

SULFIDE OXIDIZING ACTIVITY AS A SURVIVAL STRATEGY IN MANGROVE CLAM *POLYMESODA EROSA* (SOLANDER, 1786)

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ABSTRACT: The clam *Polymesoda erosa* is a large dioecious bivalve mollusk found in the mangroves restricted to the high tidal *Avicennia* sp. zone of the mangrove forest. The present study was aimed at understanding the occurrence of *P. erosa* in mangroves in relation to its ability to oxidize sulphide. In January 2011, sampling was conducted in 4 different mangrove forests viz. Siolim, Nerul, Chorao and Chapora (locations) in Goa. Sediment bacterial abundance was statistically similar at all the sites with high values at Chapora (2.5×10^9 to 4.5×10^9) and lower values were at Siolim (1×10^9 to 2×10^9). The total number of bacteria per gram of fresh tissue of *P. erosa* was highest (3.8×10^9) in Chorao station in the size class of 7.0-7.9 cm whereas; bacterial count was lowest in the same size class clams from Nerul (0.5×10^9). Cytochemical staining for sulfide oxidizing activity with Benzyl viologen resulted in the formation of purple color at the sites of sulfide oxidation in clam tissues from all collection sites. Gill tissue showed the intense purple color compared to foot and mantle. Both highest as well as lowest concentration of sediment sulfide was recorded in Nerul compared to other stations and ranged from 0.1-11.4 mM/g of sediment. Both highest (3.31 IU/g) as well as lowest (0.7 IU/g) values of sulfide oxidizing activity of clam tissue were recorded at Chorao in the specimens of 5.0-5.9 and 6.0-6.9 cm size class respectively. *P. erosa* may be a symbiotic bivalve as it contains endosymbionts in its gill tissue in addition to sulphide oxidizing regions and sulphide oxidase enzyme activity. All these characteristics may enable the clam to use it as an additional source of food or for its survival in the sulphide containing sediments of the mangroves.

Keywords: Sulphide oxidizing activity, Endosymbiosis, Mangrove clam, *Polymesoda erosa*, Goa; West coast of India.

INTRODUCTION:

Chemoautotrophic endosymbiosis, a nutritional strategy is found in an increasing number of marine invertebrates. With the aid of bacterial endosymbionts, these "chemosymbiotic" animals subsist on nutrients and energy sources, including hydrogen sulfide and carbon dioxide, which are normally unavailable to animals. Recent discoveries of sulfur-based symbioses in marine molluscs from a variety of habitats show that these animal-bacteria symbioses are not unique to hydrothermal vents but are a ubiquitous component of such hydrogen sulfide-rich habitats as anoxic basins (Felbeck *et al.*, 1981); seagrass beds (Fisher and Hand, 1984); mangrove swamps, pulp mill effluent sites (Reid, 1980) and sewage outfalls (Felbeck, 1983). Symbiotic bacteria are assumed to provide the mollusc with chemosynthetically fixed carbon dioxide via aerobic oxidation of sulfide in return of a "controlled" environment in the gills. Although this assumption is reasonable, and enzymes necessary for sulfur metabolism and the Calvin-Benson cycle have been demonstrated (Felbeck, 1983), alternative explanations such as a detoxifying role for the bacteria, with dissolved organic matter providing the bulk of animal nutrition, should not be excluded. These bacteria have enzymes of the Calvin-Benson cycle (Felbeck *et al.*, 1983; Fisher and Hand, 1984), and the energy (reducing power and ATP) needed for net CO₂ fixation (Jannasch, 1984). Enzymes involved in ATP- and NAD (P) H-generating sulfide oxidation pathways have been found in these symbioses (Felbeck *et al.*, 1981).

In order for chemosynthetic symbioses to thrive, symbionts must have access to an electron donor, used as an energy source (sulfide), and an electron acceptor (oxygen or nitrate). Chemo (thio) autotrophic associations depend upon the productivity of their symbiotic bacteria, which require sufficient sulfide availability. For this reason, both the supply of sulfide and sulfide-related physiological processes may limit production

by the symbionts or host or both. However, hydrogen sulfide (H₂S) occurs naturally in a number of marine aquatic environments, including hydrothermal vents, cold seeps, mudflats, and marshes. In each of these environments, diverse communities of animals adapted to the presence of H₂S have been found (Childress 1995; Fisher 1990). The best characterized toxic effect of sulfide is its reversible inhibition of cytochrome *c* oxidase, the terminal element of the mitochondrial electron transport chain, blocking ATP production via oxidative phosphorylation in the electron transport system.

Two main strategies of sulfide detoxification appear to be common in animals in normoxia. First, sulfide can be bound to blood proteins (Bagarinao and Vetter 1992). Second, sulfide is frequently oxidized to less toxic or nontoxic sulfur compounds, either with the help of bacterial symbionts (Wilmot and Vetter 1990) or in the animal tissue (Vetter *et al.* 1987).

As a group, thiotrophic (sulfide-utilizing) bacteria employ various enzymatic pathways for conversion of sulfide into energy, including the oxidation of sulfide, thiosulfate, sulfite, and elemental sulfur, used to fuel inorganic carbon reduction via the Calvin-Benson cycle (Nelson & Hagen, 1995). For endosymbionts, these pathways include the oxidation of sulfide by metal-containing proteins or by specific enzymes known as sulfide oxidases. During the process of bacterial sulfide oxidation, elemental sulfur, thiosulfate, and polysulfides can be formed as intermediate products, while sulfate is formed as an end product. The accumulation and/or presence of intermediate and end products of sulfide oxidation in the tissues of bivalves can provide information about specific pathways and energy yield by symbiont sulfide oxidation.

The mangrove clam (*Polymesoda erosa*) is a large dioecious bivalve mollusk found in mangroves throughout Southeast Asia (Morton, 1975). The clam is usually found in the

landward fringe of mangroves where they are generally only covered by spring tides, and able to survive extended periods of exposure and inundation by freshwater during the wet season (Morton, 1975). Mangrove clams are found commonly in exposed tidal areas dominated by mangrove tree species such as of *Avicennia matima* (Meehan, 1982). *P. erosa* can withstand long periods of exposure, during which time it can use subterranean water contained in the burrow. Any particles present in this water are taken into the mantle cavity via the pedal gape and so into the alimentary canal. This is an extreme adaptation to a semi terrestrial mode of life. Aerial respiration is also achieved via the mantle margin (Morton, 1976).

It was hypothesized that *P.erosa* contains endosymbiotic bacteria in their gill tissue in different mangrove habitats to oxidize the sulphide present in sediment. And it oxidizes the sulphide via sulphide oxidase enzyme system present in the clam tissue.

No reports of chemoautotrophic symbiotic relationship between the bacteria and mangrove clam *P. erosa* have been reported till date inspite of the fact that *this clam* inhabits one of the high sulphide-containing habitats (Ingole et al., 1994). The earlier literature on the *P. erosa* (Morton, 1976; Ingole et al., 2002; Clemente, 2007, Meehan, 1982) showed that, this clam is restricted to the high tidal zone of the mangrove forest where *Avicennia* sp. is dominant. However, none of the previous studies justify the reason responsible for the distribution of *P. erosa* in the high tidal zone. Hence the present study was aimed at understanding the occurrence of *P. erosa* in mangroves in relation to its ability to oxidize sulphide.

MATERIALS AND METHODS:

Description of the study site:Goa (3702 km²) is located on the western coast of Indian peninsula and separated from Maharashtra by the Terekhol River in the North, Karnataka in the South, the Western Ghats in the east, and Arabian Sea in the west. Goa is located between the latitudes 15°48'00" North to 14°53'54" North and longitudes 74°20'13"East to 73°40'33"East.

In January 2011, sampling was conducted in the high intertidal zone of the mangrove during a spring low tide. Sampling was conducted in 4 different mangrove forests Siolim, Nerul, Chorao and Chapora (locations) in Goa (Fig. 1). The survey was mainly concentrated in the high tide region as well as in places where *Avicennia* species of mangrove are generally dominant as preliminary studies have identified that *P. erosa* tend to be concentrated near the *Avicennia* species. Siolim is situated on the bank of Chapora River at lat. 15°37'7" N; long.73°45'39" E and mangrove area is 691 m². Chapora is situated on the bank of river Chapora River at lat. 15°37'49" N; long. 73°45'39" E and has mangrove covered area of 226 m². Nerul is situated in the same creek as Verem at lat. 15°30'36" N; long.73°46'43" E with mangrove area of approximately 1172 m². Chorao Island is situated on the bank of Mandovi River and is a protected area declared as a bird sanctuary at lat. 15°30'49" N; long.73°51'21" E and mangrove area of 1800 m².

Sampling: Collection of clams:Specimens were collected from four mangrove sites Nerul, Chapora, Siolim, and Chorao in Goa. For the quantitative analysis, the mangrove clams were collected by hand picking in a transect of 500m² long and 300

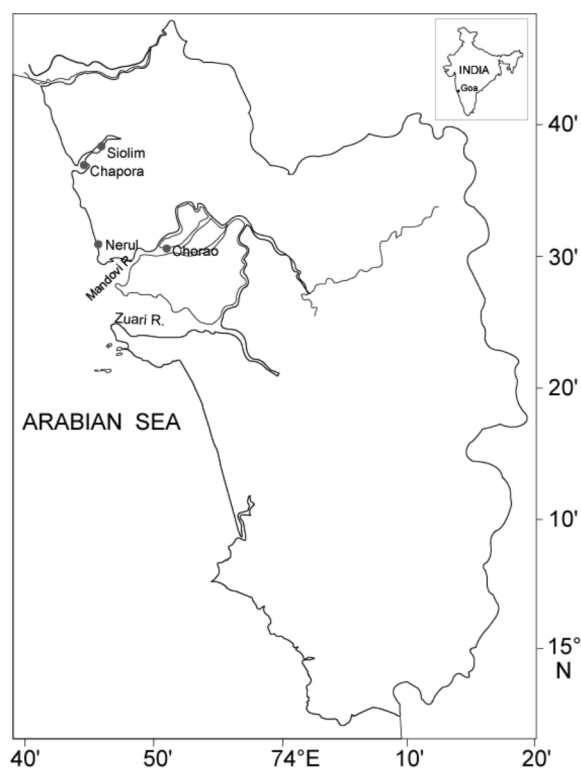


Fig. 1: Map showing the sampling locations for the distribution and abundance of *Polymesoda erosa*

M² wide from a marked area of 1 m² and live specimens were transported in plastic crates to the laboratory. Fresh clam tissues were used for each analysis. Following field collection, the bivalves were kept in running water overnight to allow any sediment to be cleared from the mantle cavity and gut. Then, the intact clams were washed, blotted dry and then left for a short time (10-15 minutes) to air dry the shell surface to dry before being measured for total length, width, height and weighed. The morphometric variables recorded were total length (TL) width and height; total wet weight of soft tissues was measured. Organs like gill, foot, and mantle were separately weighed and frozen at 80°C for enzyme analysis, for bacterial identification and for sulfur oxidizing bodies' identification (SOBs) fresh clams were collected immediately and processed and analysed within 24 hours. All variables were measured to the nearest 0.01mm with a Vernier caliper. The wet tissues were blotted and their weights were measured to the nearest 0.01g using a Mettler PB602-S electronic balance.

Sediment Collection:Sediment samples were collected by excavating around 0.125 m² area (from fixed points in duplicates) using a steel shovel in the vicinity of the clams and were fixed immediately in the field with zinc acetate. The clams were transported immediately in iceboxes to the laboratory.

Bacterial abundance in mangrove sediment:The top 5 mm of sediment was subsampled and preserved for staining with DAPI (Porter and Fieg 1980). The protocol was modified for staining of sediment samples by suspending the sediments in 10 ml of 0.2 µm filtered phosphate buffered saline (PBS: NaCl, 8 g; KCl, 0.2 g; Na₂HPO₄, 1.15 g; KH₂PO₄, 0.2 g; distilled

water, 1.0 l; pH = 7.3). The suspended sediment was preserved with formalin (2% final concentration, filtered and sodium borate buffered) and stored at 4°C until staining (within 1 wk). The slurry was diluted (200 ml into 0.8 ml PBS) and DAPI was added to a final concentration of 25-µg/ml and allowed to react for 5 min in darkness. Samples were filtered onto polycarbonate filters. Bacteria on slides were counted at 1600× on a Zeiss Axioscope epifluorescence microscope.

Identification of Gill endosymbionts in *Polymesoda erosa*:

For the identification of gill endosymbionts in *P.erosa*, the gill tissue was carefully removed from the clams and the tissue weight was measured. It was rinsed in 0.2 µm filtered seawater and homogenized (1:5 w: v) with mortar and pestle in 100 mM PO₄ buffer (pH 7.4). The extract was filtered through 600 micron Nitex screen and centrifuged (1 min at 1000g) to remove cell debris. An aliquot was diluted 1:10, and fixed in 5% formalin and stored at 4 °C. For bacterial counting, the extracts were diluted further (1:20 to 1:50) to obtain optimal counting densities. To 1 ml of extract, 1 µl of DAPI (4, 6-diamidino-2- phenyl indole, 1 mg/ ml) was added and allowed to stain for 10 min in dark. Then, 1 ml aliquots were filtered onto 0.2 µm polycarbonate filters (HA type, Costar) by vacuum using a 16 mm glass tower (Millipore). The filter was affixed on microscope slides with paraffin oil. Endosymbiotic Bacteria was counted by epifluorescence microscopy using an Olympus BH-2 microscope equipped with a 100-0.45µm-grid hemocytometer (American Optical Brightline).

Cytochemical localization of Sulfur oxidizing bodies in *P.erosa*:

Tissues were taken from animals within 30 min of capture, and fixed in freshly made 3% Glutaraldehyde in 0.1 M Phosphate buffer, pH 7.35, containing 0.35 M Sucrose, at room temperature for 90 min, and stored at 4°C. After returning to laboratory, tissues were rinsed 3 times in phosphate/sucrose buffer lacking Glutaraldehyde and post fixed for 1 h in 1% OsO₄ in phosphate/sucrose at 0°C. Tissues were then rinsed in distilled H₂O and dehydrated through graded ethanol solutions (30%, 50%, 70%, 90% Ethanol), and then the tissue was immersed in Xylene for 30 minutes and stored in paraffin wax for further use. The tissues were then embedded in paraffin wax block and were cut into 5µm section using a Weiber Spencer microtome. Sections were de-waxed in Xylene for 2-4 hrs and again dehydrated through graded ethanol solutions (90%, 70%, 50%, 30% ethanol). Finally the tissues were rinsed in distilled water and stained. Staining was done by addition of 0.2 M glycine pH 9.0, 5 mM Na₂S, 2 mM Benzyl viologen that was freshly prepared. A drop of staining solution was applied to the section and was covered with a cover slip. Ecological and physiological conditions also influence the sulfide physiology and energy immediately. Purple color developed in less than 5 min in positively staining areas and the absence of purple color indicates the absence of bacteriocytes.

Sediment sulphide analysis: Sediment sulfide analysis was done using a combination of the methylene blue and iodimetric methods from Fonselius (1985) and Standard Methods (APHA 1998).

Determination of Sulfide-Oxidizing Activity: The sulfide-oxidizing activity was determined by measuring the product of enzymatic reaction, SO₄²⁻ in the reaction mixture. The enzyme assay protocol was a modified version of Hirano *et al.* (1996) enzyme assay (Asku, Z. 1991).

RESULTS:

Bacterial abundance in Mangrove sediment: Overall higher values of sediment bacterial abundance were observed at Chapora ranging from 2.5 x 10⁹ to 4.5 x 10⁹ and lower values were at Siolim ranging 1 x 10⁹ to 2 x 10⁹. Bacterial size varied from 3µm to 5 µm in all stations. The sediment bacterial count was closer between Nerul and Chorao (Fig.2). The main feature of the bacterial abundance distribution is the almost similar values between all the sites without any indication of significant difference (ANOVA p<0.005; Table 1; Plate 1).

Gill Endosymbionts in *P.erosa*: Total number of bacteria counted at different sites and different size classes is shown in figure 3, Plate 1. The total number of bacteria per gram of fresh tissue weight was highest (3.8 x10⁹) in Chorao station in the size class 7.0-7.9 whereas the bacterial count was lowest in the same size class at Nerul (0.5x10⁹). The count was also lower at Chapora (1.8 x10⁹) in this size class. The bacteria in all stations in the size class 8.0-8.9 were similar and ranged between 2.5-3.3 at Chapora and Nerul respectively. At Chorao no clams in this size class were recorded in the present study.

Bacterial size range varied from 0.2 to 3 µm in all stations. The total count of bacteria did not show any significant variation between sites and size class (p<0.005; Table 2; Plate 1).

Cytochemical localization of sulfide oxidizing activity: In this study, cytochemical localization of sulfide oxidizing activity did not show the bacteriocytes (the cell containing sulfide oxidizing bacteria) clearly but the stains were observed on whole tissue. Cytochemical staining of sulfide and Benzyl viologen resulted in the formation of the purple color at the sites of sulfide oxidation. No colour was formed when Benzyl viologen was added without sulfide. Unstained sections show the yellow-brown color. Gill tissue shows the intense purple in color at the site of sulfide oxidation compared to foot and mantle in all stations (Plate 2).

Sediment sulfide concentration: Sediment sulfide concentration was high in Nerul compared to other station; the highest concentration level in this station was 11.4 mM/g of sediment (Fig.4). At Nerul the lowest levels of sulphides observed in the present study compared to all the sites was also recorded with a value of 0.1. In general sediment sulphide concentrations were lower at Chapora and Chorao and the lowest concentration level was 0.3 at both the sites. Highest concentration of sulphides observed at Chapora and Chorao were 6.3 and 5.2 respectively. At Siolim the highest level was 8.5 and the lowest level was 0.7 (Fig.4). There was no significant variance between station and sulfide concentration (ANOVA p<0.005; Table 3)

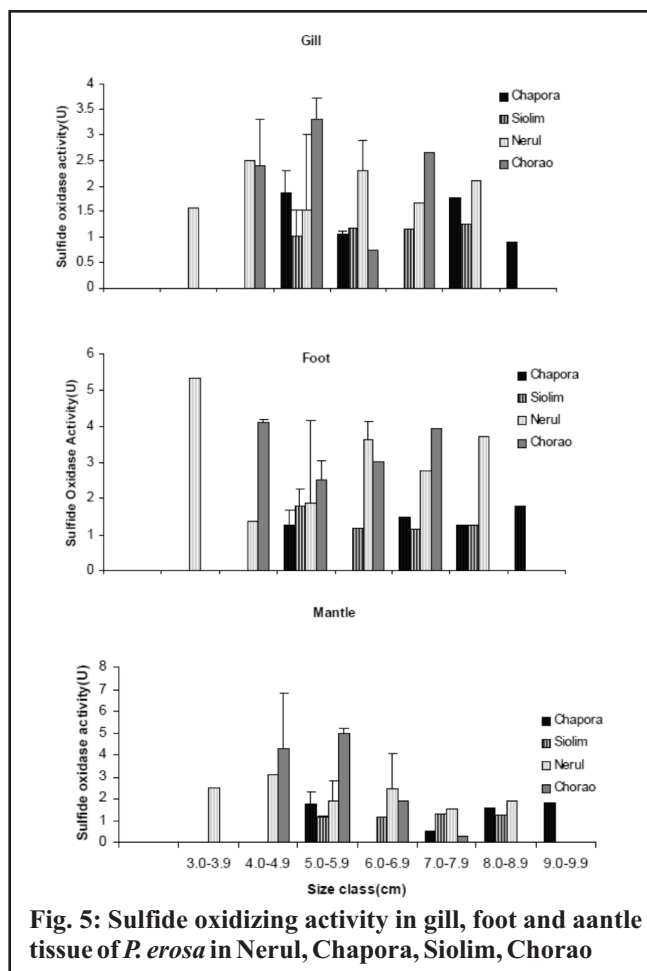
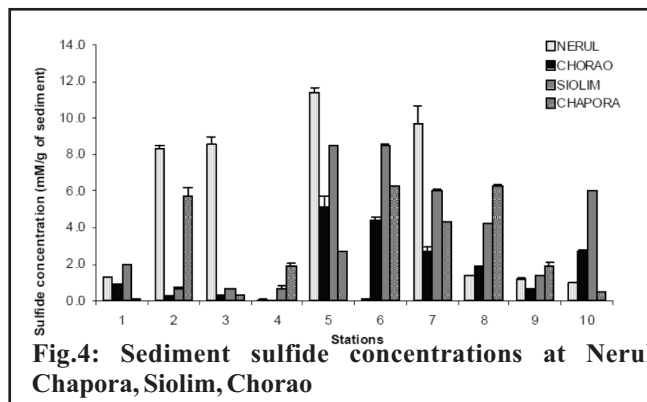
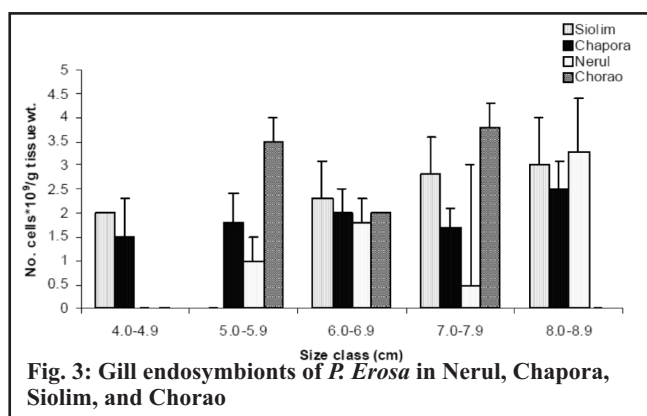
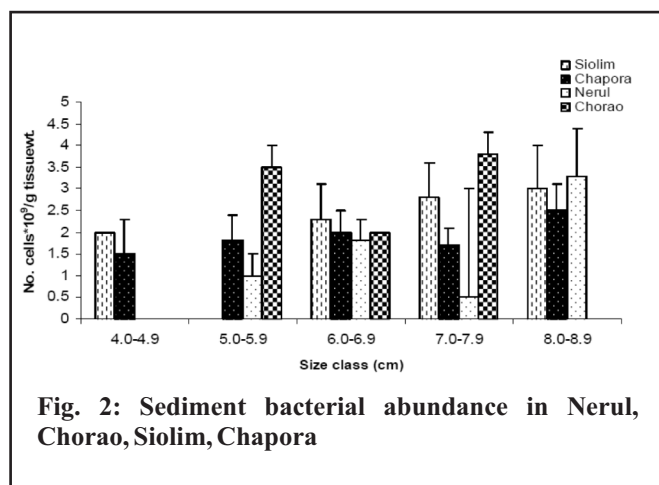
Determination of Sulfide oxidizing activity: In gill tissue, sulfide oxidizing activity was high in Chorao station, size class 5.0-5.9 cm and activity was 3.31 U/g of tissue, lowest activity also observed in 6.0-6.9 cm and activity was 0.73 U/ g of tissue. In Chapora, the highest activity was 1.88 U/g of tissue, in the size class 4.0-4.9 and lowest activity was 0.90 U/g of tissue, in the size class 9.0-9.9 and Siolim highest activity was 1.25 U/g of tissue in the size class 8.0-8.9 and lowest activity was 1.017 in the size class 5.05-5.9 cm (Fig. 5)

In foot tissue sulfide oxidizing activity was high in Nerul station 5.35 U/g of tissue in the size class 3.0-3.9 cm and

lowest activity was observed in Siolim station 1.154 U/ g of tissue in the size class 7.0-7.9 cm. In Chapora station highest activity was 1.758 U/g of tissue in the size class 9.0-9.9 and lowest activity was observed in 1.251 U/g of tissue and Siolim station highest activity was 1.761 U / g of tissue and lowest activity was 1.154 U/g of tissue observed in 7.0-7.9 cm size class and Chorao station highest activity was 4.089 U/ g of tissue observed in 4.0-4.9 size class (Fig.5).

In mantle tissue sulfide oxidizing activity was high in Chorao station 4.993U/g of tissue in the size class 5.0-5.9 cm and lowest activity was also observed in Chorao station 0.282 U/ g of tissue in the size class 7.0-7.9 cm. In Chapora station highest activity was 1.796 U/ g of tissue, in the size class 9.0-9.0 cm and lowest activity was 0.496 U /g of tissue in the size class 7.0-7.9 cm and Siolim station highest activity was 1.318 U/ g of tissue in the size class 7.0 7.9 and lowest activity was 1.115 U/ g of tissue in the size class 5.0-5.9 cm and Nerul station highest activity was 3.07 U/ g of tissue in the size class 4.0-4.9 and lowest activity was 1.49 U/ g of tissue in the size class 7.0-7.9 (Fig.5). Results of ANOVA showed significant difference in sulfide oxidizing activity of the clams between sites (p-value <0.005; Table 4).

Correlation between Clam Growth Parameters, sediment sulphide, sediment bacteria and endosymbiotic bacteria: There was no significant correlation between clam growth parameters and gill endosymbiotic bacteria at all the sites except for Nerul which showed a positive correlation of foot ($r=0.825$, $P=0.003$) and mantle tissue weight ($r=0.711$, $P=0.021$) with gill endosymbionts. When sediment sulphide concentration and sediment bacterial abundance and gill endosymbionts abundance was correlated no correlation was observed among the variables at any of the sites studied (sulphide concentration gill vs endosymbionts: Nerul $r=0.06$, $p=0.86$; Chorao $r=0.12$, $p=0.73$; Siolim $r=0.51$, $p=0.13$; Chapora $r=0.13$, $p=0.7$; sediment bacteria vs gill endosymbionts: Nerul ($r=0.11$, $p=0.75$), Chorao ($r=0.03$, $p=0.92$), Siolim ($r=0.15$, $p=0.67$), Chapora ($r=-1$, $p=0.06$). When sediment sulphide concentration and sediment bacteria were checked for correlation in all stations there was no correlation between the two (Nerul $r=0.42$, $p=0.22$; Chorao $r=0.42$, $p=0.02$; Siolim $r=0.4$, $p=0.24$; Chapora $r=0.38$, $p=0.27$).



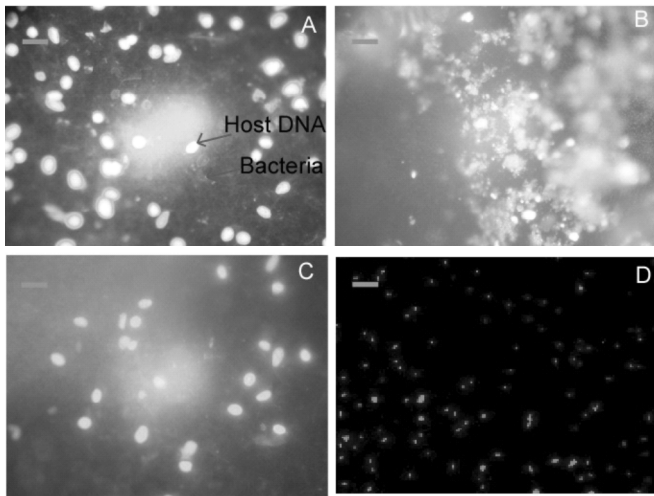


Plate 1: Epifluorescent view of DAPI staining of *Polymesoda erosa* gill endo symbionts in (A) Nerul (B) Siolim (C) Chapora (D) sediment bacteria. (Scale bar = 3µm).

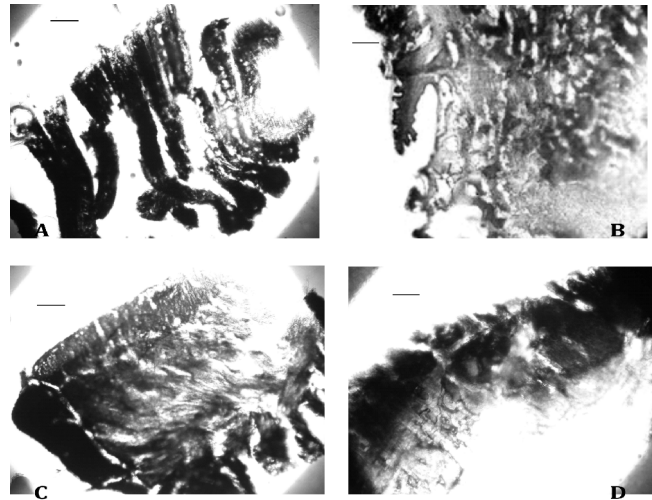


Plate 2: Sulfide oxidation activity staining of 5µm section of *Polymesoda erosa* (A) Gill Lamellae, (B) Internal view of Gill lamellae (C) Foot, (D) Mantle tissue. Scale bar = 5µm. Purple colour indicates the positively stained sulphide oxidizing regions.

Table 1: Results of ANOVA among sediment bacterial abundance between Nerul, Chorao, Siolim and Chapora.

| Source of Variation | SS | DF | MS | F | P-value |
|---------------------|----------|----|---------|----------|----------|
| Bacterial abundance | 297.57 | 1 | 297.57 | 5.81336 | 0.02113 |
| Site | 118.621 | 3 | 39.5403 | 0.772462 | 0.517033 |
| Error | 1842.744 | 36 | 51.1873 | | |

Table 2: Results of ANOVA among Gill endosymbionts of *P. erosa* in Nerul, Chapora, Siolim and Chorao:

| Source of Variation | SS | DF | MS | F | P-value |
|---------------------|----------|----|----------|----------|----------|
| Site | 1.44950 | 3 | 0.48317 | 0.27255 | 0.844034 |
| Size class | 5.09500 | 4 | 1.27375 | 0.71852 | 0.595435 |
| Site x size class | 63.01250 | 1 | 63.01250 | 35.54506 | 0.000066 |
| Error | 21.27300 | 12 | 1.77275 | | |

Table 3: Results of ANOVA among Sulfide concentration at Nerul, Chapora, Siolim, and Chorao

| Source of variance | SS | DF | MS | F | P |
|-----------------------|----------|----|----------|----------|----------|
| Sulfide Concentration | 1341.727 | 1 | 1341.727 | 5.273313 | 0.027582 |
| Sites | 523.825 | 3 | 174.608 | 0.686253 | 0.566327 |
| Error | 9159.739 | 36 | 254.437 | | |

Table 4: Result of ANOVA for Sulfide Oxidase activity of *P. erosa* on Sites, Size Class and Organs

| Source of Variation | SS | DF | MS | F | P-value |
|----------------------------|----------|----|----------|----------|----------|
| Site | 26.1602 | 3 | 8.7201 | 6.7138 | 0.000466 |
| Size class | 21.1994 | 6 | 3.5332 | 2.7203 | 0.019425 |
| Organs | 2.4443 | 2 | 1.2221 | 0.9410 | 0.395002 |
| Site X Size Class X Organs | 139.8360 | 1 | 139.8360 | 107.6624 | 0.000000 |
| Error | 93.5163 | 72 | 1.2988 | | |

DISCUSSIONS:

The results indicate that *P. erosa* contain endosymbiotic bacteria which is confirmed by the presence of sulphide oxidizing regions as well as the sulphide oxidase activity which occurs in the tissue of *P. erosa*. Oxidation of sulphide is known to result directly in ATP synthesis by the bacteria in *Riftia pachyptila* (Powell & Somero, 1986) and in the mitochondria of the gutless protobranch clam, *Solemya reidi* (Powell & Somero, 1986). The presence of sulphide-metabolizing activity (Felbeck *et al.*, 1981) suggests an affinity with the chemoautotrophic sulphur oxidizing bacteria. The presence of large internal sulphide regions in this clam are not typical of the previously described (Powell and Somero, 1985, Vetter, 1985) and suggests that *P. erosa* symbionts may be related to one of the less well studied genera which contain internal sulphur globules and are previously undescribed. The absence of solid sulphur globules from the sections with standard cytological methods suggests that descriptions of large sulphur globules in the tissues of *P. erosa* should be viewed with some caution.

Sulphide-oxidizing bacteria in a symbiotic relationship require sulphide as well as oxygen simultaneously for the symbiotic relationship to be beneficial. For this reason, bivalves with endosymbiotic bacteria are always found at the interface between oxidizing and reducing habitats. *Lucinoma annulata* and *Calyptogena elongata* occur on the silts of the Santa Barbara basin at the interface between the anaerobic, sulphide-containing bottom waters, the bivalve *Lucina floridana* occupies an interface between the aerobic overlying oxygenated surface waters (Vetter, 1985) near the rhizosphere of seagrass roots and the surrounding reduced sediments (Fisher and Hand, 1984). *P. erosa* also occupies the aerobic rhizosphere of mangrove *Avicennia* roots and the surrounding reduced sediments (Meehan, 1982; Ingole *et al.*, 2002) and could be the main reason for its occurrence only near the *Avicennia* plant. The interaction between sulphides in the mangrove environment and the mangrove vegetation itself has been studied by a number of workers. Nickerson and Thibodeau proposed that the distribution of *Avicennia* spp. and *Rhizophora* spp. may be related to the soil concentrations of hydrogen sulphide and to the different abilities of these two mangrove species to oxidize the anaerobic substrate (Nickerson & Thibodeau, 1985). McKee and co-workers in their work with *Avicennia germinans* (black mangrove) and *Rhizophora mangle* (red mangrove) on a mangrove island in the Belizean barrier reef system showed that the pore water sulphide concentration and the soil redox potentials were significantly correlated with the presence of mangrove roots (aerial roots and pneumatophores) (McKee *et al.*, 1988). It was shown that sulphide concentrations were three to five times lower near the prop roots of *Rhizophora mangle* and the pneumatophores of *Avicennia germinans* than in adjacent (< 1 meter away) unvegetated sediment. It was also discovered that sulphide concentrations in areas occupied by *Avicennia germinans* was a decreasing function of the pneumatophore density. This observation was in line with results reported earlier by Thibodeau and Nickerson (Thibodeau & Nickerson, 1986) who discovered that *Avicennia germinans* created oxidized rhizospheres substantially larger than those described for other plant species. The presence of low sulphide

levels in the regions occupied by *P. erosa* could be due to its ability to utilize sulphide. Reynolds *et al.* (2007) have reported low sediment sulphide concentration in areas that were in close proximity to lucinids, suggesting that bivalve activity reduces pore water sulphides. Large size is often considered a feature of chemosymbiotic bivalves and some species from hydrothermal vents and cold seeps are very large, particularly species of Vesicomidae, such as *Calyptogena magnifica* (Boss and Turner, 1980) 260 mm shell length, (Kennish and Lutz, 1992) or Mytilidae, *Bathymodiolus boomerang* (360 mm, Von Cosel and Olu, 1998). *P. erosa* is also a large sized bivalve with an average size of 102 mm length (Clemente and Ingole, 2009), which again points towards the possibility of this clam having chemoautotrophic bacteria.

However, none of the clam parameters in the present study could show a significant correlation with gill endosymbiont, but at Nerul the foot and mantle tissue weight correlated with gill endosymbionts. This could be because in the bivalves, the foot is used to reach into the reducing areas of the sediment to access the sulphide and transport it to the symbionts in the gills and draw oxygen from the ambient water bathing their gills (Arp *et al.*, 1984; Fisher, 1990) and it is so possible that even *P. erosa* must use the foot and mantle for similar reasons.

The role of autotrophic symbionts in the nutrition of their hosts range from being the principal source for host species lacking a functional digestive system (e.g. tubeworms) to being an additional source for host species (e.g. mussels, shrimps) (Cavanaugh *et al.*, 1981). In some species, symbionts may also play a significant role in sulphide detoxification. Bacterial density was also low in *P. erosa* compared to other bivalves (Powell and Somero, 1986) and this clam is likely to be less dependent on its symbiotic bacteria for its nutrition than other intertidal bivalve types. Similar observations were also made in a *Pectinid scallop* which lives in the periphery of vent fields (Hessler *et al.*, 1988). However, the clam *P. erosa* does not exhibit morphological characteristics of symbiosis related to the main source of nutrition. However, it does have well developed gills, which harbour endosymbionts, which points towards the fact that it may be depending on endosymbionts for its additional source of nutrition. The adjacent estuarine region where *P. erosa* lives, is known to be enriched in organic particles (e.g. phytoplankton) (Devassy & Goes (1989) which it can use as a source of food. However, *P. erosa* lives in the highest high tide level towards the landward region and feeds only during the spring tide and has to remain without food sometimes (Clemente and Ingole, 2009) wherein it must be living on the resources from the bacteria. Nonetheless, enzyme characteristics of thiotropic metabolism are detectable in gill extracts of mussels containing endosymbiotic bacteria (Cavanaugh *et al.*, 1983, 1993; Fisher *et al.*, 1993). *P. erosa* gill extract also showed sulphide metabolising activity in the present study. All these characteristics indicate the presence of sulphide oxidizing bacteria which may be used for an additional source of food or for its survival in the sulphide containing sediments of the mangroves. Presence of bacteria and high activity of sulphur-metabolizing enzymes suggest an elevated potential for energy generation by the symbionts in *P. erosa*.

The histochemical localization studies (Plate 2) indicate that the sulphide oxidizing activities are high in gill tissue and foot tissue of *P. erosa* in all stations compared to mantle tissue

which stained only at the superficial layer of tissue. All tissues studied, however showed some capacity for reducing Benzyl viologen when sulphide was present. In addition it is not clear whether the measured sulphide oxidizing activities are animal or bacterial. This "Peripheral defence" type of sulphide detoxification strategy has been previously reported in many bivalves (Powell & Somero, 1985; Vetter, 1985). For these organisms, there appears to be impermeable barrier to exclude sulphide from the outer body surface (Powell & Somero, 1985)

In animal-bacterial symbiosis from sulphide-rich environment, the flow of sulphide into cells is so great that specific enzymes, i.e., sulphide oxidases, are needed to allow the organism to cope adequately with the threat posed by sulphide and to exploit effectively the energy in sulphide. Sulphide oxidases have been found in sulphide oxidizing bacteria (Moriarty and Nicholas, 1969) but not purified and characterized. This study suggests that sulphide oxidizing activity was observed in the clams from all the sites. At Chora, the endosymbiont count as well as sediment sulphide was lower in Chora mangrove but sulphide oxidizing activity was high. This indicates that the sulphide oxidizing activity not only depends on sulphide concentration but also depends on gill endosymbionts present in the host tissue.

Chemoautotrophic symbioses live in environments characterized by substantial sulphide concentrations. All the inhabitants of these habitats whether have symbionts or not must deal with the problem of sulphide toxicity. In the case of the symbiotic molluscs, they typically oxidize sulphide to thiosulfate to reduce toxicity, and their symbionts can use thiosulfate (which is significantly less toxic) as a reductant. The primary requisite for sustaining high rates of chemoautotrophy is living in an environment with an abundant supply of sulphide and elevated temperatures in addition to oxygen (Childress and Girguis, 2010). From the present results it appears that *P. erosa* may be having chemoautotrophic symbioses but still have relatively low rates of carbon uptake. This may be partially explained by the fact that they are much more capable filter feeders (Clemente and Ingole, 2009). So, in general, they may be less well adapted to support chemosynthesis and more adapted for a mixotrophic existence. A similar type of existence has been reported for some *Bathymodiolin mytilids*. As a group these bivalves have greatly increased gill sizes and contain the bacteria within specialized cells in the surface of these gills (Childress and Girguis, 2010). Physiological and morphological attributes of the body plan as concerns oxygen uptake from the environment are a major reason for the absence of chemoautotrophic symbioses in some invertebrates (Childress and Girguis, 2010). For this symbiosis to thrive, the ability to tolerate sulphide in the environment is essential in chemically reducing environments. In many food-limiting environments, the numerically dominant bivalves are known to possess nutritional assistance in the form of photosymbionts or chemosymbionts (Richard, 1982, and Glover, 2010). Although there is some speculation regarding the presence of endosymbionts of these bivalves, their morphology and life habits can be interpreted in the knowledge of their probable chemosymbiosis.

Nevertheless, the endosymbionts could be only detoxifying the sulphide entering these clams from the surrounding

environment. Hence, it is unlikely that this clam could support a highly active population of chemoautotrophic endosymbionts. Despite the focus on chemoautotrophy as the primary nutritional source for lucinids, there is evidence that particulate feeding remains an important nutritional source for this family of bivalves (Duplessis, 2002). The nutritional health of the bivalve host may therefore be unrelated to the status of the bacterial symbiont population, at least when particulate food is readily available.

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