Density and size differences of symbiotic dinoflagellates from five reef-building coral species from Brazil

C. F. Costa[1] and F. D. Amaral[2]

ABSTRACT

The objective of this study was to compare the density and size of symbiotic dinoflagellates (zooxanthellae) living in five species of scleractinian coral from Brazil: *Agaricia agaricites, Favia gravida, Montastrea cavernosa, Porites astreoides* and *Siderastrea stellata.* Approximately 10 colonies of each species were collected from Tamandaré and Gaibu beaches on the southern coast of Pernambuco State, Brazil, between August 1997 and April 1998 from depths of 0-4m. Densities of symbiotic algae were significantly lower in *S. stellata* than in any of the other species. Symbionts of *M. cavernosa* were significantly larger, and those of *F. gravida* significantly smaller, than symbionts from any of the other coral species. The results suggest that cell size may be a useful character in systematic studies of algal symbionts. Since symbiont density and cell diameter also vary in a host species-specific manner in some species, these characters may also be useful in scleractinian coral systematics.

Keywords Zooxanthellae, *Symbiodinium*, Symbiotic dinoflagellates, Scleractinia, Brazil.

Introduction

Symbiotic dinoflagellates in the genus *Symbiodinium*, commonly referred to as "zooxanthellae", are found within the tissues of many marine invertebrates at relatively high densities (Sutton and Hoegh-Guldberg 1990). The number of symbiotic algae within the endodermal cells of cnidarians usually varies, with differences found among individuals and between different parts of the same individual (Helmuth et al. 1997). Densities of zooxanthellae change seasonally, being higher during the cool season and lower at the end of the warm season (Stimson 1997, Brown et al. 1999, Fitt et al. 2000). These changes are positively correlated with coral tissue biomass (Fitt et al. 2000). Davy et al. (1997) proposed that some hosts control symbiont density, while Warner et al. (1996) demonstrated that symbiont densities may be influenced by seawater temperature.

Little is known about the dinoflagellate symbionts of Brazilian marine invertebrates (but see Amaral and Costa 1998, Costa 1998, 2001). This paper documents the densities and size of zooxanthellae in five species of scleractinian coral from Tamandaré and Gaibu beaches along the southern coast of Pernambuco State, Brazil.

Methods

Ten independent fragments, approximately 10cm in diameter, were collected at depths of 0-4m from *Favia gravida* and *Siderastrea stellata* at Gaibu (08°20'S, 35°56'W) and *Agaricia agaricites, Montastrea cavernosa, Porites astreoides* and *Siderastrea stellata* at Tamandaré (08°47'S, 35°06'W) between August 1997 and April 1998. Coral fragments were chiseled from the substrate and brought to the surface in individual plastic bags filled with seawater.

Coral tissue was removed with a high-pressure jet of water (WaterPik) and transferred to a clean plastic bag. The extracted tissue was then homogenized mechanically. Density of zooxanthellae was determined from replicate (n=6) counts of the homogenate with a hemocytometer. The total number of symbionts was calculated and converted to number of symbionts per unit surface area. Symbiont size was measured as the mean diameter of 300 individual cells, measured using a calibrated ocular micrometer on a binocular microscope (x400).

Statistical analyses were performed using univariate analysis of variance (ANOVA), Student's t-tests, and Tukey's Studentized Range using SAS software, version 6.12. A significance level of $p<0.05$ was used for the statistical tests.

Results

Mean density of zooxanthellae ranged from $0.23\text{-}1.75 \times 10^6$ cells cm^{-2}. While densities of symbionts from *S. stellata* were not significantly different from one another between the two sites, they were significantly lower than algal densities in any of the other coral species from either site ($p<0.05$, Table 1, Fig.1). In addition, densities of zooxanthellae were significantly higher in *F. gravida* than in *A. agaricites* ($p<0.05$, Table 1, Fig.1).

The diameter of symbiotic dinoflagellates residing in *M. cavernosa* was significantly larger, and in *F. gravida* significantly smaller, than those in all other species of coral ($p<0.05$, Table 2, Fig. 2).

Discussion

Many factors appear to influence the density of symbiotic dinoflagellates in reef corals. Physical variables include light (Dustan 1979) and seawater temperature (Fitt et al. 2000), which probably combine to drive predictable seasonal changes (Verde and McCloskey 1998, Fitt et al. 2000). In addition, internal factors may also influence symbiont density. For example, Drew (1972) suggested that the number of zooxanthellae is related to the area of the coral polyp and Muller-Parker (1987) found that the host's ontogenetic state influences symbiont density

[1]Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, Campus I, João Pessoa, Paraíba 58059-900, Brazil. E-mail: fcosta@nepremar.ufpb.br
[2] Universidade Federal Rural de Pernambuco. E-mail: claufern@novaera.com.br

Table 1 Density of zooxanthellae in the scleractinian corals *Agaricia agaricites*, *Favia gravida*, *Montastrea cavernosa*, *Porites astreoides* and *Siderastrea stellata*, collected in Tamandaré and Gaibu beaches from August 1997 to April 1998. Tukey's Studentized Range (HSD) test was used to determine significance between coral species. Coral species: 1- *Agaricia agaricites;* 2- *Favia gravida;* 3- *Montastrea cavernosa;* 4- *Porites astreoides;* 5- *Siderastrea stellata.* Comparisons significant at the 0.05 level are indicated by *.

Species comparison	Simultaneous lower confidence limit	Differencebetween means	Simultaneous upper confidence limit
2 – 3	-95614	544405	1184425
2 – 4	-20995	600035	1221065
2 – 1	23405	603009	1182614 *
2 – 5	936246	1563230	2190215 *
3 – 4	-618907	55630	730166
3 – 1	-577998	58604	695206
3 – 5	338802	1018825	1698848 *
4 – 1	-614533	2974	620482
4 – 5	301014	963195	1625375 *
1 – 5	336725	960221	1583717 *

Table 2 Diameter of zooxanthellae in the scleractinian corals *Agaricia agaricites*, *Favia gravida*, *Montastrea cavernosa*, *Porites astreoides* and *Siderastrea stellata*, collected in Tamandaré and Gaibu beaches from August 1997 to April 1998. Tukey's Studentized Range (HSD) test was used to determine significance between coral species. Coral species: 1- *Agaricia agaricites;* 2- *Favia gravida;* 3- *Montastrea cavernosa;* 4- *Porites astreoides;* 5- *Siderastrea stellata.* Comparisons significant at the 0.05 level are indicated by *.

Species comparison	Simultaneous lower confidence limit	Difference between means	Simultaneous upper confidence limit
3 – 4	0.5795	1.0277	1.4760 *
3 – 1	0.7007	1.1446	1.5885 *
3 – 5	0.7772	1.1983	1.6193 *
3 – 2	1.8730	2.6923	2.6923 *
4 – 1	-0.2799	0.1169	0.5137
4 – 5	-0.2006	0.1705	0.5416
4 – 2	0.8968	1.2549	1.6130 *
1 – 5	-0.3121	0.0536	0.4194
1 – 2	0.7855	1.1380	1.4906 *
5 – 2	0.7610	1.0844	1.4078 *

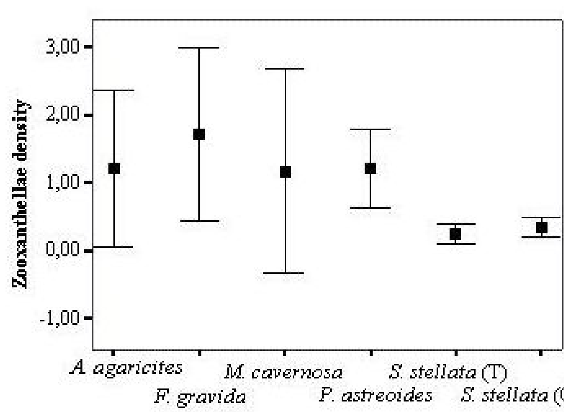

Fig. 1 Mean density of zooxanthellae in five scleractinian corals collected from Tamandaré (T) and Gaibu (G) between August 1997 and April 1998. Error bars show ± standard deviation.

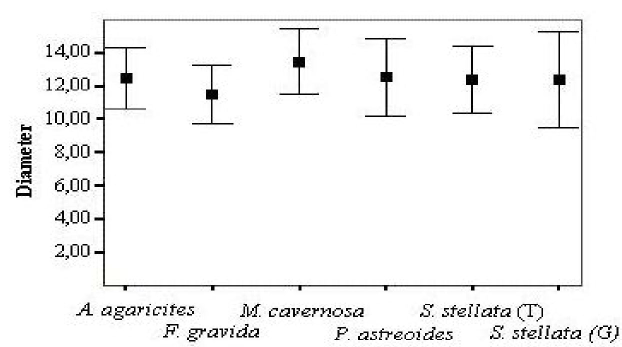

Fig. 2 Mean diameter (µm) of zooxanthellae in five coral species collected from Tamandaré (T) and Gaibu (G) between August 1997 and April 1998. Error bars show ± standard deviation.

because planula formation induces an increase in algal density which is then sharply reduced when larvae are released. Davy et al. (1997) described the maintenance of symbiont density by the host's intracellular area. However, according to Carlos et al. (2000), hosts that carry different symbiont types may have an adaptive advantage during periods of environmental extremes. Genetic differences in algal types may explain the size differences documented in this study. Amaral and Costa (1998) showed that zooxanthellae density was statistically different between different species of scleractinian coral and hydrocoral, a finding supported by this study.

Mean diameter of symbiotic dinoflagellates was different for only two of the species studied, supporting Wilkerson et al. (1988), who found significant differences in the diameter of zooxanthellae from nine species of scleractinian from the Caribbean Sea. The values reported here for the mean diameter of zooxanthellae from *Agaricia agaricites*, *Montastrea cavernosa*, and *Porites astreoides* are similar to those of Wilkerson et al. (1988) for the same species collected in Jamaica at depths of 1.5 m. Billinghurst et al. (1996) presented data suggesting that host morphology influences symbiont diameter. Different morphologies of zooxanthellae, including size, may relate to genetic differences in algal type. Baker and Rowan (1997) documented different algal types in several Caribbean corals by molecular analysis, with *M. cavernosa* harboring *Symbiodinium* in clade *C*. Whether the same species of corals in Brazilian waters harbor the same genetic symbionts has not yet been determined. Based on the above data and discussion, it is suggested that the density and size of symbiotic dinoflagellates may be used in future studies of coral systematics, providing colonies are collected at the same locality, depth and time of year.

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References

Amaral FD, Costa CF (1998) Zooxantelas dos hidrocorais *Millepora alcicornis* e *Millepora braziliensis* e dos corais *Favia gravida* e *Siderastrea stellata* de Pernambuco. Trab Oceanogr Univ Fed PE, Recife, 26 (1): 123-133

Baker AC, Rowan R (1997) Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals of the Caribbean and Eastern Pacific. Proc 8th Int Coral Reef Symp 2: 1301-1306

Billinghurst Z, Douglas AE, Trapido-Rosenthal HG (1996) Regulation of *Symbiodinium* populations in different coral species. 8th Int Coral Reef Symp (Abstracts page 19)

Brown BE, Ambarsari I, Warner ME, Fitt WK, Dunne RP, Gibb SW, Cummings DG (1999) Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs 18: 99-105

Carlos AA, Baillie B, Maruyama T (2000) Diversity of dinoflagellate symbionts (zooxanthellae) in a host individual. Mar Ecol Prog Ser 195: 93-100

Costa CF (1998) Zooxantelas de algumas espécies de corais (Cnidaria, Scleractinia) da Praia de Gaibu e da Praia de Tamandaré - PE. Monografia de Licenciatura em Ciências Biológicas. Universidade Federal Rural de Pernambuco. Recife: 66 pp.

Costa CF (2001) Zooxantelas (Dinoflagelados simbióticos) hospedados por corais (Cnidaria, Scleractinia) dos recifes do Picãozinho, João Pessoa, Paraíba, Brasil. Dissertação Mestrado. Universidade Federal da Paraíba. João Pessoa: 90 pp.

Davy SK, Lucas IAN, Turner JR (1997) Uptake and persistence of homologous and heterologous zooxanthellae in the temperate sea anemone *Cereus pedunculatus* (Pennant). Biol Bull 192: 208-216

Drew EA (1972). The biology and physiology of alga-invertebrate symbioses II. The density of symbiotic algae cells in a number of hermatypic hard corals and alcyonarians from various depths. J Exp Mar Biol Ecol 9: 71-75

Dustan P (1979) Distribution of zooxanthellae and photosynthetic chloroplast pigments of the reef-building coral *Montastrea annularis* Ellis and Solander in relation to depth on a West Indian coral reef. Bull Mar Sci 29 (1): 79-95

Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol Oceanogr 45 (3): 677-685

Helmuth BST, Timmerman BEH, Sebens KP (1997) Interplay of host morphology and symbiont microhabitat in coral aggregations. Mar Biol 130: 1-10

Muller-Parker G (1987). Seasonal variation in light-shade adaptation of natural populations of the symbiotic sea anemone *Aiptasia pulchella* (Carlgren 1943) in Hawaii. J Exp Mar Biol Ecol 112: 112-183

Stimson J (1997) The annual cycle of density of zooxanthellae in the tissue of field and laboratory-held *Pocillopora damicornis* (Linnaeus). J Exp Mar Biol Ecol 214: 35-48

Sutton DC, Hoegh-Guldberg O (1990) Host Zooxanthellae interactions in four temperate marine invertebrate symbioses: assessment of effect of host extracts on symbionts. Biol Bull 178: 175-186

Verde EA, McCloskey LR (1998). Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and season. Mar Ecol Prog Ser 168: 147-162

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef: a novel approach. Plant Cell Environ 19: 291-299

Wilkinson FP, Kobayashi D, Muscatine L (1988) Mitotic index and size of symbiotic algae in Caribbean reef corals. Coral Reefs 7:29-36