

Occurrence of Apicomplexa Infecting Queen Conch, *Strombus gigas*, From Off Shore and Near Shore in Florida

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ABSTRACT

The queen conch is a resource of ecological and economical importance in the Caribbean that suffered a widespread reduction of its populations. Aldana Aranda (2008) reported an intense and generalized sporozoan infection in the digestive gland of *Strombus gigas*. This study reports the occurrence of a Coccidian (Apicomplexa) infecting the digestive gland of conchs in the Florida Keys in two locations: offshore, where conch reproduction is common (Pelican Shoal, Eastern Sambo), and nearshore where reproduction has ceased relatively recently (East Sisters Rock, Tingler Island). Incidences of Apicomplexa in the digestive gland for conchs collected in June and February and compared the occurrence of parasites with gonadal development between conch found offshore and those found nearshore, were realized. The results showed that the Apicomplexa was present in the digestive gland of conchs in every locality sampled; conchs offshore and conchs nearshore were parasitized. Conchs from East Sisters Rock (near shore, June) and Eastern Sambo (offshore, June) had the highest incidence of infection with an average of 32.34 and 30.35 parasites, respectively; Pelican Shoal (offshore, february) and Tingler Island (near shore, february) had an average of 22.0 and 18.38 parasites, respectively. No correlation was found between number of parasites and gonadal development with Canonical Variate Analysis. Test of significance of first canonical axis: eigenvalue = 0.200, F-ratio = 87.160, P-value = 0.0020. These results raise questions: What are the environmental factors influencing this infection. What is the correlation between this parasite and gonad development. Are conchs the definitive host or do they serve as an intermediate host?

KEY WORDS: *Strombus gigas*, parasite, digestive gland, Floride, reproduction

Abundancia de la Infección de Apicomplexa en el Caracol Rosa, *Strombus gigas* en Aguas Cercanas y Lejanas a la Costa de Florida

Strombus gigas es un recurso de importancia ecológica y económica en el Caribe que ha sufrido una reducción de sus poblaciones en el Caribe. Aldana Aranda (2008) reportaron una intensa y generalizada infección de un esporozorio en la glándula digestiva de *Strombus gigas*. Este estudio reporta la abundancia de Coccidian (Apicomplexa) infectando la glándula digestiva de *S. gigas* de los Cayos de Florida en dos localidades: Offshore, donde la reproducción del caracol es frecuente (Pelican Shoal, Eastern Sambo) y nearshore, donde la reproducción ha cesado de presentarse (East Sisters Rock, Tingler Island). Se examinó la abundancia de Apicomplexa en la glándula digestiva de caracoles en Junio y Febrero con el desarrollo gonadal para caracoles offshore y nearshore. Los resultados mostraron que Apicomplexa estuvo presente en los caracoles de cada localidad; los caracoles offshore y los caracoles nearshore estuvieron fuertemente parasitados. Los Caracoles de East Sisters Rock (nearshore, Junio) y los de Eastern Sambo (offshore, Junio) tuvieron una alta incidencia de infección con una media de 32.34 y 30.35 parásitos, respectivamente; Pelican Shoal (offshore, febrero) y Tingler Island (near shore, febrero) tuvieron una media de 22.0 y 18.38 parásitos, respectivamente. La prueba de significancia del primer eje canónico: eigenvalue = 0.200, F-ratio = 87.160, P-value = 0.0020 del análisis Canonical Variate, no mostró correlación entre parásitos y desarrollo gonadal, probablemente por el bajo número de muestras analizadas. Estos resultados permiten preguntarse: ¿Cuál es el factor ambiental que esta generando esta generalizada infección en caracoles offshore como nearshore? ¿Cuál es la correlación entre incidencia del parásito y desarrollo gonadal a lo largo de un ciclo anual? ¿Son los caracoles el huésped definitivo o son huéspedes intermediarios, infectando otras especies, como la langosta?

PALABRAS CLAVES: *Strombus gigas*, parásitos, glándula digestiva, Florida, reproducción

Presence D'Apocomplexa Infectant le Lambi, *Strombus gigas*, dans de Populations Proches et Distantes de la Côte

Le lambi, ressource d'importance écologique et économique dans toute la Caraïbe, a souffert d'une surexploitation généralisée de ses populations. Aldana Aranda (2008) a montré l'existence d'une infection importante et généralisée par des Sporozoaires. La présente étude montre l'état de cette infection par des Sporozoaires (Apicomplexa) dans les populations des Florida Keys dans deux zones, l'une loin de la côte (Pelican Shoal et Sambo est) où les lambis se reproduisent normalement et l'autre, proche de la côte où la reproduction a été interrompue relativement récemment (Sister Rock est et ile Tingler). La prévalence des Apicomplexa dans les glandes digestives pour des lambis récoltés en juin et en Février, comparée avec l'état des gonades a été étudiée pour les deux localités. Les observations montrent que les Apicomplexa sont présents dans la glande digestive des lambis pour toutes les localités échantillonnées. Les lambis de Sister Rock (localité proche, échantillons de juin) et de Sambo est (localité au large également juin) ont la plus forte incidence de parasites avec une moyenne de 32.4 et 30.35 parasites respectivement. Pelican Shoal (au large février) et ile Tingler, (proche de la côte février) ont une moyenne de 22.0 et 18.38 parasites respectivement. Aucune corrélation n'a été trouvée entre le nombre de parasites et le développement des gonades en utilisant l'analyse canonique des variants. Le test du premier axe canonique donne les valeurs suivantes : eigen value = 200, F-ratio = 87.160, P-value = 0.0020. Ces résultats soulèvent différentes questions : Quels sont les facteurs environnementaux qui influent sur l'infection? Quelle est la corrélation entre ce parasite et le développement des gonades. Les lambis sont ils les hôtes définitifs ou servent ils d'hôtes intermédiaires?

MOTS CLÉS: *Strombus gigas*, parasite, glande digestive, Floride, reproduction

INTRODUCTION

Queen conch, *Strombus gigas*, represents one of the most valuable demersal resources in the Caribbean region, exceeded only by the spiny lobster. However, it is now an over exploited resource. Queen conch, has been the main source of food for the inhabitants of Caribbean coast and islands. Nowadays, conch meat is no longer an inexpensive staple food as it was some decades ago. It is now consumed most as a luxury specialty food. Conch meat prices at local markets vary between 10 and 15 US \$/kg. However, in the French West Indies, prices vary from 20 Euros/kg for imported frozen meat up to 25 Euros/kg for locally harvested fresh meat. Landings of *S. gigas* were estimated at 6 520 mt in 1992, however this specie is now an over exploited resource (3132 tons, in 2002). 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.* 2003a, 2003b, 2003c). It is from this reproduction histological work that an atypical reproduction was reported by Delgado *et al.* (2004) studied the gonad development for conchs offshore and near shore from Florida, founding a not gonad development in conchs offshore. Castro *et al.* (2005) for conchs at San Andres, Island, reported an atypical reproductive cycle. 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.* 2007). Aldana Aranda *et. al* (2007) studied the geographic distribution occurrence of Apicomplexa in conchs sampled in various sites from the Caribbean region, and found a generalized and intense infection around the Caribbean. The goal of this study was to check the presence of the parasite of queen conch from Florida in two localities: offshore and those found near shore, to determine its abundance in each population and to identify the impact of the different stages of the parasite on gonad development in two months, February and June.

MATERIAL AND METHODS

Queen conchs were sampled in the Florida Keys (Figure 1), in two localities offshore at Pelican Shoal, Eastern Sambo), and nearshore where reproduction has ceased relatively recently (East Sisters Rock, Tingler Island). All conchs sampled were adult with a shell length \geq of 22cm and a shell lip over 10 mm thick. Transverse sections of digestive gland and gonad tissues were processed for histology. The quantification to establish the occurrence of the parasite was done counting the total of every stage observed in fifteen 40 x 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.

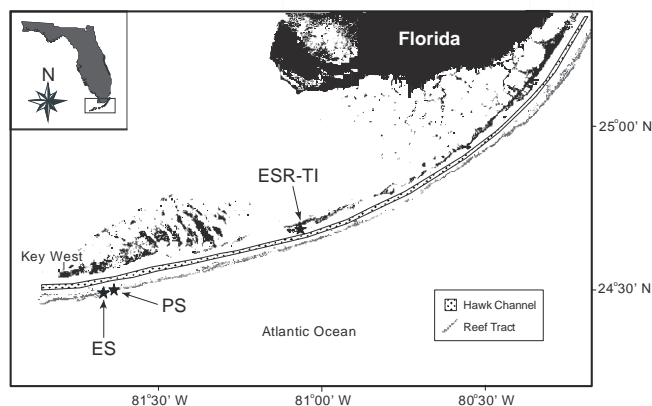


Figure 1. Sampling sites of Queen conchs in the Florida Keys. Two localities offshore ES, Eastern Sambo and PS, Pelican Shoal; and two localities nearshore ESR, East Sisters Rock, and TI, Tingler Island.

RESULTS

Different stages of an Apicomplexa like parasite were identified in the digestive gland of *S. gigas*: Trophozoites with conic apical structure, implanted in the cellular wall (Figures 2a). Gametocyst with no connection to the host cell (Figure 2b), and Gamonts characterized by their thin wall were observed (Figure 2c).

Four stages of reproductive cycle were observed; gametogenesis characterized by active cell division; mature gametes may or may not be present. Mature stage with dominance of mature gametes, although some gametogenesis may be present. Spawn stage, characterized by follicles are partially or totally emptied and broken, eggs and sperms are being reabsorbed, and undifferentiated stage, where the presence of phagocytes and the gametes may not be identified from the microscopic section.

All sampled organisms presented one or more stages of the parasite in the digestive gland. The abundance of the different stages of parasite observed in the digestive gland of conchs in a field at 40x, where observe from conchs nearshore and offshore. The highest incidence of Apicomplexa with an average of 32.3 and 30.3 parasites per field, was observed in conchs sampled near shore (Sisters), in June and conchs sampled offshore (Sambo) in June, respectively. The lowest occurrence of Apicomplexa was observed in conchs sampled offshore (Pelican Shoal) in February and conchs sampled nearshore (Tingler) in February with 22.0 and 18.0 parasites per field, respectively.

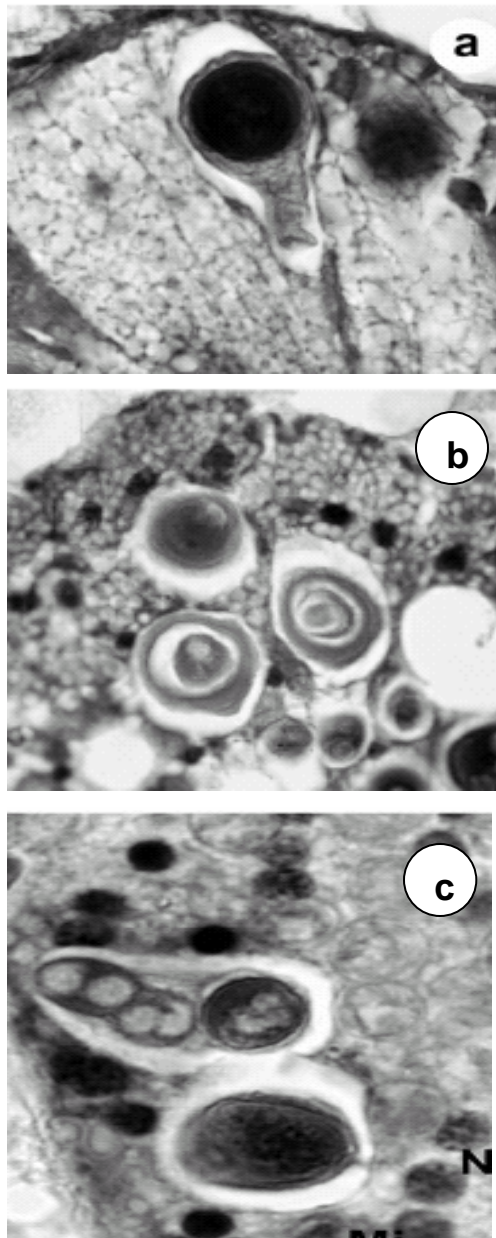
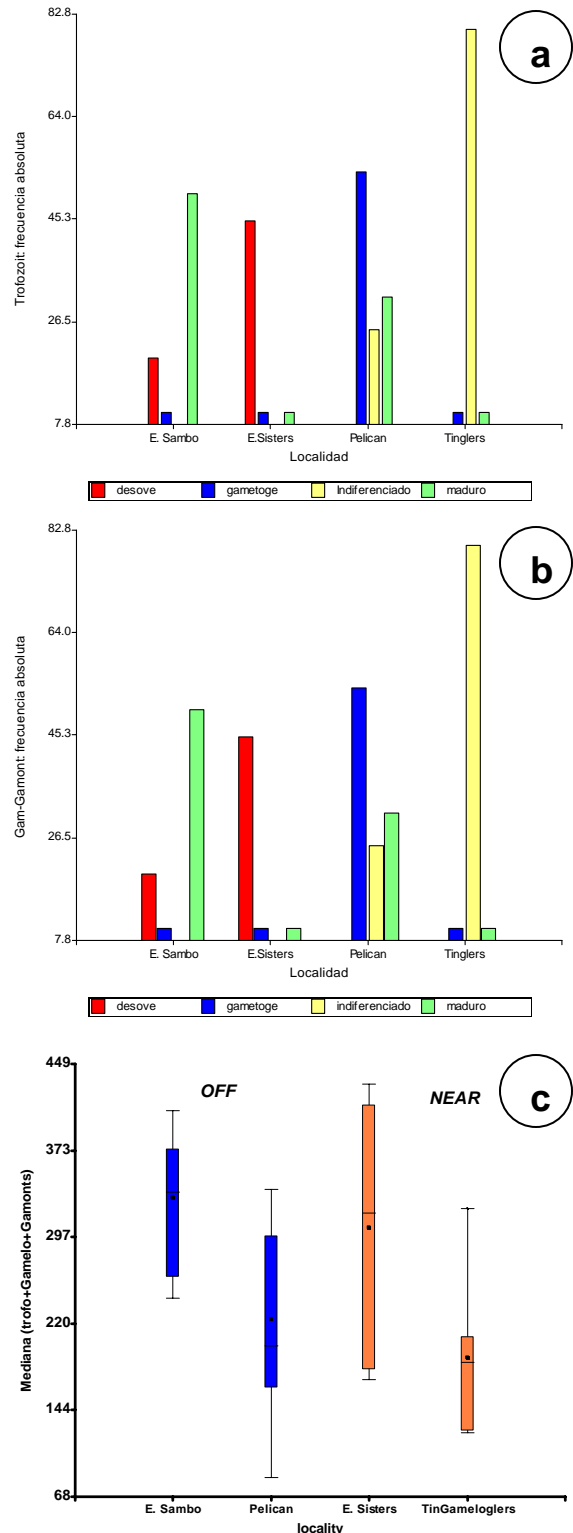


Figure 2. Different stages of Apicomplexa like parasite were identified in the digestive gland of *S. gigas*. (a) Trophozoites with conic apical structure, implanted in the cellular wall, (b) gametocyst with no connection to the host cell and (c) gamonts characterized by their thin wall.

Trophozoites was more abundant of conchs sampled at Tinglers in undifferentiated stage (Figure 3a) and gametocytes and gamonts are more abundant also at Tinglers for conchs in undifferentiated stage (Figure 3b). Median of Trophozoites, gamonts and gametocysts analyzed by sites (Figure 3c) was more abundant in conchs sampled at Sambo (offshore, June) and Sisters (near shore, June). Occurrence of trophozoites, gametozoites and gamonts by

months for conchs sampled offshore and nearshore, showed that conchs sampled in June showed the highest incidence of Apicomplexa for conchs in gametogenesis, or mature, or spawn or undifferentiated stages (Figure 3d).



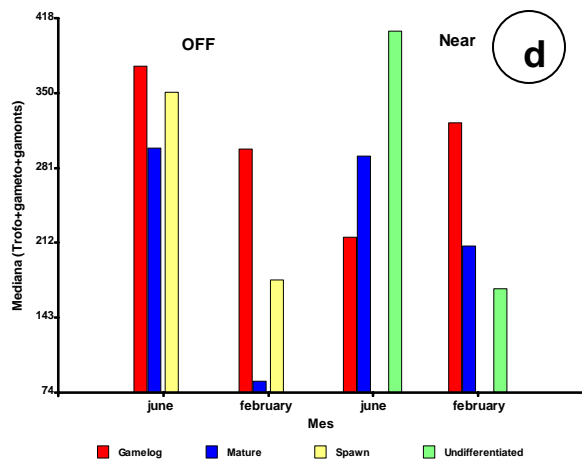


Figure 3. (a), Trophozoites abundance by site; (b), gamonts and gametocysts analyzed by sites. (c) median of trophozoites, gametocysts and gamonts by sites and (d) median of trophozoites, gametocysts and gamonts months.

Kruskal-Wallis test for occurrence of trophozoites by site does not showed difference between sites ($Kw = 0.3692$, $p = 0.9465$) (Figure 4a). Kruskal-Wallis analysis for gamonts and gametocysts by site showed difference between sites $Kw = 10.9888$, $p = 0.01178$ (Figure 4b). Kruskal-Wallis analysis for occurrence of trophozoites do not showed difference between months ($Kw = 0.08179$, $p = 0.7748$) (Figure 4c) and in figure 4d the Kruskal-Wallis analysis for occurrence of gamonts and gametozoites by months, showed a significant difference between months ($Kw = 10.1132$, $p = 0.00147$).

RDA analysis, between shell length, lip thickness and stages of Apicomplexa showed that conchs sampled offshore are biggest and they have more parasites than those sampled near shore. Test of significance of first canonical axis: eigenvalue = 0.025, F-ratio = 9.116, $p = 0.0020$. Test of significance of first canonical axis: eigenvalue = 0.025, F-ratio = 9.116, $p = 0.0020$ (Figure 5a). Figure 5b, showed RDA analysis between sites and stages of Apicomplexa. This figure illustrated that Sister and Sambo have more parasites than Tinglers and Pelican. RDA analysis between months and stages of Apicomplexa illustrated that gametogenesis, mature and spawn stages were observed of conchs sampled offshore in June. February is related with undifferentiated stage and few parasites (Test of significance of first canonical axis: eigenvalue = 0.200, F-ratio = 87.160, $p = 0.0020$) (Figure 5c). RDA Analyse between stages of Apicomplexa, sites, months and sexes showed that conchs sampled in June from Sisters and Sambo have more parasites than conchs sampled in February at Tinglers and Pelican (Test of significance of first canonical axis: eigenvalue = 0.174; F-ratio = 73.560; p value = 0.0020). (Figure 5d).

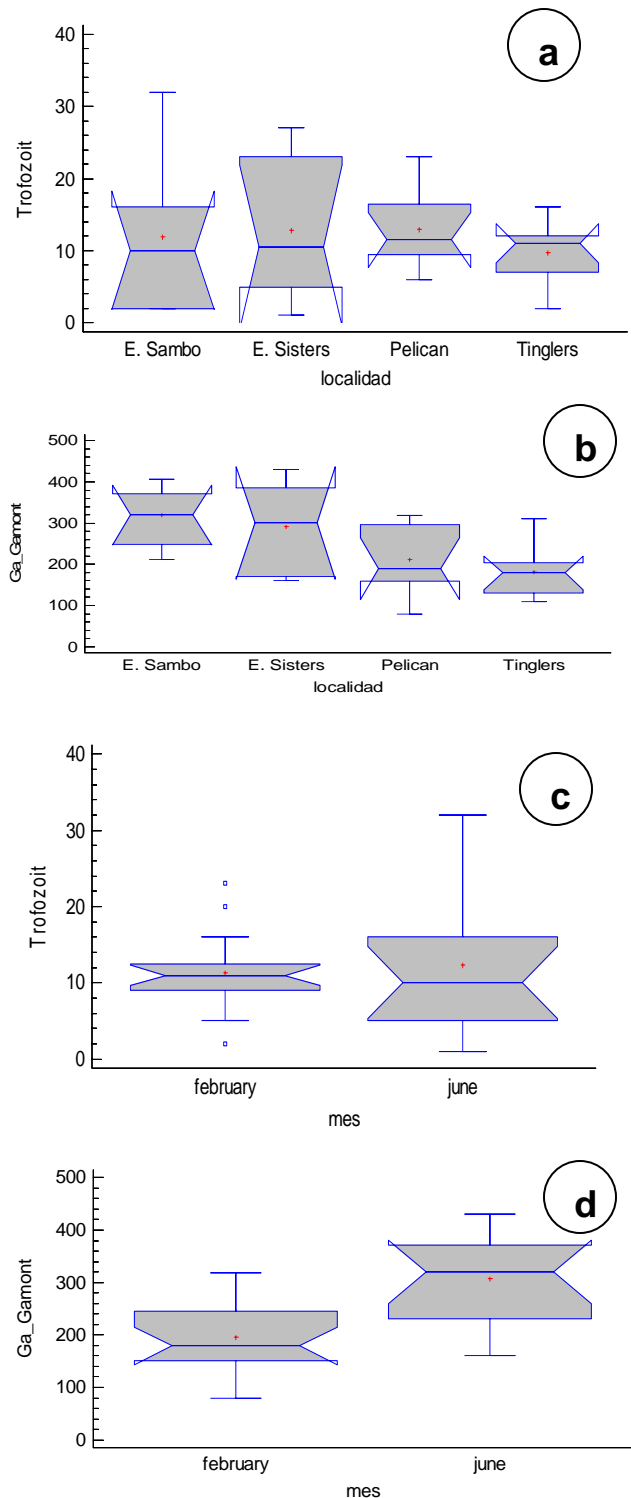


Figure 4. (a) Kruskal-Wallis test for occurrence of trophozoites by site; (b), Kruskal-Wallis analysis for gamonts and gametocysts by site; (c), Kruskal-Wallis analysis for occurrence of trophozoites and (d), Kruskal-Wallis analysis for occurrence of gamonts and gametozoites by months.

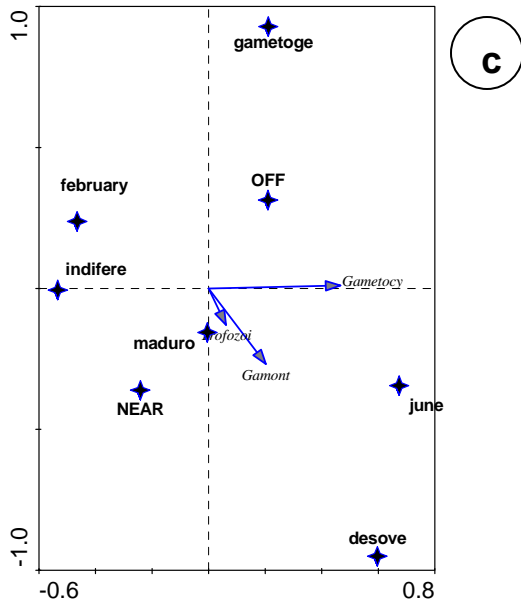
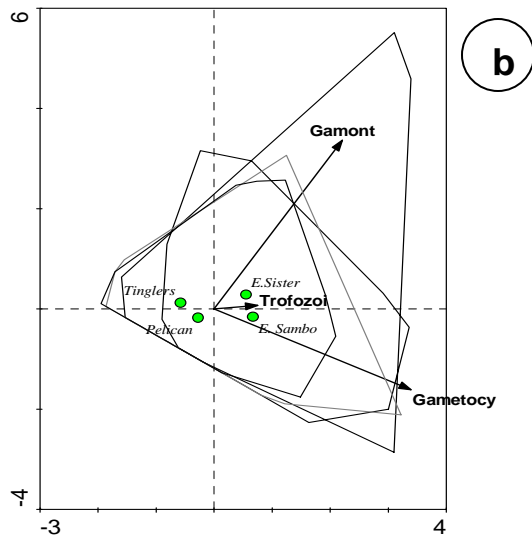
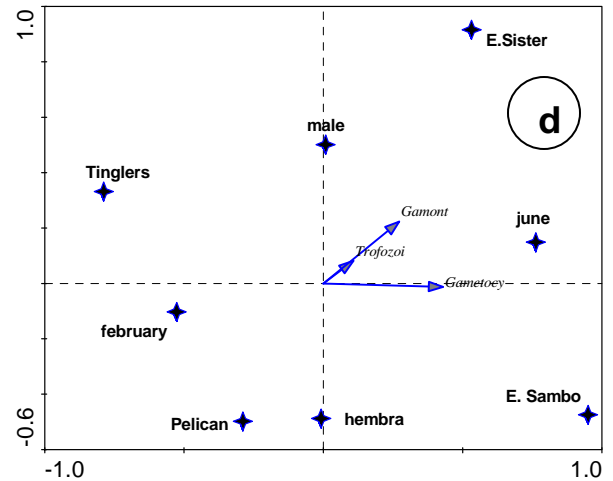
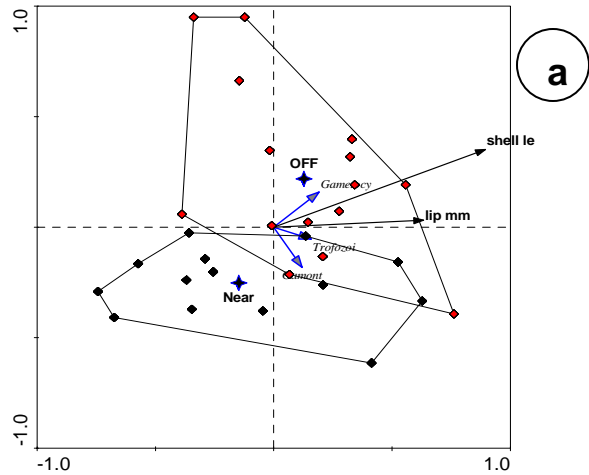


Figure 5. (a) RDA analysis, between shell length, lip thickness and stages of Apicomplexa. Test of significance of first canonical axis: eigenvalue = 0.025, F-ratio=9.116, P value =0.0020. (b), RDA analysis between sites and stages of Apicomplexa. Test of significance of first canonical axis: eigenvalue = 0.170, F-ratio = 71.690, P-value = 0.0020. (c), RDA analysis between months and stages of Apicomplexa (Test of significance of first canonical axis: eigenvalue = 0.200, F-ratio = 87.160, P-value = 0.0020). (d) RDA Analyse between stages of Apicomplexa, sites, months and sexes (Test of significance of first canonical axis: eigenvalue = 0.174; F-ratio = 73.560; P-value = 0.0020).

Figure 6 illustrates the difference of gamont and gametozoites was observed between sites (Test Tukey, $p < 0.05$).

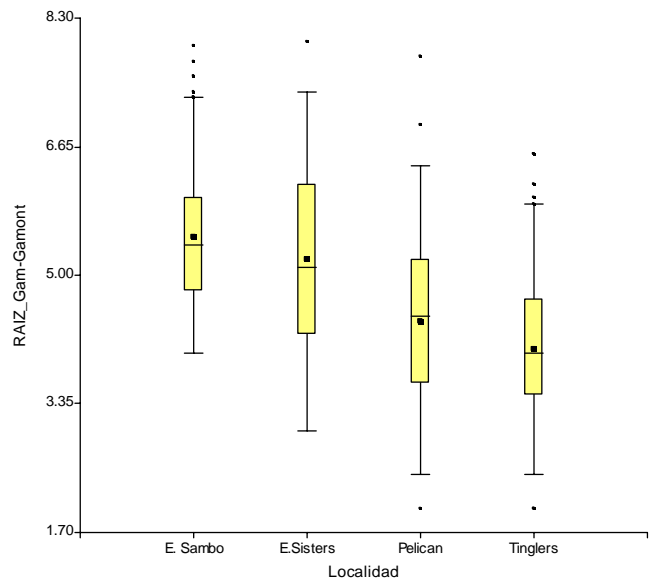


Figure 6. Anova analysis between sites and months (Test Tukey, $p < 0.05$).

Anova analyze showed an average of Apicomplexa between sites does not show a significant difference ($F_{0.05} = 0.3465$; $df = 1; 26$; $p = 0.3465$). Average of Apicomplexa between months (February and June) shows a significant difference in each site ($F_{0.05} = 12.960$; $df = 1; 26$; $p = 0.0013$) and an average of Apicomplexa does not show difference between sites in February and June ($F_{0.05} = 0.01$; $df = 1; 26$; $p = 0.9117$) (Figure 7).

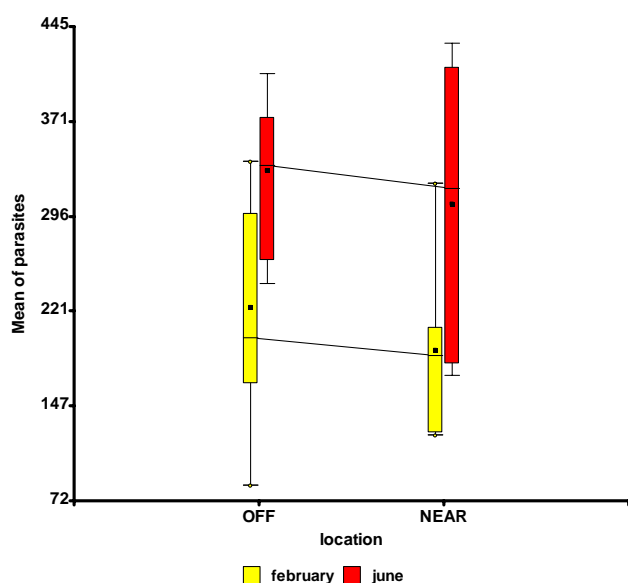


Figure 7. Anova analysis between sites does not show a significant difference ($F_{0.05} = 0.3465$; $df = 1; 26$; $p = 0.3465$). Average of Apicomplexa between months shows a significant difference ($F_{0.05} = 12.960$; $df = 1; 26$; $p = 0.0013$) and average of Apicomplexa does not show difference between sites in February and June ($F_{0.05} = 0.01$; $df = 1; 26$; $p = 0.9117$).

DISCUSSION

Apicomplexa parasites are of common occurrence in invertebrates and especially in Mollusks (Lester and Davis, 1981, Hillman *et al.* 1982, Perkins 1988, Azevedo and Padovan 2004, Duszynski *et al.* 2004). The number of different stages of Apicomplexa per field was an average of 32.3 parasites per field in the highest incidence of conchs sampled near shore (Sisters), in June and conchs sampled offshore (Sambo) in June. The lowest occurrence of Apicomplexa was observed in conchs sampled offshore (Pelican Shoal) in February and conchs sampled nearshore (Tingler) in February with 22.0. Aldana Aranda *et al.* (2007) in a study of geographic distribution of Apicomplexa around the Caribbean reported a highest incidence of parasites at Puerto Rico with 85 and Martinica with 77 parasites per field. They reported a lowest incidence at Chinchorro with nine parasites per 40x field. Abundance of Apicomplexa of conchs from Florida observed in this study corresponds to region of median incidence observed in Gulf of Honduras, Mexican Caribbean, and Campeche Bank.

Statistical analysis in this study suggests that the occurrence of Apicomplexa have a temporal effect than a spatial effect. The highest occurrence of Apicomplexa was observed in June. Although samples came from adult-conchs in both localities; percentage of gametogenesis and mature stages were always low for conchs sampled at near shore sites. Undifferentiated organisms were the dominant stage in near shore sites. Conchs sampled offshore in June showed a gametogenesis activity and a high number of parasites. However, Aldana Aranda *et al.* (2008) for conchs sampled at San Andres Colombia observed a negative correlation between the abundance of parasites and gonad cycle. These authors with a GLM analysis showed how gametogenesis, maturity, and spawning increases as the number of parasites decreases, while the number of undifferentiated organisms increases with increasing number of parasites for conchs at San Andres. They concluded that the occurrence of Apicomplexa could be a factor that affects the gametogenesis activity. In this study, we cannot establish a negative correlation between gonad activity and the occurrence of parasites. Therefore, it is necessary to investigate the relationship between the abundance of parasites and the reproductive cycle throughout the year with particular emphasis on environmental factors as temperature, food availability, and currents to determine which might influence gonad development and spawning periods, and to determine the putative impact of this parasite on conch reproduction. Therefore, it is necessary to investigate the relationship between the abundance of parasites and the reproductive cycle to successfully develop a restocking protocol for the queen conch, *Strombus gigas*.

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