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Sclerochronology – a highly versatile tool for mariculture and reconstruction of life history traits of the queen conch, *Strombus gigas* (Gastropoda)

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Abstract – The queen conch, *Strombus gigas*, is an important fisheries resource in the Western Tropical Atlantic. In order to maintain harvesting success, improve fisheries management and contribute to mariculture pursuits, a detailed understanding of the life history traits of this species is required. Traditionally, this has been achieved by tedious and time-consuming long-term field observations. This study presents a highly versatile and rapid technique to estimate the timing and rate of shell growth based on sclerochronology. The Belizean *S. gigas* specimens (N = 2) from the offshore atoll, Glovers Reef, reached their final shell size (maximum shell height: 22.7 and 23.5 cm, respectively; completed formation of the flared lip) after only two years. However, seasonal growth rates varied considerably. Shells grew up to 6 mm d^{-1} during spring (April-June) and fall (September-November) but only 1 to 2 mm d^{-1}d uring July and August. Furthermore, shell growth ceased between December and March. Fastest shell growth occurred nearly contemporaneously with times of maximum precipitation which probably resulted in increased food availability. Slowest shell growth however, occurred during times of reduced rainfall and reduced riverine runoff, i.e. during times of reduced food supply. Sea-water temperature apparently did not exert a major control on shell growth. Notably, the slow winter growth was marked by a distinct purple-colored growth line in the cross-sectioned flared lip. Formation of a second major growth line (brown) fell together with the main reproduction period (late October/early November). Shell microgrowth patterns potentially represent daily or semidiurnal periods but cannot be used to assign exact calendar dates to each shell portion, because they were not visible across the entire cross-section of the whorl. Also, the protruding spines developed on the outer shell surface do not function as time gauges. The time represented by the shell portion between consecutive spines varies greatly from 1 to 72 days. Sclerochronology can potentially facilitate maricultural strategies and aid in site pre-testing and selection to grow *S. gigas*.

Key words: Queen conch / Shellfisheries management / Life history traits / Stable isotopes / Growth patterns

Résumé – Le strombe géant ou "lambi", *Strombus gigas*, est une ressource importante pêchée en Atlantique ouest tropical. Afin de maintenir le succès de cette récolte, en améliorer la gestion et contribuer aux développement de son élevage, il est nécessaire de comprendre le cycle de vie de cette espèce de façon précise. Traditionnellement, ceci a été effectué par des observations de terrain fastidieuses et prenant beaucoup de temps. Cette étude présente une technique rapide et d'une grande souplesse d'emploi pour estimer le moment et le taux de croissance de la coquille basée sur la sclérochronologie. Les spécimens (N = 2) de *S. gigas* du Belize (Amérique Centrale) récoltés au large de l'atoll du récif Glovers, atteignaient leur taille finale (hauteur maximum de la coquille : 22,7 et 23,5 cm, respectivement ; avec formation complète de la lèvre évasée) après seulement 2 ans. Cependant, les taux de croissance saisonnière varient considérablement. Les coquilles ont augmenté de 6 mm par jour durant le printemps (avril-juin) et l'automne (septembre-novembre) mais seulement de 1 à 2 mm par jour en juillet et août. De plus, la croissance de la coquille cesse entre décembre et mars. Les croissances les plus rapides se déroulent presque simultanément avec la période des maximums de précipitations qui résultent probablement de l'augmentation de la disponibilité en nourriture. Les croissances les plus lentes ont lieu lors de pluies réduites et de faibles déversements des rivières, c'est-à-dire lors de

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faibles apports en nourriture. La température de l'eau n'exerce pas, apparemment, de contrôle majeur sur la croissance de la coquille. Notablement, la lente croissance hivernale est marquée par une ligne distincte de croissance de couleur pourpre dans les sections transversales de la lèvre évasée. Une seconde ligne importante (brune) de croissance correspond avec la période principale de reproduction (fin octobre/début novembre). Les motifs de micro-croissance de la coquille représentent potentiellement les périodes journalières ou périodes semi-diurnes mais ne peut être utilisés pour déterminer des dates exactes calendaires à chacune des portions de coquille, car elles ne sont pas visibles sur l'ensemble de la section de la volute de la coquille. De même, les épines développées sur la surface externe ne fonctionnent pas comme des indicateurs de durée (temps). Le temps représenté par une portion de coquille entre des épines consécutives varie grandement entre 1 et 72 jours. La sclérochronologie peut potentiellement faciliter les stratégies aquacoles et aider dans la sélection de premiers sites d'élevage de *S. gigas*.

1 Introduction

For centuries, the queen conch, *Strombus gigas* Linnæus 1758, a large, marine gastropod, has been an important fisheries resource in the Western Tropical Atlantic. *S. gigas* is an herbivorous gastropod, which mainly preys on epiphytes covering seagrass detritus and macro-algae (Robertson 1961; Stoner and Waite 1991). Its modern biogeographic distribution stretches from the Florida Keys to Brazil including the Caribbean and Bermuda (Randall 1964). Over the last few decades, commercial overexploitation has led to significant stock reductions. As a result, the species was listed in the Appendix II of the Convention on International Trade and Endangered Species of Fauna and Flora (CITES) in 1992 (Theile 2005). In order to maintain harvesting success and to improve management strategies, great efforts have been made to better understand the life history traits (growth, reproduction etc.) of this species (Randall 1964; Berg 1976; Appeldoorn 1985, 1988; Stoner et al. 1996).

The vast majority of existing surveys on the life history traits of the queen conch have been based on long-term mark-and-recapture of specimens (e.g., Alcolado 1976; Weil and Laughlin 1984; Appeldoorn 1985). According to these data, during the first few years of life, shell length and volume increase significantly by the accretion of shell carbonate around a coiling axis. At an age of three to four years, however, there is a transition from the juvenile growth stage to the adult growth stage during which a wing-like lip – the so-called flared lip (Fig. 2) (Appeldoorn 1988) – is formed (Alcolado 1976; Berg 1976; Wefer and Killingley 1980). Estimated life spans for the queen conch vary between six (Berg 1976) and 26 years (Coulston et al. 1989). In particular, local habitat characteristics, food availability and temperature each exert a strong control on longevity and growth rate (Alcolado 1976; Martín-Mora et al. 1995; Stoner et al. 1996). Shell morphology and size-at-age may vary greatly among different localities (e.g., slim shell = fast growth; short, bulky shell = slow growth; Martín-Mora et al. 1995). The consequence of this is that life history traits based on long-term observations at one locality may not necessarily be applicable to other specimens. Furthermore, stock assessments based on mark-and-recapture experiments and shell biometry are very time-consuming and, therefore, inappropriate for maricultural purposes and for managing stock sizes of the queen conch (Stoner 1994).

An alternative approach that can be used to determine the life history traits of organisms and to identify the environmental forcings that influence or control growth is based on variable geochemical properties of accretionary biogenic hard parts (e.g., Wefer and Berger 1991; Schöne et al. 2007). The geochemical analysis of calcium carbonate ($CaCO_3$) powder samples of the shell taken along the growth axis can provide detailed, chronologically aligned information on changes in ambient environment and growth rates that occurred during the lifetime of an organism. For example, oxygen isotope ratios of shell carbonate ($\delta^{18}O_{shell}$) can reveal important insights into the variations of ambient water temperature during the growth of mollusks such as *S. gigas* (Epstein and Lowenstam 1953; Epstein et al. 1953). Based on the number of shell oxygen isotope-derived temperature cycles, Wefer and Killingley (1980) estimated the life span of the queen conch from Bermuda. Furthermore, variable shell growth rates can be identified by cross-dating the $\delta^{18}O_{shell}$-derived and instrumental temperature records. In contrast to long-term observations and stock assessments, the isotope geochemical method is very time-efficient, and specimens from different habitats can be rapidly analyzed. As yet however, the full capability of isotope geochemistry to improve the mariculture of *S. gigas* has been underestimated.

This study presents the first high-resolution analysis of the life history traits of the queen conch, *S. gigas*. Our study focuses on (1) the subseasonal timing and rate of shell growth during different life stages and (2) the main growth controlling factors. Moreover, (3) the shell sculpture, i.e. the number of spines on the external shell surface, is analyzed in order to determine whether it can be used to estimate the age of the shells. To do this we make use of the modern microanalytical and microsampling techniques typically employed in sclerochronology (the study of growth patterns and geochemical variations of biogenic hard parts; Buddemeier and Maragos 1974). The results of this study can help to improve maricultural practices and shellfisheries management.

2 Materials and methods

Three specimens of *Strombus gigas*, SgA1, SgA2 and SgA3, were collected alive from the windward back-reef sand apron of the isolated carbonate platform, Glovers Reef (16°46'51"N, 87°46'35"W, 2m water depth), 45 km east of mainland Belize, Central America on 12 March 2005 (Fig. 1). Shell height (Fig. 2a) measured between 22.7 (SgA1) and 23.5 cm (SgA2). The width of the flared lip (Figs. 2a,b, 4a) varied between 60 (SgA1) and 45 mm (SgA2). Soft parts were removed immediately after harvesting the snails. To do this, a hole was made on the penultimate whorl (Fig. 2a) and a knife was inserted in order to separate the soft body from the shell and to pull it out.

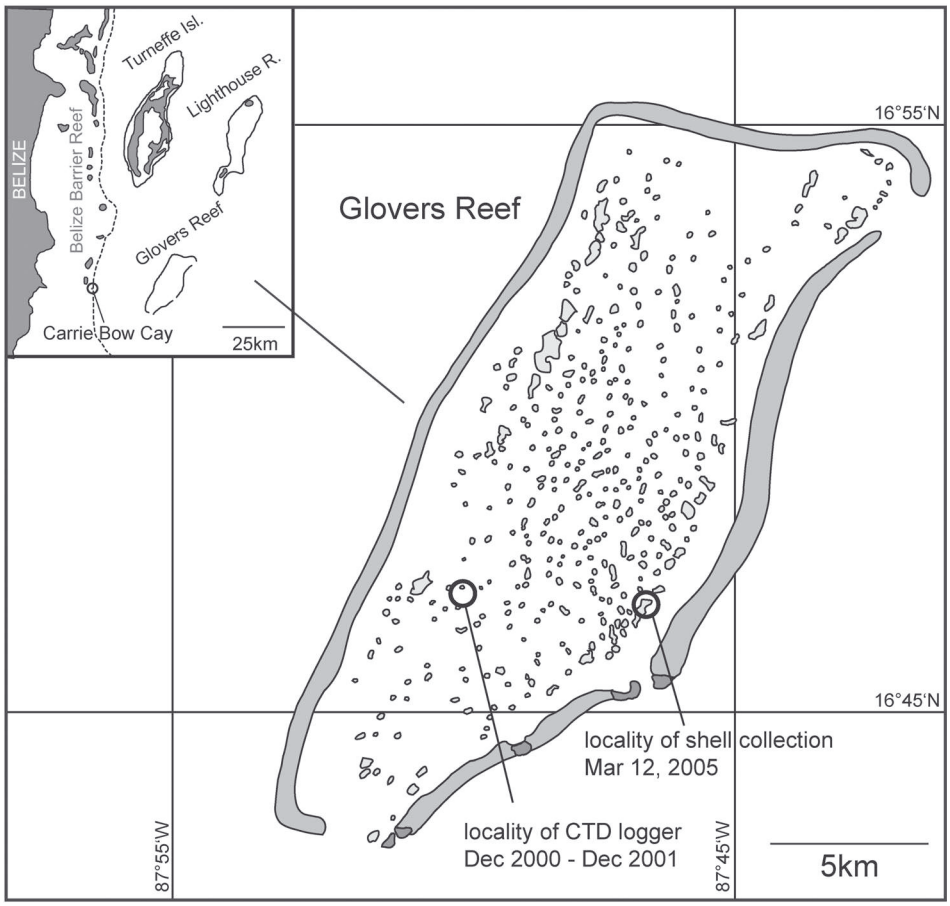

Fig. 1. Map showing the sample locality and CTD (conductivity, temperature, depth) logger site at Glovers Reef, Belize, Caribbean Sea. Also shown is the locality of the Smithsonian Field Station at Carrie Bow Cay.

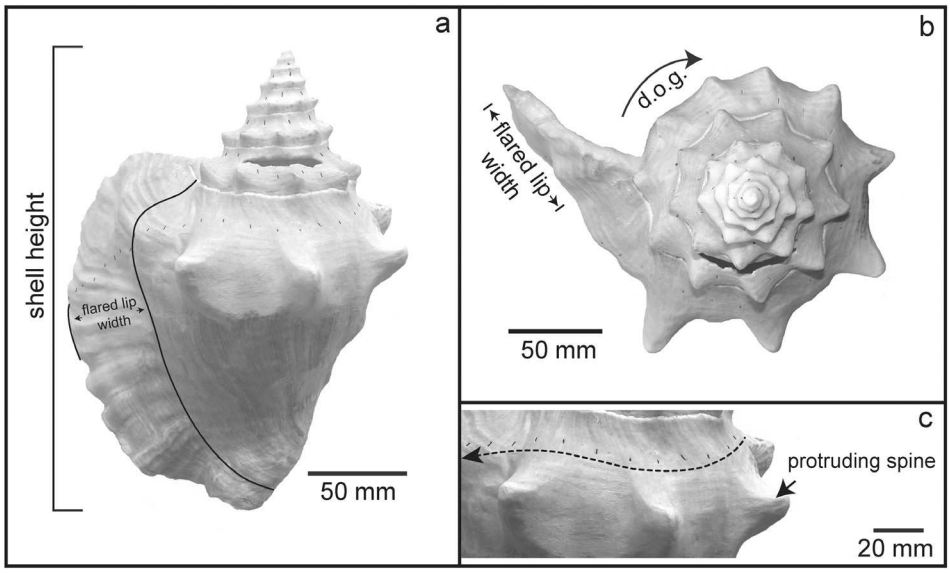

Fig. 2. *Strombus gigas*. Dorsal (a) and apical (b) view showing milling swaths on the outer shell surface of specimen SgA1. Samples of the main shell body (c) were taken from the shell portion between the spines and the whorl sutures. d.o.g. = direction of growth.

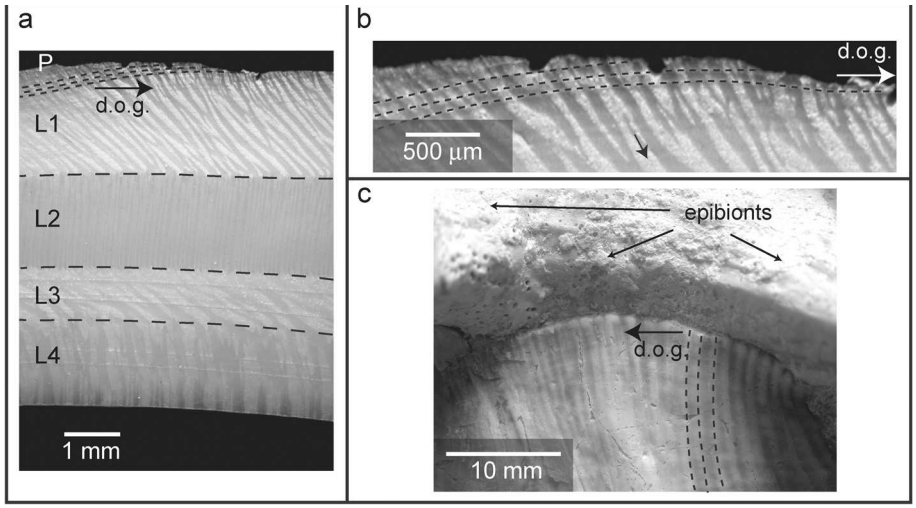

Fig. 3. *Strombus gigas*. (a) Within the main shell body four shell layers (L1 – L4) with crossed-lamellar fabric could be clearly distinguished underneath the periostracum (P). Within the outermost shell layer (L1) µm-scale growth patterns were developed. An enlargement of these growth patterns (dashed lines) is shown in (b). Arrow marks crystal fabric running perpendicular to the growth patterns. The growth lines approached the outer shell surface with a very shallow angle and formed macroscopically visible growth lines (dashed lines) there (c). These growth lines however were only visible on the last whorl. Earlier shell portions were overgrown by epibionts.

2.1 Sample locality

Glovers Reef (GLR) encompasses an area of 260 km^2, just 0.2% of which is land (Gischler and Lomando 2000) (Fig. 1). A slightly submerged oval-shaped reef crest encloses an interior lagoon with depths of up to 18 m. Approximately 860 patch reefs are scattered within the well-circulated lagoon (Fig. 1). These are composed of corals, *Montastraea annularis*, *Acropora* sp. and *Porites* sp. as well as various types of calcareous algae such as *Penicillus* sp., *Halimeda* sp., *Udotea* sp. and *Caulerpa* sp. (Gischler 2003). The sea floor is covered with patches of seagrass meadows, namely *Thalassia testudinum* and *Syringodium filiforme* (Gischler et al. 2003).

Geographically located within the trade belt, easterly winds strike the surface waters for most of the year. Northerly winds, however, are common in winter (Gischler 2003). The wave fronts also approach GLR from easterly directions, on average, from an orientation of 75° (Burke 1982). Tidal amplitudes measure ca. 30 cm (Stoddart 1962).

2.2 Shell growth of the queen conch and terminology

Juvenile shell growth refers to the formation of the main shell body (= spire + last [body] whorl) without the flared lip. The flared lip (= shell extension away from the main shell body) forms between the juvenile and adult life stages, i.e. the transitional stage. Thickening of the flared lip occurs during the adult life stage. The terms "juvenile" and "adult" as used here, merely describe shell morphological changes, since the exact point at which sexual maturity occurred was not assessed in the specimens examined.

2.3 External shell sculpture

The external shell surface of the queen conch is ornamented with protruding spines (Fig. 2). The distance between consecutive spines was measured with a digital caliper to the nearest 1 mm. Straight distances between the tip points of adjacent spines were measured.

2.4 Internal shell structures

Milling carbonate powder from the shell (Fig. 2) for stable isotope analysis requires a detailed knowledge of the number and the thicknesses of the different shell layers (Fig. 3a) and also of the orientation of the growth patterns within these layers (Fig. 3b). Therefore, a ca. 1 cm thick slab was cut from the last whorl of specimen SgA3 using a low-speed rock saw (similar to Fig. 4a). The slab was cut parallel to the whorls and perpendicular to the columella. The surface of this cross-section was ground on glass plates with 400, 800 and 1200 grit SiC powder and polished on a Buehler G-cloth with 1 µm Al_2O_3 powder. The section was then cleaned with ethyl alcohol, air-dried and immersed in Mutvei's solution for 25 min (Schöne et al. 2005). This agent helped to identify the internal growth structures and crystallographic fabrics when the shell cross-section was viewed under a reflected-light stereomicroscope.

2.5 Shell oxygen isotopes

Prior to sampling for stable carbon and oxygen isotope analysis ($\delta^{13}C_{shell}$, $\delta^{18}O_{shell}$), the periostracum and the outermost 100 µm of shell carbonate were physically removed from specimens SgA1 and SgA2 by means of a diamond-coated milling burr mounted to a dental drill device. X-ray diffraction analysis confirmed previous findings by Wefer and Killingley (1980) that the shell of this species, including the outermost shell layer, consists of aragonite.

Discrete sample swaths of shell powder were milled from the outer shell surface (= main shell body as well as across

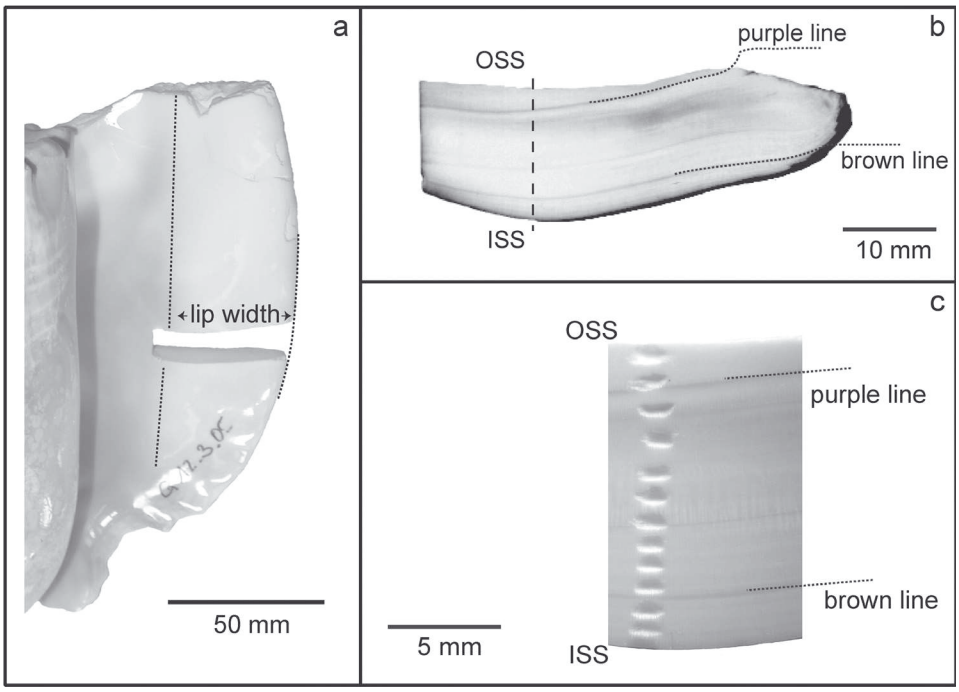

Fig. 4. *Strombus gigas* (SgA2). (a) In order to analyze the stable isotope ratios of the flared lip, a ~1 cm broad portion was cut from the shell aperture. (b) After grinding and polishing this cross-section revealed internal shell growth patterns running subparallel to the inner (ISS) and outer shell surface (OSS). Two distinct growth lines, a brown and a purple line, represent growth slowdown during winter (due to food scarcity in December–March) and during reproduction (in early to mid October). Milling swaths for stable isotope analyses are shown in (c).

the flared lip = juvenile and transitional life stages) in the direction of growth (d.o.g.) using a cylindrical diamond-coated milling bit with 1 mm diameter (Fig. 2). As such, we followed a sample trajectory from the apex and toward the margin of the flared lip (Fig. 2). Samples of the main shell body were taken from the shell portion between the spines and the whorl sutures (Fig. 2a–c). The entire spiral sample pathway amounted to ca. 700 mm. Each sample swath measured approximately 2 to 5 mm in length, 1 mm in width and less than ca. 500 µm in depth thereby assuring that only the outermost shell layer was sampled. The distance between the centers of adjacent sample swaths measured on average 9.8 ± 2.0 mm and 9.8 ± 1.6 mm (mean ± standard deviation) in SgA1 and SgA2 respectively.

The internal portion of the cross-sectioned flared lip (= adult life stage) of specimens SgA1 and SgA2 was sampled as follows. A cross-section of ca. 1 cm width was cut from the shell aperture of both specimens using a rock saw (Fig. 4a,b). These shell slabs were ground (400, 800 and 1200 SiC grit powder) and polished (1 µm Al_2O_3 powder). The samples were then ultrasonically rinsed in de-ionized water, air-dried and micromilled parallel to the outer shell surface following the internal growth patterns (Fig. 4b). The distance between the centers of individual sample swaths was less ca. 1 mm. In addition, one powder sample was obtained from the inner shell surface (i.e. the last formed shell portion prior to collection) of specimen SgA1 by superficial milling.

A total of 83 (juvenile: 63, transitional: 8, adult life stage: 12 samples) and 85 (juvenile: 68, transitional: 4, adult life stage: 13 samples) carbonate samples were milled from specimens SgA1 and SgA2 respectively (Fig. 6). Each powder sample weighed 73–107 µg and was processed in a Finnigan MAT 253 isotope ratio mass spectrometer coupled to an automated sampling device (GasBench II) following standard measurement protocols. The $\delta^{18}O_{shell}$ and $\delta^{13}C_{shell}$ values are reported relative to the Vienna Pee-Dee Belemnite (VPDB) standard based on NBS-19 calibrated Carrara marble values of –1.74‰ ($\delta^{18}O$) and +2.01‰ ($\delta^{13}C$). The average replicated precision error (1 σ = 1 SD) was better than ±0.06‰ ($\delta^{18}O$) and ±0.04‰ ($\delta^{13}C$).

The empirical paleotemperature equation for biogenic aragonite of Böhm et al. (2000) was used to calculate water temperatures $T(\delta^{18}O_{shell})$ from $\delta^{18}O_{shell}$ values, with 0.27‰ subtracted from the $\delta^{18}O_{SW}$ value (measured versus Vienna Standard Mean Ocean Water, VSMOW) to compare with VPDB (cf. Dettman et al. 1999).

$$T_{\delta^{18}O_{shell}}(^{\circ}C) = (19.7 \pm 0.6) - (4.34 \pm 0.24) \times \left[\delta^{18}O_{shell} - (\delta^{18}O_{SW} - 0.27)\right]. \quad (1)$$

Assuming no change in the oxygen isotope value of the ambient sea water ($\delta^{18}O_{sw}$), a shift in $\delta^{18}O_{shell}$ of 1‰ reflects a temperature change in the ambient water of 4.34 °C.

As seen from Eq. (1), computation of temperatures from $\delta^{18}O_{shell}$ values requires knowledge of the oxygen isotopic composition of the sea water ($\delta^{18}O_{sw}$). Because no water samples were taken at the collection locality where these gastropods lived, the $\delta^{18}O_{sw}$ values were reconstructed from instrumental salinity data (S) from GLR (see below) for the time

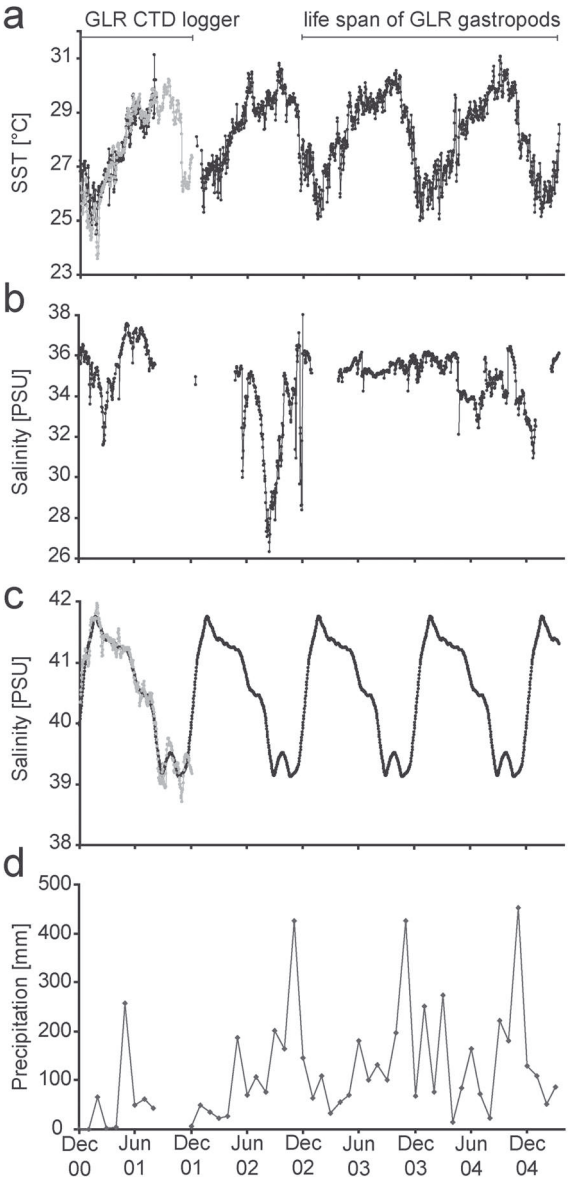

Fig. 5. Instrumental records. (a) Sea surface temperatures (SST) recorded at the Smithsonian Field Station at Carrie Bow Cay (black curve) and temperatures recorded by a CTD logger deployed at Glovers Reef (GLR, grey). Note good agreement between both curves. Because shells at Glovers Reef grew during 2002–2005, we used temperature data from Carrie Bow Cay. However, salinity data from Carrie Bow Cay (b) and Glovers Reef (c; grey = measured data, black = smoothed [center-weighted moving average, 31 days] curve) showed significant differences. Salinity at Carrie Bow Cay is therefore not representative of salinity at Glovers Reef. (d) Precipitation at Carrie Bow Cay shows a typical bimodal pattern with maximum rainfall during spring and fall.

interval of December 2000 to December 2001 using the linear S-$\delta^{18}O_{sw}$ relationship for the area (10°–30°N, 60°–90°W). The regression line between $\delta^{18}O_{sw}$ and S was derived from the NASA/GISST global seawater database (Schmidt et al. 1999):

$$\delta^{18}O_{SW}[‰_{VSMOW}] = 0.1S - 2.45. \quad (2)$$

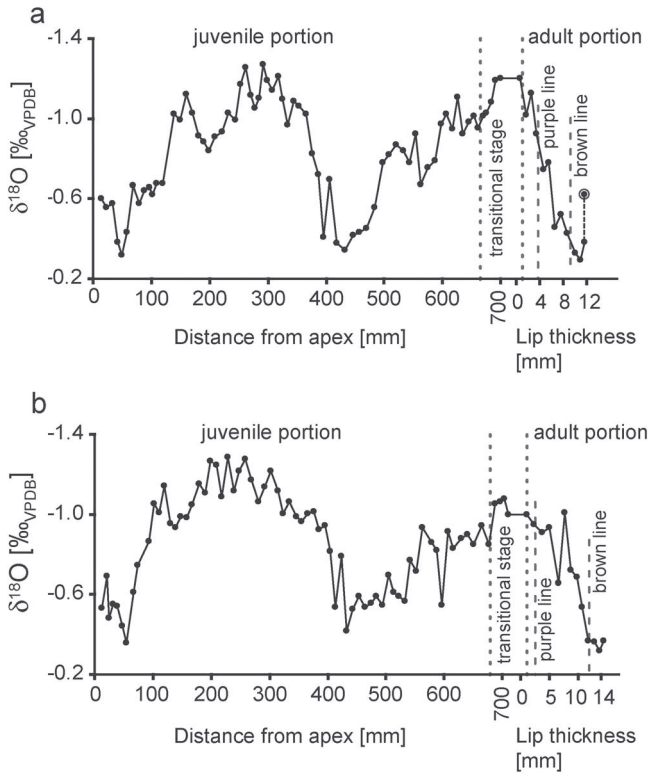

Fig. 6. Shell oxygen isotopes ($\delta^{18}O_{shell}$) profiles of *Strombus gigas* specimen SgA1 (a) and SgA2 (b). In both shells, more than two complete annual cycles were found. During the juvenile and transitional life stages (samples taken from the outer shell surface of the main shell body and across the surface of the flared lip) 1.75 annual cycles were found and 0.5 cycles during the adult life stage (cross-sectioned flared lip; see Fig. 4). Last sample (circled) of SgA1 curve comes from the inner shell surface.

2.6 Instrumental data

Water temperature and salinity were recorded from December 2000 to December 2001 at 3-hourly intervals by a CTD logger (R. Brancker, model XL210) deployed in ca. 10.5 m water depth and approx. 4 km WNW from the locality where the gastropods lived (16°47′04″N, 87°49′51″W; Gischler et al. 2003). These data were converted into daily averages (12:00 a.m. to 12:00 a.m.) (Fig. 5a–c). Daily water temperature at GLR varied between 23.6 °C in January and 30.2 °C in September. Salinity ranged from 38.7 PSU in October and 42.0 PSU in January reflecting changes of precipitation and evaporation (Hauser et al. 2007).

Unfortunately, the CTD record at GLR only covers the December 2000 to December 2001 time interval. As will be presented later (see Results section), the gastropod shells did not start growing before fall 2002. Therefore, additional instrumental data were obtained from the Smithsonian field station located at Carrie Bow Cay (CBC; 16°48′10″N, 88°04′55W, Figs. 1, 5a–c) from their webpage at http://cbc.riocean.com (last checked: 19 May 2009). The respective records recorded the environmental conditions at 10 min intervals (daily temperature and salinity were computed by arithmetically averaging the data from 12:00 to 12:00 a.m.) (Fig. 5a–c). Daily temperature data at CBC compared particularly well to that of GLR

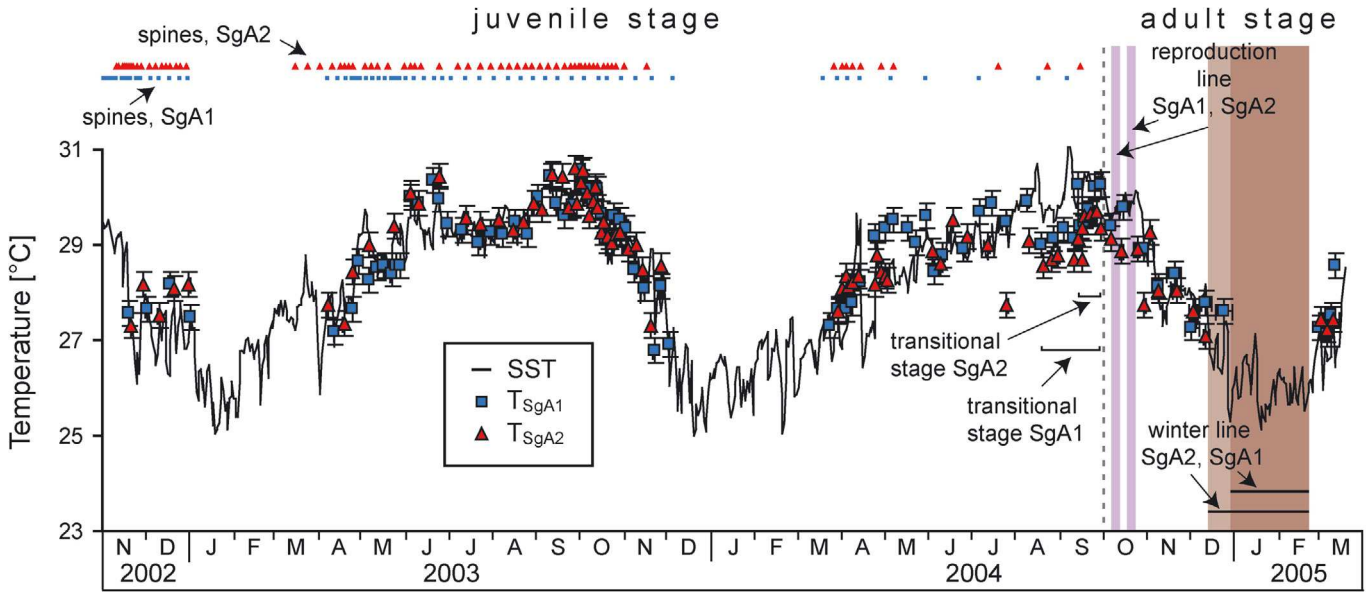

Fig. 7. *Strombus gigas*. Temporally aligned, shell oxygen isotope derived temperatures (blue squares = SgA1; red triangles = SgA2) and instrumental water temperature (black curve). Vertical bars represent analytical precision error. Small triangles at the top represent position of spines. Note that the time between two consecutive spines differs greatly and that spines were not formed contemporaneously in both specimens.

(R^2 = 0.92; p < 0.001; Fig. 5a) when shifted by +2 days. The good correlation between the two data sets implies a high connectivity of the SST in the area (Ezer et al. 2005; Tang et al. 2006). Thus, the CBC station provides a reliable temperature record for GLR.

Contemporaneous salinity records from CBC and GLR, however, differed considerably from each other (Fig. 5b,c). The strong salinity fluctuations at CBC (Fig. 5c) can probably be attributed to riverine freshwater influx from the adjacent land or to a temporal malfunctioning of the CTD logger. Extreme precipitation events that could have caused the negative PSU excursions can be excluded, because no tropical storms or hurricanes passed over Belize during the second half of 2002. Hence, salinity records from CBC cannot be used to infer salinity at GLR. Estimates of the $\delta^{18}O_{sw}$ values for 2002-2005 were therefore based on the available 12-month GLR salinity record (Fig. 5c). High-frequency oscillations were removed from this record by applying a 31-day moving average to the data. Our assumption that inter-annual salinity changes remained small is supported by the offshore position of GLR.

Annual rainfall in the atoll (Fig. 5d) amounts to ca. 1750 mm y^{-1} (Stoddart 1962) with the highest amounts falling between April and November, especially in June and September/October (Magaña et al. 1999), and lowest rates falling during winter (Gischler et al. 2003). An intermittent phase of markedly reduced rainfall – the so-called 'mid summer drought' – can occur in July and August.

3 Results

3.1 Internal shell growth patterns and crystal fabrics

Within the main shell body (shell portion in contact with the ambient water), four shell layers (all are of crossed-lamellar fabric) could be clearly distinguished underneath the periostracum (Fig. 3a). The outermost shell layer was between 0.8 and 2 mm thick. In this layer (L1; primary shell layer = formation during shell expansion), faint micrometer-scale (few tenths of μm) growth lines could be identified in some shell portions. These growth lines approached the outer shell surface with a very shallow angle (Fig. 3b). Here, they formed macroscopically visible growth lines running perpendicular to the growing direction. However, these growth patterns were only vaguely discernible and were overgrown by epibionts on all but the last whorl of the shell (Fig. 3c). The remaining three shell layers (L2-L4 = secondary shell layers) were precipitated at the time when the flared lip formed and represent shell thickening instead of shell expansion (L1).

The flared lip consisted only of one shell layer (crossed-lamellar fabric) with growth patterns running subparallel to its surface. Two distinct growth lines – slightly different in color – were found near the inner and outer shell surfaces (Fig. 4b,c). The growth line near the outer shell surface was purple, whereas the other line was brown.

3.2 Shell stable isotopes

The $\delta^{18}O_{shell}$ curves produced from the two *Strombus gigas* shells compared well to each other (Fig. 6). Both shells showed average $\delta^{18}O_{shell}$ values of –0.83‰ and similar extreme $\delta^{18}O_{shell}$ values (Table 1). Near the apex, $\delta^{18}O_{shell}$ values of ca. –0.6‰ were measured (Fig. 6). The $\delta^{18}O_{shell}$ time-series representing juvenile and transitional life stages revealed approximately 1.75 seasonal oscillations, whereas only 0.5 seasonal cycles were found in the cross-sectioned flared lip (adult growth; Fig. 6). Temperature reconstructed from the $\delta^{18}O_{shell}$ value as determined on the last formed shell portion (inner shell surface) prior to collection (–0.62‰ in SgA1; Fig. 6) closely resembled the instrumental temperature on 12 March 2005 (Fig. 7). The last powder sample of specimen SgA2

Table 1. Summary of stable isotope values determined in shells of *Strombus gigas*. All values reported in ‰ (VPDB standard).

Specimen ID	Average $\delta^{18}O_{shell}$	Max $\delta^{18}O_{shell}$	Min $\delta^{18}O_{shell}$	Amplitude	Average $\delta^{13}C_{shell}$	Max $\delta^{13}C_{shell}$	Min $\delta^{13}C_{shell}$	Amplitude
SgA1	–0.83	–0.29	–1.27	0.95	–1.05	–0.06	–1.93	1.87
SgA2	–0.83	–0.32	–1.28	0.92	–1.22	0.47	–2.28	2.75

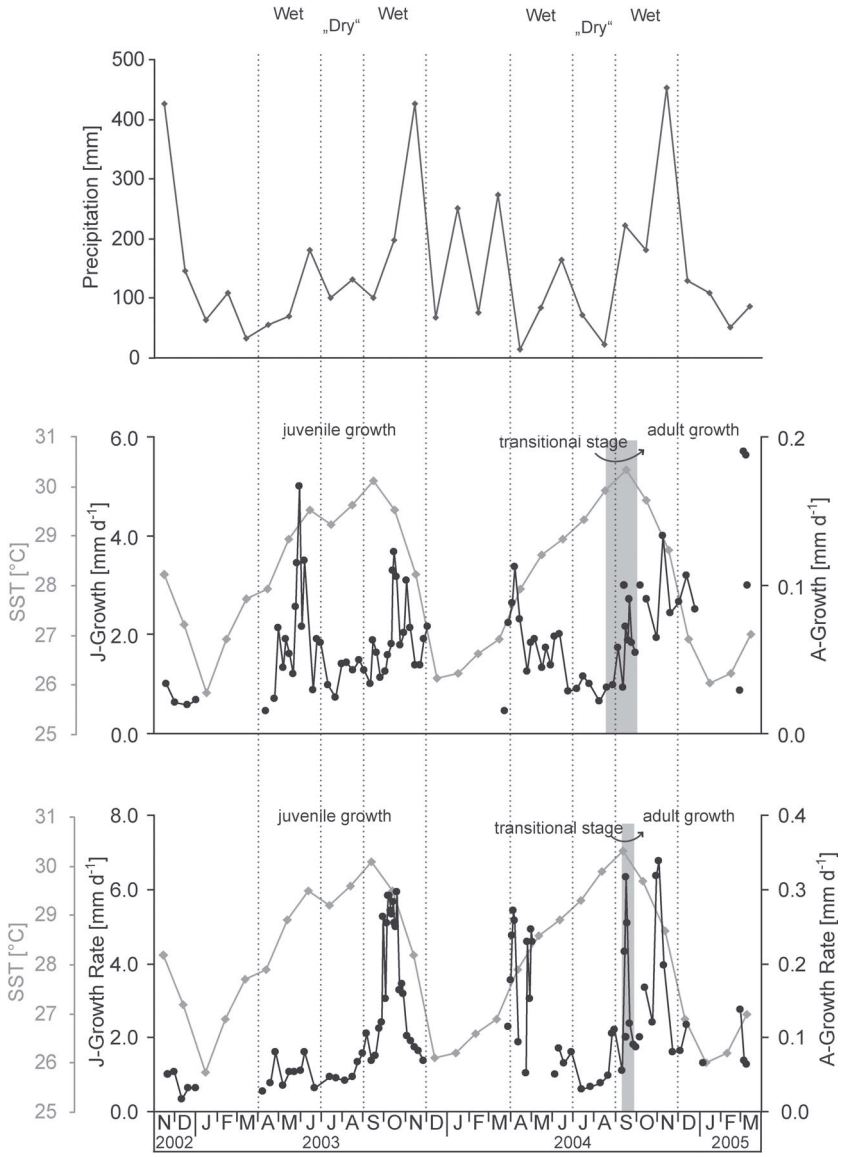

Fig. 8. Daily shell growth rate of *Strombus gigas* (black curves in lower [SgA1] and middle [SgA2] panel) is unrelated to (monthly) water temperature (grey curve; monthly averages were depicted for clearness), but positively correlated to precipitation (CBC meteorological record) and sea-level pressure (upper panel). Rainfall and shell growth show the same bimodal pattern with maximum values during spring and fall. J-Growth, A-Growth = Growth during juvenile and adult life stages.

($\delta^{18}O_{shell}$ = –0.32‰) came from a shell portion that was formed slightly before the date of collection and closely resembled the water temperature during early to mid March 2005.

The $\delta^{13}C_{shell}$ values were also measured but not analyzed in further detail in the present study. No significant correlation existed between stable oxygen and carbon isotope values (SgA1: R^2 = 0.01, p = 0.31; SgA2: R^2 = 0.03, p = 0.11). Seasonal $\delta^{13}C_{shell}$ ranges were more than double the $\delta^{18}O_{shell}$ amplitudes (SgA1: 2.07‰ SgA2: 2.75‰; Table 1).

3.3 Temporal alignment of shell portions and shell growth rate

The internal shell growth patterns of many mollusks can be used to temporally contextualize the growth record and to estimate changes in calcification rates (Schöne et al. 2007). In *S. gigas*, such growth patterns were only vaguely developed (Fig. 3) precluding a reliable calendar dating of each shell portion. Therefore, the temporal alignment of the growth

record and the reconstruction of seasonal shell growth rates were achieved by cross-dating reconstructed ($T(\delta^{18}O_{shell})$) and instrumental temperatures (SST). The $T(\delta^{18}O_{shell})$ values were arranged so as to provide the best possible fit (via linear regression analysis) with the instrumental temperature curve (Fig. 7). Moving the individual $\delta^{18}O_{shell}$ values along the temporal axis required repeated recalculation of $T(\delta^{18}O_{shell})$ data based on the current $\delta^{18}O_{water}$ value. Ultimately, each sample was assigned to a precise calendar date. R^2 values were as high as 0.81 ($p < 0.001$) and 0.76 ($p < 0.001$) in SgA1 and SgA2, respectively. The largest deviation (~1 °C) between measured and reconstructed water temperatures occurred simultaneously in both shells during August/September 2004 (Fig. 7).

According to the temporal alignment of the $\delta^{18}O_{shell}$ data, both shells started growing in November 2002 (Fig. 7). Reconstructed water temperatures ranged between ca. 26.5° and 30.5 °C. The majority of the $T(\delta^{18}O_{shell})$ data came from shell portions produced during warmer seasons, particularly during spring (April/May) and fall (October) (Fig. 7). A larger number of samples per given time interval translates into faster growth rates and vice versa (Fig. 8). Consequently, shell growth must have been significantly faster during spring and fall (up to ca. 6 mm d^{-1} during youth; Fig. 8) than during mid summer (ca. 1 mm d^{-1}; Fig. 8). Little or no growth was observed during the colder season of the year (December/January through February/March; Fig. 8). This time interval was marked by a brown growth line. In the flared lip (Fig. 4b,c, Fig. 6).

The transition from the juvenile to the adult life stage occurred during August-October of the second year of life (year 2004) with the formation of the flared lip. Formation of the flared lip (Figs. 2b, 4a) took two weeks in SgA2 and six weeks in SgA1 (Fig. 7). The purple colored growth line near the outer shell surface of the flared lip was produced around early to mid October (Fig. 6).

It was also possible to estimate the time represented by each sample swath because the time and the spatial distance between the two sample swaths were known. Sample resolution varied between subdaily in juvenile shell portions (0.2 ± 0.1 d; mean ± standard deviation) and subweekly in adult shell portions (4.5 ± 1.2 d in SgA1, and 3.2 ± 1.3 d in SgA2).

The time represented by the shell portion between two consecutive spines varied greatly from 1 to 72 days and typically increased from the apex to the aperture (Figs. 7, 9a). Furthermore, the spatial distance between adjacent spines increased exponentially from the apex to the aperture (Fig. 9b). Note that the formation of spines did not occur contemporaneously in both specimens (Fig. 7).

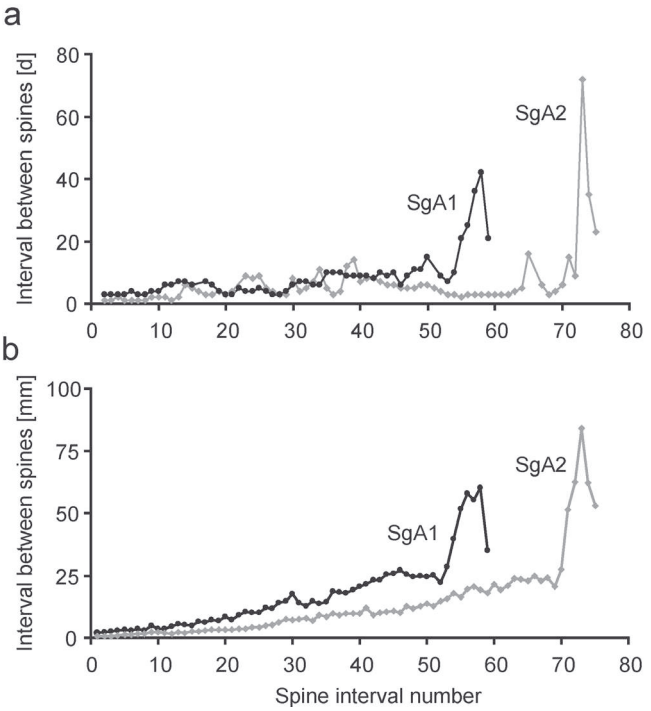

Fig. 9. *Strombus gigas*. (a) The time represented by the shell portions between two consecutive spines is highly variable. As the shell ages, however, it takes increasingly longer to form the spines. (b) The distance between consecutive spines increases exponentially from the apex to the aperture. "Spine interval number" refers to the interval between two adjacent spines; number 1 is the interval between spines 1 and 2, number 2 is the interval between spines 2 and 3 and so on. Black curve = specimen SgA1; grey curve = SgA2.

4 Discussion

High-resolution stable isotope analyses of shell carbonate can reveal important insights in to the life history traits of gastropods (Santarelli and Gros 1985; Kobashi and Grossman 2003; Schöne et al. 2007). Here, such sclerochronological techniques were applied to shells of the queen conch. Similar existing studies on *Strombus gigas* are rare and have, until now, been based on a relatively coarse sampling resolution, for example, of about ten samples per annual increment (e.g., Wefer and Killingley 1980). The present study used four times as many isotope samples per year from two contemporaneous specimens and compared these data with high-resolution instrumental environmental records. Our study is the first that presents a subseasonally resolved and precisely temporally aligned growth record for *S. gigas*, as well as an estimate of the controlling factors, timing and rate of shell growth during different seasons.

4.1 Timing and rate of shell growth and environmental controls

Shells of *S. gigas* from Glovers Reef, Belize grew at remarkably rapid rates and reached adulthood (i.e. final shell size) in less than two years (Figs. 6, 7). Comparatively high growth rates have previously been reported for specimens from the Florida Keys (Keith et al. 1964). However at many other localities, such as the US, Virgin Islands, Puerto Rico and Bermuda, the formation of the main shell body plus the flared lip took nearly twice as long (Berg 1976; Appeldoorn 1988; Wefer and Killingley 1980). Similarly, the flared lip (= transition to the adult growth stage; Figs. 2b, 4a) formed in less than six weeks at GLR, whereas in specimens from the Turks and Caicos Islands the same process took up to 2.5 months (Randall 1964). The site-specific differences in growth rates

are unlikely to be related to genetic variation. According to Campton et al. (1992), strombids in the Tropical Atlantic are genetically very similar. It is therefore more reasonable to assume that local environmental conditions – first and foremost food supply and temperature – account for the observed variations in shell growth (Alcolado 1976; Martín-Mora et al. 1995; Stoner et al. 1996).

One of the most conspicuous findings of this study was that shell growth rates varied considerably throughout the year (Fig. 8). According to previous studies shell extension rates correspond at least partially with water temperature whereby faster shell growth coincides with warmer temperatures and slower growth with colder temperatures (Alcolado 1976; Appeldoorn 1985). However, the growth curve of *S. gigas* from GLR showed a bimodal pattern with maximum extension rates of up to 6 mm y^{-1} during spring (April-June) and fall (September-November), but only 1 to 2 mm y^{-1} during July and August. Furthermore, shell growth ceased between December and March. If water temperature exerted the main control on shell growth, then why did the shells grow at their fastest rates during October 2003 but at much slower rates during August 2003 when temperatures were nearly the same (Fig. 8)? It is also unrealistic that a temperature of 26.5 °C represented the lower growth threshold because specimens of the same species reportedly grew shell between 19° and 29 °C at Bermuda (Wefer and Killingley 1980). Our findings (i.e. no good correlation between shell growth and sea-water temperature) suggests that water temperature alone does not exert a major control on shell growth of *Strombus gigas*.

Our interpretation follows Martín-Mora et al. (1995) and, in the following, suggests that shell growth of the queen conch was largely controlled by food supply rather than temperature (even if other habitat characteristics may also play a role). By visual comparison, the bimodal pattern of shell growth observed here compares particularly well to the bimodal precipitation pattern in the Western Caribbean (Magaña and Caetano 2005; Curtis and Gamble 2008; data from the Global Historical Climate Network for Belize, http://cdiac.ornl.gov/ftp/ndp041/, last checked on 15 June 2009) (Figs. 5d, 8). Maximum rainfall and fastest shell growth occurred during April through June and September through November. However, shell growth was limited during the mid summer drought (July-August) and during December through March (Fig. 8). We hypothesize that higher precipitation increased the amount of nutrients (nitrate) in surface waters and thus triggered primary productivity and, ultimately faster shell growth of the gastropods. It should be noted that this hypothesis is currently difficult to corroborate by observational data. Satellite-based records of primary productivity (ocean-color data) are typically measured over a large area (resolution: 9 × 9 km). This spatial resolution is too coarse to identify changes of primary productivity on a small atoll such as GLR. In addition, there are no long-term records of primary productivity available from GLR. However, the river-reef connectivity model of Soto et al. (2009) – based on elaborate SeaWiFS ocean color data – for Belize/Honduras supports our assumption that riverine influx from the coast (Belize) induces primary production which in turn leads to a significant growth increase in the queen conch at GLR.

4.2 Shells of *Strombus gigas* as high-resolution recorders of ambient SST

Temporally aligned $T(\delta^{18}O_{shell})$ curves of both shells compared very well to each other and also to instrumentally determined daily water temperatures. Although direct measurements of the $\delta^{18}O_{water}$ values were not available, results of this study suggest that shells of *S. gigas* are precipitated in oxygen isotopic equilibrium with the ambient water. This makes the queen conch an ideal temperature recorder. Similar findings were recently presented by Schöne et al. (2007) for a small gastropod from the North Sea, *Gibbula cineraria*.

Only during summer 2004, were the reconstructed temperatures significantly (~1 °C) lower than actual SST (Fig. 7). This deviation is best explained by more positive $\delta^{18}O_{water}$ values than assumed. It should be noted that the oxygen isotope composition of the water was not directly measured but was instead reconstructed from daily salinity data from December 2000 – December 2001; these data may therefore not be representative for 2004. According to instrumental records from the Smithsonian field station at CBC, rainfall was ~50% lower during July and August 2004 than during the previous year, i.e. less than 100 mm. It is well established that the intensity of the mid summer drought can vary from year to year (Magaña et al. 1999). During summers with low precipitation, evaporation rates increase and result in more positive $\delta^{18}O_{water}$ values. Consequently, $T(\delta^{18}O_{shell})$ values will decrease.

4.3 Are shell growth patterns of the queen conch reliable time gauges?

We observed faint micrometer-scale growth patterns in the cross-sectioned whorls of the queen conch which stroke out at the outer shell surface (Fig. 3b,c). In many other studies of bivalve mollusks and gastropods, such structures have been interpreted as semidiurnal or diurnal growth patterns. We assume that this is also likely to be the case for *S. gigas*. However, even if these growth patterns did form on a regular basis, they may not be suitable tools with which to temporally align the growth record because, firstly, the internal micro-growth patterns only occurred in few shell portions and, secondly, growth patterns visible on the external shell surface only occur on the last whorl and are often poorly developed.

Major growth lines were only studied in the cross-sectioned flared lip (Fig. 4b,c). The brown growth line probably formed as a result of slow shell growth (food scarcity) during December through March and may be termed a "winter line" (Fig. 6). The purple-colored growth line, however, formed during early to mid October (Fig. 6). Interestingly, this time interval coincides approximately with the larval stage of *S. gigas*. In a variety of mollusks, shell growth is retarded or stopped during the reproduction cycle prior to spawning. Energy resources are then allocated to the production of gametes rather than to shell formation (e.g., Jones 1980; Lorrain et al. 2002). The corresponding growth lines are referred to as reproduction breaks (e.g., Sato 1995) and may even occur in pre-mature specimens that apparently mimic the reproduction cycle of their elders ("foreshadowing", Thompson et al. 1980).

Aside from internal growth patterns, the queen conch produces distinct spines on its outer shell surface (Fig. 2) the spatial distance of which increases exponentially with ontogenetic age (Fig. 9b). However, temporal alignment of the spines revealed that the time between them differed remarkably in different shell portions (Fig. 9a). Furthermore, the spines were produced at different times in different specimens (Fig. 7). Apparently, shell sculpture is ultimately governed by genetics, not temporally controlled. Consequently, spines of the queen conch are not time gauges.

4.4 Applicability of sclerochronology for the mariculture of *Strombus gigas*

Shells of *S. gigas* contain calendar aligned records of variable ambient environmental conditions, particularly food supply and water temperature. It is possible to analyze how fast the gastropods grew during different seasons and which factors controlled shell growth. This is particularly relevant for fisheries management, maricultural purposes and stock enhancement. The shells can be used to identify localities that provide optimum growth conditions. Furthermore, shell sclerochronology can facilitate site monitoring and make time-consuming long-term observations unnecessary.

5 Conclusion

Sclerochronological analysis provides a rapid and highly versatile method with which to obtain information on the life history traits of *Strombus gigas*. Such data is particularly relevant for stock enhancement and fisheries management. In the past, data such as relative growth rates, ontogenetic age and factors controlling shell growth were typically acquired through expensive time-consuming long-term observations at various localities. Sclerochronology provides a fast and effective tool for site pre-testing and selection purposes. It may also yield important data for mariculture and aquaculture, as well as improving the current understanding of the biology of the queen conch.

The main results of our study can be summarized as follows.

(1) *Strombus gigas* is an extremely fast-growing species. At Glovers Reef, Belize, adult life stage and final shell size were reached after ca. two years.
(2) Seasonal shell growth exhibited considerable variability. Fastest growth occurred during spring (April-June) and fall (September-November) when precipitation rates and – most likely – food supply were at a maximum.
(3) Shell growth of the queen conch is apparently not controlled by temperature alone. Subsequent studies should test the hypothesis that a combination of food supply and temperature control shell growth of this species and quantify this relationship.
(4) Shell carbonate was most probably deposited close to oxygen isotopic equilibrium with ambient water.
(5) Fast-growing youth portions of the shells enable $\delta^{18}O_{shell}$–derived water temperature estimates with subdaily resolution.
(6) Microgrowth structures may potentially be useful for temporally aligning the growth record, but were only observed in some shell portions.
(7) The queen conch at GLR formed distinct growth lines during winter (brown color) and during the reproduction cycle (purple color).
(8) Spines on the outer shell surface cannot be used as time gauges, because the time represented by the distance between two consecutive spines varies considerably, i.e. the formation is not periodic but episodic. The distances between the spines increase exponentially from the apex to the aperture.

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