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Macrofouling assemblages in coastal waters adjacent to the Port of Colombo, Sri Lanka

M.P. Hendawitharana, R.R.M.K.P. Ranatunga *

Center for Marine Science and Technology, Department of Zoology, University of Sri Jayewardenepura, Sri Lanka

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ABSTRACT

Sri Lanka was an important node and a major centre of international trade in the ancient maritime silk route, and still serves a significant role in present global maritime trade. The critical role played by the country's ports on economic development has come at the cost of compromising the ecological health of the environment. The present study was to assess the status of biofouling assemblages in coastal waters impacted by the Port of Colombo. Sampling was conducted from February to December 2018 in the Dikkowita Fisheries Harbour, Kirulapone canal opening, and Panadura Fisheries Harbour. The fouling assemblages in the rocky intertidal zone were studied using photo-quadrats and environmental surveys. Photo-quadrats were obtained monthly following a protocol developed by the Marine Biological Association of the United Kingdom and modified to suit the local environments, whilst area surveys were conducted at two-month intervals. In addition, artificial settlement structures were deployed to assess subtidal fouling communities. Species abundance was estimated by individual counts, and percentage cover was determined using CPCe software. A total of 47 macrofouling taxa were recorded. High numbers were recorded from the phyla Mollusca (20), Arthropoda (06), and Porifera (4). *Saccostrea cuccullata* (Bivalvia), *Cellana radiata* (Gastropoda), *Clypidina notata* (Gastropoda), and *Balanus reticulatus* (Crustacea) were some of the common species found in all three study sites. Oysters were the dominant macrofouling organisms in terms of percentage cover in Dikkowita Fisheries Harbour and Kirulapone canal opening. Our study also recorded three globally invasive species, *B. reticulatus*, *Amphibalanus amphitrite* and *Perna viridis*, alerting us to the need for long-term monitoring.

1. Introduction

Fouling is the accumulation of matter on surfaces. If the fouling is due to organisms, it is referred to as biofouling. Biofouling can occur either through biofilms created by micro-organisms (microfouling) or macro-organisms (macrofouling). In addition, fouling can also occur through the deposition of inorganic matter. When biofouling occurs on the surfaces of living organisms, it is referred to as epibiosis. Macrofouling is a consequence of accumulating macroscopic algae and invertebrates such as hydroids, bryozoans, sponges, tunicates, soft corals, barnacles, tubeworms, or mussels (Menchaca et al., 2014). Macrofouling community development is a highly dynamic and complex process, governed by many factors including the substratum, locality, season, climate, immersion period, competition, and predation (Richmond and Seed, 1991; Callow and Callow, 2002).

Long-distance shipping has increased substantially over the decades as a consequence of modernization and globalization of world trade (Ojala and Tenold, 2017). Macrofouling has caused adverse effects to maritime activities in terms of ship hull fouling as well as the colonization of submerged structures such as cooling water intake tunnels, culverts, pump chambers, and aquaculture equipment (Pugh et al., 2005; Fitridge et al., 2012; Nair, 2013). Ship hull fouling causes detrimental economic impacts including increased fuel consumption due to drag created by biofouling mass accumulation, increased corrosion vulnerability, and excessive cost and time associated with hull maintenance (Schultz, 2007; Murray et al., 2013). In addition, biofouling is also known for the spread of non-indigenous species and invasive species across borders, mainly via commercial shipping activities (Drake and Lodge, 2007; Hewitt et al., 2009; Sylvester et al., 2011).

Sri Lanka's biofouling community is relatively understudied and has only received very limited attention to date (Marasinghe et al., 2018). The availability of literature on the biofouling community in coastal waters of Sri Lanka is also limited, where the majority of studies have mainly focused on ports (Jayasundara and Ranatunga, 2016; Marasinghe et al., 2016a; Ranatunga et al., 2017). Early studies have been conducted as pilot Port Biological

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* Corresponding author.
E-mail address: ranatunga@sci.sjp.ac.lk (R.R.M.K.P. Ranatunga).

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Baseline Surveys (PBBS). Biofouling research has received a significant boost with the deployment of artificial settlement structures to which the access of subtidal fouling communities has been enabled (Marasinghe et al., 2016a). However, most recent biofouling research has focused on the presence of exotic or invasive species limited to a few taxonomic groups (Marasinghe et al., 2015a,b, 2016b, 2018).

According to the Drewry Port Connectivity Index in the fourth quarter of 2017, the Port of Colombo which handles the majority of the country's foreign cargo was ranked 13th in the world in terms of facilitating mainline services (Sri Lanka Ports Authority, 2019). Although Port Biological Baseline Surveys have been conducted by the Marine Environment Protection Authority (MEPA) within the Port of Colombo, the absence of biofouling studies in adjacent coastal waters impacted by the Port of Colombo is a knowledge gap that needs to be addressed (Ranatunga et al., 2015; MEPA, 2019). Moreover, the detection of highly invasive encrusting bryozoans such as *Schizoporella errata* and *Watersipora subtorquata* in the Port of Colombo strongly supports the need for close monitoring of biofouling community in marine environs. (Marasinghe et al., 2015a, 2018).

In this work, we investigated the macrofouling assemblages in coastal waters adjacent to Port of Colombo to assess the present status of macrofouling community composition with reference to spatial and temporal aspects.

2. Materials and methods

2.1. Study area

The present study was conducted at three locations (Dikkowita Fisheries Harbour, Kirulapone canal opening and Panadura Fisheries Harbour) from February 2018 to December 2018 along the coastal waters adjacent to Port of Colombo in the west coast of Sri Lanka (latitudes 5°55′ – 9°51′ N and longitudes 79°41′-81°54′ E) (Fig. 1a). A pilot survey was conducted in February 2018 to identify the most suitable sampling sites to fulfil the research objectives.

Dikkowita Fisheries Harbour is situated 3 km northward from Port of Colombo and is considered to be the largest fisheries harbour in South Asia, with a basin area of 11.7 ha and 1170 m long breakwater (Fig. 1b). The Northern basin is for fishing vessels targeting local markets and the Southern basin is for export-oriented fishing vessels. For the present study, nine sampling points (D1–D9) were selected, out of which two were from the Northern basin.

The breakwater near Kirulapone canal opening, situated 3 km southward from the Port of Colombo, encloses a harbour for local fishing vessels. Four sampling points (K1–K4) were selected along the 700 m long breakwater (Fig. 1c). The monsoon brings a large influx of freshwater from the Kirulapone canal which can result in substantial salinity fluctuations.

Panadura Fisheries Harbour, situated 27 km south of the Port of Colombo, was selected as a reference point due to its distance from the main port and limited international shipping activities. It has a basin area of 2.8 ha with two breakwaters. Seven sampling points (P1–P7) were selected along the main breakwater of the Panadura Fisheries Harbour for the present study (Fig. 1d). Large quantities of freshwater received from Bolgoda River during the Southwest Monsoon (SWM) causes significant salinity fluctuations in this harbour.

The water quality parameters and environmental conditions in the west coast are highly influenced by the monsoon winds and are marked by four seasons. The Southwest Monsoon and the Second-Inter Monsoon (SIM) affect high annual precipitation in the west coast (Appendix A). Conversely, it is expected to have relatively low annual precipitation values during the First-Inter Monsoon (FIM) and Northeast Monsoon (NEM) periods. With the influx of freshwater from adjacent watercourses, high precipitation lowers the salinity in these waters while increasing the Total Dissolved Solids (TDS) and turbidity. This creates adverse environmental conditions that lead to the mass mortality of biofouling communities.

2.2. Sampling procedure

The sample collection for the present study was carried out to investigate the spatial and temporal aspects of macrofouling community assemblages in coastal waters impacted by the Port of Colombo. The sampling procedure consisted of two main components: environmental monitoring and settlement structure sampling. A modified version of "The Shore Thing - survey procedure and protocol" developed by the Marine Biological Association of the United Kingdom was followed for environmental monitoring (Marine Biological Association, 2008).

2.2.1. Environmental monitoring

A combination of digital imaging technology with field sampling was used to conduct environmental monitoring (Moysés et al., 2007). The convenience sampling method was used to select predetermined sampling points where photo-quadrats were obtained from the rocky intertidal zone of breakwaters using 24×1 m^2 belt transects (Etikan et al., 2016). Each belt transect was divided into 12 plots (2×1 m^2) and numbered 1 to 12 accordingly. Random numbers were generated among the plots using Minitab 17 and sampled one at a time during each consecutive month. Samples were obtained using a 0.4×0.4 m^2 quadrat with four replicates placed randomly in each plot. Larger quadrat sizes were avoided as the camera has to be lifted to a higher position to align the upper and lower margins of the quadrat with the camera frame and this was impractical at many sampling points. Smaller quadrat sizes were also avoided due to time and labour limitations which would have required additional replicates to be carried out. Macrofouling organisms were photographed using OLYMPUS Stylus TG-3. Photographs were obtained by aligning upper and lower margins of the quadrat with the camera frame, while maintaining a fixed distance with the subjects imaged, thus providing 20×25 cm^2 area per picture (Moysés et al., 2007).

Moreover, area surveys were conducted at two-month intervals for all three study locations along the breakwaters and in the surrounding environment to cover upper, middle, and lower intertidal zones (Connell, 1972). Each sampling location was searched for a total of 30 min and a checklist of observed fouling organisms was developed during the study period. The observed fouling organisms were identified to the lowest taxonomic level possible by observing the fine morphological features with the aid of field identification guides (CABI, 2019; Coppejans et al., 2009; WoRMS, 2019). Representative samples, that could not be identified with certainty in the field, were taken to the laboratory for further analyses. All the specimens encountered were preserved as reference specimens in the museum of the Department of Zoology, University of Sri Jayewardenepura for further studies and future references.

2.2.2. Sampling with artificial settlement structures

Artificial structures were placed at all the three sampling locations, namely Dikkowita Fisheries Harbour, Kirulapone canal opening, and Panadura Fisheries Harbour in March 2018 to investigate the succession of subtidal biofouling communities and the occurrence of Non-indigenous species (NIS) in the subtidal zone. Structures consisted of a square cross-section with vertical surface dimensions of 80 cm height and 40 cm width (Fig. 2).

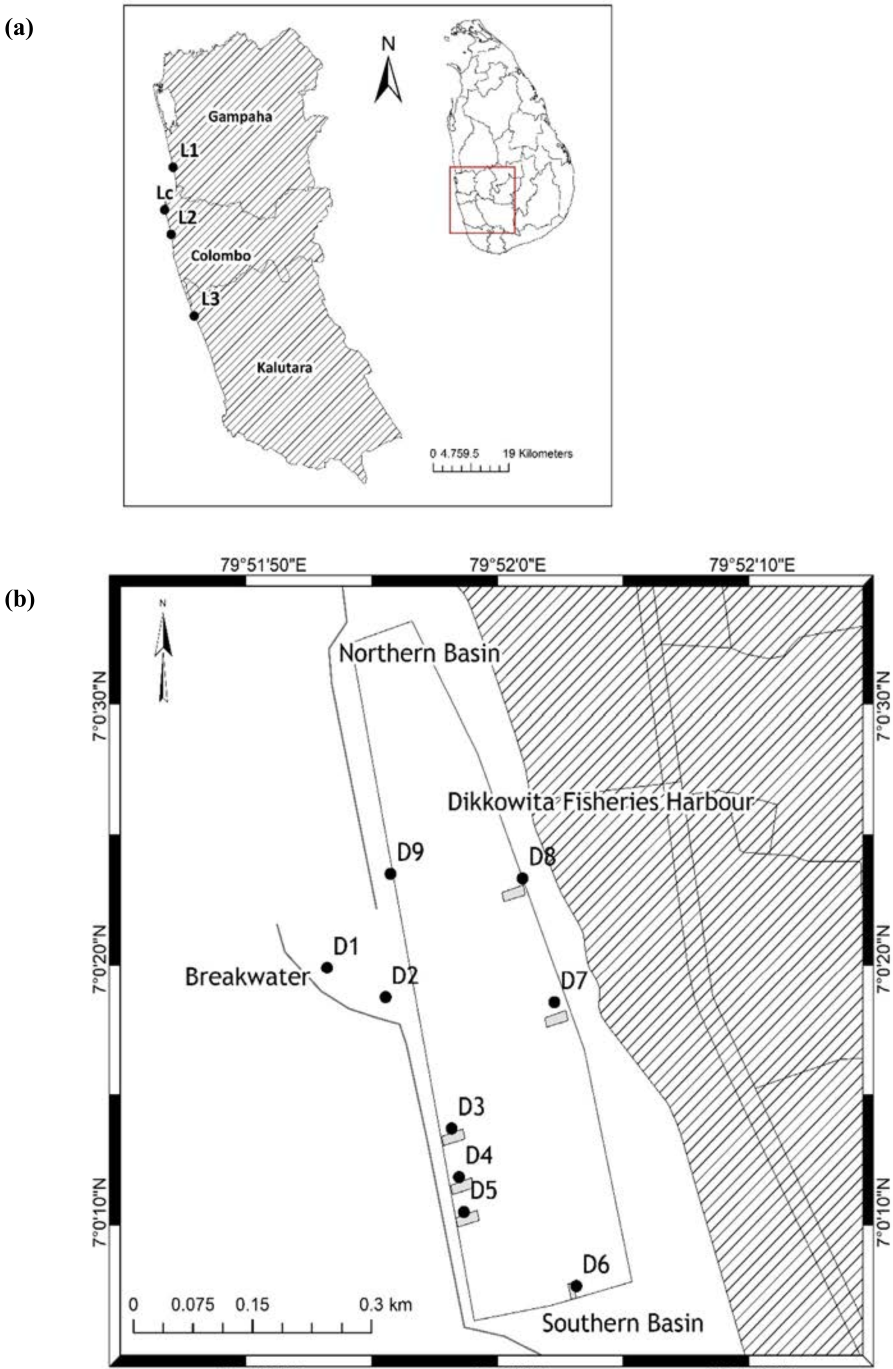

Fig. 1. Study locations in the vicinity of Colombo, Sri Lanka. (a) Map of Western Province indicating study sites Lc, L1 through L3; (b) Dikkowita Fisheries Harbour study sites D1 though D9; (c) Breakwater near Kirulapone canal opening, study sites P1 through P9; (d) Panadura Fisheries Harbour, study sites K1 to K4.

Four substrate types, namely solid wood, Fibre-Reinforced Plastic (FRP), concrete (a mixture of sand, granite, and cement in 3:2:1 ratio respectively) and steel metal plates (16 gauge) were used to cover each vertical surface whereas the top and bottom horizontal surfaces consisted of wood. These structures were placed in the lower subtidal zone at a depth of 2 m from the water surface at low tide using heavy anchors. Buoys were attached to the upper surface to keep the structure in a vertical position while submerged (Fig. 2). Three structures were placed at each sampling location, out of which one was used as a backup. At two-month intervals, all structures were sampled by retrieving them from water. The Photo-quadrat method was used to sample the settlement structures where each surface was considered as a quadrat. Macrofouling organisms on each substrate were examined and photographed without disturbing the biofouling community succession.

2.3. Data analysis

Photo-quadrats were analysed using Coral Point Count with Excel extension (CPCe) version 4.1 to quantify the abundance of the species by estimating the percentage coverage from individual counts (Kohler and Gill, 2006). The outer margin of macrofouling organisms was manually marked using the software

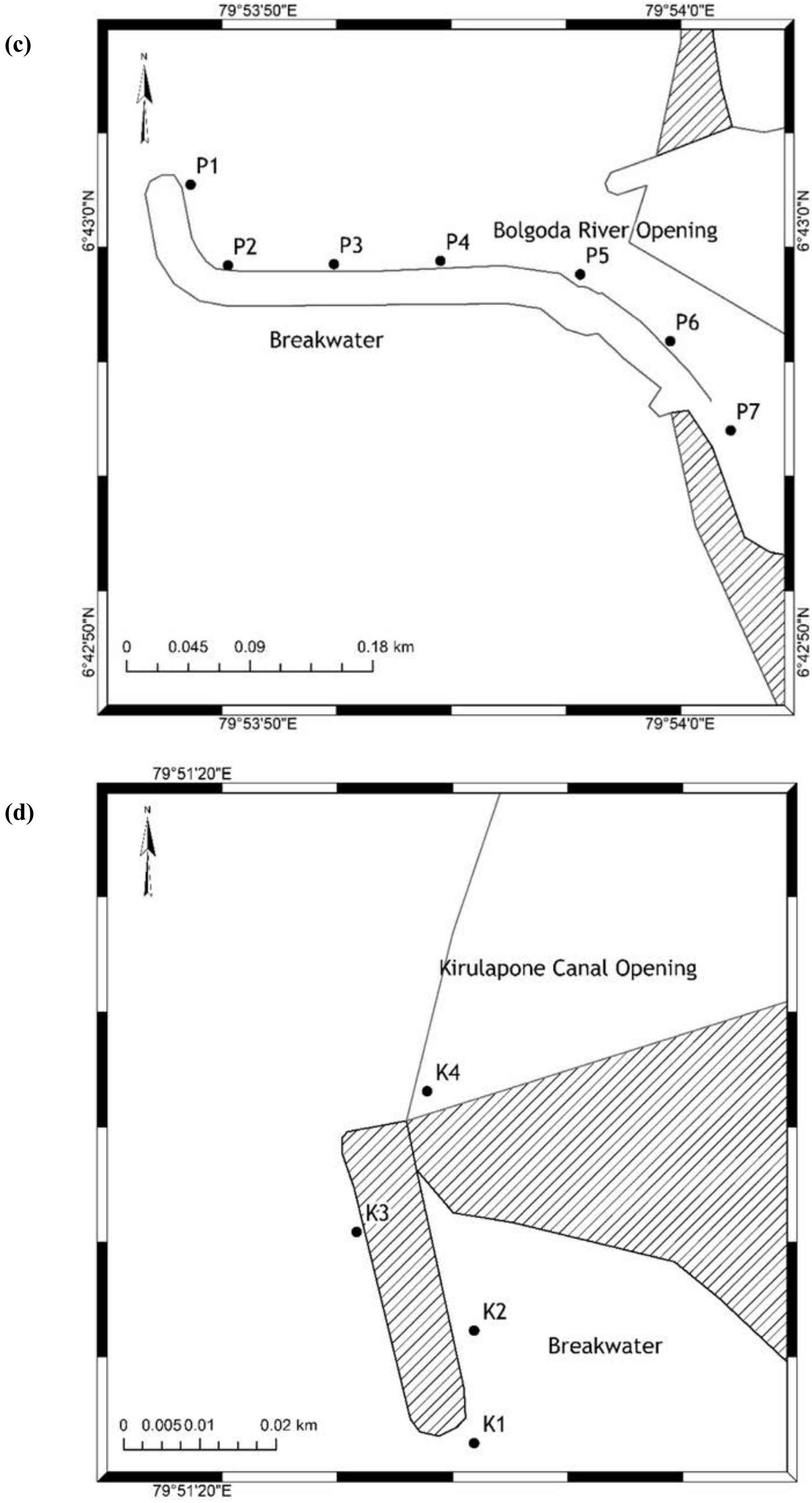

Fig. 1. (*continued*).

to estimate percentage area coverage. When organism count exceeded 100 individuals, mean area coverage of 10 random individuals was calculated and multiplied by the point count to obtain percentage area coverage for that species or organism type. Recorded fouling organisms were categorized into taxonomic groups (i.e. *S. cuccullata*, *Crassostrea* sp. and *Ostrea* sp. were pooled as oysters at Dikkowita Fisheries Harbour) to analyse the monthly variations in densities and percentage covers. Taxonomic

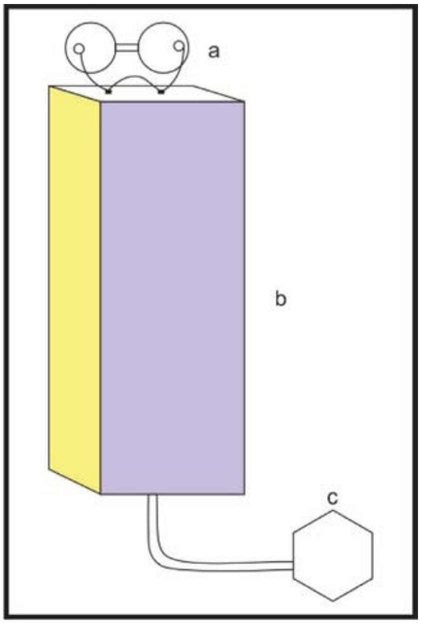

Fig. 2. Schematic diagram of the artificial settlement structure used in this study: a- floating buoys, b- vertical surfaces, c- anchor.

groups with low densities and/or percentage covers were excluded in graphical representations. Random Complete Block Design (RCBD) analysis was conducted using Minitab version 17 to investigate the relationships among the number of biofouling organisms and percentage area coverage with respect to (a) study locations and monsoon seasonal variation (Appendix A) and (b) tide hitting attributes and study locations. On each occasion, monsoon seasons and tide hitting attributes (the effect of wave action; wave strength, and direction) were selected as treatments. Since the focus was to identify the effect of wave attributes and monsoon seasons on overall biofouling assemblage in each study location, all biofouling taxa were pooled into one group and treated as a whole component. Species diversity (*H'*) (Shannon and Weaver, 1963), species richness (*R*) (Gleason, 1922), species evenness (*J'*) (Pielou, 1966) diversity indices and Hutcheson t-test (*t*) (Hutcheson, 1970) pairwise comparisons were also calculated for each study location (Appendix B).

3. Results

3.1. Assessment of fouling community structure

During the study, a total of 47 macrofouling taxa were identified from all three study locations. The list of macrofouling organisms identified is shown in Table 1. The highest number of fouling taxa (35) was recorded in Dikkowita Fisheries Harbour whereas 19 taxa in Kirulapone canal opening and 20 taxa in Panadura Fisheries Harbour were observed (Fig. 3).

3.2. The occurrence of globally invasive species.

The present study recorded three globally invasive species (Table 2) which include two barnacles (*A. amphitrite*, *B. reticulatus*), and a mussel (*P. viridis*).

3.3. Comparison of species diversity indices

The species richness (*R*) was highest in Dikkowita Fisheries Harbour (2.192) followed by Panadura Fisheries Harbour (1.302) and Kirulapone canal opening (1.168). Comparable species evenness values were observed in both Panadura Fisheries Harbour (0.524) and Kirulapone canal opening (0.562). The lowest species evenness value was observed for the Dikkowita Fisheries Harbour (0.254).

Pairwise comparison using Hutcheson t-test between Panadura reference point and Kirulapone canal opening obtained a significant P-value ($P<0.05$). A higher Shannon diversity index value was obtained for Panadura reference point (*H'* = 1.302; Confidence interval = 0.009) than for Kirulapone canal opening (*H'* = 1.168; Confidence interval = 0.007) (Fig. 4a). A significant P-value ($P<0.05$) was obtained for pairwise comparison of Hutcheson t-test between Panadura reference point and Dikkowita Fisheries Harbour. A high Shannon diversity index value was obtained for Panadura reference site (*H'* = 1.302; Confidence interval = 0.009) compared to Dikkowita Fisheries Harbour (*H'* = 0.734; Confidence interval = 0.039) (Fig. 4b).

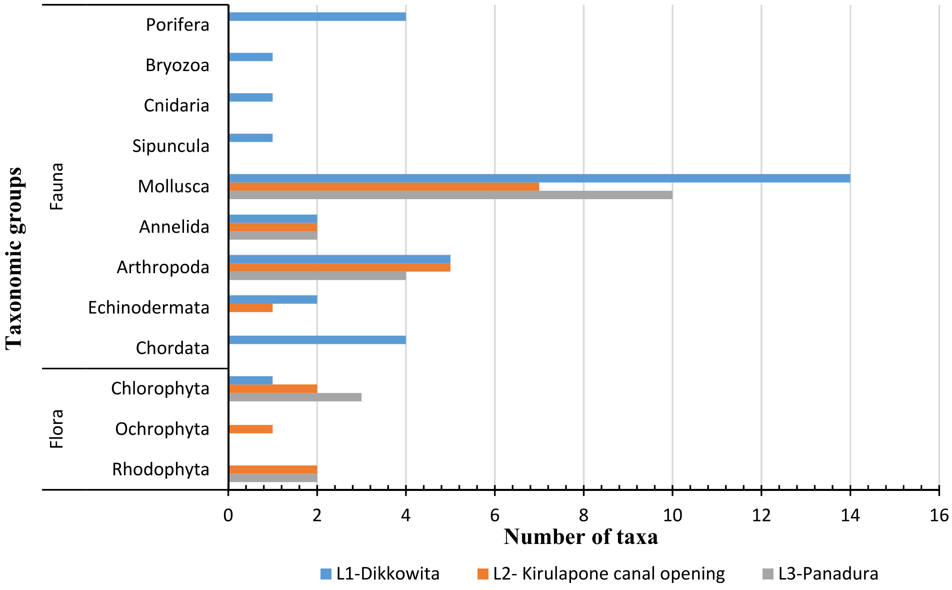

Fig. 3. Fouling community composition at three study locations near Colombo Port, Sri Lanka.

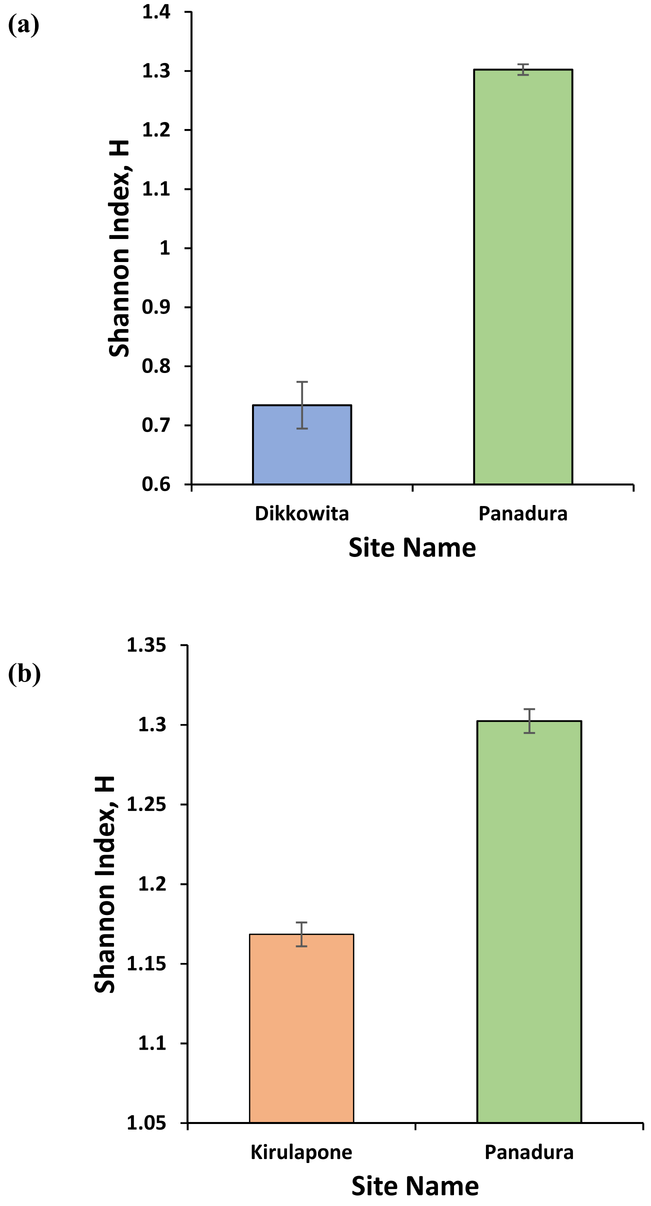

Fig. 4. Pairwise comparison of Shannon diversity index between (a) Dikkowita and Panadura fisheries harbours (b) Kirulapone canal opening and Panadura Fisheries Harbour.

3.4. Variation of fouling community structure

3.4.1. Fouling community structure in Dikkowita Fisheries Harbour

In the Dikkowita Fisheries Harbour, 35 biofouling taxa were recorded (Fig. 3 and Table 1). The highest species count was recorded from phylum Mollusca (14) followed by Arthropoda (5), Porifera (4), Chordata (3), Annelida (2), Echinodermata (2), Algae (2), Bryozoa (1), Cnidaria (1), and Sipuncula (1). *Patella* sp., *Cellana* sp., *Morula granulata* and *S. cuccullata* were the most common species recorded. The small variation in the densities of macrofouling organisms indicated minimal fluctuations during monthly samplings for all fouling organism groups except for oysters (Fig. 5a). From March to May 2018, oyster populations (*Crassostrea* sp. and *S. cuccullata*) displayed an increasing trend and reached a maximum of 693/m^2 during May. Both species then decreased in density from July (454/m^2) to September (394/m^2) and reached a minimum (112/m^2) at the beginning of November 2018. However, barnacles were absent from March to May 2018.

The percentage cover of oyster colonies exhibited similar patterns as the variation in the number of organisms (Fig. 5b). Being the dominant species, percentage area coverage of oysters fluctuated between a maximum of 33.4% (May) and a minimum of 13.0% (November). Spirorbid worms (*Spirorbis* sp.), the second most dominant species, displayed a bimodal distribution in percentage cover with one peak in June (14.7%) and another in October (5.5%). Records of marine algae were absent over three months between July to September 2018. Mussels (*P. viridis*) were only recorded during April (0.03%) and October (0.01%) with an extremely low percentage cover.

3.4.2. Fouling community structure in Kirulapone canal opening

The lowest recorded number was 20 biofouling taxa from Kirulapone canal opening (Fig. 3 and Table 1). Phylum Mollusca recorded the highest number of biofouling taxa (7) followed by Arthropoda (5), Annelida (2), Chlorophyta (2), Rhodophyta (2), Echinodermata (1), and Ochrophyta (1). *Chthamalus* sp., *Cellana* sp., *C. notata, and S. cuccullata* were the most abundant species recorded during the study. The fouling community in the Kirulapone canal opening was dominated by barnacles (*A. amphitrite* and *B. reticulatus*) in terms of individuals counts (Fig. 6a). Barnacle densities exhibited a bimodal distribution having two maxima in July (10 444/m^2) and December (9733/m^2). Barnacle numbers displayed extreme fluctuations having a maximum of 10 444/m^2 in May and a minimum of 172/m^2 in October. *Semibalanus* sp. was also recorded as sporadic patches in low densities. The highest density of gastropods was also recorded during July 2018 (24/m^2). Gastropods other than limpets (*Cellana* sp., *C. notata*, and *Patella* sp.) were absent during May, August, and October 2018.

In terms of percentage cover, the fouling community at Kirulapone canal opening was dominated by oysters (*S. cuccullata*) rather than barnacles (Fig. 6b). A maximum percentage cover of 49.8% and a minimum of 19.5% was recorded for oysters during the study. After May, oysters and algae displayed an inverse relationship in terms of monthly percentage area coverage. Percentage cover of barnacles displayed two maxima during July (18.8%) and December (15.9%).

3.4.3. Fouling community structure in Panadura Fisheries Harbour

In Panadura Fisheries Harbour, 21 biofouling taxa were recorded during the study (Fig. 3 and Table 1). Similar to the other two study sites, phylum Mollusca recorded the highest number of biofouling taxa (10) followed by Arthropoda (4), Chlorophyta (3), Annelida (2), and Rhodophyta (2). *S. cuccullata*, *M. granulata*, *Chthamalus* sp., *Chaetomorpha antennina*, and *Spyridia* sp. were recorded as the most common species during the study. Barnacles (*Chthamalus* sp.) stood out from the other macrofouling organisms in terms of individual counts at Panadura Fisheries Harbour (Fig. 7a) where it indicated a maximum of 8003/m^2 in April and a minimum of 1261/m^2 in October. From the end of April to beginning the of May, barnacles showed a decreasing trend. Other marine gastropods (*M. granulata*, *Cellana* sp. and *Patella* sp.) displayed an increasing trend starting from May and reached a maximum of 557/m^2 during August. The oyster (*S. cuccullata*) exhibited peak values of 259/m^2 and 213/m^2 during July and November months.

In Panadura Fisheries Harbour, *C. antennina* and *Spyridia* sp. were the dominant algae (Fig. 7b). The percentage cover of algae (*C. antennina* and *Spyridia* sp.) varied between 24.2% (highest) in December and 3.0% (lowest) in March. Barnacles and algae showed a decreasing trend from May to August gradually increased towards December. Oysters displayed a bimodal distribution having two peaks 10.7% (July) and 9.7% (November). Mussel species (*Xenostrobus* sp.) were only observed during July, November, and December. Polychaete worms (*Pseudonereis* sp.) were

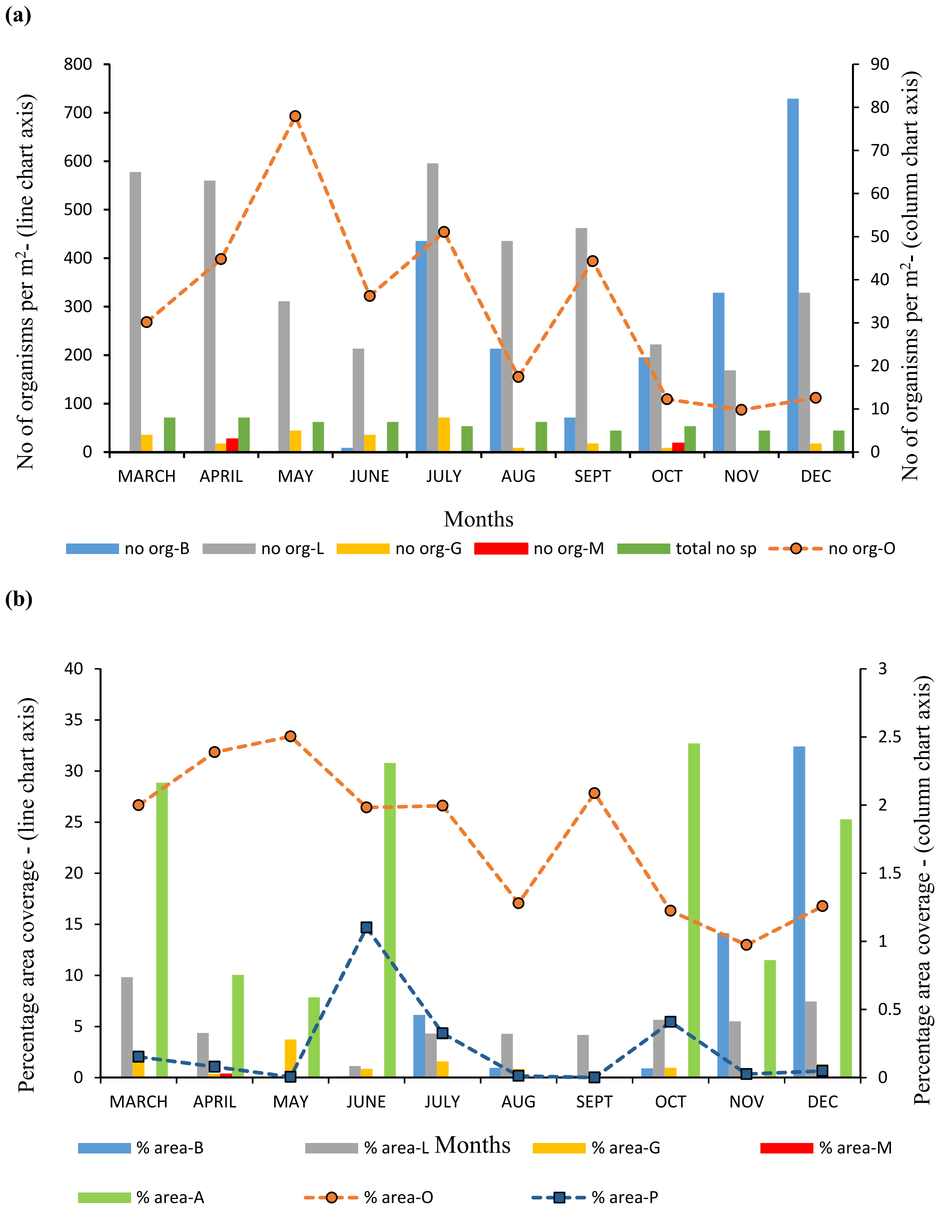

Fig. 5. Monthly variation in macrofaunal density and percentage cover at Dikkowita Fisheries Harbour between March and December 2018. (a) density; (b) percentage cover. Abbreviations: A- algae, B- barnacles, G- other marine gastropods, L- limpets, M- mussels, O- oysters, P- polychaete worms.

recorded only in March and October with extremely low percentage covers. The highest coralline algae cover recorded was 7.4% during March followed by 2.7% in October and 1.3% in November. Apart from that coralline algae were absent throughout the study.

3.5. Random Complete Block Design (RCBD) analysis

Randomized blocks were created with a treatment of tide hitting attributes, i.e., tide hitting directly on the fouling community (Hitting-1) and tide not hitting directly on the fouling community (Hitting-2). According to the ANOVA results, the paired interaction between hitting and location was significant ($P = 0.029$) at a 5% level of significance, hence the main effects of hitting and location were excluded from consideration. Natural logarithm values for the number of biofouling organisms were obtained to test normality which enabled the data to fit the ANOVA model more accurately.

From the interaction plot for the natural logarithm of response, it was evident that the mean number of fouling organisms in sampling sites with Hitting-1 treatment has high values for all three study locations than in sampling sites with Hitting-2 treatment (Fig. 8a). It was also evident when transitioning from Hitting-1 to Hitting-2 the expected number of fouling organisms on average for Panadura Fisheries Harbour was reduced. Hence, interaction plot segments being not parallel to each other explained the significance of interaction. The plot segment of location 3 not being parallel and having a higher decreasing gradient implied that there was a significant interaction between tide attributes and locations on fouling density.

A significant relationship was observed between the number of fouling organisms and a paired interaction of location with tide attributes ($P < 0.05$; 0.030). Hence relationships between main effects, i.e., location and tide attributes were excluded from consideration. The interaction plot for the response of area coverage indicated that the mean percentage area covers of fouling organisms in sampling sites with Hitting-1 treatment had high values for both Dikkowita Fisheries Harbour and Panadura Fisheries Harbour as compared to sampling sites with Hitting-2 treatment. For

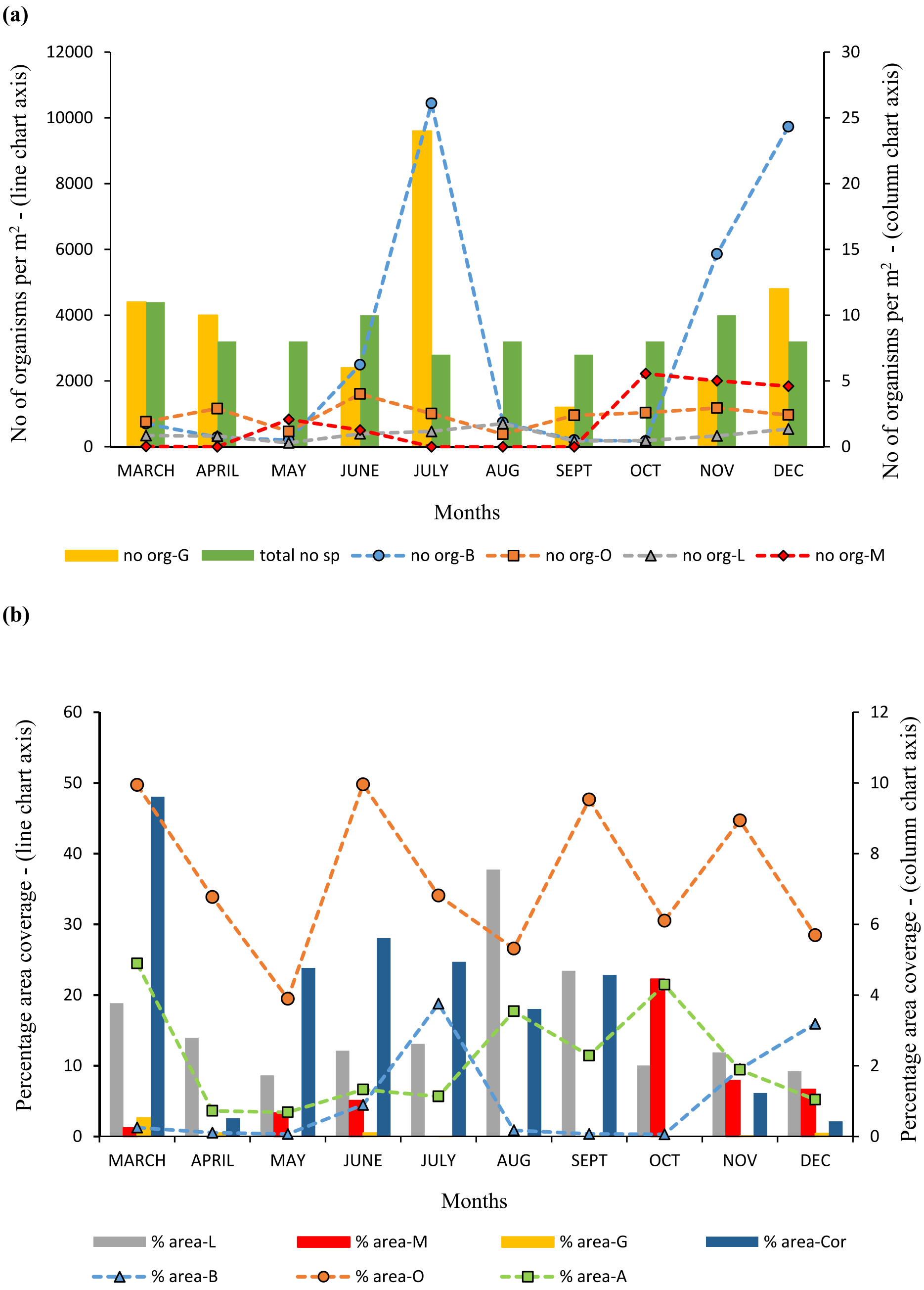

Fig. 6. Monthly variation in macrofaunal density and percentage cover at Kirulapone canal opening between March and December 2018. (a) density; (b) percentage cover. Abbreviations: A- algae, B- barnacles, Cor- coralline algae, G- other marine gastropods, L- limpets, M- mussels, O- oysters.

the Kirulapone breakwater, area coverage of fouling organisms remained relatively unchanged for both tide hitting and not hitting sampling sites (Fig. 8b). Hence interaction plot segments for Kirulapone breakwater being not parallel to other plot segments justified the significance of interaction. It implies that there was a combined significant interaction between tide attributes and locations on area coverage of fouling organisms. A P-value of P = 0.030 for the balanced ANOVA test supported this conclusion.

Randomized Complete Block Diagrams were created with four monsoon seasons as a treatment. According to the ANOVA results, paired interaction between season and location was significant ($0.007 < 0.05$) at 5% level of significance. Hence the main effects of season and location were excluded from consideration. From the interaction plot for the response, it was evident that for all four monsoon seasons Panadura reported the highest density of biofouling organisms (Fig. 9a). However, a rapid decrease of fouling organisms was indicated during the Southwest Monsoon period in Panadura. All three study locations reported a higher density of biofouling organisms during the Northeast Monsoon period. Interaction plot segments for Dikkowita Fisheries Harbour indicated slight variations in the biofouling densities during the study.

According to the ANOVA test, the paired interaction between season and location was significant ($P=0.036 < 0.05$). Hence the main effects of season and location were excluded from consideration. According to the interaction plot, the highest mean percentage area coverage of biofouling organisms was observed at Panadura Fisheries Harbour in all four seasons (Fig. 9b). Interaction plot segments for Panadura Fisheries Harbour also indicated that First-Inter Monsoon and Second Inter-Monsoon have the highest mean percentage area coverage of fouling organisms

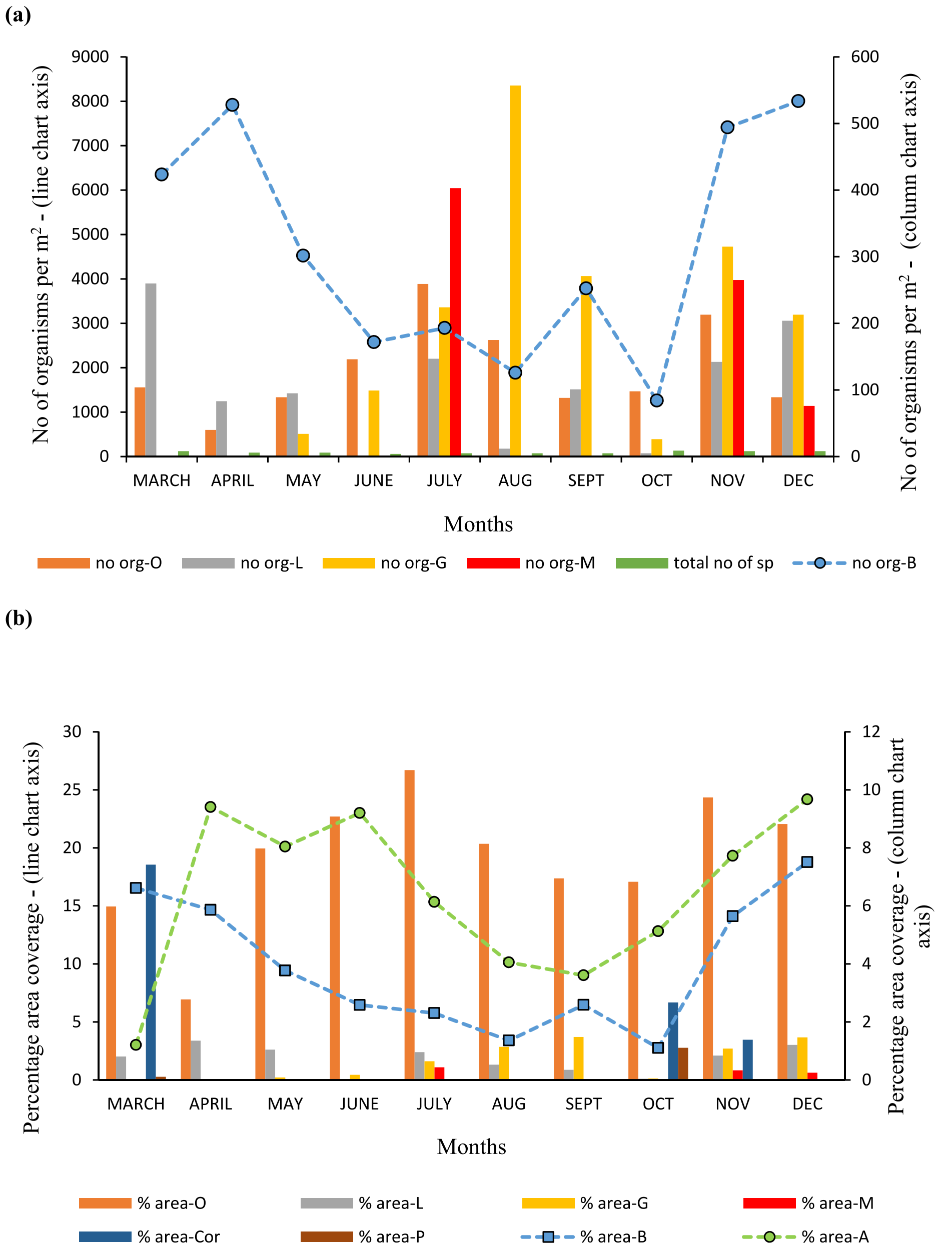

Fig. 7. Monthly variation in macrofaunal density and percentage cover at Panadura Fisheries Harbour between March and December 2018. (a) density; (b) percentage cover. Abbreviations: A- algae, B- barnacles, Cor- coralline algae, G- other marine gastropods, L- limpets, M- mussels, O- oysters P- Polychaete worms.

whereas SWM and SIM periods have the lowest. It was also evident that the percentage cover of biofouling organisms remained relatively unchanged for Dikkowita Fisheries Harbour. During the NEM period, the highest percentage cover of biofouling organisms was observed at all three study locations.

4. Discussion

The present study recorded 47 species of biofouling organisms belonging to 12 taxonomic groups, namely Mollusca (20), Marine algae (7), Arthropoda (6), Porifera (4), Chordata (3), Annelida (2), Echinodermata (2), Cnidaria (1), Bryozoa (1), and Sipuncula (1). In comparison, studies by Ranatunga et al. (2015) and Marasinghe et al. (2016a) in the Port of Colombo recorded 38 and 55 species of biofouling organisms respectively where phylum Mollusca was the dominant group in both studies. Moreover, the cheilostome encrusting bryozoan *Celleporaria volsella* which was described by Marasinghe et al. (2015b) as the first record from Sri Lankan waters, was also observed on artificial settlement structures at Dikkowita Fisheries Harbour. *S. cuccullata, Cellana* sp., *Patella* sp., and *C. antennina* were the most abundant species in all three study locations.

The obtained results display some marked differences in the diversity and abundance of species between sites. Amongst recorded species, only eight were found to be common in all three study sites. They are; *S. cuccullata*, *Cellana* sp., *Patella* sp., *Pseudonereis* sp., *Spirorbis* sp., *Semibalanus* sp., *A. amphitrite,* and *C. antennina*. The site-specific occurrence of particular species at each study site could be attributed to their adaptations or preferences to environmental conditions such as salinity, species competition, or pollution (Moran and Grant, 1993; Swami and Udhayakumar, 2010; Burrows, 2012). For instance, bivalves in the genus *Isognomon* were recorded exclusively from the Panadura Fisheries Harbour. A well-established subtidal community of *Isognomon* sp. was observed at sampling points P-6 and P-7. These byssate bivalves, also known as tree oysters, can be found in

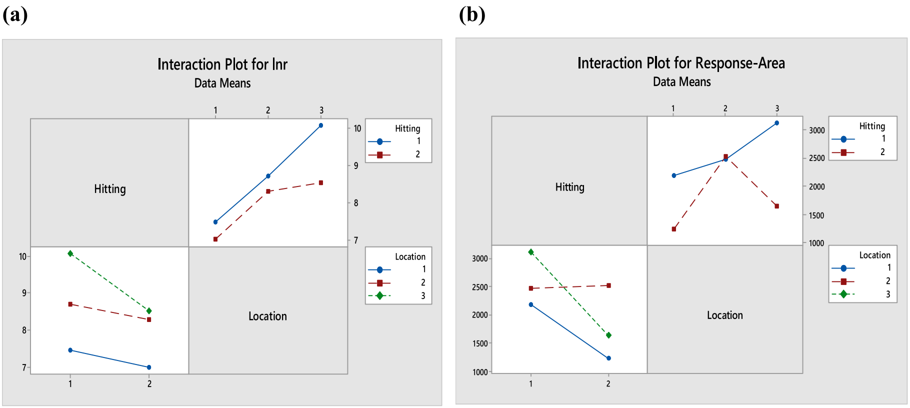
(a) (b)

Fig. 8. Interaction plot of response in macrofaunal density and percentage cover among all three study locations between March and December 2018 (a) density (b) percentage cover. Abbreviations: Hitting 1- tide directly hitting the biofouling community, Hitting 2 – tide not hitting directly on biofouling community, location 1-Dikkowita Fisheries Harbour, location 2- Kirulapone canal opening, location 3- Panadura Fisheries Harbour.

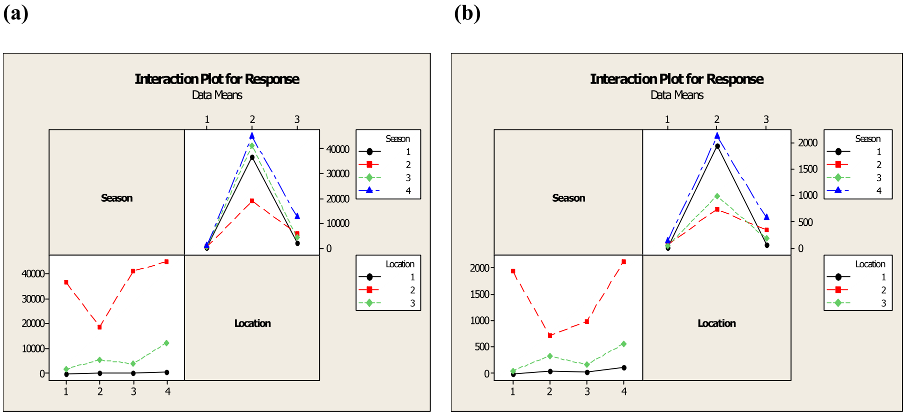
(a) (b)

Fig. 9. Interaction plot of response in macrofaunal density and percentage cover among all three study locations between March and December 2018. Abbreviations: Seasons 1 – FIM, Season 2- SWM, Season 3- SIM, Season 4 – NWM; location 1- Dikkowita Fisheries Harbour, location 2-Panadura Fisheries Harbour, location 3-Kirulapone canal opening.

dense patches mainly at intertidal and subtidal surfaces and shallow rocky areas of high sedimentation down to depths of 15 m or more (Wilk and Bieler, 2009). Hence a possible explanation for the well-established *Isognomon* sp. can be the favourable brackish water conditions created by the Bolgoda River that transports a high load of sediment deposits to sampling points P-6 and P-7.

Similarly, four poriferans belonging to the class Demospongiae were observed as subtidal assemblages exclusively in Dikkowita Fisheries Harbour during the study. Poriferans are often considered as an abundant component in marine benthic communities in the tropics (Diaz and Rützler, 2001). Yet, their specific requirements for light, substrate, water quality, sedimentation, flow rate, and abiotic factors make them highly sensitive to environmental stressors and are considered as bio-indicators (Wilkinson and Vacelet, 1979; Carballo et al., 1996; Batista et al., 2013). The environmental requirements of sponges are often constrained by the amount of sediment in the water as well as salinity fluctuations (Bell and Smith, 2004). Absence of poriferans in both Panadura and Kirulapone study sites can be directly attributed to the mixing of sediment-rich freshwater mainly from the Bolgoda River and Kirulapone canal.

In general, both Dikkowita and Kirulapone study locations were dominated by oysters (*S. cucculata*). Conversely, in Panadura Fisheries Harbour, algae species and barnacles (*Chthamalus* sp.) were the dominant organisms. Barnacles and oysters are known to compete for space in the intertidal zone, as both are filter feeders (Buschbaum, 2002; Boudreaux et al., 2009). Compared to oysters, barnacles are well adapted to avoiding desiccation along with the ability to tolerate high temperatures and low salinity levels (Barnes and Barnes, 1957; Foster, 1971). Hence, they tend to outcompete oysters in the successional pathway, and this can be attributed to the observed dominance of barnacles at Panadura Fisheries Harbour. As suggested by Buschbaum in 2002, the presence of decapods (i.e. *Carcinus maenas*) that prey on barnacles can be a possible explanation for the low barnacle densities observed in the other two study sites (Buschbaum, 2002). Since three predatory molluscs species namely, *Morula granulata*, *Stramonita* sp., and *Thais* sp. were also recorded during the study, much is needed to be investigated on the dominance between barnacles and oysters under predatory pressure (Richardson and Brown, 1990; Taylor and Morton, 1996).

The Shannon-diversity index values had wide variations among three study sites. The Hutcheson *t*-test was used to compare the species diversities of Dikkowita Fisheries Harbour and Kirulapone canal opening with Panadura Fisheries Harbour (reference point). According to the pairwise comparison of Hutcheson *t*-test, a high Shannon diversity index value was obtained for Pandaura Fisheries Harbour ($H' = 1.302$) compared to Dikkowita Fisheries Harbour ($H' = 0.734$) and Kirulapone canal opening ($H' = 1.168$). The lowest species evenness value recorded for Dikkowita Fisheries Harbour ($J' = 0.254$) can be directly attributed to the low Shannon diversity index, even though species richness ($R = 2.070$) was the highest (Zhang et al., 2012). This is in accordance with the general observations in Dikkowita Fisheries

Table 1
List of fouling organisms observed during the area surveys in all 3 study locations (D- Dikkowita Fisheries Harbour, K- Kirulapone canal opening, P- Panadura Fisheries Harbour).

Phylum	Class	Family	Species name	Locations sampled
Porifera	Demospongiae	Darwinellidae	*Aplysilla* sp.	D
		Microcionidae	*Clathria* sp.	D
		Suberitidae	*Terpios* sp.	D
		Tethyidae	*Tethya robusta* (Bowerbank, 1873)	D
Bryozoa	Gymnolaemata	Lepraliellidae	*Celleporaria volsella* (Tilbrook, 2006)	D
Cnidaria	Anthozoa	Actiniidae	Undetermined species 1	D
Sipuncula	Phascolosomatidea	Phascolosomatidae	*Phascolosoma stephensoni* (Stephen, 1942)	D
Mollusca	Bivalvia	Isognomonidae	*Isognomon* sp.	P
		Mytilidae	*Modiolus* sp.	D
			Perna viridis (Linnaeus, 1758)	D
			Xenostrobus sp.	P
		Ostreidae	*Crassostrea* sp.	D, P
			Ostrea sp.	D, K
			Saccostrea cuccullata (Born, 1778)	D, P, K
	Gastropoda	Cypraeidae	*Mauritia arabica* (Linnaeus, 1758)	D
			Monetaria annulus (Linnaeus, 1758)	D
			Monetaria caputserpentis (Linnaeus, 1758)	D
		Fissurellidae	*Clypidina notata* (Linnaeus, 1758)	P, K
		Muricidae	*Morula granulata* (Duclos, 1832)	D, P
			Stramonita sp.	D
			Thais sp.	P
		Nacellidae	*Cellana* sp.	D, P, K
		Neritidae	*Nerita albicilla* (Linnaeus, 1758)	D, K
		Pachychilidae	*Faunus ater* (Linnaeus, 1758)	P
		Patellidae	*Patella* sp.	D, P, K
		Trochidae	*Trochus* sp.	K
	Polyplacophora	Chitoninae	*Chiton* sp.	D
Annelida	Polychaeta	Nereididae	*Pseudonereis* sp.	D, P, K
		Serpulidae	*Spirorbis* sp.	D, P, K
Arthropoda	Malacostraca	Gammaridae	*Gammarus* sp.	D, K
	Maxillopoda	Archaeobalanidae	*Semibalanus* sp.	D, P, K
		Balanidae	*Amphibalanus amphitrite* (Darwin, 1854)	D, P, K
	Hexanauplia	Balanidae	*Balanus reticulatus* (Utinomi, 1967)	P, K
	Maxillopoda	Chthamalidae	*Chthamalus* sp.	D, P
	Maxillopoda	Lepadidae	*Lepas* sp.	D, K
Echinodermata	Echinoidea	Echinometridae	*Echinometra mathaei* (Blainville, 1825)	D
	Ophiuroidea	Amphiuridae	*Ophiothrix* sp.	D, K
Chordata	Ascidiacea	Corellidae	*Corella* sp.	D
		Didemnidae	*Lissoclinum* sp.	D
		Styelidae	*Botrylloides* sp.	D
Chlorophyta	Ulvophyceae	Caulerpaceae	*Caulerpa* sp.	P, K
			Caulerpa mexicana	P
		Cladophoraceae	*Chaetomorpha antennina* (Kützing, 1847)	D, P, K
		Ulvaceae	*Ulva* sp.	D
Ochrophyta	Phaeophyceae	Dictyotaceae	*Dictyota ciliolata* (Kützing, 1859)	K
Rhodophyta	Florideophyceae	Spyridiaceae	*Spyridia* sp.	P, K
		Corallinaceae	Undetermined species 2	K, P

Harbour as *S. cuccullata* was the predominant species while other taxa had a discrete and patchy distributions. This also justifies the general principle that "dominance decreases with increasing diversity and evenness" (Magurran, 1988).

In RCBD analysis, for all three study locations, a reduced number of biofouling organisms were observed in sampling points where Hitting-2 treatment was applied. Results revealed that there is a significant paired interaction of location and tide hitting attributes on the fouling communities (P value$<$ 0.05; 0.029). Similar observations have been reported in past studies, especially for barnacle species. A study conducted by Caffey in 1985 discovered that observed barnacle communities were most common on the outside exposed sites of rocky shores exposed to strong wave action than on the inside, sheltered sites. This greater density of fouling organisms on the rocky shores subjected to strong wave action can be simply due to a greater density of settlers (Caffey, 1985). In addition, percentage cover indicated the same trend in terms of the reduced number of fouling organisms at sampling sites with Hitting-2 treatment. An exception was observed at Kirulapone canal opening with both tide attributes recording similar average percentage covers of macrofouling organisms. A possible explanation for this observation can be the low relative abundance of barnacle species (*Chthamalus* sp.), limpets (*C. radiata*, *C. notata*) and high relative abundance of *S. cuccullata* in the sampling sites situated in sheltered parts of the breakwaters.

Table 2
List of globally invasive species recorded during the study.
Source: WoRMS (2019) and CABI (2019).

Species name	Native range	Reported study locations near Colombo, Sri Lanka	Status
Amphibalanus amphitrite (Darwin, 1854)	Caribbean Sea, Celtic Sea, English Channel, Gulf of Mexico, North Atlantic Ocean, North Pacific Ocean, North Sea, United States	All 3 study locations	Globally invasive
Balanus reticulatus (Utinomi, 1967)	Southern Japan, Indo-Pacific region	Panadura & Kirulapone	Globally invasive
Perna viridis (Linnaeus, 1758)	Along the Indian coast and throughout the Indo-Pacific	Dikkowita	Globally invasive

RCBD analysis also indicated significant relationships between paired interactions of seasons and locations with density ($P < 0.05$; 0.007) as well as percentage cover ($P< 0.05$; 0.036) of fouling organisms. The interaction plot indicated an increased number of macrofouling organisms in Panadura Fisheries Harbour and Kirulapone canal opening with the onset of NEM period. Increased percentage cover continued till the withdrawal of FIM for Panadura Fisheries Harbour. Conversely, organisms count remained reduced but consistent throughout other climatic seasons for Kirulapone canal opening. Low precipitation for the west coast during the NEM and FIM periods (Department of Meteorology, 2016) causing salinity levels to rise, compared to rainy seasons, can be a possible reason for reduced numbers. Similarly, RCBD analysis indicated a reduced number of fouling organisms at Panadura Fisheries Harbour during SWM and SIM periods. The freshwater influx from backwaters of Bolgoda River and Kirulapone canal which lower the salinity values in waters could be a possible reason for the low macrofouling cover observed with the onset of SWM period (Han and McCreary, 2001). These observations is supported by a study conducted by Goodbody (1961) on mass mortality of marine fauna following tropical rains. It concluded that the death of marine fauna followed immediately after unusually heavy rains and resulting low salinity (Goodbody, 1961).

The photo-quadrat sampling technique was used in the present study as it is non-destructive and generates permanent records that can be re-examined at a later date, if necessary (Bowden, 2005). The main limitation of the photo-quadrat method was the underestimation of taxa and percentage coverage of macrofouling organisms when assemblages are multi-layered (Beaumont et al., 2007). Since the occurrence of macrofouling organisms as multi-layers were sparse in all three study locations and *in situ* examinations were also conducted to identify existing taxa, errors due to underestimation were considered insignificant.

Three globally invasive species, *A. amphitrite* (Darwin, 1854), *B. reticulatus* (Utinomi, 1967), and *P. viridis* (Linnaeus, 1758), were detected during the study. All three study locations recorded the presence of *A. amphitrite* in the rocky intertidal zone. Intertidal communities of *B. reticulatus* were recorded only from Panadura Fisheries Harbour and Kirulapone canal opening. Conversely, a minor subtidal community of *P. viridis* was recorded exclusively from Dikkowita Fisheries Harbour. A study conducted by Priyadarshani and Ranatunga (2013) on biofouling assemblage of ship hulls in Port of Colombo recorded nine globally known invasive biofouling organisms including *A. amphitrite* and *P. viridis.* This emphasizes the possibility of Port of Colombo being a point source for invasive biofouling organisms. Moreover, their small and sporadic distributions in adjacent coastal waters observed during the study can be attributed to their relatively recent arrivals (Geburzi and McCarthy, 2018). A study by Lacoursière-Roussel et al. (2016) suggested that the introduction success was correlated to the number of arrivals of non-merchant ships from close regions. Hence large numbers of international fishing vessel arrivals in Dikkowita Fisheries Harbour compared to the other two study locations may be the main reason for the presence of these invasive species at this port (Ceylon Fishery Harbors Corporation, 2019).

Considering the invasive potential, even small populations of *A. amphitrite*, *B. reticulatus* or *P. viridis* can multiply and cause detrimental impacts on the native biota (Sakai et al., 2001). Periodic monitoring would help to detect a new invasion in the early stages, allowing control efforts to commence immediately. The establishment of invasive marine algae *Caulerpa taxifolia* in the Mediterranean Sea is an example of how an overlooked invasive species can become economically and ecologically detrimental (Meinesz, 1999). Therefore, the biofouling community structure should be monitored regularly at potential entry points of NIS. Moreover, the design of national policy should account for the six breaking points in the generic process of marine pest invasion (prevention, detection, quarantine, eradication, control, and mitigation) where intervention is possible (Bax et al., 2003).

5. Conclusions

During the study, a total of 47 macrofouling taxa were recorded. The highest number of fouling organisms recorded were molluscan species at all three study locations: 14 taxa in Dikkowita Fisheries Harbour, 7 taxa in Panadura Fisheries Harbour, and 10 taxa in Kirulapone canal opening. Three globally invasive species (*A. amphitrite*, *B. reticulatus,* and *P. viridis*) were also recorded during the study.

CRediT authorship contribution statement

M.P. Hendawitharana: Data collection, Writing - original draft, Visualization, Investigation. **R.R.M.K.P. Ranatunga:** Conceptualization, Methodology, Data collection, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2020.101424.

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