ANALYSIS OF MOLLUSCAN SHELLS MINERALIZATION AND MICROSTRUCTURES

[1]K.Partha Saradhi, [2]N.Manohar Reddy, [3]K.Thyagarajan

[1]Research Scholar, JNTUA, Ananthapuramu, Andhra Pradesh, India.
[2]Professor, Department of Physics, Sri Venkateswara College of Engineering, Tirupathi, Andhra Pradesh, India.
[3]Department of Physics, JNTU College of Engineering, Pulivendula, Andhra Pradesh, India.
parthasaradhidid@gmail.com , manoharreddy123n@gmail.com

ABSTRACT: Researchers from various fields, including biologists, physicists, paleontologists, and material scientists, have consistently been attracted by molluscan shells. These shells consist of meticulously arranged collections of calcite or aragonite crystals, displaying a wide range of morphologies and three-dimensional patterns. Biominerals are mineral and organic component containing biogenic mineralized products. Calcium-bearing minerals comprise about 50% of biominerals with the most common being polymorphs of calcium carbonate, e.g. calcite and aragonite. This paper presents Analysis of Molluscan shells Mineralization and Microstructures. This study looks at the mineralization and microstructures of three different species of abalone shells from the Kakinada seashore in Andhra Pradesh: Haliotis asinina, Haliotis rufescens, and Haliotis gigantea.

There is always a prismatic outer layer and a nacreous inner layer on an abalone shell. In the shell of H. asinina, it has been discovered that aragonite exclusively forms the prismatic and nacreous layers, with all crystals aligned along the c-axis. To study this, microanalysis, electron backscatter diffraction, scanning electron microscopy (SEM), and other relevant analytical techniques have been employed, or EBSD) revealed aspects of the microstructure and mineralization of molluscan shells.

KEYWORDS: Molluscan shells, Abalone shells, Biominerals, microstructures,EBSD, SEM.

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I. INTRODUCTION

Mollusks, also known as molluscans, are soft-bodied animals. As a result, many of them have developed a sophisticated method for preserving their soft tissues, defending themselves against predators, and preventing desiccation. The creation of a rigid, externally calcified shell is the foundation of this strategy. The shell is without doubt one of the studied biomineralizations and in many aspects, the most fascinating biomineralizations found in living things. The process of forming of shells are biologically-controlled mineralizations which is different from mineralization of biologically-induced, later these are converted bacterial world [1].

Biocomposites consisting of organic matrix and calcium carbonate crystals are typically found in molluscan shells. A lightweight product with elaborate morphologies and distinctive structural properties are the result of this combination. The inorganic crystals' nucleation, orientation, and growth are facilitated by an important role played by an organic matrix within the extrapallial compartment of the animal. The calcium carbonate crystallization in the shell is a lifetime process that is affected by numerous intrinsic and environmental factors.

The composition of the shell structure is widely recognized to be primarily comprised of two major phases of calcium carbonate: aragonite and calcite, which is composed of three layers: the innermost layer is the aragonitic nacre and the outer one, usually prismatic, is comprised of either aragonite or calcite. Frequently observed within the shell structure is a layer of obliquely intersecting crystals known as the crossed-lamellar layer. Additionally, the entire structure is covered from the outside by an uncalcified periostracum [3]. Because the various classes' structural patterns can be combined in many different ways, this is an oversimplified and generalized shellmodel.

For studies on biomineralization, molluscan shells are the preferred system. Due to its complex architecture and incorporation of biological macromolecules, the shell possesses superior mechanical properties compared to other composite materials (stiffness, fracture toughness, and tensile strength)[2]. It can be summed up as follows: a) The fabrication of the shell needs specialized intracellular and extracellular cellular machinery, implying that the formation of the shell is strictly controlled by gene cascades; b) The minerals that are formed are out of equilibrium with the surrounding environment; This indicates that it is possible to synthesize some minerals, which are thermodynamically unstable in natural conditions; c) The minerals that are produced differ in size and shape from the minerals that are formed naturally and most of them have complex shapes; Additionally, in contrast to minerals that are synthesized chemically, they assemble in different levels of hierarchy; d) They are formed in a delimited area, away from the direct contact with the surrounding environment; e) An extracellular organic matrix controls the entire shell construction process, part of which is included during the shell calcification.

In recent times, significant data regarding the structural characteristics and biological activity of the shell have been acquired through the utilization of modern techniques. Some of these techniques were previously restricted to biologists. It is common knowledge that the biological matrix, which is primarily composed of the polysaccharide b-chitin and glycoproteins, is closely linked to the mineral phase formation of the shell [6].

Spherical objects made of tiny crystals that radiate from a center and are referred as having a "spherulitic microstructure" (spherulitic microstructure). Spherulites serve as the starting point for prism formation in a number of cases, particularly when crystal growth is constrained in one direction. A recent discovery has revealed that spherulites are commonly formed during "emergency situations" when shell repair is required in the green ormer species Haliotis tuberculata. The shell exhibits laminar microstructures, which are flat units, oriented either parallel or close to parallel with the shell's overall depositional surface.

The nacreous and foliated microstructures are the most well-known of their many rods, laths, blades, and tablets. When an optical or electronic microscope is used to examine a microstructure, it is referred to as a homogeneous microstructure, if the crystallites do not appear to be organized in any way. In heterodont bivalves, homogeneous microstructures are extremely common. Finally, isolated crystal morphotypes, helical microstructures, and isolated spicules or spikes are all uncommon microstructures that are found as sparsely distributed crystals. Shell repair zones contain isolated crystal morphotypes, while isolated spicules frequently belong to the periostracal layer.

By gradually adding material to their inner edges and surfaces, all the molluscan shells are made. The growing edges of the shell are enlarged by the shell material that forms at the mantle margin (at the edges of an aperture in a gastropod and the edges of the shell valves in a bivalve). The mineralized organic periostracum, which may be secreted first, forms the shell's outermost layer. Contrast to the rest of the shell, which is secreted by the mantle's edge, the innermost layer of the shell is formed on the outer surface of the thin inner (or dorsal) mantle, located beneath the shell. When the shell's edge is removed from the mantle, growth at the edges stops, but the innermost shell layer continues to be deposited.

The gastropod molluscs that make up the marine snail family Haliotidae and the genus Haliotis are called abalones. Abalone is also known as muttonfish or muttonshells, ear-shells, sea ears, and ear-shells. Abalone are single-shelled, slow-growing gastropods that live in shallow, rocky waters close to algae clumps. Their muscular foot allows them to firmly attach to the stony ground. A row of apertures defines the abalone shell's dorsal margin. The animal's growth fills in the holes and makes new ones. Both respiration and outlet of waste products are accomplished through the open apertures. In order to add to this knowledge, the goal of this research is to describe the microstructure and mineralization of gastropod nacre in abalone. This study examines the mineralization and microstructure of molluscan shells were examined in three distinct abalone shell varieties: Haliotis asinina, Haliotis rufescens, and Haliotis gigantean. Detailing structural information is made possible with scanning electron microscopy (SEM).

II. LITERATURE SURVEY

Saida praveen, Anupam Chakraborthi, Dipak. Kr. Chanda, Soujitha premanik, Anandamay barik, Goutam Adiyta, et. al. [4] describes the shells of three freshwater snails, specifically Brotia costula, Pila globosa and Bellamya bengalensis, underwent comprehensive analyses conducting chemical, mechanical, and microstructure investigations. For explaining their chemical and physical characteristics, a range of techniques was used, including Fourier-transform infrared spectroscopy (FTIR), X-ray spectroscopy (EDS), X-ray diffraction (XRD), energy-dispersive and nanoindentation studies. Electron microscopy was also used to prepare the shells, and a calcium carbonate content analysis was performed. The results obtained from the XRD, FTIR, and EDS examinations demonstrated that the snail shells' microstructures were predominantly constituted of aragonite calcium carbonate crystals, which also indicated the existence of specific functional groups on the surface of the shells. The shells of B. bengalensis, P. globosa, and B. costula exhibit physical and chemical characteristics that suggest their potential as feasible biological resources for sustainable utilization across various domains. These shells offer promising opportunities for applications in bioremediation, biocatalysis, biomedical uses, and as a valuable source of lime.

David R. Lindberg, Juliet M. Ponder, Winston F. Ponder, et. al. [5] discusses Mollusca biology and evolution. Provide an overview of the molluscs external bodies, including the mantle, the shell and how it grows and forms, the epidermis and its structures, the foot and operculum (of gastropods), mucoid secretions, how they move, and general information about muscles and cartilage. The suckers that are found in a lot of coleoid cephalopods are another important external structure, but they only belong to one group.

Jaya Sharma, U. D. Sharma, Sanjay Shukla, Sanjive Shukla, Richa Shukla, Sandeep Shukla, et. al. [7] provides a report on Lucknow-based freshwater bivalves and the structure of their shells. Two new water bivalves as Parreysia favidens and

Lamellidens marginalis were identified from Lucknow, Uttar Pradesh, India, water reservoirs. Both species' shell microstructures indicate the presence of prismatic, nacreous, and periostracum layers. The nacreous layer of P. favidens's shell appeared to be thicker. Both bivalve species' behaviors and potential roles in the pearl culture industry have been discussed.

A.G. Checa et. al. [8] described about the physical and biological factors that influence, how molluscan shell microstructures are made. Now we have evidence that, beyond the influence of proteins, biophysical principles play a crucial role in interpreting and organizing molluscan microstructures, indicating the involvement of additional mechanisms. Author have specifically identified the following processes: 1) crystal nucleation on membranes that have already been formed; 2) crystal nucleation and growth take place within self-organized membranes as well as between them; and 3) contact recognition and deposition are active subcellular processes involved in the formation of crystals, the authors present a novel perspective that may make it possible to allow microstructures. Evolutionary constraints can be considered when comparing the taxonomic secretional abilities of organisms, as well as when estimating the probability of replicating microstructures for the production of functional synthetic materials.

Dauphin. Luquet, Y. Salome, G. Bellot-Gurlet, M. L, Cuif. J. P, et. al. [9] studied a nacroprismatic bivalve species' mineralogy, micro- and nanostructure, and composition: Pictorium unum. The aragonite forms a major part of the prismatic layer in Unio, and there are observable distinctions in the internal arrangement compared to the calcitic layers. The morphology of the prisms differs based on their developmental phase. Spatial representations of chemical distribution validate the lack of an organic membrane separating the nacre from the prisms. By comparing Unio pictorum with various species, the diversity of nacroprismatic shell structure, as well as the taxonomical dependence on the structure, mineralogy, and composition, is demonstrated.

III. METHODOLOGY

For the purpose of analysis, three kinds of abalone shells, Haliotis asinina, Haliotis rufescens and Haliotis gigantean were collected from the shores of Kakinada in Andhra Pradesh. The marine gastropod molluscs known as abalone belong to the family Haliotidae and the genus Haliotis. Abalone is also known as muttonfish or muttonshells, ear-shells, sea ears, and ear-shells. The unique characteristics of an abalone encompass its flattened shell, which showcases a succession of openings arranged along the spiral ridges, mirroring the shell's longitudinal growth pattern. There are open, half-closed, and closed apertures. As the shell grows, the number of apertures grows as well.

Fig. 1 illustrates instances of the shells from these three species. Small brushes were used to clean the shells, and deionized water was used to clean them in an ultrasonic bath. After that, the clean shells were dried in the air and each one was labeled with a unique sample number. The dimensions of both the inner and outer surfaces represented by the scale bars for the shells of H. asinina, H. rufescens, and H. gigantea are indicated. The scale bars measure 10, 5, and 5 mm, respectively.

Fig. 1: H. ASININE

Fig. 2: H. RUFESCENS

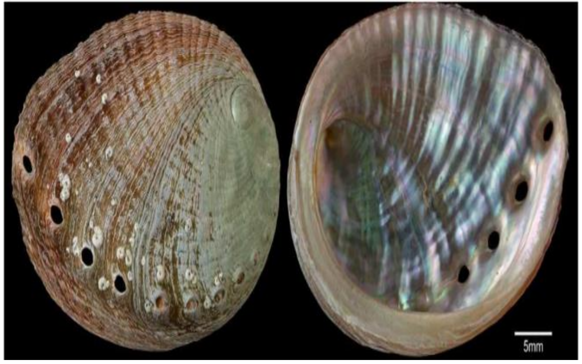


Fig. 3: H. GIGANTEAN

The chemical compound calcium carbonate has the formula CaCO3. It mostly consists of two polymorphs with different chemical structures, calcite and aragonite. Calcium carbonate's most stable polymorph is calcite. The aragonite form of calcium carbonate constitutes a significant proportion of the shells of various marine organisms, including plankton, the rigid structures of red algae, specific sponges, branchiopods, the majority of bryozoans, and certain bivalves such as oysters (Harper et al., 1997). Both warm-water and cold-water corals' calcareous endoskeletons and the shells of nearly all mollusks contain aragonite, which is found naturally in both types of coral. Aragonite covers the entire shell of some molluscs; In some cases, the bimineralic shell (aragonite plus calcite) only consists of aragonite forms only discrete parts.

Scanning electron microscopy (SEM):

Scanning electron microscopy (SEM) is a widely utilized technique for investigating and analyzing microstructures in solid samples. Providing numerous significant benefits such as superior resolution, an extensive depth of focus, and user-friendly operation (Reimer, 1985), a raster scan electron microscope is a specific variant of electron microscope that employs a high-energy electron beam to examine the surface of the sample. Through interactions between these electrons and the sample's atoms, signals are generated, offering valuable insights into surface topography, composition, and additional properties such as electrical conductivity.

The process by which the signals are interacted by the electron beam is near the surfec. An SEM has the ability to produce various signal types, such as Secondary Electrons (SE), BackScattered Electrons (BSE), characteristic X-rays, and other signal varieties. Secondary electrons are low-energy electrons that are produced within a few nanometers of the surface of the sample. Conversely, backscattered electrons contain of electrons with high energy that are discharged or deflected from the interaction volume of the specimen when the electron beam interacts with atoms within the sample. Additionally, when electrons interact with the sample, X-rays are generated (Reimer, 1985).

Electron Backscatter Diffraction (EBSD):

Electron BackScatter Diffraction (EBSD) is an extremely valuable and extensively utilized tool in the field of materials science. A specific camera and phosphor screen locator are utilized in a scanning electron microscope (SEM) under vacuum for EBSD examination. Prior to backscattering, when an electron beam interacts with a crystalline material at a low angle of incidence, it leads to electron diffraction caused by the crystal lattice. Subsequently, the diffracted electrons interact with a phosphor screen, generating specific patterns referred to as "Kikuchi bands". These bands create a pattern which is unique to the orientation of the crystal lattice. Point-to-point data is obtained by scanning the electron beam across the surface and capturing the patterns at each point. These orientation and phase maps are created for a specific surface area. EBSD provides direct evidence of the mineralogy of the prismatic layer in H. asinina, H. rufescens, and H. gigantea.

Gold coatings of 7.5 nm thickness are applied to fractured samples for SEM imaging. Samples undergo a polishing process before being carbon coated with a thickness of 2.5 nm for EBSD analysis.

IV. RESULT ANALYSIS

Morphology of prismatic layer and nacreous layers:

The main layer, which makes up about half of the shell's thickness and lies beneath the prismatic layer, is made up of tiny crystals that are hard to tell apart at low magnification. The nacreous layer, also known as mother-of-pearl, can be found here. It is the lustrous inner layer that can be seen in a number of mollusks, including the mussel, pearl oyster, abalone, and nautilus. In this layer aragonitic is always present. The nacreous layer is one of the mollusk shell microstructures that has received the most research due to its intrinsically high mechanical properties.

SEM images of the figure's H. asinine, H. rufescens, and H. gigantean shells are shown below, Fig. 4, Fig. 5 and Fig. 6, respectively. Whereas, a. An arrow is used to indicate the prismatic layer (P), which exhibits distinct crystal morphologies compared to the nacreous layer which represents the direction of longitudinal shell growth. c. The stacked tablets' nacreous layer (N) lacks smooth surfaces and has nanoscale asperities (arrows).

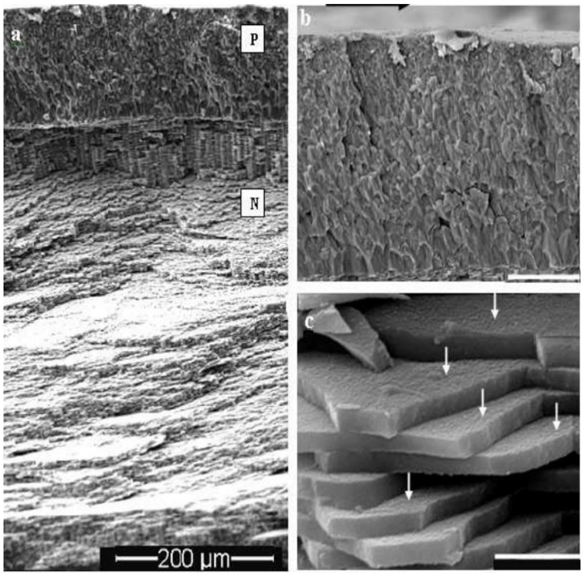


Fig. 4: SCANNING ELECTRON MICROSCOPY IMAGES OF H. ASININA SHELL

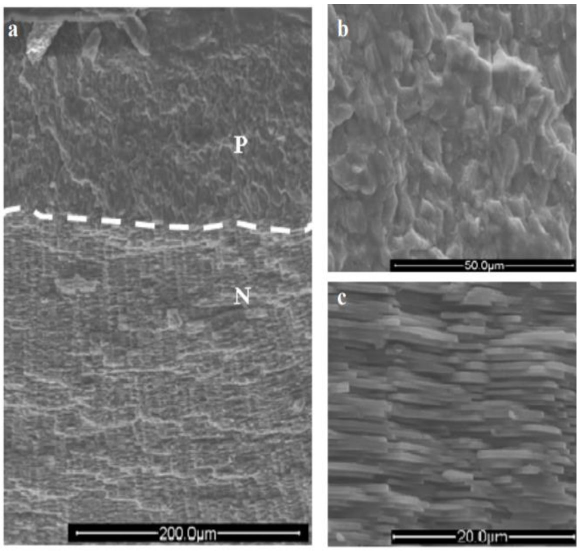


Fig. 5: SCANNING ELECTRON MICROSCOPY IMAGES OF OF H. RUFESCENS SHELL

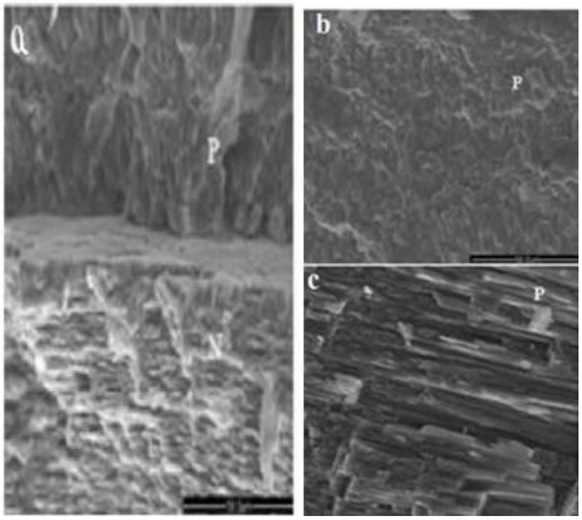


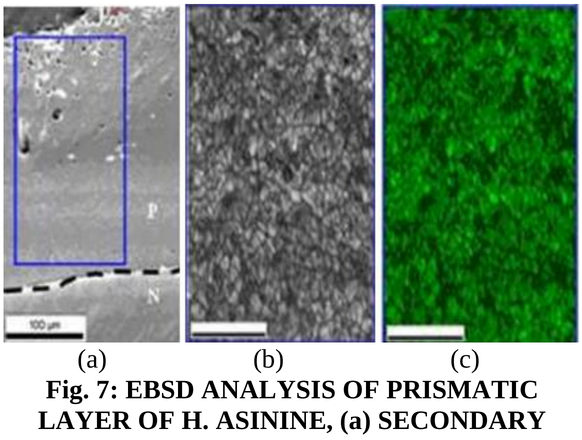
Fig. 6: SCANNING ELECTRON MICROSCOPY IMAGES OF H. GIGANTEAN SHELL

Tiled aragonite growth is linked to the interior nacre shell of H. asinina shell. On the aragonite platelet surface, the microstructure of H. asinina nacre makes it simple to see the asperities at the nanoscale. Organic "glue" is sandwiched in between the tablets. It has been discovered that H. asinina's nacreous layer contains tablets with cooriented columns ranging from 2 to 50. In H. gigantea and H. rufescens, 2 and 40 co-oriented tablets have been observed. The shells' prismatic and nacreous layers differ in proportion. When comparing the kaleidoscopic layers of the three species, there are notable differences. The calcite kaleidoscopic layer in H. rufescens accounts for approximately half of the thickness of the shell. In H. asinina, the aragonite kaleidoscopic layer makes up around 20% to 30% of the shell thickness. In H. gigantea, the kaleidoscopic layer is positioned in the center of the shell and comprises a combination of calcite and aragonite, representing over 30% of the total shell thickness.

Mineralogy of prismatic layers of H. asinina, H. rufescens and H. gigantean:

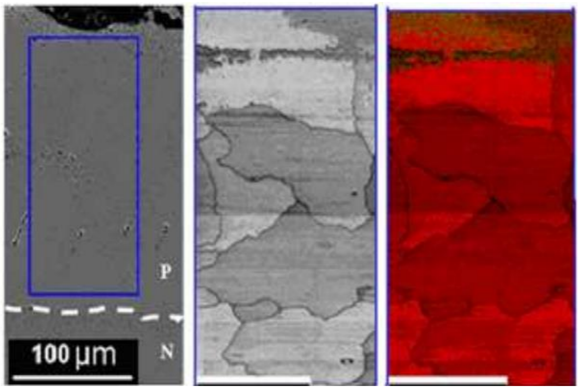
According to the findings from EBSD analysis, it has been established that the kaleidoscopic layer of H. asinina is mainly composed of aragonite. Conversely, the prismatic layer of H. gigantea exhibits a combination of aragonite and calcite, as illustrated in Fig. 7. On the other hand, the prismatic layer of H. rufescens only composed of calcite. The color key provided further supports the aragonite composition of the prismatic layer in H. asinina. The scale bar provided corresponds to 40 µm, indicating the size and dimension of the observed features.

Mollusks primarily utilize two polymorphs of calcium carbonate for their shell mineralogy: calcite, the stable form, and aragonite, the metastable form, which typically transforms into calcite through diagenetic processes. The external glazed olive-greenish color of the shell is due to the periostracum, which has not eroded throughout the animal's life. It is important to note that for some species, the color of the shell is not due to the color of the periostracum; rather the color comes from pigments that are dispersed within the mineralized layers in genetically controlled patterns. The periostracum is adjacent to a mineralized layer of elongated crystals that have grown perpendicular to the shell surface. These are crystals which make up the prismatic layer.



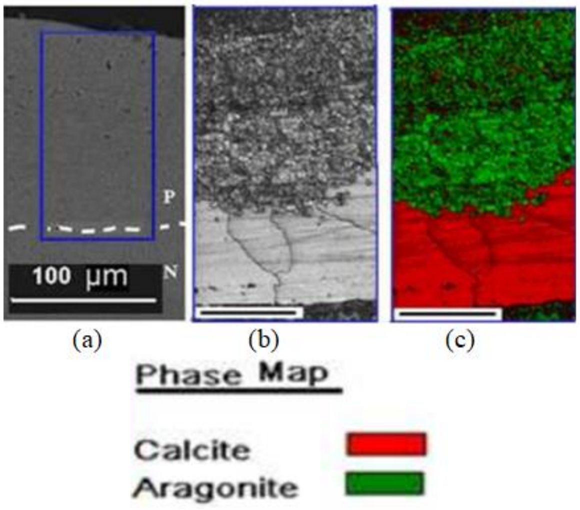
(a) (b) (c)

Fig. 7: EBSD ANALYSIS OF PRISMATIC LAYER OF H. ASININE, (a) SECONDARY ELECTRON IMAGE, (b) DIFFRACTION INTENSITY MAPS, (c) PHASE MAP



(a) (b) (c)

Fig. 8: EBSD ANALYSIS OF PRISMATIC LAYER OF H. RUFESCENS (a) SECONDARY ELECTRON IMAGE, (b) DIFFRACTION INTENSITY MAPS, (c) PHASE MAP



(a) (b) (c)

Fig. 9: EBSD ANALYSIS OF PRISMATIC LAYER OF H. GIGANTEA (a) SECONDARY ELECTRON IMAGE, (b) DIFFRACTION INTENSITY MAPS, (c) PHASE MAP

The c-axis orientation maintains a consistent alignment across both the prismatic and nacreous layers of aragonite, which constitute the shell of H. asinina. Crystallographic alignment becomes significantly more constrained as moving closer to the prismatic-nacreous interface. The nacreous layer has a tightly constrained c-axis than the prismatic layer. Calcite makes up the majority of the prismatic layer of the H. rufescens shell. Initial analyses indicate that the c-axis of crystal orientation within the prismatic layer of the H. rufescens shell is parallel to the surface of the shell. Aragonite and calcite make up H. gigantea shell's prismatic layer.

V. CONCLUSION

This investigation focuses on the microstructures and mineralization abalone shells of three different species collected from the Kakinada seashore in Andhra Pradesh: Haliotis asinina, Haliotis rufescens, and Haliotis gigantea. The gastropod molluscs that make up the marine snail family Haliotidae and the genus Haliotis are called abalones. Abalone is also known as muttonfish or muttonshells, ear-shells, sea ears, and ear-shells. In order to contribute this knowledge, the goal of this research is to describe the microstructure and mineralization of gastropod nacre in abalone. The investigation of the molluscan shell microstructure and mineralization in the three abalone species from the Kakinada seashore involved the use of scanning electron microscopy (SEM) and other analytical techniques such as microanalysis and electron backscatter diffraction (EBSD). Comparing the three species, the prismatic layer to the shell thickness of three species, several notable observations were made. The prismatic layer of H. rufescens was found to be predominantly composed of calcite, representing approximately 50% of the shell thickness. In contrast, the prismatic layer of H. asinina consisted primarily of aragonite, comprising around 20%-30% of the shell thickness. On the other hand, the prismatic layer of H. gigantea exhibited a unique composition, consisting of both calcite and aragonite. This combination of minerals accounted for over 30% of the shell thickness. It is noted that the shell of H. asinina displayed a distinct structure, consisting of both prismatic and nacreous layers, both composed of aragonite. Crystallographic alignment becomes significantly more constrained as moving closer to the prismatic-nacreous interface. The nacreous layer has a tighter c-axis constraint than the prismatic layer. According to preliminary analyses of the H. rufescens shell, in the shell of H. rufescens, In contrast, the prismatic layer of H. gigantea displayed a distinct composition, comprising both calcite and aragonite. This unique combination of minerals contributed to over 30% of the overall shell thickness. It is noted that the shell of H. asinina exhibited a notable structure, characterized by the presence of both prismatic and nacreous layers.

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