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Development and succession of sessile macrofouling organisms on the artificial structure in the Shallow Coastal Waters of Sabah, Malaysia

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Abstract. This study investigates the development and succession of sessile macrofouling organisms on PVC panels deployed in shallow coastal waters. The experimental setup includes two different sets of interconnected PVC pipe frame in a triangle shape which was attached to concrete blocks deployed at the seafloor and kept afloat vertically underwater at 2m and 8m depth, respectively. To determine the development and succession of sessile macrofouling, a total of 36 experimental PVC panels were attached on the two different frames whereby three panels were then taken for every 30 days of submersion until macrofouling had fully covered the plates at approximately after 180 days. There were nine sessile macrofouling species identified on both sides of the PVC plates with *Lyngbya* sp., being the most dominant during the first 30 days of submersion with percentage cover of nearly 70%. As macrofouling continually progress over time, *Eudendrium* sp. and *Amphibalanus* sp., surpass other species with highest percentage cover of nearly 80% and 65% respectively thereafter 180 days of development. Macrofouling are influenced by environmental parameters, CCA summarize suggest that temperature at 30.9°C is likely encouraged the progression of *Isognomon* sp. 1, *Isognomon* sp. 2, *Gracilaria* sp. and *Eudendrium* sp. while slow water movement (i.e. 5 cm/s) is more conducive for the development of *Lyngbya* sp.

1. Introduction

Biofouling is the unwanted build-up of marine plants and animals on submerged man-made structures [1]. Biofouling can be divided into two namely microfouling and macrofouling. In macrofouling, there are four succession stages that were identified [2], the macrofouling of an exposed surface of a materials that are free from any organisms, followed by the development of fast-growing marine organism (hydroids, serpulids, bryozoans, or the ascidian), which will be replaced with a slow-growing organism (molluscs, sponges and ascidians) and ended with a short-term climax stage. The concept of succession that was developed by Scheer through his experiment was further supported and developed by several authors [3-13] and summarised in a book written by Railkin [14].

Most of the study, particularly from Oshurkov in 1985 [10], 1986 [15], 1992 [16], 1993 [17] that was related to succession was carried out shallow waters, which can be classified to have an unstable environment [14]. Due to this reason, it is difficult to create a common progression of macrofouling succession, as it is not-well-known which environmental factors have the most influence on the

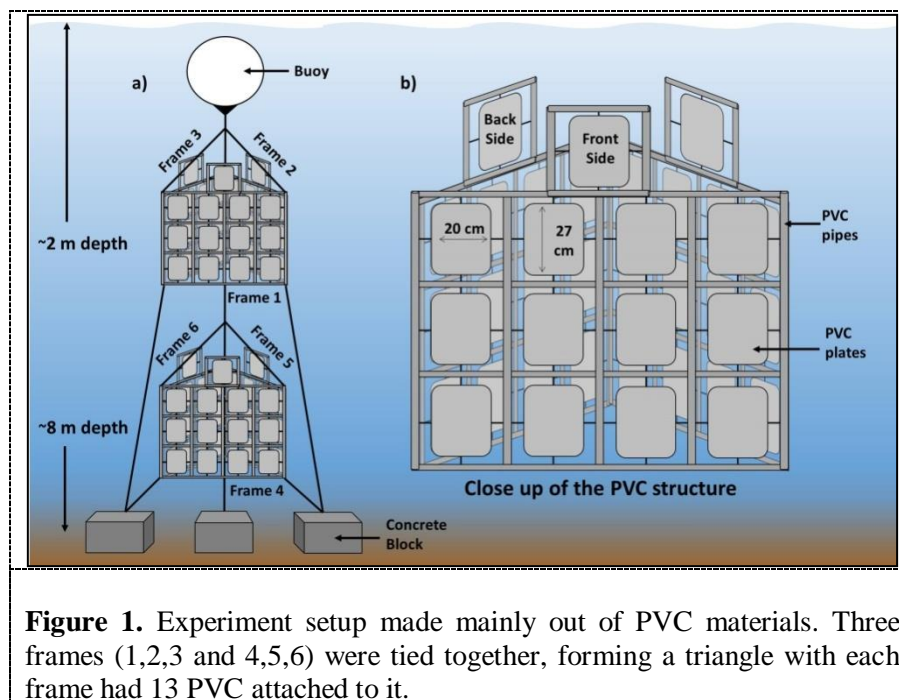


macrofouling succession process. Study on macrofouling of artificial structure in shallow coastal waters in Sabah is limited and focused more on identifying macrofouling assemblages at pontoon and sea marker. With infrastructure development along coastal area is rapid nowadays, it is essential to contribute study more on understanding the interaction between the development and succession of macrofouling assemblages with the effect of the environmental parameter so that we can understand more on the cycle of macrofouling growth. Therefore, the present work studied the sessile macrofouling community on PVC plates deployed in shallow coastal waters of Sabah. It determines the development and succession rates of sessile macrofouling species in relation to depth, the period of immersion, the different side of plates (front side and back side) and environmental parameters.

2. Materials and methods

The experiment was carried out for 180 days starting from end of April 2017 to early of October 2017 at Karambunai Bay in the west coast of Sabah, Malaysia. The seabed is sandy with soft substrate and few artificial reefs made up of car tires. Two sets of interconnected artificial structure were deployed at 2 meters (2 m) and 8 meters (8 m) (N 06.13645' E 116.11670') (**Figure 1**) and each of the structure were made of PVC pipes, that had three frames combined together, forming a triangle with each frame have 13 PVC plates with a size of 20 cm x 27 cm attached to it (**Figure 2**). PVC was used because it is non-corrosive and more durable in marine environment compared to other types of material [18]. Moreover, [18] stated that the marine growth should grow easily regardless of the type of materials.

Thereafter, at least 6 plates (3 replicate from the 2 m and another 3 from 8 m) were collected on 30 days interval (depending on weather conditions) and stored in zip lock bags for analysis. At the laboratory, each plate was weighted (in gram), photographed at both side of the plates (front side was a side that is facing net current while the back side refers to the side that was shielded from net current). Grid lines were added on the photographed plate through image processing software, ImageJ. Using the grid lines generated, the sessile biofouling organisms were subsequently separated by species, identified and enumerated through percentage cover (%) of each species present on the PVC plates.



Water samples of 2 liters for 2 replicates were collected from two different sites, one site on the north and the other site on the south of artificial structure, with each site, was approximately 30 meters

apart. The water samples were taken using a horizontal Van Dorn water sampler to determine water nutrients (ammonia nitrogen (NH₃-N), total dissolved phosphate (TDP), nitrate (NO₃-N)) concentrations. NH₃-N, TDP, and NO₃-N were measured following the guidelines [19]. Simultaneously, the hydrological parameters such as seawater temperature, salinity, pH, current speed and direction, average rainfall distribution and light attenuation (K_d) of the coastal waters were monitored throughout 180 days of experimentation. The seawater temperature, current speed, and direction were measured using Acoustic Doppler Current Profiler (ADCP) that was deployed near the study area. Additionally, seawater temperature, pH and salinity were also measured during the sampling day using YSI multiparameter probe. As for average rainfall distribution, the data were obtained monthly (March to October) from a weather station at Karambunai.

Three way-ANOVA analysis was used to determine the possible effect of submersion time, position and depth on percentage cover of each macrofouling species and environmental parameters and water nutrients. One way-ANOVA was also used to determine the significant difference between environmental parameters and water nutrients with submersion time. Other than that, multivariate analysis, Canonical Correspondence Analysis (CCA) was used to summarize the relationship between percentage cover of macrofouling species and environmental data. This method is a direct gradient analysis in community ecology for describing the major trends in species distribution and correlated environmental factors [20, 21].

To calculate such interactions, R statistical programming software was used. To calculate one way-ANOVA and three-way ANOVA, the percentage cover of each species and environmental parameters and water nutrients was subjected to arcsine transformation ($\text{Log } X+1$), where X on the equation represents the value of data [22], to normalize the data before analysis.

Likewise, several necessities need to be addressed first before CCA was calculated. First of all, the selection of species needs to be done so that we can uncover the relationship from a tangled and noisy data that may cloud the analysis [23]. In this research, this was done by dropping species with less than 5 occurrences on PVC plates at 2 meters and 8 meters. After that, each of the environmental parameters was normalized due to each of them was measured in a different reading scale. Normalizing was done using R statistical software, where the programme helped normalized the data by scaling the data to a range of 0 to 1 [23]. For the final step of data preparation, the environmental parameters were reduced via variable selection procedure, in order to select the best subset variables to include as a constraint [23]. This was done by using 'bioenv' function in R statistical software, where it picked 3 best subsets among our 10 environmental parameters. According to Oksanen [23], it is best to reduce the number of constraints to just a few for an accurate interpretation later on.

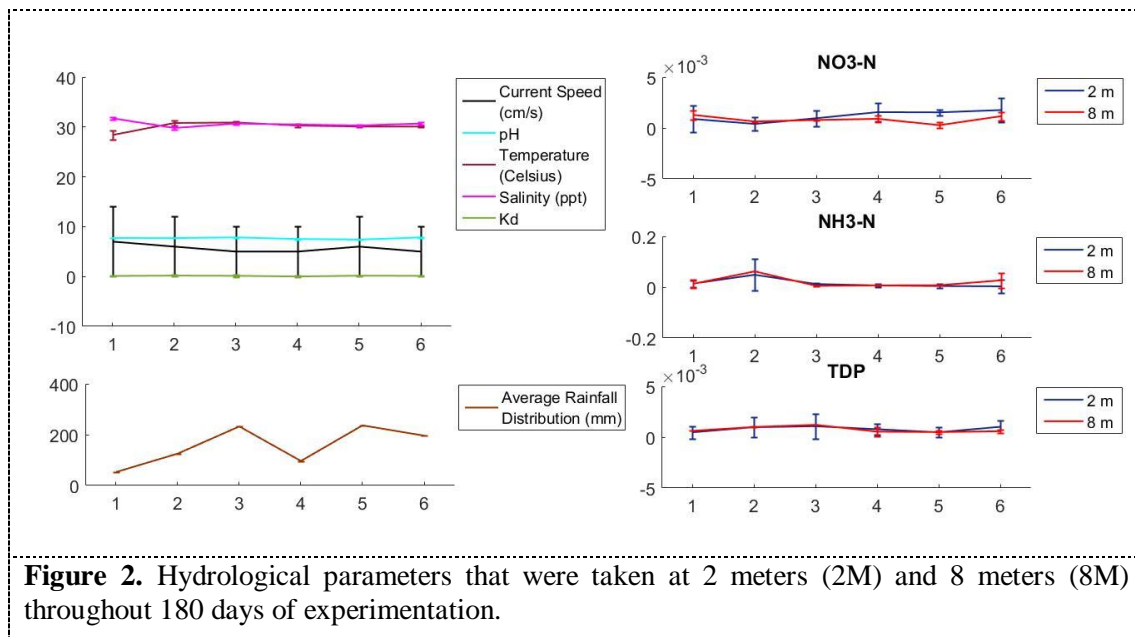
CCA biplot graph was used to explain the relationships between species and environmental parameters. This ordination diagram shows species as points and environmental variables as vectors. The vectors show the direction of maximum variation in the value of the corresponding variable [24]. In order to assess the independence of each variable, variation inflation factors (VIFs) was also calculated in R statistical programming software [25]. If the variables have small VIFs (less than 20), the analysis can be considered to have no multicollinearity issue [24]. The permutation tests for significance of the constraints was tested using "ANOVA" function for the overall test of the significance, axes of significance and significance of environmental variables tested.

3. Results and discussion

3.1 Results

3.1.1 Environmental conditions

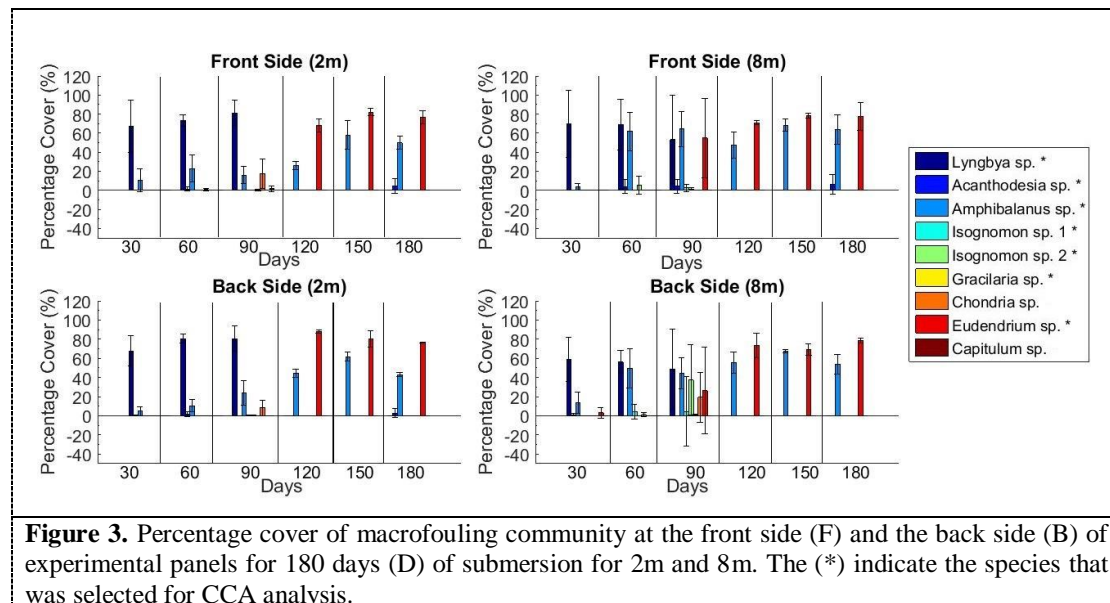
There was a significant difference between groups of hydrological parameters and water nutrients as determined by one-way ANOVA ($P < 0.05$). Based on the figure above, the reading of salinity, temperature, and pH show small variation ($< 5\%$ variation) between days of sampling and consistent with a recent study [26]. For example, the readings of temperature during April and August at Station 1, which was located at the outer part of the lagoon was around 30°C, which was similar to the readings obtained for this study (April, 28.4 °C; August, 30.1 °C).



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3.1.2 Sessile macrofouling community

There are nine species that can be found growing on the PVC plates on both side namely *Lyngbya* sp., *Acanthodesia* sp., *Amphibalanus* sp., *Isognomon* sp. 1, *Isognomon* sp. 2, *Gracilaria* sp., *Chondria* sp., *Eudendrium* sp., *Capitulum* sp., that were growing at the plate on both sides at 2 m and 8 m depth throughout 180 days of experimentation (**Figure 4**). *Lyngbya* sp. (dark blue bar) *Amphibalanus* sp. (light blue bar), and *Eudendrium* sp. (red bar) was the dominant species at both depths on both sides of the plates with the highest percentage cover, 81%, 68%, and 88%, respectively. A three-way ANOVA was run on the dominant species, on how they change over time, depth and position on the plates. There was a significant relationship between submersion time, depth and submersion time with depth for *Amphibalanus* sp., submersion time and position of plates with depth for *Lyngbya* sp., and submersion time for *Eudendrium* sp.



The earliest species to develop were *Lyngbya* sp., *Acanthodesia* sp. and *Amphibalanus* sp. with the highest percentage cover during the first sampling were 70%, 1%, and 18%, respectively. Slowly, other species such as *Isognomon* sp. 1, *Isognomon* sp. 2, *Gracilaria* sp., *Eudendrium* sp., and *Chondria* sp. started to appear, competing space with the earlier colonizer through natural succession process. The change became apparent after 90 days, as *Amphibalanus* sp. and *Eudendrium* sp. appear to occupy the majority of the PVC plate, with highest percentage cover at the end of 180 days were 79% and 64%, respectively, with *Eudendrium* sp. replacing the dominance of *Lyngbya* sp. and continued to have majority of spaces on until the end of experiment. As for the highest percentage cover for a single species, it belongs to *Eudendrium* sp. at the back side of 2 m PVC plates during 120 days with percentage cover of more than 80%. Based on the three-way ANOVA, the increase of percentage cover in relation with time was statistically significant different ($P < 0.05$).

Table 1. Three way-ANOVA of dominant species (*Amphibalanus* sp., *Lyngbya* sp. and *Eudendrium* sp., respectively) with time, depth and position of the plates.

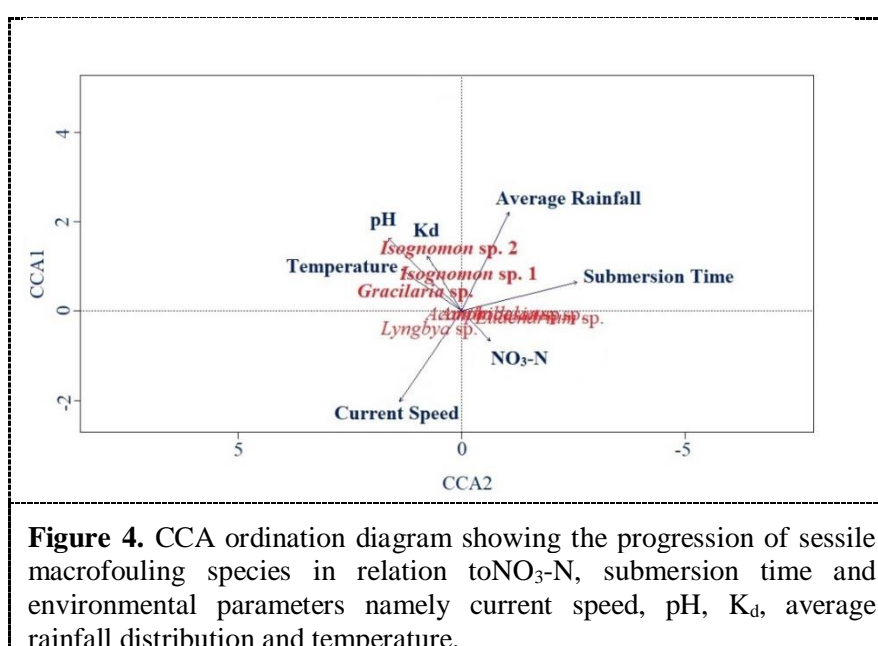
	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Time	5	3.193	0.639	32.967	0.000
Depth	1	0.756	0.756	39.057	0.000
Position	1	0.003	0.003	0.179	0.674
Time*Position	5	0.157	0.031	1.619	0.173
Time*Depth	5	0.455	0.091	4.698	0.001
Position*Depth	1	0.006	0.006	0.332	0.567
Time*Position*Depth	5	0.126	0.025	1.304	0.278
Error	48	0.930	0.019		
Total	72	37.307			

	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Time	5	12.803	2.561	27.346	0.000
Depth	1	0.008	0.008	0.088	0.768
Position	1	0.175	0.175	1.869	0.178
Time*Position	5	0.191	0.038	0.408	0.841
Time*Depth	5	0.209	0.042	0.446	0.814
Position*Depth	1	0.447	0.447	4.769	0.034
Time*Position*Depth	5	0.464	0.093	0.991	0.433
Error	48	4.495	0.094		
Total	72	31.550			

	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Time	5	18.495	3.699	99.346	0.000
Depth	1	0.018	0.018	0.476	0.493
Position	1	0.001	0.001	0.023	0.879
Time*Position	5	0.076	0.015	0.409	0.841
Time*Depth	5	0.345	0.069	1.851	0.121
Position*Depth	1	0.006	0.006	0.148	0.702
Time*Position*Depth	5	0.037	0.007	0.198	0.962
Error	48	1.787	0.037		
Total	72	44.077			

3.1.3 Summary of interaction between macrofouling and environmental factors

Seven out nine species were included in the final CCA analysis due to having more than five occurrences (**Figure 4**). Variables in the final CCA were current speed, average rainfall distribution, seawater temperature, NO₃-N, submersion time, pH and K_d. The removed variables with VIF more than 20 were current direction, NH₃-N, TDP and salinity. The relationship between the macrofouling community at 2 and 8- meter depth and the selected environmental variables are shown in **Figure 3**.



The biplot generated by abundance data (estimated using percentage cover of each species) revealed that *Eudendrium* sp., *Gracilaria* sp., *Isognomon* sp. 1 and *Isognomon* sp. 2 had a relationship with pH, temperature and K_d , suggesting that it become more abundance as pH, temperature and K_d increase. For example, as pH (7.84), temperature (30.9°C) and K_d (0.18) were high, the percentage cover of *Gracilaria* sp. (1%), *Isognomon* sp. 1 (4%) and *Isognomon* sp. 2 (37%) was at the highest. Likewise, with *Lyngbya* sp., the percentage cover of this species was at highest (81%) as the current speed was at lowest (5cm/s). Other than that, *Acanthodesia* sp. and *Amphibalanus* sp. also had a positive relationship with average rainfall distribution and NO_3-N . The percentage covers of *Acanthodesia* sp. (6%) and *Amphibalanus* sp. (68%) at the highest when the average rainfall distribution (196mm) and NO_3-N (0.00028mg/L) were also high. There was a significant relationship between abundance of macrofouling community and environmental factors in CCA ($P < 0.05$). The first two axes of CCA explained 86% and 8% of the variation in macrofouling assemblages, respectively. The eigenvalues of axis 1 and 2 accounted for 0.50 and 0.05 of the variance, respectively.

3.2 Discussion

According to Dehmordi (2011) [28], the most important members of the macrofouling community generally, were the Annelida, Crustacea, Bivalvia, and Anthozoa. In this study, we also find similar community from Crustacea (*Amphibalanus* sp. and *Capitulum* sp.) and Bivalvia (*Isognomon* sp. 1 and *Isognomon* sp. 2). Other than that, we also found the variety of seaweeds, hydroids, and cyanobacteria on our fouling community, which was also similar to the findings from Satheesh and Wesley (2008) [1], wherein their study, the major fouling groups were barnacles, ascidians, polychaetes, bivalves, and seaweeds. Other than that, there a total of nine species were found growing on PVC plates, on both sides of the plates, which was similar to the study done by Dehmordi [28], where a total of ten species belongs to 4 phyla (Molluscs, Cnidaria, Annelida, and Arthropoda) were found on piles of Deilam port, Iran. However, this was considered to be less compared to the Ong and Tan [29] where they found 27 marine organisms on jetty pilings at several nearby islands around Singapore. The reasons for the variety of species compared to this study could be due to multiple sampling locations as Ong and Tan [29] done their study on pilings situated at six different islands around Singapore. The other explanation could be due to submersion time, as the pilings on all the islands were there for many years, compared to this study, where our artificial structures were submerged for only 180 days.

There were appeared to be changed in species abundance throughout 180 days of experimentation, but overall, *Amphibalanus* sp. was the most consistent species to appear on each sampling days as *Lyngbya* sp. appeared mainly on the first 90 days, while *Eudendrium* sp. appeared majorly after 90 days. *Amphibalanus* sp. was considered to be a common fouling acorn barnacle species that can be found in the South China Sea [30]. Other dominant species, namely *Eudendrium* sp. and *Lyngbya* sp. were also common macroalgae species that can be found in the South China Sea [31]. Holistically, the fouling assemblages that were found growing on PVC plates were dominated by the fast-growing marine organism as it was dominated by algae and barnacles, different to the fast-growing organism that was found in Scheer's experiment (1945) [2]. In Scheer's experiments that were carried out in the harbor of Newport on the Pacific Coast, The first stage of macrofouling succession is developing with fast-growing marine organism was hydroids, serpulids, bryozoans, or the ascidian, whereas in this study, then it was mainly algae and barnacles.

The abundance of the macrofouling community had a significant relationship ($P < 0.05$) with pH, current speed and temperature. The temperature was considered to be the most critical factor in determining the distribution of benthic organism due it to have interaction with other physical factors such as salinity, current and air [1]. In this study, the average seawater temperature had a slight variation (29.9 to 30.9). In tropical water, the temperature was likely to be constant throughout the year due to not being influenced by seasonal changes [32]. With a constant temperature, fouling community can develop steadily. A change in temperature can be caused by fouling development to lessen. According to Railkin (2004) [15], macrofouling community succession process depreciates in development during winter due to the reduction of light, seawater temperature and in the settlement of

macrofouling organism on the substrate. Other than temperature, current speed also appears to be one of the most important environmental parameters. In our study area, the current speed range from 5 cm/s to 7 cm/s, which was similar to the findings of Hoque [33] and Osman [26], where low net currents were observed at the coastal waters of Karambunai. With low net currents and strong attachment to the PVC, plates enable the macrofoulers to resist strong water action and predation [34-36] and allow them to grow abundantly.

4. Conclusion

Overall, there are nine species found on the macrofouling assemblages that were growing on the artificial structure at the front side and the back side of the PVC plates at 2 m and 8 m. Based on the species that were found, the macrofouling community is considered to be at the fast-growing stages. As for the abundance of the macrofouling community does have a significant relationship ($P < 0.05$) with current speed, pH and temperature.

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