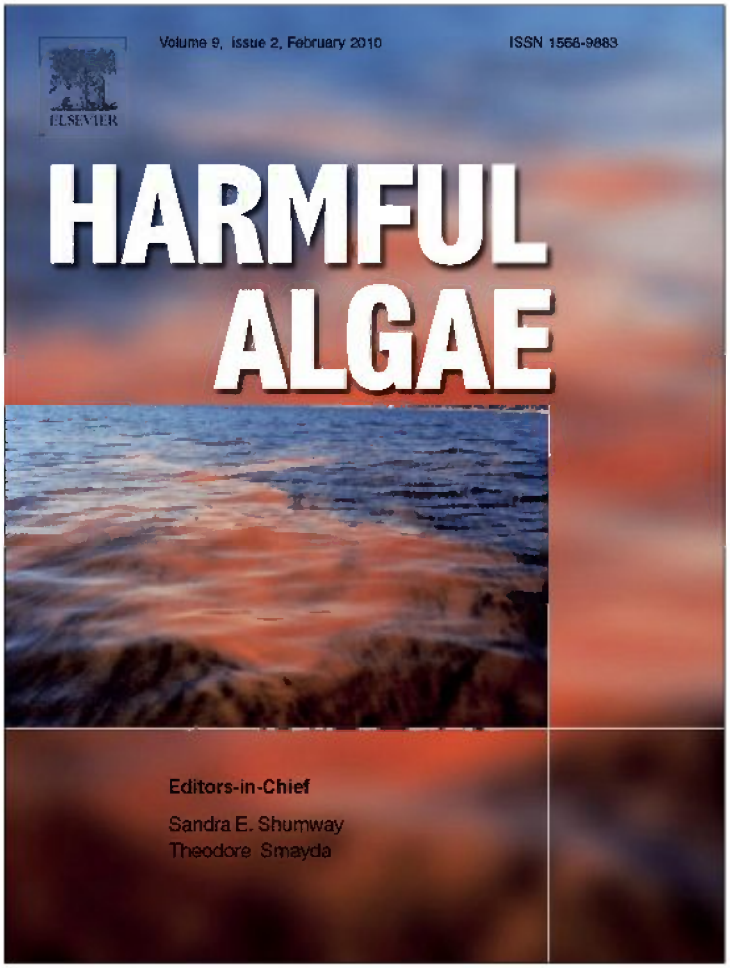

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Effects of the Florida red tide dinoflagellate, *Karenia brevis*, on oxidative stress and metamorphosis of larvae of the coral *Porites astreoides*

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ABSTRACT

Florida red tides, caused by the dinoflagellate *Karenia brevis*, produce a series of neurotoxins, brevetoxins, which have historically had a negative impact on coastal communities along the South Florida coast. While much work has examined the effects of *K. brevis* blooms or exposure to brevetoxins on a diverse array of marine organisms, there have been no reports studying the impact of *K. brevis* on coral physiology. We provide evidence that short-term exposure of naturally occurring concentrations of *K. brevis* and their associated toxins can induce oxidative stress in the coral larvae of *Porites astreoides*. Larvae of *P. astreoides* were exposed to aliquots of intact *K. brevis* cells at naturally occurring concentrations ($6 \times 10^5 - 4 \times 10^6$ cells L^{-1}) or cellular lysates (containing 5.4–15.0 $\mu g\ L^{-1}$ brevetoxin) for 20 h and a variety of larval physiological biomarkers were measured. In the presence of bloom scale concentrations of *K. brevis* larval respiration of *P. astreoides* was inhibited. This was accompanied with an increase in lipid hydroperoxide content and catalase activity indicating oxidative damage and a subsequent antioxidant response were occurring. However, when larvae were exposed to intact cells of *K. brevis* or their sonicated, lysed exudates stress indices including zooxanthellae expulsion (bleaching), larval protein carbonylation, and superoxide dismutase activity were not significantly altered. Even though there was evidence for short-term oxidative stress in larvae that were exposed to *K. brevis* there was no change in survival or settlement rates of larvae of *P. astreoides*.

1. Introduction

Toxic red tides (harmful algal blooms, HABs), caused by the dinoflagellate *Karenia brevis* (formerly, *Gymnodinium breve*, Davis) (Duagbjerg et al., 2001), occur annually along the Florida Gulf coast. Commonly referred to as the Florida red tide, these blooms occur most frequently along the southwestern Florida coast with impacts that vary from minor blooms to intensive blooms that persist up to 18 months (Landsberg, 2002). These blooms frequently pass through the Florida Keys (Fig. 1) as they are entrained into the Florida Current and Gulf Stream and transported into the north Atlantic (Tester and Steidinger, 1997). Although always present in low concentrations throughout the Gulf of Mexico with no apparent adverse effects, high concentrations (blooms) of *K. brevis* produce sufficient neurotoxins (brevetoxins) to cause human and environmental impacts including massive fish kills, marine mammal, sea turtle and sea bird mortalities and benthic community die-offs (Flewelling et al., 2005; Pierce et al., 2005; Pierce and Henry, 2008).

Brevetoxins are polycyclic ether ladder compounds that are differentiated into two similar, yet distinct backbone structures PbTx-1 (type-A) and PbTx-2 (type-B) (Poli et al., 1986; Shimizu, 1986). The suite of brevetoxins includes as many as 12 compounds (designated as PbTx-1, -2, -3, etc.) ranging in molecular weight from 868 to 936 Da (Baden et al., 2005). The primary mode of action is binding of PbTx to site 5 of voltage sensitive sodium channels (VSSC), resulting in persistent activation of cells and interfering with proper nerve transmission (Baden et al., 2005). While much work has examined the effects of *K. brevis* blooms or exposure of brevetoxins on a diverse array of higher organisms, there have been no studies on the impact of *K. brevis* on coral physiology.

Scleractinian corals are benthic organisms; however, their larvae are planktonic and spend hours to weeks (depending on the coral species) dispersing in the water column. This planktonic period represents a critical phase in the life cycle of this organism and has a direct impact on subsequent recruitment processes (Edmunds et al., 2001; Ritson-Williams et al., 2009). As coral reefs

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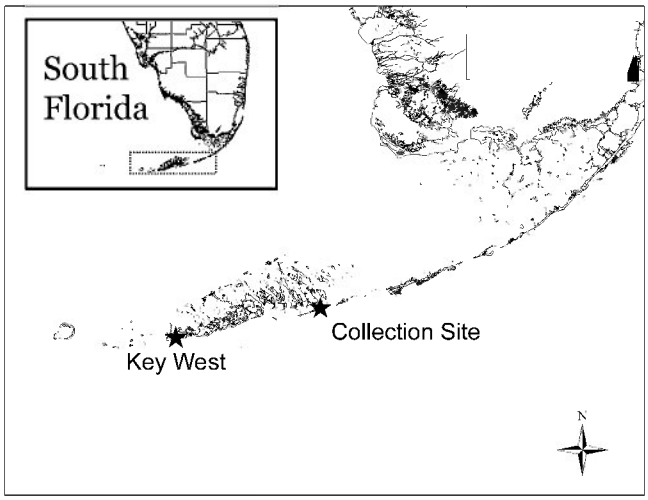

Fig. 1. Geographical location of *P. astreoides* collection and where the Florida Current transports *K. brevis* through the Florida Keys.

across the Caribbean decline in coral cover (Gardner et al., 2003; Hoegh-Guldberg, 1999), recruitment has been identified as a key process necessary for the recovery of coral reef communities. To better understand the cellular effects of brevetoxins on larval health we used the coral *Porites astreoides* as a model organism. *P. astreoides* is found throughout the Caribbean basin and has been previously studied for larval metamorphosis, mortality in response to temperature disturbance (Edmunds et al., 2001), and response to the presence of macroalgae and cyanobacteria on the benthos (Kuffner et al., 2006). In this study we assessed if naturally occurring concentrations of *K. brevis* or released toxins could compromise the physiological integrity of coral larvae and negatively impact their survival and settlement.

Within the past decade, advancements in cellular diagnostic assays have been able to demonstrate positive correlations between the onset of selected abiotic stressors and coral metabolic stress (Downs et al., 2005). Of particular interest are physico-chemical perturbations in the environment that increase the production of an organism's cellular reactive oxygen species (ROS) pool such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). The overproduction of ROS can damage a series of intracellular targets including proteins, lipids, and nucleic acids (Halliwell, 2006; Lesser, 2006). To date, the majority of the biomarker studies used for coral monitoring have focused on the stress response of adult reef-building corals (Downs et al., 2000; Morgan et al., 2001; Griffin and Bhagooli, 2004). This study tested the effects of live *K. brevis* and extracted biotoxins on the physiology, survival and settlement of larvae of *P. astreoides*.

2. Materials and methods

2.1. Collection and treatment of P. astreoides larvae

Forty colonies of *P. astreoides* were collected from the seawall at the base of the Spanish Channel Harbor Bridge in the lower Florida Keys (GPS N 24°38.9′; W 81°19.8′), and transported to Mote's Tropical Research Laboratory (Summerland Key, FL) in coolers and maintained in running seawater. Larvae were collected during the new moon ([night of May 5, 2008] McGuire, 1998). To obtain larvae, each adult colony was placed in an individual 3 L Rubbermaid Grip's Mix bowl® supplied with continuously running seawater. The bowls were tilted so the positively buoyant larvae spilled over the handles of the bowls each night into plastic tri-pour beakers fitted with a 180 μm mesh bottom supported off the seawater table bottom by 3, 1/2 cm tall PVC feet. The water level inside the tank was maintained at 15 cm so the larvae remained in the tri-pour beakers until sunrise the next morning. Larvae were pooled into 3–4 separate containers (5 L plastic buckets with 180 μm mesh as a bottom) and maintained in flow through seawater tables. Adult colonies were later returned to their site of collection and reattached with Z-Spar Splash Zone Compound® underwater epoxy.

One day old larvae were subjected to control and *K. brevis* treatment conditions. Five treatments (see below) were used with 10 replicates for each treatment. Two concentrations of *K. brevis* were incubated with coral larvae to evaluate the outcome of any biological interactions. In addition, larval samples were incubated with cellular lysates of *K. brevis* to evaluate a chemical effect. To obtain a *K. brevis* cell lysate, samples of a desired cell count were sonicated in glass scintillation vials for 15 min and subsequently centrifuged at 100 × *g* for 5 min. The resulting supernatant was used for subsequent incubation studies.

Incubation treatments were as follows: (1) low cell count (6×10^5 cells L^{-1}), which was equivalent to field concentrations during low bloom conditions; (2) high cell count (4×10^6 cells L^{-1}) that approximated the cell concentrations during high bloom conditions; (3) sonicated cellular lysate of the low cell count; (4) sonicated cellular lysate of the high cell count; (5) control (no *K. brevis* or sonicated lysate).

Each replicate contained 200 larvae of *P. astreoides* in a total volume of 200 mL of 0.45 μm filtered seawater (with respective concentrations of live cells of *K. brevis* or cell lysates from *K. brevis*). Each replicate was held in an individual, open 400 mL plastic tri-pour beaker. All experimental incubations lasted for 20 h at a constant temperature of 25.6 °C and ambient light level of 4 μmol m^{-2} s^{-1}.

Post-treatment, 50 larvae from each replicate were removed for subsequent settlement/metamorphosis assays, and eight additional larvae were removed for oxygen respiration studies or bleaching experiments. The remaining individuals were collected, flash frozen in liquid N_2 and stored at −80 °F for cellular stress assays.

2.2. K. brevis culture and brevetoxin analysis

K. brevis (Manisota Key strain) was obtained from the Mote Marine Laboratory Phytoplankton Culture Facility at a stock concentration of 11×10^6 cells L^{-1}. Aliquots of this stock culture were diluted as needed and fixed with Utermohls solution to determine the appropriate cell concentration required for larval exposure studies.

Prior to and post-coral larvae exposure, incubation treatments were analyzed for brevetoxin content to determine the actual concentration of brevetoxin analogs in the treatments. Two hundred milliliters of incubation media was extracted through a C-18 reverse-phase extraction disk (Ansys Technologies Inc., Lake Forest, CA, USA) and recovered with methanol. Subsequently, the composition of the brevetoxin mixture was determined by LC–MS using a ThermoFinnigan AqA LC-Ms (Thermo Electron Corp., Manchester, UK) single quad system scanned from 204 to 1216 AMU with a Phenomenex Luna C-18 5Fm 250 mm × 2 mm Analytical Column with solvent gradient. The instrument was calibrated with a standard PbTx mix containing PbTx-2 and PbTx-3 obtained from the Center for Marine Science, UNCW, Wilmington, NC.

2.3. Larval oxygen consumption

To evaluate the impact of the concentration of *K. brevis* (or the effects of brevetoxin analogs) on larval respiration, larvae of *P.*

astreoides were analyzed for their oxygen uptake post-treatment. Respiration studies were conducted using an Oxygraph system outfitted with a DW3 liquid-phase electrode chamber (Hansatech Instruments, Norfolk, UK). Three haphazardly selected replicates from each treatment group were used. Each replicate contained 3 larvae in a 2.5 mL reaction volume of O_2 saturated filtered seawater (0.45 μm). Samples were dark adapted for 2 h prior to any measurement. Oxygen uptake was measured over the course of 20 min for each experiment. Respiration was calculated as nanomoles of oxygen consumed per minute per larva.

2.4. Larval bleaching

The density of zooxanthellae symbionts per larva was determined according to a protocol previously described by Edmunds et al. (2005) with slight modification. Briefly, five larvae were collected from three replicates of each treatment (post-exposure). Each replicate of five larvae was homogenized with a small Teflon tissue homogenizer in a microcentrifuge tube. The algal symbionts were pelleted by centrifugation (14,000 × *g*), resuspended in 500 μL of filtered seawater, and 3 subsamples were counted via hemocytometer. Final zooxanthellae counts were averaged to express symbiont density as algal symbionts larva^{-1}.

2.5. Oxidative stress assays

Previously frozen samples of larvae were thawed to room temperature and each extracted in 2.5 mL of buffer (50 mM potassium phosphate buffer (pH 7.0) containing 10% (w/v) polyvinylpolypyrrolidone (PVP)-40, 0.25% Triton X-100, and 1% (v/v) plant cell protease inhibitor cocktail [Sigma–Aldrich, St. Louis, MO, USA]). Samples were homogenized with mortar and pestle and centrifuged at 16,000 × *g* for 10 min. The resulting supernatants were normalized for protein content with the Quick Start™ Bradford Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Extracts were subsequently evaluated by a suite of commercially available biochemical assays following the manufacturer's specifications. Assays were used to evaluate the direct damage caused by increasing ROS levels and the cell's ability to respond to oxidative stress by measuring induced antioxidant enzyme activity.

Superoxide dismutases (SOD) are considered ubiquitous metalloenzymes that catalyze the dismutation of the reactive superoxide anion ($O_2^{\bullet -}$) into the less damaging H_2O_2 (Wu et al., 1999). SOD activity was measured using a Superoxide Dismutase Assay Kit (Cayman Chemical, Ann Arbor, MI, USA). Catalase (CAT) is another widely distributed enzyme that destroys H_2O_2 by dismutation to O_2 and H_2O (Halliwell, 2006). Upregulation of SOD and CAT reflects an organism's response to counteract the presence of damaging ROS. For CAT activity, a 10 mM potassium phosphate buffer (pH 7.0) was used to prepare a stock solution of 0.036% H_2O_2 (Sigma–Aldrich). One hundred microliters of coral larvae supernatant was brought to a total volume of 2000 μL using the H_2O_2 solution. Samples were mixed and incubated for 30 s at room temperature. Subsequently, CAT activity was determined by the decrease in absorbance at 240 nm as a function of time based upon the decomposition of hydrogen peroxide using a Genesys 10 UV/VIS spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). CAT activity for each sample was reported as specific activity (unit/mg protein) where one unit would decompose 1.0 μmol of H_2O_2 per minute at pH 7.0 at 25 °C.

To evaluate the damaging effects of ROS on cellular integrity two endpoints were measured, protein carbonylation and lipid peroxidation. Protein carbonylation can serve as a reflection of protein oxidative damage (Nystrom, 2005). Divalent redox cycling cations such as Fe^{2+} or Cu^{2+} can bind to cation binding locations on amino acid residues. Subsequent attack by ROS can transform side-chain amine groups on several amino acids (i.e. lysine, arginine, proline, or histidine) into unrepairable carbonyls. Protein carbonylation was measured with a Protein Carbonyl Assay Kit (Cayman Chemical).

Unsaturated lipids of cell membranes or other membranous systems are common targets of oxidative attack by ROS (Girotti, 1998). The end result is lipid peroxidation, a destructive process that compromises normal cellular function. Quantification of lipid peroxidation is one valuable biomarker to assess the role of this damage. Lipid peroxidation was measured using a Lipid Hydroperoxide Assay kit (Cayman Chemical).

2.6. Larval survival and settlement

Post-treatment, 50 larvae were placed in customized plastic tripour beakers in outdoor seawater tables. The bottom of each 300 mL beaker was replaced with a 180 μm nitex mesh bottom to permit exchange of seawater with the free flowing water tables. In addition, each beaker contained a single terracotta tile (4.5 cm × 4.5 cm × 1 cm; Sunshine Pavers®), which served as a settlement substrate. All tiles were preconditioned at a depth of 6 m offshore for 5 weeks. Larvae were left in the settlement assays for 48 h. After 48 h the number of larvae that remained as swimmers (still in planula phase) and those that settled and underwent metamorphosis onto the settlement tile were counted. The total (swimmers + settlers) were divided by 50 (the initial number of larvae) to determine the percentage survival, and the number of larvae that had settled and metamorphosed were divided by 50 to determine the percentage settlement.

2.7. Statistical analysis

Data for larval respiration was analyzed with a one-way ANOVA. Data for larval bleaching and CAT activity were not normally distributed so were rank transformed and analyzed with a one-way ANOVA. Data for SOD, lipid peroxidation and protein carbonylation also were not normally distributed and were log(x) transformed and analyzed with a one-way ANOVA. Data for percent mortality and percent settlement were arcsine square root transformed since they were proportions and then analyzed with a one-way ANOVA. In all cases a Tukey's post hoc test was performed to determine significant groupings.

3. Results

Coral larvae were exposed to two different concentrations of live cells or cellular lysates of *K. brevis* to assess short-term acute stress. Before and after this 20-h exposure period the incubation media was analyzed for brevetoxin analog content. Exposure concentrations for coral larvae are reported in Table 1 for both high (4×10^6 cells L^{-1}) and low (6×10^5 cells L^{-1}) levels of *K. brevis* or equivalent concentrations of cell lysate. *K. brevis*-based media showed the presence of PbTx-1, PbTx-2, PbTx-3, PbTx-CA, and negligible levels of brevenal. Larvae that were exposed to the low concentration of *K. brevis* or equivalents of cellular lysate had brevetoxin concentrations below the detection limit of the instrumentation and are reported as such.

Post-exposure, larvae of *P. astreoides* were monitored to determine if the presence of *K. brevis* (or associated toxins) had a negative impact on respiration (Fig. 2). Larvae in the control had an average oxygen consumption rate of 0.12 nmol O_2 min^{-1} larva^{-1}, and there were significant differences in the consumption rates among treatments (one-way ANOVA, $F = 16.72$, $p < 0.001$). Larvae that were exposed to a low cell count of *K. brevis* or the low levels of cellular lysate were not different from the controls (Tukey's post hoc test), but larvae that were exposed to high cell counts and a high

Table 1
Combined concentrations of brevetoxin analogs in incubation media [PbTx-1, PbTx-2, PbTx-3, PbTx-CA, brevenal]. Brevetoxin content ($\mu g\,L^{-1}$) [mean (SE)]; high, high cell count (4×10^6 cells L^{-1}); low, low cell count (6×10^5 cells L^{-1}). Low concentrations of *K. brevis* or equivalents of cellular lysate had brevetoxin concentrations below the detection limit of the instrumentation (0.05 $\mu g\,L^{-1}$ for PbTx).

Exposure period	Brevetoxin content
Pre-exposure (intact *K. brevis*—high)	6.29 (0.68)
Post-exposure (intact *K. brevis*—high)	4.38 (0.20)
Pre-exposure (intact *K. brevis*—low)	Below detection limit
Post-exposure (intact *K. brevis*—low)	Below detection limit
Pre-exposure (lysed *K. brevis*—high)	15.03 (5.17)
Post-exposure (lysed *K. brevis*—high)	5.4 (1.24)
Pre-exposure (lysed *K. brevis*—low)	Below detection limit
Post-exposure (lysed *K. brevis*—low)	Below detection limit
Controls (no exposure)	0

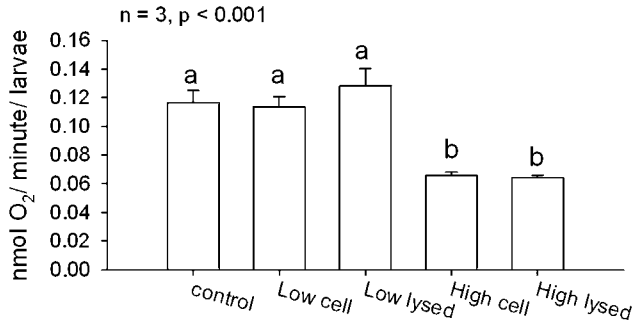

Fig. 2. Cellular respiration of *P. astreoides* larvae after a 24-h exposure to live cells or cellular lysates of *K. brevis*.

concentration of lysed cells had lower rates of respiration (0.07 nmol O_2 min^{-1} $larva^{-1}$ for both treatments).

The density of zooxanthellae in the larvae was measured in each treatment (Fig. 3). None of the treatments altered the density of zooxanthellae in larvae of *P. astreoides* (one-way ANOVA, $F = 2.56$, $p = 0.071$); however there was a trend for decreasing zooxanthellae density associated with the highest concentration of *K. brevis* and its lysate.

Superoxide dismutase activity did not differ among treatments (one-way ANOVA, $F = 0.47$, $p = 0.759$). All values ranged between 100 and 165 U/mg protein (Fig. 4a). Conversely, there was a difference in the upregulation of catalase (one-way ANOVA, $F = 3.61$, $p = 0.013$), with significantly more catalase activity in treatments where larvae were exposed to high cell counts or high levels of cellular lysate (Tukey's post hoc test; Fig. 4b). Lipid peroxidation showed a dose-dependent response with respect to the concentration of *K. brevis* cells and the concentration of cellular

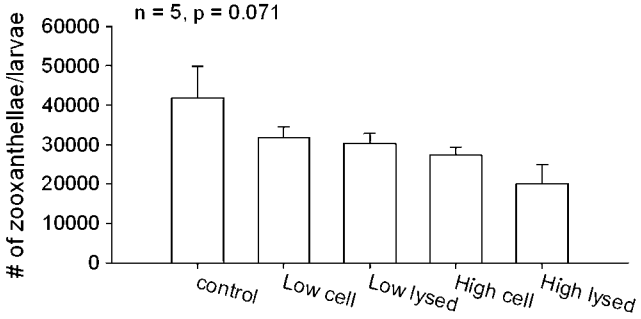

Fig. 3. Zooxanthellae density of *P. astreoides* larvae after a 20 h exposure to live cells or cellular lysates of *K. brevis*.

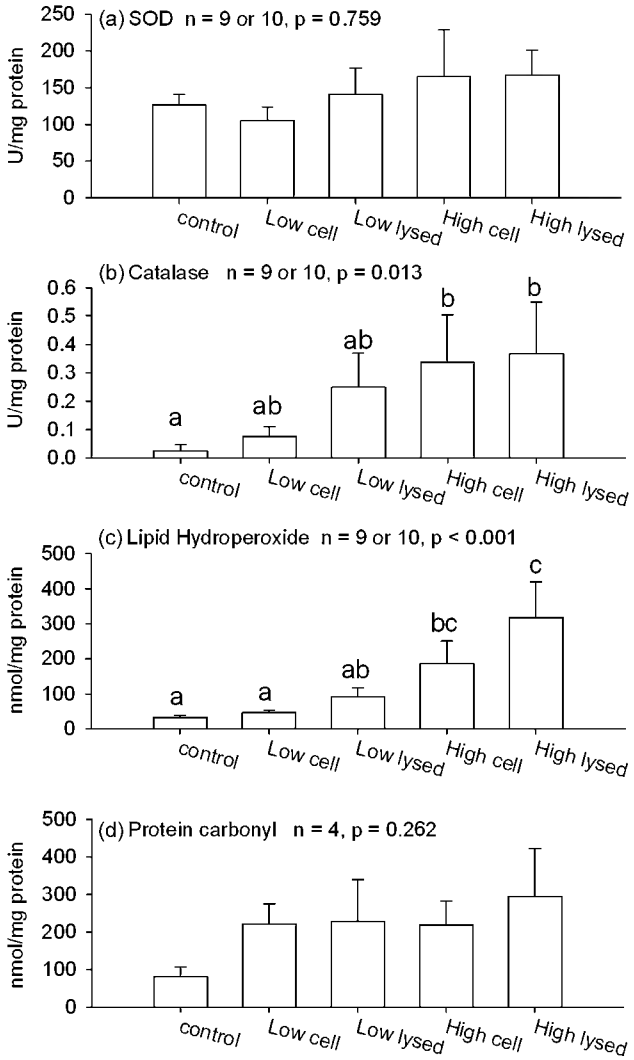

Fig. 4. Biomarkers of oxidative stress in *P. astreoides* larvae after a 20-h exposure to live cells or cellular lysates of *K. brevis*: (a) superoxide dismutase, (b) catalase, (c) lipid hydroperoxides, and (d) protein carbonylation.

lysates (one-way ANOVA, $F = 13.26$, $p < 0.001$; Fig. 4c). Larvae exposed to low cell concentrations or low levels of cellular lysate did not show a significant increase in lipid peroxidation compared to controls. Larvae exposed to high levels of *K. brevis* or high levels of *K. brevis* lysate showed a significant increase in lipid peroxidation levels when compared to control values (Tukey's post hoc test; Fig. 4c). There was no difference in the protein carbonylation levels among the treatments (one-way ANOVA, $F = 1.46$, $p = 0.26$; Fig. 4d).

After exposure to *K. brevis* (or associated lysates released by sonication) larvae were evaluated for survivorship and settlement (Fig. 5). Increasing concentrations of *K. brevis* or cellular lysates had no effect on the proportion of larval survival (one-way ANOVA, $F = 0.62$, $p = 0.651$; Fig. 5a). There was also no difference in the proportion of settlement and metamorphosis among the treatments (one-way ANOVA, $F = 1.12$, $p = 0.357$; Fig. 5b).

4. Discussion

The larval phase is crucial for the dispersal and subsequent recruitment of adult coral populations; however, the biological interactions that negatively influence pelagic coral larvae remain poorly understood (Ritson-Williams et al., 2009). In this study we measured oxidative injury (lipid peroxidation and protein

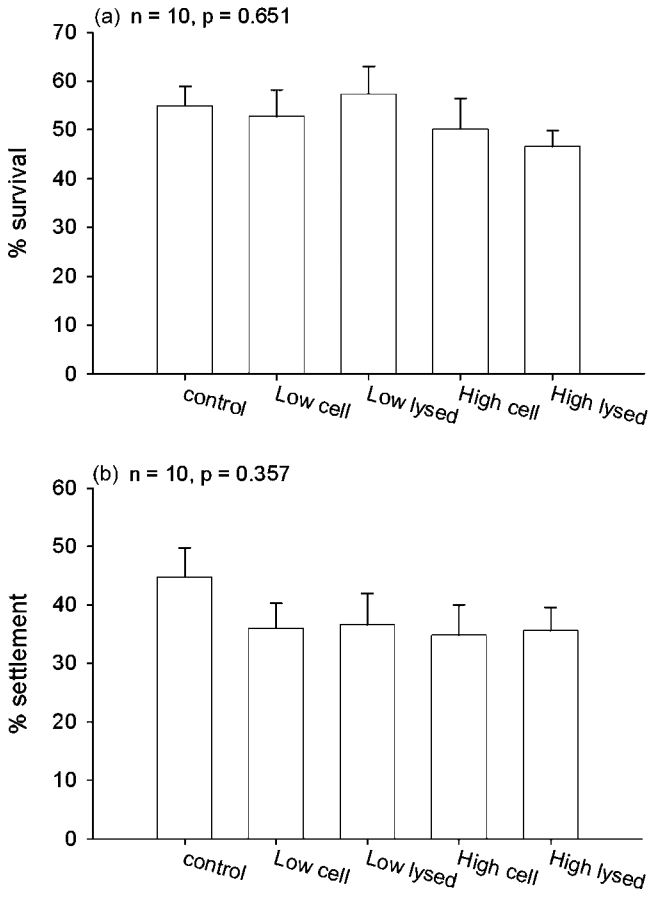

Fig. 5. (a) Percent survival and (b) settlement of *P. astreoides* larvae after a 20 h exposure to live cells or cellular lysates of *K. brevis*. Larvae were offered settlement tiles and survival and metamorphosis were measured after 48 h.

carbonylation) as well as the upregulation of antioxidant enzymes (SOD and CAT) that might ameliorate the oxidative stress encountered in coral larvae. The results show that larvae of *P. astreoides* undergo sublethal oxidative stress when exposed, under short-term conditions, to monospecific blooms or cellular lysates of *K. brevis*. This stress is evident immediately after exposure to *K. brevis*, but these levels of sublethal stress had no subsequent effect on survival, settlement and metamorphosis.

Reports regarding the exposure of brevetoxins on the sublethal cellular responses of marine organisms are limited (Landsberg, 2002). *K. brevis* produces a suite of brevetoxins that can have ecosystem scale consequences during a bloom (Paul et al., 2007), but it is not understood how these toxins might impact the larval stages of benthic organisms. Work by Keppler et al. (2005, 2006) demonstrated that upon short-term exposure of the eastern oyster *Crassostrea virginica* to brevetoxin-producing strains of raphidophytes lysosomal destabilization rates increased, suggesting cellular damage was occurring. This type of damage has been reported to significantly reduce gamete viability and delay embryonic development (Ringwood et al., 2004).

Both toxic and non-toxic blooms of phytoplankton can induce hypoxic conditions which could have a negative impact on the cellular respiration of any aerobic organism (Shumway et al., 1985; Wu, 2002; Treasurer et al., 2003). After a 20-h incubation with high concentrations (4×10^6 cells L^{-1}) of both live and lysed cells of *K. brevis*, coral larvae exhibited a notable decline in oxygen consumption. Control respiration rates of larvae of *P. astreoides* were comparable to previous reports of 0.13 nmol O_2 min^{-1} $larva^{-1}$ obtained by Edmunds et al. (2001). Reduced respiration rates after exposure to *K. brevis* shows that these coral larvae are stressed in the presence of *K. brevis* and suggests that they might not survive a longer exposure to *K. brevis* and its toxins. The relationship between reduced respiration (associated with negative biotic interactions or hypoxic events) and long-term growth and viability has yet to be evaluated in *P. astreoides*.

When the larvae of *P. astreoides* are released, they contain endosymbiotic zooxanthellae acquired through vertical transmission. Expulsion of these symbionts is an indicator of stress that is commonly measured in adult corals. Exposure to *K. brevis* at low and high concentrations did not cause significant levels of bleaching in larvae of *P. astreoides* when compared to controls. However, general trends suggest that all treatments decreased zooxanthellae concentrations when compared to controls (Fig. 3). The zooxanthellae densities in larvae in these experiments were approximately three times higher than the values reported by Edmunds et al. (2001). However, this difference can be attributed to natural variation as a function of temperature or other abiotic influences on the larvae (Edmunds et al., 2005).

The presence of zooxanthellae in these larvae might make them more susceptible to oxidative damage as has been suggested by Yakovleva et al. (2009). At the higher concentrations of whole cells and lysed *K. brevis* there was mixed evidence for the antioxidant defense system. There was no significant increase in SOD activity after exposure to *K. brevis*; however, there was an increase in CAT activity demonstrating that these larvae activated a component of their antioxidant defense system. Yakovleva et al. (2009) reported that coral larvae that were exposed to increased temperatures required a lag time of 2 days from the onset of the stress until the upregulation of SOD. The short exposure time of the *K. brevis* incubation may not have been of sufficient duration to induce elevated SOD activity in larvae of *P. astreoides*.

Ramos and Garcia (2007) demonstrated that when adult *Montastraea faveolata* were exposed to the organic contaminant benzo(*a*)pyrene CAT and SOD activities increased not only in the host but in the zooxanthellae. The production of ROS by coral larvae, when exposed to brevetoxin-containing extracts, could be a strategy for detoxifying (via oxidative addition) what would otherwise be considered lethal compounds (Hua and Cole, 1999). Work by Farina et al. (2008) demonstrated that when *P. astreoides* larvae were exposed to benzo(*a*)pyrene the total thiol content significantly increased as a reflection of oxidative stress. To our knowledge this is the first study of brevetoxins inducing ROS related stress in coral larvae.

Increased lipid peroxidation has been observed in marine gastropods and arthropods in response to metal, endosulfan, and atrazine exposure (Downs et al., 2001a,b). Similarly, larvae of *P. astreoides* had higher concentrations of lipid hydroperoxides after 20 h of exposure to high concentrations of *K. brevis*. In larvae of *Acropora intermedia*, lipid peroxidation was observed after 3 days of exposure to a thermal stress (Yakovleva et al., 2009). Tchernov et al. (2004) suggested that the lipid composition of thylakoid membranes of zooxanthellae is a critical factor regulating thermal stress sensitivity as thermally labile clades of zooxanthellae possess a higher proportion of major polyunsaturated fatty acids. *P. astreoides* from the Florida Keys consistently contains A_4 zooxanthellae (LaJeunesse, 2001; Thornhill et al., 2006), but it is unknown if this strain is thermally tolerant or sensitive. Nonetheless, there may be a relationship between the degree of polyunsaturated fatty acid content and the susceptibility to chemical irritation leading to subsequent ROS production.

Another commonly used marker of oxidative damage is protein carbonyl content, which is an indicator of protein oxidation. The introduction of carbonyl groups into selected amino acid side chains may render the protein useless and degrade physiological function (Nystrom, 2005). Selected proteins may be considered redox sensitive due to their proximity of ROS generating sites such

as electron transport chains or photosystems. After exposure to *K. brevis* there was no evidence of increased protein carbonylation.

Some but not all of the biomarkers that were measured were upregulated in response to *K. brevis* exposure. While brevetoxins elicit an oxidative stress response, larvae of *P. astreoides* may not be emptying their reservoir of oxidative stress tolerance. For example, the fact that SOD activity did not vary significantly among treatments suggests that SOD levels are already maintained at a concentration high enough to counteract the damaging photosynthetic by-products generated from *in hospite* zooxanthellae. In addition, the fact that recruitment is not affected suggests that the stress does not overly tax the adaptive response to oxidative stress that is already in place. Comparative work by Richier et al. (2005) demonstrated that symbiotic and non-symbiotic anemones show notable differences in their response to oxidative stress. In particular symbiotic organisms showed constitutively higher levels of SOD expression when compared to aposymbiotic individuals. When organisms were exposed to elevated temperature or hyperoxic conditions the aposymbiotic anemones were the only group to show an upregulation in SOD activity. The symbiotic individuals were suggested to be primed to manage the effects of oxidative stress. Additional work comparing the effects of brevetoxins on aposymbiotic larvae would undoubtedly aid our understanding of how different coral larvae respond to stress.

Signs of sublethal stress are rarely measured in new coral (spat or young recruits) so the impact on later life history fitness is poorly understood. After larvae of *M. faveolata* were exposed to lower salinities, they had decreased post-settlement growth, showing a latent effect of larval stress (Vermeij et al., 2006). A better understanding of latent effects in new recruits of corals is necessary to understand how larval sublethal stress might affect post-settlement ecology (Ritson-Williams et al., 2009; Pechenik, 2006). Fortunately, research on the larval ecology of corals is increasing, and there is enhanced understanding of the positive and negative factors that influence this critical life history stage (Ritson-Williams et al., 2009). Planktonic coral larvae are especially vulnerable to water quality issues including elevated temperatures (Edmunds et al., 2001, 2005; Bassim and Sammarco, 2003; Putnam et al., 2008; Yakovleva et al., 2009) and UV exposure (Wellington and Fitt, 2003; Gleason et al., 2006; Kuffner, 2001), but they are also exposed to harmful planktonic organisms, especially during the high concentrations experienced during a bloom event. For larvae of *P. astreoides* the toxins and live cells of *K. brevis* caused sublethal stress to multiple aspects of their physiology that would not have been apparent without the use of cellular biomarkers. Cellular biomarkers promise to be an important method of assessing stress in multiple life history stages of corals as they are exposed to more local and global scale stressors.

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