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Effects of cyanide on coral photosynthesis: implications for identifying the cause of coral bleaching and for assessing the environmental effects of cyanide fishing

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ABSTRACT: Modulated chlorophyll fluorescence techniques were used to examine the effects of cyanide (NaCN) from cyanide fishing on photosynthesis of the symbiotic algae (zooxanthellae) located within the tissues of the zooxanthellate hard coral *Plesiastrea versipora*. Incubating corals for 3 h in a cyanide concentration of $>10^{-5}$ M NaCN under a saturating light intensity (photosynthetically active radiation [PAR] intensity of 250 µmol quanta m^{-2} s^{-1}) caused a long-term decrease in the ratio of variable to maximal fluorescence (dark-adapted F_v/F_m). The effect of cyanide on dark-adapted F_v/F_m was light dependent; thus F_v/F_m only decreased in corals exposed to 10^{-4} M NaCN for 3 h under PAR of 250 µmol quanta m^{-2} s^{-1}. In corals where dark-adapted F_v/F_m was significantly lowered by cyanide exposure, we observed significant loss of zooxanthellae from the tissues, causing the corals to discolour (bleach). To further examine the light-dependent effect of cyanide and its relation to loss of zooxanthellae, corals were exposed to 10^{-4} M NaCN or seawater only (control), either in darkness or under 250 µmol quanta m^{-2} s^{-1}. A significant decrease in dark-adapted F_v/F_m and loss of zooxanthellae only occurred in corals exposed to cyanide in the light. These results suggest cyanide causes the dissociation of the symbiosis (bleaching) by affecting photosynthesis of the zooxanthellae. Quenching analysis using the saturation-pulse technique revealed the development of high levels of non-photochemical quenching in cyanide-exposed coral. This result is consistent with the known property of cyanide as an inhibitor of the dark reactions of the Calvin cycle, specifically as an inhibitor of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Therefore, chronic photoinhibition and an impairment of photosynthesis of zooxanthellae provides an important 'signal' to examine the environmental effects of cyanide fishing during controlled releases *in situ*.

KEY WORDS: Bleaching · Cyanide · Zooxanthellae · Coral · Chlorophyll fluorescence

INTRODUCTION

Cyanide has been used on coral reefs in the Asia-Pacific region to facilitate the capture of fish for the aquarium trade for several decades. More recently, cyanide usage has grown considerably to supply a rapidly growing restaurant-based demand for live reef fish (Johannes & Riepen 1995). Methods of cyanide fishing and other destructive fishing practices associated with the live reef fish trade have been discussed in Johannes & Riepen (1995) and Jones & Steven (1997). Cyanide fishing is banned in many Asia-Pacific countries; however, widespread illegal fishing continues. There is particular concern over the environmental effects of cyanide on hard corals since they provide the framework for the reef structure and homes for fish and reef biota (Johannes & Riepen 1995).

In a recent laboratory-based study, it was shown that brief exposure to elevated cyanide concentration caused the corals *Pocillopora damicornis* and *Porites lichen* to lose their symbiotic photosynthetic algae

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(zooxanthellae, Jones & Steven 1997). Similar loss of zooxanthellae from corals has been observed in response to variation in a wide range of physical and chemical parameters (Brown & Howard 1985, Hoegh-Guldberg & Smith 1989, Jones 1997). Loss of zooxanthellae causes corals to discolour, and the stress response has been called 'coral bleaching'. This term is normally associated with the discolouration of corals following periods of elevated seawater temperatures (see, for example, Hoegh-Guldberg & Salvat 1995, Brown 1996). Why cyanide causes coral to bleach is presently unknown and is the subject of the present communication.

Cyanide is a respiratory poison; its toxicity is based upon a high affinity for the ferric heme form of cytochrome a3 (cytochrome oxidase). In addition, cyanide also affects photosynthesis, through formation of a stable complex with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, Wishnik & Lane 1969) or inhibition of plastoquinone-oxidoreductase (Buchel & Garab 1995). Thus, cyanide may cause the loss of zooxanthellae from corals by suppressing host respiration and/or suppressing algal photosynthesis. Previous studies have shown that exposure of corals to high doses of cyanide (10^{-1} to 10^{-3} M NaCN), such as those used during cyanide fishing, causes a temporary reduction in respiratory rate (Jones & Steven 1997). Chalker & Taylor (1975) and Barnes (1985) report that photosynthetic oxygen evolution in *Acropora cervicornis* and *A. formosa* is reduced following exposure to 10^{-5} M NaCN.

In this study, we use pulse-amplitude-modulated (PAM) chlorophyll fluorescence techniques (Schreiber et al. 1986) to examine the effect of cyanide on photosynthesis of coral, and to determine its relationship with coral bleaching. Fluorescence at ambient temperature stems almost exclusively from chlorophyll associated with the antennae of photosystem II (PSII). One of the most useful parameters that can be measured using PAM fluorometry is the ratio of variable (F_v) to maximum fluorescence (F_m). $F_v = F_m - F_0$, where F_0 is the initial fluorescence when all reaction centres in PSII are open and F_m is the maximal fluorescence determined after the application of a saturating white light pulse, i.e. when all PSII reaction centres are closed. When determined in a dark-adapted state, the ratio is a measure of the maximum potential quantum yield of PSII. Changes in F_v/F_m can be used to evaluate reductions of PSII activity caused by acute stress (Schreiber & Bilger 1987). Photoinhibition by excessive light is the main cause for reduction of F_v/F_m (Krause & Weis 1991), but other stress factors, such as heat and cold stress in the light, can lead to photoinhibition and lowering of PSII quantum efficiency.

MATERIALS AND METHODS

Coral selection, collection and preparation procedures. All experiments were conducted with the hard coral *Plesiastrea versipora* (Lamarck, 1816) a faviid coral which occurs on tropical reef systems throughout the Indo-Pacific (Veron 1993). Colonies were collected from 5 to 6 m depth from Fairlight and Middle-Head, Port Jackson (NSW, Australia), and transported to the re-circulating seawater system at The University of Sydney. Several days after collection the coral fragments were cut into small pieces (surface area of 3 to 5 cm^2) and mounted onto numbered holders with marine epoxy (Vepox, Vessey Chemicals). The coral colonies were placed in aquaria under photosynthetically active radiation (PAR, 400 to 700 nm; measured with a Li-Cor 190SA quantum sensor) of 30 to 40 µmol quanta m^{-2} s^{-1}. Corals were held for 14 to 21 d under a 12 h light:12 h dark cycle before experimentation.

Experimental program. *Plesiastrea versipora* were exposed to various concentrations of cyanide under different light intensities in several experiments conducted over 3 mo. During these experiments, light was provided by fluorescent cool white tubes recessed within a light reflector array to increase irradiance intensity. Light levels were further adjusted by inserting neutral density filters (50% normal density) between the corals and the light banks. Cyanide solutions were made immediately before each experiment using analytical grade NaCN (Sigma Chemicals) dissolved in seawater from the re-circulating seawater aquarium. Cyanide or control (seawater-only) solutions were stirred throughout the incubations using magnetically coupled stir bars. All experiments were conducted in a constant temperature room at 22°C, and started between 12:00 and 13:30 h (6 h after the start of the daily illumination period). When chlorophyll fluorescence parameters were measured over several days, readings were taken at 17:00 h.

Photosynthesis versus irradiance (*PI*) curves were measured for 7 corals using a 'photo-respirometer' comprising 4 separate 90 ml water-jacketed acrylic chambers. Each chamber had a false bottom enclosing a stir-bar powered by a magnetic stirrer. Actinic light was provided by 50 W quartz-halogen spotlights, illuminating each chamber from opposite sides. Respiratory O_2 consumption and photosynthetic O_2 production were measured using Clark-type electrodes (Strathkelvin Instruments, Glasgow, UK) inserted into the chamber tops. Sensors were connected via oxygen polarizing units to an analogue to digital converter (ADC-1, Remote Measurement Systems, Seattle, USA) which was controlled by data acquisition software

(DATACAN IV, Sable Systems, Los Angeles, USA). Oxygen concentrations were measured every 6 s from an average of 2 consecutive voltage readings from each sensor. The voltage of the sensors was calibrated using air-saturated seawater at the incubation temperature (22°C) and salinity (34‰) and oxygen purged (nitrogen-bubbled) seawater.

Corals were exposed to darkness or to 7 different irradiance levels (~8 to 500 µmol quanta m^{-2} s^{-1}). Different irradiance levels were obtained by inserting neutral density filters between the lights and incubation chambers housing the corals. The maximal rate of gross photosynthetic production at saturating light intensities ($P_{m(gross)}$; µmol O_2 cm^{-2} s^{-1}), the light intensity at which the initial slope of the *PI* curve intersects the $P_{m(gross)}$ value (I_k; µmol quanta m^{-2} s^{-1}), and respiration of the symbiosis at irradiance (I) = 0 (R; µmol O_2 cm^{-2} h^{-1}) were estimated by fitting a non-linear hyperbolic tangent function to the data comprising the *PI* curve (Jassby & Platt 1976). The model has the form:

$$P = P_{m(gross)} \cdot \tanh(I/I_k) + R$$

where P is the production at any photon irradiance. The goodness of fit for the function was assessed using a least squares regression of predicted versus observed values (r^2). Residual variances were minimized with multiple iterations of altered parameter sets using the Solver Utility of Microsoft Excel 1997.

To study the influence of different cyanide concentrations, corals were exposed to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M NaCN for 3 h under a PAR of 250 µmol quanta m^{-2} s^{-1}. Cyanide solutions were poured into 3 replicate 200 ml containers at each cyanide concentration, and 2 corals placed in each of the test containers. At the end of the incubations, corals were dark-adapted before measuring F_v/F_m (see 'Chlorophyll fluorescence measurements'). Dark-adapted F_v/F_m was measured daily for 11 d and the corals were then sacrificed to determine the density of zooxanthellae.

To determine the influence of different light intensities on dark-adapted F_v/F_m, corals were exposed to 10^{-5} M NaCN cyanide or seawater (controls) for 3 h under irradiances of 0, 62.5, 125 and 250 µmol quanta m^{-2} s^{-1}. Corals were then dark-adapted before measuring F_v/F_m.

To study the relationship between a decrease in dark-adapted F_v/F_m and loss of zooxanthellae, corals were exposed to either 10^{-4} M NaCN or ambient seawater (control) in either darkness or 250 µmol quanta m^{-2} s^{-1} PAR for 3 h. Three replicate containers with 4 corals in each container were used for each treatment. F_v/F_m was measured in dark-adapted samples immediately after the experiment, and then daily for 20 d. Corals were then sacrificed to determine the density of zooxanthellae.

To study the effect of cyanide on maximum effective quantum yield, photochemical quenching (qP) and non-photochemical quenching (qN), corals were exposed to 10^{-4} M NaCN or ambient seawater (control) under 250 µmol quanta m^{-2} s^{-1} light for 0.5 h. Maximum effective quantum yield, qP and qN were determined using a TEACHING-PAM fluorometer.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured using PAM fluorometry (Schreiber et al. 1986) using either of 2 recently developed chlorophyll fluorometers (DIVING-PAM and TEACHING-PAM, Walz, Effeltrich, Germany). The TEACHING-PAM fluorometer was used for determining photochemical quenching and non-photochemical quenching in cyanide-exposed corals; all other measurements were made with the DIVING-PAM fluorometer. In both fluorometers, 3 µs pulses of a light-emitting diode (LED) are used as the measuring light (peak emission at 650 nm). In the DIVING-PAM fluorometer, fluorescence is detected at wavelengths above 710 nm. Saturation pulses (8000 µmol quanta m^{-2} s^{-1} PAR, 800 ms pulse width) are provided by a halogen lamp. In the TEACHING-PAM fluorometer, an LED (660 nm) is used to provide actinic light illumination and saturation pulses (3500 µmol quanta m^{-2} s^{-1} PAR, 800 ms pulse width). During analysis with the TEACHING-PAM, small pieces of coral (~3 cm^2 surface area) were placed on a glass slide with a few drops of seawater over the 2 mm exit point of the measuring head (Schreiber et al. 1997). During analysis with the DIVING-PAM fluorometer, the fibre-optic light guide was gently pressed on the surface of the coral, which was held in seawater.

The photochemical energy conversion in PSII can be evaluated by both fluorometers by using saturating pulses of light. These cause a temporary saturation of energy conversion at the PSII reaction centres (Schreiber et al. 1986, Genty et al. 1989). Two consecutive measurements can be used to estimate the maximum potential quantum yield in a dark-adapted sample (i.e. placed in darkness for 20 min) or the maximum effective quantum yield of a sample in an illuminated state. Firstly, weak pulsed red light (<1 µmol quanta m^{-2} s^{-1}) was applied to determine F_0 in a dark-adapted state or F in an illuminated state. Secondly, a saturating pulse was applied to determine the F_m value (in a dark-adapted state) or F_m' (in an illuminated coral). The change in fluorescence (ΔF) caused by the saturating pulse in relation to the maximal fluorescence yield (F_m or F_m') has been shown to be a good measure of quantum yield (Genty et al. 1989). Thus $\Delta F/F_m$ (dark-adapted sample) = F_v/F_m = maximum potential quantum yield, and $\Delta F/F_m'$ (illuminated sample) = maximum effective quantum yield.

In addition to maximum effective quantum yield, photochemical [qP = $(F_m' - F)/(F_m' - F_0)$] and non-photochemical [qN = $(F_m - F_m')/(F_m - F_0)$] quenching coefficients were calculated using the TEACHING-PAM fluorometer. During quenching analysis we used the pre-programmed sequence of commands and instrumental settings available with the DA-TEACH software (Protocol No. 4 in 'saturation pulse mode', DA-TEACH v.1.00g, Walz). In this procedure, corals were dark-adapted before measuring F_0 and F_m. The actinic light was turned on, and the fluorescence (F) measured. A series of saturation flashes were applied at 20 and 40 s intervals, the new F_m value (F_m') determined and qP and qN calculated.

Biomass determination. Coral tissues were stripped from the skeletons with a jet of re-circulated filtered seawater using a WaterPik™. The slurry produced from the tissue-stripping process was homogenized in a blender for 30 s and the volume of the homogenate (~100 ml) recorded. The number of zooxanthellae in 10 ml aliquots of the homogenate was measured using a hemacytometer (8 replicate counts). Total zooxanthellae per coral was calculated after correcting for the volume of the homogenate. Density of zooxanthellae was expressed as number per unit surface area. Surface area was measured using an image analysis program (NIH-image) from digital pictures of the coral calibrated against images of graph paper of known surface area. Densities of zooxanthellae in a subset of corals (field controls) were also measured in order to examine whether the handling and preparation procedures caused any significant loss of zooxanthellae. Field controls were measured whenever fresh corals were collected over the experimental program.

Data are presented as mean ($\bar{x}$) ± standard deviation (SD). Data are analyzed ($\alpha = 0.05$) using analysis of variance (ANOVA). Assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Welch's test) were tested, and where appropriate the data transformed (arc sine). Dunnett's test of significance was used to examine the nature of significant differences.

RESULTS

Plesiastrea versipora display a clearly defined saturating *PI* response (Fig. 1), with a $P_{n/gross}/R$ ratio of 2.7 ± 0.3 and I_k = 67 ± 31 µmol quanta m^{-2} s^{-1}.

Corals exposed to 10^{-2} and 10^{-3} M NaCN under 250 µmol quanta m^{-2} s^{-1} PAR retracted deeply within their calices. At the other cyanide concentrations, no tentacle retraction was observed. Dark-adapted F_v/F_m of corals exposed to $\geq 10^{-5}$ M NaCN decreased as a function of increasing cyanide concentration (Fig. 2). Mean dark-adapted F_v/F_m in corals exposed to concentrations $\geq 10^{-5}$ M NaCN were significantly different from control corals (Fig. 2, $p < 0.05$, ANOVA).

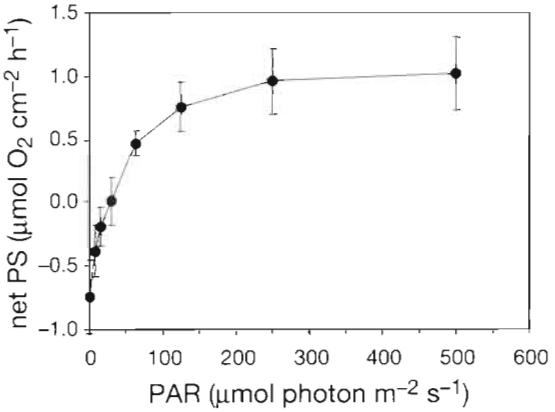

Fig. 1 *Plesiastrea versipora*. Net photosynthesis (PS) versus irradiance (PAR) (*PI*) curve for the corals used in the study. Fitted curve is a hyperbolic tangent function (see text). Data are expressed as $\bar{x}$ ± SD, n = 7

All corals exposed to 10^{-2} NaCN died within 24 h. Twenty-four hours after experimentation, dark-adapted F_v/F_m of corals exposed to 10^{-4} M NaCN was lower than that of corals exoposed to 10^{-3} M NaCN and remained lower for the rest of the monitoring period (Fig. 3). After the initial decrease, dark-adapted F_v/F_m of corals exposed to 10^{-3}, 10^{-4} and 10^{-5} M NaCN increased rapidly during the monitoring period. After 11 d, only F_v/F_m in corals exposed to 10^{-4} M NaCN was lower than control corals.

During the first 4 to 5 d of the monitoring period, corals exposed to 10^{-3}, 10^{-4} and 10^{-5} M NaCN discoloured from a dark to a pale green/brown. After 11 d, the number of zooxanthellae of corals exposed to

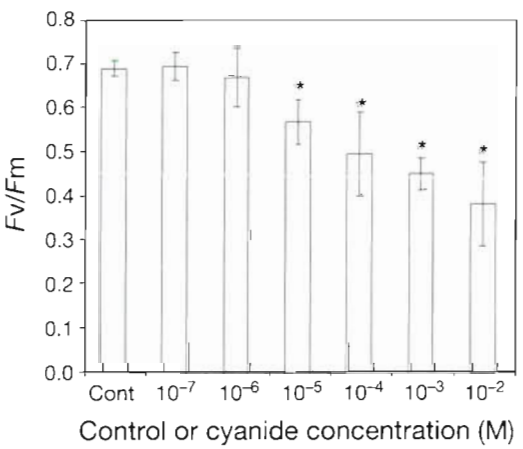

Fig. 2. *Plesiastrea versipora*. Dark-adapted F_v/F_m in corals 20 min after exposure to 10^{-7} to 10^{-2} M NaCN for 3 h under PAR of 250 µmol quanta m^{-2} s^{-1} Data are expressed as $\bar{x}$ ± SD, n = 6. Significantly different from control: *$p < 0.05$ (ANOVA)

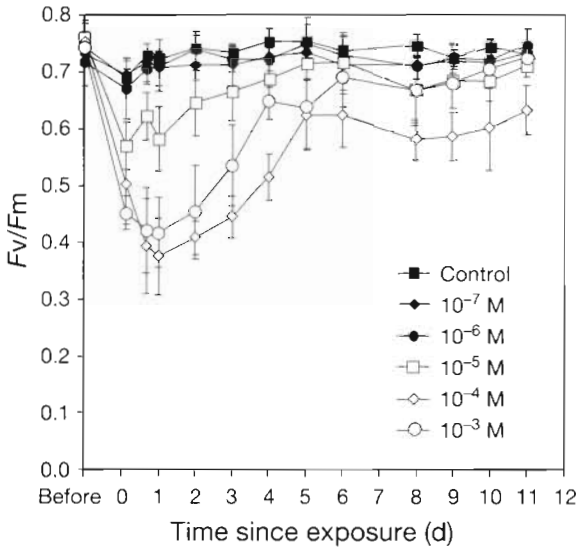

Fig. 3. *Plesiastrea versipora*. Dark-adapted F_v/F_m in corals following exposure to cyanide concentrations in the range 10^{-7} to 10^{-3} M NaCN for 3 h under PAR of 250 µmol quanta m^{-2} s^{-1}. Data are expressed as $\bar{x}$ ± SD, n = 6

these cyanide concentrations was lower than of control corals (exposed to seawater only) or of freshly collected corals (Fig. 4), corresponding to significant differences between control and experimental treatments ($p < 0.05$, ANOVA). There was no significant difference in the number of zooxanthellae between control corals and freshly collected corals ($1.7 \pm 0.2 \times 10^7$ zooxanthellae cm^{-2}, n = 12), suggesting that the preparation and manipulative procedures had no measurable effect, in terms of loss of zooxanthellae, on the corals.

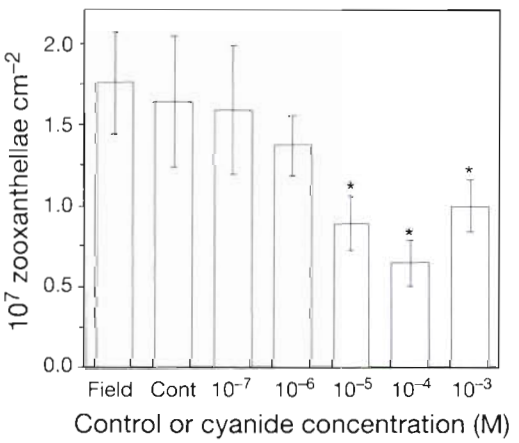

Fig. 4. *Plesiastrea versipora*. Mean numbers of zooxanthellae in corals following exposure to cyanide concentrations in the range 10^{-7} to 10^{-3} M NaCN for 3 h under PAR of 250 µmol quanta m^{-2} s^{-1}. A mean number for freshly collected corals is also given. Data are expressed as $\bar{x}$ ± SD, n = 6. Significantly different from control: *$p < 0.05$ (ANOVA)

Dark-adapted F_v/F_m of corals exposed to 10^{-5} M NaCN under 250 µmol quanta m^{-2} s^{-1} PAR (0.57 ± 0.41, n = 5) was 20% lower than control corals (0.69 ± 0.013) which were exposed to ambient seawater under the same irradiance; these values were significantly different ($p < 0.05$, ANOVA, Fig. 5). There were no significant differences in dark-adapted F_v/F_m of cyanide-exposed and control corals at the other irradiances tested.

Twenty-four hours after experimentation, dark-adapted F_v/F_m of corals exposed to cyanide (10^{-4} M NaCN) in the light was lower than levels measured before the experiment or in the other treatments (Fig. 6A). Dark-adapted F_v/F_m of corals exposed to cyanide in the light returned to levels measured in control corals over the subsequent 20 d monitoring period. There were 2 distinct phases of the recovery, a fast phase from Day 1 to Day 6, and a second slower phase from Day 7 until the end of the experiment. Discolouration of the corals (bleaching) was observed during the first phase. The number of zooxanthellae in corals exposed to cyanide in the light was ~40% of the densities in other treatments or in colonies freshly collected from the field, corresponding to a significant difference ($p < 0.05$, ANOVA, Fig. 6B).

Representative original dark-light induction curves of control or cyanide-treated corals are shown in Fig. 7. Saturating pulses were applied at regular intervals to assess qP and qN and to measure maximum effective quantum yield ($\Delta F/F_m'$). Maximum potential quantum yield (F_v/F_m) was measured before each recording by the application of a saturation pulse to a previously dark-adapted sample. Control corals show high levels of qP and yield, indicating high PSII activity (Fig. 7A). The initial rise of qN reflects the build-up of a ΔpH, as

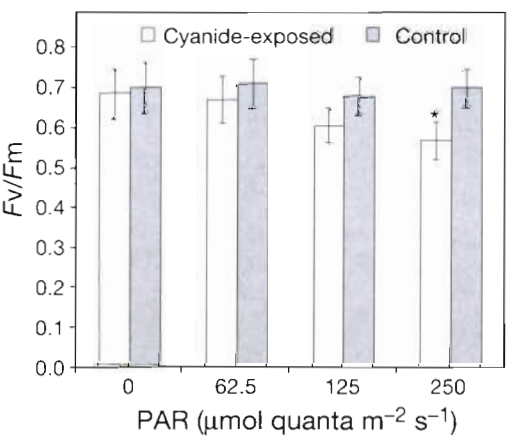

Fig. 5. *Plesiastrea versipora*. Dark-adapted F_v/F_m in corals exposed to 10^{-5} M NaCN or seawater (control) for 3 h under PAR of 0, 62, 125, or 250 µmol quanta m^{-2} s^{-1}. Data are expressed as $\bar{x}$ ± SD, n = 5. Significantly different from controls: *$p < 0.05$ (ANOVA)

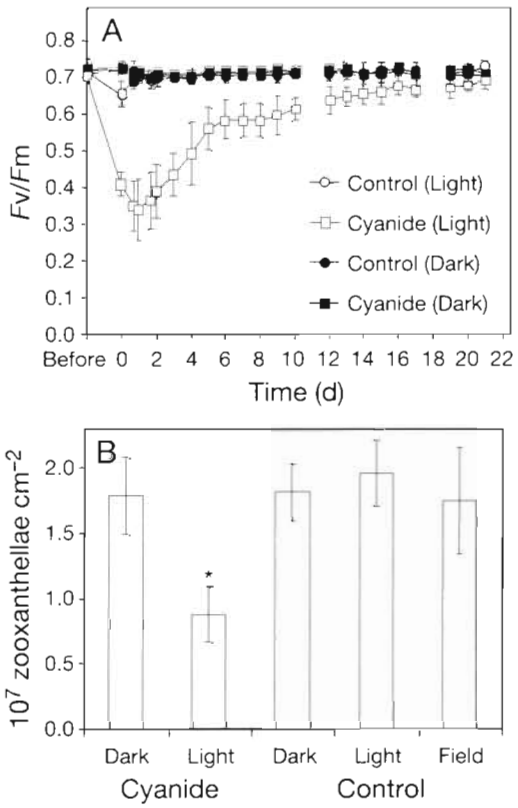

Fig. 6. *Plesiastrea versipora*. (A) Dark-adapted F_v/F_m in corals following exposure to 10^{-4} M NaCN or seawater (control) for 3 h under PAR of 250 µmol quanta m^{-2} s^{-1} or darkness. Data are expressed as $\bar{x}$ ± SD, n = 12. (B) Mean number of zooxanthellae at the end of the 20 d experiment. Significantly different from control: *p < 0.05 (ANOVA)

Calvin cycle enzymes are not yet light activated and ATP accumulates. Calvin cycle activation by increased pH consumes ATP, and the ΔpH is dissipated, corresponding to a decrease in qN. In cyanide-treated coral light-induced non-photochemical quenching of fluorescence yield was considerably enhanced (Fig. 7B). At the same time, the effective quantum yield decreased with respect to the control. qN did not relax during illumination, but increased in an initially rapid and then slower phase. Hence, there was no induction of Calvin cycle activity as occurs in control corals (Schreiber & Bilger 1987).

DISCUSSION

Our data confirm previous observations that corals lose their symbiotic algae (zooxanthellae) when exposed to elevated concentrations of cyanide (Jones & Steven 1997), and provide several important insights into the mechanism associated with the dissociation of the coral-algal symbiosis (bleaching).

Dark-adapted F_v/F_m in zooxanthellae of *Plesiastrea versipora* was 0.7 to 0.75, slightly lower than observed in higher plants, but typical for marine algae (Falkowski et al. 1994). Exposure of *P. versipora* to cyanide concentrations $\geq 10^{-5}$ M caused a significant decrease in dark-adapted F_v/F_m. Chalker & Taylor (1975) and Barnes (1985) reported a decrease in photosynthesis in staghorn corals *Acropora cervicornis* and *A. formosa* exposed to 10^{-5} M NaCN. Our studies show that the effect of cyanide is light dependent. Thus, exposure of corals to cyanide at an irradiance intensity sufficient to saturate photosynthesis caused a significant decrease in dark-adapted F_v/F_m, whilst exposure to the same concentration at lower intensities or in darkness had no measurable effect. Importantly, a significant decrease in dark-adapted F_v/F_m preceded a reduction in density of zooxanthellae in the tissues and subsequent tissue discolouration (bleaching; for example, compare Fig. 2 with Fig. 4). This is most clearly highlighted in the experiment in which corals were exposed to the same cyanide concentration in the dark or in the light. In this experiment, a significant decrease in dark-adapted F_v/F_m and density of zooxanthellae only occurred in corals exposed to cyanide in the light (Fig. 6). Collectively, these results suggest that cyanide causes the dissociation of the coral-algal symbiosis by affecting photosynthesis of the zooxanthellae as opposed to host or symbiont respiration.

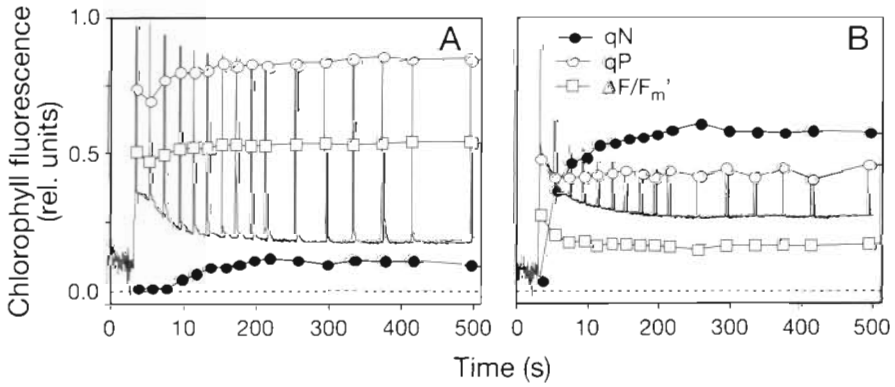

Fig. 7. *Plesiastrea versipora*. Original recordings of dark-light induction curves with fluorescence quenching analysis by the saturation pulse technique of (A) control coral and (B) coral exposed to 10^{-4} M NaCN under 250 µmol quanta m^{-2} s^{-1} light for 0.5 h. Quenching analysis was conducted with a TEACHING-PAM chlorophyll fluorometer. qP: photochemical quenching, qN: non-photochemical quenching and $\Delta F/F_m'$: maximum effective quantum yield (see text)

The light- and cyanide-dependent decrease in dark-adapted F_v/F_m is symptomatic of photoinhibition. Photoinhibition encompasses processes associated with the lowering of the efficiency of photosynthetic energy utilisation, reversibly or irreversibly, in the short term or long term (Osmond 1994). Two types of photoinhibition, dynamic and chronic, have been distinguished, separable by their relaxation times. Dynamic photoinhibition encompasses short-term, rapidly reversible decreases in quantum yield, associated with PSII antennae-based dissipation of excess light as heat. Chronic photoinhibition involves a slowly engaged and slowly reversible decline in yield, associated with the loss of PSII reaction centre function (Osmond & Grace 1995). The long-term (>24 h) decrease in dark-adapted F_v/F_m measured in this study suggests zooxanthellae were chronically photoinhibited by the elevated cyanide concentrations.

To further examine the nature of the impairment of algal photosynthesis we used the saturation pulse technique to examine photochemical (qP) and non-photochemical (qN) components of fluorescence quenching. Corals exposed to low levels of cyanide developed high levels of qN. Non-photochemical quenching is considered to reflect a mechanism for photoprotection, i.e. to prevent over-reduction of the photosynthetic electron transport chain by dissipation of excess absorbed light energy in the PSII antenna system as heat (Demmig-Adams 1990). Non-photochemical quenching occurs when the rate of light-driven electron transport exceeds the rate of ADP/Pi recycling by the dark reactions of photosynthesis (Schreiber & Neubauer 1990). The development of qN in cyanide-treated corals is consistent with the ability of cyanide to act as an inhibitor of the dark reactions of the Calvin cycle, specifically as an inhibitor of Rubisco (Wishnick & Lane 1969). In fact, cyanide is routinely used to inhibit Calvin cycle activity during studies of chlorophyll fluorescence and plant physiology (see, for example, Kobayashi & Heber 1994). When Calvin activity is affected, the supply of NADP+ for reduction and ADP and Pi for phosphorylation is slowed. If incoming light is continually funneled into the electron transport chain, then this may lead to its over-reduction, and subsequent damage to the PSII reaction centre (Styring & Jegerschöld 1994).

Under conditions in which assimilatory electron flow is impaired, for example, under heat stress (Weis 1981, Schreiber & Bilger 1987), photoprotective mechanisms are available to prevent photoinactivation of PSII under high light levels. Recently, it has become clear that in addition to its role in ATP formation, the formation of a ΔpH, which accompanies vectorial proton transport, functions to lower PSII with respect to PSI (Weis & Berry 1987). Both cyclic electron flow at PSI and O_2-dependent electron flow have been implicated as contributing to the formation of the regulatory ΔpH when assimilatory electron flow becomes limiting. O_2-dependent electron flow consists of 2 tightly linked light-driven partial reactions, the Mehler reaction (in which oxygen reduction results in superoxide and H_2O_2 production) and photoreduction of monodehydroascorbate (MDA), which is formed by the ascorbate peroxidase reaction (the enzyme catalysed reduction of H_2O_2 by ascorbate). Collectively, these reactions are referred to as the Mehler-ascorbate-peroxidase (MAP) cycle. Cyanide also inhibits ascorbate-peroxidase activity (Asada & Takahashi 1987, Kobayashi & Heber 1994). The extent of an active MAP cycle in zooxanthellae is presently unknown; however, cyanide has the potential not only to cause an over-reduction of the electron transport chain by blocking assimilatory electron flow, but also to limit photoprotective down-regulation of PSII by inhibiting ascorbate-peroxidase activity. During cyanide fishing, corals may experience considerably higher concentrations than those used in the present study. In these situations the effect on photosynthesis should be viewed in terms of multiple effects of cyanide on several processes associated with photosynthesis, including inhibition of the oxidation of plastoquinone-oxidoreductase (Buchel & Garab 1995), as well as detoxification mechanisms associated with the removal of active oxygen species (i.e. ascorbate-peroxidase, Asada & Takahashi 1987).

Dark-adapted F_v/F_m in cyanide-damaged coral returned to normal levels during the post-exposure recovery period, possibly reflecting repair and/or assembly of new centres in the PSII repair cycle (Aro et al. 1993). However, this interpretation is complicated by the loss of zooxanthellae observed during the study. Previous studies have shown that the reduction of zooxanthellae during heat stress and elevated copper concentrations is largely caused by export of the zooxanthellae from the tissues (Hoegh-Guldberg & Smith 1989, Jones 1997). If corals preferentially lost 'impaired' zooxanthellae (i.e. those with lower yields), then this may also result in an increase in dark-adapted F_v/F_m, as the population becomes progressively dominated by 'healthy' zooxanthellae (i.e. those with higher yields). On further inspection, it can be seen that the dark-adapted F_v/F_m recovered in an initial fast phase (occurring over the space of ~6 d) and a subsequent slower phase (see Figs. 3 & 6). Discolouration of the corals only occurred in the first phase. We suggest that the initial rapid recovery of dark-adapted F_v/F_m in the first phase is primarily associated with the selective export of impaired zooxanthellae. The second phase may signify the PSII repair cycle and/or an increase in the number of healthy zooxanthellae through algal division (Jones & Yellowlees 1997).

Dark-adapted F_v/F_m in corals exposed to 10^{-3} M NaCN was lower than in corals exposed to 10^{-4} M NaCN immediately after the 3 h experiment, consistent with a normal dose-response relationship (Fig. 2). However, 24 h after the experiment, dark-adapted F_v/F_m in the corals exposed to 10^{-4} M NaCN was lower than in corals exposed to 10^{-3} M NaCN, and remained lower for the rest of the monitoring period (Fig. 3). Discolouration (bleaching) of the tissues was more pronounced in the corals exposed to 10^{-4} M NaCN than in corals exposed to 10^{-3} M NaCN, consistent with a lower density of zooxanthellae in the tissues after 11 d (Fig. 4). Thus, overall, exposure of corals to a cyanide concentration of 10^{-4} M NaCN appeared to have a greater effect than exposure to a cyanide concentration an order of magnitude higher. One possible explanation for this unusual effect is that corals exposed to 10^{-3} M NaCN retracted within their calices during the experiment. In all other treatments, and during the recovery period, the polyps from all corals appeared expanded. *Plesiastrea versipora* has large polyps and a very deep tissue layer (~10 mm). Retraction of the polyps within the calices is likely to cause shading of zooxanthellae at the base of the polyp and perhaps exclusion of cyanide from the inner tissues. When chlorophyll fluorescence parameters were determined after the 3 h incubation, measurements would have been made from zooxanthellae in the coenosarc and upper parts of the tentacles which were exposed to the full experimental irradiance and/or cyanide concentration. However, when measurements were taken in the recovery period, when the polyps were expanded, fluorescence measurements would include previously shaded zooxanthellae. Given the light-dependent effect of cyanide, this may have had a significant effect on average dark-adapted F_v/F_m measured by the fluorometer. If this is the case, then polyp retraction affords protection to the coral from photochemical damage, as has been suggested during sub-aerial exposure (Brown et al. 1994).

Recent technical advancements in the development of a fibre-optic microprobe in combination with a modified PAM fluorometer have allowed measurements of chlorophyll fluorescence characteristics of cells within leaves of higher plants (Schreiber et al. 1996). Such systems have an adequate resolution (20 µm) for examining fluorescence characteristics of zooxanthellae within different parts of the coral polyp. These techniques may prove particularly insightful in measuring self-shading in cyanide-exposed corals, or during studies of photoinhibition in corals exposed to high photosynthetic photon flux density.

It has been suggested from studies on the effect of heat stress on photosynthesis of cultured zooxanthellae that when the symbionts become a net burden to the host they are expelled (Iglesias-Prieto 1997). Our results support this proposition. Interestingly, the effects of cyanide on zooxanthellae are very similar to the effects of elevated water temperature. For example, in both instances there are high levels of non-photochemical quenching (Fig. 7, see also Warner et al. 1996 and Jones et al. 1998a) and a lowering of dark-adapted F_v/F_m, symptomatic of damage to PSII (Fig. 2, see also Fitt & Warner 1995, Warner et al. 1996, Jones et al. 1998a). During cyanide-mediated toxicity, light is a secondary variable that is essential to elicit loss of zooxanthellae (Fig. 6B); a similar interaction between light and temperature has also been reported for corals during laboratory experiments (Coles & Jokiel 1978) and in observations of bleaching on the upper sunlight-exposed surfaces of corals during bleaching events (Harriott 1985). It has recently been suggested that elevated seawater temperature causes bleaching in coral by primary damage to PSII (Warner et al. 1996). Whilst damage to PSII appears to be the case with cyanide-induced bleaching, it is likely not to be the primary site of action but a secondary effect, which is light-dependent and subsequent to 'sink' limitation in electron transport

Beyond certain minimal irradiances, cyanide has the potential to cause chronic photoinhibition of zooxanthellae within the tissues of coral. Loss of zooxanthellae and subsequent bleaching of the tissues reported during cyanide exposure appears to be closely associated with damage to photosynthesis of the zooxanthellae. In this laboratory-based study, we exposed coral to static cyanide concentrations under carefully controlled incubation periods and light intensities. *In situ* during cyanide fishing, corals are likely to experience rapidly fluctuating cyanide levels depending upon the starting cyanide concentration and proximity to the target fish (Jones & Steven 1997). Light levels are also likely to be highly variable depending on weather conditions and sea state. Our results suggest that a decrease in dark-adapted F_v/F_m and changes in photosynthetic electron transport provide important signals with which to assess the effects of cyanide on corals *in situ*. Future studies on the effect of cyanide on corals should therefore consider impairment of photosynthesis as an important 'effect criterion' (see Jones et al. 1998b).

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