

The ancient giant clam shells from Balobok Rockshelter, Philippines as potential recorders of environmental changes: a diagenesis assessment and sclerochronology study

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Abstract

The study explores the preservation state of ancient giant clams excavated from the Balobok Rockshelter Archaeological Site, Philippines. The *Hippopus hippopus* and *Tridacna maxima* are from the cultural layer of Early Occupational Phase (8760 +/- 130 BP) and Later Occupational Phase (5140 +/- 100 BP), respectively. The research presents the mineral composition, microstructure characteristics and microgrowth patterns of the Tridacnidae shells. Both species were subjected to Fourier Transform Infrared Spectrometry (FTIR) and Scanning Electron Microscopy (SEM). These two methods aim to recognize its mineralogy and microstructure of the studies material. Only the *H. hippopus* was analyzed through sclerochronological method to understand its growth patterns. The results of the analyses imparted a record on the diagenesis alteration of the Tridacnidae shells. Both shells are still aragonite; however, altered prismatic and crossed-lamellar microstructure can be observed, which is not favorable for geochemical study. On the other hand, sclerochronology study on *H. hippopus* determines the age and internal growth changes of the shell, which is approximately one year and started to development in the last quarter. This concludes that sclerochronology is feasible because of the shell's preserved inner growth increments. However, larger (older) shells should be selected for study to obtain palaeoenvironmental information.

Keywords: diagenesis, FTIR, microstructure, mineralogy, SEM, sclerochronology, *Tridacna*.

Introduction

Ancient giant clams from the Tridacnidae family located in the Indo-Pacific region are potentially useful in understanding environmental changes. The shells provide good environmental records namely in paleoclimatology and geological history because they existed from the Eocene epoch to the present time (Bonham 1965; Hean & Cacho 2003; Jaubert 1977; Klump & Lucas 1994). Moreover, it's one of the largest bivalves that can grow over a meter in length and can have a lifespan of more than a century (Watanabe & Oba 1999). The shell is composed of hard and dense aragonite that makes it less sensitive to diagenetic processes (Aharon & Chapell 1986; Pätzold *et al.* 1991; Watanabe &

Oba 1999). Lastly, it has a fast growth rate with an approximate annual thickness increment of a centimeter (Bonham 1965). These mentioned characteristics allow studying the living environment of the mollusk from its original place and time (Aharon 1983, 1985; Aharon & Chapell 1986; Watanabe & Oba 1999).

This study evaluates the diagenesis state of the *Tridacna* shells, specifically *Tridacna maxima* and *Hippopus hippopus* excavated in the Balobok Rockshelter Site, Philippines dated 8000 to 5000 BP. If diagenesis has altered the shell, any geochemical study for palaeo-environmental reconstructions is precluded. To assess the diagenesis of the shells, these were examined by observing the mineralogy, microstructure and microgrowth of the selected Tridacnas, inquiring the following characteristics: (1) are the shells

still composed of aragonite; (2) are the outer crossed-lamellar and inner prismatic layers well preserved; (3) can shell growth patterns record past environmental changes, particularly past sea surface temperature.

The study area

The Balobok Rockshelter is one of the archaeological sites in the Philippines where numerous shell remnants of the Tridacnidae family were found. The site is located along the western margin of the Sanga-Sanga Island under the Bongao Municipality in the province of Tawi-Tawi (Fig. 1). The province of Tawi-Tawi is one of the three provinces of the Sulu Archipelago. It is located in the southwestern tip of Philippines bounded by the Sulu Sea in the west and the Celebes Sea in the east and south. The island group of Tawi-Tawi is situated facing to the Alice Channel off the northeast coast of Borneo with an approximate distance of 60 km.

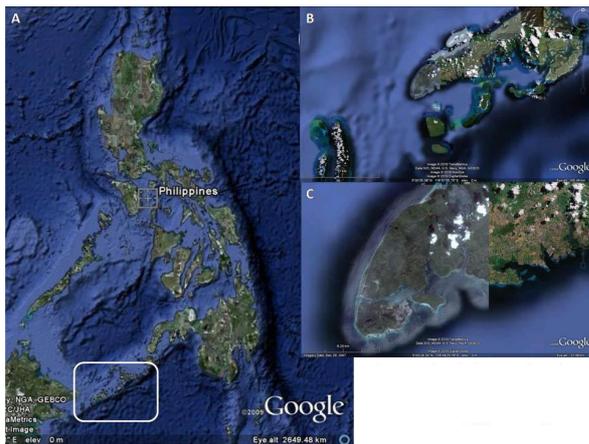


Fig.1. The archaeological site location in the Philippines. (A) Map of the Philippines, indexing the Tawi-Tawi Province; (B) The Tawi-Tawi Province; (C) The Sanga-Sanga Island.

The name "Balobok" originated from a local freshwater fish (English, small tarpon; *Megalops cyprinoides*). The rockshelter is part of a large Miocene limestone formation. It is in the form of an amphitheater 19 m in length with an overhang of 10 m. Inside, the surface slopes slightly for 12-15 m and then drops abruptly to the shore. The arms of the rockshelter extend into the water to form a cove and contain carbonate sediments deposits. The floor of the rockshelter is 10 m above sea level.

The site accumulated significant archaeological findings since it was excavated in 1969. It was first reported in 1966 to the National Museum (Casiño 1966). The excavation was initiated in 1969 by Spoehr (1973). Afterwards, it was re-excavated in 1992 (Cabanilla & Cabanilla 1992; Ronquillo *et al.* 1992). From the several excavations, shell adzes, stone adzes, lithic flake tools, earthenware sherds and a shell midden yielding a considerable amount of animal remains were unearthed.

From the excavations conducted, the site was divided in 10 arbitrary layers called Spit 1, Spit 2, until Spit 10. Based on these, three cultural layers were identified from the shell samples taken from the spits. These shell samples produced Carbon-14 dates of 5140±100 BP (Spit 3), 7290±120 BP (Spit 5), 8760±130 and 8000±110 BP (Spits 7 and 9, respectively). The three cultural layers were labeled as Early Occupational Phase (8760±130 and 8000±110 BP), Middle Occupational Phase (7290±120 BP) and Later Occupational Phase (5140±100 BP). The given dates were processed at the Gakushuin Radiocarbon Laboratories, Tokyo, Japan.

Five squares of 2x2 m² were opened for excavation in 1972 and 1992. The excavated squares fall on the grid map at NE 42, 62 and 82, and SE 61 and 62. In these squares *Tridacna gigas*, *Tridacna maxima*, *Tridacna crocea*, *Tridacna derasa*, *Tridacna squamosa*, *Hippopus hippopus* and *Hippopus porcellanus* were retrieved except in square NE 42. *T. maxima* is the most abundant Tridacnidae species in the site and is present in almost all cultural layers.

Materials and methods

The giant clams are bivalve mollusks, of the Order Veneroida. The taxonomy of the giant clam is as follows:

Phylum :	Molluska
Class :	Bivalvia (Linné, 1758)
Order :	Veneroida
Super family :	Tridacnoidea or Cardioidea (Keys & Healy 1999- still debatable)
Family :	Cardiacea
Sub-family:	Tridacnidae

Two excavated valves sample species were studied. The *Hippopus hippopus* (left valve) and

Tridacna maxima (right valve) from the Square SE61 of Early and Late Occupational phases, respectively, were subjected to several types of analysis. Both are collections of to the National Museum of the Philippines. The *Tridacna maxima* has an accession number IX-1992-K-112 assigned by the National Museum of the Philippines (Fig. 2). It belongs to the Later Occupation Phase dating 5140 +/- 100 BP. The right valve measures 6.3 cm in height and 10 cm in length. The anterior length of the valve is 4.8 cm and posterior length of the valve is measured at 5.7 cm.

On the other hand, the *Hippopus hippopus* has an accession number IX-1992-K-379 of the Early Occupational Phase (8760 +/- 130 BP). The left valve has a height of 9 cm and length of 12.4 cm. The anterior length of the valve measured 8.3 cm and the posterior length measured 6.1 cm. The given measurements were aided with a digital caliper.

Sample preparation

The two giant clams utilized were prepared for diagenesis assessment, particularly mineralogical and microstructural studies and sclerochronological study (only for *Hippopus*

hippopus). The *H. hippopus* had visible growth lines in contrast to the *T. maxima*. The two shells went through a series of processes in the Paléotrope laboratory of the Institut de Recherche pour le Développement (IRD) laboratory. Both shells were cut using a precision saw to see the cross sections, wherein several sections were obtained for analysis.

In making several sections, the transverse sections of both shell valves were sliced numerous times in order to have several samples for different analyses. The apparatus used for cutting was Buelher Isomet 5000, a motorized apparatus with a disc saw (diameter of 12.7 cm or 20.3 cm). Before initiating the sample cutting, the parameters were regulated numerically on the machine to determine the desired thickness of the sample. When the modifications were carried out, the machine commenced adjusting the saw rotation around 4250 rpm and with an acceleration of 4.8mm/minute. Afterwards, the water-cooling system was turned on where cold water flowed on the sample's cutting level. When the cut was done, the samples were polished and cleaned in an ultrasonic bath. Finally, all samples made were labeled.



Fig.2. Cut shell of *Tridacna maxima* IX-1992-K-112 (left) and *Hippopus hippopus* IX-1992-K-379 (right) with two transverse sections.

Approaches to the samples for analysis

The two giant clam shells were studied through Fourier Transform Infrared Spectrometry (FTIR), Scanning Electron Microscopy (SEM) and sclerochronology. This is to determine the mineralogy, and characterize the microstructure and microgrowth patterns of the samples.

(1) FTIR is one of the methods of infrared spectroscopy. It provides information on the mineralogy of unknown materials at a high

spatial resolution. This method was applied to determine if the shells were completely composed of pure aragonite or if the aragonite could have been transformed into calcite through diagenetic processes.

The spectrometer equipment used is a Bruker Fourier Equinox 55, coupled to a microscope (Bruker A-590) allowing high resolution analysis of samples between 20 and 350µm diameter (with an adjustment precision of approximately 1µm of the measured spot). The

opening used (or the size of sample studied) for analysis is 1.8 mm^2 (equivalent to $120\mu\text{m}$ diameter). The apparatus of spectrometer has an infrared radiation source, a network and a system making it possible to divide the infra-red beam into two. A part of the beam is used as reference while the second part is used for the studied substance (University Southern Paris XI, 2007). The samples analyzed by FTIR were IX-1992-K-112-A2b (anterior part of the *T. maxima*), IX-1992-K-379-EP2a (posterior edge of the *H. hippopus*) and IX-1992-K-379-UP2a (umbo posterior part of the *H. hippopus*). For the infrared study, the surface of the samples must be as plane and as smooth as possible in order to optimize light reflection. The samples were polished with successive polishing discs with grains of 45, 26, then of $15\mu\text{m}$. Final polishing was carried out using diamond suspension of $6\mu\text{m}$, $3\mu\text{m}$ and $1\mu\text{m}$.

The samples were rinsed in an ultrasonic bath in between each polishing step. This is necessary to eliminate any abrasive powders that could be left on the sample. Once polished and dried, the samples were analyzed under the FTIR.

When the sample sections were ready for analysis, the points of interest for FTIR analyses were carefully chosen. This was done under the optical microscope. For each sample, the chosen points were patterned in a manner of transects. The analyses were carried out in a vertical way from the internal zone towards the external zone. FTIR analyses were realized with H. Boucher (URO55-IRD).

(2) SEM is a secondary electron microscope that obtains various images by focusing a high energy beam of electrons onto the surface of the sample, detecting signals from the interaction of the incident electrons with the sample's surface. It gives a large depth of field, which allows a large amount of the sample to be in focus at one time. It also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. The SEM study was undertaken to answer the second question of the study; that is, to characterize the microstructure of the shell, namely, the internal (prismatic) and external (crossed-lamellar) area. SEM was also used to determine if primary microstructures of the shell were conserved or not by comparing them with microstructures of modern specimens of the same shell species.

For SEM observation, the other parts of the

same samples were used. This time, the posterior right valve of IX-1992-K-112 and anterior left valve of IX-1992-K-379 were used. The two valves were cut again using the diamond saw and were labeled IX-1992-K-112-P2ab and IX-1992-K-379-A2ab.

To observe the microstructure in an optimal way, one works with an irregular piece. The sections from the samples were broken manually into several pieces. The break was made for the two shells in a place where both the external and the internal shell layers have a thickness of $100\mu\text{m}$ and an average size of $1.5 \text{ cm} \times 1 \text{ cm}$. Only this size can enter the SEM compartment. When the fracture was done, it was necessary to cut the irregular edge with the saw to have a better view and placement when it is being observed under the SEM.

Because SEM utilizes vacuum conditions and uses electrons to form an image, special preparations must be done to the sample. The shell fragments need to be made conductive by covering the sample with a thin layer of conductor material like gold. This was done using a device called a *sputter coater*.

The sputter coater uses an electric field and Argon gas. The sample is placed in a small chamber that is in a vacuum. Argon gas and an electric field cause an electron to be removed from the Argon, making the atoms positively charged. The Argon ions then become attracted to a negatively charged gold foil. The Argon ions knock gold atoms from the surface of the gold foil. These gold atoms fall and settle onto the surface of the sample producing a thin gold coating. The final sample preparation for SEM analysis was assisted by C. Lazareth and S. Caqueneau of the Paleotropique Laboratory.

(3) Sclerochronology deals with the study of shell growth increments. It is an approach that synchronizes the variability of wide and narrow rings in time, between numbers of individual series and establishes the age of the sample.

The internal shell lines of *H. hippopus* were exposed using the methods described by Brousseau (1984) and Aubert (2007). The remaining valve of IX-1992-K379-UP specimen was mounted on a glass slide and sectioned longitudinally along a plane passing through the umbo and approximately bisecting the posterior margin. The cross-section was polished again and was labeled IX-1992-K-379-UP3b.

After polishing, the cross-section of IX-1992-K-379-UP3b was etched using the Mutvei's

solution consisting of 1% acetic acid with a 25% glutaraldehyde solution and an alcian blue colorant at 37–40°C in Marie bath along with constant agitation for almost an hour (Schöne *et al.* 2003a; Aubert 2007). Then, it was carefully rinsed in demineralized water and allowed to air-dry. While acetic acid dissolves the carbonate, glutaraldehyde dries the organic matrix and alcian blue stains mucopolysaccharides in the shell. This treatment results in a three-dimensional preservation of the growth structures and reveals distinct, etch-resistant, blue-colored growth lines. This solution is commonly applied to bivalve mollusks (Dauphin *et al.* 2003; Schöne and Giere 2005; Schöne *et al.* 2003b; Aubert 2007; Valdovino & Pedreros 2007).

In counting the growth lines, the sample was photographed under an optical microscope (on the same scale) coupled to a camera. This was done using the computer program named Visilog (Visilog® V. 6.841). The magnification was at x10 (100 times its real size). These were taken in diffuse light to observe the color attacked on the surface of the shell. The photographs were assembled together to have the totality of the internal layer. With each photograph taken, a benchmark is selected in assembling the shell's internal layer.

When the photographs of the internal layer were put together, they were subjected to various methods using the Visilog software. The Paléotrope laboratory in IRD developed a special module for sclerochronology which was

added to the software Image Analysis Visilog basic. This module is used to mark each growth line observed. The data extracted from the marked lines produce a graph illustrating the distance between each growth lines from the beginning of the internal layer until the end of the profile.

Afterwards, the software calculates the distance between the marked line, in pixels, with the following formula: $DAB = \sqrt{(Xa - Xb)^2 + (Ya - Yb)^2}$. The distances are measured in μm and were calibrated using a micrometer. The data gathered were converted under an Excel program to comprehend the growth rate and growth rate variations of the *H. hippopus* shell.

Results

High-resolution mineralogy of the ancient T. maxima and H. hippopus

Mineralogy of the *T. maxima* shell. The spectra obtained on the *T. maxima* (IX-1992-K-112-A2b) is aragonitic based from the several points chosen to determine the shell's mineralogy. The points 6 and 9 show spectra that tend to go in the direction of the calcitic reference spectrum. However, they are still interpreted as aragonitic points. The rest of the spectra (not shown) are located between points 4 and 9 on top of aragonitic reference spectrum (Fig. 3).

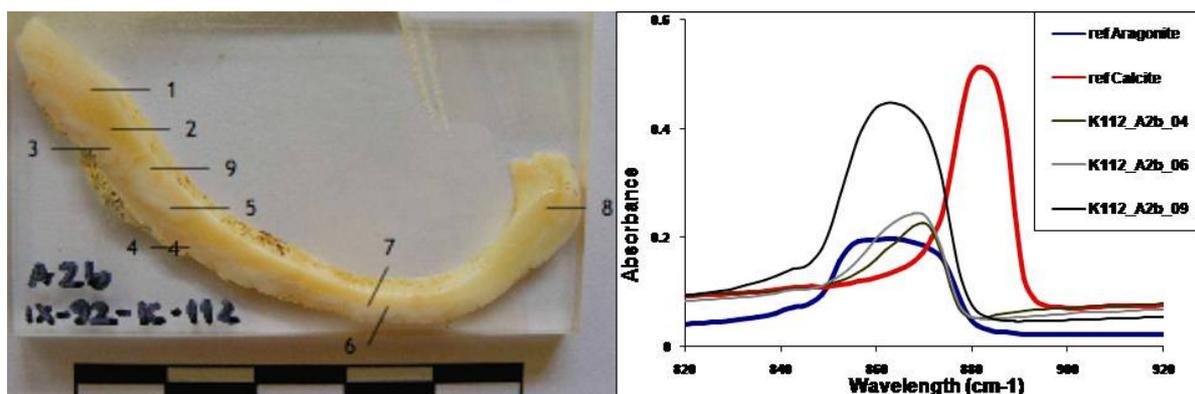


Fig.3. Location of the points to be analyzed (left) and the obtained spectra being compared with the aragonite and calcite reference spectra (right).

Mineralogy of the *H. hippopus* shell. The sample of *H. hippopus* shell was divided into two sections, the umbo posterior and edge posterior parts. These were labeled IX-1992-K-379-UP2a

and IX-1992-K-379-EP2a, respectively.

Among the spectrum obtained from the IX-1992-K-379-UP2a, points 0 and 2 are leaning to the calcite. Point 3 reaches the highest peak of aragonite spectra. All points of IX-1992-K-379-

UP2a analyzed are within the bounds of the reference aragonite spectrum whereas the IX-1992-K-379-EP2a shows different levels of aragonitic spectrum. Point 3 is below the aragonitic spectrum reference, while the peaks of points 4 and 10 are heading to the calcitic spectrum reference. Most of the spectra (not shown) are concentrated in point 5 (Fig. 4).

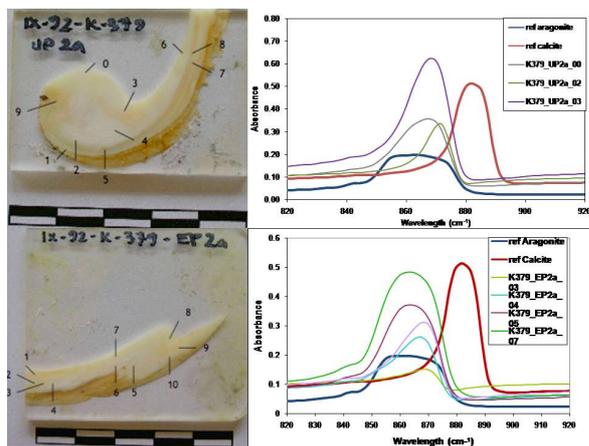


Fig. 4 . Location of the points to be analyzed (left column) and obtained spectra being compared with the aragonite and calcite reference spectra (right column).

Microstructure of *T. maxima* and *H. hippopus*

The shells of giant clams have a crossed-lamellar feature for external layer and prismatic for internal layer (Bonham 1965; Pätzold *et al.* 1991). Crossed-lamellar feature is characterized by perpendicular blocks of crystals that have distinct axis of crystallization and prismatic is characterized by parallelogram shaped crystals.

In determining the preservation state of the *Tridacna*, one should compare the internal and external microstructures of a modern and fossil shell.

Microstructure of the ancient *T. maxima* (IX-1992-K-112-P2ab). The microstructure of the ancient *T. maxima* was characterized based from Pieces 3 and 4. The cross-lamellar external layer of Piece no. 3 shows dissolution and recrystallization. Piece no.4 has a unified crystal orientation in the prismatic zone. However, the end parts of the prism are in a molten state. The crossed-lamellar microstructure of the fragment is also altered. (Fig. 5).

Microstructure of the ancient *H. hippopus* (IX-1992-K-379-A2ab). The microstructure of the ancient *H. hippopus* was characterized based on Pieces 2 and 4. Piece no. 2 has ‘molten’ patches in the crossed-lamellar and prismatic shell layer. Piece no. 4 shows the transition between internal and external structural layers. It also shows some patches in the crossed-lamellar and prismatic area. (Fig. 5).

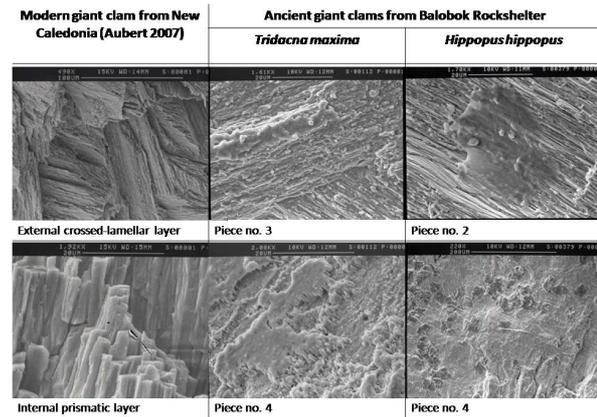


Fig.5. Microstructures comparison between modern and ancient Tridacnidae observed under SEM (up to 200µm).

Description of the IX-1992-K-379-UP3b growth lines

The examined sample for sclerochronology has a width of 5.76mm divided into three profiles (Fig. 6). 367 growth lines were counted. The first profile has 73 lines. The second profile has 141 lines and the last profile has 153 lines. Each increment (distance between growth lines) is equivalent to a day, which makes the sample a year old (Watanabe & Oba 1999; Aubert 2007; 2009).

The average thickness of the sample growth lines is 15.78µm with a standard deviation of 6.00µm. The range of the growth line thickness is between 2.30 and 51.00µm. These results agree with those obtained by Watanabe and Oba (1999) and Aubert (2007) on the *Hippopus hippopus* species. The early growth lines (beginning of life) have a mean thickness of 14.22µm with a standard deviation of 3.28µm.

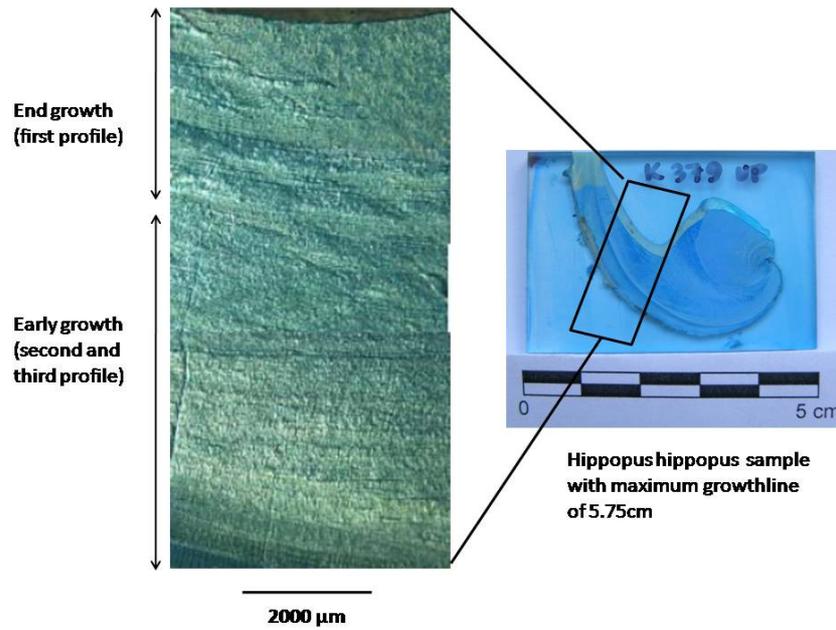


Fig.6. Right: The shell's examined area for sclerochronology (1:5cm); Left: Assembly of three photographs of the shell, IX-1992-K-379-UP3b, after etching (x4).

Growth increments thickness variations

The early growth of the *H. hippopus* presents a regular thickness pattern as observed from the first two profiles. However, the first profile which is equal to three months had wider

thickness variations as compared to the second profile which corresponds to four and a half months. At the end-growth of the sample, it was observed that the growth increments are wider and their thickness varied more.

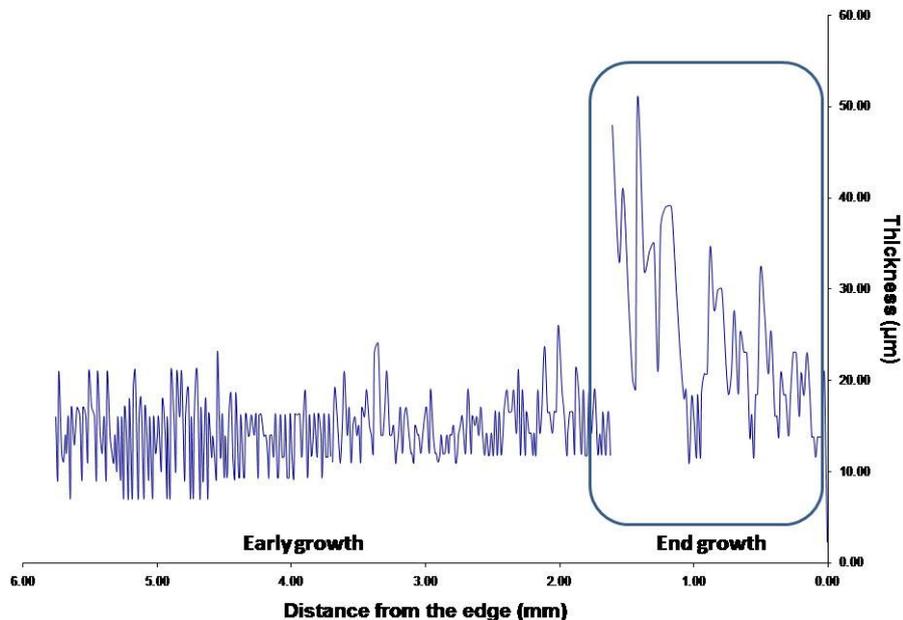


Fig.7. Thickness variations of the sample growth increments (IX-1992-K-379-UP3b) compared to the distance from the edge (mm).

The results of the daily increment thickness variation measurements of the *Hippopus hippopus* sample shows that the sample started to develop more at the end of its first year (Fig. 7).

Before that time, growth is lower and almost constant. Unfortunately, the data provided are not sufficient to establish a relationship between the growth of the *H. hippopus* and the environment. Indeed, the sample is juvenile and only demonstrated more significant growth variations at the last quarter of its life. This study shows that sclerochronological work on juvenile samples cannot give information on environmental parameters. Older specimens (thus bigger) should be selected for future sclerochronological work.

Discussion

In general, the fossil shells studied are still composed of aragonite. Nevertheless, these three samples have been modified through the course of time after its death. The aragonitic spectra are slightly different from the reference spectra. This might be interpreted as the beginning of transformation into calcite particularly for those spectra leading towards the calcitic reference spectra.

The zones that correspond to places leaning to the calcite spectrum reference appear more translucent with a coloring slightly darker than the remainder of the shell. These zones may have been the shell's weakpoints that may have favored the start of the diagenesis process.

Hence, the *T. maxima* and *H. hippopus* mollusks are aragonitic based on the observation gathered from FTIR analysis. Both species are resistant to deterioration, except for zones of weaknesses, such as the discoloration of the interior part of the shell, where secondary calcite can precipitate. These changes provide information regarding how a shell deteriorates after its death.

Nevertheless, if the mineralogy was conserved, the microstructures could have changed. It is thus necessary to check if the original shell microstructures were preserved for further confirmation of the shell state regarding diagenesis.

The SEM was used to verify if the original shell microstructures were well-preserved or not. It demonstrated the arrangement and shape of the crystals in each piece. It also distinguished the sub-units from the prismatic structure from the internal layer in a more precise manner. Moreover, it presented diagenetic features of a bivalve shell that have not been described before.

Under the SEM, the limit between the internal and external layers appears clear. It

seems to consist of small prismatic crystals. Both shells underwent quite intensive diagenetic changes, in both shell layers. The original shell microstructures (namely, cross-lamellar and prismatic) are still visible but highly modified. The major diagenetic change observed is crystal dissolution. In addition numerous 'patches' were observed. At the moment, they are not characterized in a more precise way sufficient to determine what kind of material these patches are composed of. High-resolution geochemical analysis is needed to characterize these 'patches' material.

SEM study highlighted the important diagenetic changes underwent by the shells through time, even if the mineralogy is still aragonite. These diagenetic microstructural modifications could not have been possible without geochemical changes. Consequently, geochemical investigations on these shells are unfortunately precluded.

Sclerochronological analysis of the shell microstructure provides an effective tool in describing and examining internal growth patterns. Daily growth increments were easily measured in the inner layer of the *Hippopus hippopus* archaeological shell after etching using the Mutvei's solution. It was possible to determine the sample's age and its period of development. Moreover, it demonstrates the capacity of this kind of sample for further studies namely, palaeoenvironmental reconstruction, if one select older (bigger) specimens.

Conclusion

In summary, the composition of the *Tridacna maxima* and *Hippopus Hippopus* displayed a substantial amount of variation due to its mineralogy, structure and growth patterns. Both sample species show that they retained their aragonitic mineralogy. In general, on the other hand, the microstructure of the two sample species revealed altered characteristics on its crossed-lamellar and prismatic areas. These characteristics show the preservation state of a bivalve mollusk.

Finally, studying the growth band of the *H. hippopus* through sclerochronology provided the age and growth development of the shell. The method can also be a parameter to know if the sample is viable for geochemical analysis.

These three methods employed are proven interconnected with each other. To further

comprehend the diagenesis of bivalve mollusks especially from the Tridacnidae, one has to study the mineralogy, microstructure and microgrowth patterns of the shells. It imparts precise characteristics of how these mollusks fossilized and may be correlated to its original environment.

The only limitation of the study is that the samples subjected into analysis are young especially in the case of the *H. hippopus*. *H. hippopus* is estimated to be one year old. Moreover, the *T. maxima*'s internal structure is heavily altered. It failed to conduct geochemical studies like isotope analysis or even to reconstruct the paleoenvironment and paleoclimate of the area. For these reasons, it is highly suggested to have bigger and older samples to analyze.

The study of ancient giant clams, *T. maxima* and *H. hippopus*, from the Balobok Rockshelter, Philippines contributed significantly to scientific studies. Besides the *Tridacna gigas*, these two species are popularly excavated in most archaeological sites in the Philippines as well as in the Southeast Asian region. Most archaeological studies on Tridacnidae in this region are related to subsistence and tool-making technologies. To date, no studies have ever conducted using Tridacnidae for paleoenvironment reconstruction in Southeast Asian countries.

Characteristics of Tridacnidae have established great potential for paleoenvironmental reconstruction. It opens the field for approaches and improvements especially for Southeast Asian region which has abundant resources of Tridacnidae. It has potential in studying old environments. The biological organization controlled by complex internal rates/rhythms, particularly the role of zooxanthelles, is interesting to study for the growth and geochemistry of the shell. Such studies may bring new research and prospects in the field of paleoenvironment.

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