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ECOSYSTEMS

Biofouling initial succession on offshore artificial substrate under subtropical conditions

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Abstract: This study presents the initial stages of the macrofouling community on artificial substrate exposed to the offshore subtropical marine environment, and the contribution of depth (3 and 22m), exposure time (1-2-4-7-10-13-weeks), UV-radiation, rainfall, temperature, pH, salinity, water chlorophyll-*a*, and zooplankton supply to organism establishment. Steel substrates were placed horizontally on the structure of a pipeline monobuoy off the southern shore of Brazil (Tramandaí beach), and the ecological succession was monitored by six random removals per depth during the summer-autumn of 2011. Approximately 88.5% of the quantified settled individuals comprised fouling fauna and 11.5% vagile and sedentary fauna, although the taxa richness was higher for non-sessile invertebrates. Species richness and organism density up to four weeks were significantly higher at 3m-depth. After this period, a higher density of organisms was found at 22m, while during the whole study the species richness and diversity remained higher at 3m-depth. Zooplankton composition did not show a simultaneous temporal relationship with invertebrate recruitment at any depth; however, increasing the exposure time, the similarity between the planktonic and benthic communities also increased. Meroplankton, tychoplankton, and holoplankton were recorded on the substrates. This study showed that the depth of available substrates affects the macrofouling establishment, which is mainly associated with UV-radiation, exposure time, and ecological interspecific interactions.

Key words: Settlement, macrofouling, artificial substrate, pelagic–benthic coupling, UV radiation, zooplankton.

INTRODUCTION

Biofouling refers to the accumulation of biological deposits on hard substrates, including fouling, sedentary, and vagile organisms (Scheer 1945, Agostini et al. 2018). This benthic community is important from an ecological and socio-economic point of view, because it acts as a structuring agent (Krohling et al. 2006) and plays an expressive functional role in marine systems. Generally, this process results in increased diversity and local biological productivity, providing resources exploited by fishing and tourism (*i.e.* artificial reefs) (Dowling & Nichol 2001, Agostini et al. 2017a, b, c, 2018). In contrast, fouling communities, especially macrofouling communities, are known to cause economic damage to oceanic structures. On ships, they increase the surface roughness of the hull, leading to increased fuel consumption, frictional resistance, and decreased maximum range speed (Leer-Andersen & Larsson 2003, Schultz 2007, Schultz et al. 2011), while they can cause clogging in marine pipelines and increase corrosion. Additionally, biofouling action can make submarine cables more brittle, reducing their durability (Coutinho 2002). Fixed installations, such as platforms, piers, and docks, are also greatly affected by this phenomenon (Gama et al. 2009).

The study of biofouling communities and their succession processes is essential to controlling or at least minimising their harmful effects on fixed oceanic engineering or vessels (Agostini et al. 2018). It also allows the assessment of possible environmental impacts, interspecific interactions, the potential for installing artificial reefs, and the introduction of exotic species (Gama et al. 2009). Therefore, it is important to know precisely how environmental factors influence colonisation on hard substrates.

Biological and physicochemical parameters, such as salinity, temperature, and light penetration, are known to vary greatly with depth, distinctly affecting the attachment of organisms to the substrate (Lehaitre et al. 2008, Abdelsalam et al. 2012, Forbord et al. 2020). Another important factor, according to Thorson (1950), Pineda (1994, 2000), and Pineda et al. (2010), is the availability of larvae in the water column (meroplankton), which is associated with the potential of benthic recruits. However, few studies have found a consistent relationship between recruitment and the presence of planktonic organisms (Menge & Farrell 1989, López & Coutinho 2008). However, when the study involved community pelagic–benthic coupling on hard substrates, many authors did not find a match between larvae and recruits (Olivier et al. 2000, Porri et al. 2006, Rilov et al. 2008, Agostini et al. 2017a, 2018). These patterns were often associated with a low meroplankton sampling frequency (Rodrigues et al. 2019), or deemed a result of disregarding possible interactions between other zooplanktonic organisms (tychoplankton and holoplankton) and biofouling (Agostini et al. 2017a, 2019). These organisms may be associated with biofouling either actively (feed) or passively (sedimentation) (Minchin & Gollasch 2003, McCollin & Brown 2014, Watson et al. 2015, Agostini et al. 2017a, 2019).

The characterisation of the settlement process of marine invertebrates on artificial substrates, as well as the evaluation of the influence of depth on species recruitment, can assist in surface manipulation to promote or prevent the colonisation of organisms, selecting favorable or unfavorable environmental factors. Thus, this study aims to describe the early stages of macrobiological colonisation on steel surfaces under subtropical offshore conditions and evaluate the influence of exposure time, depth, UV radiation, rainfall, temperature, pH, salinity, water chlorophyll-*a*, and zooplankton supply in the biofouling process.

MATERIALS AND METHODS

Study area

The study was conducted in Tramandaí beach on the northern coast of Rio Grande do Sul, Brazil, which is adjacent to the Atlantic Ocean. The study site is considered from the geomorphological point of view as straight and uniform with few openings, dominated by sandy bottoms dominated by waves with predominantly northeast-southwest orientation (Toldo et al. 1993, Pereira et al. 2005). A micro-tidal regime is present, although it can often be flooded by meteorological tides (Pereira & Calliari 2005). The coast is influenced by a strong seasonal pattern of winds and marine currents, having as main forces the circulation associated with the western currents, the position of the Subtropical Convergence and the volume of the continental discharge (Braga & Niencheski 2006). During the summer, the region is dominated by northeast and east winds and the temperature is higher (~24° C), due to the entry of the Brazil Current. In winter, southeast winds predominate and Sub-Antarctic Water flows north, lowering the temperature (~16° C) (Giannini & Garcia, 2009).

To describe the initial process of colonisation on metallic substrate, an experiment was carried out off Tramandaí beach, on the north coast of Rio Grande do Sul state, Brazil, using an MN-602 pipeline monobuoy of the TRANSPETRO company (Petrobrás S.A.) as a support for substrate fixation (Figure 1a). The MN-602 is located 4,445 meters away from the coast, where the depth is 24 meters. The substrates were represented by low-carbon steel (SAE 1020) discs (ø 215 mm × 16 mm thickness) because this material is by far the most frequently used in marine engineering and industrial facilities (Aramide 2009).

Sampling

The experimental design consisted of the discs being horizontally arranged at 3 m (monobuoy skirt) and 22 m (monobuoy pipeline end manifold [PLEM]) of depth on MN-602 pipeline monobuoy structure (Figure 1b). The experiment lasted three months (January to April 2011) with six samplings, represented by the random removal of two discs from each depth. The

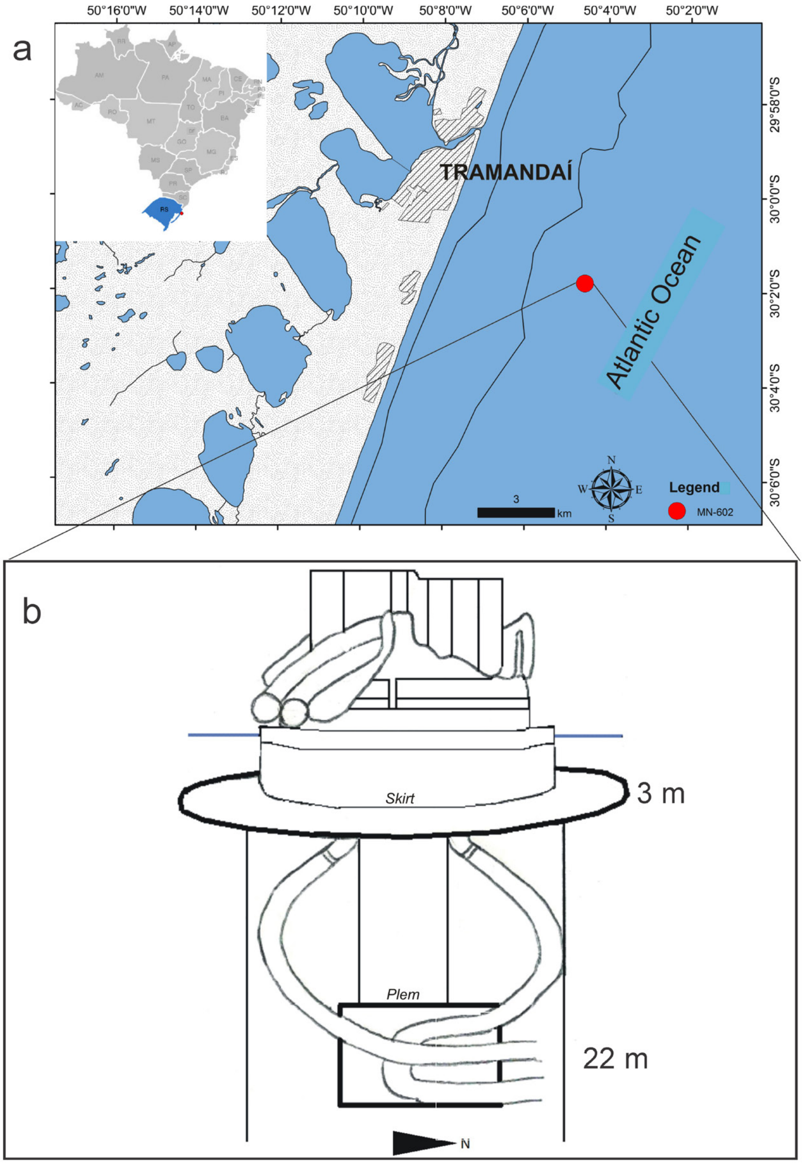

Figure 1. a: Biofouling experiment location in Tramandaí beach, RS, Brazil, b: MN-602 pipeline monobuoy (TRANSPETRO, Petrobrás S.A.) where the SAE 1020 steel substrates were fixed.

frequency of sampling was as follows: 1, 2, 4, 7, 10, and 13 weeks of exposure. As this study was conducted in a subtropical region, it was decided to start the experiment in the summer to ensure a satisfactory supply of larvae with potential for colonisation on hard substrates (Meekan et al. 1993).

Water temperature (°C), salinity, predominant current direction, and pH were monitored at both depths. Addiotionally, water samples were collected to chlorophyll-*a* determination. Regarding ultra violet (UV) radiation (KJ m^{-2}) and rainfall (mm), the values were obtained from the Imbé Meteorological Information Center of Centro de Previsão de Tempo e Estudos Climáticos (CPTEC/INPE; https://www.cptec.inpe). The water concentration of chlorophyll-*a* (µg L^{-1}) was estimated by spectrophotometer analyses (665 and 750 nm) performed by the Water, Sediment and Fish Biology Analysis Laboratory (CECLIMAR-UFRGS), according to the methodology of APHA (2012).

Zooplankton sampling was performed through collections simultaneous with the disc removals, with the aid of a cylinder-conical plankton net (150 µm mesh and 0.30 m diameter). The sampling method consisted of a two-independent horizontal tow of the net at the subsurface of the sea for 3 min at 1.5 miles h^{-1} around the monobuoy. A mechanical flowmeter (2030R; General Oceanics, Florida, USA) was attached to the mouth of the net to determine the volume of filtered water.

Laboratory analysis

Once collected, the discs were immersed in seawater and transported to the laboratory. As the specimens were positioned horizontally, only the surface-facing side was analysed, although descriptions of the opposite side were made when necessary. Then, initially, the upper surface of each disc was photographed and then the cover area of species was measured. Afterwards, each half substrate was scraped with a spatula, totalizing two replicates and two pseudoreplicates per treatment. The material was fixed with 70% ethyl alcohol, and the present species were screened qualitatively and quantitatively under binocular loupe.

The species coverage was estimated by photos, before the scraped, with the aid of a 215-mm diameter wire circumference, subdivided into eight equal parts, which was arranged over the removed disc. In each disc, the percentage of taxa coverage was estimated, using the following values: 0%, 15%, 35%, 50%, 75%, 90%, and 100%.

The zooplankton organisms obtained were fixed with 4% formaldehyde and analysed in a Bogorov chamber under 40× magnification. For identification and counting of the organisms, aliquots made with a sub-sampler with known volume (10 mL) were used.

Data analysis

The biological descriptors analysed by the experiment were species richness and density (org m^{-2}), *S* being the density data transformed [$\log_{10}(x + 1)$]. Shannon–Weiner index and the equitability (Pielou) were calculated to compared the communities of the different depths. Factorial variance analysis was applied, namely depth (fixed factor) and exposure time (random factor), the first with two levels (3 and 22 m) and the second with six (1, 2, 4, 7, 10, and 13 weeks). Tukey's post-hoc test was used. For zooplankton supply/potential (invertebrates present in plankton with potential of colonization on the substrates), the average density of organisms (org m^{-3}) of each sample was calculated and, for comparison with the settled organisms, presence/absence was used. The one-way permutational multivariate analysis of variance (PERMANOVA) (999 permutations) was applied to test for possible differences among communities' composition (zooplankton, biofouling at 3 m, and biofouling at 22 m). Non-metric multidimensional scaling (MDS) was used to investigate possible similarities between the composition of organisms in each community (zooplankton, biofouling at 3 m, and biofouling at 22 m) using the Bray–Curtis index. The MDS shows the effect of environmental conditions to biological data ordination. As longer the variable line more influence it provides to the community evaluated.

RESULTS

Regarding the environmental conditions, it was observed that the predominant current was in the northeast direction. The maximum water temperature recorded at 3 m depth was 26 °C (second week) and the minimum was 17 °C (thirteenth week). At 22 m depth, the maximum temperature was 20 °C (second week) and the minimum was 14 °C (fourth week). For salinity, differences were also observed between 3 and 22 m deep, being the higher depth holder of the highest values in most weeks. At 3 m, the maximum recorded value was 34 (first week) and the minimum was 23 (second week), while at 22 m, the maximum recorded salinity was 35 (tenth week) and the minimum was 22 (first week). The pH almost did not differ between the depths, ranging from 8.12 (first week) to 8.20 (thirteenth week). The average UV radiation and chlorophyll-*a* concentration at 3 m (20,858.2 KJ m^{-2} and 3.43 µg L^{-1}, respectively) was higher than at 22 m (0 KJ m^{-2} and 3.18 µg L^{-1}). The influence of rainfall values was considered the same for the depths with an average of 0.93 mm over the experiment (see Supplementary Material - Table SI).

The biological community documented during the experiment was mostly composed of cnidarian recruits and/or juveniles (*Ectopleura* sp., Octocorallia, and Actiniaria); the amphipods *Stenothoe valida* Dana, 1852, *Jassa* cf. *falcata* (Montagu 1808), *Apohyale media* (Dana, 1853), and *Elasmopus rapax* Costa, 1853; and other crustaceans, such as crabs from the families Grapsidae and Xanthidae, barnacles of the family Balanidae, and crustaceans from the families Tanaidacea and Pantopoda. For molluscs, six gastropod taxa were found, including family members of Lottiidae and Littorinidae, and five bivalve taxa were found, including *Brachidontes* sp., *Perna perna* (Linnaeus, 1758), *Isognomon bicolor* (C. B. Adams 1845), and *Crassostrea* sp. The polychaete families Amphinomidae, Sabellariidae, Eunicidae, Capitellidae, Polynoidea and Nereididae were also registered.

During the 13 weeks of the experiment, 40 invertebrates and two seaweed taxa were recorded on the substrates, accounting for a total of 22,877 individuals, 14,082 at 3 m and 8,795 at 22 m depth (see Table SII). With one week of exposure, the most superficial discs were already colonised by invertebrates (gammarids); however, the discs at 22 m were only colonised by invertebrates from the second week of exposure (gammarids, bivalves, and decapods). Approximately 88.5% of the quantified individuals comprised fouling fauna (*i.e.,* sessile organisms) and 11.5% comprised vagile (*i.e.,* organisms with high mobility) and sedentary (*i.e.,* organisms with low mobility) fauna, although the taxa richness was higher for non-sessile invertebrates. The Table I shows the occurrence and coverage percentage of the organisms recorded during the experiment.

According to the Shannon–Weiner index, the community established at 3 m depth was more diverse (1.90) than that established at 22 m (0.40). Regarding equitability (Pielou), the following values were observed: 0.53 for the 3 m community and 0.12 for the 22 m community.

Table I. Description of the colonisation process on a steel substrate exposed to subtropical coastal marine water (Tramandaí beach, RS, Brazil) from January to April 2011. Gm: gammarids, B: bivalve, H: hydrozoan, Ba: barnacle, D: decapod, P: polychaeta, G: gastropod, Br: bryozoan, N: nematode, An: anthozoan, Ph: Phaeophyceae, Rh: Rhodophyta.

Exposure	Depth	Observation
1 week	3 m	juvenile: Gm
	22 m	
2 weeks	3 m	seaweed (Ph) - larvae: B, N, Ba, D - juvenile: Gm
	22 m	larvae: B, D - juvenile: Gm
4 weeks	3 m	seaweed (Ph) - larvae: B, H, Ba, D, G - juvenile: Gm
	22 m	larvae: Gm, B - juvenile: Gm
7 weeks	3 m	seaweed (Ph) - larvae: H, Ba, G, B, R, D - juvenile: Gm, B
	22 m	larvae: H, Ba, D, B - juvenile: H, B
10 weeks	3 m	seaweed (Rh) - larvae: P, B, Ba, H, D, G, N, H - juvenile: P, B, Br, H, Ba, Gm, An
	22 m	larvae: P, B, Ba, H, D, G, N - juvenile: P, B, Br, H, Ba, Gm, An
13 weeks	3 m	seaweed (Rh) - larvae: P, B, Ba, H, D, G, N - juvenile: P, B, Br, H, Ba, Gm, An
	22 m	larvae: P, B, Ba, D, G, N, H - juvenile: B, H, L

The total density of invertebrates recorded on the substrates showed significative differences between depths and exposure times ($F_{(1,4)}$ = 176.0; $p < 0.001$). There was an increase in organism density between the first and 10th week. Until the fourth week, the density at 3 m depth was significantly higher than 22 m, while at the 10th and 13th weeks, the pattern was reversed and the density was higher at 22 m (Figure 2a). In contrast, species richness did not show differences between depths, despite the exposure time; the interaction between this factor and depth affected the benthic community ($F_{(1,4)}$ = 27.0; $p < 0.001$). At both depths, there was a gradual and significant increase in the number of taxa up to the seventh week. After this period, the benthic community at 3 m showed no increase in species richness, while that at 22 m had its largest increase, which did not remain until the end of the experiment (Figure 2a). The PERMANOVA for total density and species richness can be seen in Table SIII.

When evaluating the density of each of the invertebrate groups recorded on the substrates, it was observed that the cnidaria presented colonisation variations in relation to exposure time and in relation to the interaction between exposure time and depth. The anthozoans were not recorded on the discs until the seventh week of exposure, but at the 10th week, these cnidaria had similar high densities at both depths ($F_{(1,1)}$ = 0.95; p = 0.349) (Figure 2b). The hydrozoans presented different densities between the two depths depending on the exposure time ($F_{(1,2)}$ = 346.7; $p < 0.001$). Until the second week, none of the substrates were colonised by hydrozoans. From the fourth week, the 3 m community showed a higher density of *Ectopleura* sp. than the 22 m community; this was repeated in the 10th week. In contrast, in the seventh and 13th weeks, the 22 m community had a higher density of hydrozoans (Figure 2b) than the 3 m community.

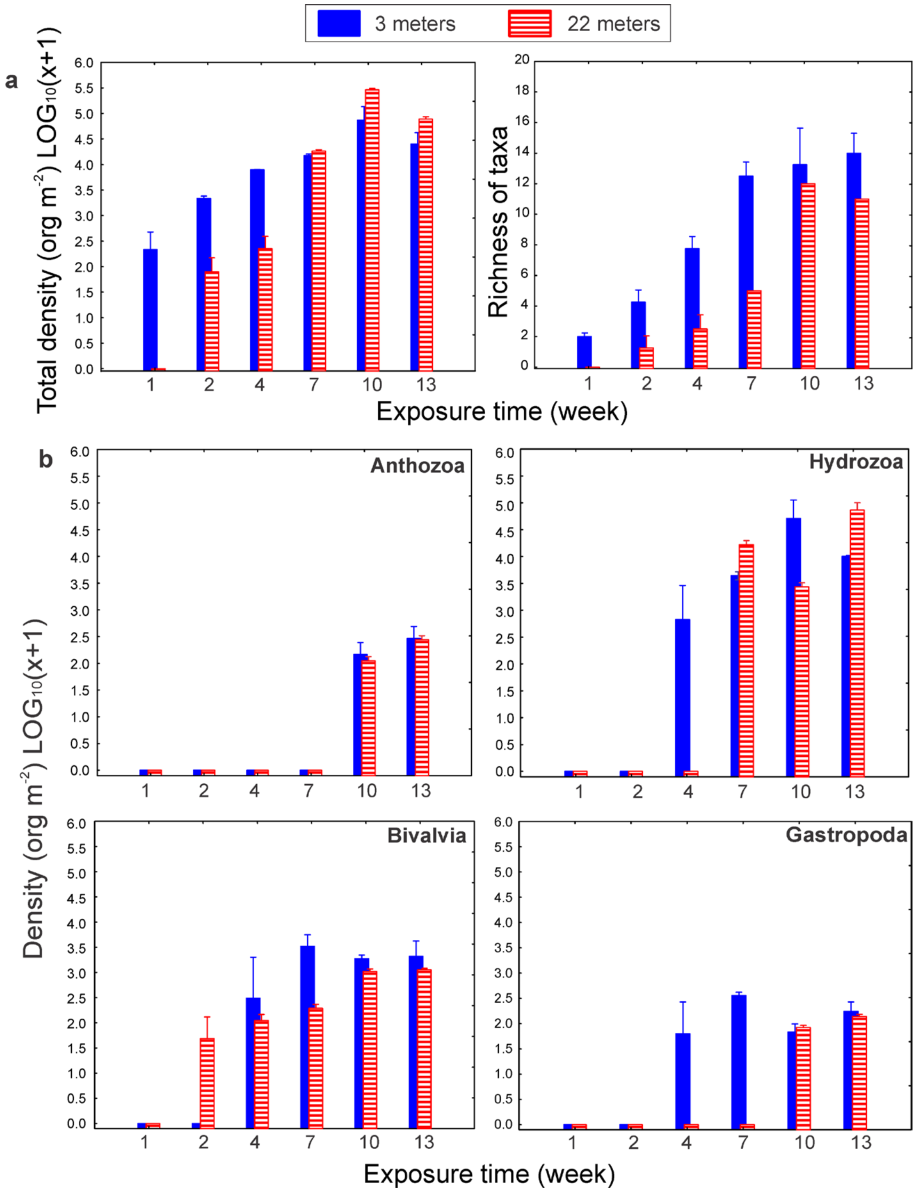

Figure 2. a: Total density (org m^{-2}) $\log_{10}(x + 1)$ and Richness of taxa; b: Densities (org m^{-2}) $\log_{10}(x + 1)$ of the invertebrates recorded on the steel discs at 3 and 22 m depths during the weeks of the biofouling experiment on the MN-602 monobuoy (Tramandaí beach, RS, Brazil) from January to April 2011. The vertical lines denote standard error × 1.96 (95% confidence).

In relation to molluscs, significant differences were found in the density of organisms only in relation to exposure time. Bivalves were first recorded at 22 m depth and were not observed at 3 m. However, from the fourth week onwards, the highest average bivalve densities were recorded at 3 m, although significant differences were observed only from the seventh week ($F_{(1,3)} = 10.70$; $p < 0.001$) (Figure 2b). The gastropods settled on the substrates from the fourth week at only 3 m depth, and the gastropod density at 3 m was significantly different that at 22 m ($F_{(1,3)} = 10.04$; $p < 0.001$) (Figure 2b). The result of the PERMANOVA for the total density of cnidaria and molluscs can be seen in Table SIV.

The order Malacostraca was observed as early as the first week of the experiment, however, only at 3 m. This order was recorded at 22 m depth from the second week; however, the densities were lower than those observed at 3 m depth until the end of the experiment. There was significant difference in the density of Malacostraca between the two depths in all exposure times ($F_{(1,4)} = 38.3$; $p < 0.001$), with exception of 10th week (Figure 3). Maxillopoda crustaceans occurred from the second week at both depths, with the community at 3 m holding a higher density until the 10th week. However, in the 13th week, the 22 m community showed a higher density of crustaceans of this order than the 3 m community, with a clear decrease in Maxillopoda density in the 3 m community in the last week of the experiment ($F_{(1,2)} = 3795.0$; $p < 0.001$) (Figure 3). Pantopoda only occurred from the seventh week at a depth of 3 m and from the 10th week at a depth of 22 m ($F_{(1,1)} = 4.652$; $p = 0.052$) (Figure 3). The result of the PERMANOVA for the total density of crustaceans can be seen in Table SIV. Cyclopoida (*Halicyclops* sp.) and Harpacticoida (*Euterpina acutifrons* (Dana, 1847)) copepods were also observed on the substrates at both depths but were not quantified.

The Nematoda phylum was only observed in the seventh week of the colonisation experiment at 3 m depth. In the deeper community, there was settlement of nematodes only in the 10th week. At 3 m depth, the nematode density was similar between the seventh and 13th weeks, with the

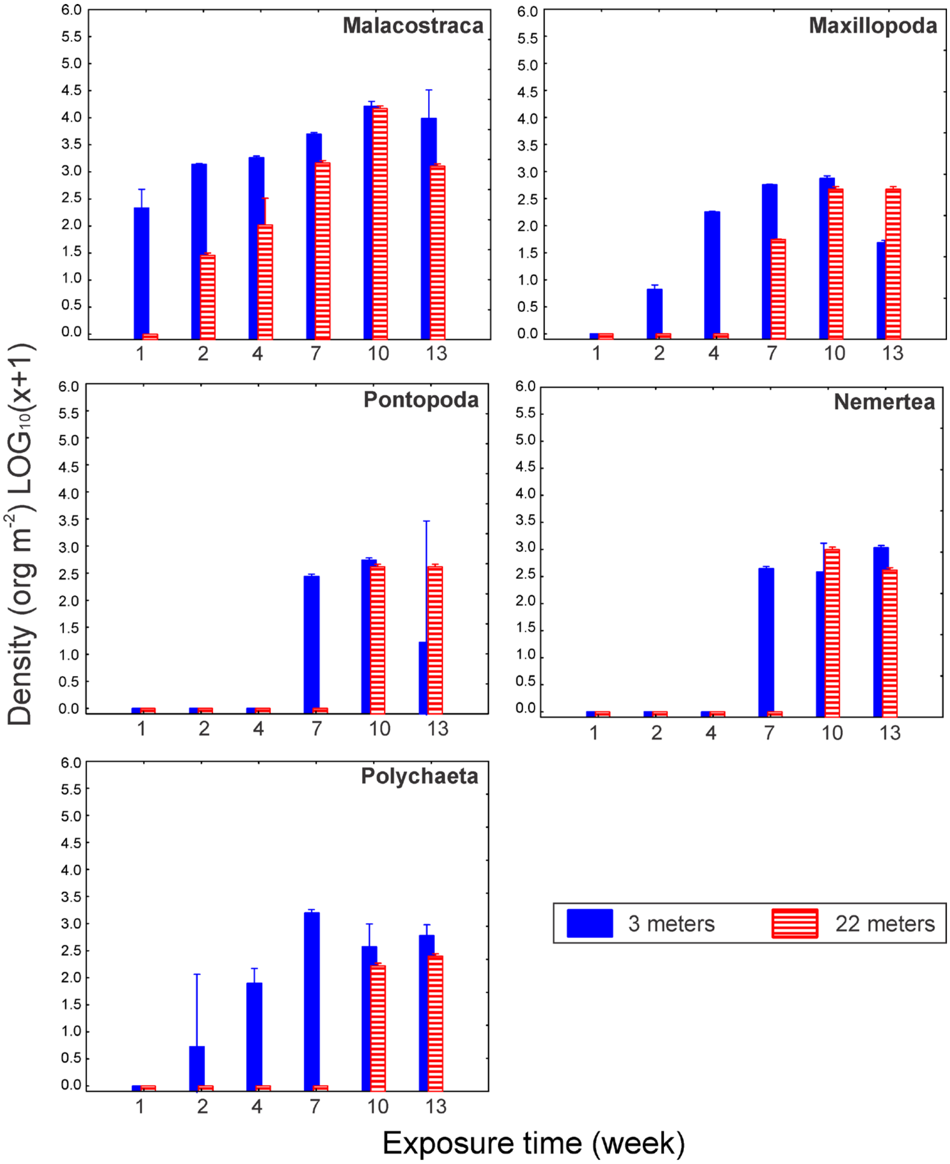

Figure 3. Densities (org m^{-2}) log$_{10}$(x + 1) of the invertebrates recorded on the steel discs at 3 and 22 m depths during the weeks of the biofouling experiment on the MN-602 monobuoy (Tramandaí beach, RS, Brazil) from January to April 2011. Vertical lines denote standard error × 1.96 (95% confidence).

highest density recorded in the last week (Figure 3) ($F_{(1,1)} = 24.10$; $p < 0.001$). The polychaetes only settled on the substrate from the second week in the 3 m community. In the deeper community, the settlement occurred from the 10th week, with a similar density of polychaetes at both depths ($F_{(1,1)} = 0.04$; $p = 0.838$) (Figure 3). The result of the PERMANOVA for the total density of nematodes and polychaetes is shown in Table SIV.

At a depth of 3 m, seaweed emerged after the second week of exposure. At this depth, hydrozoans were only recorded after the fourth week. However, it was found that on the opposite side to the analysed disc, which was facing the sea floor, *Ectopleura* sp., covered 90% of the surface, while seaweeds were absent. After this period, another species of seaweed (Rhodophyta) was also observed. At the end of the experiment, this seaweed completely occupied the surface of the substrates installed at 3 m depth, decreasing the coverage of *Ectopleura* sp.

In terms of biofouling coverage, at a depth of 3 m, with one week only amphipods are recorded, covering 5% of the discs. With two and four weeks of exposure, more invertebrates taxa were recorded covering less than 5% of the discs, while the seaweed Phaeophyceae had been covered 60% and 95%, respectively. On the other hand, with seven weeks of exposure, Phaeophyceae had been covered 90% of the discs and *Crassostrea* sp., *Megabalanus coccopoma* (Darwin, 1854), and anthozoans occupied each one on average 5% of the discs. At ten weeks of exposure there was a 35% coverage by *Ectopleura* sp., 15% by *Crassostrea* sp., bryozoans, and *M. coccopoma*, and 5% by anthozoans, while the seaweed Rhodophyta appeared covering 40% of the substrates. Finally, with 13 weeks of exposure, Rhodophyta had been covered 95% of the surfaces, *Ectopleura* sp. continued to occupy 15% of the disc, while *I. bicolor* occupied 5%, anthozoans 25%, bryozoans and *M. coccopoma* 5%, and *Crassostrea* sp. 15%. At a depth of 22 m, the first week did not show colonization. With two and four weeks some invertebrates were recorded covering 5% of the surfaces (see Table I). While with seven weeks *Ectopleura* sp. had been covered 50% of the discs, while *Crassostrea* sp. and bryozoans were also present, representing 10% of the colonization. *Ectopleura* sp., *Crassostrea* sp. and bryozoans occupied 90%, 5%, and 5% at ten weeks; and 50%, 5%, and 15% at 13 weeks, respectively. Other invertebrates represented 5% of occupation (see Table I).

Regarding zooplankton supply, 56% of the total sampled organisms comprised holoplankton, while meroplankton and tychoplankton accounted for 44% together. The zooplanktonic peak density in the water was 893.42 org m^{-3} (second week). The lowest density recorded was 211.26 org m^{-3} (thirteenth week) (see Figure S1a). The most abundant group was Copepoda (holoplankton). The Figure S1b shows the zooplankton groups occurrence frequency over time. The average density of organism colonisation potential was 107 org m^{-3}. The peak density of meroplanktonic and tychoplanktonic organisms was 350 org m^{-3} in the second week of evaluation, while the lowest density of these organisms recorded was 15 org m^{-3} in the 10th week. The group that presented the highest density of organisms (org m^{-3}) was *Ectopleura* sp. (family Tubulariidae). The SM Figure 1c shows the meroplanktonic and tychoplanktonic composition over time.

Of the meroplanktonic and tychoplanktonic organisms that are often associated with hard substrates, 21 taxa were found in zooplankton. Although meroplanktonic (e.g. barnacles, mussels), tychoplanktonic (e.g., nematodes, amphipods), and holoplanktonic (e.g., copepods) organisms were recorded on the substrates, in general, there were no direct temporal relationships between the presence of organisms in the superficial water and those settled on the substrates (Table II, see Figure S2), except for the family Mytilidae and the order Malacostraca. The other groups generally presented settlement on average one week after its occurrence in the water column. Maxillopoda was recorded first on substrates and after as larvae in plankton samples. Captelidae, Pantopoda, Decapoda, Nematoda, and Tanaidacea only were recorded on the substrates. In contrast, calanoid copepods (holoplankton) were recorded in plankton samples and at both depths but were not counted. The three community compositions (zooplankton, biofouling at 3 m and biofouling at 22 m) were different from each other (PERMANOVA: F = 8.207, $p < 0.001$). However, it is possible to observe that the species composition among the three communities tended towards becoming similar with increasing exposure time. Regarding the environmental variables analysed, UV radiation was the most important for all communities, because influenced the community composition more than the other environmental conditions tested in the MDS ordination. The UV radiation values are directly associated to the depths, resulting in light and dark situation and consequently to chlorophyll-*a* concentration (Figure 4).

Table II. Presence or absence of invertebrates in the planktonic (meroplankton and tychoplankton potential) and benthic communities (3 and 22 m) during the biofouling experiment on the MN-602 monobuoy (Tramandaí beach, RS, Brazil) from January to April 2011.

Community	Exposure time	Anthozoa	Hydrozoa	Polychaeta	Bivalvia	Gastropoda	Maxillopoda	Malacostraca	Pantopoda	Nematoda
Zooplankton	1 week	■	■	■	■	■		■		
Zooplankton	2 weeks	■	■	■	■	■		■		
Zooplankton	4 weeks	■		■	■	■	■	■		
Zooplankton	7 weeks	■	■	■	■					
Zooplankton	10 weeks	■			■	■	■			
Zooplankton	13 weeks	■		■	■		■			
Biofouling (3 m)	1 week							■		
Biofouling (3 m)	2 weeks			■			■	■		
Biofouling (3 m)	4 weeks		■	■	■	■	■	■		
Biofouling (3 m)	7 weeks		■	■	■	■	■	■	■	■
Biofouling (3 m)	10 weeks	■	■	■	■	■	■	■	■	■
Biofouling (3 m)	13 weeks	■	■	■	■	■	■	■	■	■
Biofouling (22 m)	1 week									
Biofouling (22 m)	2 weeks				■			■		
Biofouling (22 m)	4 weeks				■			■		
Biofouling (22 m)	7 weeks		■		■		■	■		
Biofouling (22 m)	10 weeks	■	■	■	■	■	■	■	■	■
Biofouling (22 m)	13 weeks	■	■	■	■	■	■	■	■	■

PERMANOVA (Bray-Curtis: 9999 permutation): F = 8.207, $p < 0.001$

	Zooplankton	Biofouling (3 m)	Biofouling (22 m)
Zooplankton		0.001	0.001
Biofouling (3 m)	0.001		0.003
Biofouling (22 m)	0.001	0.003	

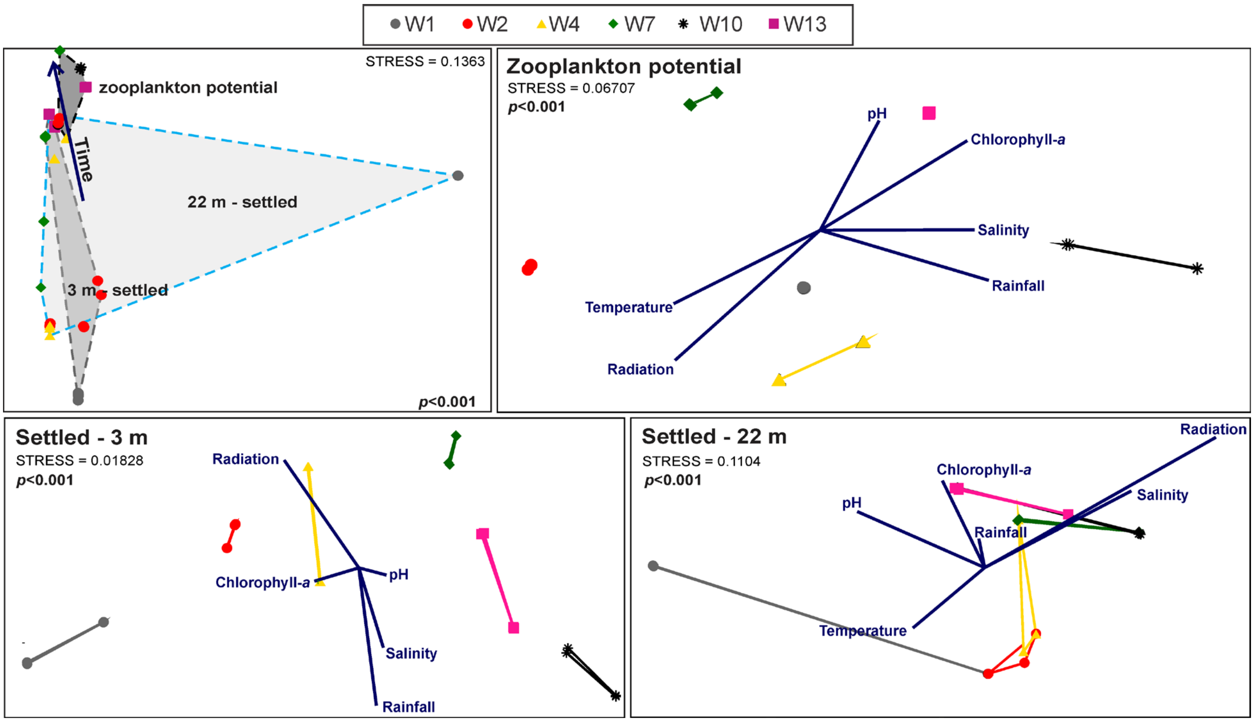

Figure 4. Non-metric multidimensional scaling (Bray–Curtis: 9,999 permutations) with the planktonic and benthic (3 and 22 m) communities relative to the exposure time (W = week) and the environmental variables during the biofouling experiment on the MN-602 monobuoy (Tramandaí beach, RS, Brazil) from January to April 2011.

DISCUSSION

The current work presents the evaluation of the initial stages of macrofouling community on artificial substrate (SAE 1020) exposed to the offshore subtropical marine environment, as well as the contribution of depth, exposure time, UV radiation, rainfall, temperature, pH, salinity, water chlorophyll-*a*, and zooplankton supply for organism establishment.

Fouling fauna were more abundant on the substrates compared to sedentary and vagile fauna. According to Chalmer (1982), vagile and sedentary fauna need microenvironments (i.e., seaweed) to settle and the colonisation process observed was still in its initial stage. However, contrary to what was expected, vagile species (e.g., gammarids) settled on the substrates before fouling species. This may be related to the roughness generated by surface corrosion of the substrate, creating depressions that serve as microhabitats (Agostini 2011, Agostini et al. 2017a). According to Bourget et al. (1990) and Agostini et al. (2018), the ability of an organism to detect the biological, physical, and chemical characteristics of the hard surface determines the structure of the initial community. These topographic changes caused by corrosion and biocorrosion (microorganism activity) initiate the process of structural deterioration, or are aggravated by fouling invertebrates, visibly altering the material surface (Videla 2002) (Figure 5). The topographic characteristics of the substrate play a key role in the settlement of many invertebrate species (Alfaro & Jeffs 2002, Aldred et al. 2006, Gribben et al. 2011).

Species richness and organism density up to four weeks were significantly higher at 3 m depth. After this period, a higher density of organisms was found at 22 m, while during the whole study, the species richness and diversity remained higher at a depth of 3 m. From the

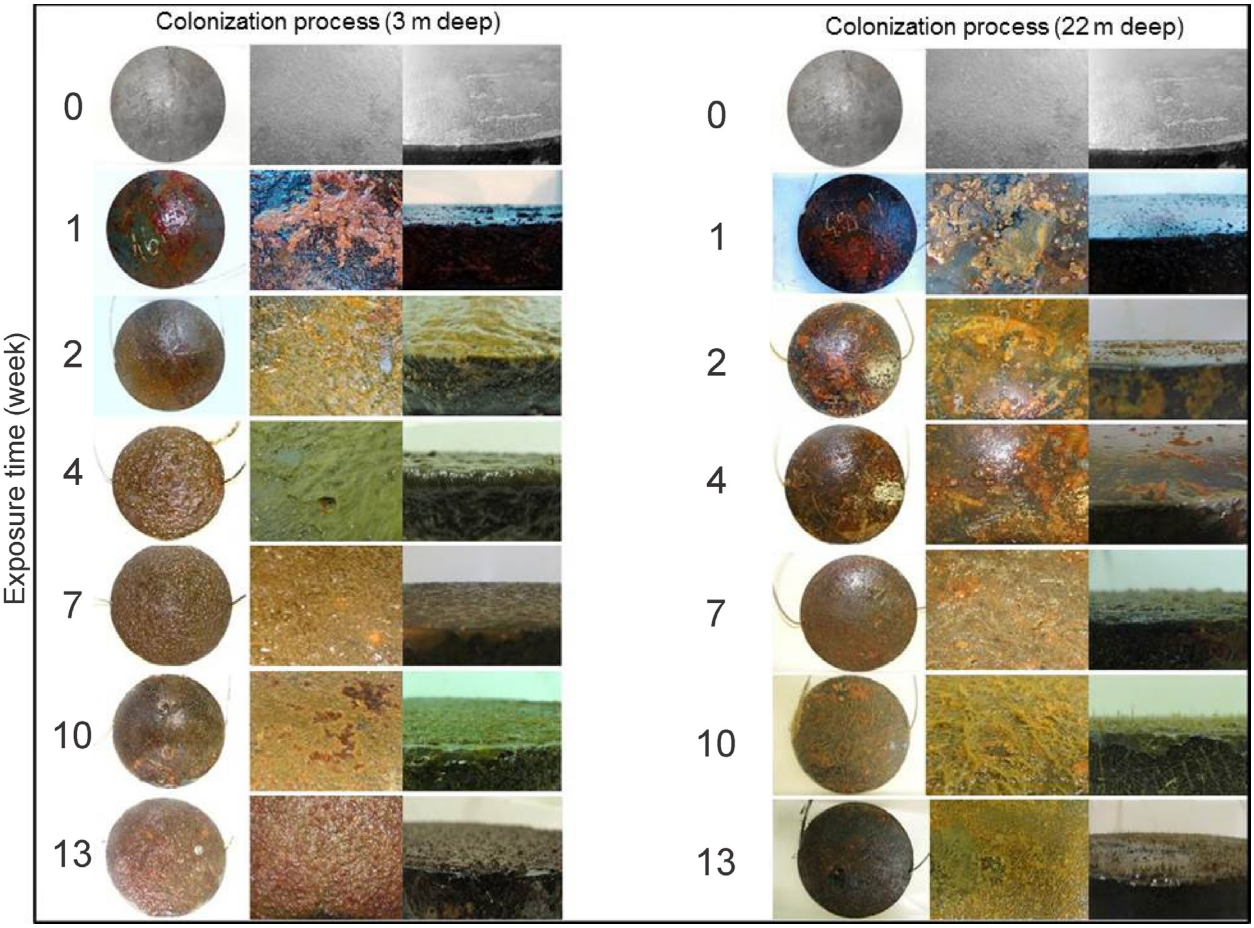

Figure 5. Photos of the stages of the biofouling colonisation process on the SAE 1020 steel under marine subtropical conditions (Tramandaí beach, RS, Brazil) with different details: whole disc, disc surface, and disc side.

10th week onwards, the similarities increased, suggesting a distinct initial colonisation process that will result in similar community compositions in the future. For both depths, up to the 10th week of exposure there was a gradual increase in colonisation, which was not maintained in the 13th week. Such a result may eventually be due to interspecific ecological interactions, such as competition for space (Strong 1980). The colonisation of organisms was slower at a depth of 22 m; however, juvenile forms of hydrozoans occurred primarily at this depth. The species *Ectopleura* sp. (Hydrozoa) showed greater coverage at a depth of 22 m, especially in the seventh and 13th weeks, which after the seventh week had already covered 50% of the substrate surface in the absences of light and, consequently, seaweed, as observed by Fejtek et al. (2011).

Concomitant with this change in ecological succession, a decrease in temperature was observed, which may have favoured the appearance and development of Rhodophyta. The Phaeophyceae seaweed also disappeared after colonisation by Rhodophyta. The inverse occurrence observed between hydrozoans and algae suggests a competitive exclusion, since *Ectopleura* sp. settled satisfactorily in places where seaweed was not present. The density variation of *Ectopleura* sp. at both depths reflected in the diversity and equitability of the background community.

Bumbeer & Rocha (2012) conducted a colonisation experiment in a tropical region, describing the process of colonisation at different depths and at different seasonal periods. Recruitment plates were submerged for approximately three months at 10 different depths between 8 and 17 m during spring, summer, and autumn. Fifty-nine species associated with hard substrates were found, and it was observed that in summer, the percentage coverage was lower in the shallowest strata of the water column, in contrast to the results of the current experiment in the first four weeks. Regarding the colonisation by hydrozoans, Bumbeer & Rocha (2012) verified a greater abundance on the deepest plates, associating this result with the negative phototrophic character of the larvae.

Differences in zooplankton composition were observed over time. According to Hansen et al. (2012) and Agostini et al. (2019) the zooplankton community is sensitive to minimum variation of the environmental conditions and can change in just a few hours or days. Rodrigues et al. (2019) observed differences in zooplankton density and composition among short-term sampling (three consecutive days in the same week). However, the highest zooplankton densities were recorded in the first four weeks, problably associated with the highest values of UV radiation and temperature (see Table SI and Figure 4). The proportion observed among holoplankton, meroplankton and tychoplankton is corroborate by other studies (Agostini et al. 2017a, 2019). Crustacea, Mollusca and Cnidaria were the groups more frequently found in the zooplankton samples (see Figure S1b), problably associated to copepods contribution and the bivalves, gastropods, and hydrozoans reproduction time. According to Longhurst (1985) and Cornils et al. (2018), copepods can represent between 80 and 90% of the zooplankton samples some times, being considered the most successful metazoan group known (Schminke 2007). About benthic bivalves, gastropods, and hydrozoans, they produce pelagic larvae, meroplankton, mainly in spring and summer (Reitzel et al. 2004, Michelsen 2017), that will searching for a hard substrate to settle.

Regarding zooplankton supply, there was a lack of correspondence between plankton and benthos. It was found that the zooplankton supply did not show simultaneous temporal relationship with invertebrate recruitment at any depth. Larvae may be available in the water column but not prepared for settlement (Agostini et al. 2019). This may be related to the conditions required for establishment in the substrate, such as the larval energy content, presence of specific or microbial biofilm, substrate type, orientation, and competition (Steinberg et al. 2002, Jarret 2003, Jenkins 2003, Todd 2003, Thiyagarajan et al. 2006, Agostini et al. 2018). According to López & Coutinho (2008), little more than 50% of studies on larval nesting did not find a strong relationship between number of larvae and settled organisms. Some researchers associated the absence of positive correlation between larvae and settlement to larval mortality and/or dispersal (Caley et al. 1996), while others suggested that submerged artificial substrates may initially be occupied by taxa that could affect the ecological succession by repelling the recruitment (Smith & Rule 2002), which could explain the weeks of delay between the presence of the organism in zooplankton samples and its settlement on the substrate. Azevedo et al. (2006) correlated low colonisation with the sedimentation of water particulate matter on the substrate surface, which can be more intense on horizontal structures (Agostini et al. 2018), such as those used in the current work.

It is noteworthy that in all the aforementioned studies, the recruits were only correlated with meroplankton. In this study, not only meroplankton, but also tychoplankton and holoplankton were recorded on the substrates, the same pattern as that observed by Agostini et al. (2019). The holoplankton may contribute to biofouling through of opportunistic association (by food) (McCollin & Brown 2014, Rodrigues et al. 2019) while tychoplankton by opportunistic association passive resuspension (Agostini et al. 2019). In the current work, vagile and sedentary fauna presented a higher species richness than the fouling fauna, mainly because of the contribution of tychoplankton. This demonstrates the importance of evaluating the association of zooplankton as a whole in the biofouling process (Agostini et al. 2018, 2019).

Moreover, it has been observed that the initial community immigration and extinction rates are linear, and not dependent on the larval population density, with the highest colonisation rate in available spaces (Simberloff & Wilson 1969). In the course of the process, the relationships between settlement and extinction may be determined by ecological interactions resulting from spatial modifications of the substrate, as observed in this study. In contrast, Menge (1991) states that variations in adult communities often reflect established patterns in the early stage of settlement, while Somsueb et al. (2000) and Agostini et al. (2018) reported that horizontally oriented substrates facilitate colonisation for some organisms, but make it difficult for others, which could be related to the absence of matches between larvae and recruits. These authors have reported that horizontal substrates are more attractive to oysters, mussels, and volatile fauna, but less attractive to barnacles. The results obtained in this study corroborate this, because barnacles presented a lower abundance in the benthos compared to that of plankton, while molluscs, such as *P. perna*, *Brachidontes* sp., and *Crassostrea* sp., and vagile fauna were more abundant on the substrate.

It is noteworthy that the colonization of the exotic species *Isognomon bicolor* was registered, showing the offshore artificial substrate as a vector for invasion species. This specie is known as an exotic invader, with a high dispersal capacity, control measures must be employed to prevent its spread (Martinez 2012). The occurrence of I. *bicolor* in the faunistic surveys performed in the Rio Grande do Sul coast represented the first documented record for this species in this region (Agostini & Ozorio 2016). It was found that the zooplankton supply did not show a simultaneous temporal relationship with invertebrate recruitment at any depth; however, increasing exposure time also increased the similarity between the planktonic and both benthic communities. The three communities were mainly affected by UV radiation (see Figure 5). Nevertheless, the UV radiation did not influence the communities alone, but with covariables (e.g., depth, light). In the 1990s, our understanding of the effects of ultraviolet radiation on aquatic ecosystems increased (Leech et al. 2005). Irradiance reflects in seasonal cycles of abundance and species composition for both planktonic and benthic organisms (Beman et al. 2011). In most of the samples, zooplankton abundance was associated with higher values of UV radiation, except for in the 10th and 13th weeks. For benthos, the same zooplankton pattern was observed in the 3 m community. For invertebrates, UV could be considered relatively biologically harmless or biologically damaging, influencing the distribution, behaviour, and physiology (Speekmann et al. 2000).

The highest density of organisms, as well as the highest species richness and diversity of organisms, were recorded at 3 m depth.

Light is a limiting factor for several fouling species, such as seaweed, so the community is structured differently according to its intensity. However, Lehaitre et al. (2008) believed that the relationship between depth and invertebrate settlement is more related to food availability than to light. This feature may also be involved in the colonisation delay at a depth of 22 m, in addition to the ecological relationships between species.

The other environmental variables evaluated followed the expected seasonal pattern for summer-autumn, although it was observed that the temperature and salinity showed differences between 3 and 22 m. The low temperature and salinity recorded at the 22 m depth is related to a lower influence of UV radiation (Garcia-Corral et al. 2015) and the density of water mass that tends to sink, leaving a higher salinity at lower depths (Maslin et al. 1995). According to Maslin et al. (1995), in general when it rains, there is an expected decrease in surface salinity that, per density difference, forms a saltier layer on top and fresher water below. These variables contributed to the macrofouling community establishment with differences in composition between 3 and 22 m deep.

CONCLUSIONS

The benthic community established on substrates at 3 m depth showed faster colonisation, higher taxa richness, and higher diversity compared to the community at 22 m depth;

The ecological succession was different between the 3 and 22 m benthic communities; the main composition differences were associated with the occurrence of seaweeds and hydrozoans (*Ectopleura* sp.), respectively, that proved the importance of exposure time, depth (and light), and ecological interactions. Regarding the zooplankton supply, there was a lack of correspondence between zooplankton and biofouling. It was found that the zooplankton supply did not show simultaneous temporal relationship with invertebrate recruitment at either depth; a delay was observed between the presence of the organism in zooplankton samples and its settlement on the substrate. Not only meroplankton, but also tychoplankton and holoplankton, were recorded on the substrates, contributing to vagile fauna in macrofouling communities representing the higher species richness recorded on the substrates. The invertebrate compositions between the three communities (zooplankton, biofouling at 3 m, and biofouling at 22 m) were different; however, increasing exposure time also increased the similarity between them. So, the first colonizers are the most affected by biological, physical and chemical surface characteristics. The three communities were main affected—positively or negatively—by UV radiation.

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SUPPLEMENTARY MATERIAL

Figures S1, S2.
Tables SI-SIV.

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