Available online at www.sciencedirect.com
SCIENCE DIRECT
Marine Pollution Bulletin 52 (2006) 515–521
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Patterns of inorganic phosphate uptake in *Cassiopea xamachana*: A bioindicator species

Brian D. Todd [a,*], Daniel J. Thornhill [b], William K. Fitt [b]

[a] *The University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA*
[b] *The Institute of Ecology, The University of Georgia, Athens, GA 30602, USA*

Abstract

Nutrient levels in the nearshore waters of the Florida Keys have increased over the past few decades concomitant with a decline in the health of Florida's reef system. Phosphorus is a particular concern in the Florida Keys as it may be the limiting nutrient in nearshore waters. We demonstrate that the upside-down jellyfish, *Cassiopea xamachana*, decreases its rate of phosphate uptake following exposure to elevated levels of dissolved inorganic phosphate. We also show that this subsequent suppression of uptake rates persists for some time following exposure to elevated phosphates. Using these attributes, we experimentally investigated the use of *C. xamachana* as a bioindicator for dissolved inorganic phosphates in seawater. Our results show that these animals reveal comparative differences in environmental phosphates despite traditional testing methods yielding no detectable phosphates. We propose that *C. xamachana* is a bioindicator useful for integrating relevant information about phosphate availability in low nutrient environments.

Keywords: *Cassiopea xamachana*; Phosphates; Florida Keys; Coral reefs; Nutrients; Zooxanthellae

1. Introduction

Florida's coral reef ecosystem is declining in coral cover and is at risk for further degradation (Porter and Meier, 1992; Porter et al., 2002). One cause for the decline is increased turbidity and nutrient loading resulting from interconnectedness between the offshore reefs and nearshore waters, including Florida Bay (Porter et al., 1999; Cook et al., 2002). Elevated nutrients are implicated in the localized deterioration of coral reefs and they act together with other stressors to contribute to the decline of coral reefs globally (Szmant, 2002). Increased nutrient inputs reduce coral growth and reproduction and contribute to macroalgae proliferation and overgrowth, leading to changes in reef community structure (Simkiss, 1964; Hughes, 1994; Koop et al., 2001; Loya et al., 2004).

The source of rising nutrients in the Florida Keys remains unresolved (Boyer and Jones, 2002; Lapointe et al., 2002). Nutrients are intimately associated with sewage and the dissolved organic forms found in sewage are easily remineralized to more readily available inorganic forms favored by plants and algae (Szmant, 2002). Shinn et al. (1994) showed that injected wellwater rapidly migrates both vertically and horizontally in porous Florida limestone; nutrient-rich contaminated groundwater seeps into coastal and ocean waters. Viral tracer studies corroborate this rapid movement of wastewater from on-site sewage disposal systems toward the coral reef systems in the lower Florida Keys (Paul et al., 2000). Additionally, Patterson et al. (2002) reported that the white pox coral disease afflicting the Elkhorn Coral, *Acropora palmata*, is caused by *Serratia marcescens*, a bacterium commonly found in human intestines. However, other authors offer the contrasting evidence that elevated nutrient levels found on reefs are likely not a result of shore-based or Florida Bay-influenced sources, but instead derive from resuspension of

* Corresponding author. Tel.: +1 803 725 0422; fax: +1 803 725 3309.
E-mail address: todd@srel.edu (B.D. Todd).

0025-326X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2005.09.044

nutrient-rich sediments by offshore upwelling (Szmant and Forrester, 1996; Leichter et al., 2003). Regardless of the source, it is important to understand the distribution and impact these nutrients have on reefs in the Florida Keys.

Phosphorus is a particular concern in the Florida Keys as it may be the limiting nutrient in nearshore waters (Lapointe, 1989; Cook et al., 2002). However, if uptake of phosphates by the surrounding biota is rapid, then measurement of nutrient concentrations alone may not adequately assess nutrient availability in the habitat (Koop et al., 2001). Thus, a potentially more informative way of monitoring for increased environmental nutrients is through the use of bioindicators. Bioindicators are commonly used for detecting in situ pollution or the extent of anthropogenic influence on an environment. Examination of stable isotope ratios of $\delta^{15}N/\delta^{14}N$ in reef organism tissues is one method of determining the level of human sewage inputs to reef systems (Yamamuro et al., 2003). Other analyses that rely on bioindicators to determine relative nutrient enrichment include examining chlorophyll *a* activity in coastal waters (Harding and Perry, 1997), detecting alkaline phosphatase in phytoplankton (Lapointe, 1989), and assaying seaweeds and seagrasses to determine nutrient enrichment and limitation in surrounding seawater (Lapointe et al., 1994).

Cassiopea xamachana is a jellyfish symbiotic with zooxanthellae, unicellular dinoflagellates of the genus *Symbiodinium* (Freudenthal, 1962). Like other symbiotic cnidarians, these jellyfish acquire dissolved nutrients from the surrounding seawater to meet the needs of their photosynthetic partners (Pomeroy and Kuenzler, 1969). Additionally, high levels of available nutrients have been shown to negatively affect the subsequent nutrient uptake rates by both host animals and the symbionts (Jackson and Yellowlees, 1990; Belda and Yellowlees, 1995; Kelty and Lipschultz, 2002). We tested the hypothesis that exposure to elevated dissolved inorganic phosphate (DIP) causes a decrease in the subsequent uptake rate of *C. xamachana.* In a second experiment, we used lower concentrations of elevated DIP in the experimental seawater, but increased the length of time that *C. xamachana* were exposed to the solutions in order to test the hypotheses that uptake rates would decrease with increasing phosphate concentrations and increasing lengths of exposure to those concentrations. We performed a third experiment to determine the length of time that reduced uptake rates would persist following exposure to elevated DIP. We performed our fourth experiment to demonstrate the application of *C. xamachana* as a bioindicator of DIP in seawater by transplanting the animals to local reef environments and comparing subsequent uptake rates following four days of exposure.

2. Materials and methods

2.1. Animal collection

C. xamachana were collected from a nearshore mangrove lagoon on the Atlantic coast of Key Largo, FL (24°59′N, 80°22′W) near highway US 1 mile marker 99.5. This mangrove lagoon is ocean fed from both ends, allowing tidal currents to enter and exit. Ambient levels of DIP were typically less than 0.1 μM as measured using the methods described below. All animals were collected within a few meters from each other with the assumption that they had a shared nutrient history. Animals were transported to the lab in water collected at the site and used in experiments within the hour unless otherwise indicated. Medusae were selected within a narrow size range (4.5–6.0 cm bell diameter) in order to reduce size-related variability in uptake rates. Bell diameters were recorded to the nearest tenth of a centimeter while extended. Animals were blotted dry once and wet weights were obtained by weighing on a Metler scale to the nearest hundredth of a gram.

2.2. Quantifying phosphate uptake rates

Phosphate uptake rates for cnidarians are typically calculated as the rate of phosphate depletion from seawater (Pomeroy and Kuenzler, 1969; D'Elia, 1977; Belda and Yellowlees, 1995). These methods were followed with minor modifications as described below. *C. xamachana* jellyfish were placed into acid-washed glass Petri dishes of adequate size so that the bell of the animal could completely extend. Each Petri dish contained 200 ml of Instant Ocean(r) seawater (35 ppt). The seawater was mixed to contain approximately 2.0 μM phosphate in the form of dissolved KH_2PO_4. This concentration was chosen to minimize the likelihood that an animal would completely deplete all DIP from the seawater before the end of the 10-min assay. Three 50 ml samples of the starting solution were collected to determine the precise initial concentration for the mixture. Animals were allowed to deplete the dissolved KH_2PO_4 from the medium for only 10 min to minimize the likelihood that exposure to the 2.0 μM DIP assay solution would affect the final calculated uptake rates. Duplicate 25 ml water samples were collected at the conclusion of the assay and all samples were immediately placed in a −20 °C freezer. Less than 24 h later, samples were thawed and prepared for DIP determination using the addition of a molybdate compound and following the heteropoly blue formation method (Strickland and Parsons, 1972). The change in the concentration of DIP over 10 min was used to determine the uptake rates of the animals.

2.3. Experiment 1: Exposure to elevated DIP for 1 h

This experiment was conducted to test the hypothesis that exposure to elevated concentrations of DIP in seawater causes a reduction in the phosphate uptake rates of *C. xamachana*. Eighteen *C. xamachana* were collected and randomly divided into six equal groups of three animals. Each group was then exposed to a different concentration of dissolved KH_2PO_4 in Instant Ocean(r) seawater (35 ppt) for 1 h. The six predetermined concentrations

were 0, 0.2, 0.5, 1.0, 2.0, and 20 μM DIP. These values encompass known DIP concentrations on eutrophic reefs as well as concentrations that have been shown to reduce phosphate uptake in a clam-zooxanthellae interaction (Belda and Yellowlees, 1995; Szmant and Forrester, 1996; Szmant, 2002). Phosphate concentrations in the salt mix are known to be negligible and were undetectable by our methods (<0.03 μM). Specimens were placed in large glassware and kept outdoors in a naturally lit area at 25 ± 1 °C. Animals were incubated in their respective solutions for 1 h and then removed. All specimens were rinsed gently in filtered seawater to prevent phosphate carry-over from incubation solutions. Assays were then performed to quantify the uptake rates as described above. To test the hypothesis that incubation at elevated phosphate levels for one hour would have an effect on phosphate uptake parameters, data were analyzed using one-way ANOVA ($\alpha = 0.05$) with the analytical software Statistica (Statsoft, 1999). Where a significant effect was found, Fisher's protected least significant difference test was used to identify differences between groups. Tests of the assumptions underlying the analyses were made prior to performing each analysis (Zar, 1998). No serious violations of the assumptions were evident at the level $\alpha = 0.05$.

2.4. Experiment 2: Increased length of exposure to moderate levels of DIP

This experiment was conducted to determine if lengthier exposures to moderately elevated DIP levels would negatively affect subsequent uptake rates. Forty-five medusae were collected for the experiment and three separate environmental tanks were established with 0.1, 0.2, and 0.5 μM dissolved KH_2PO_4. These levels are lower than those used in the first experiment and they were chosen because they are more representative of actual phosphate levels recorded in reef environments (e.g., Szmant and Forrester, 1996; Szmant, 2002). The environmental tanks were placed outdoors in a naturally lit area and kept at 25 ± 1 °C. Repeated addition of KH_2PO_4 to the three tanks throughout the experiment kept phosphate concentrations relatively constant as verified through periodic water testing. The 45 individuals were divided into three equal groups of 15 and randomly assigned one of these tanks. Exposure was initiated at 10:00 AM and three individuals from each tank were removed and assayed to determine uptake rates 1, 3, 6, 12, and 24 h after placement in the tank. Data were analyzed using two-way ANOVA ($\alpha = 0.05$) with the analytical software Statistica (Statsoft, 1999) to test the hypotheses that DIP uptake rates would decrease with increasing phosphate concentrations and with increasing lengths of exposure to elevated phosphates. Where a significant effect was found, Fisher's protected least significant difference test was used to identify differences between groups. Again, no serious violations of the assumptions were evident at the level $\alpha = 0.05$.

2.5. Experiment 3: Duration of nutrient uptake signature

Fifteen *C. xamachana* were collected in the mangrove lagoon 10 m from shore and transported in a cooler filled with water from the collection site to Athens, GA, USA where this experiment was conducted the following day. *C. xamachana* were kept at 25 ± 1 °C under constant artificial light for the duration of this experiment. *C. xamachana* were exposed to 2.0 μM dissolved KH_2PO_4 for 1 h. Following 1 h of exposure, the animals were removed and rinsed gently with artificial seawater to prevent phosphate carryover before being placed into a holding tank of Instant Ocean(r) artificial seawater with no dissolved phosphates. Three animals were immediately used to determine phosphate uptake rates following elevated DIP exposure, and three additional animals were randomly removed 1, 5, 24, and 48 h later to determine their phosphate uptake rates. Data were analyzed using one-way ANOVA ($\alpha = 0.05$) to test the hypothesis that removal from exposure to elevated DIP would result in a return to normalcy of phosphate uptake rates. Where a significant effect was found, Fisher's protected least significant difference test was used to identify differences between groups. No serious violations of the assumptions were evident at the $\alpha = 0.05$ level.

2.6. Experiment 4: Using C. xamachana as a bioindicator

Twenty-seven *C. xamachana* were collected in the mangrove lagoon 10 m from shore and separated into three random groups of equal sample size for transplantation to one of three different environments: (1) Florida Bay 10 m from shore, (2) Admiral Reef, a patch reef approximately 4.8 km offshore, and (3) Little Grecian Reef, a fore reef 8 km offshore. The medusae were contained in clear plastic chambers 10 cm × 10 cm × 15 cm with numerous 1 cm diameter holes drilled in the sides and screened ends to readily allow exchange of water and exposure to the surrounding environment. Animals were collected after four days and assayed immediately upon collection aboard the boat to determine uptake rates. Forty animals were simultaneously collected from the mangrove lagoon and immediately assayed (<1 h) to determine reference phosphate uptake rates. Three water samples from each of the four environments were also collected. Phosphate uptake rates were analyzed with one-way ANOVA ($\alpha = 0.05$) to test the hypothesis that uptake rates of animals close to shore would be lower than those of animals farther from shore. Where a significant effect was found, Fisher's protected least significant difference test was used to identify differences between groups. Tests of the underlying assumptions for all analytical procedures were performed prior to analysis and no significant violations were found at the level $\alpha = 0.05$.

3. Results

3.1. Experiment 1: Exposure to elevated DIP for 1 h

Phosphate uptake rates of *C. xamachana* decreased following exposure to increasing concentrations of DIP (Fig. 1). Specimens incubated at 20 μM excreted phosphate during the course of the assay while those incubated at 2.0 μM showed no significant uptake or excretion. Animals incubated at phosphate concentrations less than 2.0 μM took up phosphate during the course of the assay. The uptake rates for the 20, 2.0, and 0.5 μM groups were significantly lower than those from the unexposed control group (p-values < 0.05). Additionally, uptake rates for animals incubated in 20 and 2.0 μM groups were significantly lower than those from all other groups (p-values < 0.05). The 1.0 μM exposure group did not follow the general trend, apparently due to an anomalous animal or problems with an erroneous measure of that animal.

3.2. Experiment 2: Increased length of exposure to moderate levels of DIP

Exposure to elevated phosphates significantly affected the uptake rates of *C. xamachana* ($p = 0.042$; Fig. 2). At each interval, jellyfish maintained in 0.5 μM phosphate exhibited uptake rates significantly lower than those of animals incubated at 0.1 μM phosphate ($p = 0.013$), and animals exposed to 0.2 μM had intermediate uptake rates. Uptake rates also varied significantly with length of exposure ($p = 0.007$; Fig. 2). However, uptake rates did not decrease progressively with corresponding increases in length of exposure as predicted.

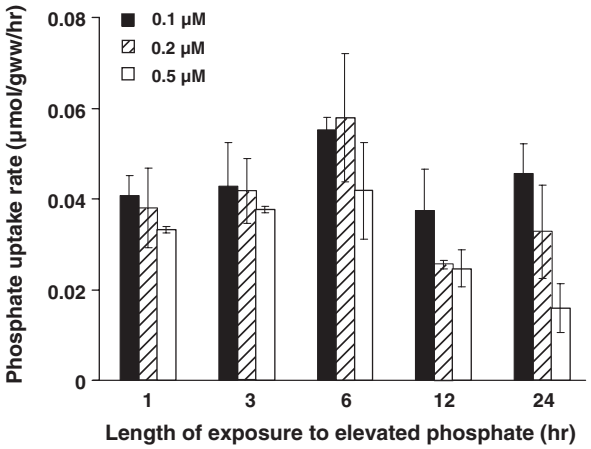

Fig. 2. Mean uptake ± 1 SE of *C. xamachana* exposed to elevated phosphates. Means represent three animals. Exposure to the highest phosphate concentration produced a significant reduction in uptake rates across all lengths of exposure ($p < 0.05$). Uptake rates did not decrease progressively with corresponding increases in length of exposure.

3.3. Experiment 3: Duration of nutrient uptake signature

C. xamachana exhibited diminished phosphate uptake rates following 1 h of exposure to 2.0 μM dissolved KH_2PO_4, but returned to active uptake within 24 h (Fig. 3). The three jellyfish assayed immediately upon removal from the DIP solution had significantly reduced uptake rates compared to jellyfish allowed to recover for 5 or more hours (p-values < 0.01). This reduction in uptake rates was still apparent after 1 h of recovery when compared to the uptake rates of animals allowed to recover for 24 and 48 h (p-values < 0.05). Major adjustments in the uptake rate occurred in as little as 5 h after exposure to elevated phosphate.

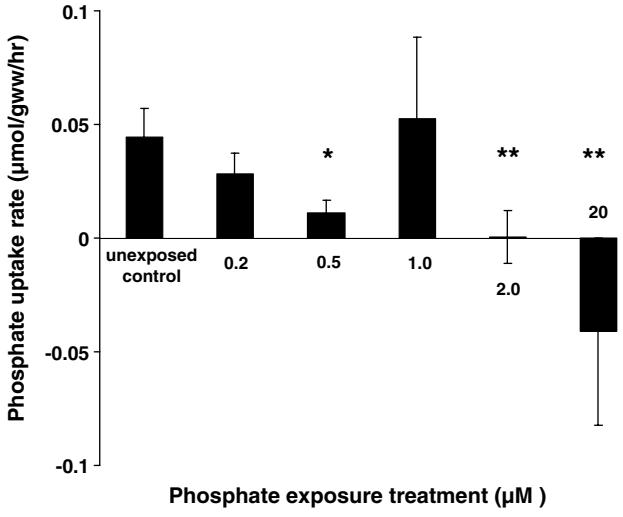

Fig. 1. Mean uptake of dissolved inorganic phosphate ± 1 SE for *C. xamachana* exposed to elevated phosphates for 1 h. Phosphate concentrations for each treatment are listed. * Significant effect of treatment compared to unexposed control group ($p < 0.05$). ** Significant effect of treatment compared to all other groups ($p < 0.05$).

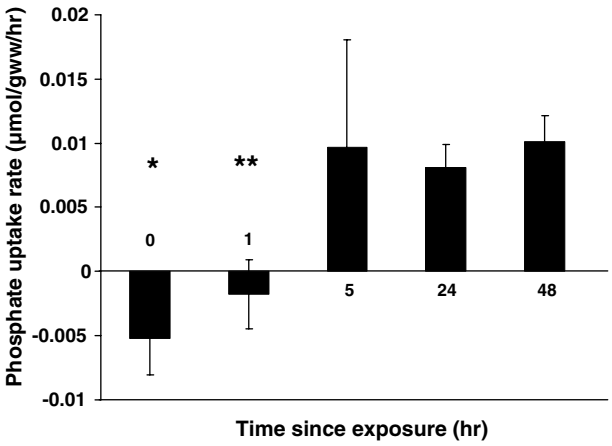

Fig. 3. Mean uptake ± 1 SE of *C. xamachana* following exposure to 2.0 μM dissolved inorganic phosphate. * Significantly lower uptake rates than all other groups ($p < 0.05$). ** Significantly lower uptake rates than groups removed 5, 24, and 48 h later ($p < 0.05$).

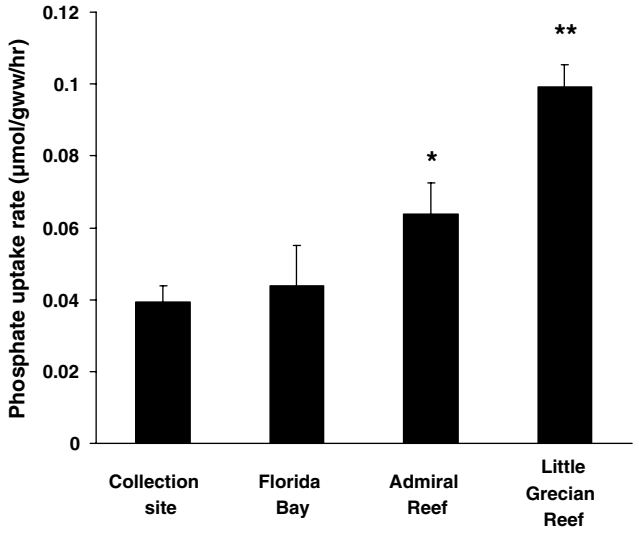

Fig. 4. Mean uptake ± 1 SE for *C. xamachana* following environmental exposure for four days. * Significant difference between Admiral Reef and Atlantic Canals ($p = 0.01$). ** Significant differences between Little Grecian Reef and all other environments ($p < 0.05$).

3.4. Experiment 4: Using C. xamachana as a bioindicator

Translocation habitat had a highly significant effect on phosphate uptake rates of *C. xamachana* (Fig. 4; Table 1). Translocating jellyfish from the Atlantic side of Key Largo into Florida Bay did not significantly affect their uptake rates compared to reference animals from the original collection site ($p = 0.67$). However, translocating animals from shore and onto coral reefs produced significant increases in uptake rates (see Table 1 for *p*-values). Those jellyfish that were moved 8 km from shore to Little Grecian fore reef exhibited the highest phosphate uptake rates of all treatments while those placed 4.8 km from shore at Admiral patch reef exhibited intermediate uptake rates (Fig. 4; Table 1). Water samples from Little Grecian Reef and Admiral Reef revealed undetectable phosphate concentrations (<0.03 µM DIP; Table 1) compared to the collection site on the Atlantic side (0.09 µM DIP; Table 1) and the translocation site in Florida Bay (0.05 µM DIP; Table 1).

4. Discussion

4.1. Physiological effects of nutrients on C. xamachana

Atkinson and Bilger (1992) speculated that changes in the physiology of phosphate uptake by cnidarians, specifically corals, could be a function of past history of nutrient availability. Our results indicate that exposure of *C. xamachana* to elevated phosphates reduces their subsequent uptake rates and this physiological adaptation can occur in as little as 1 h if dissolved phosphates concentrations are high enough. Previous studies show that both zooxanthellae and their associated hosts have reduced uptake rates following exposure to increased environmental phosphates (Dean and O'Brien, 1981; Kelty and Lipschultz, 2002). For example, Belda and Yellowlees (1995) showed that isolated zooxanthellae and host clams (*Tridacna* spp.) respond similarly, with reduced uptake rates occurring after the organisms were exposed to elevated phosphates. These physiological adaptations occur presumably as the organisms become saturated during periods of high nutrient availability. Kelty and Lipschultz (2002) demonstrated that zooxanthellae isolated from a host anemone (*Aiptasia pallida*) for 24 h were able to absorb phosphate 75 times faster per cell than were freshly isolated zooxanthellae and they attributed this uptake suppression to higher phosphate concentrations measured within host tissue compared to that of filtered Bermudan seawater (Kelty and Lipschultz, 2002). Our findings corroborate these results and suggest that this phenomenon is widespread among invertebrate-*Symbiodinium* mutualisms. Additionally, we demonstrate that the effects of elevated phosphate exposure on subsequent uptake rates persist for at least one hour and are capable of returning to a typical pre-enrichment pattern in as little as 5 h.

It is important to note that phosphate concentrations of 2.0 µM are an order of magnitude greater than those often encountered in coastal and reefs waters (see Szmant, 2002 for discussion). Therefore, after establishing the response observed in the first experiment, we tested whether exposure to low levels of phosphate for lengthier periods would increase the sensitivity of uptake rates in *C. xamachana*. While animals exposed to the highest phosphate treatment did exhibit significantly lower uptake rates than those incubated at the lowest treatment across all time intervals, increasing the duration of phosphate exposure did not progressively affect uptake rates as predicted. One possible reason for this response may have been that the animals were kept outside and fluctuations in light intensity with the progression of the day may have influenced uptake rates. Previous authors have demonstrated that light affects nutrient uptake rates in anemones and giant clams (D'Elia, 1977; Muller-Parker et al., 1990). This has been attributed to the photosynthetic needs of the endosymbiotic zooxanthellae (Belda and Yellowlees, 1995). In contrast, the animals

Table 1
Comparative phosphate levels and uptake rates as measured for the four transplant environments

Site	PO_4 concentration	Mean uptake rates (µmol/gww/h)	*p*-values for uptake rate comparisons relative to collection site
Collection site	0.09	0.039438	–
Florida Bay	0.05	0.043881	0.67
Admiral Reef	Not detectable	0.063707	0.01
Little Grecian Reef	Not detectable	0.099076	<0.001

in the third experiment were kept indoors under continual artificial light and their uptake rates appeared to normalize 5 h after exposure and remained constant 24 and 48 h later. Our data do not allow us to specifically address the effects of light on phosphate uptake in *C. xamachana*. However, we caution that future work using these animals as comparative bioindicators should be conducted contemporaneously under similar lighting to avoid any confounding influence that light may have on nutrient uptake rates.

4.2. Nutrient levels in Floridian nearshore environments

C. xamachana are capable of integrating past nutrient history into a detectable uptake signature that persists for a period of time. Animals translocated from a common collection site and to Florida Bay and coral reefs revealed comparative differences in environmental phosphates between these sites. *C. xamachana* displayed the lowest uptake rates following environmental exposure at sites with the highest phosphate concentrations as measured by traditional instantaneous time-point sampling. Jellyfish translocated from the collection site on the Atlantic side of Key Largo and into Florida Bay experienced little change in phosphate uptake rates, consistent with the similar dissolved phosphate levels in both habitats. Animals placed on the most distant reef, Little Grecian, had significantly higher uptake rates than those from Admiral Reef despite the fact that phosphates were below detectable levels at both locations using traditional analytical methods. These findings suggest that average ambient phosphate levels were lower at the reef farther from shore. However, it is important to clarify that we did not replicate sites at equal distances from shore, therefore we caution against generalizations about phosphate levels and their relation to distance from shore based on these current data. Also, it is not possible to precisely quantify background phosphate levels using these data, however, inferences can be made about the comparative phosphate levels from one location to another based on the observed relative uptake rates. Future investigations should focus on establishing a phosphate uptake response curve capable of providing more exact approximations of environmental phosphates.

4.3. C. xamachana as a bioindicator

There are several important advantages to using *C. xamachana* as a bioindicator of dissolved inorganic phosphates. Direct measurement of seawater samples provides information that is inherently time-specific, and therefore may not be indicative of overall conditions at the tested environments. In contrast, the information obtained by analyzing phosphate uptake rates of *C. xamachana* represents an integration of local phosphate conditions over a lengthier period of time, and thus provides a more accurate portrayal of recent phosphate levels in coastal waters. Furthermore, organismal bioindicators such as *C. xamachana* enable researchers to obtain ecologically relevant information about the physiological effects of phosphate levels on cnidarian–zooxanthellae symbioses in addition to providing comparative information about environmental phosphates. Traditional methods of seawater analysis produce information regarding the ambient phosphate levels at a specific time, but offer no insight as to the ecological impact the nutrient may be having on local animals. *C. xamachana* are particularly useful to researchers because they provide a dynamic model that closely resembles corals. Both *C. xamachana* and hermatypic corals exhibit similar patterns of phosphate uptake related to the nutrient needs of their endosymbionts (D'Elia, 1977). However, *C. xamachana* medusae are not attached benthic organisms, thus making them easily transplantable to different environments for testing purposes. Unlike many corals, *C. xamachana* jellyfish are also readily abundant and are not a protected species. Researchers can therefore examine the effects of increased environmental phosphates on the physiology of a symbiotic cnidarian without contributing to the detriment of threatened coral species. Lastly, using *C. xamachana* allows researchers to make comparisons on the relative concentrations of phosphates in different habitats even though conventional testing methods may not yield distinguishable differences.

Acknowledgements

We thank J.W. Porter and K.G. Porter for their valuable comments and insights on earlier drafts of this manuscript. This manuscript was improved with the assistance of two anonymous reviewers. We also thank Kevin Todd, Luke Presley, Geoff Chilcoat, and Josh Vinson for their assistance with data and animal collection. This research was supported in part by grants NSF-9906976 and NSF-0137007 from the National Science Foundation. Manuscript preparation was aided by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, US Department of Energy through Financial Assistant Award No. DE-FC09-96SR18546 to the University of Georgia Research Foundation.

References

Atkinson, M.J., Bilger, R.W., 1992. Effects of water velocity on phosphate uptake in coral reef-flat communities. Limnology and Oceanography 37 (2), 273–279.

Belda, C.A., Yellowlees, D., 1995. Phosphate acquisition in the giant clam-zooxanthellae symbiosis. Marine Biology 124, 261–266.

Boyer, J.N., Jones, R.D., 2002. A view from the bridge: external and internal forces affecting the ambient water quality of the Florida Keys National Marine Sanctuary. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, NY, pp. 609–628.

Cook, C.B., Mueller, E.M., Ferrier, M.D., Annis, E., 2002. The Influence of nearshore waters on corals of the Florida Reef Tract. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, NY, pp. 771–788.

Dean, E.M., O'Brien, R.W., 1981. Uptake of phosphate by symbiotic and free-living dinoflagellates. Archives of Microbiology 128, 307–310.

D'Elia, C.F., 1977. The uptake and release of dissolved phosphorus by reef corals. Limnology and Oceanography 22 (2), 301–315.

Freudenthal, H.D., 1962. *Symbiodinium gen. nov.* and *Symbiodinium microadriaticum* sp. *nov.*, a zooxanthella: taxonomy, life cycle and morphology. Journal of Protozoology 9 (1), 45–52.

Harding, L.W., Perry, E.S., 1997. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950–1994. Marine Ecology Progress Series 157, 39–52.

Hughes, T.P., 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265, 1547–1551.

Jackson, A.E., Yellowlees, D., 1990. Phosphate uptake by zooxanthellae isolated from corals. Royal Society of London Proceedings Series B 242 (305), 201–204.

Kelty, R., Lipschultz, F., 2002. Phosphorus uptake and allocation by *Symbiodinium bermudense* isolated from *Aiptasia pallida*. In: Proceedings of 9th International Coral Reef Symposium.

Koop, K., Booth, D., Broadbent, A., Brodie, J., Bucher, D., Capone, D., Coll, J., Dennison, W., Erdmann, M., Harrison, P., Hoegh-Guldberg, O., Hutchings, P., Jones, G.B., Larkum, A.W.D., O'Neil, J., Steven, A., Tentori, E., Ward, S., Williamson, J., Yellowlees, D., 2001. ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Marine Pollution Bulletin 42, 91–120.

Lapointe, B.E., 1989. Caribbean coral reefs: are they becoming algal reefs? Sea Frontiers 35, 82–91.

Lapointe, B.E., Matzie, W.R., Barile, P.J., 2002. Biotic phase shifts in Florida Bay and back reef communities of the Florida Keys: linkages with historical freshwater flows and nitrogen loading from Everglades runoff. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, NY, pp. 629–648.

Lapointe, B.E., Tomasko, D.A., Matzie, W.R., 1994. Eutrophication and trophic state classification of seagrass communities in the Florida Keys. Bulletin of Marine Science 54, 696–717.

Leichter, J.L., Stewart, H.L., Miller, S.L., 2003. Episodic nutrient transport to Florida coral reefs. Limnology and Oceanography 48, 1394–1407.

Loya, Y., Lubinevsky, H., Rosenfeld, M., Kramarsky-Winter, E., 2004. Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction. Marine Pollution Bulletin 49, 344–353.

Muller-Parker, G., Cook, C.B., D'Elia, C.F., 1990. Feeding affects on phosphate fluxes in the symbiotic sea anemone *Aiptasia pallida*. Marine Ecology Progress Series 60, 283–290.

Patterson, K.L., Porter, J.W., Ritchie, K.B., Polson, S.W., Mueller, E., Peters, E.C., Santavy, D.L., Smith, G.W., 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. Proceedings of the National Academy of Sciences, USA. 99 (13), 8725–8730.

Paul, J.H., McLaughlin, M.R., Griffin, D.W., Lipp, E.K., Stokes, R., Rose, J.B., 2000. Rapid movement of wastewater from on-site disposal systems into surface waters in the Lower Florida Keys. Estuaries 23 (5), 662–668.

Pomeroy, L.R., Kuenzler, E.J., 1969. Phosphorus turnover by coral reef animals. Proceedings from the Conference on Radioecology 2, 474–482.

Porter, J.W., Meier, O.W., 1992. Quantification of loss and change in Floridian reef coral populations. American Zoologist 32, 625–640.

Porter, J.W., Jaap, W.C., Wheaton, J.L., Kosmynin, V., Tsokos, C.P., Yanev, G., Hackett, K., Patterson, K.L., Marcinek, D.M., Dotten, J., Eaken, D., Brill, M., Lybolt, M., Patterson, M., Meier, O.W., Porter, K.G., Dustan, P., 2002. Detection of coral reef change by the Florida Keys Coral Reef Monitoring Project. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, NY, pp. 749–769.

Porter, J.W., Lewis, S.K., Porter, K.G., 1999. The effect of multiple stressors on the Florida Keys coral reef ecosystem: a landscape hypothesis and a physiological test. Limnology and Oceanography 44, 941–949.

Shinn, E.A., Reese, R.S., Reich, C.D., 1994. Fate and pathways of injection-well effluent in the Florida Keys. Department of the Interior, US Geological Survey, pp. 94–276.

Simkiss, K., 1964. Phosphates as crystal poisons of calcification. Biological Reviews 39, 487–505.

Strickland, J.D.H., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis, second ed. Bulletin of Fisheries Resource Board of Canada, pp. 1–310.

Szmant, A.M., 2002. Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? Estuaries 25 (4b), 743–766.

Szmant, A.M., Forrester, A., 1996. Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. Coral Reefs 15, 21–41.

Yamamuro, M., Kayanne, H., Yamano, H., 2003. $\delta^{15}N$ of seagrass leaves for monitoring anthropogenic nutrient increases in coral reef ecosystems. Marine Pollution Bulletin 46, 452–458.

Zar, J.J., 1998. Biostatistical Analysis, fourth ed. Prentice-Hall, Englewood Cliffs, NJ.