

Octocoral colonies as spawning ground for the cuttlefish *Sepiella inermis* (Cephalopoda: Sepiidae)

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Abstract:

Corals are key ecosystem engineers, supporting marine biodiversity by providing nursery ground and living habitat. Among corals, octocorals are a diverse group of soft corals that are an integral part of reef ecosystems. In the present study, the association between two octocoral corals *Pseudopterogorgia* sp. Kükenthal, 1919 and *Menella* sp. Gray, 1870, and the cuttlefish *Sepiella inermis* were described from the intertidal region of the highly urbanized seascape of Mumbai, India. *S. inermis* used the octocorals as spawning substratum during their spawning season in October-November, 2020. Out of 92 octocoral colonies from two sites, 48.9% were found with cuttlefish egg capsules attached. 36 (57.14 %) out of 63 colonies and 9 (31.03 %) out of 29 colonies were hosted with cuttlefish eggs capsules at Marine Drive (a protected bay) and Worli (a site exposed to wave action). This suggests that cuttlefish prefer to lay their eggs in protected areas irrespective of the octocoral species. The presence of cuttlefish eggs on octocorals and absence on other possible structures highlights the ecological importance of octocorals, the necessity of proactive conservation measures, and comprehensive studies to protect this crucial intertidal habitat in urban coastlines.

Keywords: Octocorals; Habitat formation; Cuttlefish; Spawning; Indian Ocean

1. Introduction

Associations of different kinds (e.g., mutualism, commensalism, and parasitism) are ubiquitous in coral reef ecosystems (Stella et al. 2011). Reef-building corals are key ecosystem engineers, supporting biological diversity (~25% of known marine diversity) by providing shelter for numerous organisms (Fisher et al. 2015; Wagner 2020). Corals also contribute to habitat formation and complexity, thereby enhancing ecosystem services by supporting reef biodiversity (Graham and Nash 2013; Woodhead 2019). Many different organisms, such as fishes, bryozoans, crustaceans, and molluscs, associate with branching hard corals and other reef-building invertebrates (Castro 1988; Stella et al. 2011).

Among corals, octocorals (Cnidaria: Anthozoa: Octocorallia) are the second most abundant corals after hard corals (Shoham and Benayahu 2017). The octocorals are a broader group of soft corals that occur in a number of reef habitats, from shallow inter-tidal regions to deep mesophotic reefs (Pérez et al. 2016; Shoham and Benayahu 2017). Octocorals are known to associate with highly diverse invertebrates such as sponges (Calcinai et al. 2013), fishes (Epstein and Kingsford 2019), hydroids (e.g., Montano et al. 2017), molluscs, polychaetes, crustaceans, bryozoans, and echinoderms (Bayer 1961; Goh et al. 1999), which often determine the overall fitness and long-term survival of host colony (Stella et al. 2011; Montano 2020). The octocorals *Pseudopterogorgia* sp. and *Menella* sp. belong to the family Plexauridae, order Alcyonacea and are relatively abundant throughout the tropical waters. They mainly live in the intertidal zones or on inner reefs such as bay and creeks (Alarif et al. 2019). Compared to their reef-building Scleractinian coral counterparts, there is a knowledge gap about the relationships between octocorals and their associated fauna (Stella et al. 2011; Calcinai et al. 2013).

Cuttlefishes are a group of cephalopod molluscs (Phylum Mollusca, Class Cephalopoda), and distribution ranging from temperate to tropical seas, mainly in shallow depths. The cuttlefish *Sepiella inermis* Van Hasselt [in Férussac and d'Orbigny], 1835, is one of seven species in the genus *Sepiella* Gray, 1849 belonging to the family Sepiidae (MolluscaBase 2020). *S. inermis* is a small-sized cuttlefish (maximum mantle length about 12.5 cm) with narrow fins and a mantle with a pore at the posterior extremity (Jereb and Roper 2005). The species is widely distributed through Indo-West Pacific region (31°N - 19°S, 32°E - 121°E) from the Arabian Sea to Indonesian water and in the tropical waters of the southern South China Sea (Jereb and Roper 2005). In Indian waters, it is widely distributed along both east and west coasts up to depths of about 40 m (Sundaram and Khan 2011). *S. inermis* is a vital food source for a large number of predators like perches, tunas, swordfishes, dolphins, toothed whales, sharks, and sea birds, and thereby plays a vital role in marine food webs (Chakraborty et al. 2013). In addition, *S. inermis* is also an economically important marine species in India, where cephalopods are a major marine fishery export commodity, with increasing demand in various parts of

the world (Sundaram and Khan 2009; 2011). *S. inermis* constitutes 2-4% of the total cephalopod landings of India (CMFRI 2006) and presently experiencing overexploitation threats (Madhu 2021).

Typically, cuttlefishes attach their eggs to a hard and anchored substrate in shallow coastal areas, such as plants, algae, sponges, and artificial objects like rope and cuttlefish traps (Larcombe and Russell 1971; Boletzky 1998). A few studies have also reported that cuttlefishes use hard corals and octocorals as habitats for mating and substratum for egg deposition (Bouligand 1961; Darmaillacq and Osorio 2017; Pratasik et al. 2017; Sam et al. 2018). In the present study, we report that the cuttlefish *S. inermis* utilizes the octocorals *Pseudopterogorgia* sp. and *Menella* sp. as egg-laying substrate along the highly urbanized coastline of Mumbai, India.

2. Material and Methods

2.1. Study sites

This study was conducted in the rocky intertidal regions of the Mumbai coast, Eastern Arabian Sea (Fig. 1). The study sites (Marine Drive and Worli) are highly impacted by human activities, such as rapid coastal development, the discharge of domestic sewage, nutrients, pollutants, and sediment, as well as fishing pressure. The two study sites are characterized as a rocky intertidal habitat with the presence of several large tidal pools (maximum depth 0.46 m at -0.1 m low tide level and 2-14 m in length, 1-5 m wide), covered with sediment, algal turf, macroalgae, encrusting stony corals (Scleractinia), sponges and zoanthids (Zoantharia). Marine Drive is situated in a protected bay, where the wave breaks outside of the bay due to low slope and depth, and the intertidal region of Worli is directly exposed to constant wave energy.

2.2. Survey and data collection

The survey was conducted in October-November, 2020, during low tide. A thorough search was conducted to locate cuttlefish eggs and identify the substratum they were attached to. For quantification of octocoral and cuttlefish egg density, surveys along a 10 m × 2 m long belt transect covering a 20 m² area laid perpendicular to the shore were conducted in triplicate at each study site. All octocoral colonies with and without cuttlefish eggs attached and attached egg capsules (Figs. 2a-b) within the transect area were counted and photographed using camera Olympus TG-3 and Samsung M31 (64 MP). Two egg capsules were collected from each site for identification purposes without damaging the octocorals (Fig. 2d). Close-up photographs were also taken to ensure the presence of cuttlefish in egg capsules (Fig. 2c). All organisms that were found with eggs attached were photographed in high resolution for later identification and identified to the lowest possible taxonomic level using taxonomic keys (Bayer 1981; Sánchez and Wirshing 2005; Devictor and Morton 2010; Williams and Chen 2012).

These specimens were not collected due to restrictions in the Indian Wildlife (Protection) Act 1972 (Schedule I, Part IVA).

2.3. Statistical analysis

Data were checked for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. A Mann Whitney test was performed (at $p = 0.05$) between the number of capsules at each of the two sites and the two species of octocoral as the data were non-normally distributed. All values are given as means \pm SE. All the test and graphical illustrations were performed using Minitab v17.1.0.

3. Results and Discussion

The present study sheds light on the association of cuttlefish with the octocoral community in a shallow intertidal region of the Eastern Arabian Sea. Clusters of *S. inermis* egg capsules were found attached to colonies of two octocoral species such as *Pseudopterogorgia* sp. and *Menella* sp. (Figs. 2a-b) in two coastal areas of Mumbai. Moreover, we did not observe any association between cuttlefish eggs and other potential egg-laying substrates such as poriferans, pennatulaceans, ascidians, zoantharians, etc., or on any other type of substratum in the study area. In total, we observed 92 individual octocoral colonies across both study sites. The highest number of octocoral colonies with a total of 63 individuals and mean density of 1.05 ± 0.14 SE ind./m² was recorded at Marine Drive (Fig. 3a), while 29 octocoral colonies with a mean density of 0.48 ± 0.07 SE ind./m² were recorded at Worli. Of the 92 individual colonies, 45 were found with attached egg capsules, corresponding to 48.91% of total octocorals colonies across both study sites (Fig. 3b). At Marine Drive, 36 colonies (57.14 %) out of 63 colonies had cuttlefish eggs, and at Worli, nine colonies (31.03 %) out of 29 colonies were found with egg capsules.

Pseudopterogorgia sp. was more dominant than *Menella* sp. at both sites. *Pseudopterogorgia* sp. contributed to 63.49% and 58.62% of total octocoral composition at Marine Drive and Worli, respectively. 49.12 % (n=28 out of 57 colonies) of the *Pseudopterogorgia* colonies and 48.57% (n=17 out of 35 colonies) of the *Menella* colonies were found to have cuttlefish eggs. A total of 1449 egg capsules were counted during the study, out of which 85.44% (n=1238) were at Marine Drive, and 14.56% (n=211) were found at Worli. At substrata level, 31.19% (n=452) of the egg capsules were attached to *Menella* colonies, and 68.81% (n=997) of the egg capsules were found deposited on the *Pseudopterogorgia* colonies. This difference between the number of egg capsules attached to the octocoral species was not statistically significant (Mann Whitney test: $p > 0.05$). Therefore, our results

suggest that the cuttlefishes do not show any specificity towards the octocoral species as a spawning substrate.

At Marine Drive, the mean density of *S. inermis* egg capsules was recorded as 37.96 ± 4.07 SE and 28.08 ± 2.57 SE egg capsules per colony on *Pseudopterogorgia* sp. and *Menella* sp. respectively (Fig. 3c). Whereas, at Worli, the mean density of *S. inermis* eggs was 24.8 ± 3.57 SE and 21.75 ± 7.78 SE number of egg capsules per colony on *Pseudopterogorgia* sp. and *Menella* sp. respectively. The Mann Whitney test showed a significant ($p = 0.007$) difference between the sites in the number of egg capsules attached to the octocorals. This could potentially be due to a preference for a sheltered bay with low wave exposure (Marine Drive) than more exposed sites (Worli) for laying eggs. Furthermore, studies indicated that local hydrodynamics and protection from wave exposure are the key structuring forces that regulate the distribution of the octocoral community (Birkland 1974). The high density of octocorals colonies at the Marine Drive could be attributed to the protected nature of the area from wave energy, which may positively influence higher cuttlefish egg deposition. Our results indicate that the prevalence of *S. inermis* egg capsule clusters on octocoral colonies was highest at the site with the higher density of host octocorals (Marine Drive), suggesting a habitat-dependent relationship for the egg-laying of *S. inermis*. Our observation did not show any adverse effect, and association-related tissue damage of the octocoral colonies and the coral polyps were able to extend without being hampered by the *S. inermis* egg capsules. Therefore, we excluded the option of parasitic interaction in this relationship. However, understanding of the underlying physiological and biological processes of the Octocoral colonies that were influenced by this association is beyond the scope of the present study.

Despite the lack of robust physical protection in the highly competitive marine environment, the octocorals primarily depend on their chemical defensive system by a wide range of secondary metabolites accumulating in their bodies or releasing to their ambient environment. Similar to corals, this chemical defense system of octocorals serves as antipredatory, antimicrobial, allelopathic, and antifouling agents (Changyun et al. 2008). Furthermore, octocorals contain several bioactive compounds, such as alkaloid, flavonoid, steroid, and saponin, that protect them from predators (Abdel-Lateff et al. 2019; Alarif et al. 2019). It is possible that cuttlefish selectively lay their eggs on these octocorals for the protection it provides. For instance, Pratasik et al. (2017) hypothesized that hard corals provide physical and chemical protection to the cuttlefish *Sepia latimanus* eggs during their early life cycle. However, it is unclear if this is the case here for *S. inermis*, and awaits further study. Previous studies suggested cuttlefishes uses a wide range of organisms and objects for egg-laying purpose including macrophytes, sponges, corals, rope, cuttlefish traps, etc. (Boletzky 1998; Sam et al. 2018). Further, octocoral-cuttlefish associations are also known. For instance, *Sepia elegans* lays eggs

on the octocoral *Alcyonium palmatum* (Bouligand 1961). Results of the present study indicate that *S. inermis* is only laying on the dominant octocoral in the study sites, which indicates habitat preferability of *S. inermis* towards the octocoral species. However, further experimental studies using different spawning substratum are required to confirm the habitat preference.

The nearshore corals of the Central West coast of India are highly threatened due to multiple stressors, including global climate change-driven coral bleaching events and pressures from rapid coastal development activities (De et al., 2020a, 2021; Nanajkar et al. 2019). Additionally, these corals are regularly exposed to drastic salinity fluctuations from monsoonal dilution of seawater, high sedimentation, organic and inorganic pollution, commercial fishing, and extensive recreational activities (De et al., 2020b; Kamble and Vijay, 2011). Coastal seawater along the Mumbai coast is experiencing severe sewage pollution due to the release of partially treated domestic and industrial wastewater and discharges from various point and non-point sources (Vijay et al., 2015). The present study areas are surrounded by west-flowing rivers, estuaries, and creeks, which cause continuous turbidity of the seawater around this region (see figure 1). Despite the fact that juveniles *S. inermis* prefer habitats with high current velocity and turbid waters and low underwater brightness levels (Nabhitabhata and Nilaphat 2000). The dissolved oxygen levels in nearshore inland waters (bays, estuaries, and creeks) have been found lowest (< 2 mg/L) than stipulated standards during low tide. Nevertheless, the Marine Drive area was within limits for aquatic life (SW II standard) (Kamble et al. 2010). Habitat availability for reproduction is critical for organisms to attain sexual maturity, maintain a life cycle that ensures the sustainability of species population (Stoner 2003). In summary, the present study indicates that the octocoral population in Mumbai's rocky intertidal region provide essential nursery habitat to *S. inermis* for spawning, safe shelter for juveniles and may enhance survivorship. Thus, loss or degradation of octocoral habitat could negatively affect *S. inermis* population as well, adding further impetus to protect these important organisms. However, the factors that regulate the ecological interaction need further investigation to identify the possible detrimental or positive effect of this association on fragile coral ecosystem.

Acknowledgments

Authors are thankful to the Scientist in charge, CSIR-NIO, Regional Centre, Mumbai, for the facilities. We want to thank three anonymous reviewers for their valuable comments for improving the article. The author (SG) would like to acknowledge the Council of Scientific and Industrial Research (CSIR) for providing the CSIR-Senior Research Fellowship (CSIR-SRF). This is NIO contribution number

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Declarations

Funding: No funds, grants, or other support was received.

Conflicts of interest/Competing interests: Authors declared that they have no competing interests.

Availability of data and material: All data generated or analyzed during this study are included in this published article and will be available with the corresponding author on request.

Code availability: Not applicable

Authors' contributions: All authors contributed to the conceptualization and design of the study. Material preparation, data collection, and analysis were performed by Santosh Gaikwad, Kalyan De, and Sabyasachi Sautya. All authors reviewed, edited, and approved the finalized manuscript.

Ethics approval: No approval of research ethics committees was required to accomplish the goals of this study because the work was conducted with an unregulated invertebrate species. Two *Sepiella inermis* egg capsules were collected for identification as the species is an unregulated invertebrate species as per the Indian Wildlife (Protection) Act 1972. And none of the octocorals were harmed during the study.

Consent to participate: Not applicable

Consent for publication: All authors have given their consent to publish the data

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Fig. 1 Geographic location of the study sites (Marine Drive and Worli) situated at low intertidal zone along the Mumbai coast, Eastern Arabian Sea. The map was produced using QGIS v2.14.

Fig. 2 Octocoral colonies with egg capsules of the cuttlefish *Sepiella inermis* attached: **a** *Pseudopterogorgia* sp.; **b** *Menella* sp.; **c** close-up of egg capsules of the cuttlefish *S. inermis*; **d** two juvenile individual of *S. inermis* with ovate shell and red spot on the posterior end.

Fig. 3 Plots of analytical parameters related with octocorals and egg capsules of the cuttlefish count in Marine Drive and Worli sites: **a** mean density of octocorals; **b** percentage composition of octocorals which are used as substratum; **c** mean count of cuttlefish egg capsules per species per site. Octocoral species are denoted as *Pseudopterogorgia* sp. (Ps sp.) and *Menella* sp. (Me sp.).

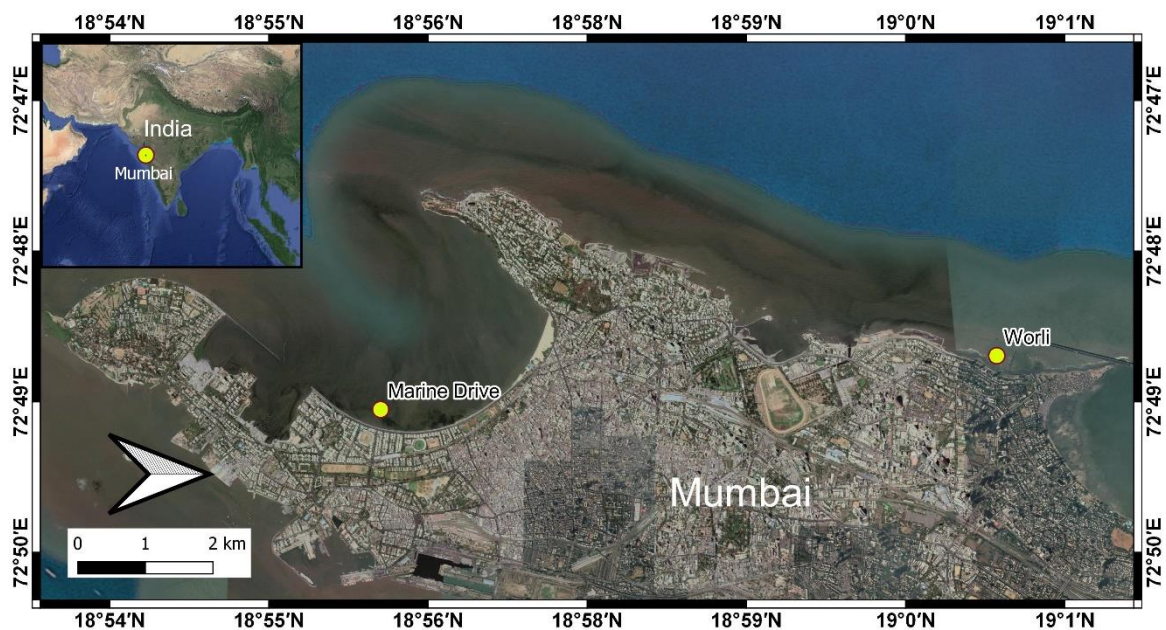


Figure 1

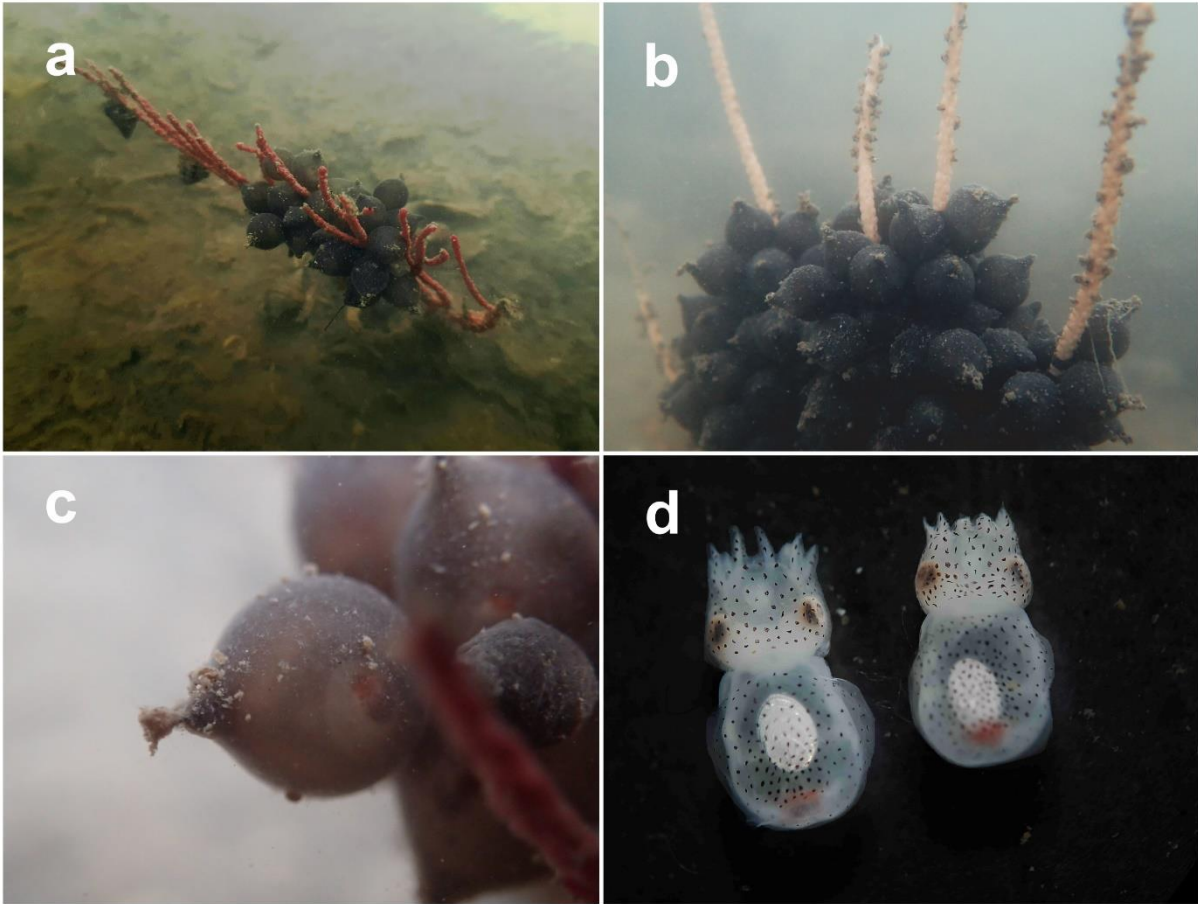


Figure 2

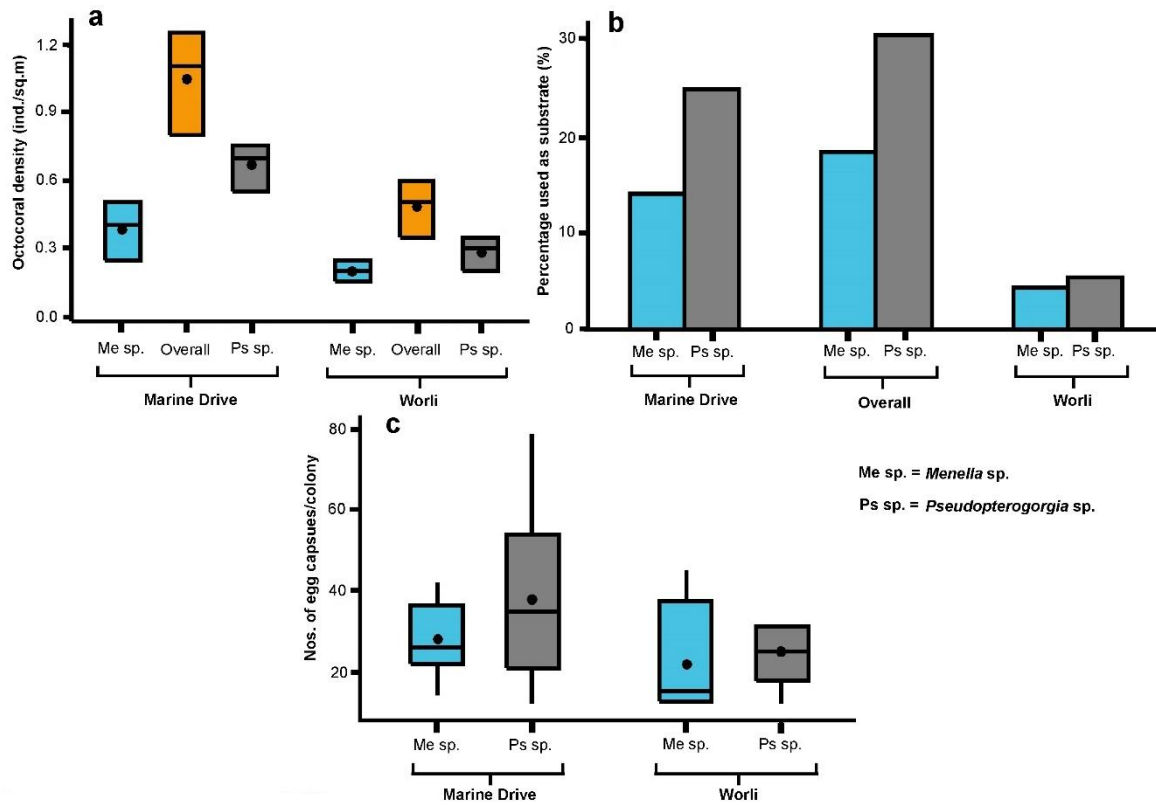


Figure 3