Does the Age of the Queen Conch Female (*Lobatus gigas*) Affect Steroid Hormones and Fecundity?

¿La Edad de la Hembra del Caracol Rosado (*Lobatus gigas*) Afecta las Hormonas Eesteroides, y la Fecundidad?

¿Est-ce que l'âge de la reine conque femelle (*Lobatus gigas)* Affecter les hormones stéroïdes et la fécondité?

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ABSTRACT

The queen conch, *Lobatus gigas* occupies an important place in the fishing economy as it is the second most economically important fishery in the Caribbean, but this resource is over exploited. Thus, in the face of the scarcity of resources, it is important to put in place appropriate and effective management plans. From this perspective, it is necessary to understand its reproductive pattern, specially the fertility rate and its variations influenced by biological factors of the organism. The Goal of this study was to know the fertility rate and the age of queen conch females. The concentrations of estrogen, testosterone and progesterone were measured by high performance liquid chromatography and a UV detector. The three sex hormones were present in this species, and their concentrations increased with the age/size of the conch, being higher in the oldest organism. Old females showed the highest concentration of testosterone and Estrogen (1.8 ± 0.3 and 2.1 ± 0.4 ng/ml, respectively). Progesterone concentration in this category of females was, 1.2 ± 0.7 ng/ml). A positive correlation was observed between the fecundity rate and the older females that present a thicker lip of their shells. Pearson correlation between estrogen and spawning activity were r = 0.32 and for testosterone, r =0.70 and progesterone, r=0.64. This study constitutes the first use of a non-invasive method to analyze steroids hormones, showing that sex hormones may be controlling this species' reproductive events, as occurs in other gastropods. These results could support new measures for the sustainable management of the queen conch, prohibiting the fishing of deep-sea organisms.

KEY WORDS**:** Steroid Hormones, reproduction, fecundity, spawning, protection

INTRODUCTION

Reproduction in mammals and non-mammals is regulated by complex, overlapping regulatory circuits by gonadotropin -releasing hormone (GnRH), which indirectly activates production of steroids hormones. Sex steroids play an essential role in sexual differentiation and gamete maturation (Ogino et al. 2016, Vardanyan and Hruby 2016). Studies of these hormones traditionally employ tissue (Taves et al. 2010). However, tissues extraction can endanger the organism (Amaral 2010). An alternative, is to use a non-invasive techniques that involve collection of feces and urine (Sheriff et al. 2011; Busso and Ruiz 2011).

Sex or steroid hormones such as testosterone, estrogen, and progesterone as well as GnRH have been reported in at least three molluscan classes: Cephalopoda (D'aniello et al. 1996), Bivalvia (Gauthier-Clerc et al. 2006) and Gastropoda (Alon et al. 2007). Several authors have demonstrated that gastropods, cephalopods and bivalves are able to synthesize sex steroids through the activity of enzymes involved in biosynthesis using as precursors cholesterol or pregnenolone (Lafont and Mathieu 2007, Fernandes et al. 2011).

The queen conch, *Strombus gigas* occupies an important place in the fishing economy in the Caribbean, but this resource is over exploited. Scarcity of this resource require of appropriate and effective management plans, being necessary to understand the dynamics of population and its reproductive pattern (Aldana Aranda et al., 2005, Aldana Aranda, et al 2014). An important parameter to consider is the fertility rate and its variations influenced by environmental and biological factors of the organism. In particular, the relationship between the fecundity and the age of queen conch females. In fishes, fertility is directly related to the age of the species (Remiz-Llodora 2002). On the other hand, the oldest conch is found in deeper waters, between 30 and 50 m. Moreover, in some countries such as Dominican Republic, French West Indies, Martinique, Jamaica, Colombia, and Honduras conch of deep waters is authorized, mentioning that they are a non-fertile population. However, to date, no research has been conducted on the fertility of old females found in deep water. The objective of this study was to determine if older females are reproductive organism and to determine the influence of age on fecundity. Levels of the steroid hormones as testosterone, estradiol and progesterone in females of queen conch using a non-invasive technique were determined. These results could support new measures for the sustainable management of the queen conch, prohibiting the fishing of deep-sea organisms.

MATERIAL AND METHODS

Conch sampling. Adult queen conchs were collected in the Yucatan Peninsule, Mexico (20°18'N and 87°21'W), this is a reproduction site of conch reported by (Aldana Aranda et al. 2005). Specimens were collected by free diving. On the surface, organisms were sexed, observing the egg grove or the penis. Conch were not hurt or remove from the shell. Each conch was placed in plastic aquariums (80 l volume) filled with marine water. Organisms were left in the aquariums for 4 to

6 hours, during which feces from each animal was collected and placed on ice, then they were frozen at -20 °C until processing. After sample collection, all organisms were returned to their habitat.

A total of 100 females were marked and measured of shell length and lip thickness. Individuals were categorized in three categories of lip thickness: 6-10mm; 11-20 mm and 21-35mm. Spawning females were monitored. When the female separates from her egg mass it is considered that the spawning is over. Each egg mass spawned was weighed. For each egg mass, five replicates of 10 cm of the cord of eggs were sampled. Eggs cordon was placed an incubation to determine fecundity rate, counting eggs for each cordon. Egg quality was determined using diameter of eggs. Larval growth and calcification of conch were also used as indicators of the quality of eggs, of the different age classes of conch females.

Steroids hormones analysis. Testosterone, 17-β-estradiol, and progesterone (99.6% purity), HPLC-grade methanol and water (99.8% purity) were supplied by Sigma (St. Louis, MO, USA). The HPLC system consisted of a Dual Reciprocating Plunger Design (LC-20AT; Shimadzu, Japan), Autosampler (SIL-20A; Shimadzu, Japan), with temperature control (CCTO-20A; Shimadzu, Japan), a UV detector (SpD-20A; Shimadzu, Japan) and an Ultra C18 column (5 µm, 150 x 46 mm, Restek, Bellenfonte, PA, USA). The column and HPLC system were kept at 22 °C.

One gram of each sample was weighed out and mixed with 80% methanol (1 ml/0.1 g) (Palme et al. 2013). This mixture was stirred manually, allowed to settle and the liquid phase recovered and preserved at -20 °C. The stock solutions of estrogen, testosterone and progesterone were prepared in methanol and serially diluted. Stock solutions were used to record the flow rate, retention time and peak. The mobile phase was a mixture of methanol and water in different proportions for each hormone. For estrogen the mobile phase was 50% methanol and 50% water at 0.08 ml / min flow rate and analysis conditions was 230 nm. Testosterone mobile phase was 80% methanol / 20% water at 1 ml/min flow rate and sample analysis conditions was 245 nm. For progesterone the ratio was 70% methanol / 30% water at 1 ml/min flow rate and analysis conditions was 254 nm. The resulting chromatograms were analyzed with the Shimadzu LC Solutions software to calculate hormone concentrations.

Statistical analyses. The Mann-Whitney U/ Wilcoxon Rank test (Sokal 1995) was applied to assess differences for each hormone of females and males. Kruskal–Wallis test with peer-to-peer comparison was used to analyze hormone concentration of female per each size category and spawning activity. Pearson correlations were performed between hormone concentration and fecundity rate.

RESULTS

The average of shell length of females was 215±15 mm, with a minimum of 166 mm and a maximum of 235 mm and lip thickness variated from 6 to 31mm. A positive correlation was observed between the sizes of lip thickness and the number of egg masses spawned by females. The older females showed the higher fecundity and biomass of eggs spawned (Figures 1 and 2). Diameter of eggs of old females was larger than young conch. In conch of 6 - 10 mm of lip thickness, the average egg size was 200 ± 70 µm. Old conch, of 21 - 35mm of lip thickness showed an average egg size of 300 ±50 µm. The hatching rate was similar in both size categories of conch; old and young females with an average of 85-90%.

Sex hormones concentration exhibited differences depending on the size classes. Old females showed the highest concentration of testosterone and estrogen (1.8 ± 0.3 and 2.1 ± 0.4 ng/ml, respectively). Progesterone concentration in old females was 1.2 ± 0.7 ng/ml compared to 0.9 ng/ml present in young females (Figure 3). A positive correlation was observed between the fecundity rate and steroids hormone concentrations. Regarding the concentration of the three steroid hormones and the

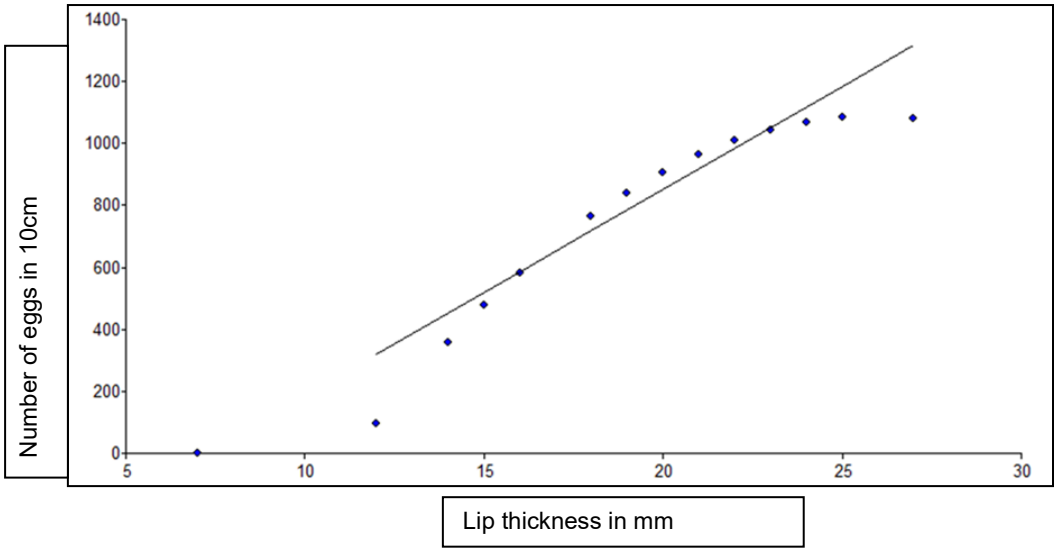

Figure 1. Fertility of the queen conch, *Lobatus gigas* in number of eggs versus of the thickness of the lip.

thickness of the lip, significant difference was observed (Figure 4). Pearson correlation between estrogen and fecundity rate were r = 0.64 for progesterone, r = 0.70 for testosterone and r = 0.32 for estrogen.

DISCUSSION

This study constitutes the first study to evaluate hormone steroids in female's old conch. We showed the presence of testosterone, estrogen, and progesterone in conch of various ages and their levels of hormones showed difference between ages/sizes of the females. It is also important to highlight the use of a non-invasive technique, which allows the analysis of hormones without the sacrifice of conch, using feces (Amaral 2010). Fecal progesterone and estrogen data have also been used for ovarian activity in captive female bottlenose dolphins. The knowledge of endocrine functions of steroids in mollusks is still fragmentary. However, results obtained so far from different mollusk strongly suggests the involvement of steroids in the control of reproduction in this species

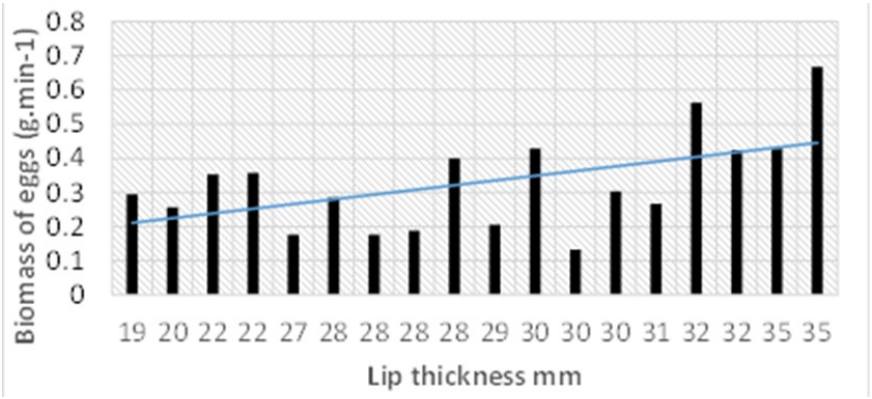

Figure 2. Biomass in grams of eggs spawned per minute by conch females presenting different lip thicknesses in mm.

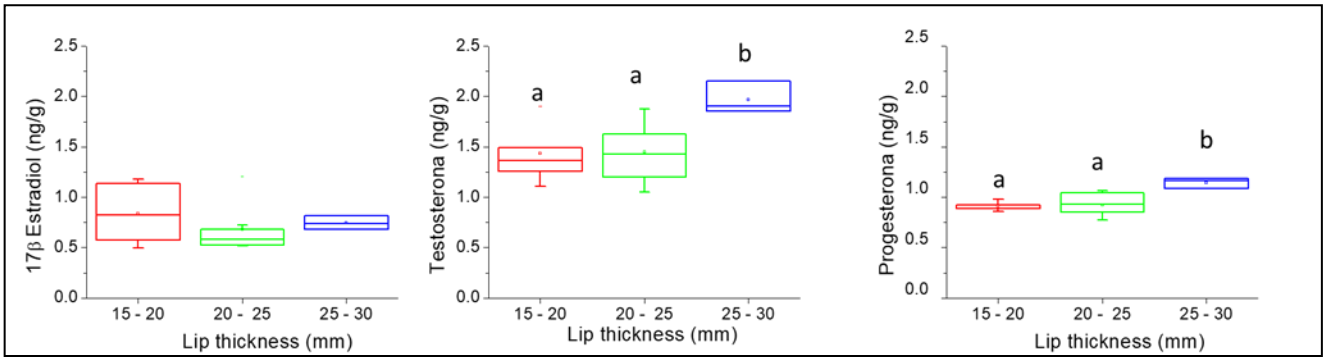

Figure 3. Fluctuations in mean concentration and standard deviation bars of the sex hormones, estrogen, progesterone and testosterone in females of the queen conch *Lobatus (Strombus) gigas* for females with various lip thickness. Different letter indicates Kruskal–Wallis statistic differences between lip thickness ($p < 0.05$).

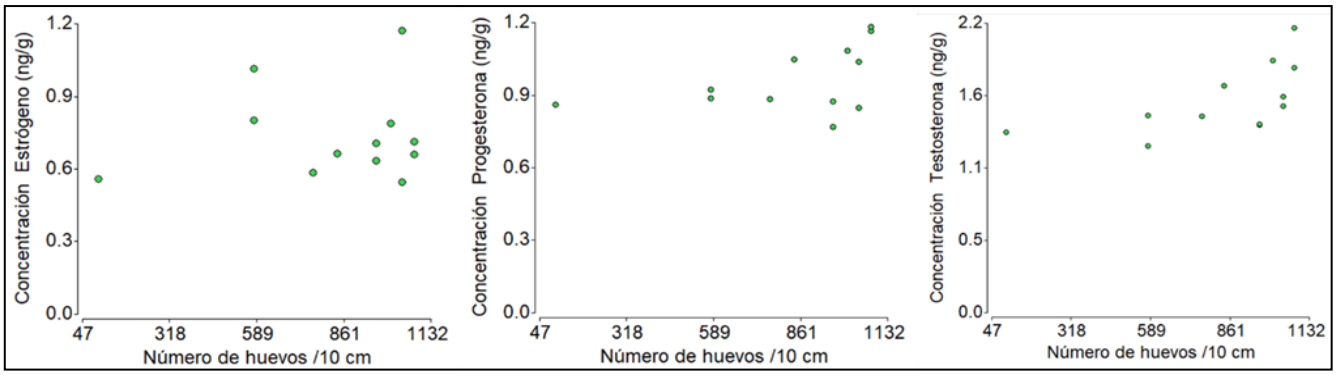

Figure 4. Pearson correlation of three steroid hormones and the fecundity showed a positive correlation. Values of correlation between estrogen and fecundity was r = 0.32, for progesterone, r = 0.64 and testosterone r = 0.70.

(Fernandes et al. 2011). Correlation between estradiol and mean oocyte diameters (r = 0.54) were performed in a clam, *Mya arenaria* (Gauthier-Clerc et al. 2006) and *S. constricta* (Yan et al. 2011). In this work, we found also a relationship between the highest presence of sex hormones concentrations in relation to oocyte diameter and fecundity rate, indicating that sex hormones in this organism are linked to reproductive processes. It was observed a correlation between the number of eggs per conch and the lip thickness. Wendell et al. (2003) observed that fecundity and size of the eggs was higher in the older females of mussels. In this study, the old females of conch are still fertile. The fecundity of the old females was greater than that of the young females. The hatching rate of old females was slightly higher than that of young females. Based in these original results, the old females in general placed in depth waters (30 - 60 m) are contributing to the recruitment of the conch population. For this reason, it is necessary to protect this population and not allow more the catch in deep waters with scuba or nets. It is possibly that this stock permit to conserve the conch population in the Caribbean.

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