www.gondwanags.org.in
Research Article

ISSN : 2455-1953
JGSR
Journal of Geosciences Research
Vol. 9, No.2, July, 2024, pp. 153-160

Morphology, Biochemistry and Distribution of *Villorita cyprinoides* and *Meretrix casta* (Bivalve) Shells in Vembanad Estuary, Kerala, India

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Abstract

Estuaries are among the most commercially significant ecosystems on the earth, and they provide different habitats for numerous bivalve species. The Clams are one of the most widely distributed and used aquatic bivalves, providing much more protein-rich food than mussels and oysters. The Vembanad Estuary is one of the richest clam fisheries coastal wetland in Kerala. The present study focusing on comparatives ofmorphology and biochemistry of *Villorita cyprinoides* and *Meretrix casta* shells in Vembanad Estuary. It also attempts to understand the variations in clam distribution caused by salinity fluctuations and sediment texture. According to the morphological and morphometric analysis, medium size shells were found in greater abundance in both species. A total of 306 shells of *V. cyprinoides* and 169 shells of *M. casta* were obtained from the ten sample locations of Vembanad Estuary. The density of *V. cyprinoides* shells (192) was higher in the southern part while the northern portion of Vembanad Estuary represented by *M. casta* shells (108) with comparatively lower density. *V. cyprinoides* has a negative linear correlation with salinity, as indicated by R^2 of 0.96. *M. casta*, on the other hand, exhibits a positive correlation with salinity, with 0.94 linear coefficient. *V. cyprinoides* was more prevalent in clayey and silty sediments, while *M. casta* was more common in sandy sediments. The X-ray Fluorescence (XRF) analysis showed that the Calcium Oxide percentage of *V. cyprinoides* and *M. casta* shells were 39.47% and 38.72%, respectively, while all other metal oxides were present only in trace amount.

Keywords: Bivalves, *Villorita cyprinoides, Meretrix casta,* Major Oxides,Vembanad Estuary

Introduction

Estuaries are among the most commercially important environments on the planet and provide a variety of habitats for various bivalve species (Costanza *et al.*, 1997). The living forms of bivalves are entirely aquatic, with the highest abundance in both seawater and freshwater (estuarine or backwater). The organisms in this class have lived since the Cambrian period and continue to Present day. The bivalves are also known as Pelecypods or Lamellibranchiata, are the second largest class of Mollusca. Linnaeus (1758) was the first to give this group the name "bivalvia". Bivalvia consist of two valves (shells) that are normally identical, equal in size, but mostly inequilateral. Valves are located laterally on either side of the organism and are held together by a strong muscle. The right and left valves are identical and shows bilateral symmetry by passing the symmetry plane parallel to the ventral margin of the shell (Senthil, 2019). Many environmental factors, including latitude (Beukema and Meehan, 1985), depth and type of bottom (Wie *et al.*, 2020), tidal level (Tran *et al.*, 2020), sediment type (Kanaya *et al.*, 2005), and burrowing behaviour (Knaust, 2015), are known to influence shell morphology, size, shape and selective proportions of bivalve species. The hard calcareous shells are the result of a mineralization process that is physiologically and genetically designed by the species. The shells consist diverse types of $CaCO_3$ such as aragonite, calcite *etc.* Aragonite is found in prismatic, nacreous, crossed lamellar, complex crossed lamellar, and homogenous structures. Calcite is commonly found in prismatic or foliated structures (Adarsh and Senthil, 2018). For bivalves, growth increments or rings in shells are used to record the time. Because development rate of animals are mostly determined by environmental factors, alterations in skeleton biochemistry provide a dependable archive of environmental variables encountered by species (Kennish and Olsson, 1975). Shells are thus exploited as biological archives for paleo-environmental and paleo-climatic conditions. Additionally,the nutritional value of edible molluscs can be estimated using biochemical composition (Celik *et al.*, 2014). All the biochemical conditions of shells are influenced by various factors like salinity, sediment composition, temperature, water flow, larval transport and chemical pollutants (Mckeon *et al.*, 2015; Galtsoff, 1947; Korringa and Postma, 1957).

Clams, scallops, mussels, oysters, and shipworms are among the most common bivalve species. Among them clams are one of the most widely distributed and used aquatic molluscs, offering

(Received : 05 April 2024 ; Revised Form Accepted : 18 June 2024)
https://doi.org/10.56153/g19088-024-0201-62

significantly more protein-rich food than mussels and oysters (Arun, 2015). Clams are economically significant as both a food and an industrial raw material. Barter, tools, decorations, pottery, cement, lime industries, chemicals, fertilizers, and flux material in iron and steel, ferro-alloy, and other metallurgical industries are all made from clam shells (Ajonina *et al.,* 2005). Aside from these, the shells are employed in biomedical applications such as artificial dental root implantations, orthopedic applications in bone restoration, and so on. Some bivalves are utilized in the treatment of diseases such as anemia, hypertension, labour pain, and constipation. (Ademolu *et al.*, 2015).

India has abundant clam resources along its coastline, including inshore seas, bays, backwaters, and estuaries (Mohite and Mohite, 2012). Clams are a commercially important Molluscan fishery resource in India, fished for their meat and shell. Clam shells occur extensively along the East Coast of India and in a few places along the West Coast of India. Clam shells occur in a few Indian states such as Kerala, Tamil Nadu, Goa, Maharashtra, Gujarat, Odisha, Group of Andaman and Nicobar Islands (Narasimham, 1991).Vembanad and Ashtamudi Lakes in Kerala are the richest clam-producing sites in India. Clam and Finfish fisheries are the main source of income for coastal communities near the lake (Sathiadhas and Hassan, 2004). Most of the annual production of *Villorita cyprinoides (*black clams), about 25,000 t, comes from Vembanad Lake **(**Suja and Mohamed, 2014). The black clam grows to a length of 30 mm by the end of its first year, and an additional 11 mm during its second year (Laxmilatha *et al.*, 2005). The black clam reaches sexual maturity at a length of 11 to 15 mm. The construction of the Thanneermukkom Bund across the Vembanad Lake, has had a significant impact on the reproduction pattern of various clams especially *V. cyprinoides* (Arun, 2009). The other clams harvested in the lake are the *Meretrix casta* (grey clam), *Paphiama labarica* (yellow clam), *Etroplus suratensis* (Pearl spot) and *Sunetta scripta* (Kurup and Samuel,1983). Vembanad Lake also includes huge sub-fossil clam shell deposits that are harvested for commercial purposes (Kripa *et al.*, 2004). The shells can also be found beneath some of the regions that surround the lake, such as rice paddy farms. This demonstrates that the lands were previously part of the lake but were covered by sediments (Rasalam and Sebastian, 1976). Apart from the previous studies, the current study focused on morphological and biochemical comparisons between *V. cyprinoides* and *M. casta* shells in Vembanad Estuary. It also aims to comprehend variations in clam distribution caused by salinity fluctuations and sediment texture.

Study Area

Vembanad is the largest estuarine system and most productive life-supporting coastal wetland in Kerala. Vembanad was designated as the second-largest Ramsar site on India's southwest coast in November 2002 because to its high biodiversity and ecological worth. The area spreads over Kottayam, Alappuzha and Ernakulam districts in Kerala, between Latitude 09°25′30″-10°10′30″N and Longitude 76°21′00″-76°31′30″E (Fig.1). It lies parallel to the coastline with a maximum length of 96.5km and covers an area more than 2033.02 km^2. The estuary opens into Arabian Sea at Cochin bar mouth and separated into northern and southern portion by Thanneermukkom Bund (Kolathayar *et al.*, 2021), a manmade barrier to prevent the seeping of saline water by the tidal action. The southern portion has fresh water, while the northern section possesses brackish water. The rivers which discharge fresh water into the estuarine system are Periyar and Muvattupuzha on the north and Manimala, Meenachil, Pamba and Achenkovil on the south (Haldar *et al.*, 2019).

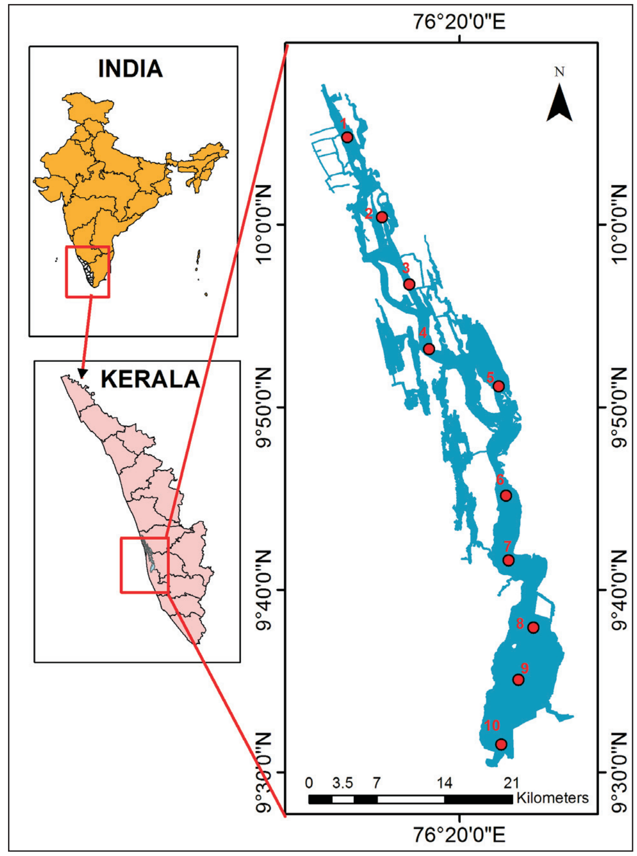

Fig.1. Location map of the study area

Methodology

Sample Collection and Pre-treatment

The clams were collected from 10 locations (Table 1) along northern and southern part of the Vembanad Estuary using van-Veen grab sampler during the month of October 2023. The bivalve shells in sediment samples were sieved onboard, numbered, and washed with water (Aswathy and Parvathi, 2018). The sediments and shells were then carefully placed in separate clean plastic bags for further investigation. The sampling locations were geographically determined in the field using the Global Positioning System (GPS).

Table 1: Geographic coordinates of sample locations

Sample No	Longitude	Latitude
1	76.227862	10.08477
2	76.242139	10.01862
3	76.28604	9.945567
4	76.299425	9.908983
5	76.373783	9.853363
6	76.372248	9.720839
7	76.393378	9.686004
8	76.383371	9.626288
9	76.420155	9.579061
10	76.375208	9.522683

Identification of Bivalve Species

The major bivalve species found in the Vembanad Estuary include *V. cyprinoides* (black clam), *M. casta* (grey clam), *P. labarica* (yellow clam) and *S. scripta* (Christy and Jayalakshmi, 2023). The collected bivalves were identified based on their exterior and interior shell morphological traits. The observed characteristics were the shell's shape and outline, sculpture, exterior colour, umbo, hinge ligament, internal colour, pallial line, pallial sinus, adductor muscle scars, and dentition.The morphological features of *V. cyprinoides* were studied in detail using the characteristics (Gray, 1825; Jijina *et al.*, 2023). *M. casta* shells were identified in accordance with Gmelin (1791) and Hamli (2015).

Measurement of Morphometric Parameters

The length, height, and width measurements are the morphometric parameters of a bivalve shell (Ambarwati *et al.,* 2021). Digital calliper was used to measure the morphometry of the collected shells.

Shell Length

The maximum distance from the anterior to the posterior end of the bivalve shell was measured. It provides insights into the overall size and growth of each specimen.

Shell Height

The maximum distance from the dorsal to the ventral side of the bivalve shell was measured. It indicates the vertical dimension and shape of the shell

Shell Width

The maximum distance between the valves when they were closed was considered as height.

Shell size

After the morphometric analysis, the collected specimens were sorted on the basis of their size such as big, medium and small. *V. cyprinoides* shell above 3.5cm size was considered a big shell. If the height of the shell ranges between 2.5 and 3.5cm, it is considered medium size shell, whereas the shell with a height less than 2.5 cm was considered a small shell. *M. Casta* shell with 1.5cm height was considered as medium size and the shells with height higher or lesser than 1.5 cm was considered as big or small shell, respectively. The size of all the shells were counted separately and recorded for distribution study.

Sample Preparation and Analytical Studies

The morphological analysis of shells needs various cleaning processes. The shells were immersed in clean water for one night before being cleaned with a hand brush. After that, the valves were immersed in a 2.5% sodium hypochlorite (NaClO) solution for 7 minutes to remove organic contaminants. They were cleaned with deionized water and dried in the sun for a day (Giordano *et al.*, 2022).The dried shells were crushed and ground into a fine powder using agate mortars. The powdered samples were sieved using a 230-mesh sieve, and 20g powder was extracted for analytical studies (Senthil, 2019*). The* X-ray fluorescence (XRF) analytical techniques were used to determine the biochemical properties and elemental composition of collected bivalve shells. The XRF analysis was conducted at the National Centre for Earth Science Studies, Thiruvananthapuram, using a Bruker model S4 Pioneer sequential wavelength-dispersive X-ray spectrometer equipped with a goniometer and a 4 kW Rh X-ray tube. The supporting software used for computer analyses can handle matrix effects, dead time correction, background, line overlap corrections, and other issues. After using a calibration curve to convert counts into concentration, the output was recorded directly as the concentration in weight percent or ppm.

Water Salinity and Sediment Texture Analysis

Salinity of water was measured in-situ using Hanna instrument- HI98319 Digital Salinity Tester on parts per thousand (ppt). Each surface sediment sample is completely dried in a hot air oven to eliminate the moisture content. A suitable quantity (weight determined) of each sample is then treated with 0.025 N sodium hexametaphosphate (Calgon) solution in order to facilitate deflocculating. The sample thus disaggregated is washed through a 230 ASTM sieve (mesh opening = 0.063 mm) until clear water passes through, taking care that the washings do not exceed 1000 ml. The portion of the sample retained on the sieve was dried and weighed for obtaining the weight of the sand fraction. The fine fraction (silt and clay) in the washings was analysed by the pipette method in accordance with the procedure adopted by Krumbein and Pettijohn (1938).

Results and Discussion

Morphology

Based on taxonomic characterization (Souji, 2018), 21 landmarks were identified in the bivalve (clam) shells. The morphological features of *V. cyprinoides* and *M. casta* shells were identified and their taxonomic hierarchy was ascertained based on these features (Fig. 2; Table 2).

Shell Morphology of Villorita cyprinoides

Moderately large, thick, ovately triangular, inflating oblique

Table 2: Taxonomic hierarchy of *Villorita cyprinoides* and *Meretrix casta* (Mollusca Base, 2024; World Register of Marine Species; https://www.molluscabase.org on 2024-01-14)

Kingdom	Animalia	
Phylum	Mollusca	
Class	Bivalvia	
Subclass	Autobranchia	
Infraclass	Heteroconchia	
Subterclass	Euheterodonta	
Superorder	Imparidentia	
Order	Venerida	
Superfamily	Cyrenoidea	Veneroidea
Family	Cyrenidae	Veneridae
Genus	Villorita	Meretrix
Species	Villorita cyprinoides	Meretrix casta

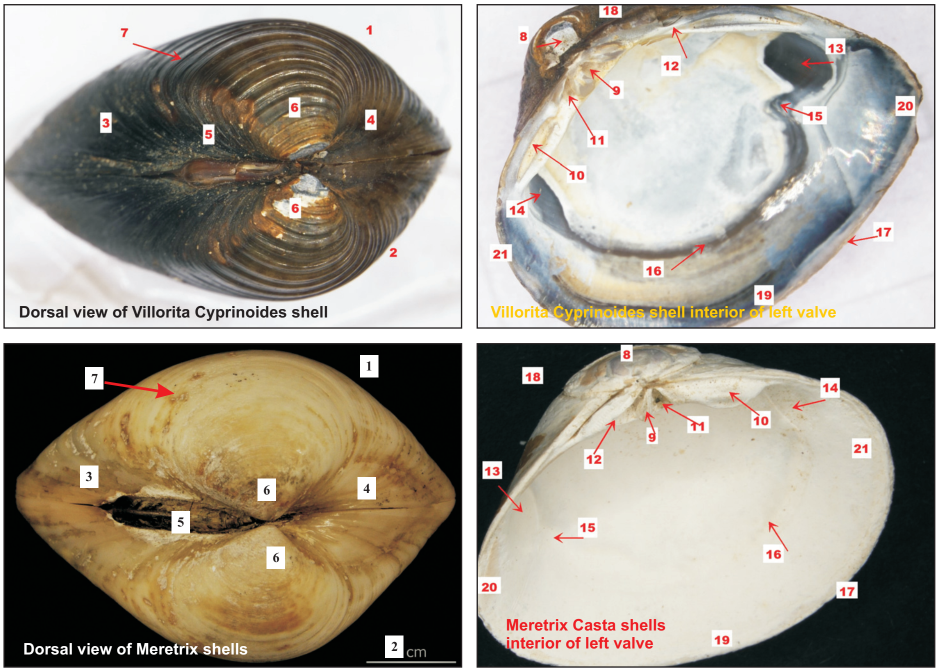

Fig.2. The identified morphological features of *Villorita cyprinoides* and *Meretrix casta* shells (1) Left valve, 2) Right valve, 3) Escutcheon, 4) Lunule, 5) Ligament, 6) Umbo, 7) concentric growth lines, 8) Beak, 9) Cardinal Tooth, 10) Lateral Tooth, 11) Socket, 12) Hinge plate, 13) Posterior Adductor Muscle, 14) Anterior Adductor Muscle, 15) Pallial sinus, 16) Pallial line, 17) Commisure, 18) Dorsal margin, 19) Ventral margin, 20) Posterior margin, 21) Anterior margin).

shell, swelled in the umbonal and central regions. Shell was scultured with concentric growth lines and ridges. The valves are inequilateral and have bilateral symmetry. Scars on the adductor muscles and the pallial line are visible. Pallial sinus: thin and rounded at the bottom. When the clams are alive, the shells are black; when they are buried, they turn white.

Shell Morphology of Meretrix casta

Shell is thick, moderately large, equivalve, and smooth. The umbo is prominently anterior, as are the beaks; the outer shell surface is pale yellowish grey, with a brown sticky periostracum; the inner shell surface is white, with a posterior dark purple ray; the shells are grey when the clams are alive, but turn white after burial. Scars from the adductor muscles and the pallial line are visible. Pallial sinuses are small and rounded at the bottom. Hinges and teeth are strong, and the umbones are inflated.

Morphometric Parameters

Length, Height and Width of all the collected samples from Vembanad Estuary were measured. After taking morphometric data of all collected specimens, their maximum, minimum and mean values were displayed using a bar-diagram (Fig. 3 a-c).

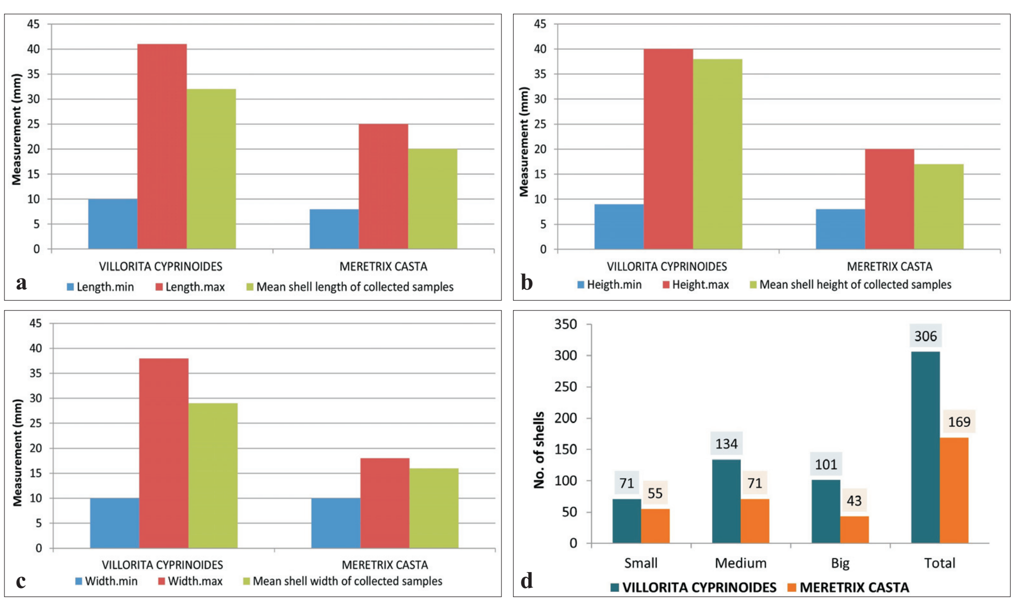

Fig.3. a) Variation in a) length, b) height, c) height and d) size wise distribution of *Villorita cyprinoides* and *Meretrix casta* shells in Vembanad Estuary

Shell Length

The maximum length of *V. cyprinoides* and *M. casta* shells in Vembanad Estuary is 41cm and 25cm, respectively. The minimum length of *V. cyprinoides*is 10cm and *Meretrix casta* shell is 8cm. The mean shell length for *V. cyprinoides* and *M. casta* shells was 32cm and 20cm, respectively.

Shell Height

The mean shell height of *V. cyprinoides* and *M. casta* shells was 38cm and 17cm, respectively. The maximum and minimum height of *V. cyprinoides* shells were 9cm and 40cm, respectively and 20cm and 8cm, respectively for *M. casta.*

Shell Width

V. cyprinoides showed minimum 10cm and maximum 38cm width. The mean width of collected *V. cyprinoides* shells was 29cm. *M. casta* shells showed 18cm maximum width and 10cm minimum width with the mean width of 16cm.

Shell Size

The size distribution of *V. cyprinoides* and *M. casta* shells in the Vembanad Estuary is depicted in figure 3(d). In both species medium size shells were found in more numbers.

The total number of 134 and 71 medium sized shells of *V. cyprinoides* and *M. casta*, respectively were observed. Small size *M. casta* shells (55) were more common than the large sized shells (43). On the other hand, big size *V. cyprinoides* (101) were more abundant than small size clam shells (71) in Vembanad Estuary (Fig. 4a-b).

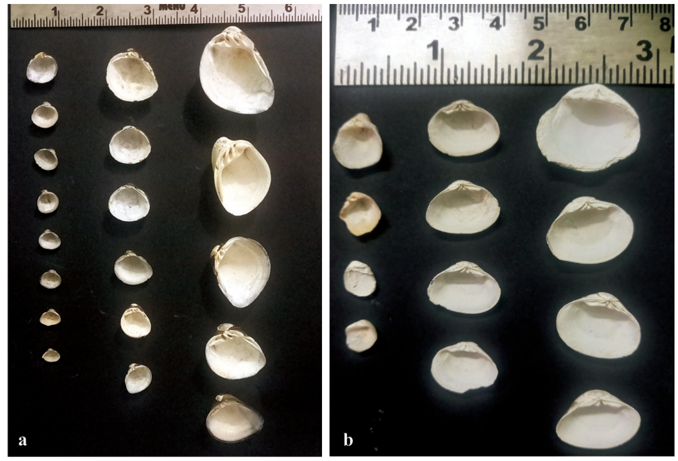

Fig.4. Size variation of a) *Villorita cyprinoides* and b) *Meretrix casta* shells in Vembanad Estuary

Clam Shells Distribution

The distribution of bivalves is determined by hydrodynamic variables, sediment types, and depth of substrates (Whetstone *et al.*, 2005). Sediment features influence the number and form of clams, whereas salinity influences the physiological and productive properties of clams (Cao *et al.*, 2022). A total of 306 shells of *V. cyprinoides* and 169 shells of *M. casta* shells were obtained from the ten sample locations of Vembanad Estuary. The location 10 showed the highest record of 73 shells of *V. cyprinoides* and location 1 records the highest numbers of 29 shells of *M. casta*. *V. cyprinoides* shells were absent in location 2 and *M. casta* shells were very less (2) in location 9. The density of *V. cyprinoides* shells were high in the southern portion (192) and *M. casta* shells (108) in the northern portion (Fig. 5). The population of *V. cyprinoides* in northern region

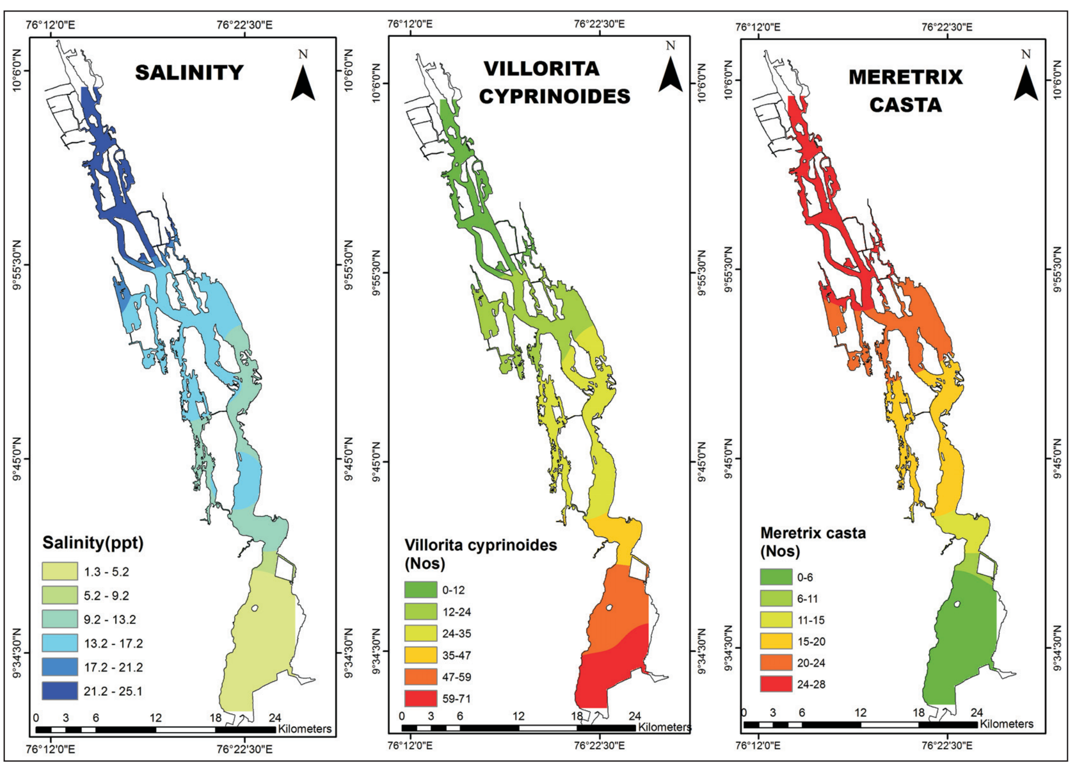

Fig.5. Salinity and clam shell distribution in Vembanad Estuary

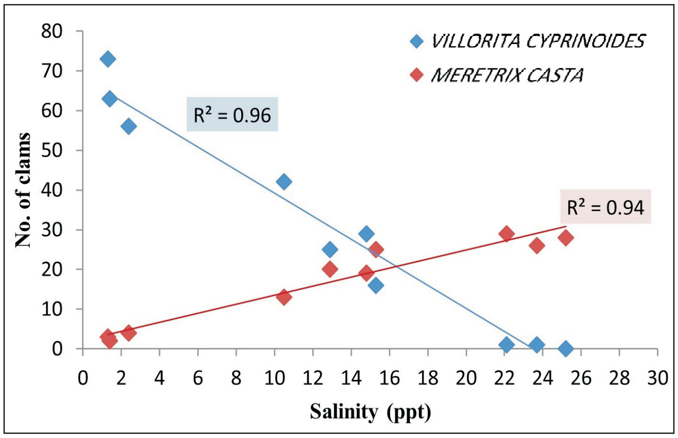

Fig.6. Correlation between Salinity and Clam shell distribution in Vembanad Estuary.

is about 18 shells and *M. casta* in southern region was about 9 shells. 96 shells of *V. cyprinoides* and 52 shells of *M. casta* were obtained from the central portion of the estuary.

Salinity and Clam Shell Distribution

Salinity is a key component that determines the distribution and population of clams (Ouseph and Jayalakshmi, 2023). The northern portion of the estuary has higher salinity (22.1-22.2 ppt) than the southern region due to the fresh water flux from main rivers contributes significantly to the low salinity (1.3-2.4 ppt) of the southern section (Fig. 5). According to the current study, the population of *V. cyprinoides* is higher in the estuary's southern fresh water section than in its brackish region. *M. casta*, on the other hand, is more prevalent in the northern brackish area. *V. Cyprinoides* showed a negative linear relationship with salinity with R^2 of 0.96, while *M. casta* had a favourable connection with salinity (0.94 R^2) (Fig. 6).

Sediment Texture and Clam Shell Distribution

According to Kripa and Salih (1999), clam density is greatest in clayey, sandy, or muddy estuary substratum. Based on the current study, majority of the clams live in estuary zones with clayey and sandy substratum. The northern half of the estuary was dominated by sand, whereas the southern portion was dominated by clay and silt (Fig. 7). *V. cyprinoides* was more common in clayey and silty sediments, while *M. casta* was abundant in sandy sediments (Fig. 7).

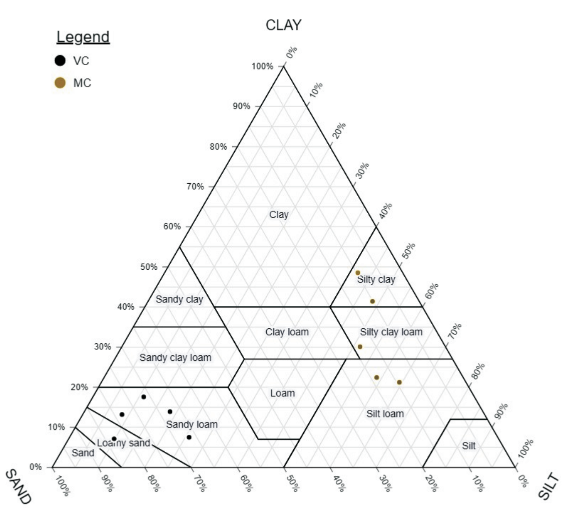

Fig.7. Sand, silt, clay ratio in the study area, according to the USDA Soil texture triangle (Soil Survey Division Sta , 1993)

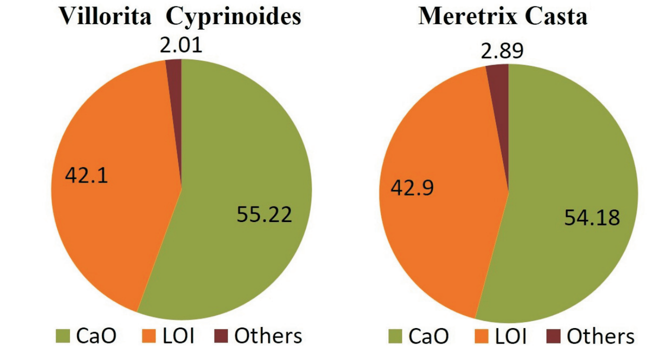

Fig.8. Major oxides in *Villorita cyprinoides* and *Meretrix casta* shells in the Vembanad Estuary.

Biochemistry

Oxides

The XRF biochemistry of shells showed the percentage of Calcium Oxide (CaO) of *V. cyprinoides* and *M. casta* in the study area (Table 2). The results showed that the CaO concentration is higher in both clam species. The primary oxides of shells in the study area revealed that *V. cyprinoides* accounts for approximately 55.22% of the total, whereas *M. casta* accounts for approximately 54.18% (Fig. 8). All other oxides were present in low concentrations and contribute roughly 2.01% and 2.89% in the shells of *V. cyprinoides* and *M. casta*, respectively (Fig. 8).

Loss on ignition (LOI)

LOI includes gases, contaminants such as inorganic materials, water and others. These contaminants are frequently found in organic shells (Adarsh and Senthil, 2018). LOI is approximately 42.1% in *V. cyprinoides* and 42.9% in *M. casta* shells of the study area.

Elements

The elemental content (wt.%) of *V. cyprinoides* and *M. casta* shells in the Vembanad Estuary (Table 3) showed that Ca is

Table 3: Major oxides and element concentrations in *Villorita cyprinoides* and *Meretrix casta* shells of the study area.

Oxides	*Villorita cyprinoides* (m/m%)	*Meretrix casta* (m/m%)	Element	*Villorita cyprinoides* (Weight %)	*Meretrix casta* (Weight %)
CaO	55.22	54.18	Ca	39.47	38.72
Al_2O_3	0.21	0.07	Al	0.11	0.04
Fe_2O_3	0.23	0.14	Fe	0.16	0.10
SiO_2	0.43	0.21	Si	0.20	0.10
MgO	0.39	1.21	Mg	0.24	0.73
Na_2O	0.42	0.24	Na	0.31	0.18
K_2O	0.12	0.001	K	0.10	0.00
SrO	0.21	1.02	Sr	0.18	0.86
LOI	42.1	42.9			

particularly abundant in both species when compared to other elements. The other elements found in shells are insignificant and have only been found in trace levels. The comparative study of elements in *V. cyprinoides* with *M. Casta* indicated that the former has Ca concentration of 39.47%, while 38.72% in latter.

Conclusions

The present study demonstrates that the distribution patterns of *Villorita cyprinoides* and *Meretrix casta* in the Vembanad Estuary have a strong link with the salinity of the water and the texture of the sediments. *V. cyprinoides* lives in the upper and middle sections of the estuary, where salinity is low, but *M. casta* lives near the bar mouth, where salinity is high. This indicates that *V. cyprinoides* had a negative linear correlation with salinity, while *Meretrix casta* had a positive correlation with salinity. Not only salinity, but also sediment texture, influences the distribution and density of clam shells in Vembanad Estuary. *V. cyprinoides* was present in clayey and silty sediments, whereas *M. casta* was found in sandy areas. When compared to the whole clam population, the density of *V. cyprinoides* shells was greater than that of *M. casta* shells. Furthermore, medium-sized shells were found to be more abundant in both species. The XRF study showed that Calcium Oxide (CaO) was most abundant in both the shells and all other elements were only discovered in trace amounts. Ca concentrations in *V. cyprinoides* and *M. casta* were 39.47% and 38.72%, respectively. This analysis can be used to evaluate the changes in quantity and quality of clam shells in the Vembanad Estuary accordance with the salinity and textural changes of sediments.

Acknowledgements

The authors sincerely thankful to Head of Department, Department of Geology, University of Madras for providing lab facilities to carry out this work

Conflict of Interest

There will be no conflict of Interest for the authors.

Authors' Contributions

AMJ: Sample Collection, Sediment and Geochemical Analysis, Methodology, Manuscript Drafting. **MSG**: Species Identification, Interpretation and Data Analysis, Reviewing and Editing.

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