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Seasonal Variation in Growth and Survival of *Strombus canarium* (Linnaeus, 1758) Larvae

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Abstract: This study was conducted to analyze variation in *Strombus canarium* larvae development, growth and survivals when cultured during wet (main reproductive period) and dry seasons. Larvae were reared at 200 larvae L^{-1} in filtered seawater (0.22 μm) and fed with *Isochrysis galbana* at 1000 cells mL^{-1} *ad libitum*. The culture environment was maintained at 29±1°C, salinity of 30±1 PSU and photoperiod of 12:12 light dark cycle. Growth of the larvae was described on a length-at-age basis using the modified Gompertz regression. There was high correlation in shell length-at-age relationship for both wet season (R^2 = 0.99) and dry season (R^2 = 0.98) culture experiments. The maximal growth rate (M) and survival rate (S) were higher for larvae cultured during wet season (M = 62.44 μm day^{-1}, S = 14.36±2.31%), compared with dry season (M = 43.05 μm day^{-1}, S = 5±1.15%). The maximal attainable larval size (a) was however lower during wet season (950.19±66.93 μm shell length) compared with dry season (1343.05±586.51 μm shell length), which might be due to significantly low larvae density in the latter. Further studies are needed to investigate variation in bio-chemical composition of the egg mass, which was suggested as the main reason for the differences.

Key words: Mollusk, veliger, dog conch, larval growth rate

INTRODUCTION

The dog conch, *Strombus canarium* Linnaeus, 1758 is one of the most important benthic resources within the Johor Straits, Malaysia (Cob *et al*., 2008). It is an economically important species for the locals where the conch collecting activity has long been reported in the area (Poutiers, 1998). They are considered a delicacy and collected for their high quality meat, as well as for their shell that also has considerable ornamental value (Poutiers, 1998). The species is highly associated with sea grass beds (Cob *et al*., 2008, 2009a), which is very extensive within the sheltered coasts of the study area (Japar *et al*., 2006). *Strombus canarium* is an ecologically successful conch species within sea grass bed ecosystem where they were normally very abundant and usually formed one of the dominant taxa when present.

The study area, i.e., the Merambong shoal, is a traditional conch fishing ground in Malaysian waters. Unfortunately, it is located within a highly sensitive and strategic area. There is Port of Tanjung Pelepas of Malaysia in the North and Tuas Port of Singapore in the South and are subjected to heavy shipping traffics en-route the Malacca Straits. Many development activities already took place in the vicinity where large areas of sea grass have been reclaimed and there are planned for more reclamation (Japar *et al*., 2006). Replenishing the natural stock and transplanting to other area is one of the possible methods in conserving the conch fishery, which currently cannot be applied without proper understanding of the larviculture technique of the species.

The family Strombidae in general has widely been studied, particularly concerning the economically important species of the Caribbean such as *Strombus gigas*, *Strombus costatus* and *Strombus pugilus* (Brownell, 1977; Stoner, 1997; Aldana Aranda and Patiño Suárez, 1998; Aldana Aranda *et al*., 2001). Many aspects of their early life history e.g., feeds and physical

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requirements, development stages, growth rates, etc., have been documented and advances have been realized in mariculture potential of these species (Heyman *et al*., 1989; Rodriguez Gill, 1996; Stoner, 1997; Navarrete *et al*., 2007). In contrasts information regarding *S. canarium* larvae is very limited. Although, larval development stages of this species are studied (Cob *et al*., 2008). However, informations regarding growth and survival rates of this species are not reported yet. Such information is very important for aquaculture of the species. It has also been reported that the species can reproduce all year round, but major recruitment normally occurred at specific time of the year i.e., during wet season (Cob *et al*., 2008). Therefore, the objective of this study was to analyze *S. canarium* larval development, growth and survivals and to compare larval performance between wet and dry seasons.

MATERIALS AND METHODS

The larval culture experiments were conducted at two different time of the year i.e., within the main spawning season from November 2006 to March 2007 (wet season) and outside this main spawning season from May 2006 to September 2006 (dry season). Since, both experiments were conducted separately, variability in culture environment was kept at minimum by maintaining a strict culture protocol. For each experiment, 3 freshly laid egg masses (still underneath the females) were collected at Merambong sea grass bed, Johor Straits, Malaysia (01°19.778'N, 103°35.798'E). The egg masses were cleaned from debris and kept in separate containers filled with 0.22 µm filtered seawater, with salinity of 30±1 PSU, temperature of 29±1°C and moderate aeration. Salinity was measured using a refractometer while temperature within the incubator was measured using a thermometer. The culture medium was changed every 24 h with freshly filtered seawater until the larvae hatched.

After hatching actively swimming veligers were immediately siphoned off and filtered out using a 63 µm mesh size filter. Larvae from each egg mass were then transferred into 4 culture replicates (2-liter round bottom glass containers) with concentrations of 200 larvae L^{-1}. Three egg masses were collected for each experiment (dry and wet season), thus the total numbers of culture replicates were 12 for each season. Natural seawater filtered to 0.22 µm was used as culture medium, with salinity of 30±1 PSU and only very mild aeration. All culture vessels were kept in an incubator with temperature setting at 29±1°C and photoperiod of 12:12 h light-dark condition. Total seawater exchanges were performed every 2 days with freshly filtered (0.22 µm) natural seawater. To minimize variation in quality of feed, the larvae were only given single algae species *Isochrysis galbana* at 1000 cells mL^{-1} *ad libitum* throughout the culture period.

Observations on larval growth and development were performed daily. Few larvae were removed at random from each replicates and were examined under both dissecting and compound microscopes equipped with an image analysis system (MOTIC™). Measurements of larvae shell length (from the tip of shell spire to the end of siphonal canal) were made from the digital images using the image analysis system, to the nearest 0.01 µm. Stages of veliger larvae development was determined following criteria that previously been used by Brito Manzano *et al*. (2000), Brito Manzano and Aldana Aranda (2004) and Cob *et al*. (2009b), which includes: number of velar lobes; number of shell whorls; the development of proboscis, radula, tentacles and adult heart; development of eye stalk and migration of eyes up the tentacles; pigmentation; swim-crawl behavior and finally metamorphosis and settlement.

Growth rate of the larvae was described using the modified, four parameters Gompertz type model, which has previously been used to describe molluscan growth on a length-at-age basis (Devillers *et al*., 1998; Chicaro and Chicaro, 2000; Rodriquez *et al*., 2001; Harding, 2006). The length-at-age relationship was determined using the equation:

$$L = L_0 + a * \exp\left(-\exp\left(\frac{-(t - t_0)}{b}\right)\right)$$

Where:

- L_0 = Veliger shell length (µm) at hatch
- t = Time post-hatch or age in days
- t_0 = Time corresponding to the midpoint of the rise
- a = Maximum attainable larval size
- b = Rate constant

Maximum growth rate during the larval period was then calculated according to Harding (2006) as maximum growth rate (M) = 0.368 (a/b) µm day^{-1} where, a is the maximum attainable larval size, while b is a rate constant. Growth rates at age intervals were also estimated according to Harding (2006) using the formula: Growth rate at age (µm day^{-1}) = (average shell length at day_{n+1} - average shell length at day_n)/[(n+1) - n)], where, n is number of days between the two age intervals. Mean daily growth rate (µm day^{-1}) was calculated according to García Santaella and Aldana Aranda (1994) as: (average shell length at the end of experiment - average shell length at the beginning)/total growth period in days.

Data analysis: All data were checked for normality and homogeneity of variance. Larval developments were compared using one-way ANOVA with season (wet and dry) as factor. A Kruskal-Wallis nonparametric analysis was conducted for data that depart from the assumptions of normality and homogeneity of variance. Significant levels for all analyses were established at 0.05 probability levels. Statistical analysis were conducted using MINITAB® release 14.1 statistical software.

RESULTS

At hatching time the larvae only had 2 small velar lobes with smooth and rounded margin. The velar lobes were expanded and divided as larvae grow (Table 1). The larvae had 4-lobed velum around day 4 to 6 and 6-lobed velum around day 8 to day 12. The larvae generally spent between 18 to 24 days in the plankton before metamorphosed. Schematic diagram of a competent *S. canarium* larva is presented in Fig. 1.

Comparisons between veligers cultured during wet season and dry season found no significant difference during the early stage of larvae development (day 1 to 9) (ANOVA, $p>0.05$). However, there were significant differences detected in various development characters upon reaching metamorphic competence stage (Table 1). Larvae cultured during wet season grew faster where significant differences were detected in: the development of 6-lobed velum (ANOVA, $F = 16$, $p<0.05$); the pigmentation of the mantle (ANOVA, $F = 18$, $p<0.05$); the pigmentation of the foot (ANOVA, $F = 32$, $p<0.05$); the migration of eyes (ANOVA, $F = 128$, $p<0.05$); the development of swim-crawl behavior (ANOVA, $F = 196$, $p<0.05$) and the development of dark green pigmentation on the foot (ANOVA, $F = 45$, $p<0.05$). There was however no significant difference in terms of larval size (shell length). The larvae shell length at the time of hatching (ANOVA, $F = 0.79$, $p>0.05$) and at metamorphic competent stage (ANOVA, $F = 2.25$, $p>0.05$) were not significantly differed between the larvae cultured during wet season and dry season.

The shell length-at-age relationships determined using the modified Gompertz regression showed very high correlations between shell length and age for both culture experiments (wet season, $R^2 = 0.99$; dry season $R^2 = 0.98$) (Table 2). The estimated larval size at the time of hatching (L_0) for both larvae was quite similar, i.e., 224.83±25.09 µm shell length for wet season and 210.38±88.24 µm shell length for dry season larvae. However, the maximum attainable larval size (a) estimated for both larvae was markedly different, i.e., 950.19±66.93 µm for wet season and 1343.05±586.51 µm for dry season larvae.

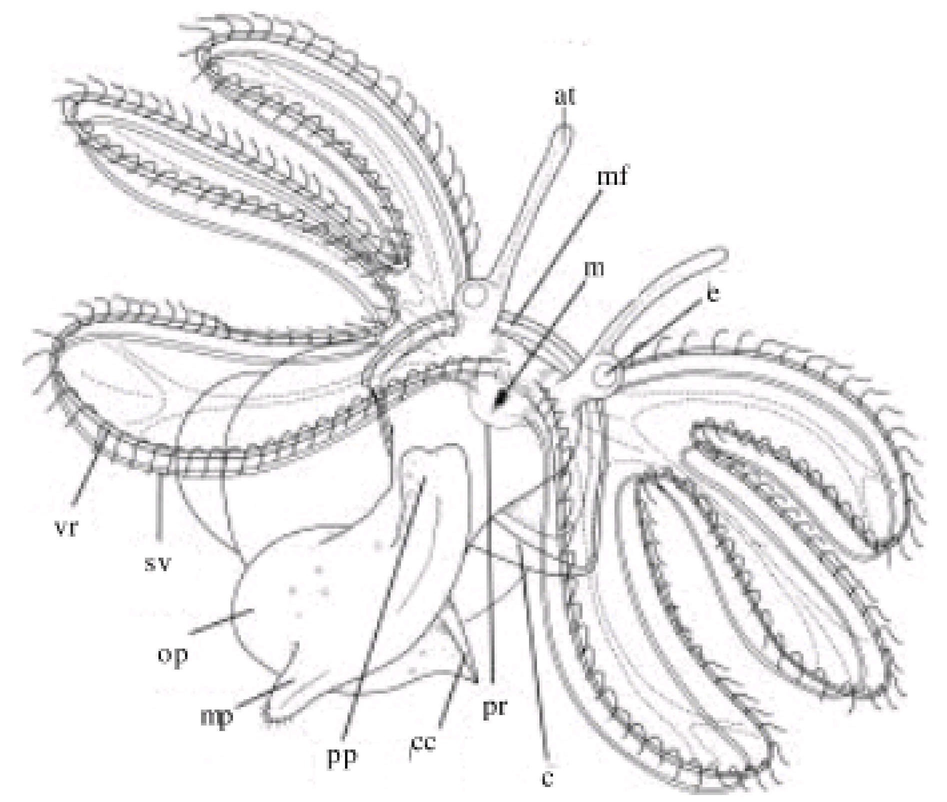

Fig. 1: Schematic diagram of a competent *Strombus canarium* larva. at: antennae, c: columella, cc: conchiolin claw, e: eye, mf: mantle fold, m: mouth, mp: metapodium, op: operculum, pp: propodium, pr: proboscis, sv: sub-velar ridge, vr: velar ridge. Scale bar: 100 µm

Table 1: Day to appearance of development characteristics of *Strombus canarium* larvae cultured during wet season and dry season

	Wet season	Dry season
Two velar lobes and 1.5 shell whorls	1	1
2 velar lobes and 2.0 shell whorls	2.34±0.34 (2-3)	3
4 velar lobes and 2.0 shell whorls	4.34±0.33 (4-5)	5.33±0.68 (4-6)
4 velar lobes and 3.0 shell whorls	5.67±0.33 (5-6)	7.33±0.67 (6-8)
Adult heart appears	5.67±0.33 (5-6)	-
Left tentacle stalks begin to form	6.33±0.33 (6-7)	8.00±0.58 (7-9)
6 velar lobes and 3.0 shell whorls	9.00±0.58 (8-10)*	11.67±0.33(11-12)*
Migrations of eyes up the tentacles stalks begin	9.00±0.58 (8-10)	-
6 velar lobes and 4.0 shell whorls	13.33±0.33 (13-14)	15.00±0.58(14-16)
Pigmentation of the mantle	11.33±0.33 (11-12)*	13.33±0.33(13-14)*
Pigmentation of the foot	10.68±0.33 (10-11)*	13.33±0.33(13-14)*
Proboscis appear	16.33±0.88 (15-18)	-
Radula appear	17.00±1.15 (15-19)	-
Eyes on top of stalks	17.33±0.33 (17-18)*	22.67±0.33(22-23)*
Swim crawl behavior	18.33±0.88 (17-20)*	23.00±0.58(22-24)*
Foot with dark green pigmentation	17.67±0.88 (16-19)*	23.67±0.33(23-24)*
Settlement	20.00±1.15 (18-22)*	0*

Values are Mean±SE with ranged in parentheses. Asterisks (*) denotes a significant difference when compared between months, at probability levels of 0.05, N = 12

Table 2: Gompertz regression coefficients to describe *Strombus canarium* larval growth using length-at-age data

	Gompertz equation parameters				
Seasons	L_0 (µm)	b	t_0 (day)	a (µm)	R^2
Wet	224.83±25.09	5.60±0.77	10.08±0.46	950.19±66.93	0.99
Dry	210.38±88.24	11.48±5.72	15.26±3.86	1343.05±586.51	0.98

Values are expressed as Mean±SE. a: Maximum attainable larval size (length, µm), b: Rate constant, L_0: Veliger size at hatch (length, µm), t_0: time corresponding to mid-point of the rise (day)

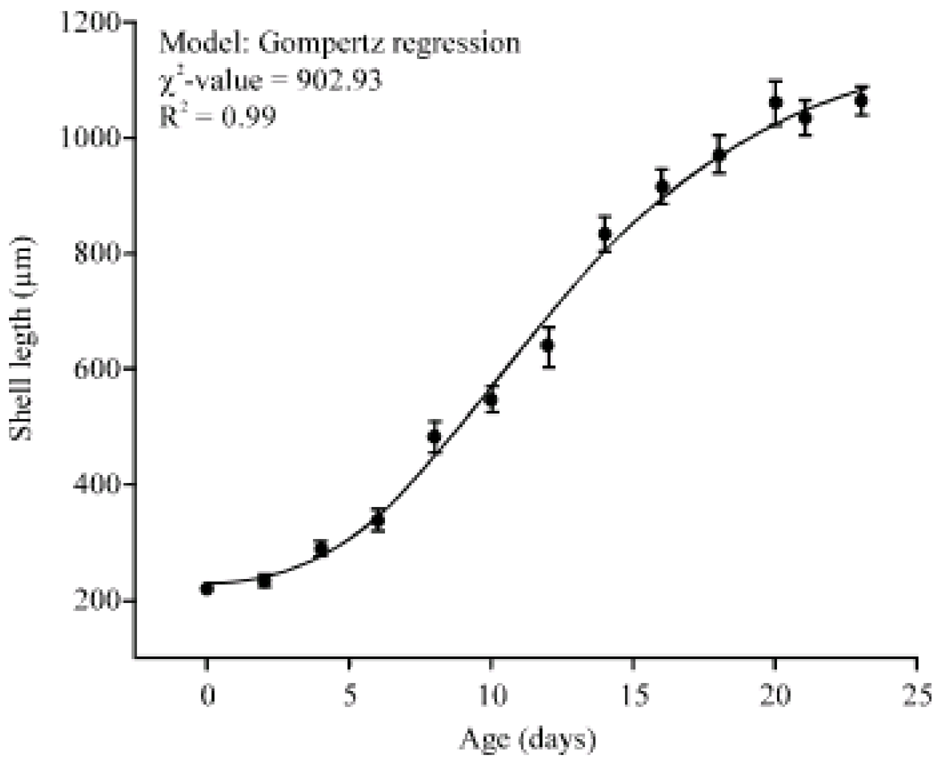

Fig. 2: Shell length at age relationship for *Strombus canarium* larvae cultured during wet season plotted using Gompertz model

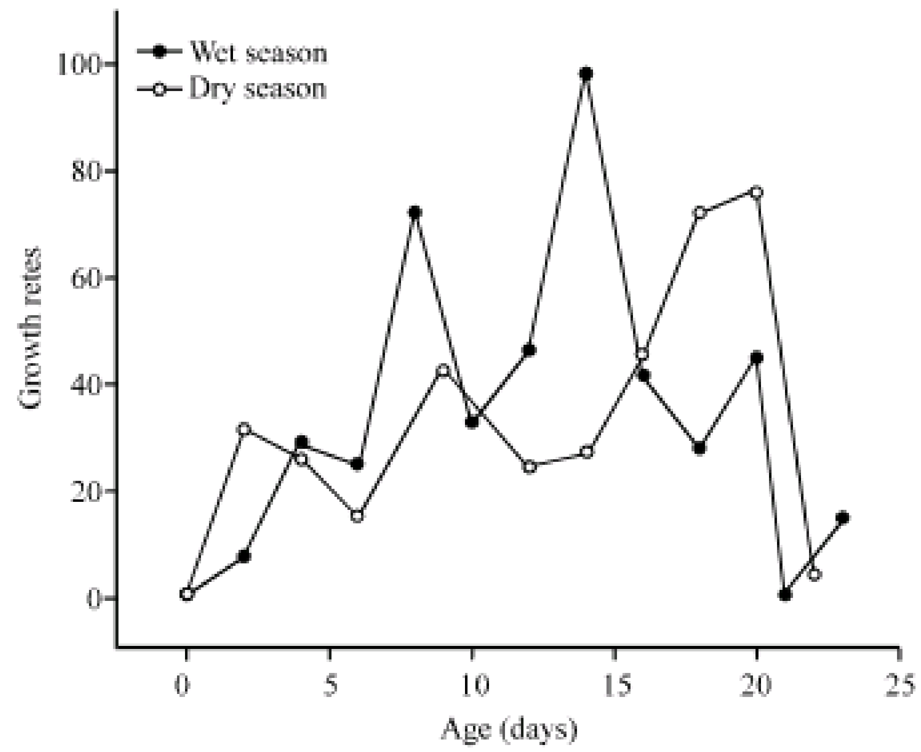

Fig. 4: Growth rate at specific age intervals for *S. canarium* larvae cultured during wet season and dry season

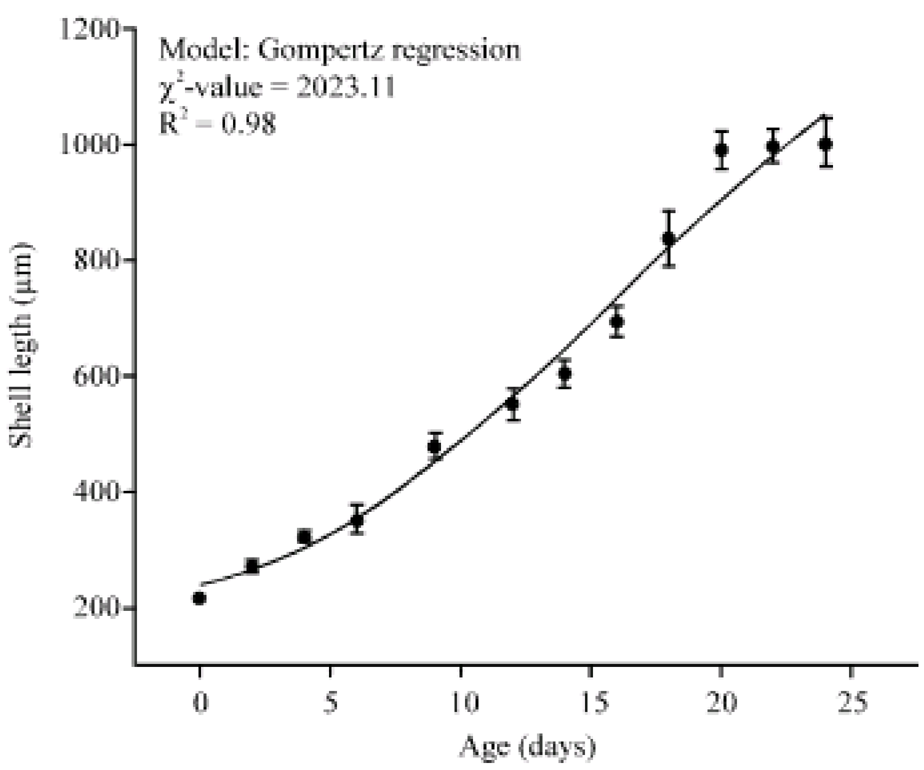

Fig. 3: Shell length at age relationship for *Strombus canarium* larvae cultured during dry season plotted using Gompertz model

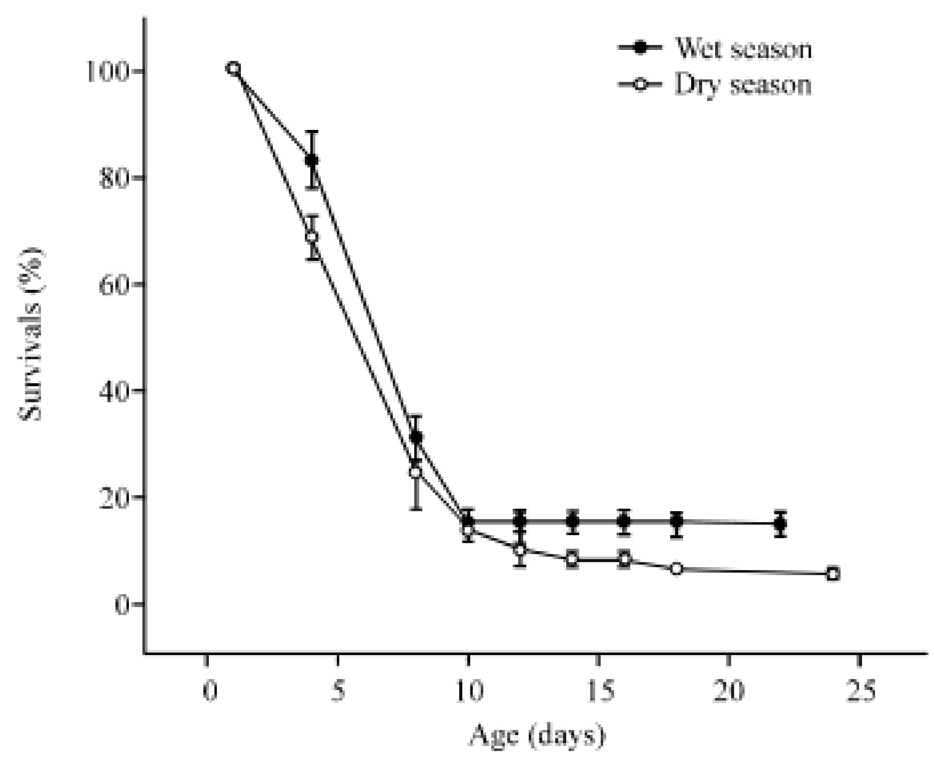

Fig. 5: Percent survivals of *Strombus canarium* larvae cultured during wet season and dry season

The shell length for larvae cultured during wet season increased more rapidly (Fig. 2) compared to the larvae cultured during dry season (Fig. 3). The latter took significantly longer time to reach the competent stage (Table 1) ($p<0.05$). The larvae from wet season showed higher growth rates between day 8 to 16, whereas, larvae from dry season recorded peak in growth rates at much later period (day 18 to 20) (Fig. 4). The Maximam growth rate (M) was estimated at 62.44 µm day^{-1} for larvae from wet season, which was much higher than the rate for larvae from dry season (43.05 µm day^{-1}). The Mean daily growth rates also showed much higher value for larvae from wet season (at 42.18 µm day^{-1}) compared with larvae from dry season (at 32.92 µm day^{-1}).

The Mean larval survivals at metamorphosis for larvae from wet season ranged from 7.5 to 29% (Mean 14.36±2.31%; N = 12), whilst for dry season larvae ranged from 3 to 7%, (mean 5±1.15%; N = 3). The percentage larval survivals throughout the experimental period for both experiments are presented in Fig. 5. Larvae from both seasons showed same decreasing trend where heavy mortality occurred during the early stage of larval development (the first 10 days), which correspond to stages prior to the development of 6-lobed velum. After this period (day 10 onwards) the mortality rates remained relatively more constant. Larvae cultured during wet season showed significantly higher survival rates (Kruskal-Wallis, H = 12.60, $p<0.05$), as well as better overall growth performance. Culture experiments conducted for larvae from dry season resulted in mass larval mortality (total collapsed) in most of the culture replicates (3 out of 12 culture replicates) and only a few larvae reached the metamorphic competent stage.

DISCUSSION

Strombus canarium larvae have 1.5 shell whorls and 2 velar lobes at the time of hatching, which is similar to other *Strombus* species (Brownell, 1977; Davis *et al*., 1993; Brito Manzano and Aldana Aranda, 2004). This characteristic appeared as common for the genus. However, larval size at hatching time recorded for *S. canarium* was slightly inferior compared with other *Strombus* larvae. Grana-Rafucci and Appeldorn (1997) reported an initial larval shell length of 300±6.17 µm, while Brito Manzano and Aldana Aranda (2004) reported initial larval size of 325±35.36 µm for *S. gigas*. Grana-Rafucci and Appeldoorn (1997) reported Mean initial larval size of 345±32.6 µm for *S. costatus*.

The Mean shell length of *S. canarium* larvae during metamorphosis was also inferior compared with other *Strombus* species. Reported values of *Strombus* veliger size (shell length) at metamorphic competent stage were about 1200 µm for *S. gigas* (D'Asaro, 1970; Brownell, 1977; Davis *et al*., 1993; Garcia Santaella and Aldana Aranda, 1994; Brito Manzano and Aldana Aranda, 2004); about 1270 µm for *S. costatus* (Aldana Aranda *et al*., 1989; Davis *et al*., 1993) and about 1150 µm for *S. pugilis* (Brito Manzano and Aldana Aranda, 1998; Brito Manzano *et al*., 2000).

This study showed that developmental characters previously used to describe growths in many *Strombus* larvae were also applicable to *S. canarium*. However, the rates of development of these characters were faster in *S. canarium* larvae (Table 3). For example the adult heart appeared as early as day 5; the left tentacle stalk starts to develop by day 6; the division of velum into 6-velar lobes starts by day 8 and metamorphosis and settlement as early as day 18. The metamorphosis and settlement stage reported for other *Strombus* species required longer time, ranged between 20 to a maximum 60 days for *S. gigas* (Brownell, 1977; Davis and Hesse, 1983; Corral and Ogawa, 1985; Ray and Davis, 1989; Brito Manzano and Aldana Aranda, 2004); between 29 and 32 days for *S. costatus* (Aldana Aranda *et al*., 1989; Davis *et al*., 1993); and between 27 and 31 days for *S. pugilis* (Brito Manzano and Aldana Aranda, 1998; Brito Manzano *et al*., 2000).

Earlier studies have demonstrated a wide variation in growth rates of *Strombus* larvae cultured in laboratory.

Table 3: Day to appearance of development characteristics of *Strombus canarium* larvae in comparisons with other *Strombus*

Development characters	*S. canarium*	*S. gigas**	*S. pugilis***
2 velar lobes and 1.5 shell whorls	1	1	1
2 velar lobes and 2.0 shell whorls	2	3	-
4 velar lobes and 2.0 shell whorls	4	4	5
4 velar lobes and 3.0 shell whorls	5	9	11
Adult heart appears	5	11	9
Left tentacle stalks begin to form	6	11	11
6 velar lobes and 3.0 shell whorls	8	13	15
Migrations of eyes up the tentacles	8	12	-
6 velar lobes and 4.0 shell whorls	13	15	15
Pigmentation of the mantle	11	15	19
Pigmentation of the foot	10	15	21
Proboscis appear	15	19	21
Radula appear	15	21	19
Eyes on top of stalks	17	21	-
Swim crawl behavior	17	27	-
Foot with dark green pigmentation	16	27	-
Settlement	18	29	27

*Brito-Manzano and Aldana-Aranda (2004), **Brito-Manzano *et al*. (2000)

Various factors e.g., larval density, temperature as well as the quality and quantity of food have an important influence on the survival and growth of the larvae (Aldana Aranda *et al*., 1989; Boidron-Metairon, 1990; Davis, 1994; Garcia Santaella and Aldana Aranda, 1994). The growth rates reported for *S. gigas* is between 5 µm to 93 µm day^{-1} (Brownell, 1977; Davis *et al*., 1993; Garcia Santaella and Aldana Aranda, 1994), between 17 to 111 µm day^{-1} for *S. costatus* (Brownell, 1977; Aldana Aranda *et al*., 1989; Davis *et al*., 1993) and between 23 to 104 µm day^{-1} for *S. pugilis* (Brownell, 1977). The growth rate of *S. canarium* larvae was well within the reported values for other *Strombus*.

The most vulnerable period in *S. canarium* larval development is during the early larval stage where high percentages of larvae died. Brownell (1977) also reported heavy mortality during the early period of *S. gigas*, *S. costatus* and *S. pugilus* larval development. Another critical stage is during the period of active feeding and rapid growth in preparation for metamorphosis, which started approximately by day 7 or 8 for the species studied. This period is actually corresponds to the development of larger and more elaborate velum to facilitate higher feeding rates and higher nutritional requirements in preparations for the metamorphosis and settlement. This is a very important phase as lack of nutritional reserves might delay metamorphosis, or might prevent larvae from metamorphosing successfully (Davis, 1994). Larvae that grow quickly in the plankton and reach competence quickly reduce their exposure to planktonic sources of mortality.

Results obtained in this study showed a seasonal phenomenon in *S. canarium* larval development. Since, the larvae were cultured in similar environmental condition it is suggested that the differences might be related to parental physiological condition as expressed in the egg mass quality. Although, the pattern of development was similar (as showed by the Gompertz plot), the rates at which each character developed was obviously different between the two seasons. This is in agreement with earlier study by Brito Manzano and Aldana Aranda (2004) on *S. gigas*, where they attributed it to the differences in biochemical compositions of the egg masses. Gastropod species that deposit eggs relied on energy reserves within the oocytes and in some cases on the organic substances dissolved in the capsular fluid bathing the embryos. The capsular fluid may contain amino acids, proteins and polysaccharides (Miloslavich, 1999). Even the inner capsule walls could also provide some extra nutrients to the embryos (Ojeda and Chaparo, 2004). Therefore, it might be interesting to further investigate the possible relationships among the seasonal quality of gametes and viability of culture of *S. canarium* larvae. In addition the optimum and minimum requirements still have to be determined to ascertain the length of time the larva could spend in the plankton prior to metamorphosis.

CONCLUSION

Strombus canarium larvae showed similar development characteristics compared with other *Strombus* species. However, there is difference in rate of kinetic development where the species studied showed faster development rates compared with reported data on other Strombacea. The results also indicated a probable seasonal phenomenon in larval development where larvae produced within the peak reproductive season showed better growth and survivals. Further studies are needed to investigate variation in bio-chemical composition of the egg mass, which is suggested as the main factors contributed to the differences in the larvae performances.

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