

Salinity changes may influence dinoflagellate cyst morphometry: data from monsoon-influenced tropical coastal ecosystems

Roy Valentino Rodrigues^{1,2}, Jagadish Siddalingappa Patil*¹

¹ CSIR – National Institute of Oceanography, Dona Paula – 403 004, Goa, India.

Tel: 91(0)832-2450413; Fax: 91(0)832-2450615

² School of Earth, Ocean, and Atmospheric Sciences, Goa University,

Taleigao Plateau - 403 206, Goa, India

*corresponding author: patilj@nio.org

Abstract

Generally, dinoflagellate cyst morphology is species-specific. Their variability due to environmental factors (temperature and salinity) make them potential proxies for such factors. However, there is a dearth of information on the variability of cyst morphology from monsoon-influenced coastal ecosystems. This study on distribution and variability in the dinocyst morphometry of *Pyrophacus steinii* and *Protoperidinium pentagonum*, from estuarine (Cochin-port and Zuari estuary) and marine (New-Mangalore port) ecosystems along the Indian west-coast revealed that both are common and euryhaline species. However, variability in cyst morphometry was observed and correlated salinity values between the ecosystems. Other parameters (i.e., the prevalence of narrow temperature ranges and nutrient- rich conditions) may not be dominant in influencing cysts morphometry. Cyst length, breadth, and processes length (only in *P.steinii*) of both were relatively larger and smaller at salinity ranges higher and lower than 25psu, respectively. The data presented for different ecosystems are comparable in most parameters except salinity variations. This study extends the ranges of records for both species. *P.steinii* cysts were significantly larger (up to 120µm in Zuari estuary) than literature reports on cysts from higher salinity systems and salinity related variability in *P.pentagonum* cyst size indicates notable potential proxy indicator of salinity.

Keywords: Dinoflagellate; Cyst morphometry; *Pyrophacus steinii*; *Protoperidinium pentagonum*; salinity; estuarine; marine.

Introduction

Dinoflagellates are one of the important primary producers in the marine ecosystem. About 12 - 13 % of total dinoflagellates are known to produce resting or pellicle cysts as part of their life cycle (Head, 1996). These cysts are important for their ability to form seed banks for blooms (including harmful algal blooms), sources of toxins, unwanted marine bioinvaders, and also as proxies for climate reconstruction and pollution (Marret et al., 2009, Hallegraeff and Gollasch, 2006, Mertens et al., 2009b, Pospelova et al., 2005). Generally, dinoflagellate cyst morphology i.e. shape, size, types of processes, wall structure, color is species-specific. Dinoflagellates produce species-specific cyst types with different morphological features (Fensome et al., 1993; Matsuoka and Fukuyo, 2000; Zonneveld and Pospelova, 2015). The shape, color, wall structure, paratabulations, ornamentation, and archeopyle (aperture) structure were considered essential features for cysts identification. Such morphological characteristic diversity is observed more in organic-walled cyst morphotypes than in the calcareous cysts (Narale, 2016). Notably, there are marked variations in the cyst morphological characteristics even in a single species. Recently few studies have confirmed that the environmental parameters such as salinity, temperature, and nutrients are some of the critical factors responsible for causing the variations in cysts morphology (e.g., process length) of the cosmopolitan dinoflagellate, for example, *Protoceratium reticulatum* (Mertens et al., 2009a, 2009b, 2010, 2012). Further, such variations in the size of the cysts processes have been used as proxies to reconstruct past environmental conditions such as salinity (Mertens et al., 2009b, 2010).

Several studies have reported an environmentally controlled variability in morphological features in the cysts of certain dinoflagellates. Wall et al. (1973) noted a positive relationship between variable process lengths of the resting cyst of *Lingulodinium polyedrum* and salinity in the Black Sea. Most cysts bearing short processes have been linked with low salinity found near coastal and estuarine environments (Nehring, 1994; Dale, 1996; Nehring, 1997; Ellegaard et al., 1998). Resting cyst processes length have been used semi-quantitatively for down-core salinity reconstructions in the Black Sea (Dale, 1996; Mudie et al., 2001; Marret et al., 2009), the Baltic Sea (Dale, 1996; Brenner, 2005; Head, 2007), the Limfjord in NW Denmark (Ellegaard, 2000), in the SW Black Sea (Verleye et al., 2009) and the Cariaco Basin (Mertens et al., 2009a). Cysts bearing shorter processes with reduced salinity conditions have also been recorded in other dinoflagellate cyst species. These species include cyst of *Protoceratium reticulatum* (Nehring, 1994, 1997; Dale, 1996; Ellegaard et al., 1998), *Pentapharsodinium dalei* (Nehring, 1994, 1997), and several *Spiniferites* (Gonyalux) species (Wall et al., 1973; Gundersen, 1988; Dale, 1996; Lewis et al., 1999;

Ellegaard et al., 2002). In the cyst of *Protoceratium reticulatum*, salinity and temperature were the influencing factors for morphological variation, especially the process length in the tropical and Southern Hemisphere Oceans (Verleye et al., 2012). However, it is still unclear whether such variations occur in other cysts, which are cosmopolitan in the distribution in various ecosystems. Nevertheless, the studies on viability and morphological details of the cysts encountered at different environmental gradients will provide more insights on their tolerance capabilities, biogeography, and ecological proxies.

In light of this, the morphological variation in some dinoflagellate cysts located in different geographic ecosystems (estuarine and marine) along the Indian coast is studied from the sediment samples collected under Ballast Water Management Program-India. Here, the cysts of *Pyrophacus steinii* and *Protoperidinium pentagonum* were selected as they were predominant in both estuarine [Cochin port (Kerala) and Zuari estuary (Goa)] and marine port (New Mangalore port, Karnataka) ecosystems. Though available literature does confirm their presence in good numbers along the coast of India, encompassing different ecosystems (D'Costa et al., 2008; D'Silva et al., 2012; Narale et al., 2013, 2015; Narale and Anil, 2017; Prabhudessai and Rivonker 2020; Rodrigues et al., 2019) but the existence of morphometric variations to environmental settings, have not been addressed.

Methodology

Sediment and water samples (surface and sub-surface) were collected at several stations from three major ports viz Cochin (23 stations), New Mangalore (19 stations) and Kandla (26 stations), as well as Zuari estuary (5 stations), located on the west coast of India (Fig.1). The sampling locations are influenced by monsoon reversals, a freshwater influx of varying magnitude, and anthropogenic pressures, particularly in ports wherein a more significant proportion of all cargo traffic is being handled. Therefore the sampling in each port was undertaken on a seasonal basis (i.e. two during post-monsoon and one each during pre-monsoon and monsoon), while in Zuari estuary, sampling was at monthly intervals for one year. The details of water sampling for environmental parameters such as temperature, salinity, and inorganic nutrients and sediment for dinoflagellate cysts, processing, and cyst analysis were the same as described in Rodrigues et al., 2019. The environmental parameters were recorded using standard procedures for temperature, salinity, and inorganic nutrients, and the palynological method was employed for cyst analysis from the sediments (Rodrigues et al., 2019). Based on cyst data, cysts of *Pyrophacus steinii* and *Protoperidinium pentagonum* were selected as they were predominant in the studied regions (Cochin port, New Mangalore port, and Zuari estuary), except Kandla port. In this study, morphometric variations in

the selected cysts of *P. steinii* and *P. pentagonum* were evaluated. Though the selected dinoflagellates are predominant, their average abundance per gram dry weight is <80 and <50 for *P. steinii* and *P. pentagonum*. Therefore, approximately 25-30 intact cysts for each dinoflagellate from ecosystems were selected for the morphometric measurements. All the measurements of both the cyst were done when cysts were in the dorsal view. For measurement, fragments of the cyst, and the broken cysts were not considered for the analysis in this study. *P. steinii* is an autotrophic dinoflagellates cyst belonging to the order Gonyaulacales and family Pyrophacaceae. The *P. steinii* cysts are generally large, spherical, and disk-shaped with large, bulbous, or barrel-shaped processes. They have a hypocystal archeopyle, which is sub-trapezoidal in outline. Cysts comprise an inner (endophragm) and outer membrane (ectophragm) and are connected with structures called processes. The base and tip of the processes are attached to the endophragm and ectophragm, respectively (Fig. 2. a). *P. pentagonum* is a heterotrophic dinoflagellates cyst belonging to the order Peridinales and family Protoperidiniaceae. Cysts are pentagonal or peridinoid shaped and dorso-ventrally compressed with short, needle-shaped processes. In these cysts, hexagonal archeopyle is observed (Fig. 2. b). In this study, *P. steinii* and *P. pentagonum* cyst distribution and their size variability with respect to salinity were studied. The pooled total cyst abundance (i.e. data from no of stations X 4 seasons for ports and no. of stations X 12 months for Zuari estuary) from each sampling region were then categorized into seven different salinity ranges (i.e. 0-4.9, 5-9.9, 10-14.9, 15-19.9, 20-24.9, 25-29.9 and >30 psu) based on their occurrences. All the measurements, based on cysts morphology as illustrated in Figures 2.a and 2.b, were made using an Olympus BH-2 light microscope, equipped with a digital camera and Cell F Software Imaging System, respectively, and 40x objectives. For *P. steinii*, the length and breadth of the cysts endophragm (inner membrane) and ectophragm (outer membrane), were measured along with the length of the three most extended visible processes as shown in figure 2. a. The process length was calculated from the base to the tip (Fig. 2. a). In *P. pentagonum* the length and breadth were measured as illustrated in figure 2. b. The *P. pentagonum* variant used in the study is given below (Fig. 2. b).

Results

Environmental conditions

The environmental settings in each of the ports are different, and thereby the selected locations represent diverse ecosystems. In each of the chosen areas, environmental data (Table.1) collected during the port biological sampling as part of the Ballast Water Management Program India (BAMPI) program showed a distinct seasonality (for details please see Rath et al. 2019 for New

Mangalore port, Rodrigues et al. 2019 for Cochin port, Sathish et al., 2020 for all three ports). The average water temperature changes in between the locations were broadly comparable, i.e., 28.7 ± 1.7 °C, 27.9 ± 3.6 °C, 29.1 ± 2 °C were recorded from Cochin port, New Mangalore port, Zuari estuary, and Kandla port, respectively. However, the salinity showed a wide variation between the locations and was mainly due to the riverine influx and precipitation. The maximum salinity variations were recorded at Cochin port (4- 36 psu) followed by Zuari estuary (26 to 36 psu), New Mangalore port (30.53 to 36.70 psu), and Kandla port (30 to 40 psu). Interestingly the nutrient concentrations were high in all the locations during sampling (Table. 1).

Pyrophacus steinii

P. steinii cyst abundance and its contribution to total cyst assemblage from Zuari estuary (Goa), New Mangalore, and Cochin ports are provided in figure 3. a. The results revealed lower *P. steinii* cyst abundance (2- 21 avg cyst g⁻¹ of (Dry Weight) DW sediment) and contribution (i.e., 6 -23 % to the total cyst abundance; Fig. 3.a) in Zuari estuary (Goa) compared to other locations. In New Mangalore (i.e., 18- 81 avg cyst g⁻¹ of DW sediment; 20-30% to total cyst abundance) and Cochin (34-68 avg cyst g⁻¹ of DW sediment; contribution 10-16% to total cyst abundance), the cysts were observed during all the sampling seasons (Fig. 3.a)

Salinity wise distribution of *P. steinii* cyst for each ecosystem was different. For instance, in Cochin ports, cysts were encountered at wider salinity (5-35 psu) ranges, and the maximum contribution (up to ~55%) was recorded at lower salinities (<30 psu) (Fig. 3. b). Whereas in New Mangalore (100%) and Zuari estuary (~80%) locations, the maximum cyst contribution was recorded at salinity >30 psu (Fig. 3.b).

The morphometric measurements revealed that the dimensions of *P. steinii* cyst sizes between the locations were different. The dimensions of the cysts ectophragm (the outer covering) and endophragm (the inner body of cyst) was found to be larger in Zuari estuary (Goa) (ectophragm l= 114.76 ± 7.5 µm and b= 108.36 ± 5.76 µm; endophragm l = 74.43 ± 13.77 µm and b= 69.29 ± 2.1 µm) followed by New Mangalore (ectophragm l= 78.80 ± 9.76 µm and b= 71.76 ± 8.36 µm; endophragm l= 57.26 ± 6.93 µm, b= 50.64 ± 4.79 µm) and Cochin (ectophragm l= 72.27 ± 9.07 µm, b= 65.78 ± 7.96 µm; endophragm l= 51.97 ± 6.42 µm, b= 50.64 ± 4.79 µm) ports (Fig. 4. a. b.). Even the process length of *P. steinii* was also found to be larger in Zuari estuary (Goa) (11.92 ± 2.1 µm) followed by New Mangalore (8.78 ± 1.23 µm) and Cochin (8.15 ± 1.38 µm) ports (Fig. 4. c). ANOVA followed by post hoc test (Tukey's HSD (Honest Significant Difference); $p < 0.05$) revealed that the cysts from the Zuari estuary (Goa) region were significantly larger (cyst size and processes

length) than those found in other ecosystems. Between New Mangalore and Cochin ports *P. steinii* cyst size was relatively larger, but not significant, in the former port than those encountered in the latter port.

Protoperidinium pentagonum

The *P. pentagonum* cyst contribution to total cyst abundance was least in Goa i.e. 0.5-3 % (1-3 avg cyst g⁻¹ of DW sediment), compared to New Mangalore (8-12%; 5-24 avg cyst g⁻¹ of DW sediment), and Cochin (4-10%; 25- 53 avg cyst g⁻¹ of DW sediment) ports (Fig. 5. a). Similar to *P. steinii* cyst, the salinity-wise distribution of *P. pentagonum* cyst abundance for each ecosystem was also different. In Cochin ports, cysts were encountered at wider salinity (5-35) ranges, but the maximum contribution (~55%) was recorded at lower salinities (<30 psu) (Fig. 5. b). While in New Mangalore port (100%) and Zuari estuary (~72%), the contribution was highest at >30 salinity (Fig. 5. b). The morphometric measurement of *P. pentagonum* revealed that the cyst length) was slightly larger in New Mangalore (l= 93.33 ± 9.76 µm, b= 76.67 ± 4.88 µm) followed by Zuari estuary (l= 90.50 ± 8.66 µm, b= 80.98 ± 7.28 µm) and Cochin (l= 72.27 ± 9.07 µm, b= 65.78 ± 7.96 µm) ports (Fig. 6. a. b), while breadth was relatively larger in Zuari estuary followed by New Mangalore and Cochin ports. However, ANOVA revealed insignificant variation in the size of the cyst between the ecosystems.

Discussion

Dinoflagellate cysts are expected to resist the conditions outside the range tolerated by motile stages. However, cyst morphology variations were known to be influenced by environmental parameters such as salinity, temperature, light, and nutrient in certain dinoflagellates (Zonneveld and Susek, 2007; Mertens et al., 2009a; 2009b; 2010; 2012). For instance, Mertens et al. (2009b), after analyzing core-top samples from 144 locations having a global distribution, showed a relationship between the average process lengths of *L. machaerophorum* with both salinity (positive) and temperature (negative). According to observation and theoretical considerations (Kokinos and Anderson, 1995; Hallett 1999; Hemsley et al., 2004; Mertens et al., 2009b) explained the formation of processes in dinocyst, which begins with the outer membrane expansion, a monomer is formed. It starts to amalgamate on the cytoplasmic membrane, depending on the environmental conditions such as temperature and salinity, a lot of smaller (at low temperature and salinity) or a few larger (at high temperature and salinity) colloids of monomers are formed. The visco-elastic dinosporin is produced on the globules, which stretch the membrane and polymerized coated under the monomer in a radial direction. The membrane expansion often comes to a stop before radical stretching ends. The

formation of a longer process takes a longer duration at high temperature and salinity than the shorter process at low temperature and salinity. Although laboratory experiments have been previously addressed in some dinoflagellate species, environmental data is needed to uncover factors explaining cyst morphology variability, establish proxies for species identification, and delimit species distribution niches.

Available reports suggest that *P. steinii* and *P. pentagonum* cysts are wide-spread spatially along the west coast of India (e.g., D'Costa et al. 2008, D'Silva et al. 2011, D'Silva et al. 2012, Rodrigues et al. 2019) and are also present in all the seasons, including during monsoon (Figs. 3a and 5a). The freshwater influx (through precipitation and river discharge) brings in drastic environmental changes, particularly salinity fields. However, the magnitude of salinity field variations (particularly duration) is dependent upon the proximity of the location of the river. For instance, the salinity field variations was significant in Cochin port compared to New Mangalore port (Table 1). Since *P. steinii* and *P. pentagonum* cysts were predominant in nutrient-rich estuarine and marine ecosystems, salinity could be an important factor in determining the cysts' dimension and distribution. Interestingly, though both the cysts were present throughout the samplings in all three ecosystems, distinct seasonality was not observed. Although the cyst abundance of both the species showed similar abundance in Cochin and New Mangalore port their percentage contribution to the total abundance was higher in later port in all seasons. These two dinocysts were also reported at wider salinity ranges than previously reported especially in Cochin port (Fig. 3.b, 5b). Therefore the life stages of *P. steinii* and *P. pentagonum* are classified as euryhaline type as they were recorded in all the salinity ranges (Fig. 3a and b), which suggests one of the possible reasons for their predominance in the region. It was observed that the cyst of *P. steinii* and *P. pentagonum* found in New Mangalore port and Zuari estuary (Goa) were encountered at salinity above 30 psu while in Cochin port cysts were found at wider salinity i.e. 5 to 36 psu. The prevalence of freshwater influx for most of the year and the occurrence of planktonic motile-stages of *P. steinii* in high numbers at wide salinity ranges (5->30 psu) are the main reasons for the proliferation in Cochin port (Rodrigues et al., 2019). Even in New Mangalore port, a marine system with less salinity variation (>30 psu), planktonic motile stages were observed in good numbers at higher salinity. Whereas in Zuari estuary, unlike Cochin port is an open system with less freshwater influx might harbor less abundance of motile stages as evidence from lower cyst production. In *P. pentagonum*, planktonic motile-stage were found in above 30 and 15-20 psu ranges in Cochin port and above 30 psu in New Mangalore port. Therefore, different cyst size classes observed in Cochin port could be explained by the salinity changes experienced by their planktonic motile stages, resulting in cyst morphometry variability.

The morphometric measurements of *P. steinii* and *P. pentagonum* cysts showed distinct variation between the studied ecosystems. For instance, cysts of *P. steinii* and *P. pentagonum* in Cochin port were generally smaller than New Mangalore and Zuari estuary (Goa). Further, within Cochin port, the size of both the cysts types also showed larger variations. Zonneveld and Susek (2007) through culture experiments reported that the changes in *P. steinii* cyst sizes were observed to salinity but not statistically significant, especially at the higher salinity range wherein a large cyst size was observed. Generally, *P. steinii* cyst length and breadth ranged between 85 -115 μm and 75-95 μm , respectively, in salinity ranging from 20 to 45 psu. Large and small cyst sizes were observed at higher and lower salinities (Zonneveld and Susek, 2007). These findings, when related with the present study revealed that the salinity effect can be possible as (i) the variation was very large (i.e. 0-36 psu), (ii) the seasonal distribution of the planktonic form of *P. pentagonum* and *P. steinii* indicated that the former is absent during monsoon and the latter present throughout the year with highest during monsoon and pre-monsoon season, (iii) spatially abundance of cysts and planktonic forms are higher in the region where the variations in salinity field is very large eg. Cochin port. Interestingly, the cysts of *P. steinii* found in the Zuari estuary (Goa) were larger when compared to the information available in the literature (Zonneveld and Susek, 2007). However, to our knowledge, specific studies on morphometry variations in *P. pentagonum* and *P. steinii* for lower salinity (<20 psu) are not available. Like *P. steinii*, cysts of *P. pentagonum* also showed variation in cell length. The relatively bigger-sized cysts were observed in the Mangalore port, followed by Zuari estuary (Goa) and Cochin port, but the differences were insignificant. The prevalence of low salinity for a longer period in Cochin port could be the factor responsible for producing cysts of smaller size.

Additionally, certain dinoflagellate cyst processes have been considered a potential proxy for salinity variation (Mertens et al., 2010). Generally, *L. machaerophorum* and other cysts processes are considered a primary biological function as a clustering device to improve sinking rates (Mertens et al., 2009b). The influence of salinity variation on the length of the process in *Lingulodinium* is well documented through laboratory experiments. For instance, cysts without or with processes showed reduced length when grown at a low (15 psu) salinity, and at 40 psu slight reduction of process length has been observed (Hallett, 1999). Hallett (1999) also confirmed positive and negative relationships to salinity and temperature, respectively, but in combination could show morphological variation. Although studies related to interactive effects (eg. salinity and temperature) had shown morphological variation in some dinocyst (eg. *Lingulodinium polyedrum* and *Protoceratium reticulatum*) (Hallett, 1999; Verleye et al., 2012) but the information on the studied organisms are scarce. Among the two dinoflagellates, the temperature effect on *P. steinii* cysts' morphometry is

also reported by Zonneveld and Susek, 2007. According to their study, temperature affected the *P. steinii* cyst morphology wherein the length of the cyst processes formed at a temperature of 16.5 °C and 34.8 °C were small and flattened. In comparison, between 20°C to 30 °C were significantly longer. Even the influence of changing temperatures on the cyst morphological variations in *Lingulodinium machaerophorum* and *Spiniferites baltica* are also reported (Hallett, 1999; Ellegaard et al., 2002). The *P. steinii*, cell size length, and width showed a larger variation i.e., smaller cells were formed at lower (16.5°C) and highest temperature (34.8 °C) as compared to intermittent temperatures (Zonneveld and Susek, 2007). This confirms that the temperature effect on the cyst morphometry of both the dinoflagellates in the study region will be negligible due to the prevalence of warmer waters (avg temperature 28°C) and the broadly comparable in the water temperature between the ecosystems. In the Cochin, New Mangalore ports, and Zuari estuary, the temperature ranged between 28.7 ± 1.7 , 27.9 ± 3.6 , and 29.1 ± 2^0 C, respectively. Since the ranges for the water temperature in the studied locations are similar, salinity variations could be the driving factor for cyst encystment. Also given that nutrient depletion triggers the encystment of some dinoflagellates (eg. *Scrippsiella cf. lachrymosa*) (Olli and Anderson, 2002), the information on the effect of nutrient availability on the cyst morphology needs further investigations. Given these, here, an attempt has been made to understand the variability. However, in our study, compared to other ports, large size cysts were found in Zuari estuary where salinity was >30 . Interestingly 45% of *P. steinii* cyst recorded from Goa showed a cell length of $>115 \mu\text{m}$, which is larger, not only from those reported from other ports but also to that available in the literature. The body measurement and process length of *P. steinii* showed a significant difference in cyst found in Goa as compared to others. The process length of *P. steinii* was smaller in Cochin port with wider salinity ranges than New Mangalore and Zuari estuary (Goa), where they were found in stations with salinity above 30 psu. There also exists a variation in the species phenotype, as seen in Zonneveld and Susek (2007) wherein 22 strains of *Pyrophacus steinii*, only three of them produced cysts suggest all phenotypes species do not produce cyst or their triggering mechanism for cyst formation was different. The possible reason for this variation could be that the strains of *P. steinii* recorded in Goa are different from other ports. For example, different sexual behaviour such as homothallism (self-fertilization) or heterothallism (intercrossing of +/- strains) is possible within strains of the same species. However, there are few species with both homothallic and heterothallic sexuality (e.g., *Gymnodinium catenatum* - Figueroa et al., 2006; Steidinger and Garccés, 2006; *Scrippsiella trochoidea* -Montresor et al. 2003; *Alexandrium tamarense* and, *Karenia brevis* - Blackburn and Parker, 2005; *Polykrikos hartmannii* - Chai et al., 2020) including *P. steinii* (Zonneveld and Susek, 2007). Previously *P. steinii* was

considered to have a heterothallic sexual reproduction (Pholpunthin et al., 1999) but Zonneveld and Susek (2007) suggested that (i) homothallic sexual reproduction do exist in some strains and (ii) identified temperature as one of the variable influencing cysts morphometry. However, specific studies through molecular approaches are a need of the hour to understand the strain variability, and such an approach will boost dinoflagellate eco-biological studies.

Conclusion

This study concludes that the *P. steinii* and *P. pentagonum* cysts are euryhaline species. They encountered a much wider salinity range compared to available reports, thereby adding newer information to their biogeographic distribution. Further variability in cysts morphometry was also observed between the ecosystems. It was found to be following salinity values as the influence of other environments (i.e., the prevalence of narrow temperature range and nutrient-rich conditions) may not be dominant. Cyst morphometry, i.e., length, breadth, and process length (only in *P. steinii*) of both the dinoflagellates were relatively larger at higher (>25 psu) and smaller at lower salinity (<25 psu) ranges. The occurrence of large size *P. steinii* cysts and salinity-wise variability in *P. pentagonum* cysts size also adds newer information to the eco-biology of dinoflagellate cysts. This study confirms that, in addition to cysts with processes, cysts without processes exhibit variability in their morphometry in different environmental settings. Such cysts (if dominant) can form alternative indicators in regions where known indicator species are less predominant. However, specific studies similar to those available for certain cysts with processes need to be undertaken for the cysts without processes exhibiting morphometric variations. Further, documenting the cysts morphometry in the studies related to distribution along with the environment as well as controlled experiments to understand the responses to environmental triggers/cues and the reasons for variability will be useful for robust data generation and interpretation to strengthen the utility of cyst morphometry of other dinoflagellates as potential environmental indicators.

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Table No.1. The annual ranges for each environmental parameters recorded during sampling from different ecosystems.

Environmental parameters	Ecosystems			
	New Mangalore Port	Cochin Port	Kandla Port	Zuari estuary (Goa)
Temperature (°C)	27.9 ± 3.6	28.7 ± 1.7	26.5 ± 3.6	29.1 ± 1.8
Salinity (psu)	35.2 ± 0.8	20.8 ± 11.3	36 ± 6	33.1 ± 2
Dissolved Oxygen (mg/L)	3.4 ± 1.8	4.6 ± 2	5.7 ± 0.8	NA
Phosphate (µM)	2.6 ± 1.3	3.3 ± 1.3	4 ± 1.2	1.8 ± 0.7
Silicate (µM)	22.1 ± 14.2	37.7 ± 16.7	72.7 ± 37.3	17.4 ± 14.6
Ammonia (µM)	28.3 ± 10	28.2 ± 12.9	5.7 ± 5.9	31.3 ± 14.5
Nitrite (µM)	2.8 ± 2	0.9 ± 0.7	5.4 ± 2.1	0.8 ± 0.4
Nitrate (µM)	4.7 ± 4.2	4.7 ± 3.1	12.6 ± 5.6	1.1 ± 0.9
Chl a (µg/L)	8.8 ± 9.3	20.6 ± 22.2	0.6 ± 0.3	2.2 ± 1.5

Fig. 1. Location of sampling stations in different ecosystems along the west coast of India. (a) Zuari estuary (5 stations), (b) New Mangalore port (19 stations), and (c) Cochin port (23 stations), along the west coast of India.* Samples from Kandla port were collected and analyzed but the cysts of *P. steinii* and *P. pentagonum* species were not encountered. Therefore, the map showing the location of sampling stations from Kandla port is not included.

Fig. 2 Cyst of (a) *Pyrophacus steinii*, and (b) *Protoperidinium pentagonum* with morphometry details (total length and breadth) used for the measurements. In *P. steinii*, the length and breadth of ectophragm and endophragm along with processes length were recorded. The dotted line shown in 'b' for *P. pentagonum* is used only for length measurements. All the measurements were performed in the same orientation i.e. dorsal view.

Fig 3. Seasonal distribution of *Pyrophacus steinii* cysts in different ecosystems. (a) Bar represented the average abundance of total and *P. steinii* cysts in the Cochin port (n=23), New Mangalore port (n=19), and Zuari estuary (n=20). The numbers above the bars represented the total cyst abundance and the numbers in parenthesis represent the total abundance of *P. steinii* cysts. (b) The percentage contribution of *P. steinii* cyst was obtained based on their occurrences in six different salinity ranges calculated for each sampling region. PoMI, PrM, Mon, and PoM II corresponded to Post Monsoon I, Pre Monsoon, Monsoon, and Post Monsoon II seasons respectively.

Fig 4. Morphological measurements of *Pyrophacus steinii* from Cochin port, New Mangalore port, and Zuari estuary. (a) Averaged length and breadth of the cysts ectophragm, (b) Averaged length and breadth of the cysts endophragm breadth, and (c) averaged length of the cysts processes. Error bars indicate standard deviation. One-way ANOVA and Tukey's multiple comparison test ($\alpha=0.05$) were conducted to evaluate the cyst size differences recorded between ecosystems. Only the significant differences between the ecosystems are shown. '*' indicates that the cyst size recorded from the Zuari estuary is significantly different from other regions.

Fig 5. Seasonal distribution of *Protoperidinium pentagonum* cysts in different ecosystems. a. Bar represents the average abundance of total and *P. pentagonum* cysts in the Cochin port (n=23), New Mangalore port (n=19), and Zuari estuary (n=20). The numbers above the bars represent the total cyst abundance and the numbers in parenthesis represent the total abundance of *P. pentagonum* cysts. (b) The percentage contribution of *P. pentagonum* cyst obtained based on their occurrences in six different salinity ranges calculated for each sampling region. PoMI, PrM, Mon, and PoM II corresponded to Post Monsoon I, Pre Monsoon, Monsoon, and Post Monsoon II seasons respectively.

Fig 6. Morphological measurements (a. average length and b. average breadth) of *Protoperidinium pentagonum* cysts from Cochin port, New Mangalore port, and Zuari estuary. Error bars indicate standard deviation. One-way ANOVA was conducted to evaluate the cyst size differences recorded between ecosystems. Since analysis revealed insignificant differences Tukey's multiple comparison test ($\alpha=0.05$) was not performed.

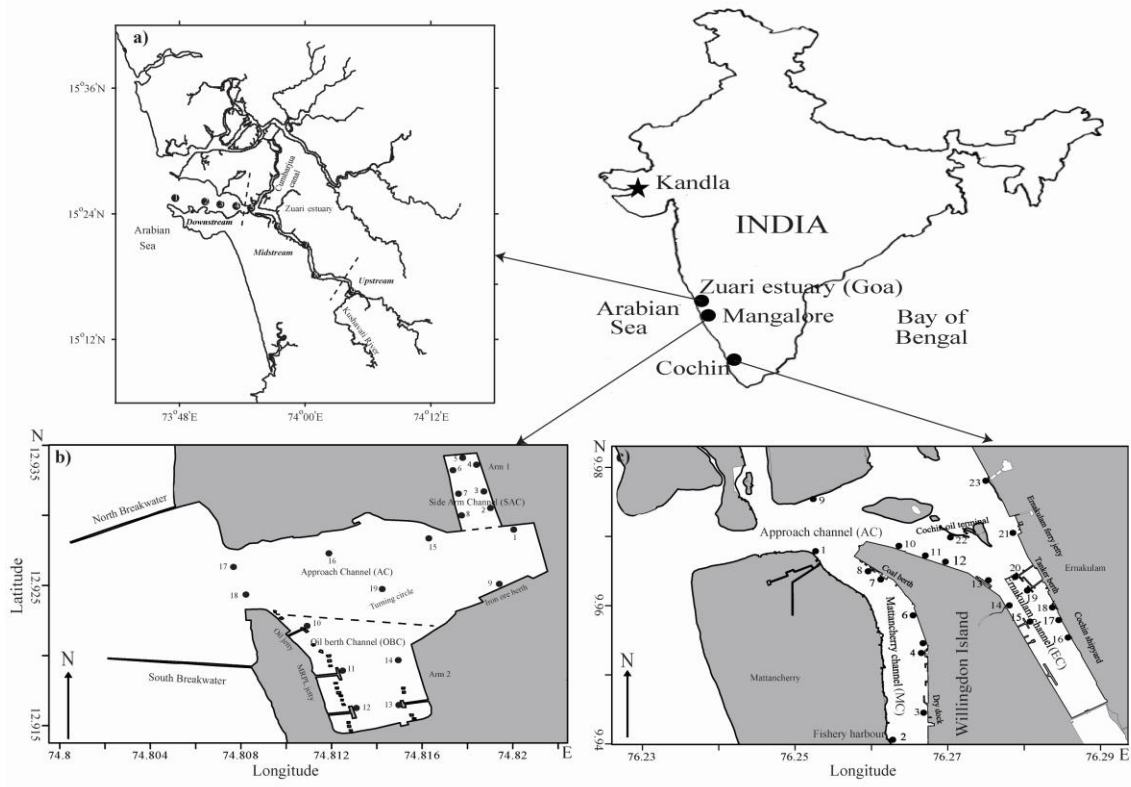


Fig. 1.

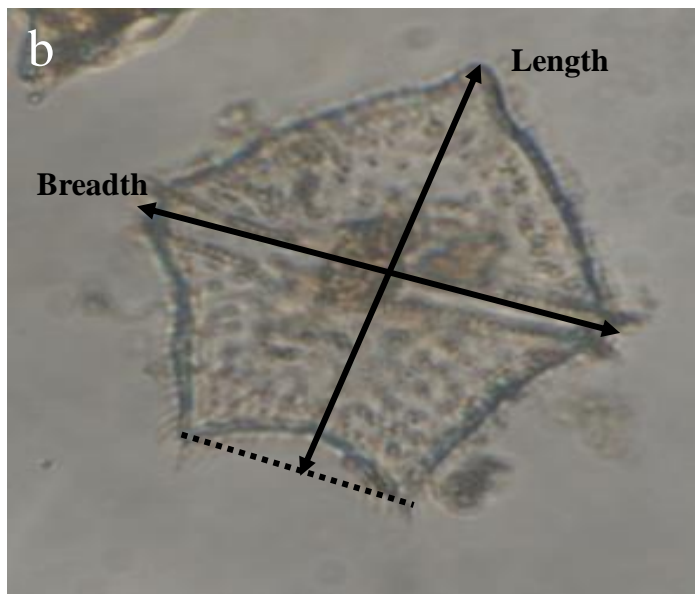
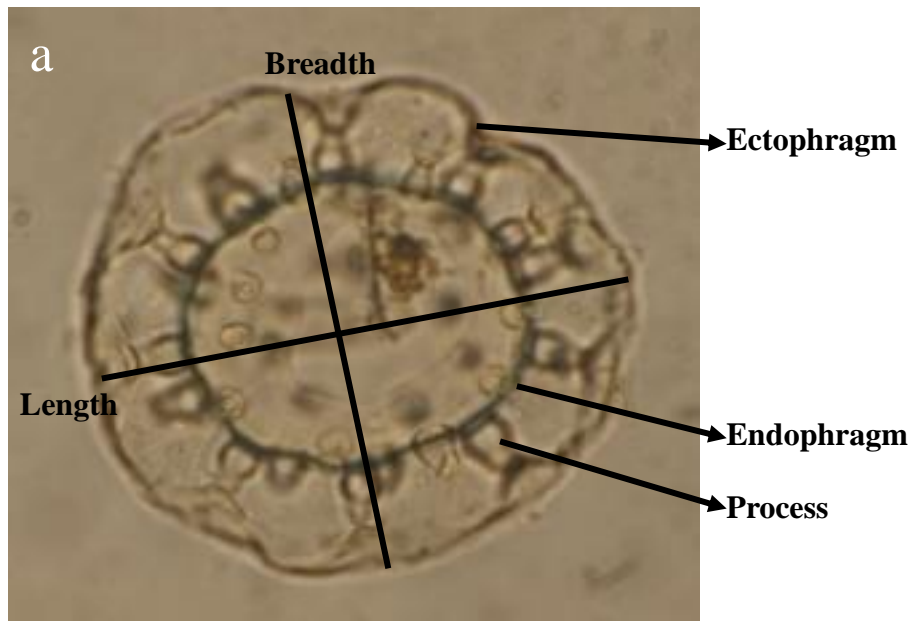


Fig.2.

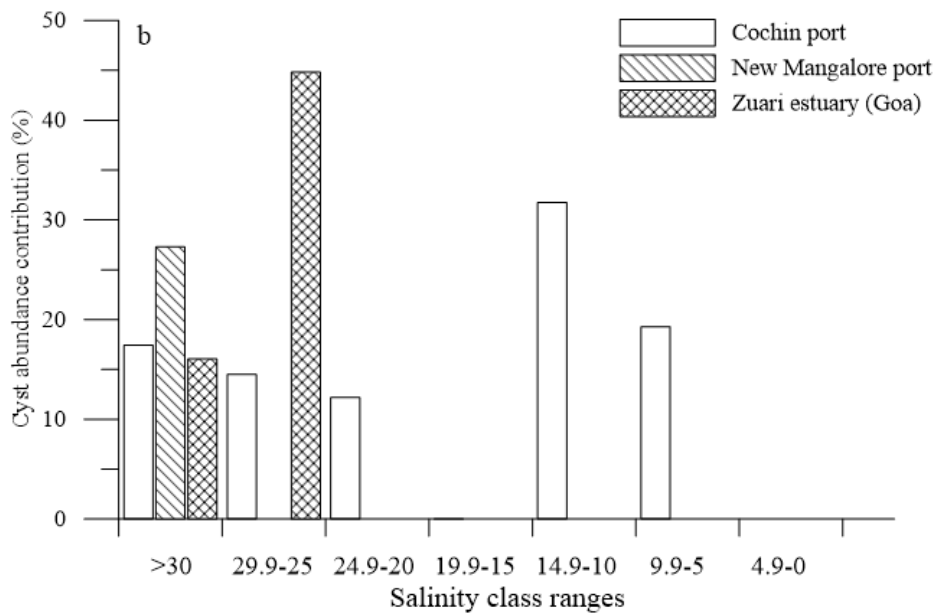
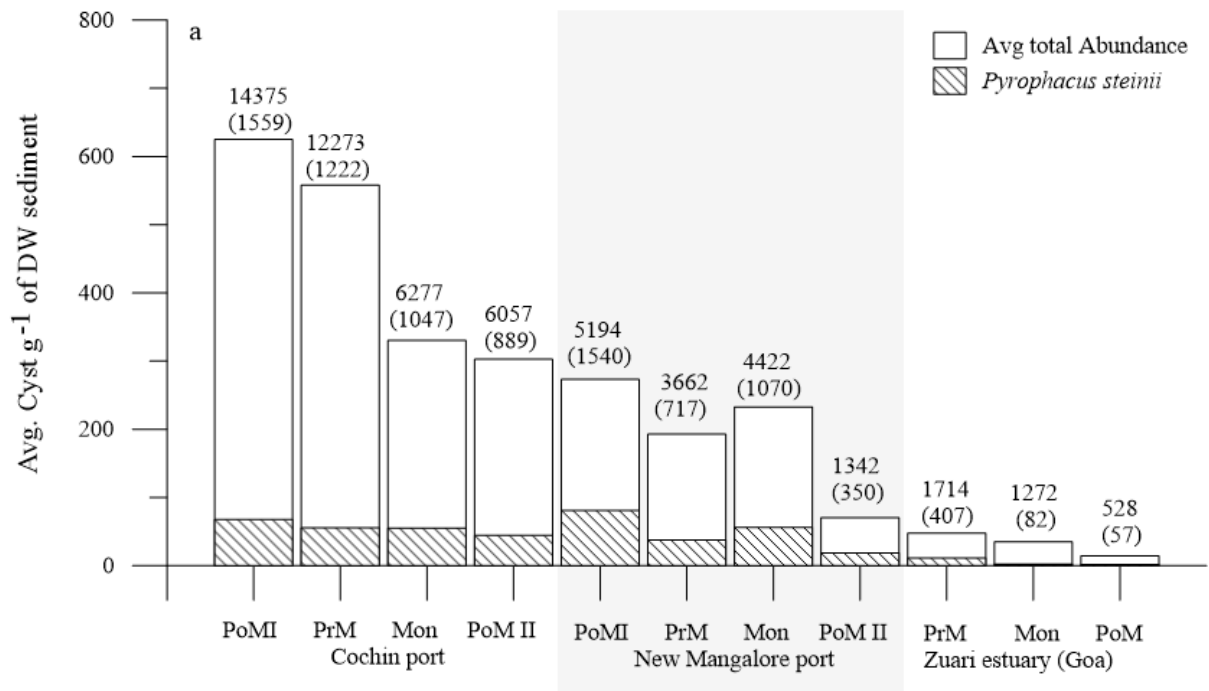


Fig 3.

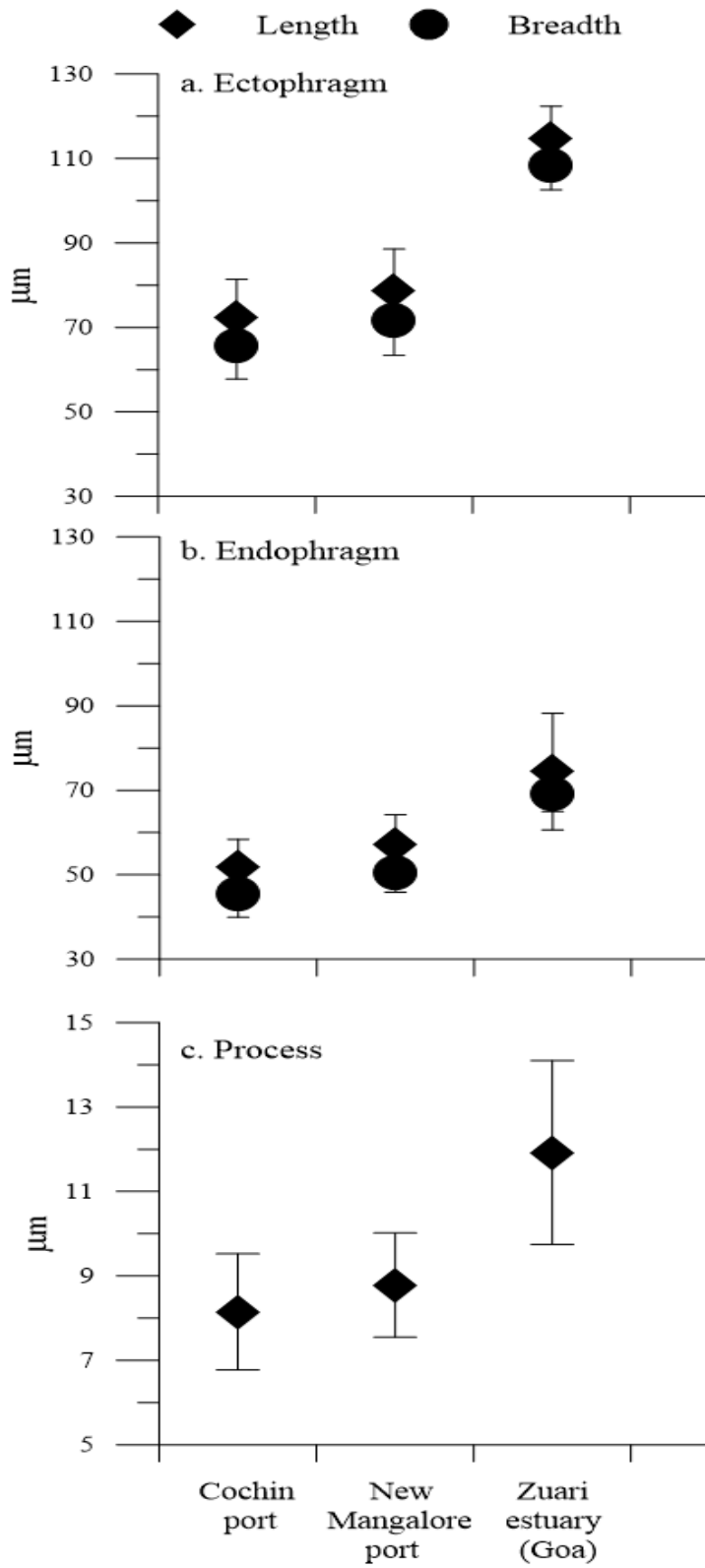


Fig 4.

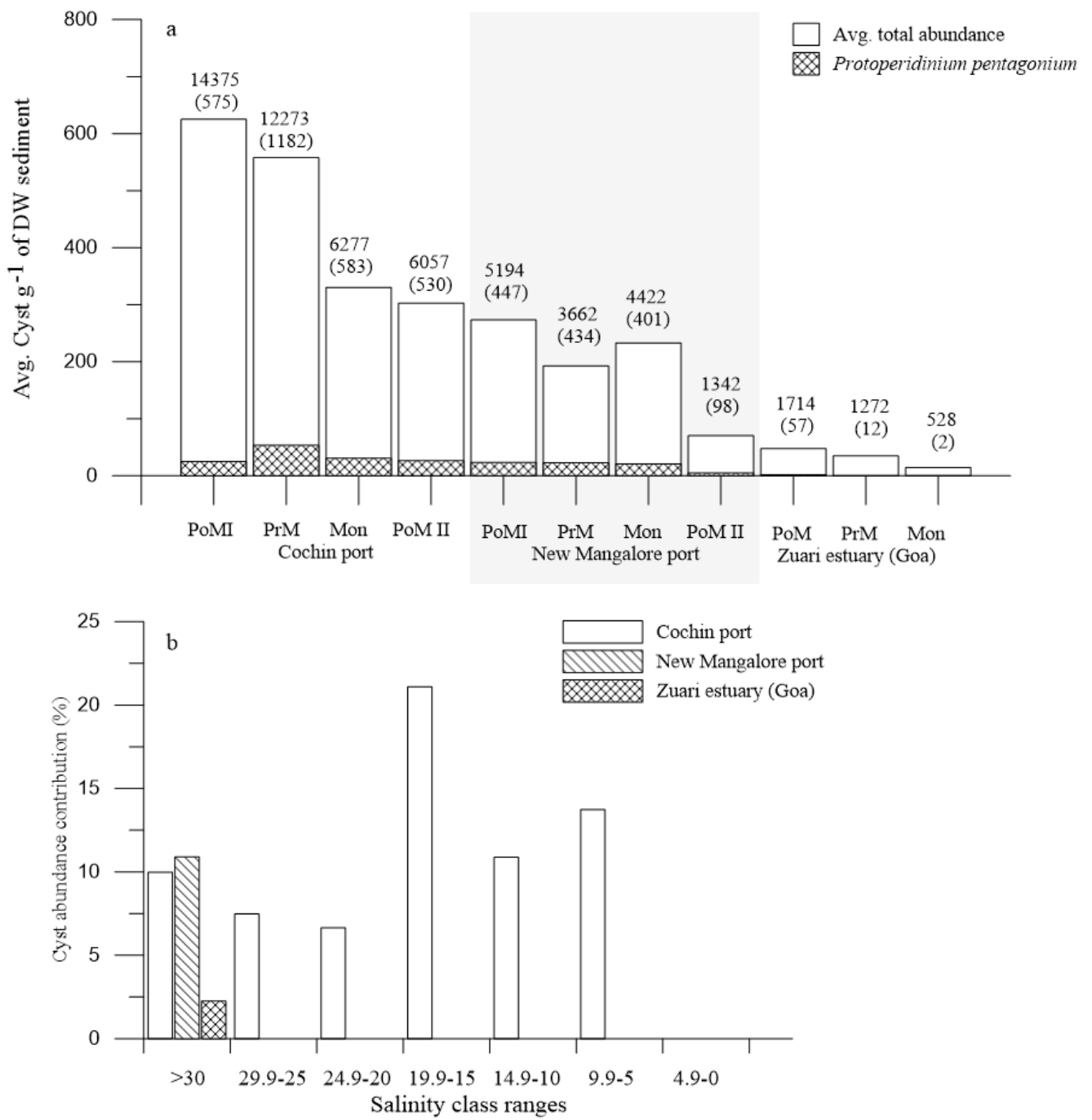


Fig 5.

Protoperidinium pentagonum

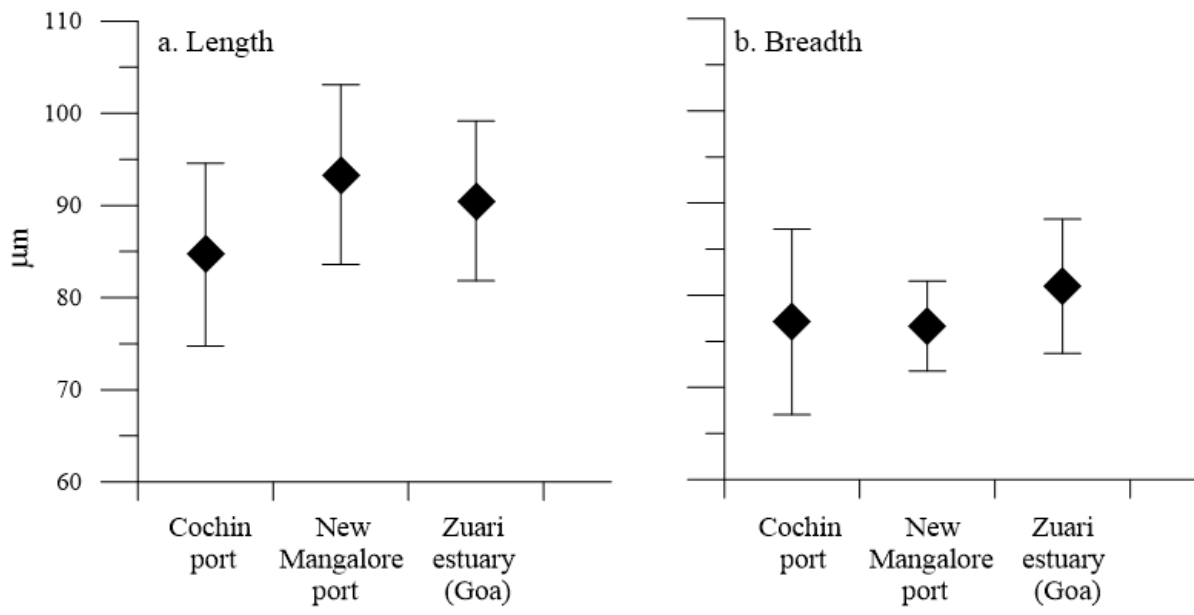


Fig 6.