Journal of Marine
Technology and Environment
DOI:10.53464/JMTE.03.2026.07

ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

PREFERENCES OF *HIPPOCAMPUS GUTTULATUS* INDIVIDUALS FROM THE BLACK SEA FOR IRON AND STAINLESS STEEL ARTIFICIAL REEFS

Cristian-Gabriel DUMITRU[1], Iulian RUSU[2] & Simona GHIȚĂ[3]

[1]Constanța Maritime University, Faculty of Naval Electro-Mechanics, 104 Mircea cel Batran Street, 900663, Constanta, Romania, e-mail address: cristian.dumitru1514@gmail.com
[2] Club Marine Explorer NGO, Ovidiu, Romania, e-mail: clubmarineexplorerconstanta@gmail.com
[3] Lecturer Ph.D. Biol., Constanța Maritime University, Faculty of Naval Electro-Mechanics, 104 Mircea cel Batran Street, 900663, Constanta, Romania, ORCID ID 0000-0002-1165-9631, e-mail address: simona.ghita@cmu-edu.eu

Abstract: The implementation of artificial habitats made from recycled materials can significantly enhance marine habitats for seahorses and other species, especially in areas where the natural habitat has been altered by anthropogenic factors such as pollution, trawling, beach enlargement, eutrophication, invasive species, and climate change. The "Seahorse House" (Romanian: "*Casa căluților de mare*") is a small project conducted by Club Marine Explorer NGO in partnership with academic institutions that aims to help conserve *Hippocampus guttulatus* (Cuvier, 1829) individuals on the Romanian shore through the "seahorse hotel" model of artificial reefs. The seahorse hotel replicas were made with the target individuals' behaviour in mind; they cling to natural or man-made structures with their prehensile tails. The project established four key distinct locations for these reefs along the coast of Constanța city on different substrate types: sand, rocky, and mixed. Two distinct types of artificial habitats were placed at each installation sector, which were made from recycled iron, stainless steel, and rope. In addition, the nutrient levels for each placement zone were analysed using the rapid kits Viscolor HE and Viscolor ECO for phosphate and nitrate ions, respectively; in addition, the pH was monitored with the help of Merck MQuant™ Universal pH Indicator Solution. Moreover, the presence of total coliform and faecal coliform bacteria was assessed using CompactDry™ TC and CF culture dishes. Overall, the placement areas were in no peril of eutrophication or any bacterial infestation from the point of view of the rapid kits and the dry culture dishes, except for one of the zones, which is the closest to the piers and the touristic port. Ultimately, the iron reefs were more successful than their stainless-steel counterparts. Thus, it has been concluded that marine life may be attracted to underwater iron structures.

Keywords: artificial reefs, ions, iron, nutrients, seahorses, stainless steel.

1. INTRODUCTION

The Black Sea is considered a "Unicum Hydrobiologicum", as the marine oceanographer Nikolay M.Knipovich put it, meaning its ecosystem is one of a kind; therefore, it needs to be protected from anthropogenic harmful influences.

The research was conducted within the "Seahorse House" project, which provides students from Constanța County the chance to explore and protect the marine environment of the Black Sea through educational and scientific activities. Participants designed, built and installed artificial reefs by the shore of the Romanian coast, creating safe habitats for seahorses (Hippocampus gutulatus) and other marine species. Carried out between May and November 2025, the project is implemented by Club Marine Explorer NGO, in partnership with high school and university students, covering a large part of the public regarding species conservation.

2. METHODOLOGY

The artificial reefs of the project followed the „seahorse hotel" model [l] to help seahorses attach to them with their prehensile tail. Seahorses live at a short distance from the shore, on sandy and rocky bottoms and usually cling with their prehensile tail to marine plants and other anthropogenic structures [2]. The placement of the reefs was followed by water sampling and analysis to determine the nutrient levels and the possibility of a eutrophication threat.

The reefs are in four distinct locations; the coordinates and other specifications are given in Table 1 and Figure 1 (the areas were numbered in the order of the reefs' placement from Z1 to Z4 and were located on

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)

Journal of Marine
Technology and Environment
DOI:10.53464/JMTE.03.2026.07
ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

the map using Google Earth). The first location area (Z1) is near the pier at the Malibu Hotel beach, located at the deepest of the four areas, 8.3 meters below sea level, on a sandy substrate; Z2 is inside the pier in the area of the former fisher zone, located at the shallowest point of all, 3 meters, on a rocky substrate; Z3 is located the furthest offshore, perpendicular to the Malibu Hotel, at a depth of approximately 8 meters on a combined sandy and rocky substrate; and the last location area, Z4, is located right at the mouth of the tourist port, at a short distance from the breakwater, with a depth of around 4 meters, on a mostly rocky substrate. The reefs were monitored in 2025 in August-September, but the Z1 reef could no longer be found at the end of September.

The water samples were collected in plastic containers, which were individually labelled immediately after sampling. For each sample, essential details about the sampling site were recorded to ensure monitoring and facilitate subsequent inventories within the Engineering and Environmental Protection Laboratory of the Maritime University of Constanta. The wind speed did not exceed level 2 on the Beaufort scale during monitoring.

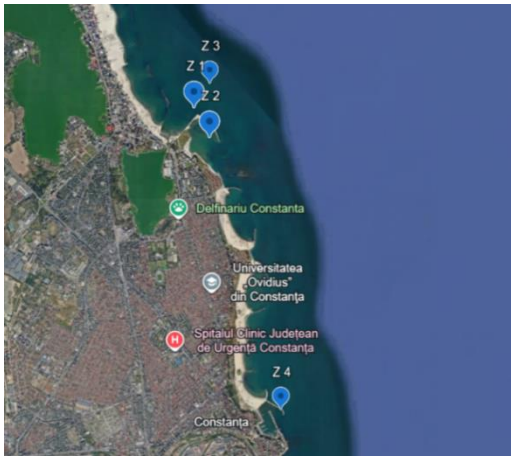


Figure 1. Placement areas of the reefs

Table 1. Specifications for artificial reefs location

Location number	Coordinates		Depth (m)	Substrate
	Latitude	Longitude		
1	44°13’19” N	28°38’46” E	8.3	Sandy
2	44°13’03” N	28°39’02” E	3	Rocky
3	44°13.3’32” N	28°38’58” E	8	Sandy and Rocky
4	44°10’39” N	28°39’51” E	4	Rocky

To determine the pH value, Merck MQuant™ Universal pH Indicator Solution was used. To a 5mL sample of water, 5 drops of indicator solution were added, then the sample was analysed calorimetrically. Chemical analyses to determine the level of eutrophication were performed by the colorimetric method, using the rapid Viscolor HE and Viscolor ECO kits for phosphate and nitrite ions, respectively. The method involves staining the samples through chemical reactions using 6 drops of 15-30% sulfuric acid and 10-25% sodium disulphate for phosphate ions, and for nitrite, 4 drops of $(NO_2)^{-1}$ and a measuring spoon of $(NO_2)^{-2}$ were added. After adding the reagents, both samples were left for 10 minutes to react. After the staining time, the samples were analysed calorimetrically using coloured cards, blue for phosphates and yellow-pink for nitrite.

For the bacteriological determination, we used CompactDry™ TC (total aerobic microorganisms) and CF (coliform bacteria) culture plates. The cultures were incubated for 24 hours at 37°C, after which the colony spots on the Petri dishes were counted. Once the colonies had been counted, the following formulas were used to determine their density:

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{number of CFUs}}{\text{sample volume} * \text{dilution factor}} \quad (1)$$

$$\frac{\text{CFU}}{\text{cm}^2} = \frac{\frac{\text{CFU}}{\text{mL}}}{\text{plate surface area}} \quad (2)$$

The reefs were built by high school students with the guidance of staff from the NGO Club Marine Explorer. The iron reef has a three-dimensional size of 50x50x50 cm, with a horizontal bar for stability connecting the edges on each side face approximately 17 cm from the base. One face of the cage has only the stability bar, while two of them also have two stainless steel wire cables parallel to the substrate, arranged at almost equal distances from each other. The third plane of the artificial habitat has three vertical and four horizontal stainless-steel wires, and the top of it has an iron rod in the middle, along with a matrix of eight stainless alloy wires connected horizontally and vertically to the edges of the cube (Figure 2 and Figure 4). The stainless steel one has a size of 70x70x70 cm,

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)

Journal of Marine
Technology and Environment
DOI:10.53464/JMTE.03.2026.07
ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

with fabric ropes on the faces of the cage, connected to its edges (Figure 3 and Figure 4).



Figure 2. The iron reef



Figure 3. The stainless-steel reef

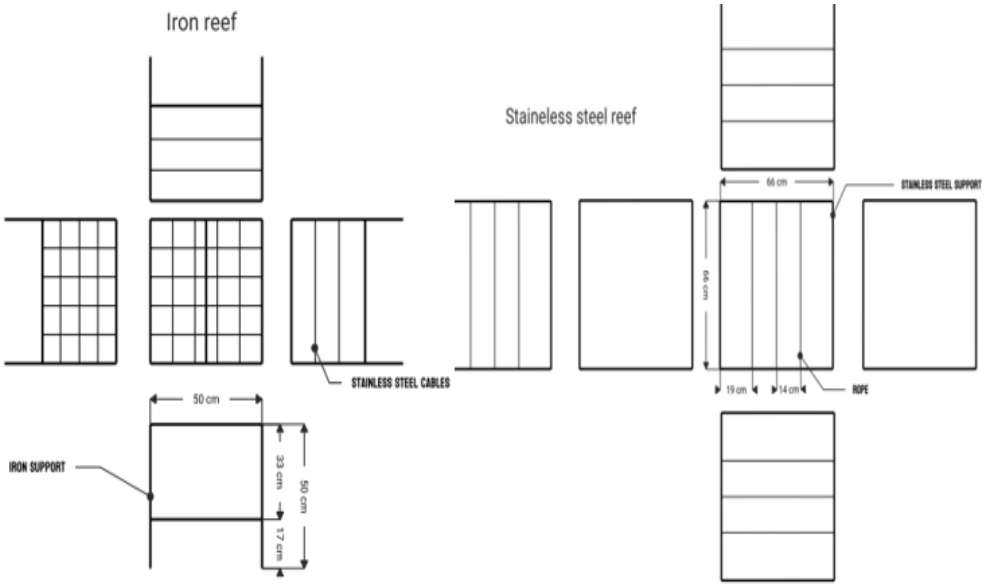


Figure 4. The iron reef (left) and the stainless steel (right) diagrams

3. DISCUSSIONS

Following the monitoring, from the point of view of analysing the nutrient level, it can be said that the reef locations are clean areas. The average concentration of nitrite ions is 0.04 mg/L NO_2^-, with the first three zones having a value of 0.02 mg/L NO_2^-, and Z4 recording 0.1 mg/L NO_2^-. The average of phosphate ions is higher than that of nitrite ions by 0.2 mg/L P. Surprisingly, Z3 contains the highest concentration, of 0.8 mg/L P, followed by Z4 with 0.1 mg/L P, while Z1 and Z2 have a concentration similar to that of nitrite ions, of 0.2mg/L P / mg/L NO_2^- (Figure 5). In addition, the waters of the Dobrogea region have an alkaline character; as a result, all the analysed samples registered 8.5 pH units with slight, insignificant variations.

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)

Journal of Marine
Technology and Environment
DOI:10.53464/JMTE.03.2026.07
ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

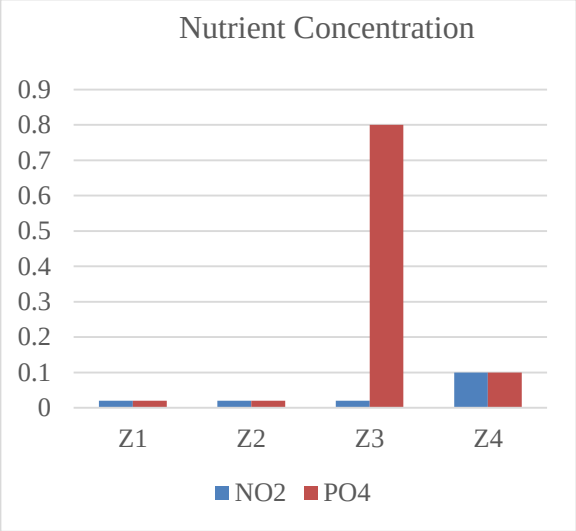


Figure 5. The nutrient concentration of each zone

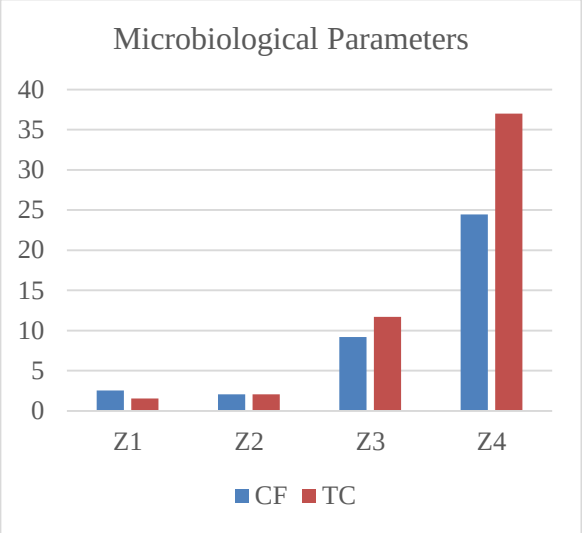


Figure 6. CF and TC parameters

CompactDry™ TC and CF cultures were used to determine the presence of coliform bacteria, which thrive in fresh or low-salinity waters—such as the Black Sea, where salinity reaches a maximum of 18 PSU during periods of drought. These bacteria can also be introduced by rivers, such as the Danube, and transported to the coastal zone by the sea's circular currents. Z4 was the only site with high levels of CF (24.45 CFU) and TC (37 CFU). This value can be linked to the specific area where the reef was installed: located at the entrance to the Tomis tourist harbour, the site receives wastewater discharged directly from boats, and the presence of bathers in the area may also contribute to the introduction of these bacteria. Z3 follows, with the next highest levels of CF and TC, 9.17 CFU and 11.7 CFU, respectively. This area is located offshore, where the sea's circular currents may transport these types of bacteria from the mouth of the Danube Delta toward the coastline. Z2 and Z1 show normal levels of FC and TC: 2.04 CFU and 2.04 CFU for Z2, and 2.55 CFU and 1.53 CFU for Z1 (Figure 6).

The iron habitats undergo an electrochemical corrosion process that produces rust. The rusting process occurs in two stages:

Stage I consists of the iron oxidation (in which atoms give up two electrons and form ferrous ions) and the electrolyte reduction (the corrosive agents that affect reefs are seawater and oxygen dissolved in water), according to the following formulas and scheme:

Iron oxidation:

$$Fe^{0} \rightarrow Fe^{+2} + 2e^{-} \quad (3)$$

Electrolyte reduction:

$$H_2O + \frac{1}{2} O_2 + 2e^{-} \rightarrow 2(OH)^{-} \quad (4)$$

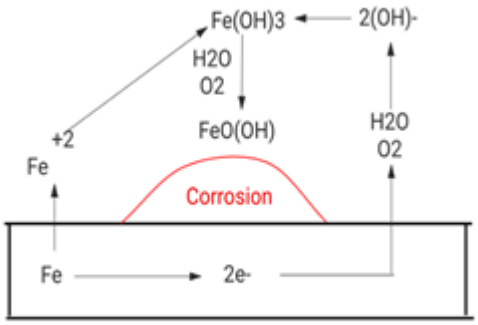


Figure 7. Diagram of rust formation on iron surfaces

In the second stage, the removed ferrous ions are oxidised in turn and form ferric ions ($Fe^{+2} \rightarrow Fe^{+3}$). The ferrous and ferric cations removed by the metal rods fall into the category of trace elements (elements that are part of the composition of living organisms) and attract marine animals to the reefs.

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)

Journal of Marine
Technology and Environment
DOI:10.53464/JMTE.03.2026.07
ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

The other type of reefs has not been of great interest to any marine species, as stainless steel is an inert alloy, made of iron in combination with other semi-noble elements (nickel, chromium, aluminium, etc.) that stop oxidation. The redox reaction does not occur because non-oxidising metals form a thin passivating layer of metal oxide on the surface of the material that stops oxygen molecules from interacting with the other compounds in the structure.

Habitat Z3 was the most successful of the four areas, with a population of seven seahorses attached to the iron reef (Figure 7), while the stainless reef was not populated by any individuals. Moreover, not only are seahorses present at the reef, but it is also populated by other syngnathids, clams and benthic fish. The preference of individuals of the target species towards the iron reef solidifies the idea of a trace element attracting marine animals.



Figure 8. The Z3 iron habitat

4. CONCLUSIONS

The zones in which the reefs are localised are conventionally clean, free of eutrophication threats, except for the third area, where phosphate concentration was highest, and the fourth one where a high quantity of bacteria has been registered. As for the reef's yield, iron reefs showed a greater preference between the two types, not only for the targeted species, but for other marine animals in general. This is further strengthened by the release of trace elements such as ferrous ions and the presence of various species at the reef.

5. FUNDING

The project was funded by the *Științescu Fund* - a national strategy to rekindle STEAM education, developed by the Federation of Community Foundations in Romania (FFCR) with the support of the Romanian American Foundation (RAF). The Constanța *Științescu Fund* is administered by the Galați Community Foundation and funded locally by the Ameropa Romania Foundation.

6. CREdit authors statement

Conceptualisation: Cristian Gabriel-Dumitru and Iulian Rusu

Data curation: C.-G.D.;

Formal analysis: C.-G.D

Funding acquisition: Iulian Rusu

Investigation: C.-G.D., I. R.;

Methodology: S.G.; I.R.;

Project administration: I. R.;

Resources: S.G.; I.R.; C.-G.D.;

Software: C.-G.D.;

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)

ISSN (Print): 1844-6116
ISSN (Online): 2501-8795
https://www. jmte.eu

Supervision: .S.G.; I.R.;

Validation: C.-G. D.; S.G.; I.R.;

Visualization: C.-G. D., S. .G.;I. R.;

Writing – original draft: C.-G. D.;

Writing – review & editing: C.-G. D.; S. .; I. R.

7. REFERENCES

[1] Simpson M., Coleman A. R., Morris R., Harasti D. (2019). *Seahorse Hotels: Use of artificial habitats to support populations of the endangered White's seahorse Hippocampus whtei*. Marine Environmental Research Volume 157.

[2] Niță V., Nenciu M., Galațchi M., & Diaconu D. (2024). S*peciile de pești de la litoralul românesc: Atlas actualizat / Fish Species of the Romanian Coast: Updated Atlas*. CD Press. pp. 171-173.

[3] Ghita, S., Hnatiuc, M., (2018). *Studies concerning flora from different protected areas in Constanta county by applying ecological and statistical methods*, Journal of Marine Technology and Environment, vol.1, pp 23-28, Nautica Publish House, Constanta, Romania.

[4] https://www.researchgate.net/figure/H-guttulatus-individual-in-its-natural-habitat-southern-Romanian-Black-Sea-coast_fig1_330654796

[5] Magda-Ioana NENCIU, George Emanuel HARCOTA, Aurelia TOTOIU, Elena BISINICU, Adrian FILIMON, Victor Nicolae NITA, Carmen Georgeta NICOLAE, (), *PREY PREFERENCE OF THE LONG-SNOUTED SEAHORSE (Hippocampus guttulatus Cuvier, 1829) AT THE ROMANIAN BLACK SEA COAST*, ISSN 2285-5750, ISSN CD-ROM 2285-5769, ISSN-L 2285-5750, ISSN Online: 2393 – 2260.

[6] Mazza, G., (2023), MONACO NATURE ENCYCLOPEDIA. Available at: https://www.monaconatureencyclopedia.com/hippocampus-guttulatus/?lang=en

[7] M.Correia , J.Palma, H. Koldewey, J. P.Andrade, (2013). *Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (Hippocampus guttulatus Cuvier)?*, Journal of Experimental Marine Biology and Ecology, 448 (2013) 258-264

[8] Ambrose, R.F., Anderson, T.W., (1990). *Influence of an artificial reef on the surrounding infaunal community*. Mar. Biol. 107 (1), 41–52.

[9] Bell, J.D., Westoby, M., Steffe, A.S., (1987). *Fish larvae settling in seagrass: do they discriminate between beds of different leaf density?* J. Exp. Mar. Biol. Ecol. 111 (2), 133–144.

[10] Bell, S.S., Hall, M.O., Soffian, S., Madley, K., (2002). *Assessing the impact of boat propeller scars on fish and shrimp utilizing seagrass beds*. Ecol. Appl. 12 (1), 206–217.

Creative Commons Attribution BY 4.0 International Licence (CC BY-4.0)