Geographic distribution of Apicomplexa infecting Strombus gigas

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ABSTRACT

The queen conch *Strombus gigas* is a marine resource of ecological and economical importance in the Caribbean region that suffers a reduction of its populations in most Caribbean countries. An intense and generalized sporozoan infection was detected in *Strombus gigas* populations from various Caribbean countries. The parasite, apparently a Coccidian, Apicomplexa was observed on histological sections of the digestive gland of every sampled organism throughout the various sites: in the samples from San Andres, Colombia; Guadeloupe and St. Barthelemy, French West Indies; Alacranes reef and Chinchorro bank, Mexico; Parguera, Puerto Rico; Margarita Island, Venezuela. To the present light-microscopy analysis, the parasites from all localities could be the same and apparently, they complete their cycle in the digestive gland tissue. The parasite was abundant in Puerto Rico, Colombia and Mexico samples, coinciding with observed anomalies in reproductive cycle, reduced gametogenesis and maturity in Alacranes, Mexico (20-40%) and no gonad activity was observed in conchs from San Andrés. The damage to the digestive diverticula begins in the cryptic cells and attains secretory cells later, giving way to discharge of cysts to the stomach through the digestive gland ducts. Various stages are identified. Five different stages were identified: Apicomplexa-like trophozoites, gametocysts, gamonts, gamets and oocysts. Given the presence of multiple stages at any time a re-infection and the life cycle is asumed to occurred within the same host. These results raise several questions: Given the generalized infection at so distant sites, if the parasite is the same, are *S. gigas* populations more connected than it has been supposed? What are the environmental factors inducing such an intense and generalized infection? What is the impact on recruitment and therefore on the fishery? It is necessary to identify the geographic distribution of this parasite, to discriminate damages to the digestive gland from the physiological cycle destruction of digestive gland cells; to evaluate its impact on the reproductive activity.

KEY WORDS: *Strombus gigas*, parasites, queen conch, Caribbean

Distribución Geográfica de Apicomplexa Infectando a *Strombus gigas*

El caracol *Strombus gigas* es un recurso marino con importancia económica y ecológica en el Caribe. Una intensa y generalizada infección de un esporozooario presentan varias poblaciones de *Strombus gigas* en varias localidades del Caribe: Turmaline, Puerto Rico; San Andrés, Colombia; Guadalupe y St. Barthelemy, Antillas Francesas; arrecife Alacranes y banco Chinchorro, México; Isla Margarita, Venezuela. El parásito es probablemente un Apicomlexa encontrado en la glándula digestiva de *S. gigas* en diferentes localidades, presentándose una invasión frecuente del tejido alveolar de la glándula digestiva. El daño de los divertículos digestivos incluye tanto a las células crípticas como las secretoras. A nivel microscópico el parásito parece ser el mismo en las localidades estudiadas y pareciera completar su ciclo en el mismo organismo. La infección fue abundante en los organismos de Puerto Rico, Colombia y México, coincidiendo con anomalías en el ciclo reproductor. Los organismos de Alacranes presentaron una reducción de la gametogénesis y madurez (20-40%), mientras que los de San Andrés, no presentaron actividad gonádica. El daño en la glándula digestiva se inicia en las células crípticas, invadiendo posteriormente las células secretoras, liberando quistes al estómago a través de los ductos digestivos. Se identificaron varios estadios: Trofozoito tipo Apicomplexa, gametocistos, gamontes, gametos y ooocistos. Dada la presencia de los diferentes estadios en un mismo organismo, se presupone una posible re-infección y el ciclo completo en el hospedero. Los resultados anteriores generan varias preguntas: ¿Dada la infección generalizada, eso implicaría que las poblaciones de *S. gigas* están más interconectadas que lo que se supone? Cuáles son las condiciones ambientales que favorecen esta infección? Cuál es su impacto en el reclutamiento?

PALABRAS CLAVES: *Strombus gigas*, parásitos, caracol rosa, Caribe

INTRODUCTION

Given the regional importance of *Strombus gigas* Linne, 1758 in the Caribbean, and the critical state of some of its populations (Appeldoorn, 1987, Aldana Aranda et al. 2003a), its populations dynamics and reproductive biology have been studied (Reed, 1995a, 1995b; Aldana Aranda et al, 2003b; 2003c; 2003d; Delgado et al 2004; Castro *et al.* 2005). It is from this reproduction related histological work that the presence of a parasite in the digestive gland was detected. Apicomplexa parasites are of common occurrence in invertebrates and especially in Mollusks (Lester & Davis, 1981; Hillman et al, 1982; Perkins, 1988; Duszynski et al., 1999, 2004). The goal in this study was to identify the abundance of the different stages of the Apicomplexa like parasite from the digestive gland of *S. gigas* from different localities in the Caribbean region.

MATERIAL AND METHODS

Samples for histological study of *S. gigas* were made at San Andres, Colombia; Alacranes, Cozumel and Chinchorro from Mexico; Tourmaline, Puerto Rico; Guadaloupe and San Berthelemy, French Antilles and Margarita Island from Venezuela (Fig. 1). They were sampled one or more times to ascertain the presence and abundance of the parasite, including some laboratory reared juveniles from Florida that were imported to Xcaret, Mexico. All conchs sampled were adult with a shell lip over 6 mm thick. A sample of 1 cm^3 of the spire, including digestive gland (DG) and gonad (G) was processed through standard histological techniques (Gabe, 1968). Sections were stained with a modified Goldner trichrome method, including Hematoxylin, acid fuchsine and phosphomolybdic orange G with Alcian blue. Digital images were taken with a Sony CCD-IRIS video-camera mounted on the Carl Zeiss microscope or a Nikon DXm 1200F digital camera mounted on the Nikon microscope. The quantification to establish an incidence of the parasite was done counting the total of every stage observed in two 40x fields per slide, ten slides of different organisms per month and locality were used.

RESULTS

The different stages identified in the digestive gland of *S. gigas* were an Apicomplexa like, bottle shaped trophozoites, 25-30 µm long and 15-20 µm wide, with conic apical structure, it is always found within the cells (Pl. 1 a-b). The apical structure is implanted in the cellular wall (Pl. 1b). These trophozoites become cysts (gametocyst) with no connection to the host cell (Pl. 1c). Gamos 15 to 20 µm with two to four macrogamets 12 to 15 µm. (Pl. 1c) or microgamets 5-8 µm (Pl. 2b) and Oocyst with a single trophozoite (Pl 1d), and Oocyst with four trophozoits (Baqueiro, et al. in press).

All sampled organisms presented one or more stages of the parasite in the digestive gland. Figure 2 presents the variation in abundance of the different stages of the parasite at the localities studied. The dominant stage of the parasites varies with the site and in time, even though, the gametocystes are the dominant stage through the Caribbean the whole year. The lowest incidence was registered in the laboratory reared organisms from Xcaret with a total of 9 parasites per field, and the highest were registered at Puerto Rico with 85 parasites per field and San Andres with 82 parasites per field. The mean number of parasites at San Andres and Alacranes through the year was similar, 54 and 50 respectively (Fig. 3).

The different stages vary much in number through the year, with variations for trophozoits of 1 to 7 individuals per field, gametocysts form 3 to 47, gamonts from 1 to 45 (Fig. 4). Gametes and oocysts are absent during several moths but they can be very abundant, gametes are found in groups of over 30 with several groups per field, while oocysts were registered up to 25 per field. Oocystes start from the conjugation of a macro and microgamet (Duszynski, et al. 2004), originating a small cyst, easily distinguished form gametocysts that are originated from the encystment of a trophozoit, but as they mature and enlarge they can be mistaken with gametocyst.

The variation of stage abundance and chronology was investigated at Alacranes, Mexico and San Andres, Colombia where a year cycle was sampled. Relative abundance of the different stages between the two localities is no similarity in these localities. The dominant stage is the gametocyst, followed by gamonts, except for December at Alacranes that the dominant stage is the trophozoits. The largest number of gametocysts was detected at Alacranes from August to November, while a San Andres it was from January to June.

DISCUSSION

The dominant stage at any moment are generally the gametocysts, but their importance seem to vary with the time of the year, presenting different patterns at every locality, which may be influenced by the prevailing environ-

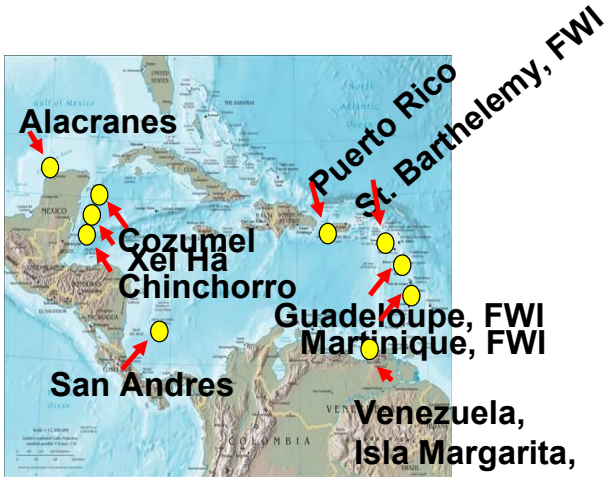

Figure 1. Samples for histological study of *Strombus gigas* in different localities from the Caribbean region.

mental conditions. This is clearly seen when Alacranes and San Andres populations were compared. But it is also possible that a variation in abundance is associated with the physiological stage of the conch, taking advantage of the renewal of the digestive cell.

The presence of cysts and spores of an intracellular parasitic organism in the digestive gland cells is constant, although it varies in intensity according to individuals. Their presence in the lumen of the digestive gland ducts is much more variable, probably according to the discharge

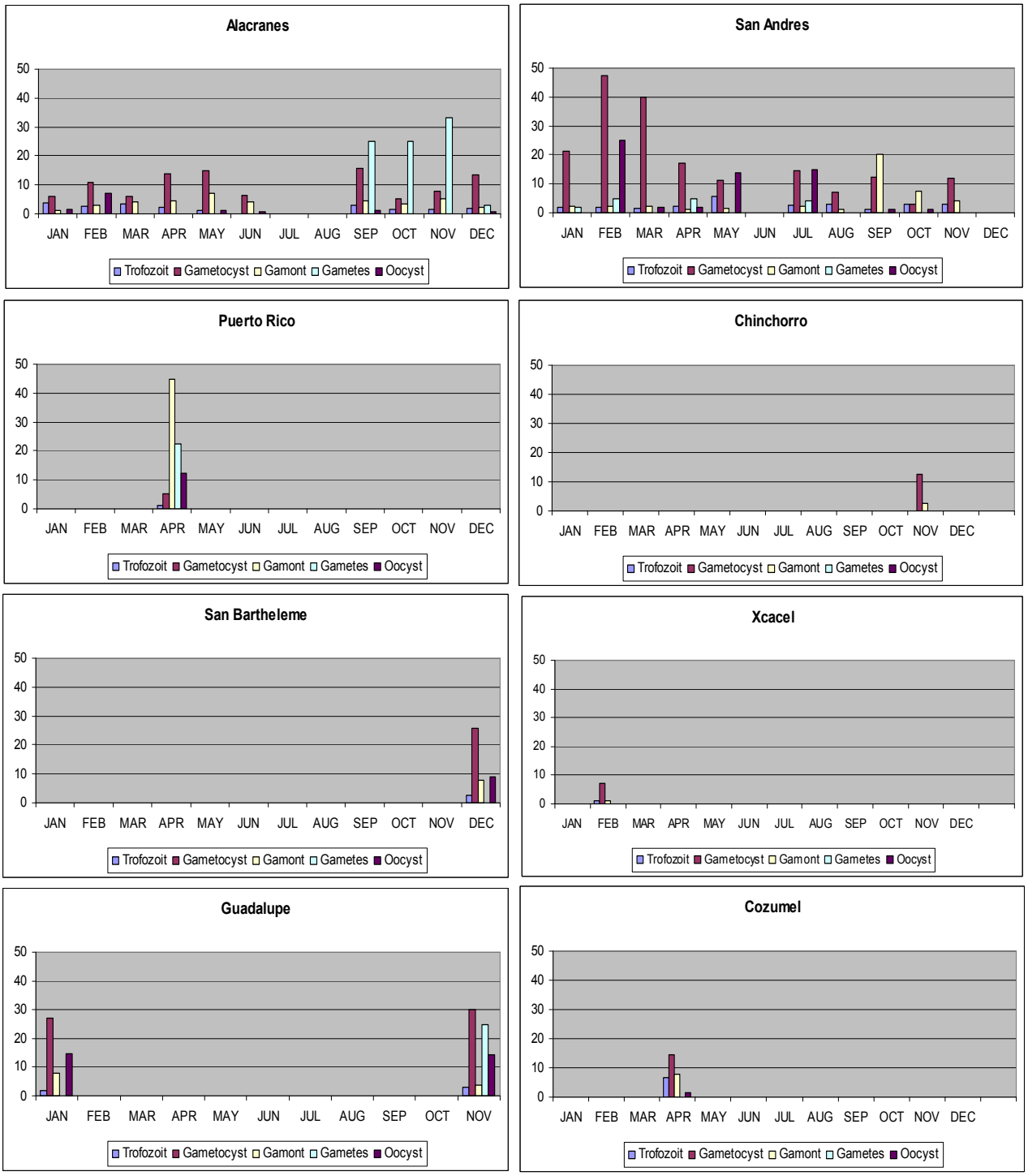

Figure 2. Variation in abundance of the different stages of the parasite at the localities studied.

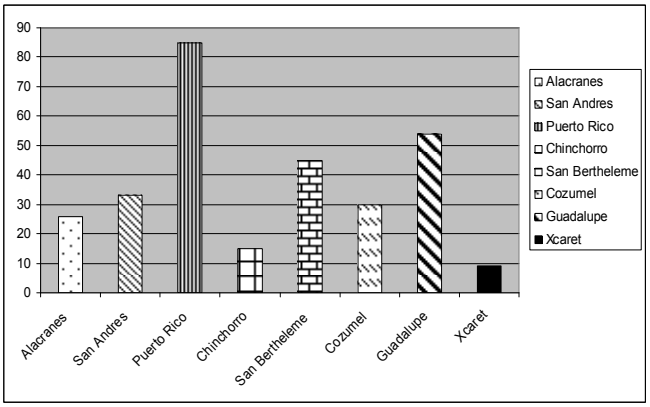

Figure 3. Incidence of the Apicomplexa infecting the digestive gland of *S gigas* in different localities in the Caribbean.

cycle of secretory cells. Gestala et al. (2002) described on the life cycle of *Aggregata* (Apicomplexa, Aggregatidae).

The constant presence of the different stages of the parasite implies that infection occurs all the year round. As maturing gametocysts and gamots were detected emerging in the same organism, it can be interpreted as a re-infection from the ingestion of gametocysts and oocysts, or that they complete the life cycle without leaving the host. The second hypothesis is more likely to be the regular process, as just in a few cases a total destruction of the digestive diverticula cells was detected, with a massive liberation of every present stage. The different stages identified through out the Caribbean suggest that the parasite may be an Emeriidae, which require of two hosts to complete their life cycle (Duszynski et al. 2004). But it is not rare that they complete the life cycle within the same host with the gametocysts expelled with the fesses as a dispersion stages (Duszynski, et al. 1999). Apparently, the organisms found in the digestive gland of *S. gigas* belong to the second modality, as all the stages have been found within the digestive gland tissue. It is possible that we are dealing with more than one species, as suggested by an oocyst found at Guadaloupe with four trophozoits within, compared to the frequently found one trophozoit oocyst at all other localities (Pl. 3).

The intracellular characteristic of the infection, the presence of an apical feeding structure of the trophozoite, and of sporozoite stages with macro and microgamonts, suggest this may be a Coccidia, however they are reported mostly for vertebrates. Pl. 4a and 4d could be spores of Nematopsis spp. No reference has been found of such large intracellular trophozoites, being similar only to extracellular parasites of the family Eimeridae, which is characterized for having more than one host (Bower, 2001)

The presence of such a generalized and intense infection and its coincidence with reduced reproductive activity raises several questions: What are the environmental factors inducing such an intense and generalized infection? What is the geographic extension of the disease and its impact on recruitment and long term health of impacted populations? Is it possible to revert to a healthy state? And last but not least, what is the putative danger of transmission to natural predators and humans when the queen conchs are eaten raw?

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