Larval Organogenesis and Growth of *Strombus gigas* During the Reproductive Season in Alacranes Reef, Mexico

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ABSTRACT

Larval organogenesis and growth of *Strombus gigas,* from hatching to settlement was analyzed from March to September, corresponding to the reproductive season of *S. gigas* in Alacranes Reef. All cultures were maintaianed in four litre containers with three replicates and with the same methodology: density of 200 larvae per litre, feeding with *Tetraselmis suecica*, concentration of 1,000 algal cells per millilitre, temperature 28° ± 1°C, and natural photoperiod.

At hatching the larvae presented two velar lobes, 1.5 shell whorls and right tentacle. The adult heart appeared on the 7th day in the larvae from June, July, and August and by the 11th day in larvae from March, April, May, and September.

The proboscis appeared between 16 to 20 days in all cultures. The larvae from June, July, and August, reached the metamorphosis in 21 and 22 days, whilst that from the March, April, May, and September larvae reached metamorphosis at 22 and 24 days. Settlement was observed in 26 days (50 %) for larvae from June, July, and August *versus* 29 days (26 %) for larvae from March, April, May, and September.

Strombus gigas larvae, obtained an average shell growth to metamorphosis between 860 to 900 µm, the higher shell length was registered in June, July, and August, with a growth rate of 27 to 33 µm per day.

The growth of *S. gigas* larvae from hatching to settlement for June, July and August was much better from hatching to settlement, than in March, April, May, and September ($F = 4.01$, $p < 0.05$, Tukey < 0.01) .

KEY WORDS: *Strombus gigas*, organogenesis, growth, experimental cultures

Organogénesis y crecimiento larvarios de *Strombus gigas* durante la temporada reproductiva en el Arrecife Alacranes

La organogénesis y el crecimiento larvarios de *Strombus gigas,* de la eclosión al asentamiento, son analizados de marzo a septiembre, que corresponde a la época de reproducción de *S. gigas* en el Arrecife Alacranes. Todos los cultivos se realizaron en recipientes de cuatro litros con tres réplicas y siguiendo la misma metodología: densidad 200 larvas por litro, alimentación con *Tetraselmis suecica*, concentración 1000 células algales por mililitro, temperatura 28° ± 1°C y fotoperíodo natural.

Al momento de la eclosión las larvas presentaron dos lóbulos del velum, 1.5 vueltas de espira de la concha y tentáculo derecho. El corazón adulto apareció al día siete en las larvas de junio, julio y agosto y al día 11 en las de marzo, abril, mayo y septiembre. La aparición de la probóscis se observó entre los días 16 a 20 en todos los cultivos. Las larvas de junio, julio y agosto, alcanzaron la metamorfosis a los 21 y 22 días, mientras que las de marzo, abril, mayo y septiembre, lo lograron en 22 y 24 días. El asentamiento se observó al día 26 día (50 %) en las larvas de junio, julio y agosto, por el contrario las larvas de marzo, abril, mayo y septiembre tardaron 29 días en asentarse (26 %).

Las larvas de *S. gigas*, alcanzaron en promedio una longitud sifonal a la metamorfosis entre 860 y 900 µm, registrando las mayores tallas en junio, julio y agosto, con tasas de crecimiento de 27 a 33 µm por día.

El crecimiento de las larvas de *S. gigas*, de la eclosión al asentamiento, de acuerdo a la prueba estadística ($F = 4.01$, $p < 0.05$, Tukey < 0.01), fue más rápido en junio, julio y agosto, que en marzo, abril, mayo y septiembre.

PALABRAS CLAVES: *Strombus gigas*, organogénesis, crecimiento, cultivos experimentales

INTRODUCTION

The queen conch, *Strombus gigas*, is one of the most important benthic resources in the Caribbean region. It is one of six species of conchs distributed throughout the Caribbean inshore waters on sandy bottoms (Berg, 1976). Along the Yucatan Peninsula, Mexico, conches are an important economic resource to the inhabitants of the region. Total conch production reached in 1983 a maximum of 1,250 metric tons for the three states of Yucatan Peninsula, which generated a direct income of US$ 200,000 to the fishermen. At present, most conch stocks are thought to be fully or overexploited. For Yucatan, Mexico a permanent ban on conch catches was established in 1988 (Aldana Aranda et al. 2003). In response to declining stocks and intensive fishing for *Strombus gigas* in Mexico, various management strategies were reviewed and implemented for improvement of stocks.

Advances have been realized in order to understand the biology and mariculture potential of queen conch; these include the development of various management programs, with regulations ranging from complete closure of the fishery, to the use of size limits, closed seasons, harvest quotas, and gear restrictions (Appeldoorn and Rodríguez, 1994). Although not threatened with extinction, the queen conch has been placed on the World Wildlife Foundation Red Book as "commercially threatened" (Creswell, 1994).

In the present work are presented the results obtained on development and growth for *S. gigas* larvae reared in the Marine Laboratory of CINVESTAV IPN, during 1999.

MATERIALS AND METHODS

The egg masses were sampled from March to September, which includes the reproductive cycle of *Strombus gigas* in Alacranes Reef (Aldana Aranda et al. 2003). For the experiment three fertilized egg masses were taken each month, from under a spawning female conch to ensure species identity and to help estimate hatch date (Davis et al. 1987), from a depth of 4 m at Alacranes Reef, Yucatan (a 22°29' LN and 89°45' LW). These were transported individually in a closed thermic container with seawater to the laboratory, where sand particles were removed. Cleaned eggs were placed over a 300µm mesh and kept immersed in a 25 L tank with seawater filtered through 2 µm cotton filters and UV sterilized. For each egg mass, three experimental cultures were set up in 4 L containers with a density of 200 larvae/L. Larvae were fed with the algae *Tetraselmis suecica* at a concentration of 1,000 cells/ml. The experiments were run at 29° ± 1°C of seawater. The water was renewed totally every 24 hours. Thirty larvae were collected daily at random from each replicate for observation of development. Each morning veligers were transferred to new containers with fresh seawater filtered through 10 and 15 µm cotton filters.

Larval development was examined with these criteria: number of lobes and disappearance of velar lobes, number of shell whorls, presence of proboscis and radula, tentacles, adult heart, eyes stalks, swim-crawl behavior, and settlement (Brito-Manzano et al. 1999).

The sample size taken every two days for growth during the experiment was n = 30 from each replicate. Growth was assessed by recording increments of shell length. Larvae were measured using a compound microscope with a calibrated ocular micrometer to the nearest 0.10 µm. Growth rates were calculated according to García Santaella and Aldana Aranda (1994) as: average growth rate in µm/day = (average shell length at the end of the experiment - average shell length at the beginning) / total growth period in days.

ANOVA was used to determine if shell length were significantly different for veligers grown in different months. Cochran's test was used to test for homogeneity of variances. Tukey's multiple comparison tests of means was used to compare shell length data. The statistical program STATISTICA was used for the statistical analyses. Significance was assumed when $p < 0.05$.

RESULTS

Development

In Table 1 are presented the developmental characteristics for the larvae from hatching to settlement. Twenty-one development characteristics and their percentage are described, considering the moment of first appearance of one or several characteristics.

At hatching the larvae showed two different characteristics: two velar lobes and 1.5 shell whorls (for larvae of March, April, May, and September) or four velar lobes with two shell whorls (for June, July, and August larvae).

The adult heart appeared at 7th day in 54 % of the larvae from June to

August and on the 11th day in 38 % from March, April, May, and September larvae. By 13 days, began the migration of eyes up the tentacles for the larvae of all months. At 13 days the larvae from March, April, May, and September shows six velar lobes and three shell whorls, whilst the larvae of June, July and August never presented this characteristics. At 18 days in March, 15 % the larvae developed six velar lobes and four shell whorls; however the larvae from July presented 82 % of this characteristic. The proboscis appeared on the 17th day in 48 %, 53 %, and 40 %, respectively for June, July, and August larvae. However, for larvae of March, April, May, and September this characteristic appeared on the 19th day. Settlement was observed after 26 days in 50 % of the larvae of June, July, and August and after 29 days in 26 % for larvae of March, April, May, and September.

After 30 days the larvae of all months are competent for metamorphosis having velums with six lobes, shell with 4.5 whorls, fully developed adult heart, eyes on top of stalks, foot with a dark green pigmentation, proboscis, and radula fully developed.

Growth

Table 2 shows shell length at hatching, minimum and maximum shell length and mean shell length at metamorphosis, and growth rate. At hatching the larvae showed at shell length from 290μm to 300μm and average of 300 ± 6.17μm (n = 630 larvae).

Larvae showed an average growth rate between 22.33μm/day and 30.00μm/day and reached an average shell length at metamorphosis of 867μm-904μm, with an average of 883 ± 1.23 μm.

Figure 1 shows the growth for larvae of *S. gigas* cultured from March to September. Larvae from August and September showed the highest growth with 908μm. Statistical difference was observed between growth of larvae from April, May, and July. No differences were found for larval growth of March and June and for larvae grown in August and September.

Table 1. Developmental characteristics for *Strombus gigas* larvae from Alacranes Reef (n = 90 larvae for each month).

Developmental characteristics	March		April		May		June		July		August		September	
	Day	%	Day	%	Day	%	Day	%	Day	%	Day	%	Day	%
2.0 velar lobes, 1.5 shell whorls	1	100	1	100	1	100	----	---	----	---	----	---	1	100
2.0 velar lobes, 2.0 shell whorls	3	100	3	100	3	100	----	---	----	---	----	---	3	100
4 velar lobes, 2.0 shell whorls	5	50	4	54	4	59	1 a 3	100	1 a 3	100	1 a 3	100	5	48
4 velar lobes, 2.5 shell whorls	8	51	8 a 9	49	4	41	5	100	5	100	5	100	8	43
4 velar lobes, 3.0 shell whorls	9	52	9 a 10	57	9	55	7	100	7	100	7	100	9	57
4 velar lobes, 3.5 shell whorls	11	51	11	55	9	45	9	38	9	44	9	36	11	38
Appears adult heart	11	36	11	39	11	37	7	56	7	58	7	52	11	46
Appears left tentacle	11	20	11	21	11	37	11	39	11	39	11	31	11	22
6 velar lobes, 3.0 shell whorls	13	49	13	46	13	52	----	---	----	---	----	---	13	40
Migration of eye tentacle	13	18	12-13	22	12	25	13	37	13	39	13	35	13	25
6 velar lobes, 3.5 shell whorls	16	66	15-16	60	15	48	11	18	11	33	11	28	15	57
Mantle pigmentation	15	28	15	35	15	44	15	33	15	37	15	30	15	33
Foot pigmentation	15	28	15	30	17	35	15	31	15	35	15	30	18	28
6 velar lobes, 4.0 shell whorls	18	15	17	25	15	45	17	78	17	82	17	71	15	33
Proboscis appear	19	19	19	19	19	51	17	48	17	53	18	40	19	23
Outward migration of eyes	21	96	23	98	23	100	21	100	21	100	23	98	21	95
6 velar lobes, 4.5 shell whorls	23	33	23	31	23	35	21	39	21	49	21	37	23	80
Radule appear	23	31	20	19	21	29	25	43	21	100	21	41	23	35
Swim-crawl behaviour	27	44	27	42	27	48	25	48	25	57	27	33	27	37
Pigmentation dark-green of foot	27	49	27	39	27	45	25	45	25	54	27	38	27	27
Settlement	29	25	29	27	29	26	27	55	26	59	26	52	29	26

Table 2. Growth of shell length for the larvae of *Strombus gigas* in the cultures realized in 1999. Shell length at hatching, minimum, maximum, average and growth rate at 30 days.

	Shell length growth (µm)				
Months	**Hatching**	**Minimum**	**Average**	**Maximum**	**Growth rate (µm/day)**
March	290 ± 5	881 ± 1.05	883 ± 1.47	887 ± 1.31 [a]	22.33
April	300 ± 8	865 ± 1.16	867 ± 1.14	871 ± 1.75 [b]	24.00
May	300 ± 7	877 ± 4.17	881 ± 0.94	885 ± 2.10 [a]	26.66
June	300 ± 7	879 ± 2.10	882 ± 1.44	887 ± 6.11 [a]	27.33
July	300 ± 5	888 ± 2.31	894 ± 1.05	898 ± 5.11 [c]	30.00
August	300 ± 6	902 ± 1.44	904 ± 1.69	908 ± 1.69 [d]	24.66
Septem-ber	300 ± 5	898 ± 2.01	902 ± 1.57	908 ± 1.03 [d]	24.33

Average ± Standard deviation for three replicates
Value between column with different letter are statistical different (Tukey = 0.01).

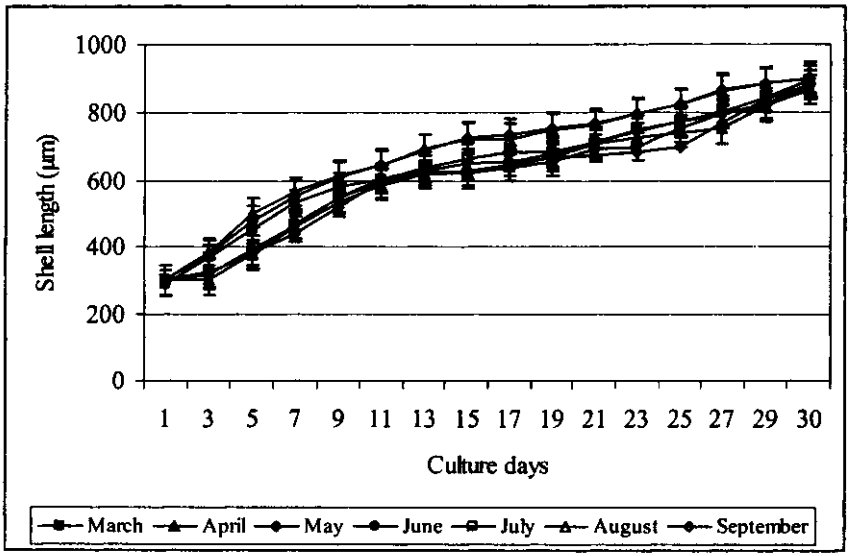

Figure 1. Shell length growth curves for all cultures of *Strombus gigas* larvae from Alacranes Reef

DISCUSSION

At hatching the larvae of March, April, May, and September had 1.5 shell whorls and two velar lobes, which is the same as *S. gigas* and *S. costatus* (Brownell 1977, Davis et al. 1993), whilst larvae from June to August presented four velar lobes and 2.0 whorls of the shell, characteristics observed by D'Asaro (1965), Brownell (1977), Davis et al. (1993) at four or five days.

Brownell (1977) reported that the proboscis appears as early as the 25th day in *S. gigas* and *S. costatus*, and begins to function in grazing on algae within two days. In this study, proboscis appears at the 17th day and begins to function within three days for the larvae of June, July, and August. D'Asaro (1965) and Brownell (1977) observed the metamorphosis between 28 to 33 days, Siddall (1983) in 20 to 25 days, Davis and Hesse (1983) in 28 days, whilst Corral and Ogawa (1985) obtained the metamorphosis between 25 to 60 days and Ray and Davis (1989), observed this characteristic bewtween 21 to 40 days. In this study, metamorphosis took from 27 to 30 days, with 30 % of larvae that had not settled in June.

Larvae development in this work demonstrates that larvae of March, April, May, and September are smaller than those obtained ones in June, July, and August.

In this study the average growth rate varied from 20.25µm/day to 21.53µm/day at a density of 200 larvae/L. Ballantine and Appeldoorn (1983) reported an optimal growth rate of 50-55µm/day, when the larval density was 100 larvae/L. Aldana Aranda and Torrentera (1987) reached a growth rate of

40μm/day at 200 larvae/L. Aldana Aranda et al. (1989) working at same larval density with *S. costatus* larvae obtained 16.7μm/day, Heyman et al. (1989) report 24μm/day at a larval density of 500 - 600 larvae/L. Davis et al. (1993) working at a larval density of 100 - 200 larvae/L report a growth rate of 39μm/day, Domínguez Tec (1993) reached 5 - 13μm/day at a larval density of 60 larvae/L, and García Santaella and Aldana Aranda (1994) obtained a growth rate of 24-37μm/day, when the larval density was 275 larvae/L. So, these results only could be compared using larval density of 60 to 600 larvae/L. The results obtained in this work demonstrate that the growth rate was 10μm/day below the reported average, but similar or even better, to the reported ones by the investigators of Mexico.

In this work the *S. gigas* larvae reached a mean shell length at metamorphosis between 867 to 902 μm. This value is similar to data reported in literature for other *Strombus* spp., except for the values reported by Brownell, which are above the average (2,200μm at metamorphosis). Ballantine (1981) reported 900 μm for *S. gigas*. García Santella and Aldana Aranda (1994) obtained a maximal shell length of 800 μm for *S. gigas* larvae. Larval growth varies according to the culture conditions and also to the author's criteria. Nevertheless, in this work, the best growths were obtained in August and September.

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