IJEGEO
International Journal of Environment and Geoinformatics 11(1):030-035 (2024)

Research Article

Pre-study of the evaluation of ecological sessile succession and their relationship with bacteria on concrete artificial reef material

Benal Gul[1], Tuba Unsal[2*]

[1] Faculty of Aquatic Sciences, Istanbul University, Vezneciler Fatih, Istanbul 34134, Türkiye
[2] Institute of Marine Sciences and Management, Istanbul University, Vefa Fatih, Istanbul 34134, Türkiye

* Corresponding author: T. Unsal
E-mail: tunsal@istanbul.edu.tr

Received: 12.02.2024
Accepted 19.03.2024

How to cite: Gul and Unsal (2020). Pre-study of the evaluation of ecological sessile succession and their relationship with bacteria on concrete artificial reef material, *International Journal of Environment and Geoinformatics (IJEGEO)*, 11(1): 030-035. doi. 10.30897/ijegeo.1435748

Abstract

Artificial reefs are human-made structures built for promoting marine life. Long-term monitoring and research regarding the use of artificial reefs in terms of ecological and economic perspective is essential. In this study, the ecological succession and their relationship with bacteria on concrete artificial reef material was investigated. Heybeliada artificial reef site was selected as sampling area. After three years period, the visual examination of ecological succession and sessile marine bacteria count were performed on the concrete artificial reef material. All the results showed that the strength of the artificial reef material is also affected by the biofilm layer and the creatures in the microecosystem. Therefore, monitoring studies should be carried out to determine the service life of artificial reef materials as well as to reveal the existence of the ecosystem formed and developing in these areas qualitatively and quantitatively.

Keywords: Artificial reef material, Ecological succession, Marine bacteria, Biofilm

Introduction

Ensuring the sustainability of the marine ecosystem and resources and protecting biodiversity are issues that have increased in importance in recent years (Gazioğlu, 2018). Although some sectors such as fishing, transportation and tourism directly benefit the sea, the developing industrial sector poses a significant problem for the marine ecosystem. Moreover, practices such as landfilling, littering, illegal fishing activities and the global effects of climate change lead to the loss of underwater habitats (Brander, 2013; Bracho-Villavicencio et al.,2023; O'Reilly and Willerth, 2024). These damages to water quality and habitat opportunities lead to irreparable damage to the aquatic ecosystem balance. Especially, to improve damaged habitats and support the ecosystem many scientific studies are carried out and various tools and methods are used (Borja et al. 2020). Artificial reefs are also among the tools used for this purpose (Toring-Farquerabao et al. 2021; Bracho-Villavicencio et al. 2023). Artificial reefs, which are frequently used for habitat restoration especially on the Mediterranean coasts, have been implemented in more than 70 applications on the coasts of our country.

Due to human impact and climate change, the Sea of Marmara has lost its ecosystem balance and sustainability and faced the habitat loss (Keles et al. 2020). One of the biggest disasters caused by these problems manifested itself as the mucilage disaster in 2021. In the same period, a large-scale artificial reef project was implemented off the coast of Istanbul in order to improve the ecosystem and habitat restoration. Artificial reefs are expected to function like natural reefs after being placed underwater (FAO, 2015). Succession features are critical in realizing this expectation. It is extremely important to monitor the processes developing on the reef in the long term from the moment the artificial reef material first contacts with the water and to determine the efficiency of the application in order to reveal whether the targeted goals are achieved (Bracho-Villavicencio et al. 2023).

The artificial reef materials should be durable and safe for marine environment. The mechanical properties of artificial reef materials such as strength, chemical composition, water absorbability need to be suitable for marine life. The metabolic activities of marine organisms can cause corrosion problems on artificial reef materials and it leads lowering service life of reef materials (Yang et al. 2018a). Therefore, the selecting appropriate materials is essential step for artificial reef durability. There are limited studies that have demonstrated the relationship between benthic organisms and bacteria communities on artificial reef materials (Guo et al. 2021). In this work, the ecological succession and their relationship with bacteria on artificial reef material was evaluated. After three years period, the visual examination and the enumeration of sessile marine bacteria were performed on the artificial reef material.

Materials and Methods

Sampling area

Artificial reefs located off the coast of Heybeliada was selected as sampling area (Figure 1). Artificial reefs were deployed at 24m depth and muddy ground in 2020.

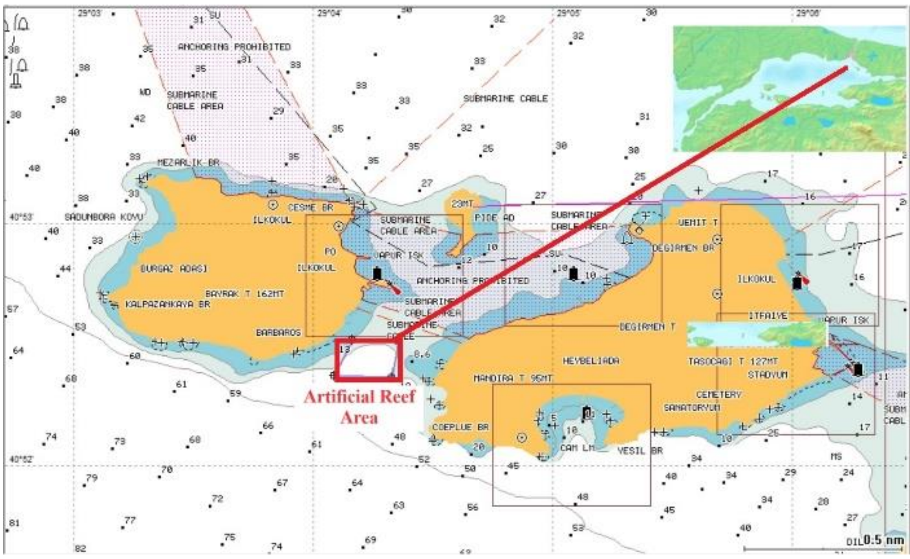

Fig. 1. Sampling point of Heybeliada.

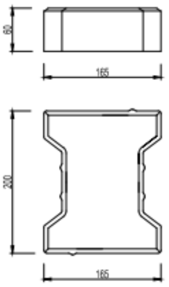

Fig. 2. The dimensions of Interlocking Paving Stones (Iston, 2021).

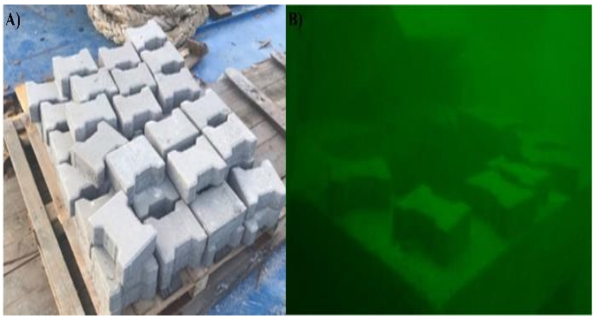

Fig. 3. Pre-placement view of paving stones and their positioning on artificial reef blocks .

Artificial reef specimens and deployment plan

Interlocking Paving Stones (Iston, 2021) were used for sampling (Figure 2). Paving Stones are made of concrete material, placed on artificial reef sets consisting of concrete cubic blocks, were placed in the region in December 2020, positioned above the artificial reef blocks (Figure 3).

Sampling

The stones placed on the reef blocks were placed in ziplock bags underwater without touching by scuba diving researchers and brought back to the boat. Samples were kept in the dark and at the seawater temperature until arrival at the lab. Then, they were returned to the laboratory as soon as possible.

Enumeration of marine bacteria

Water samples were taken from study area and filtered by a sterile (142 mm diameter) 0.22 μm pore size (Sartorius) nylon membrane filter. Afterwards, the samples were re-suspended in 20 ml of sterile seawater using Stomacher Lab Blender (Interscience BagMixer) for 2 min. All suspansions were diluted 10^{-1} to 10^{-7} to enumerate marine bacteria in seawater.

For sessile bacteria counting, the sample was rinsed with sterile seawater to remove planktonic bacteria and to avoid sample contamination. Then the bacterial samples were collected carefully from three different sites of stones with sterile swabs. The constituents of biofilm were put in a 10 mL sterile seawater and vortexed. The bacterial suspensions were diluted to 10^{-7}. Marine agar medium (Merck) was used to enumerate bacteria. Inoculated plates were incubated at 28 °C for 7 days. All plates were inoculated in triplicate. The colonies were counted by a colony counter (Funke Gerber, Colony Counter) after incubation period and expressed as colony forming unit (CFU) (Reasoner ve Geldrich, 1985).

Results

Physicochemical parameters

The chemical parameters of water samples that were taken from the study area were given Table 1. These chemical data were obtained within the framework of the research project (TUBITAK 121G102) carried out in the region in October 2021-October 2022 (Gül et al. 2023).

According to project (Tubitak 121G102) results, the average of the temperature values were around 12.6 ± 1.5°C. The minimum and maximum pH values of the water were found as 7.67 and 8.03 respectively. The average concentration of DO was observed as 7.48 mg/l and the salinity of water was found as ‰ 23.63. The lowest value of $NO_2 + NO_3$ was found as 0.88 mg/l and the highest value was 26.66 mg/l. Also, the values of PO_4-P reached 251.05 mg/l at winter and the average chlorophyll-a found as 1.34 μg/l.

Table 1. Chemical parameters from sampling point at 20m depth between October 2021-October 2022

Parameters	Min	Max	Average
Temperature °C	8	20	12.6
pH	7.67	8.03	8.09
DO (mg/l)	1.89	9.59	7.48
Salinity	21.2	26.5	23.63
NO_2+NO_3	0.88	26.66	10.28
PO_4-P	2.31	251.05	86.34
Chlorophyl-a	0.13	3.19	1.34

Fig. 4. Macroscopic images of paving stone.

Fig. 5. Macroscopic images of Serpulidae members.

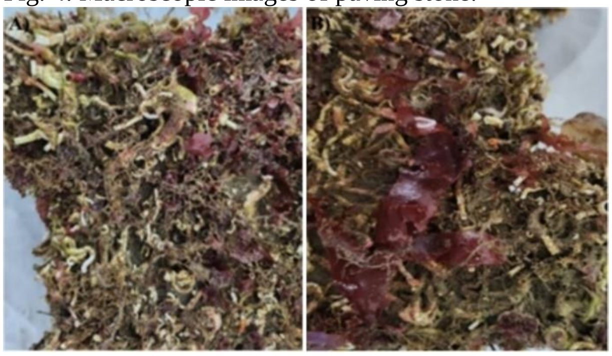

Fig. 6. Macroscopic images of *Bougainvillia muscus* (A) and Rhodophyta species of algae (B)

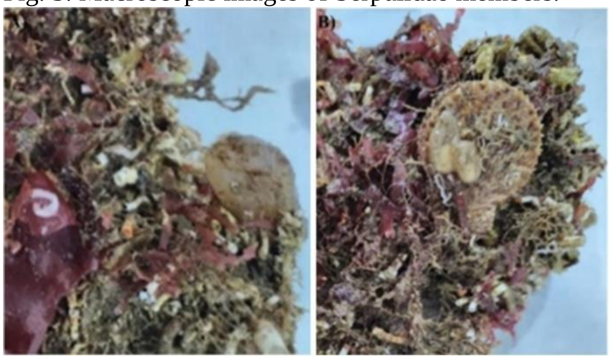

Fig. 7. Macroscopic images of *Ciona intestinalis* and *Mimachlamys varia* (A) and *Corella* sp.(B)

Visual examination

According to visual examination of the sample material showed that the entire area of the material was covered with benthic organisms. Also, the most dominant species are determined as Polychaeta on the artificial reef material surface (Figure 4).

The data related to the species and groups identified through the examination conducted with the naked eye on the paving stone surface have been presented in figures. It has been determined that the most dominant group on the stone surface is members of the Serpulidae family (Figure 5).

Bougainvillia muscus (Figure 6A) takes the second place in dominance, Rhodophyta species of algae (Figure 6B) have also been recorded.

The sea squirt *Ciona intestinalis* and the scallop named *Mimachlamys varia* (Figure 7A) are represented by a single individual, whereas very small individuals of *Corella* sp. (Figure 7B) tunicates have also been observed.

Enumeration of total marine bacteria

The levels of total marine bacteria on artificial reef material and in seawater were given in Figure 8. The total marine bacteria on the artificial reef material was determined as 2.1×10^5 cfu/cm^2 and the value in the seawater was found as 2.3×10^4 cfu/ml (Figure 8).

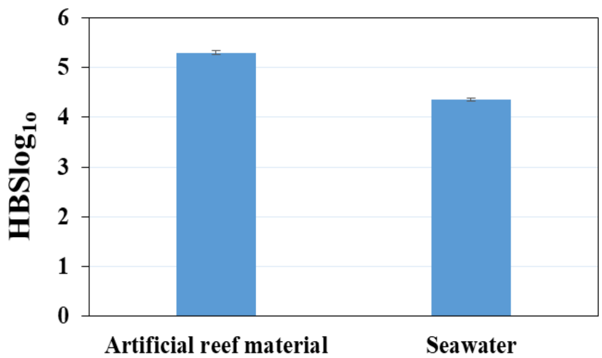

Fig. 8. The levels of total marine bacteria in artificial reef material and seawater.

Discussion and Conclusion

Chemical composition and physical properties of the reef material surface affect bacterial attachment and formation of biofilm (Georgakopoulos-Soares *et al*. 2023). The characteristics of the biofilm also determines the development of succession. In this study, after three years, the material was completely covered by sessile organisms. The upper part of material was scraped to evaluate micro and macro organisms. The most dominant species was the members of Serpulidae. It is known that the members of the Serpulidae provide calcium carbonate enrichment in the area through the tubes they form. Although there is limited studies about Serpulidae, it is known that the members of this family contribute to the enrichment of mineralization in the area where they are located, and also create habitats on their own due to the physical space they create (Hill, 2013). Also, *Bouganville muscus*, a hydrozoan species, was densely detected on the surface. Hydrozoans, which have a wide distribution and high diversity in the world, have found the chance to live on the cobblestone representing the artificial reef and have taken their place in biodiversity. The continuous fecal production of these organisms lead to occur low-oxygen gradients and support the development of anaerobic heterotrophic bacteria on artificial reef materials (Guido et al. 2014). In this study, the number of marine bacteria in sessile form, was found as $2.1x10^5$ cfu/ml (Figure 8). It indicated that bacteria and other organisms have symbiotic relationship and the extracellular polymeric subtances (EPS) promote them to adhere to each other or any surface. This finding has also been corroborated by various studies (Costa *et al.* 2018, Yang *et al.* 2018b). The other two individuals detected are described as sea squirts: *Ciona intestinalis* and *Corella* sp. Both species are filter feeding creatures (Emerson *et.al.* 2022). Although they were found as a individual on reef material, the researchers who carried out the underwater sampling also noted the presence of dense clusters on the artificial reef sets. Especially the density in the number of *Ciona intestinalis* indicates that the region is under algal pressure. Different studies have studied the filtration rates of this species in different regions (Petersen and Riisgârd, 1992).

Considering the long-term mucilage disaster that has occurred in the region in recent years, it is thought that *Ciona intestinalis* and *Corella* sp. provide a benefit in reducing algal pressure with their filter feeding feature. Also, *Ciona intestinalis* has been used as a model organism in molecular biology (Blasiak *et al.* 2014). Members of Rhodophyta were detected on the artificial reef material. Additionally, the diver researchers who conducted the sampling observed that members of this family were abundantly found on and around the artificial reef sets. Rhodophyta members are indispensable for the marine ecosystem with their oxygen production, biogeochemical effect, contribution to carbon synchronization and important role in the food web (Minhas *et al.,* 2020). Also, many studies showed that bacteria-algae interactions has a great impact on algal development (Ramanan *et al.* 2016).

Juvenile Chlamys was also detected on reef material. Being a filter-feeding creature, this scallop, like all its other similarly feeding relatives, feeds on phytoplankton, particulate organic matter, inorganic particles and planktonic larvae of some marine invertebrates from the water column and filters them and excretes biodeposits. In this way, the water column becomes clear by removing phytoplankton, biotic and abiotic particles, and organic and nutrient-rich particles are transferred to the bottom (National Research Council, 2010). Considering that all these detected creatures feed on particles in the water, this suggests that the nutrient level of the environment is high. Moreover, the mucilage disaster experienced in recent years occurred when the increase in algae and wastes due to pollution in the environment exceeded the carrying capacity. By providing hard ground, reefs provide an environment for such sessile creatures to live and reproduce, and these creatures play an important role in ecological balance with their water filtering properties.

All this ecological development provides real benefits only with the long-term durability of the artificial reef. FAO (2015) was prepared a guide for artificial reef applications, the importance and types of monitoring studies and important criteria are discussed. Many applications are subject to biological and ecosystem monitoring, and some applications are supported by socio-economic monitoring. However, studies on the underwater durability of artificial reefs are limited. These studies mostly aimed to reveal the connection between movements such as waves, currents, etc. and the stability of artificial reefs (Fauzi *et al.,* 2017, Düzbastılar and Tokaç, 2003). However, the strength of the artificial reef material is also affected by the biofilm layer formed on the surface and the creatures in the microecosystem. For this reason, it is also essential to monitor the biological development process on the material. Therefore, sustainable benefits can be achieved for the longest period of time in these ecosystem-forming areas. Otherwise, the destruction of reef materials may cause the collapse of this system, which was created by spending intense effort, time and cost.

The following conclusions can be drawn from the obtained data.

- The microbiota develops on artificial reefs may have both a constructive and a destructive effect on the ecosystem. Therefore, the studies need to be conducted on their impact on the ecosystem. There is no study to evaluate the effect of the sessile ecosystem, such as their mineral transformation and physical drilling-carving on reef materials. These gaps should be addressed with future studies.
- The ecosystem on the reef material has a dynamic structure. To achieve the expected benefit, not only the succession stages but also the life processes of organisms in the ecosystem must be taken into account.
- Since the initial formation of the biofilm on the reef material to model all the processes that will

develop is important in determining the reef material composition to be used in the region.

- In artificial reef studies to be carried out specifically for a species or a group, the benthic ecosystem that this microbiota will attract should be foreseen, as well as knowing the behavioral and metabolic needs of the target species. For example; if an artificial reef project is to be prepared for filter feeding creatures in order to improve water criteria in waters under pollution pressure, the mineral composition of the reef material to be used should be carefully selected, taking into account the microbiota in the water.

Acknowledgements

We thank Hayati Yağlı for his contributions to diving safety during this study.

References

Blasiak, L., Zinder, S., Buckley, D. et al. (2014). Bacterial diversity associated with the tunic of the model chordate *Ciona intestinalis*. ISME J 8, 309–320 https://doi.org/10.1038/ismej.2013.156.

Bracho-Villavicencio, C.; Matthews-Cascon, H.; Rossi, S. (2023) Artificial Reefs around the World: A Review of the State of the Art and a Meta-Analysis of Its Effectiveness for the Restoration of Marine Ecosystems. *Environments* 10, 121. doi.org/10.3390/environments10070121

Brander, K. (2013). Climate and current anthropogenic impacts on fisheries. *Climatic Change,* 119(1), 9-21. doi.org/10.1007/s10584-012-0541-2

Borja A., Andersen J. H., Arvanitidis C. D., Basset A., Buhl-Mortensen L., Carvalho S., Dafforn K. A., Devlin M. J., Escobar-Briones E. G., Grenz C., Harder T., Katsanevakis S., Liu D., Metaxas A., Morán X. A. G., Newton A., Piroddi C., Pochon X., Queirós A. M., Snelgrove P. V. R., Solidoro C., St. John M. A., Teixeira H. (2020) Past and Future Grand Challenges in Marine Ecosystem Ecology. Frontiers in Marine Science Vol:7 DOI=10.3389/fmars.2020.00362.

Calder, D.R., Choong, H.H.C., Carlton, J.T., Chapman, J.W., Miller, J.A., Geller, J. (2014) Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. Aquatic Invasions 9:425-440 doi:10.3391/ai.2014.9.4.02.

Costa, O.Y.A., Raaijmakers, J.M., Kuramae, E.E. (2018). Microbial Extracellular Polymeric Substances: Ecological Function and Impact on Soil Aggregation. Front Microbiol. 23; 9:1636.

Düzbastilar, F., Tokaç, Adnan. (2003). Determination of effects of artificial reef size on local scouring phenomena resulting from wave action.. Su Ürünleri Dergisi. 20. 10.12714/egejfas.2003.20.3.5000157086.

Emerson, R. W., Kieran. J. M., Russel, C. W. (2022) Ecological Review of the Ciona Species Complex. The Biological Bulletin 242:2, 153-171.

Fauzi, M. A. R., Armono, H. D., Mustain, M., Amalia, A., R. (2017). Comparison Study of Various Type Artificial Reef Performance in Reducing Wave Height. IPTEK Journal of Proceedings Series. Regional Conference in Civil Engineering (RCCE) 430 *The Third International Conference on Civil Engineering Research (ICCER)* August 1st-2nd 2017, Surabaya – Indonesia

FAO. (2015) Practical guidelines for the use of artificial reefs in the Mediterranean and the Black Sea,by Gianna Fabi, Giuseppe Scarcella, Alessandra Spagnolo, Stephen A. Bortone, Eric Charbonnel, Juan J. Goutayer, Naoufel Haddad, Altan Lök, and Michel Trommelen. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 96. Rome, Italy.

Gazioğlu, C. (2018). Biodiversity, Coastal Protection, Promotion and Applicability Investigation of the Ocean Health Index for Turkish Seas. *International Journal of Environment and Geoinformatics,* 5(3), 353-367. https://doi.org/10.30897/ijegeo.484067

Georgakopoulos-Soares I, Papazoglou EL, Karmiris-Obratański P, Karkalos NE, Markopoulos AP. (2023). Surface antibacterial properties enhanced through engineered textures and surface roughness: A review. Colloids Surf B Biointerfaces. 231:113584.

Guido A, Mastandrea A, Rosso A, Sanfilippo R, Tosti F, Riding R, Russo F. (2014). Commensal symbiosis between agglutinated polychaetes and sulfate-reducing bacteria. *Geobiology*. 2014 May;12(3):265-75. doi: 10.1111/gbi.12084. Epub 2014 Mar 17. PMID: 24636469.

Guo Z, Wang L, Cong W, Jiang Z, Liang Z. (2021). Comparative Analysis of the Ecological Succession of Microbial Communities on Two Artificial Reef Materials. *Microorganisms*. 6;9(1):120.

Gül, B., Turan Aktan, Y., Çağlar, N., Balcıoğlu, E. B., Gönülal, O. (2023) The Effect of Mucilage on Artificial Reef Succession and Fishing Productivity. Project Report (Tubitak 121G102)

Hill, R. (2013) The importance of serpulid reefs and their vulnerability to ocean acidification: editorial comment on the feature article by Smith et al. Mar Biol 160, 2279–2280. doi.org/10.1007/s00227-0132221 9

Iston (2021) Technical document. https://iston.istanbul/urun/asik-tasi (Ret: 08.02.2024).

Keleş G., Yilmaz S., Zengin M. 2020. Possible Economic Effects of Musilage on Sea of Marmara Fisheries.. *Int. J. Agric. For. Life Sci.,* 4(2): 173-177.

Mendoza-Becerril, M.d.l. A., Marques, A.C. (2013) Synopsis on the knowledge and distribution of the family Bougainvillidae (Hydrozoa, Hydroidolina). Lat. AM. *J. Aquat. Res.,* 41(5): 908- 924 doi: 103856/vol41-issue5-fulltext-11.

Minhas, A., Kaur, B., Kaur, J. (2020) *Genomics of algae: Its challenges and applications. Pan-genomics: Applications, Challenges, and Future Prospects*, Eds. Debmalya Barh, Siomar Soares, Sandeep Tiwari, Vasco Azevedo, Academic Press. Pages: 261-283. ISBN 9780128170762, doi.org/10.1016/B978-0-12-817076-2.00013-5

National Research Council, (2010) *Ecosystem services of bivalves: ımplications for restoration overvıew of ecosystem servıces provıded by bıvalve molluscs. ecosystem concepts for sustainable bivalve mariculture.* Washington, DC: The National

Academies Press. doi.org/10.17226/12802 ISBNs: Paperback: 978-0-309-14695-1, Ebook: 978-0-309-15231-0.

O'Reilly LM., Willerth SM (2024) Evaluating the biocompatibility of ceramic materials for constructing artificial reefs.*Front. Mar. Sci*. 10:1292584. doi: 10.3389/fmars.2023.1292584.

Petersen, J.K., Riisgârd, H.U. (1992) Filtration capacity of *Ciona intestinalis* and its grazing impact in a shallow fjord. *Marine Ecology Progress Series* Vol 88:9-17.

Ramanan, R., Kim, B. H., Cho, D. H., Oh, H. M., & Kim, H. S. (2016). Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnology advances*, *34*(1), 14-29.

Reasoner, D.J. and Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from Potable Water. *Applied and Environmental Microbiology,* 49, 1-7.

Toring-Farquerabao, M. L. B., Domingo. E. R., Tahiluddin A. B. (2021). Effectiveness and impact of artificial reef project in Tigbauan, Iloilo, Philippines: A case study. Menba *Journal of Fisheries Faculty,* 7(2), 77-89.

Yang, Y., Ji, T., Lin, X., Chen, C., Yang, Z. (2018a). Biogenic sulfuric acid corrosion resistance of new artificial reef concrete. *Construction and Building Materials,* 158, 33-41.

Yang L, Xiao S, Luan T, Tam NFY. (2018b) Overproduction of microbial extracellular polymeric substances in subtropical intertidal sediments in response to endocrine disrupting chemicals. *Sci Total Environ.* 15;624:673-682.