoculated with cultured ciliates (3-5 cells/ml) remained healthy throughout the experimental period of 120 hours. However, corals kept at 29 °C began to experience tissue loss within three hours of the start of the experiment. There was 40% ± 5% tissue loss within 24 hours of the start of the experiment. The ciliates engulfed the zooxanthellae cells and eventually degraded them. The infection spreaded and killed coral pieces of 5cm in length in approximately 48 hours. Microscopic observations showed destruction of polyps, coenosarc, and the surface of the coenosteum. As the infection spread and the ciliate abundance increased in the aquaria, all coral fragments were killed. Our results show that ciliates act as opportunists feeding on zooxanthellae from physiologically compromised *A. formosa*. Our results suggest that temperature stress plays a role in this coral becoming susceptible to ciliate infection.

Introduction

In recent years there have been many reports concerning diseases occurring in corals (Peters 1997; Harvell *et al*. 1999; Green & Bruckner 2000; Harvell *et al*. 2001; Rosenberg & Ben-Haim 2002; McClanahan *et al*. 2002; Sutherland *et al*. 2004). Many diseases that affect corals have been documented, such as Black Band Disease (BBD) (Antonius 1981), Shut-Down-Reaction (Antonius 1977), microbial infection (Ducklow & Mitchell 1979), and White Band Disease (WBD) (Antonius 1981; Gladfelter 1982). However, with most of the diseases, the etiology is not yet fully understood. Apart from diseases that are caused by bacteria and fungi, there are many other biological agents that are known to affect coral health (Peters 1997;

1. Laboratory of Environmental Conservation, Graduate School of Kuroshio Science (GRAKUS), Kochi University, Nangoku, Kochi 783-8502, JAPAN
2. Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Senbaru, Nishihara, Okinawa 903-0213, JAPAN
3. Research Program for Marine Biology and Ecology, Extremobiosphere Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, JAPAN

* *Corresponding author*; present address: Biodiversity Research Center Academia Sinica, Section 2, 128 Academia Road, Nankang, Taipei, 115, TAIWAN
e-mail: shashank@gate.sinica.edu.tw

Richardson *et al*. 1997; Knowlton & Rohwer 2003). In most cases, the pathogens and their pathogenicity still remain unknown (Sutherland *et al*. 2004).

The first coral-killer ciliate (*Halofolliculina corallasia*) was identified in the Indo-Pacific (Antonius & Lipscomb 2000). The effect of this disease is similar to "band" diseases, such as BBD or WBD (Antonius 1985). This protozoan disease is known as Skeleton Eroding Band (SEB), and is caused by advancing masses of ciliates resulting in tissue loss and damage of the oral skeleton. Winkler *et al*. (2004) also reported this disease caused by the same ciliate species from the Gulf of Aquaba, and later Croquer *et al*. (2006) reported on SEB from the Caribbean.

There have been reports of ciliate infections in corals (*Montastraea cavernosa*) kept in aquariums and the causative agent is also believed to be a protozoan belonging to *Helicostoma*, the infection of which results in the formation of a jelly-like tissue mass in the infected corals (Borneman 2001) and is termed brown jelly infection. However, studies indicate *Helicostoma* sp. to be of freshwater origin (Ishida & Ishibashi 2006). In 2004, two groups (Bourne *et al*. 2004; Willis *et al*. 2004) reported a new syndrome affecting *Acropora* spp. in the Great Barrier Reef and named it Brown Band Syndrome. In this disease, there is loss of tissue from the coral skeleton leaving brown healthy tissue. The disease was found to be restricted to *Acropora* spp. in the Great Barrier Reef.

There have been unsubstantiated reports of similar infections caused by ciliates in branching corals from Okinawa (M. Omori, pers. Comm.). Despite many reports on coral diseases, the phenomena remain generally poorly understood. Here we examine the infection process by simulating temperature stress in corals in laboratory experiments and then microscopically observing zooxanthellae and ciliate interactions.

Materials and methods

Samples: Coral branches of *Acropora formosa* were collected at a depth of 3 meters from coral communities at Otsuki, Kochi Prefecture, Japan (Fig. 1) in August 2006. The

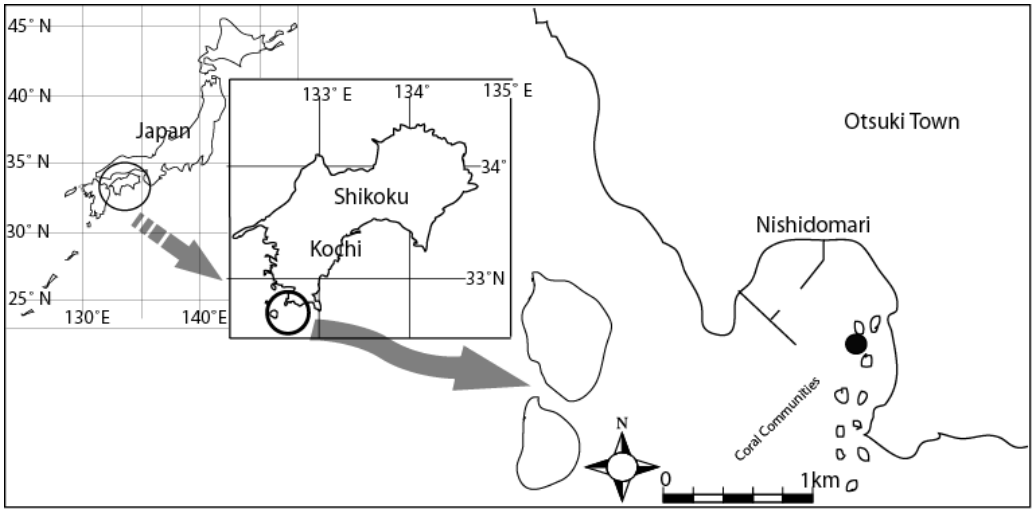


Fig. 1. Map showing the coral communities located in Nishidomari, Otsuki, southern part of Kochi Prefecture, Japan. Sampling location of coral is shown by a circle.

branches were kept in large aquarium tanks (200 liters) for acclimatization for 3-4 days. The ciliates in the seawater were isolated from micro-plate bioassays (Keshavmurthy *et al*. 2007) containing freshly isolated zooxanthellae from *A. formosa*. The ciliates were maintained in the laboratory in a medium (Daigo IMK medium-Wako, Japan) used for marine micro-algae at 120 μEm^{-2} (12 h Light: 12 h Dark) and were supplemented with freshly isolated zooxanthellae.

Experimental setup: After acclimatization, the coral branches were further cut into 4-5cm in length and placed in smaller 5-liter experimental aquaria (Pl. 1) and allowed to acclimatize for an additional two days. Each experimental tank had four coral pieces. All the coral branches used in this experiment were taken from one coral colony ensuring that all were of the same genotype. The experiments were conducted in duplicate aquaria (in all 8 coral branches per treatment) with the following setup (Pl. 1); a) control aquaria 1: only coral (24 $^{\circ}$C, seawater temperature during the sampling); b) control aquaria 2: coral and ciliates (24 $^{\circ}$C, seawater temperature during the sampling); c) experiment aquaria 1: coral and ciliates (28 $^{\circ}$C, summer average seawater temperature at the sampling site); d) experiment aquaria 2: coral and ciliates (29 $^{\circ}$C, +1 $^{\circ}$C more than the summer average) and e) experiment aquaria 3: coral and ciliates (30 $^{\circ}$C, +2 $^{\circ}$C more than the summer average). The ciliate cultures were added into the tanks at the beginning of the experiment using 1ml auto-pipettes. The experiments were carried out in the aquaria with or without ciliate inocula (3-5 cells/ml or 15000-25000 ciliates per tank). Temperatures were maintained using temperature controllers and were checked for temperature differences every 2 hours using a laboratory thermometer. The experiment was carried out for 120 hours. The condition of the coral fragments was monitored every 2 hours throughout the observation period.

Microscopic observations: Coral condition was noted by observing sloughed off tissue samples obtained from the coral fragments under a dissecting microscope. We also checked for the presence or absence of the ciliates in this manner. Coral fragments with ciliates were kept in a small glass dish with seawater and observed under a light microscope (Olympus BX 60 with a Canon EOS digital camera mount) at 200X / 400X, and external video camera (Sony Co.).

Results

Tank experiment: Plate 2 shows the condition of the individual coral branches kept in the experimental aquaria. Ciliates were observed to initially mass on one branch and then subsequently moved to the other branches in the tank. Fragments where ciliates massed then turned white within 6 hours. Coral branches kept at 24 $^{\circ}$C and at 28 $^{\circ}$C remained unbleached and ciliates were not observed to mass on the coral branches throughout the experimental period (Pl. 2a), although they were inoculated with ciliates (3-5 cells ml^{-1}). The corals branches kept in the aquaria at 29 $^{\circ}$C and inoculated with ciliates started to experience tissue loss within three hours of the start of the experiment (Pl. 2b). The corals branches kept at 30 $^{\circ}$C bleached before any massing of the ciliates was observed.

At 29 $^{\circ}$C, the infection started with one branch experiencing massing ciliates, bleaching and experiencing tissue loss (5cm) within 48 hours. After 48 hours, the density of ciliates was almost 3 times (10-15 ± 3 cells ml^{-1}) that of the initial density (3-5 ± 2 cells ml^{-1}) (Pl. 3), which

was determined by counting the ciliates in 1 ml aliquots of seawater sampled from the experimental aquaria. Our observation showed that the ciliates started the infection by dissolution of the coral tissue resulting in the release of zooxanthellae as a loose dense tissue mass.

Microscopic observations: The spread of ciliates was noticed as the swarming movement along the base of the coral branch (Pl. 3d). Our microscopic observations showed that the increases in the ciliate density (Pl. 3 f and h) corresponded with bleached polyps, loss of tissue and finally bare coral skeletons in the affected coral fragments (Pl. 3g. Also, the affected coral skeletons showed that the coenosarc and the surface of the coenosteum were degraded (Pl. 3i). Ciliates were observed to engulf zooxanthellae. The number of zooxanthellae inside each individual ciliate ranged from 50-300 zooxanthellae cells depending on the ciliate size (Pl. 4a). The presence of ciliates feeding on zooxanthellae was observed in the sloughed tissue (Pl. 4b). Photographs taken at different times showed various stages of the spread of the ciliates along with the absorption (endocytosis) of zooxanthellae into the ciliate body and finally, degradation of the zooxanthellae cells (Pl. 5).

Discussion

Reports on ciliate infection in corals have only recently appeared compared to reports on other diseases affecting coral. Colonization of ciliates (Class Oligohymenophora, Subclass Scuticociliatia) in/on coral colonies often results in the death of the coral host (Willis *et al*. 2004; Boyett 2006). It is also speculated that ciliates may not be the direct cause of this disease and that they may be a secondary pathogens after other agents cause tissue necrosis (Boyett 2006). Recently, it has been shown that in ciliate-caused Brown Band Syndrome in infected *A. muricata*, the initial tissue necrosis is caused by *Vibrio fortis* followed by a secondary infection from the ciliates (Boyett 2006).

In this study, we observed that high temperatures (29 $^{\circ}$C) alone may be enough to cause the spread of ciliates in the coral *Acropora formosa*. In this experiment ambient temperatures (24.0 $^{\circ}$C) and summer average ocean temperatures (28 $^{\circ}$C) treatments did not correspond with any appearance of massed ciliates. However, at 29.0 $^{\circ}$C, the coral surface was covered with ciliates and all the branches in these experimental tanks were killed within 120 hours of the start of the experiment (Figs. 3 and 4e).

Similar experiments (Boyett 2006) on the effect of elevated temperatures (27, 28.5 and 30.5 $^{\circ}$C) on the progression of ciliate-caused brown band syndrome in *Acropora muricata* did not show any significant differences in the progression of the disease, suggesting that high temperatures do not enhance the progression of this disease. However, we speculate that corals that become weak due to temperature stress may be easily infected by the ciliates. The spread of ciliates among the stressed corals is fast enough that the effect of bacteria may be minimal. Boyett (2006) found that the time taken for Brown Band Syndrome to transmit from diseased coral branches to healthy coral branch was 1.7 days.

Ciliates also apparently damage the coral skeleton (Pl. 3). Similar erosion of coral skeleton due to ciliate infection was seen in corals affected with a Skeleton Eroding Band (SEB) and has been reported to progress 1mm per day (Antonius & Lipscomb 2000).

Ulstrup *et al*. (2007) have noted that zooxanthellae that are engulfed by the ciliates during the infection process are photosynthetically competent and do not become compromised during the progression of the brown band zone and thus the question remains as to the fate of these zooxanthellae. In our study, the photographs taken during this process showed the polyps completely bleached and devoid of any zooxanthellae (Pl. 3g) and further observations of the affected coral fragments showed that the ciliates were extensively feeding on the zooxanthellae from the tissue and finally degrading the zooxanthellae (Pl. 5). Since we do not know the specific identity of the ciliates infecting corals in this experiment, we cannot confirm if the ciliate is similar to that previously observed in Australian waters (Boyett 2006).

In conclusion, in this study we show that ciliates infect by dissolution of the coral tissue to engulf the zooxanthellae only in those corals subjected to temperature stress and that the engulfed zooxanthellae are ultimately completely degraded. The mechanism(s) by which the ciliates enter inside the coral to feed on the zooxanthellae remains to be studied. Clearly more research is needed on this important topic.

Acknowledgements

KS would like to thank F. Iwase for permitting us to use the laboratory facilities at the Biological Institute on Kuroshio, Otsuki, Kochi, Japan and also helping in the field sampling, and Y. Matoba for transportation support to the field. KS was supported by a Japanese Government (MEXT) scholarship. This work was partly supported by a Sasakawa Scientific Research Grant from the Japan Science Society to KS.

References

Antonius, A. 1977. Coral mortality in reefs: a problem for science and management. In: D. L. Taylor (ed.), Proc. 3rd Int. Coral Reef Symp., Miami, USA. 2: 617–623.

Antonius, A. 1981. The “band” diseases in coral reefs. In: (E, D, Gomez) (ed), Proc. 4th Int Coral Reef Symp, Manila, Phillipines. 2: 7-14.

Antonius, A. 1985. Black band disease infection experiments on Hexacorals and Octocorals. Proc. 5th Int. Coral Reef Congr., Tahiti, 6: 155- 160.

Antonius, A. and D. Lipscomb. 2000. First protozoan coral-killer identified in the Indo-Pacific. Atoll Res. Bull. 481: 1-21.

Bourne, D. G., H. V. Boyett and B. Willis. 2004. Microbiology of Brown Band disease (BRB) affecting *Acropora* corals of the Great Barrier Reef. Abstract 10th International Symposium on Microbial Ecology. Cancun, Mexico August 22-27, 2004.

Borneman, E. H. 2001. Aquarium corals: Slection, husbandry and natural history. TFH Publications. 464pp.

Boyett, H. V. 2006. The ecology and microbiology of black band disease and brown band syndrome on the Great Barrier Reef. M.sc. Thesis, James Cook University, Australia, 136 pp.

Croquer, A., C. Bastidas, D. Lipscomp, R. E. Rodriguez-Martinez, E. Jordan-Dahlgren and H. M. Guzman. 2006. First report of folliculinid ciliates affecting Caribbean scleractinian corals. Coral Reefs 25: 187-191.

Ducklow, H. and R. Mitchell. 1979. Observations on naturally and artificially diseased tropical corals: a scanning electron microscope study. Microbial. Ecol 5: 215-223.

Gladfelter, W. B. 1982. White band disease in *Acropora palmata:* implications for the structure and growth of shallow reefs. Bull. Mar. Sci. 32:639-643.

Green, E. P. and A. W. Bruckner. 2000. The significance of coral disease epizootiology for coral reef conservation. Biol. Conserv. 96: 347-361.

Harvell, C. D., K. Kim, J. M. Burkholder, R.R. Colwell, P. R. Epstein, D. J. Grimes, E. E. Hofmann, E. K. Lipp, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith and G. R. Vasta. 1999. Emerging marine diseases - Climate links and anthropogenic factors. Science 285: 1505-1510.

Harvell, C. D., K. Kim, C. Quirolo, J. Weir and G. W. Smith. 2001. Coral bleaching and disease: contributors to 1998 mass mortality in *Briareum asbestinum* (Octocorallia, Gorgonacea). Hydrobiologia 460: 97-104.

Ishida, H. and M. Ishibashi. 2006. Seasonal changes in species composition of ciliate in the Lake Nakaumi. Jpn. J. Protozool. 39: 29-35. (In Japanese with English summary)

Keshavmurthy, S., K. Fukami and E. Nakao. 2007. Algicidal bacteria in a high-latitude coral community and their effect on zooxanthellae (*Symbiodinium* spp.) isolated from the coral *Acropora formosa*. Galaxea, JCRS 9:13-21

Knowlton, N and F. Rohwer. 2003. Multispecies microbial mutualisms on coral reefs: The host as a habitat. Am. Nat. 162 (Supplement 4): 51-62.

McClanahan, T., N. Polunin and T. Done. 2002. Ecological states and the resilience of coral reefs. Conserv. Ecol. 6: 18 (online) URL: http://www.consecol.org/vol16/iss2/art18.

Peters, E. C. 1997. Diseases of coral-reef organisms. In: Birkeland C (ed) Life and death of coral reefs. Chapman and Hall Publishers, pp 114-119.

Richardson, L. L. 1997. Occurrence of the black band disease cyanobacterium, on health corals of the Florida Keys. Bull. Mar. Sci. 61: 485-490.

Rosenberg, E. and Y. Ben-Haim. 2002. Microbial diseases of corals and global warming. Environ. Microbiol. 4: 318-326.

Sutherland, K. P., J. W. Porter and C. Torres. 2004. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar. Ecol. Prog. Ser. 266: 273-302.

Ulstrup, K. E., M. Khul and D. G. Bourne. 2007. Zooxanthellae harvested by ciliates associated with Brown Band syndrome of corals remain photosynthetically competent. App. Environ. Microbiol. 73: 1968-1975.

Willis, B. L., C. A. Page and C. Dindsdale. 2004. Coral disease on the Great Barrier Reef. In: Rosenberg E, Y Loya (eds) Coral Health and Disease. Springer-Verlag, Berlin, Heidelberg. pp 69-102.

Winkler, R., A. Antonius and D. A. Renegar. 2004. The skeleton eroding band disease on coral reefs of Aqaba, Red Sea. P. S. Z. N. Mar. Ecol. 25: 129-144.

Explanation of Plates

PLATE 1

Experimental setup for the ciliate infection experiment (see Materials and Methods section for details).

PLATE 2

Spread of ciliates and percentage tissue loss in the branches of *A. formosa* infected with ciliates over a period of 120 hours; (a) corals kept at 24 ºC or 28 ºC, (b) corals kept at 29 ºC (n=8 coral branches for each setup). Photographs to the right show the condition of the coral branches in the respective aquaria at the end of the experimental period.

PLATE 3

Progression of ciliates and coral condition in the experimental aquaria kept at 29 ºC; (a) Coral branches at the start of the experiment; (b) Start of the infection in one branch; (c) Tissue loss of 5 cm in 24 hours; (d) Transfer of the infection starts at 36 hours (see Pl. 2b) and (e) Death of all coral fragments kept in the respective aquaria.

Effect of ciliates in the polyp and skeleton at the end of the infection period; (f) Ciliates swarming around the coral branch; (g) Polyps devoid of zooxanthellae; (h) Increase in the ciliate density after 48 hours; (i) High ciliate density around the coral fragment and (j) Degraded coral skeleton.

PLATE 4

Accumulation of zooxanthellae inside the ciliate body. (a) Zooxanthellae inside the ciliate body and (b) Sloughed tissue samples containing the ciliates. Scale bar = 50µm.

PLATE 5

Photographs of zooxanthellae being engulfed. Left: zooxanthellae being engulfed indicated by circle. Right: degraded zooxanthellae indicated by circle. Scale bar = 50µm.

PLATE 1

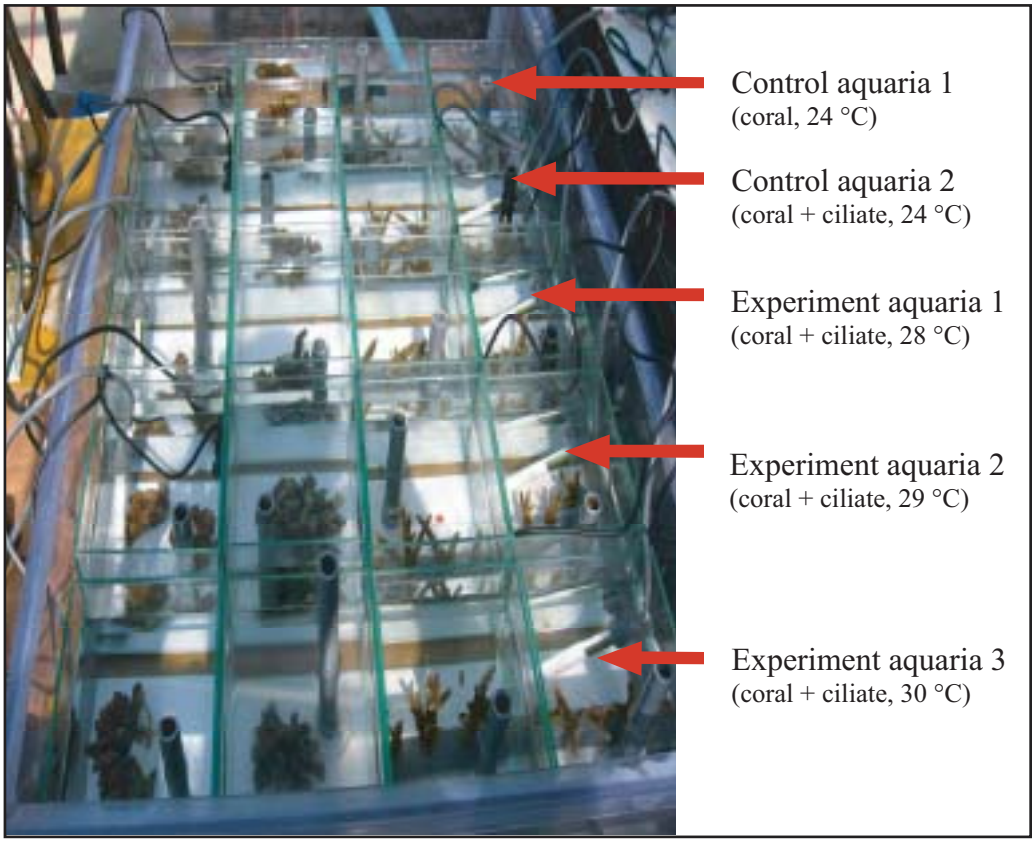

Control aquaria 1
(coral, 24 °C)
Control aquaria 2
(coral + ciliate, 24 °C)
Experiment aquaria 1
(coral + ciliate, 28 °C)
Experiment aquaria 2
(coral + ciliate, 29 °C)
Experiment aquaria 3
(coral + ciliate, 30 °C)

PLATE 2

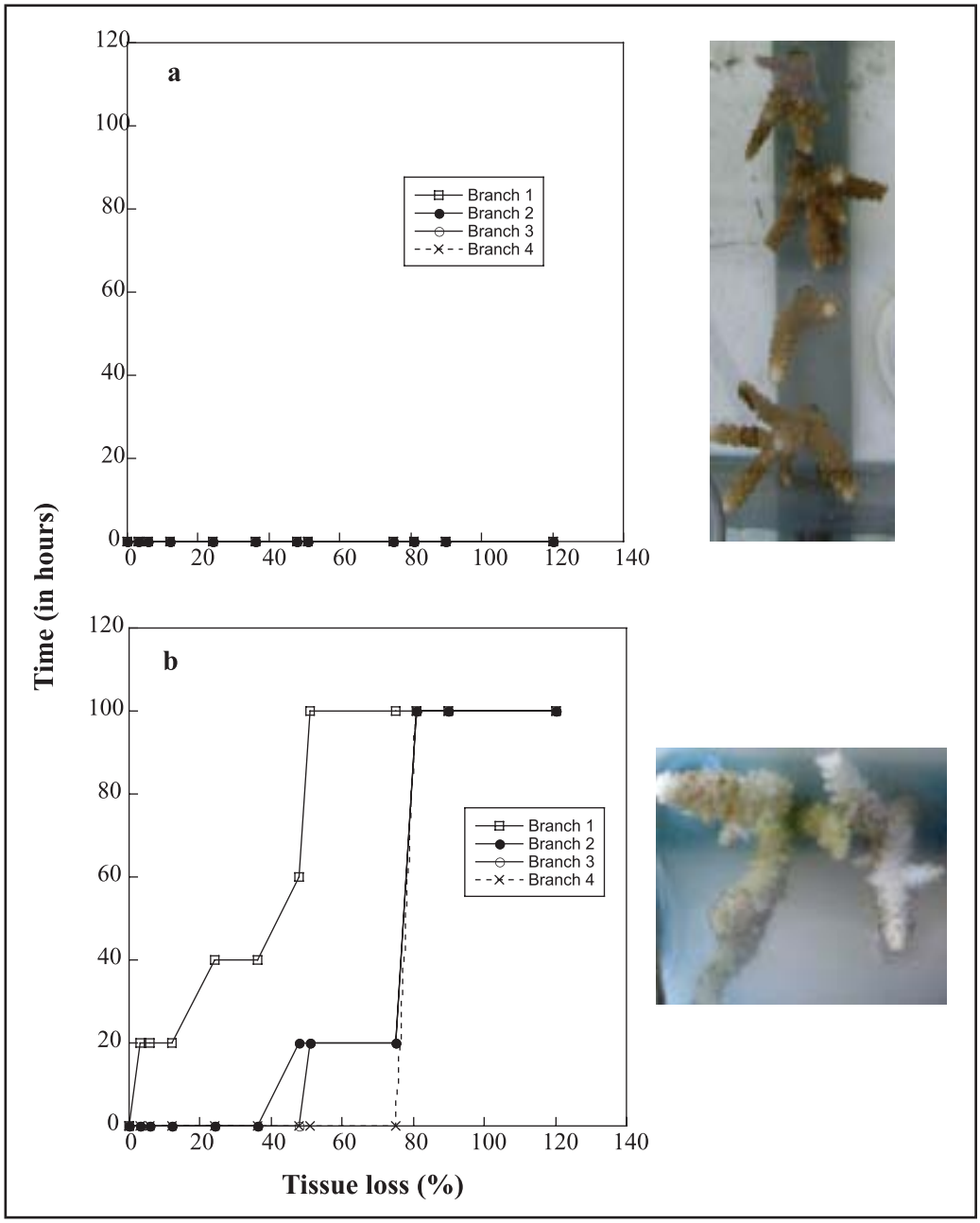
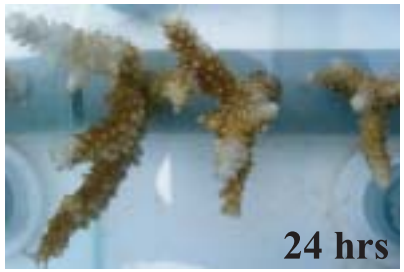
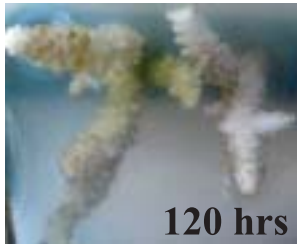

a
b
Branch 1
Branch 2
Branch 3
Branch 4
Time (in hours)
Tissue loss (%)
0
20
40
60
80
100
120
140

PLATE 3

Progression of disease, kept at 29 ˚C with ciliates



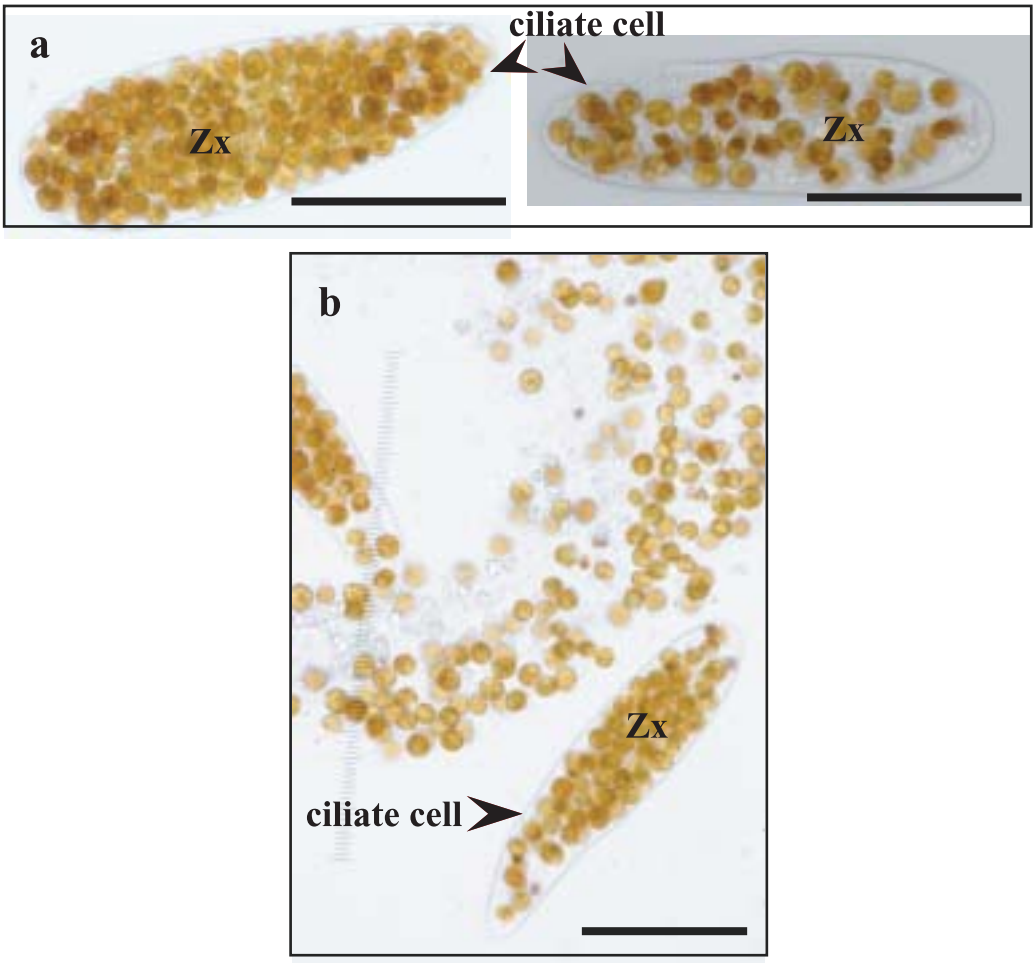


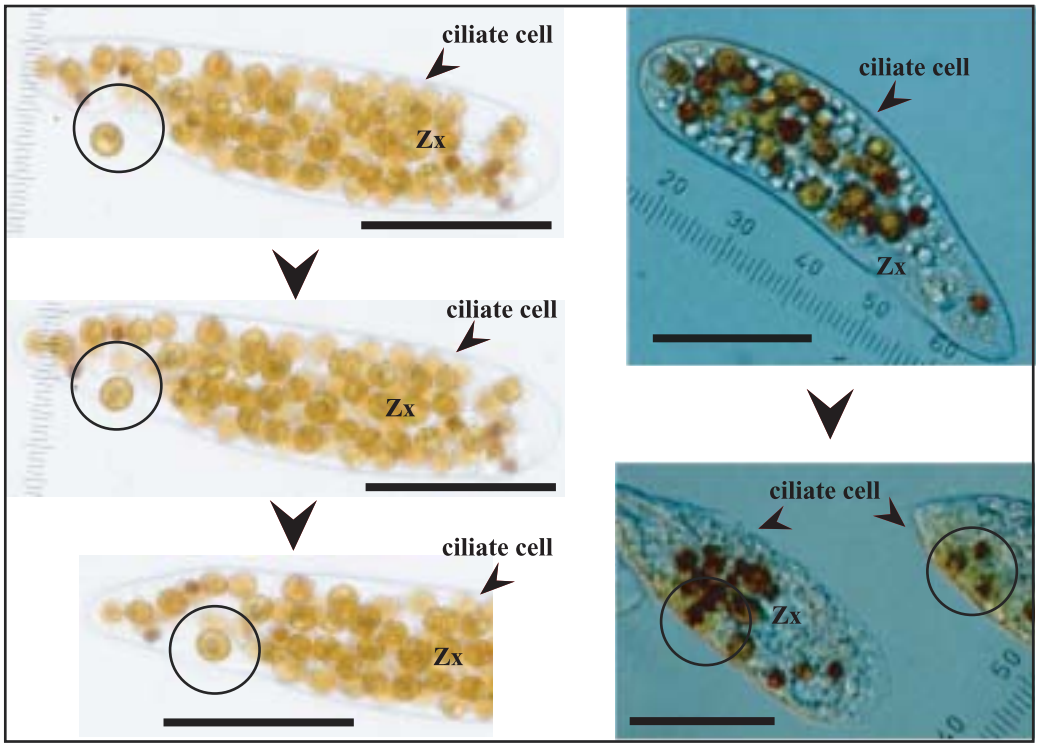
a **b** **c** **d** **e**

Effect on the polyp and skeleton, 48 hrs after the infection starts

f **g** **h** **i** **j**

PLATE 4




ciliate cell
Zx
ciliate cell
Zx
ciliate cell
Zx
ciliate cell
Zx
ciliate cell
Zx