NOTE

Water flow facilitates recovery from bleaching in the coral *Stylophora pistillata*

T. Nakamura[1], H. Yamasaki[1], R. van Woesik[2,*]

[1]**Laboratory of Cell and Functional Biology, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan**
[2]**Department of Biological Sciences, Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901-6975, USA**

ABSTRACT: Exposing partially bleached samples of *Stylophora pistillata* coral colonies to controlled water-flow treatments revealed differential recovery rates that varied in accordance with flow rate. Recovery of the number of zooxanthellae per unit area and chlorophyll *a* concentrations increased rapidly in moderate-flow treatments (of 20 cm s^{-1}), after an initial 3 wk of stasis. Colonies in low-flow treatments ($\leq$3 cm s^{-1}) remained pale for the entire 7 wk experimental period and showed only slight increases in zooxanthellae per unit area. In an earlier study, we showed that when corals were subjected to high temperature and high irradiance, coral bleaching occurred in low-flow but not in moderate-flow treatments; the present study shows that recovery from bleaching is facilitated by flow treatment. In combination, prevention of and rapid recovery from bleaching by enhanced water flow suggest that the mechanisms that lead to dysfunction of the algae-coral symbiosis are driven by mass-transfer-limited processes.

KEY WORDS: Corals · Bleaching · Water flow · Recovery

Coral bleaching is the loss of symbiotic algae (i.e. zooxanthellae) and/or pigment because of stress at and above the corals' acclimation capacity (Hoegh-Guldberg & Smith 1989, Glynn 1991, 1993, Fitt & Warner 1995, Brown 1997, Kuroki & Van Woesik 1999). Coral bleaching may be temporary or lead to coral mortality. Higher-than-average sea surface temperatures (SSTs) have been suggested as the primary causative agent of large-scale coral bleaching events, as seen in 1997 and 1998. High irradiance seemingly acts as a secondary factor (Jones et al. 1998, Hoegh-Guldberg 1999, Warner et al. 1999), and in some coral species high SSTs can cause the onset of virulence in coral-associated bacteria which reduce endosymbiotic zooxanthellae populations (Kushmaro et al. 1996).

In contrast, rapid water flow facilitates coral survival under high irradiance and high SSTs (Nakamura & Van Woesik 2001). Differential rates of water flow are largely a consequence of the effect of frictional drag across the corals' boundary layers thereby changing mass-transfer rates of gases and metabolites (Patterson et al. 1989, Atkinson & Bilger 1992, Patterson 1992). When water-flow rates are low, diffusive boundary layers are thick and passive diffusion is restricted and slow. Several coral studies have shown a positive correlation with water-flow rates and rates of (1) photosynthesis (Dennison & Barnes 1988, Lesser et al. 1994), (2) growth (Jokiel 1978), (3) phosphate uptake (Atkinson & Bilger 1992, Hearn et al. 2001), (4) micosporine-like amino acid accumulation under ultraviolet light exposure (Kuffner 2001, 2002) and (5) survival under high irradiance and SSTs (Nakamura & Van Woesik 2001). There has been little study of the effects of water motion on coral recovery from bleaching. The present study used partially bleached coral samples to investigate the effect of differential water flow upon recovery of chlorophyll concentrations and symbiotic algae populations.

Materials and methods. In March 2002, 3 colonies of *Stylophora pistillata* were collected from Cape Kyann and 3 from Oku in the Ryukyu Islands, southern Japan (>10 m depth, irradiance about 100 µmol photons m^{-2} s^{-1}). After 1 wk in an open flow-through water table with light at <300 µmol photons m^{-2} s^{-1} (a neural shading mesh reduced photosynthetically active radiation to 23% of the mid-day maximum), the colonies were separated into small 3 to 4 cm nubbins. They were immediately attached to small polycarbonate screws using gel high-speed super glue ('alone-alpha'); colony origin was tracked using color-coded screws. Samples were kept in this condition (i.e. $\leq$3 cm s^{-1} flow, <300 µmol photons m^{-2} s^{-1}, average water temperature 27.7°C) for the next 4 mo prior to experimental manipulation. Irradiance levels were measured
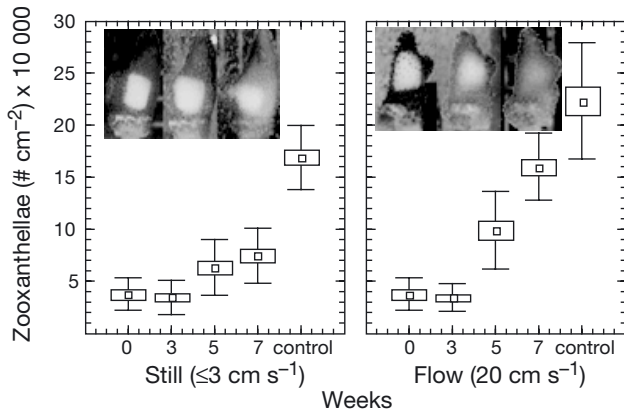
*Corresponding author. Email: rvw@fit.edu

Fig. 1. *Stylophora pistillata.* Number of zooxanthellae (no. of cells cm^2 surface area of living tissue) in still (≤3 cm s^{-1}) and flow (20 cm s^{-1}) treatments over 7 wk (n = 3). (□) means, (▭) SE, whiskers: SD. Photos show initial (left), 5 wk (middle), and 7 wk (right) samples. Control groups were not bleached and were placed in each flume for same experimental time period (7 wk, n = 3)

with a micro-photon sensor on a DIVING-PAM fluorometer (Waltz).

Artificial bleaching was induced by maintaining the corals under water and exposing a small (1.0 cm^2) smooth surface of each sample to 1500 µmol photons m^{-2} s^{-1} of light while covering the rest of the coral surface with aluminum foil for 6 h (method adapted from that of Yamasaki et al. [1995] for tropical higher plants). Partial colony bleaching, similar that induced by this experimental procedure, is common during sublethal thermal stress events, as evidenced on the Great Barrier Reef (Heron Island) in February 2002 (van Woesik pers. obs.). To generate this high light level, we used cold-lighting fiber-optic tubes equipped with a UV filter (NPI, Picl-nex; Phillips, projection lamp 150 W) placed 3 cm above the surface of the water. Samples were immersed and held close (1 cm) to the surface of a 5 l water bath (TAITEC, SM-05R) supplied with filtered seawater; temperature was maintained at 27°C by a thermostat and homogenizing propeller. After artificial bleaching, 3 samples (n = 1 for each colony) were frozen and subsequently analyzed for chlorophyll *a* and *c*$_2$ concentrations, and zooxanthellae numbers were counted and recorded as Week 0.

Perspex miniflumes (length 140 cm, width 10 cm, height 10 cm, depth 5 to 6 cm) were adapted from those described by Atkinson & Bilger (1992). Two miniflumes were supported on concrete blocks within an open aquarium (length 2.0 m, width 1.5 m, depth 0.5 m) supplied with running seawater. Water flowrate treatments were maintained at (hereinafter classified as 'still') ≤3 cm s^{-1} (hereinafter classified as 'flow') 20 cm s^{-1} and at an irradiance level of 300 µmol pho-

tons m^{-2} s^{-1}. On June 29, 2002, 36 samples (6 samples from each colony per treatment) were placed in the flumes following artificial bleaching treatment. Additionally, 6 unbleached control samples (3 for each treatment; n = 1 for each colony) were placed in the flumes for 7 wk. The water-flow rates were generated and maintained with submersible pumps (REI-SEA, Type RSD-40).

On July 20 (Week 3) and August 4 (Week 5), 2 samples were taken from each colony (i.e. a total of 12 samples, 6 from each treatment). On August 21 (Week 7), 6 samples and 3 controls were taken from each treatment (n = 18). These nubbins were immediately frozen in –20°C, and taken to the laboratory to measure the number of zooxanthellae per tissue area, and chlorophyll *a* and *c*$_2$ concentrations. To determine the number of zooxanthellae, bleached tissue was removed from skeletons with a Water-pik (Johannes & Wiebe 1970). The water-piked liquid was centrifuged at $15\,000 \times g$ for 15 min (10°C), the top clear layer was discarded after centrifugation, and the remaining zooxanthellae pellets were resuspended in 10 ml of filtered seawater. Centrifugation was repeated 3 times. Subsamples were then taken for hemocytometer counts. Three replicates were taken from each waterpik product. The number of zooxanthellae was expressed as the number of cells cm^{-2} surface area of living tissue. Surface area was estimated with the aluminum foil method (Marsh 1970). For chlorophyll extraction, 90% acetone was added to zooxanthellae pellets after 3 repeated washing centrifugations. The samples were left in the dark at 7 to 10°C for 48 h. The extracted solution was centrifuged to exclude the sus-

Table 1. *Stylophora pistillata.* Two-way analysis of variance (ANOVA) of changes in number of zooxanthellae cm^2 surface area of living tissue, chlorophyll *a*, and chlorophyll *c*$_2$, where both flow and colony are fixed effects

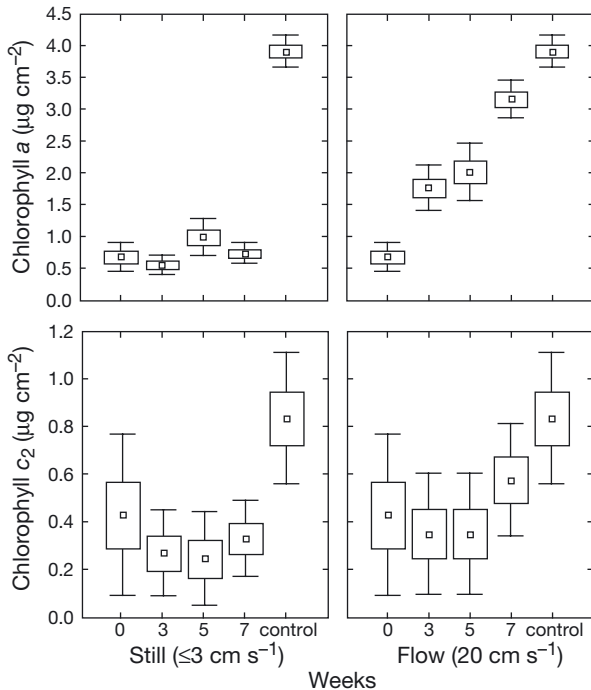| 2-way ANOVA	df	MS	F	p-level
Zooxanthellae nos.				
Flow (F)	1	280×10^8	33.640	0.001
Colony (C)	2	633×10^6	0.760	0.001
F × C	2	143×10^7	1.720	0.257
Error	6	833×10^6		
Chlorophyll *a*				
Flow (F)	1	17.558	352.851	0.001
Colony (C)	2	0.089	1.792	0.245
F × C	2	0.047	0.940	0.441
Error	6	0.050		
Chlorophyll *c*$_2$				
Flow (F)	1	0.181	6.179	0.047
Colony (C)	2	0.031	1.059	0.404
F × C	2	0.084	2.854	0.135
Error	6	0.029		

Fig. 2. *Stylophora pistillata.* Changes in chlorophyll a and c_2 concentrations in still and flow treatments over 7 wk (n = 3). Symbols as in Fig. 1

pended matter prior to spectrophotometer measurements. Chl a and c_2 concentrations were calculated using the equations of Jeffrey & Humphrey (1975).

Using the final Week 7 measurements, 2-way ANOVAs were used to examine the effects of flow and genotype (colony) on (1) the number of zooxanthellae per area, (2) chlorophyll a concentration, and (3) chlorophyll c_2 concentration.

Results. High-light treatments caused sample surfaces to pale within 6 h, except the tentacles, which paled within 24 h. Immediate sampling revealed that chlorophyll concentration per zooxanthella did not change, which suggests that paling was a consequence of zooxanthellae loss. The number of zooxanthellae per unit area remained similar for 3 wk for both treatments (i.e. still and flow conditions, at ≤ 3 and 20 cm s^{-1} respectively), and then gradually increased in the flow-treated colonies only (Fig. 1; Table 1). Notably, there were considerable differences in the number of zooxanthellae per unit area for the controls, with still-treatment controls supporting fewer zooxanthellae (Fig. 1). Flow-treated colonies regained as much as 70% of the control group chlorophyll concentration within 7 wk, while still-treated colonies stayed pale for the entire experimental period (Fig. 2); this difference was significant, particularly for chlorophyll a (Table 1).

Pigmentation encroached from the periphery of the bleached area in flow treated samples. Chlorophyll a

increased in flow-treated colonies throughout the study and changed little in still treatments. Chlorophyll c_2, however, increased slightly in flow treatments in the last 2 wk of the experimental period. Notably, there were no differences in total chlorophyll c_2 concentration between still and flow controls (Fig. 2). The flow treatment also showed a strong positive (logarithmic) relationship between the number of zooxanthellae per unit area and chlorophyll a concentration per unit area (Fig. 3). Chlorophyll a per zooxanthella increased only in flow treatments during the first 3 wk, and then declined, while chlorophyll a per zooxanthella declined over time in still treatments (Fig. 4). Chlorophyll c_2 per zooxanthella showed declines over time in both still and flow treatments (Fig. 4). Fig. 5 shows that there was little change in chlorophyll a:c_2 in still conditions, while flow conditions induced a rapid increase in a:c_2 during the first 5 wk, followed by a decline.

Discussion. Chlorophyll concentration and number of zooxanthellae per area were reduced in patches of *Stylophora pistillata* colonies exposed to high and prolonged light exposure. Recovery of chlorophyll concentrations and number of zooxanthellae was most apparent in flow-treated colonies. Recovery occurred firstly through an increase in chlorophyll a followed by an increase in the zooxanthellae population. Although full recovery was not achieved in 7 wk, the recovery rate was significantly higher in the flow-treated samples; however, the chlorophyll concentration per zooxanthella decreased as an inverse function of zooxanthellae number per area. These results agree with those of Fitt et al. (1993) and Jones (1997), who both showed an inverse relationship between algal cell number and chlorophyll a concentrations during recovery from coral bleaching. Furthermore, the flow conditions induced a rapid increase in a:c_2 during the
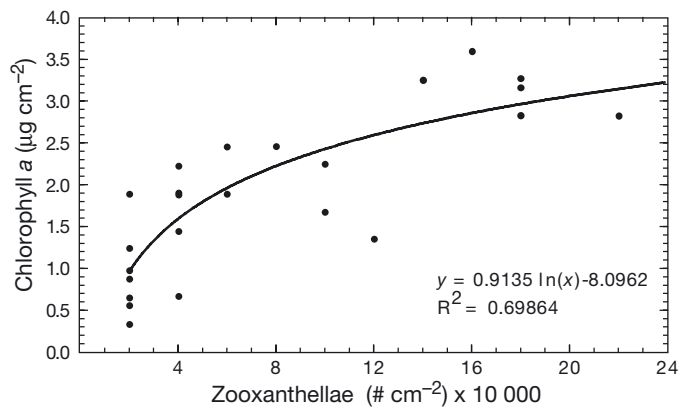

Fig. 3. *Stylophora pistillata.* Chlorophyll a concentration in relation to number of zooxanthellae in flow (20 cm s^{-1}) treatment. Logarithmic response curve fitted using least-squares
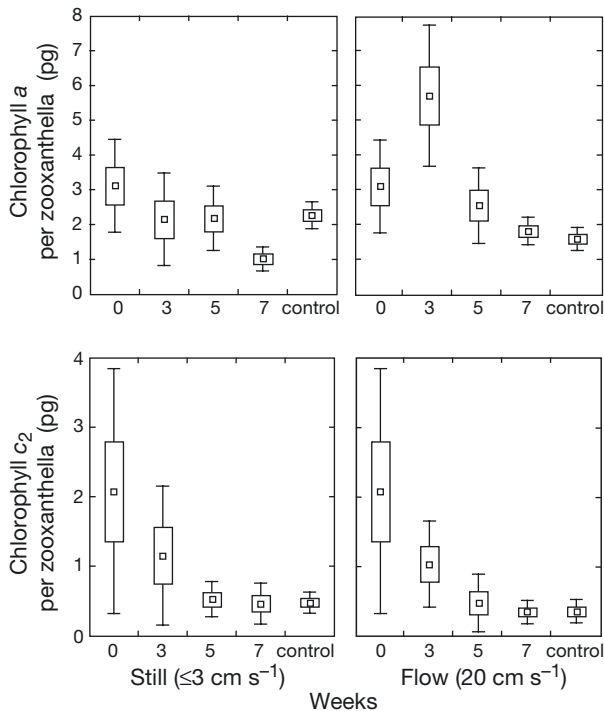
Fig. 4. *Stylophora pistillata.* Changes in chlorophyll *a* and *c*$_2$ concentration per zooxanthella in still and flow treatments over 7 wk (n = 3). Symbols as in Fig. 1

first 5 wk followed by a decline (Fig. 4), while there was little change in chlorophyll *a*:*c*$_2$ in still conditions. The changes in *a*:*c*$_2$ in the flow treatment suggests the algae are initially more high-light acclimated, and then photoacclimate to lower light as cell shading occurs by an increase in the number of zooxanthellae. These results agree with those of Brown (unpubl. data reported in Brown [1997]), who hypothesized that reduced chlorophyll *c*$_2$ values are associated with exposure to high radiant flux.
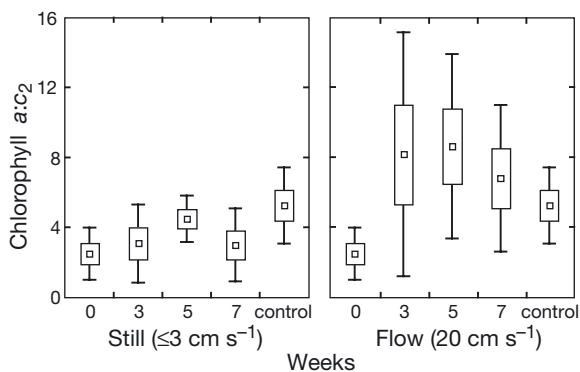

Fig. 5. *Stylophora pistillata.* Changes in chlorophyll *a*:*c*$_2$ in still and flow treatments over 7 wk (n = 3). Symbols as in Fig. 1

Within the coral host, zooxanthellae numbers can recover by 1 of 2 processes: (1) mitosis of remnant zooxanthellae within photodamaged tissue, or (2) encroachment of zooxanthellae from adjacent non-photodamaged tissue. While chlorophyll degradation products are not discriminated from intact chlorophyll *a* using this conventional spectrophotometer technique (a facet that could result in artificially high pigment concentrations), there were clear differences in chlorophyll concentrations between the 2 flow treatments. Therefore, we suggest that recovery, in this study, was mainly through new peripheral algae encroaching into the open space (Fig. 1).

Previously, Nakamura & Van Woesik (2001) showed that bleaching is prevented under moderate-to-high water-flow rates when corals are subjected to high SSTs (up to 33°C) and high irradiance. In the present study, flow facilitated the recovery of zooxanthellae populations, most probably by enhancing mass-transfer rates of gases and molecules. Under high temperature and high irradiance, low water-flow rates cause slow mass transfer, and carbon fixation may be reduced significantly, either through the dark reactions of the Calvin cycle (see Jones et al. 1998) leading to stress at Photosystem II (PS II) and consequential photoinhibition of photosynthesis and bleaching, or because D1 proteins in the thylakoid membranes may become dysfunctional leading to direct photoinhibition of photosynthesis in PS II (see Warner et al. 1999). The present study has shown that the mechanisms of coral bleaching and recovery extend beyond mere temperature-light effects, since the rate of water flow alters the rate of mass transfer which, in turn, modifies any adverse effects of temperature and light. Indeed, the prevention of and rapid recovery from bleaching induced by moderate-to-high flow rates suggests that mechanisms that lead to dysfunction of the algae-coral symbiosis are driven by mass-transfer-limited processes.

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