Attenuated Reproduction of *Strombus gigas* by an Apicomplexa Parasite

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ABSTRACT

The queen conch is a marine resource of ecological and economical importance in the Caribbean region that suffers a reduction of its populations. An intense and generalized sporozoan infection was detected in populations from various Caribbean countries. The goal of this study was to establish a relationship between occurrence of an Apicomplexa parasite and reproduction. Conchs sampled were adults with a lip thickness 6 mm. A sample of 1 cm^3, including digestive gland and gonad was processed through standard histological techniques. Sections were stained with a trichrome technique. A quantification of occurrence of the parasites was done counting the total of the different stages observed in three fields on five sections at 400x magnification. ANOVA and Tukey test were used to establish the significance of the differences in occurrence between months. Canonical variate analysis between stages of parasites and frequency of reproductive stages was made. Gametogenesis and spawn were always low, undifferentiated was the dominate stage. With the principal component analysis a positive correlation was established between parasite abundance and undifferentiated stage, and negative correlation was observed of parasite abundance with maturity and spawn stages. A General Liberalized Model analysis showed that undifferentiated organisms are the dominant stage the year through. This analysis shows how gametogenesis, maturity and spawn increase as the number of parasites decreases. These findings suggest that the occurrence of Apicomplexa could be a factor that affects the gonad development in *S. gigas* from San Andrés Inland.

KEY WORDS: *Strombus gigas*, apicomplexa, reproduction, parasite

Reproducción Atenuada de *Strombus gigas* por Apicomplexa

EL caracol rosa es un recurso de importancia económica y ecológica en el Caribe cuyas poblaciones están reducidas. Por otra parte una intensa y generalizada presencia de un parasito Apicomplexa (coccidea) fue puesta en evidencia en varias poblaciones en el Caribe. El objetivo de este trabajo es establecer una relación entre abundancia de Apicomplexa y la reproducción. Los caracoles utilizados fueron organismos adultos con un labio superior a 6 mm. Muestras de 1 cm3, incluyendo glándula digestiva y gónada fueron procesados por técnicas clásicas de histología. Los cortes fueron coloreados con la técnica de tricromo. La abundancia del parásito fue realizada contando el número total de parásitos en tres campos y en cinco cortes a 400x. ANOVA y test de Tukey fueron realizados para establecer diferencias significativas de la abundancia del parásito en el tiempo. Un análisis canonico fue realizado entre estadios del parásito y la frecuencia de los estadios reproductivos. Gametogenesis y desove fueron siempre bajos mientras el estadio indiferenciado fue el dominante. Con análisis de componentes principales se estableció una correlación positiva entre abundancia del parasito y el estadio indiferenciado, y una correlación negativa entre abundancia del parasito con los estadios de madurez y desove. El análisis con General Liberalized Model mostró también que son los organismos en estadio indiferenciado los dominantes. Este análisis mostró que la gametogenesis, madurez y desove incrementan a medida que el número de parásitos disminuye. Estos resultados sugiere al menos para el caracol rosa *S. gigas* de San Andrés que una de las causas que podría estar afectando la actividad reproductiva de esta especie es la abundancia de Apicomplexa.

PALABRAS CLAVES: *Strombus gigas*, apicomplexa, reproducción, parasito

Reproduction Atténuée de *Strombus gigas* par des Parasites Apicomplexes

Le lambi, ressource marine ayant une importance écologique et économique dans la région Caraïbe, présente une réduction de sa population. Une infection intense et généralisée a été détectée dans les populations de diverses région de la Caraïbe. Le but de cette étude était d'établir une relation entre la présence de ces parasites Apicomplexes et la reproduction. Les lambis échantillonnés étaient des adultes avec une épaisseur de lèvre de 6mm. Un échantillon d'1cm3, incluant la glande digestive et la gonade était analysé selon les techniques standard d'histologie. Les coupes étaient colorées avec une technique trichrome. Une estimation de la quantité des apicomplexes a été faite par comptage de l'ensemble des stades de parasites observés sur trois champs sur 5 coupes à un grossissement de 400. Des tests Tukey et ANOVA ont été utilisés pour établir s'il y avait une différence entre les mois analysés. Une analyse canonique entre les stades de parasites et la fréquence des stades de reproduction a été faite. La gamétogénèse et la ponte sont toujours faibles avec les stades indifférenciés comme étant le plus représentatif. Avec l'analyse de composante principale, une corrélation positive a été mise en évidence entre l'abondance des parasites et les stades indifférenciés et une corrélation négative a été observée entre la présence des parasites et les stades de maturité et de ponte. Une analyse GLM a montré que les oganismes indifférenciés représentent le stade dominant tout au long de l'année. Cette analyse montre comment la gamétogenèse, la maturité et la ponte augmentent lorsque le nombre de parasites diminue. Ces résultats suggèrent que la présence des parasites Apicomplexes pourrait être un facteur qui affecte la gamétogenèse chez *S. gigas.*

MOTS CLÉS: *Strombus gigas*, Apicomplexa, reproduction, parasite

INTRODUCTION

The queen conch, *Strombus gigas* represents one of the most valuable demersal resources in the Caribbean region, exceeded only by the spiny lobster. Nowadays, conch meat is no longer an inexpensive food as it was some years ago. Conch meat prices at local markets vary 10 to 15 US $/kg until 25 Euros/kg in the French West Indies, for locally fresh meat.

Given the regional importance of *S. gigas* in the Caribbean, and the critical state of some of its populations the dynamics and reproductive biology have been studied (Aldana Aranda *et al.* 2001, 2003b; 2003c; 2003d. Delgado *et al.* 2004, Castro *et al.* 2005).

It is from this reproduction histological work that the presence of an Apicomplexa like parasite was detected in the digestive gland of *S. gigas* in the population of San Andres island (Baqueiro *et al*. 2005 and 2007). Apicomplexa parasites are of common occurrence in invertebrates and especially in Mollusks (Azevedo 2004, Lester and Davis 1981, Hillman *et al*. 1982; Duszynski *et al*. 2004, Perkins 1998)- Castro *et al*. (2005) reported an atypical reproductive cycle for *S. gigas* from San Andres Inland; it was characterized by a height percentage of undifferentiated stage. The goals of this study were to study the reproductive cycle of Queen conch *Strombus gigas* at San Andres Inland in the Caribbean sea and to identify the impact of the different stages of the Apicomplexa (Coccidea) like parasite on the reproductive cycle.

MATERIALD AND METHODS

Queen conchs were sampled at San Andrés Inlands that is situated at 12° 28'N and 81°39'W. All conchs sampled were adult with a shell length ≥ of 22 cm and a shell lip over 10 mm thick. Transverse sections of digestive gland and gonad tissues were processed for histology. For the reproductive study 30 organisms were observed monthly. The quantification to establish the occurrence of the parasite was done counting the total of every stage observed in fifteen 40x fields per slide, thirty slides of different organisms per month were used. Sections were stained with a modified Goldner three chrome method (Gabe, 1968). Digital images were taken with a Sony CCD-IRIS video-camera mounted on the Carl Zeiss microscope.

RESULTS

Different stages of an Apicomplexa like parasite were identified in the digestive gland of *S. gigas*: Trophozoites

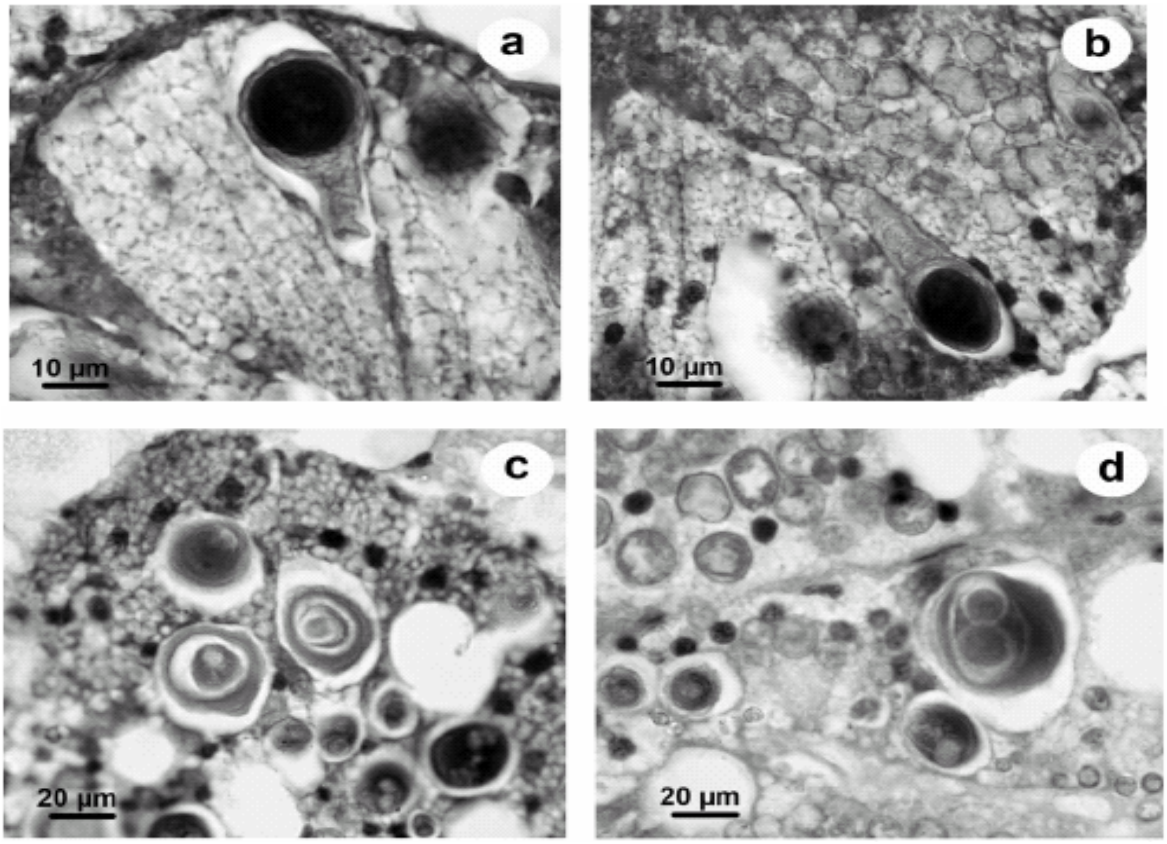

Figure 1. Different stages of Apicomplexa (Coccidea) like parasite were identified in the digestive gland of *Strombus gigas*. Trophozoites with conic apical structure, implanted in the cellular wall (a and b), Gametocyst with no connection to the host cell (c), and gamonts characterized by their thin wall were observed (d).

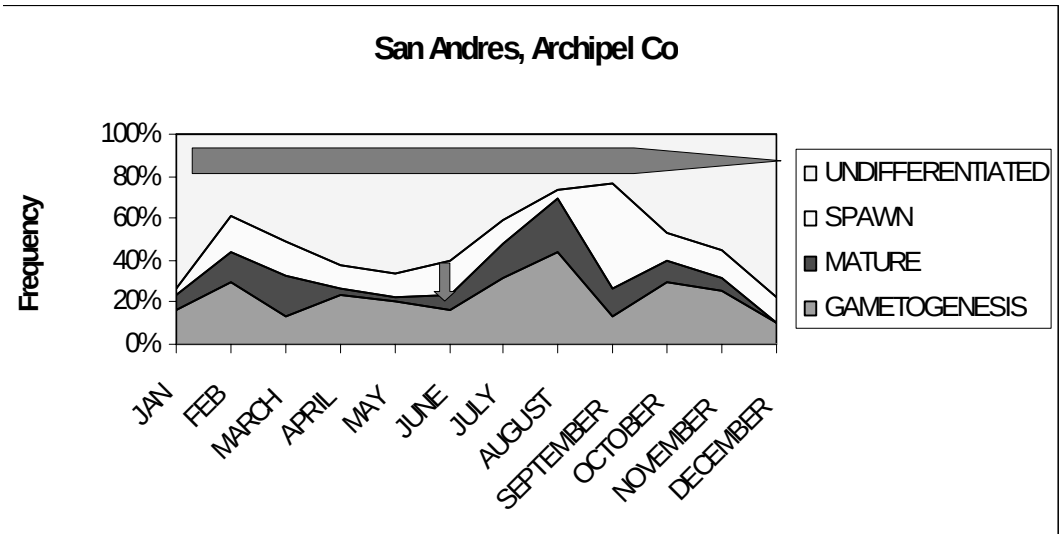

Figure 2. Reproductive cycle of Strombus gigas from San Andres Inland. Undifferentiated Gametogenesis, mature and spawn stages were used for gonad development.

with conic apical structure, implanted in the cellular wall (Figures 1a and 1b), Gametocyst with no connection to the host cell (Figure 1c), and Gamonts characterized by their thin wall were observed (Figure 1d).

Figure 2, showed the reproductive cycle of *S. gigas* at San Andres Inland. Conchs from San Andres showed an undifferentiated stage profuse through the year. Gametogenesis stage was observed through the year but in a low percentages; less than 20% and mature stage was observed in low percentage (10%). The relationship between the abundance of parasite and reproductive cycle is showed in Figure 3; where bars represent the different percentage of reproductive stages and lines represent number of parasites. Even though conchs samples come from adults, gametogenesis and spawn were always low. Undifferentiated organisms are the dominant stage the year through. With the principal component analysis a positive correlation was established between parasite abundance and undifferentiated stages, and negative correlation was observed of parasite abundance with maturity and spawn stages (Figure 4). The General Liberalized Model (GLM) analysis shows how gametogenesis, maturity and spawn increase as the number of parasites decreases, while the number of undifferentiated organisms increases with increasing numbers of parasites. It is interesting to note that in this and the previous analysis angles between vectors represent levels of correlation, with angles below 90º representing positive correlation between vectors and, angles over 90º represent negative correlation (Figure 5). Figure 6 shows No metric Multidimentional Scaling analysis (NMDS). The

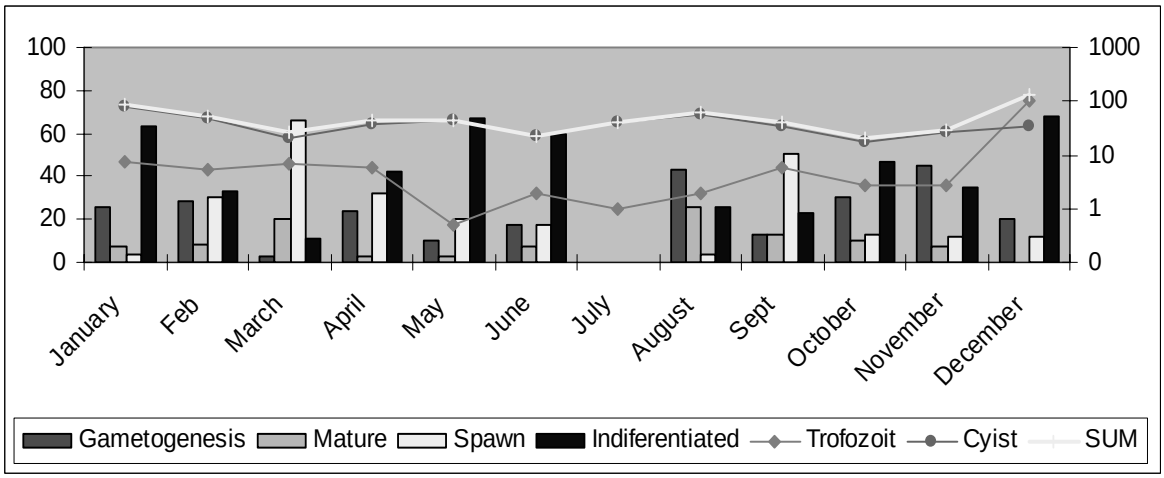

Figure 3. Relationship between the abundance of parasite and reproductive cycle.Bars represent the different percentage of reproductive stages and lines represent number of parasites.

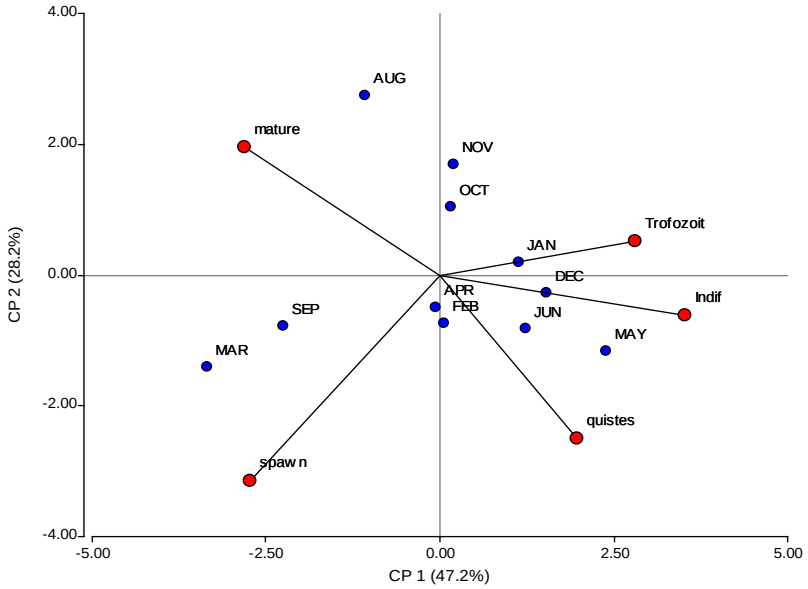

Figure 4. Principal component analysis shows a positive correlation between parasite abundance and undifferentiated stages, and negative correlation was observed between parasite abundance with maturity and spawn stages.

circles represent the abundance of the parasite through the year. Green circles represent the abundance of cysts, and red circles abundance of trophozoits. Vectors show the same results as on the previous analysis. Trophozoits are more abundant during February, May and June.

DISCUSSION

At San Andres, the percentage of animals in gametogenesis is minimal the year through, with minimal maturity and spawning. The undifferentiated stage prevails the year through. Frequency of gametogenesis, maturity and spawn stages diminish with increasing number of parasites. These findings and statistical analysis suggest that the occurrence of Apicomplexa could be a factor that affects the gametogenesis activity in *S. gigas.* The presence of such a generalized and intense infestation raises several questions: What are the environmental factors inducing such an intense and generalized infestation? Are all the populations around the Caribbean infested by various stages of the same parasite or by various species that may be identified through DNA analysis?. Therefore, it is necessary to investigate the relationship between the abundance of parasites and the reproductive cycle of conchs in others sites in the Caribbean.

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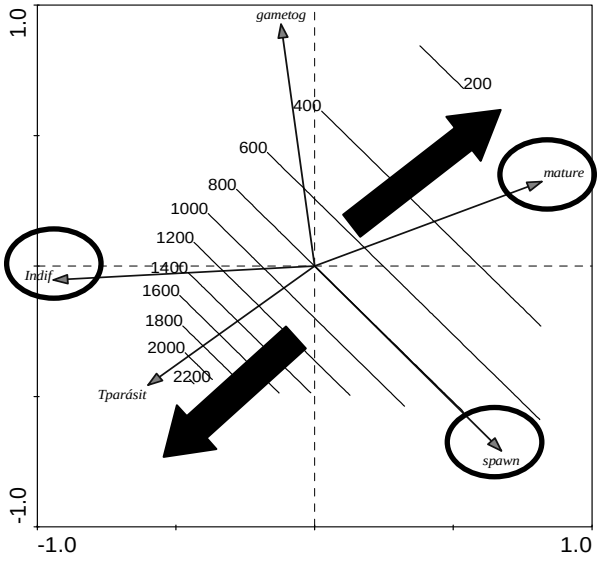

Figure 5. The General Liberalized Model (GLM) shows how gametogenesis, maturity and spawn increase as the number of parasites decreases.

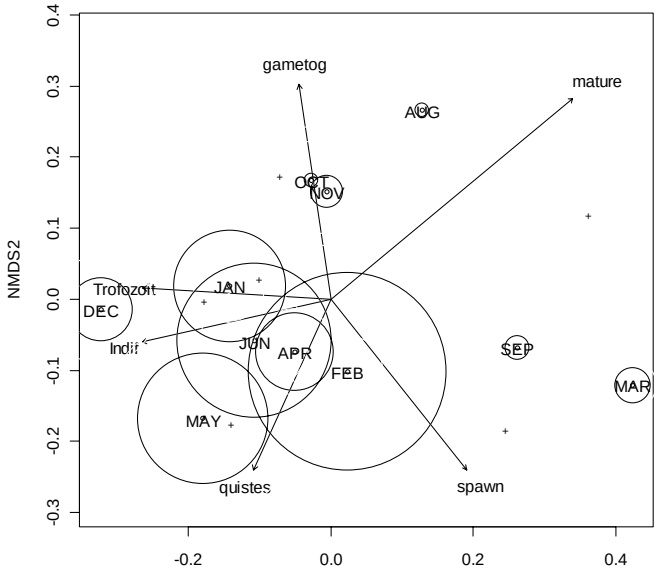

Figure 6. No metric Multidimentional Scaling analysis (NMDS) The circles represent the abundance of the parasite through the year and vectors the reproductive stages. Trophozoits are more abundant during February, May and June.

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