The Distribution of the Dinoflagellate *Symbiodinium* in the Conch *Strombus gigas*

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ABSTRACT

The dinoflagellate, *Symbiodinium,* forms symbiotic associations with a wide variety of marine invertebrates. In mollusks, *Symbiodinium* has been documented in bivalves and nudibranchs and recently has been identified in the gastropod *Strombus gigas.* Conch originating from Puerto Morelos, Cozumel, and Banco Chinchorro from the Mexican Caribbean were dissected, and dinoflagellates were found in the digestive gland, stomach, nephridium, gills, mantle, and foot, while no evidence of Sy*mbiodinium* was found in the gonad or muscle tissue. The number of dinoflagellates present in the conch reaches up to 5.22 x 10^8 *Symbiodinium* cells per organism in adults. The dinoflagellates show no signs of digestion or damage suggesting that they are not a dietary source for the conch. In addition, the ability of the dinoflagellates to do photochemistry implies that they are capable of providing the host with photosynthetically-derived carbon. Using histology, the distribution of *Symbiodinium* was determined to not be homogeneous in the different organs and tissues of the conch. The presence of the dinoflagellate in structures that are not directly related to the digestive system suggests that *Symbiodinium* plays an important role in the life cycle of the conch.

KEY WORDS: *Strombus gigas, Symbiodinium,* symbiosis

La Distribución del Dinoflagelado Symbiodinium en el Caracol *Strombus gigas*

El dinoflagelado *Symbiodinium* se encuentra en asociaciones simbióticas con una gran variedad de invertebrados marinos. En los moluscos se ha encontrado *Symbiodinium* en bivalvos y nudibranquios, y recientemente se ha identificado en el gasterópodo *Strombus gigas.* Se realizó la disección de caracoles provenientes de Puerto Morelos, Cozumel y de Banco Chinchorro del Caribe Mexicano, observando que el dinoflagelado se encuentra en la glándula digestiva, el estomago, el nefridio, la branquia, el manto y el pie, no encontrando evidencia de la presencia de *Symbiodinium* en la gónada y el músculo. La cantidad de dinoflagelados presentes en adultos se encontró llega a ser hasta 5.22 x 10^8 células por organismo. Los dinoflagelados no presentan daño aparente ni señales de digestión lo cual significa que no son una fuente alimenticia para el caracol. Además, su capacidad de realizar fotoquímica sugiere que pueden proveer carbono fotosintético al hospedero. Mediante técnicas histológicas se determinó que la distribución de *Symbiodinium* en el caracol no es homogénea en las diferentes estructuras en donde se localiza. La presencia del dinoflagelado en estructuras no relacionadas con el sistema digestivo sugiere que *Symbiodinium* juega un papel importante en el ciclo de vida del caracol.

PALABRAS CLAVES: *Strombus gigas, Symbiodinium,* simbiosis

INTRODUCTION

The success of coral reefs has been attributed to the symbiotic associations that many coral reef-dwelling organisms form with the dinoflagellate genus, *Symbiodinium*. Symbiosis, defined as the coexistence of two or more genomes of distinct phyletic origins that persist over subsequent generations (Trench 1979), includes, therefore, the spectrum of associations ranging from mutualism (both species benefit) to parasitism (one species benefits to the detriment of the other species). *Symbiodinium* has been found in mutualistic association with a variety of coral reef species such as Foraminifera, Cnidaria, including anemones, gorgonians, scleractinian corals, and hydrozoa, as well as Mollusks, including the bivalves such as *Tridacna* species and nudibranchs (Trench 1979, Banaszak *et al.* 1993).

Dinoflagellates have been identified in *Strombus tricornis* and other gastropods from the Red Sea (Berner *et al*. 1986a, 1986b). Berner *et al*. (1986a) found that adult *Strombus tricornis* contained dinoflagellates in the gonad and hepatopancreas (digestive gland) and determined that they were photosynthetically active, postulating that these algae could translocate photosynthetic carbon to the gastropod host. More recently, dinoflagellates isolated from the digestive gland and the digestive tract of *Strombus gigas* from the Caribbean (La Jeunesse 2002) were identified as *Symbiodinium* spp. using molecular biology techniques.

The ability of *Symbiodinium* not only to carry out photosynthesis, but also, to translocate the photosynthetic products to their host species, is believed to be the foundation of the success of coral reef dwelling species such as corals and mollusks. For example, in the bivalve mollusk species, *Tridacna crocea* and *T. gigas*, the translocation of photosynthetically derived products provides the host with a carbon source that exceeds its

daily requirements and thus gives the host a competitive advantage in the oligotrophic waters characteristic of coral reefs (Banaszak 1985, Fisher *et al.* 1985). The loss of dinoflagellate symbionts, such as in recently documented world-wide bleaching events on coral reefs, has placed these relationships as well as coral reef ecosystems in jeopardy (Hoegh-Guldberg 1999, Banaszak *et al.* 2003).

The association that exists between *Symbiodinium* and *Strombus gigas* has not been studied to date, however, due to the economic importance of conch in the fisheries industry, in particular in Mexico, and the importance of the dinoflagellate in the ecology of coral reefs, this symbiosis deserves to be investigated further.

MATERIALS AND METHODS

Adult *Strombus gigas* were collected from three sites along the Mexican coast of the Mesoamerican Barrier Reef System: five adults from the lagoon associated with the coral atoll, Banco Chinchorro at a depth of 10 m, one adult from the island of Cozumel at a depth of 25 m and one adult from the coral reef lagoon associated with the Puerto Morelos Reef National Park at a depth of 3 m. After each collection, the strombids were brought to the Photobiology Laboratory located in the Unidad Académica Puerto Morelos of the Universidad Nacional Autónoma de México. The gastropods were weighed, the visceral mass was removed from their shells, they were weighed again and then dissected into different component parts: the gonad, columellar muscle, digestive gland, stomach, nephridium, rectum, mantle, gills and foot. Each component was separately fixed in 4% formalin in sea water, subsampled (approximately 0.5 g wet weight) and homogenized with a Tissue-Tearor (Biospec Products, USA) until the sample was homogeneous. The number of dinoflagellate cells per sample were counted using a Bright-Line Improved Neubauer Hemocytometer (Hausser Scientific, USA) using an optical microscope (Olympus CH-2, USA). Ten subsamples were counted for each sample, the counts averaged and extrapolated to the total tissue or organ weight. The number of dividing cells in each sample was also counted to calculate the mitotic indices.

RESULTS

All seven adult *Strombus gigas* collected, from the three different sites of Banco Chinchorro, Cozumel, and Puerto Morelos, contained evidence of the dinoflagellate *Symbiodinium*. The dark brown to black coloration of the most of the visceral mass as shown in Figure 1 is due to the presence of large numbers of *Symbiodinium* cells as well as an unidentified black pigment. The only tissue that contained no evidence of these cells was the columellar muscle and the only organ that did not contain any cells was the gonad (Figure 1).

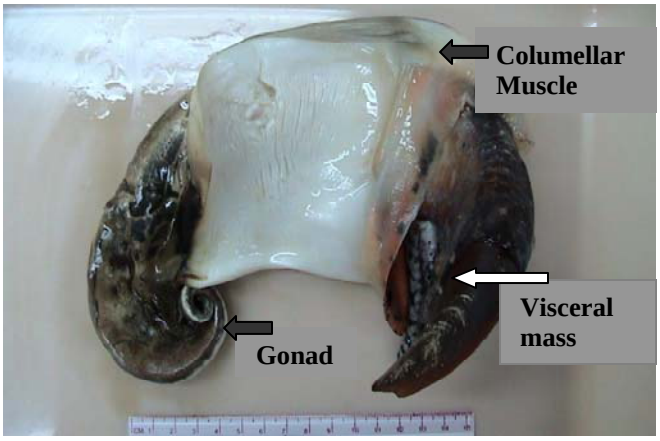

Figure 1. The presence of the dinoflagellate species, *Symbiodinium* in *Strombus gigas* is characterized by the dark brown coloration of the visceral mass due to the large number of golden brown cells as well as an unidentified black pigment.

The total number of cells per individual is highest in the *S. gigas* collected from the reef lagoon at Puerto Morelos reef (5.22 x 10^8 cells), whereas it is lowest in the individual collected from a rocky patch at Cozumel (1.15 x 10^8 cells) (Table 1). The average density of the cells per gram of wet weight of the visceral mass (excluding the weight of the shell) followed the same pattern as the total number of cells, with the individual from Puerto Morelos containing the highest density of *Symbiodinium* at 5.58 x 10^6 cells/g and the conch from Cozumel containing the lowest density of cells at 8.23 x 10^5 cells/g.

Table 1. The total number and density of *Symbiodinium* cells in *Strombus gigas* collected from the three different sites, a reef lagoon at Banco Chinchorro, a rocky patch on the island of Cozumel and the reef lagoon at Puerto Morelos Reef .

Location	Banco Chinchorro	Cozumel	Puerto Morelos
Total *Symbiodinium* per host (cells x 10^8)	2.26	1.15	5.22
Density of *Symbiodinium* per host (cells x 10^5 g^{-1})	24.7	8.23	55.8

The tissues and organs that contained *Symbiodinium* (Table 2) were the digestive gland where the majority of the cells (average for the three sites = 94.76%) are contained, followed by the stomach (average for the three sites = 2.62%), mantle (average for the three sites = 0.94%), rectum (average for the three sites = 0.69%), nephridium (average for the three sites = 0.45%), foot (average for the three sites = 0.39%)and gills (average for the three sites = 0.15%). Dividing *Symbiodinium* cells were only found in the digestive gland, mantle and stomach (Table 3). The division rates of *Symbiodinium* in *S. gigas*

followed a similar pattern for all adults collected from the three different sites, with the highest mitotic indices observed in the mantle (ranging from 20 to 39%), followed by the stomach (ranging from 5 to 28%) and finally by the digestive gland (ranging from 2 to 4%) (Table 3). All other tissue and organs that contained *Symbiodinium* did not contain any dividing cells.

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Table 2. The distribution of *Symbiodinium* cells as a percentage (%) of the total number of cells found in the organs and tissues of *Strombus gigas* from the three different collection sites, a reef lagoon at Banco Chinchorro, a rocky patch on the island of Cozumel and the reef lagoon at Puerto Morelos Reef.

Tissue or organ	Banco Chinchorro	Cozumel	Puerto Morelos	Average for the 3 sites
Digestive gland	95.57	92.40	96.32	94.76
Stomach	1.08	5.90	0.88	2.62
Mantle	1.47	0.25	1.09	0.94
Rectum	0.65	0.52	0.90	0.69
Nephridium	0.41	0.44	0.51	0.45
Foot	0.54	0.07	0.56	0.39
Gills	0.29	0.04	0.12	0.15
Columellar muscle	0	0	0	0
Gonad	0	0	0	0

Table 3 . Mitotic indices of *Symbiodinium* (ratio of dividing to non-dividing cells expressed as a percentage) found in the organs and tissues of *Strombus gigas* from the three different collection sites, a reef lagoon at Banco Chinchorro, a rocky patch on the island of Cozumel and the reef lagoon at Puerto Morelos Reef.

Tissue or organ	**Banco Chinchorro**	**Cozumel**	**Puerto Morelos**	**Average for the 3 sites**
Digestive gland	3.02	1.38	2.71	2.37
Stomach	21.74	6.06	13.64	13.8
Mantle	30.52	37.50	37.50	35.2
Rectum	0	0	0	0
Nephridium	0	0	0	0
Foot	0	0	0	0
Gills	0	0	0	0
Columellar muscle	0	0	0	0
Gonad	0	0	0	0

DISCUSSION

All *Strombus gigas* collected from Banco Chinchorro, Cozumel and Puerto Morelos contained *Symbiodinium* cells in large quantities (up to 5.22 x 10^8 cells per adult, Table 1). The samples collected in Puerto Morelos on average contained 4.5 times more cells than those from Cozumel and 2.3 times more cells than those from Banco Chinchorro. This variation may be related to the habitat differences at the three collection sites. The adult collected from the reef lagoon in the Puerto Morelos Reef National Park at a depth of 3 m, was found in a relatively high-light environment in comparison to the other two sites (10 m for Banco Chinchorro and 25 m for Cozumel), which may be an important factor for maintaining the photosynthetic activity of the dinoflagellate cells. The adult conch collected from Cozumel at a depth of 25 m, where light intensities are very low, contained the fewest cells, both in total number of cells as well as in terms of density. In addition the adult populations of conch at Cozumel are found on rocky patches rather than in coral-derived sand due to the distance to the nearest coral reefs. The absence of corals nearby may contribute to the lower number of dinoflagellate cells per individual in comparison to those collected close to a coral reef, such as the samples from Puerto Morelos and Banco Chinchorro.

The mitotic index (ratio of dividing to non-dividing cells) of *Symbiodinium* cells is significantly higher in the mantle than in the other tissues and organs (Table 3). The behavior of *Strombus gigas* to extend its mantle out over the substrate beyond the limits of its shell results in the

exposure of the mantle to higher light intensities conducive to the photosynthetic activity of the dinoflagellate cells. A higher rate of photosynthetic activity would result in higher division rates of the algal cells. It is interesting to note, however, that the mantle does not contain the highest number of cells in total but actually contains less that 1% of the total cells (Table 2). This may mean that the cells grow and divide preferentially in the mantle but do not remain in this region but move elsewhere. The *Symbiodinium* are not found within the host cells but rather are in ducts (Garcia Ramos and Banaszak Unpublished data), which may facilitate the movement of these cells from one part of the host to another. This movement of algal cells within an invertebrate host has important implications in terms of the association between *S. gigas* and *Symbiodinium*.

The entire life cycle of *Strombus gigas* is within the coral reef system. Juveniles tend to be found in sea grass beds whereas the adults are found on sandy bottoms (Randall 1964). Due to the benthic life style and the habit of *S. gigas* to rasp seagrass blades for epiphytes (Randall 1964), makes it highly likely that *S. gigas* coincidentally ingests dinoflagellates from the surrounding environment, which then enter the digestive tract. The abundance of symbiotic dinoflagellates such as *Symbiodinium* in other species of coral reef dwelling-organisms, which expel excess symbionts into the surrounding water column means than *Symbiodinium* can be found free-living at least temporarily, until another host is found. These dinoflagellates could be found either in the water column during their motile phase or on substrates during their sedentary phase either as active cells or as cysts. The large number of *Symbiodinium* cells in the digestive tract may be explained, at lest in part, by incidental ingestion, however, the presence of *Symbiodinium* cells in organs and tissues that are not directly part of the digestive tract, such as the nephridia, mantle, gills, and foot, indicates that the presence of *Symbiodinium* in *S. gigas* is not incidental and that there exists a symbiotic association between them. In addition, the dinoflagellates, found within the tissues as well as those expulsed via the anus, do not show any signs of digestion (Trench, Banaszak and LaJuenesse, Unpublished data) and are photosynthetically active (Iglesias and Banaszak, unpublished data). This signifies that *Symbiodinium* are not a direct dietary source for *S. gigas*.

Strombus gigas, due to the presence of the very large number of cells of *Symbiodinium* within its tissues, organs and digestive tract may play a very important role in coral reef ecosystems. The dinoflagellate cells are expelled in a healthy state and, therefore, are available for other hosts, such as various species of coral larvae that are spawned without symbionts or adult colonies that have bleached, to acquire them. This highlights the fact that *Strombus gigas* plays a very important role not only due to its economic importance but also to its ecological importance in coral reef ecosystems, in particular because bleaching of coral reef dwelling organisms is predicted to be a more common event due to global climate change (Hoegh-Guldberg, 1999).

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LITERATURE CITED

Banaszak, A.T. 1985. The importance of zooxanthellae at various stages of development of the intertidal, burrowing clam, *Tridacna crocea* Lamarck 1819. Honours Thesis. James Cook University, Australia. 80 pp.

Banaszak, A.T., R. Iglesias-Prieto, and R.K. Trench. 1993. *Scrippsiella velellae* sp. nov. (Peridiniales) and *Gloeodinium viscum* sp. nov. (phytodiniales), dinoflagellate symbionts of two hydrozoans (Cnidaria). *Journal of Phycology* **29**:517-528.

Banaszak, A.T., N. Ayala-Schiaffino, A. Rodríguez-Román, S. Enríquez, and R. Iglesias-Prieto. 2003. The response of *Millepora alcicornis* to two bleaching events in the Mexican Caribbean. *Revista de Biologia Tropical* **51**:57-66.

Berner, T., A. Wishkovsky, and Z. Dubinsky. 1986a. Endozoic algae in shelled gastropods – a new symbiotic association in coral reefs? I. Photosynthetically active zooxanthellae in *Strombus tricornis*. *Coral Reefs* **5**:103-106.

Berner,T., A. Wishkovsky, and Z. Dubinsky. 1986b. Endozoic algae in shelled gastropods – a new symbiotic association in coral reefs? II. Survey of distribution of endozoic algae in Red Sea snails. *Coral Reefs* **5**:107-109.

Fisher, C.R., W.K. Fitt, and R.K. Trench. 1985. Photosynthesis and respiration in *Tridacna gigas* as a function of irradiance and size. *Biological Bulletin* **169**:230-245.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world´s coral reefs. *Marine and Freshwater Research* **50**:839-866.

LaJeunesse, T.C. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141**:387-400.

Randall, J.E. 1964. Contribution to the biology of the queen conch, *Strombus gigas. Bulletin of Marine Science* **14**:246-295.

Trench, R.K. 1979. The cell biology of plant-animal symbiosis. *Annual Review of Plant Physiology* **30**:485-531.