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Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of 'low-salinity bleaching'

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ABSTRACT: The response of the scleractinian coral *Stylophora pistillata* to short-term (min to h) and long-term (d) exposure to reduced-salinity seawater was examined. Pulse Amplitude Modulated (PAM) chlorophyll fluorescence techniques were used to assess the photosynthetic efficiency of the symbiotic dinoflagellates (dark-adapted F_v/F_m) in the coral tissues (*in hospite*) before, during and after exposure. Exposure to reduced-salinity seawater caused a marked reduction in efficiency (the ratio of variable [F_v] to maximal [F_m] fluorescence), and there was an apparent link between a reduction in dark-adapted F_v/F_m and a loss of symbiotic dinoflagellates from the corals. The reduction in F_v/F_m of the symbiotic algae and subsequent dissociation of the coral-algal symbiosis (coral bleaching) occurred during exposure to reduced-salinity seawater in either the light or dark. The results demonstrate that bleaching in response to low-salinity seawater is a truly sublethal response, contrary to a recent suggestion. The study also suggests that bleaching of corals in response to low-salinity seawater may not involve the passive loss of algal symbionts and that an impairment of the capacity of the algal symbionts for photosynthesis represents a common 'cue' initiating the dissociation of the coral-algal symbiosis during exposure to sub-optimal conditions. This study demonstrates how exposure to low-salinity seawater alone can cause some of the symptoms commonly attributed to temperature anomalies and anthropogenic pressures on coral reefs.

KEY WORDS: Coral bleaching · Symbiotic dinoflagellates · Salinity · Chlorophyll fluorescence

INTRODUCTION

Coral reefs are generally considered to exist in areas where salinity is stable over long timescales (Coles & Jokiel 1992). Nevertheless, major rainfall events are common in tropical regions, and during such episodes corals may experience significant reductions in salinity. In the short term (min to h), decreases in salinity levels can occur in shallow reef areas following the coincidence of heavy rainfall with low tides. Over longer periods (i.e. d to wk), salinity levels may be lowered when reefs are inundated by flood plumes of coastal rivers.

Numerous studies have documented short- and long-term reductions in salinity on coral reefs (reviewed in Coles & Jokiel 1992). For example, Orr & Moorhouse (1933) recorded a salinity level of 17 ppt in a shallow tide pool on the reef flat at Low Isles (Great Barrier Reef) during the 1928–1929 Great Barrier Reef Expedition. Moberg et al. (1997) recorded a salinity level of 10 ppt in coral-containing tidal pools on the reefs of the inner Gulf of Thailand. Cloud (1952) recorded a salinity level of 4 ppt on a reef flat in Kiribati (formerly the Gilbert Islands, Central Pacific Basin) for the duration of low tide following heavy rains. Salinity levels returned to normal on the following high tide. Over longer periods, Goodbody (1961) reported that heavy rainfall and coastal runoff reduced surface salinity levels in the inshore waters of Jamaica to 5.4 ppt during 1958, and it was 3 mo until all affected areas returned to normal levels of salinity. Goreau (1964) also reported markedly reduced seawater salinity in coastal areas of Jamaica following Hurricane Flora. On the Great Barrier Reef plumes of reduced salinity seawater have been reported to persist for up to 3 wk following cyclones (Van Woesik et al. 1995, Devlin et al. 1998). Berkelmans & Oliver (1999)

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reported that salinity levels in Cleveland Bay (inner, central Great Barrier Reef) were reduced to 28–32 ppt for 4 wk following heavy rain.

Many studies report the appearance of discoloured or white corals and anemones after exposure to flood waters (Goreau 1964, Egana & DiSalvo 1982, Engebretson & Martin 1994, Van Woesik et al. 1995). Both Goreau (1964) and Van Woesik et al. (1995) report striking cutoffs between discoloured and normally pigmented corals coinciding with the depth to which floodwaters penetrated. Laboratory-based studies have also confirmed that corals discolour during exposure to low-salinity seawater (Marcus & Thorhaug 1981, Coles 1992, Titlyanov et al. 2000). Discolouration of the corals in these studies has traditionally been interpreted as 'coral bleaching', i.e. a sublethal response of corals involving loss of endosymbiotic dinoflagellate microalgae from the coral tissues and/or loss of the pigments of the algae (Fitt et al. 2001). However, an alternative viewpoint has been suggested. Corals that have recently died, leaving only a white calcium carbonate skeleton, superficially resemble heavily bleached corals. This has led to the suggestion that corals exposed to low-salinity seawater do not bleach but rather die, leaving tissue-free skeletons that are mistaken for live corals that have lost their symbiotic algae (Hoegh-Guldberg 1999). This suggestion adds some confusion as to the nature of the relationship between exposure to low-salinity seawater and coral bleaching.

Corals are osmoconformers, rapidly absorbing water to become iso-osmotic with their surroundings (Rankin & Davenport 1981). Since water movement is faster than salt diffusion, osmoconformers gain water upon contact with low-salinity water (Rankin & Davenport 1981). This may result in damage to the animal tissue. On the Great Barrier Reef, Van Woesik et al. (1995) observed tissue swelling and damage to colonies of *Acropora formosa* exposed to floodwater of the Fitzroy River. Tissue necrosis has also been observed in anemones exposed to reduced salinity (Engebretson & Martin 1994). Host cells, still containing symbiotic algae, have been observed released from *Stylophora pistillata* after hypo-osmotic shock (Titlyanov et al. 2000). These authors suggest that the animal host is primarily affected by reductions in salinity.

Marine algae are also affected by reduced salinity. In seaweeds, hypo-osmotic stress causes increases in cell volume and turgor, resulting in the loss of ions and organic solutes as well as damage to membranes and organelles, culminating in cell rupture (Lobban & Harrison 1994). Damage to cellular structures will inevitably disrupt metabolic function, and changes in ion concentrations may have an inhibitory effect on enzymes systems (Lobban & Harrison 1994). A number of reports have described a reduction in the photosynthetic rate of symbiotic dinoflagellates *in hospite* following exposure to reduced-salinity seawater (Muthiga & Szmant 1987, Moberg et al. 1997, Ferrier-Pages et al. 1999). Sakami (2000) has found that low salinity causes a reduction in photosynthetic activity in isolated symbiotic dinoflagellates (*in vitro*). Muthiga & Szmant (1987) and Moberg et al. (1997) suggest that the observed reduction in photosynthesis may have been due to cellular damage to the algae.

Bleaching of corals in response to low-salinity seawater is the second most commonly cited cause of coral bleaching (Glynn 1991). Despite this, there is no consensus as to the mechanism associated with reduced-salinity bleaching. In contrast, the mechanism associated with the bleaching of corals in response to warm water (the most common form of bleaching) is gradually being elucidated. Numerous studies, using photorespirometry and chlorophyll fluorescence techniques, have now suggested that an impairment of photosynthesis of the algal symbionts is an important component of the bleaching response (Iglesias-Prieto et al. 1992, Jones et al. 1998, Warner et al. 1999). The *in vitro* and *in vivo* studies with chlorophyll fluorescence techniques have been particularly informative. One of the most useful chlorophyll fluorescence parameters that can be measured is the ratio of variable (F_v) to maximal fluorescence (F_m). $F_v = F_m - F_o$, where F_o is the initial fluorescence when all reaction centres in Photosystem II (PSII) are open, and F_m is the maximal fluorescence determined after the application of a saturating pulse of white light, i.e. when all PSII reaction centres are closed. When determined in a dark-adapted state, the ratio F_v/F_m is a measure of the maximum potential quantum yield of PSII and changes in F_v/F_m can be used to evaluate reductions of PSII activity caused by acute stress (Schreiber et al. 1986, Krause & Weis 1991). Marked long-term reduction in F_v/F_m in symbiotic algae of corals *in hospite* appears to precede the dissociation of the coral-algal symbiosis. This has been observed in response to many stressors, and led to the suggestion that impairment of algal photosynthesis may be a cue (universal) initiating the dissociation of the coral-algal symbiosis (i.e. bleaching) (Jones et al. 1999).

How bleaching in response to reduced-salinity seawater fits into this model is unclear. Certain studies have highlighted damage to the animal host (Engebretson & Martin 1994, Titlyanov et al. 2000) whilst other studies report impairment of the algae (measured as marked reductions in photosynthetic rates) (Ferrier-Pages et al. 1999, Sakami 2000). In this study, we explore the nature and cause of freshwater bleaching of corals. Firstly, we address the issue of whether the discolouration of corals following exposure to reduced-salinity seawater is in fact coral bleaching, as

opposed to coral death (Hoegh-Guldberg 1999). Secondly, using chlorophyll fluorescence techniques, we examine the relationship between the impairment of algal photosynthesis and the dissociation of the coral-algal symbiosis in *Stylophora pistillata* subjected to short- and longer-term reductions in seawater salinity.

MATERIALS AND METHODS

Coral collection and preparation. All experiments were conducted with the coral *Stylophora pistillata* (Esper 1797) collected from Heron Reef (23° 26' S, 151° 55' E) in the Capricorn-Bunker Group of reefs in the southern Great Barrier Reef, Australia. Corals were collected from the protected intertidal reef flat at a depth of <1 m (MLWS). Small (~40 mm long), vertically oriented fragments of *S. pistillata* were isolated from the centre of parent colonies (2 explants per colony) using surgical bone forceps. Branch fragments were returned to a 500 l flow-through holding/observation tank at the Heron Island Research Station (HIRS) and mounted into plastic holders with non-toxic modelling clay. Corals were left to recover from the handling procedures for 24 h before experimentation.

All experiments were conducted under natural light conditions. Photosynthetically Active Radiation (PAR, 400 to 700 nm) was measured using a cosine-corrected photosynthetic irradiance sensor, and mean PAR over 10 min intervals was recorded onto a waterproof 392 data recorder (Dataflow Systems). Light recorders were calibrated against a LI-190SA quantum sensor (LI-COR). Water temperature (°C) was recorded with an Optical Stowaway® temperature logger (Onset computer Corporation, accuracy ±0.1°C) calibrated against a NATA-certified (National Association of Testing Authorities, NSW, Australia) thermometer. Salinities were measured using a hand-held refractometer (ATAGO, accuracy ±0.5 ppt).

For each experiment, solutions of the necessary salinity were made by diluting water from the seawater system at HIRS (37 ppt drawn from a subtidal inlet at 6 m depth on the protected reef slope) with distilled water (MilliQ). Fresh solutions were made immediately before each experiment. To minimise temperature fluctuations, all experiments were conducted in 20 l aquaria semi-submerged within the holding/observation tank (water bath) receiving a continuous supply of seawater. This minimised temperature fluctuations to <2°C and water temperatures during experiments did not rise above 26°C. Corals were incubated in 5 l of water during static test conditions and stirring within the containers was achieved using miniature submersible aquarium pumps (Mini-Pro). Evaporation was negligible over a 12 h period, and salinities did not deviate more than 0.5 ppt over the exposure time. After exposure, corals were returned to the holding/observation tank for an observation period.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence parameters were measured using Pulse Amplitude Modulated (PAM) chlorophyll fluorometry (DIVING-PAM, Walz; Schreiber et al. 1986). Where necessary, corals were held in the dark (dark-adapted) for 30 min prior to fluorescence measurements (Jones & Hoegh-Guldberg 2001). During measurements, the fibre-optic light guide was held <2 mm from the coral, 2 cm from the tip on a vertical plane. Initial fluorescence (F_o) was measured by applying a weak pulse of red light (<1 µmol quanta m^{-2} s^{-1} PAR). A saturating pulse (8000 µmol quanta m^{-2} s^{-1}; 800 ms pulse width) was applied to determine maximal fluorescence (F_m). Variable fluorescence (F_v) was calculated as $F_m - F_0$ and maximal potential quantum yield as F_v/F_m. During all experiments, chlorophyll fluorescence parameters were measured prior to and at the completion of treatments. Where corals were left for an observation period in the holding/observation tank, fluorescence measurements were made at dawn (06:00 h) and dusk (18:00 h).

Experimental design. During the experimental sequence, 3 types of controls were used. An initial set of freshly excised branches from the parent colonies (parent colony controls, PC) was frozen immediately after collection. A subset of corals (handling controls, HC) was mounted into plastic containers and maintained in the holding/observation tank over the duration of experiments. A third set of controls, (treatment controls, TC) was exposed to ambient seawater only during the experiments.

Short-term exposure experiments. Short-term exposure experiments began at dawn (06:00 h) and finished after 12 h. Following treatments, corals were returned to the holding/observation tank and allowed to recover in ambient salinity for 6 to 11 d before being sacrificed for biomass determination. Chlorophyll fluorescence parameters were measured twice daily during the exposure and recovery periods.

Short-term Expt 1: To determine the response of *Stylophora pistillata* to a wide range of low-salinity seawater, coral explants were exposed to ambient seawater (37 ppt) or reduced-salinity seawater (33.5 to 15 ppt). Four explants were exposed to each of 7 salinities for 12 h. Following exposure, corals were returned to the holding/observation tank for a 6 d observation period before being sacrificed.

Short-term Expt 2: The above experiment was repeated with 3 low-salinity treatments (31.5, 26, 20.5 ppt) and ambient salinity (37 ppt) in 3 replicate tanks with 3 corals per tank.

Light-interaction experiment: To determine whether the effect of reduced salinity on *Stylophora pistillata* is light-dependent, corals were exposed to either ambient seawater (37 ppt) or reduced-salinity seawater (20.5 ppt) in near darkness or light for 12 h. 'Light' treatments were exposed to natural light conditions whilst 'dark' treatments were covered by black plastic that reduced light levels to <10 µmol quanta m^{-2} s^{-1}. Corals were sacrificed 10 d after return to the holding/observation tank.

Long-term exposure experiment. To examine the chronic (longer-term) response to low-salinity stress, *Stylophora pistillata* was exposed to ambient seawater (37 ppt) or reduced-salinity seawater (33.5, 29.5, 26 ppt) for 12 d. Three replicate tanks with 3 corals per tank were used for each treatment. The salinity in each tank was monitored daily and freshwater added if the salinity had risen due to evaporation and replaced every 3 d to prevent stagnation. Salinities did not vary more than 1 ppt over the exposure period. Chlorophyll fluorescence parameters were measured twice daily, and after 12 d exposure corals were sacrificed for biomass determination.

Time-course experiment. The response of *Stylophora pistillata* to exposure to very low salinity (10 ppt) was investigated over a 5 to 120 min period. Tests were conducted over the time of peak irradiance, from 11:00 h until 13:00 h. Nine 2 l aquaria were filled with seawater diluted to 10 ppt and 5 corals placed into each. One coral was removed from each aquarium after 5, 10, 30, 60 and 120 min exposure and returned to the holding/observation tank. Three 2 l aquaria served as controls and were filled with ambient seawater. Three corals were placed into each of these tanks and returned to the holding/observation tank after 120 min. All corals were held for a 7 d observation period before being sacrificed for biomass determination. Chlorophyll fluorescence parameters were measured before and after exposure and throughout the observation period.

Dinoflagellate density determination. Tissue was stripped from coral skeletons with a jet of recirculating seawater using a Water-Pik™ (Johannes & Wiebe 1970). Subsamples of the homogenate were taken and the density of symbiotic dinoflagellates determined from 8 replicate counts using a hemacytometer. Chl *a* concentration in three 13.5 ml subsamples was determined according to the equations of Jeffrey & Humphrey (1975). Total symbiotic dinoflagellates and chl *a* were determined after correction for the total homogenate volume and are expressed per unit surface area. Surface area was determined by the paraffin wax technique (Stimson & Kinzie 1991). Techniques are fully described in Jones et al. (2000).

Data analysis. All data are presented as the mean ($\bar{x}$) ± 95% confidence intervals. Treatments were compared using 1-way ANOVAs. In all instances (except the time-course experiment) there was no significant difference between field, handling and treatment controls ($p < 0.001$) and thus, experimental values are compared to treatment controls (TC).

RESULTS

Salinity tolerance

Stylophora pistillata fragments were dead 1 d after exposure to salinity levels of 15 ppt for 12 h and 10 ppt for 120 min. Mortality occurred as tissues sloughed away from the coral skeleton. Corals exposed to salinity ranging from 26 to 18 ppt for 12 h or 12 d and 10 ppt for 10 to 60 min remained alive but discoloured within 3 d of exposure to the low salinity. During the exposure and observation periods, the polyps of corals (which eventually discoloured) remained fully extended and responded to tactile stimulation by contracting into their calices.

Chlorophyll fluorescence

In affected corals, chlorophyll fluorescence measurements of dark-adapted F_v/F_m followed a general pattern of an initial rapid decrease followed by a more gradual increase to levels within 5 to 10% of both control and pre-treatment values. Corals from all 12 h treatments that were exposed to 26 ppt or less displayed an initial reduction of up to 50% in F_v/F_m over the 12 h exposure period. This decreased further overnight and reached the lowest value 12 h after a return to ambient salinity. The greatest reduction in dark-adapted F_v/F_m was in corals exposed to 18 ppt (65% reduction, Fig. 1A). Following the initial rapid decline, F_v/F_m gradually increased over the recovery/observation period and was within 5% of pre-exposure and control levels by Day 5 (Figs. 1A, 2 & 3). Corals exposed to 10 ppt for 30 and 60 min followed the same pattern of decrease and recovery of dark-adapted F_v/F_m as the 12 h treatment corals (Fig. 4).

Corals exposed to low-salinity seawater for 12 d also experienced a sudden decrease in dark-adapted F_v/F_m over the first 36 h of exposure. F_v/F_m in corals exposed to 26 ppt was 0.48, while corals exposed to 29.5 and 33.5 ppt had no change in fluorescence parameters. F_v/F_m gradually increased to within 10% of control values by Day 5 and remained at this level for the duration of the 12 d treatment (Fig. 5).

Dark-adapted F_v/F_m decreased in all corals exposed to salinity of 26 ppt or lower and to 10 ppt for 30 to 120 min and differences were significant when com-

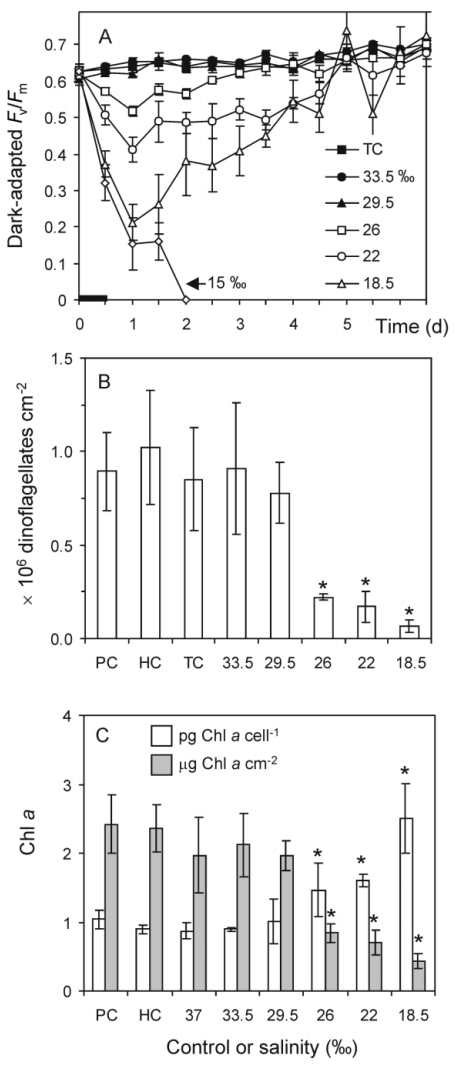

Fig. 1. *Stylophora pistillata.* (A) Mean dark-adapted F_v/F_m of symbiotic dinoflagellates in coral exposed to salinity levels of 37 to 15 ppt for 12 h and during a 6 d observation period (in ambient seawater). The horizontal bar above the *x*-axis represents the exposure period. (B) symbiotic dinoflagellate density ($\times 10^6$ cm^{-2}). (C) Chlorophyll *a* (pg algal cell^{-1}) and µg cm^{-2}) in coral 6 d after exposure to salinity levels of 37 (TC) to 18.5 ppt for 12 h. PC = parent colony controls, HC = handling controls and TC = 100% (ambient) seawater controls. All data are ±95% CI, n = 4. Asterisks denote significant ($p < 0.001$, ANOVA) differences from treatment controls TC

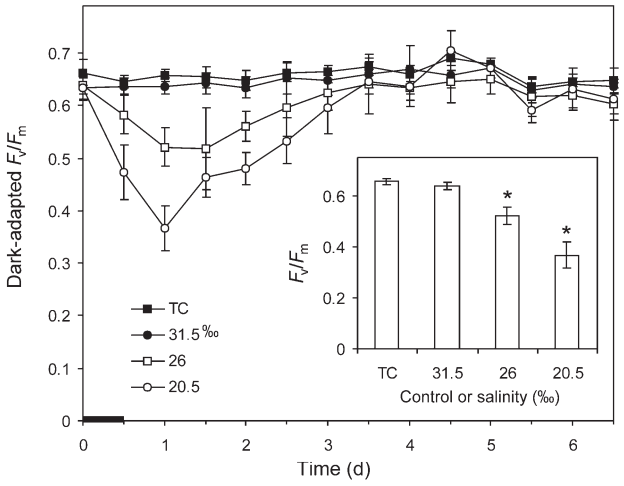

Fig. 2. *Stylophora pistillata*. Mean dark-adapted F_v/F_m of symbiotic dinoflagellates in coral exposed to salinity levels of 37, 31.5, 26 or 20.5 ppt for 12 h and during a 6 d observation period (in ambient seawater). The horizontal bar above the *x*-axis represents the exposure period. Inset: lowest recorded dark-adapted F_v/F_m (after 12 h observation). All data are ±95% CI, n = 9. Asterisks denote significant ($p < 0.001$, ANOVA) differences from treatment controls (TC) exposed to ambient seawater

pared to TC corals (ANOVA, $p < 0.001$, n = 9). Minimum values of F_v/F_m were reached 24 h after the commencement of experiments (Figs. 2 to 5). In short-term Expt 2, F_v/F_m in corals exposed to 20.5 ppt was 25% lower than control values. The 24 h period over which this decrease occurred included 12 h at reduced salinity, which was followed by 12 h at ambient salinity. The minimum dark-adapted F_v/F_m in corals from the long-term experiment was also 25% lower than control corals, however the experimental corals were maintained at 26 ppt for the entire exposure period. F_v/F_m in corals exposed to 20.5 ppt in full sunlight was lower (0.38) than in corals exposed to low salinity in darkness (0.48) 12 h after a return to ambient salinity (Fig. 3). F_v/F_m from both treatments was significantly different from controls (ANOVA, $p < 0.001$, n = 9). After 12 h at ambient salinity, dark-adapted F_v/F_m in corals exposed to 10 ppt for 30 min was 55% less than control values and 70% less than control values in corals exposed for 60 min.

Dinoflagellate density

At the end of experiments, *Stylophora pistillata* exposed to salinity of 26 ppt or less had fewer symbiotic dinoflagellates remaining *in hospite* than control corals. The differences in dinoflagellate density in these corals were significant when compared to control corals (ANOVA, $p < 0.001$, n = 4 or n = 9, Figs. 1B & 6A to C). Exposure to 20.5 ppt for 12 h resulted in a loss of ~80% of dinoflagellates from coral tissues (Fig. 6A,B). Exposure to 26 ppt for 12 h resulted in a 50% loss of dinoflagellates, while exposure to the same salinity for 12 d resulted in a 90% decrease (compare Fig. 6A with Fig. 6C). Corals exposed to 10 ppt for 10 to 60 min also lost up to 90% of symbiotic dinoflagellates. Dinoflagellate density in these corals was significantly different

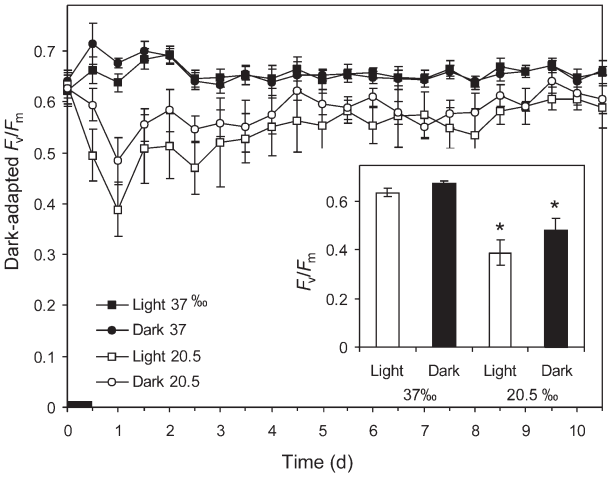

Fig. 3. *Stylophora pistillata*. Mean dark-adapted F_v/F_m of symbiotic dinoflagellates in coral exposed to salinity levels of 37 ppt (TC) or 20.5 ppt for 12 h in near darkness or under natural daylight and during a 10 d observation period (in ambient seawater). The horizontal bar above the *x*-axis represents the exposure period. Inset: lowest recorded dark-adapted F_v/F_m (after 12 h observation). All data are ±95% CI, n = 9. Asterisks denote significant ($p < 0.001$, ANOVA) differences from treatment controls (TC) exposed to ambient seawater

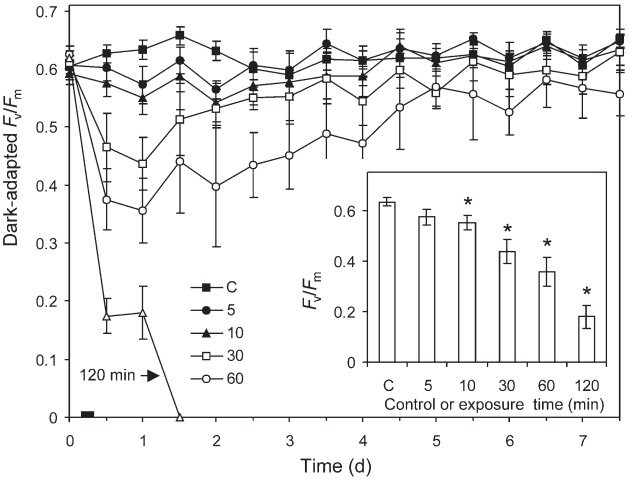

Fig. 4. *Stylophora pistillata*. Mean dark-adapted F_v/F_m of symbiotic dinoflagellates in coral exposed to a salinity level of 10 ppt for 5 to 120 min or to 37 ppt for 120 min (control, C) and during a 7 d observation period (in ambient seawater). The horizontal bar above the *x*-axis represents the exposure period. Inset: lowest recorded dark-adapted F_v/F_m (after 12 h observation). All data are ±95% CI, n = 9. Asterisks denote significant ($p < 0.001$, ANOVA) differences from controls (C) exposed to ambient seawater

from control corals (ANOVA, $p < 0.001$, n = 9, Fig. 6D). In all experiments, corals which experienced a decline in dark-adapted F_v/F_m after 24 h also lost significant proportions of symbiotic dinoflagellates (compare Figs. 2–5 with 6A–D).

Chl *a* concentrations were determined for corals in short-term Expt 1. Corals which lost symbiotic dinoflagellates also experienced a 50% reduction in areal chl *a* concentration. However, symbiotic dinoflagellates in remaining in host tissues had chl *a* concentrations of between 1.5 and 2.5 pg cell^{-1}, which was approximately 2 to 3 times the concentration of chl *a* in cells from corals with unchanged dinoflagellate density (Fig. 1C). Differences in chl *a* concentrations were significant when compared to control values (ANOVA, $p < 0.001$, n = 4).

DISCUSSION

The most obvious physiological response of *Stylophora pistillata* to exposure to reduced-salinity seawater was a discolouration of the coral tissues. This discolouration was, in most instances, a sublethal response and involved a loss of the endosymbiotic dinoflagellate microalgae, thus conforming to current definitions of coral bleaching (see Fitt et al. 2001). Recently, it has been suggested that the discolouration of corals following osmotic stress is not caused by the dissociation of the coral-algal symbiosis (bleaching), but rather by death of the coral (Hoegh-Guldberg 1999). However, the results of this study refute Hoegh-Guldberg's (1999) suggestion. A loss of symbiotic dinoflagellates occurred in *S. pistillata* subjected to both dramatic decreases in salinity for short periods (i.e. min to h) and slight decreases in salinity for long periods (i.e. d). Mortality and sloughing of the tissues from the skeleton was observed, but only in corals exposed to extremely low-salinity seawater. In these instances, all that remained was a bare, white coral skeleton which very superficially resembled a heavily bleached coral. However, in the treatments where bleaching occurred, it was via a loss of symbiotic dinoflagellates from the tissues of the corals, which were otherwise alive and responding to tactile stimuli. Thus bleaching, or the dissociation of the coral-algal symbiosis, was a quantifiable sublethal response to hypo-osmotic stress.

In response to reduced-salinity seawater, bleaching occurred through a loss of symbiotic dinoflagellates from coral tissues, which resulted in a reduction in areal chl *a* concentration. However, the concentration of chl *a* per algal cell remaining inside bleached corals increased to twice that of normally pigmented (control) corals. Many other studies have reported higher chl *a* concentrations per algal cell in corals recovering from bleaching events (Fitt et al. 1993, Le Tissier & Brown 1996, Jones 1997, Brown et al. 1999b), although earlier studies have indicated lower algal chl *a* concentrations

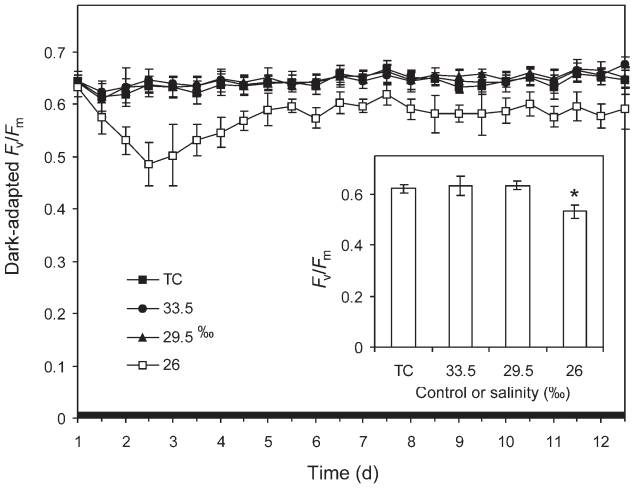

Fig. 5. *Stylophora pistillata*. Mean dark-adapted F_v/F_m of symbiotic dinoflagellates in coral exposed to salinity levels of 37, 33.5, 29.5 or 26 ppt for 12 d. The horizontal bar above the *x*-axis represents the exposure period. Inset: lowest recorded dark-adapted F_v/F_m (after 36 h exposure). All data are ±95% CI, n = 9. Asterisks denote significant ($p < 0.001$, ANOVA) differences from treatment controls (TC) exposed to ambient seawater

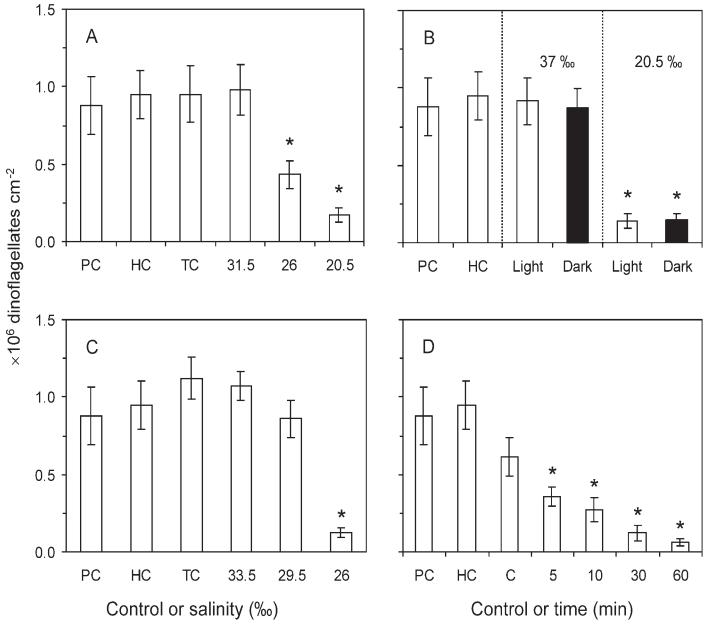

Fig. 6. *Stylophora pistillata*. Symbiotic dinoflagellate density (× 10^6 dinoflagellates cm^{-2}) in coral (A) 6 d after exposure to a salinity level of 37 ppt (TC) or seawater diluted to 31.5, 26 or 20.5 ppt for 12 h; (B) 10 d after exposure to 37 ppt (TC) or 20.5 ppt in near darkness or under natural daylight for 12 h; (C) after 12 d exposure to 37 ppt or seawater diluted to 33.5, 29.5 or 26 ppt; (D) 7 d after exposure to 10 ppt for 5 to 60 min or 37 ppt for 120 min. PC = parent colony controls, HC = handling control, TC = ambient seawater controls and C = control (120 min exposure to ambient seawater). All data are ±95% CI, n = 9. Asterisks denote significant ($p < 0.001$, ANOVA) differences from treatment controls (TC)

(Kleppel et al. 1989). Why chl *a* concentrations are higher in algae remaining in the coral tissues following bleaching is presently unclear (see Le Tissier & Brown 1996, Jones 1997). However, it is clear that any attempt to define coral bleaching by reference to the chlorophyll content of the algae will quickly become confused when (1) corals with fewer symbionts have higher chl *a* $cell^{-1}$ concentrations than control corals and (2) this trend is not consistent across all studies.

In this study, chlorophyll fluorescence techniques were used to assess the photosynthetic capacity of algal symbionts *in hospite*. In some cases, exposure to low-salinity seawater caused a marked long-term reduction in the PSII efficiency, assessed as the ratio of variable to maximal fluorescence in a dark-adapted sample (F_v/F_m). This was measured in *Stylophora pistillata* exposed to a range of low-salinity treatments over the short-term (min to h) and long-term (d). Reductions in the rate of photosynthesis, measured as a decrease in oxygen evolution, have also been documented in several coral species exposed to low salinity over similar timescales (Muthiga & Szmant 1987, Moberg et al. 1997, Ferrier-Pages et al. 1999, Alutoin et al. 2001). Both behavioural and physiological responses have been proposed to account for these changes. Several studies suggest that the retraction of animal tissues into the skeleton, thereby preventing excessive water uptake, may restrict gas exchange and reduce the light levels experienced by the dinoflagellate (Muthiga & Szmant 1987, Moberg et al. 1997, Ferrier-Pages et al. 1999). Muthiga & Szmant (1987) suggest changes in photosynthesis are more physiological, involving damage to the algal cell ultrastructure. In seaweeds, hypo-osmotic stress causes increases in cell volume and turgor, which damages membranes and organelles and can culminate in cell rupture (Lobban & Harrison 1994). Changes in ion concentrations, associated with osmotic fluctuations, may have an inhibitory effect on enzyme systems (Lobban & Harrison 1994) and many reactions involved in photosynthesis are dependent on enzymatic processes (Falkowski & Raven 1997).

There was an apparent link between a reduction in the dark-adapted F_v/F_m of symbiotic dinoflagellates *in hospite* during the experiments and the subsequent loss of symbionts measured at the end of the observation period. Decreases in dark-adapted F_v/F_m in corals exposed to low salinity preceded the loss of symbiotic dinoflagellates from coral tissues across all experiments. Within each experiment, corals that experienced the largest reduction in dark-adapted F_v/F_m lost the most symbiotic dinoflagellates. It is particularly noticeable that corals exposed to 29.5 ppt seawater for 12 h and 12 d did not suffer a decrease in F_v/F_m and suffered no subsequent loss of algae; however, in

corals exposed to a few ppt less (i.e. 26 ppt) dark-adapted F_v/F_m was reduced and a significant loss of algae occurred. The corals exposed to 29.5 ppt were likely to have become iso-osmotic to the surrounding seawater within minutes of exposure (Engebretson & Martin 1994).

An impairment of algal photosynthesis (measured as a long-term decrease in dark-adapted F_v/F_m) and loss of algal symbionts has been observed in corals following exposure to many stressors. These include heat stress (Fitt & Warner 1995, Warner et al. 1996, Jones et al. 1998), cold stress (Saxby 2001), cyanide ions (Jones & Hoegh-Guldberg 1999) and elevated light levels (Jones & Hoegh-Guldberg 2001), as well as elevated copper and effluents from the offshore oil and gas industry (R. J. Jones unpubl. data). Reductions in dark-adapted F_v/F_m in these studies have typically been discussed in terms of photoinhibition, the light-dependent inhibition of the light reactions of photosynthesis (Long et al. 1994, Osmond 1994). In this study, decreases in dark-adapted F_v/F_m also occurred in corals exposed to reduced-salinity seawater (20.5 pt) in darkness. The reduction in photochemical efficiency of the algae seen in corals exposed to low salinity cannot, therefore, be attributed to photoinhibitory processes alone, as photoinhibition is, by definition, a light-dependent process (Long et al. 1994, Osmond 1994).

Earlier examinations of the response of corals to low-salinity seawater suggested that the cause of bleaching was primarily associated with the host while the algal symbionts played a more passive role in bleaching (Van Woesik et al. 1995). Bleaching was interpreted as being caused by the swelling and perhaps rupture of host cells (Van Woesik et al. 1995). The results from this study, although correlative, suggest that the algae are not passively lost from the coral during hypo-osmosis: the dissociation of the coral-algal symbiosis only occurred when there was a reduction in the PSII efficiency measured *in hospite*. However, this does not preclude some effect of reduced salinity on the animal host cells.

Management implications

This study demonstrates how exposure to low-salinity seawater alone can cause some of the symptoms commonly attributed to temperature anomalies and anthropogenic pressures on coral reefs. It was found that decreases in the photochemical efficiency of the algal symbionts, as well as bleaching, can simply be caused by osmotic effects. These results highlight the difficulty of differentiating between the effects of warm water, herbicide-contaminated water and reduced-salinity seawater on corals when exposure is simultaneous. Contamination of the Great Barrier Reef Marine Park with PSII inhibiting herbicides has recently been reported (Haynes et al. 2000). These herbicides, which may enter the marine environment in river runoff associated with floods, also cause decreases in F_v/F_m and bleaching in corals (Jones et al. 2003). On the Great Barrier Reef, heavy rain associated with monsoons, tropical depressions and cyclones occur during the summer months when maximum seawater temperatures also occur. The bleaching of reefs around Magnetic Island during the 1998 Great Barrier Reef mass-bleaching event also coincided with heavy rainfall and a reduction in surface salinity levels to <30 ppt for several weeks (Berkelmans & Oliver 1999). Questions remain as to whether the bleaching of corals occurred because of the low-salinity seawater or elevated temperatures, or both (Berkelmans & Oliver 1999). The power of PAM chlorophyll fluorescence techniques lies in the ability to rapidly assess the passage of a stress factor(s) over coral reefs and, due to the apparent link between a decrease in F_v/F_m and loss of dinoflagellates, predict if coral bleaching may occur.

This study has established that the discolouration of corals commonly observed after contact with low-salinity seawater is due to a loss of symbiotic dinoflagellates from host tissues and not coral death as recently suggested. The use of PAM fluorometry has revealed that an impairment of algal photosynthesis precedes the loss of dinoflagellates from hypo-osmotically stressed corals. Reasons for the impairment of algal photosynthesis may include damage to cellular structures and/or enzymatic systems within symbiotic dinoflagellates exposed to low-salinity seawater. The implications of these results are important when considering that corals may experience salinity stress in combination with other factors such as elevated water temperature and pollutants from coastal runoff. Thus, the effects of low salinity on corals and coral reefs must be considered in the wider context of both mass bleaching and increasing anthropogenic stressors.

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