STABILITY OF EPIBIOTIC COMMUNITIES ON THE METAL SURFACES OF SHALLOW-WATER WRECKS IN THE MALTESE ISLANDS

Perla Galatolo, Patrick J. Schembri

Abstract: The epibiotic assemblages on three wrecks in Maltese waters were studied by taking photoquadrats on the metal hull of each wreck, and on nearby natural hard substrata for comparison. The taxon richness and cover area of the colonising organisms was quantified, and organisms were classified into functional groups based on structural features. Bare patches of hull surface were present on all wrecks and the epibiota occurred in a mosaic of assemblages. Taxon richness was higher on the hulls than on natural substrata. It is suggested that the wrecks have a disclimax community supported by disturbances, such as uneven sloughing of the corroding metal, resulting is a mosaic of patches at different stages of succession. The natural hard substrata showed a high proportion of tall arborescent forms compared to hulls, supporting the hypothesis of the impeded development of a climatic climax community on the wrecks. NMDS ordination showed that the three wrecks were distinguishable from each other based on the relative cover of the different functional groups.

Keywords: Disclimax, Succession, Infralittoral Rock, Colonisation, Functional groups.

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Introduction

In the oceans, the presence of artificial metal structures, including vessels, gives rise to new underwater habitats on the seabed. Occurrence of such structures can be attributed to historical events, as for example the First and Second World Wars, but in modern times, intentional scuttling has transformed defunct vessels ('wrecks') and other structures into popular diving sites, thereby boosting the tourism industry of coastal nations. In many countries, regulatory requirements demand the removal of hazardous objects, pollutants and harmful materials from the vessels, to safeguard the ecosystem from potential negative effects and to ensure safety of wreck divers. Additionally, site selection for scuttling takes into account ecological considerations, often focusing on areas with sandy seabeds devoid of attached macroscopic organisms [24].

Artificial reefs are submerged structures composed of natural or synthetic materials which serve to protect, enhance, or restore components of marine ecosystems [20]. Sunken metal vessels in the photic zone are commonly categorized as 'artificial reefs' by the general public, due to their tendency to attract a higher density of fish compared to the surrounding habitat. However, while epibionts may flourish due to the establishment of bacterial and microalgal communities which provide the foundation for new biomass on the metal surface, fish populations do not usually exhibit a substantial surge. Instead, fish often seek refuge within the extensive spaces of the wreck, leading to a redistribution of biomass, rather than a significant increase [3].

Despite their potential consequences, the ecological implications of scuttled vessels and shipwrecks remains understudied. In particular, research concerning the colonization of wrecks by sessile organisms and the subsequent ecological succession occurring on their surfaces, is notably lacking on a global scale, with a scarcity of published works focused on the Mediterranean region.

Microbial communities adhering to wreck surfaces initiate marine biofouling processes, and these biofilms provide an ideal substratum for the attachment and settlement of larval forms and spores of higher-order sessile organisms such as invertebrates and algae [22]. The development of a biofilm underwater involves a series of distinct and regulated stages which follows a specific sequence of chemical and biological events, although the exact mechanisms remain elusive [22]. Planktonic bacteria in seawater are thought to initiate the process by interacting with organic and inorganic particles on the metallic surface, thus laying the foundation for the initial community. Subsequently, these pioneering microorganisms facilitate the growth and reproduction of other bacterial colonies, leading to modifications in the surface characteristics of the substratum. This, in turn, facilitates the colonization by secondary microorganisms. The third phase involves interactions between established colonies and other free-living bacteria, ultimately resulting in the formation of an initial biofilm. Over time, this biofilm matures through antagonistic or symbiotic interactions with other existing bacteria, as well as the recruitment of additional colonizers [5].

The colonization and ecological progression on metal surfaces within marine ecosystems are dynamic processes influenced by an array of factors, including the physicochemical properties of the metal surface and environmental variables such as depth, temperature, nutrient levels, hydrodynamics, illumination, and water chemistry [2][11][16][23][25][27]. Moreover, physical interactions, seasonal fluctuations, and geographical differences further shape ecological succession on marine wrecks [1][4][15]. As a result, marine succession is a complex phenomenon that cannot be universally applied, even within a given geographical area [11][16]. While research into the ecological succession associated with artificial substrata in marine habitats has largely focused on objects submerged in the upper layers of the ocean (epipelagic zone) [4][10][12][13], there have been few investigations on the long-term temporal aspect of ecological succession on wrecks.

The formation of a bacterial film is a universal event; however, subsequent phases of the succession depend on the physicochemical characteristics of the environment, resulting in varying outcomes. For instance, Henschel et al. (1990), in their study conducted in South Africa, observed minimal settlement of algae during the initial three months of submersion. Subsequently, the algae were largely outcompeted by barnacles and other organisms such as bryozoans, mussels, hydrozoans, and polychaetes [12]. In contrast, López Garrido et al. (2015) documented a colonization sequence starting with algae and barnacles, where the former eventually outcompeted the latter, leading to an algal-dominated metal surface [16]. A study by Choi et al. (2006) in Japan revealed a sequence of diatoms preceding algae colonization on metal surfaces; this study also emphasized the role of seasonality and the recruitment of spores and other propagules from mature algae in close proximity to the study site [4].

In the existing literature, a significant gap remains in terms of the long-term monitoring of ecological succession on wrecks, spanning multiple years. Consequently, empirical evidence regarding the potential long-term changes within these communities is lacking. Nevertheless, some studies have evaluated the epibiotic diversity on wrecks submerged for decades [14][26]. These studies indicate that the principal groups of organisms that settle after microbes, such as algae, bryozoans and mussels, tend to persist over the long term, although the actual species and their coverage may undergo alterations over time. An increase in the structural complexity of epibiotic assemblages was observed on natural marine substrata as succession proceeds [6][7][8][21]. However, in contrast to terrestrial ecosystems, no definitive climax community appears to conclude the succession. Instead, the original colonisers, which may include algae, bryozoans, crustaceans and molluscs, tend to remain dominant throughout the succession, with potential changes in coverage and abundance among different organism classes over time, also due to variations in environmental conditions [11][23][25].

The present study aimed to examine the stability of epibiotic communities inhabiting the metal hulls of wrecks at different locations around the Maltese islands and to determine whether wrecks that have been submerged for an extended period exhibit consistent epibiotic assemblages among themselves and when compared to nearby natural hard substrata.

Materials and Methods

The investigations were carried out on three wrecks situated off the Maltese coast:

- MV 'Um el Faroud' located off Zurrieq at 35.8188° N 14.4492° E. The ship was scuttled in 1998 and originally measured ca 110m in length, but today the wreck has broken into two pieces. Currently the vessel lies at a depth of approximately 36 m.
- Patrol boat 'P29' located off Cirkewwa at 35.9885° N 14.3261° E. The ship was scuttled in 2007 and measures ca 52m in length; up to the present it is intact on the sea bed. Currently the vessel lies at a depth of approximately 34 m.
- MV 'Karwela' located off ix-Xatt l-Ahmar, Gozo, at 36.0168° N 14.2864° E. The ship was scuttled in 2006 and measures ca 50m in length, and up to the present it is intact on the sea bed. Currently the vessel lies at a depth of approximately 45 m.

In preparation for this study, recognition dives were conducted to identify optimal sections of the hull for assessment, considering factors like surface uniformity and verticality.

The sampling areas for hulls were determined based on the hull size: 1.5 m x 1.5m for the MV Karwela and P29 wrecks (depth ranges 39-42 m and 31-34 m, respectively), and 3 m x 3 m for the Um el Faroud wreck (depth range 27-31 m).

Ten photoquadrats of dimensions 30 cm x 30 cm were imaged within each hull's designated sampling area. Previous to sampling, grids representing the sampling area on each hull were generated digitally, with random photoquadrat positions assigned using a random number generator. In the field, divers used vertical and horizontal lines to locate the predetermined positions of the photoquadrats on the hulls. A PVC frame delineated the photoquadrats and maintained a fixed camera distance above each quadrat. Data collection was from August to October 2022. In the laboratory, Image J software [19] was employed to analyse images and quantify the area covered by variously coloured epibiota.

Organisms for later identification were collected by taking scrape samples from the wreck surfaces. Comparable assessments were carried out on three rocky surfaces as close as possible to each wreck site. The rocky surfaces were shallower than the wrecks (Table 1) as no rock was present at the depth of the scuttled vessels. Scrape samples of rock epibiota were procured from the same locations as the photoquadrats. In the laboratory, the collected organisms that also appeared in the photoquadrats were identified using published keys and manuals. Statistical analyses were carried out with R Studio [18].

Table 1 – Depth of natural rock surfaces sampled in the vicinity of each wreck site.

Site	Depth (m)
Um el Faroud	25
MV Karwela	32
P29	26

Results

Taxon richness (Table 2) and Simpson's diversity index (Table 3) were calculated for the hulls and neighbouring natural substrata.

For all wrecks, absolute taxon richness surpassed that of nearby rocky surfaces. Among the wrecks, MV Karwela exhibited the highest taxon richness, while Um el Faroud had the lowest. Simpson's diversity index was lower for the rocky substrata adjacent to the Um el Faroud and MV Karwela sites compared to the wrecks, indicating higher hull surface diversity. Notably, P29 was an exception, displaying lower diversity on the hull surface than the corresponding rocky substrate. The taxa recorded at each site are shown in Table 4.

Welch's two-sample t-test corroborated higher biodiversity on the Um el Faroud hull compared to the nearby natural surface ($p<0.05$). MV Karwela's hull presented a higher, and P29 a lower, Simpson's diversity index on the hull than on nearby natural surfaces, although statistical significance was not achieved (independent samples t-test, $p>0.05$).

Table 2 – Absolute taxon richness (total number of different taxa) on the wrecks' hulls and on nearby rocky surfaces.

Site	Hull	Rock
Um el Faroud	4	2
MV Karwela	7	2
P29	5	4

Table 3 – Mean Simpson's diversity index (± standard deviation) of the wrecks' hull and nearby rocky surfaces.

Site	Hull	Rock
Um el Faroud	0.55 ± 0.10	0.48 ± 0.02
MV Karwela	0.45 ± 0.09	0.38 ± 0.09
P29	0.43 ± 0.13	0.47 ± 0.04

Table 4 – Taxa collected form the hulls of the three wrecks and from adjacent natural hard substrata.

	Um el Faroud		MV Karwela		P29	
	Wreck Hull	Natural Substratum	Wreck Hull	Natural Substratum	Wreck Hull	Natural Substratum
Dasycladus vermicularis	x					
Gigartinales	x					
Ceramium sp.	x		x		x	
Turf	x	x	x	x	x	x
Cyanobacteria			x			
***Sphacelaria* sp.**			x			
Encrusting Coralline Algae			x			
Sporochnales			x			
Porifera			x		x	x
***Sargassum* sp.**					x	
***Cladophora* sp.**					x	
***Cystoseira* sp.**		x		x		x
***Titanoderma* sp.**				x		
Rhodymeniales				x		x
***Ulva* sp.**				x		

Functional group categorization (Encrusting, Turf, Filamentous, Short Arborescent, Tall Arborescent epibiota) was employed to explore succession on the wrecks (Figure 1). Turf and filamentous algae were ubiquitously present on the wrecks. Encrusting organisms were negligible on P29 and Um el Faroud, while the MV Karwela had a significant abundance of this functional group (Wilcoxon test, $p<0.05$) compared to the other wrecks. Turf cover showed no significant difference between wrecks (Kruskal Wallis test $p>0.05$), with MV Karwela having the highest value. Filamentous algal cover also showed no statistical difference between wrecks (Kruskal Wallis test, $p>0.05$). Short arborescent epibiota occurred solely on Um el Faroud, while tall arborescent algae were unique to P29, obviating statistical tests. Figure 1 also shows an evident difference between the cover area of the different functional groups on the wrecks and the respective nearby rocky surfaces. From Figure 1 it is also evident that the three rocky substrata had very similar cover of epibiont functional groups.

An NMDS ordination, based on functional group cover, was generated to evaluate similarity between wrecks (Figure 2).

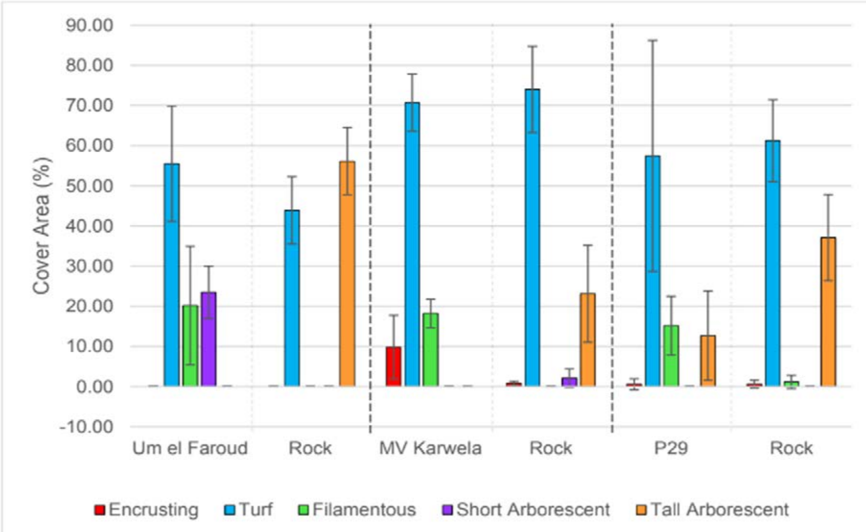

Figure 1 – Bar graph of Cover Area (%) of epibiont functional groups on the surface of the wrecks' hulls and nearby rocky surfaces. Error bars represent ±1 standard deviation.

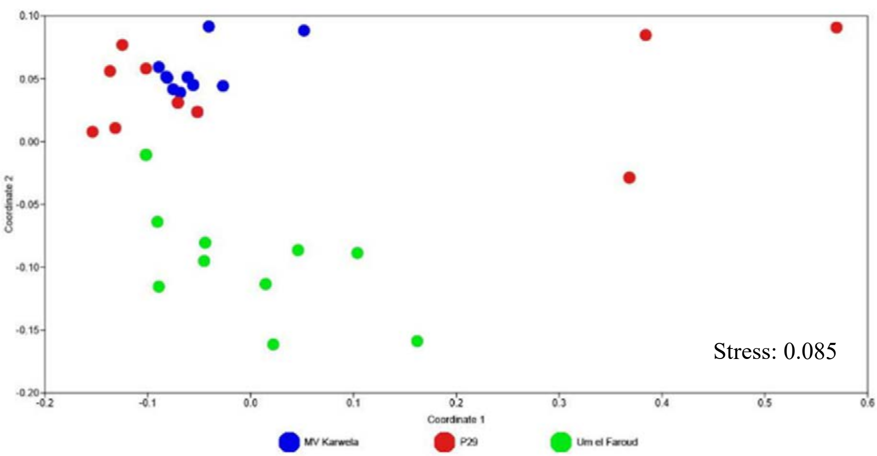

Figure 2 – Non-metric Multidimensional Scaling (NMDS) ordination diagram based on functional groups cover area (cm^2) in the photoquadrats sampled from the three wrecks.

Despite taxon differences, there is slight overlap in functional groups coverage between MV Karwela and P29. This implies greater taxonomic than functional group disparity among wrecks. While Um el Faroud does not overlap with the other wrecks, the photoquadrats samples for this wreck display a more dispersed distribution, indicating greater functional group variability compared to P29 and MV Karwela. Three photoquadrats, on the top right corner of the figure, are well separated from the rest. These three photoquadrats had a higher amount of bare surface area, a lower abundance of turf, and a slightly higher cover area of tall arborescent algae, compared to the other P29 photoquadrats. These three photoquadrats were collected from the lower region of the P29 hull sampling area, which may explain the observed distinctions [25]. Different parts of a wreck superstructure may be subject to different conditions and, as consequence, have different epibiotic assemblages.

Discussion

The observed pattern of colonisation by epibiota on wreck surfaces supports the hypothesis that the ecological succession on these surfaces reaches a specific stage but does not progress to a climax due to flaking off. Such a situation can be described as a "disclimax," resulting from the continuous disturbance caused by the conversion of metal surfaces into rust, leading to the shedding of old surfaces and the exposure of new metal substratum [11][17]. As a result, overall, the hull is characterised by a mosaic of bare patches and others with biota representing various stages of succession [9]. Bare surfaces were observed on all wrecks, consisting of rust on Um el Faroud and MV Karwela, and more extensive uncolonized areas, primarily painted surface, on P29. The presence of bare surfaces suggests frequent ecological succession interruptions and restarts.

Comparing the communities on the wreck hulls to nearby natural rocky surfaces provides supports the existence of a disclimax community and a dynamic situation rather than stability. Taxon richness on wrecks exceeded that on rocks, reflecting the mosaic of dissimilar assemblages in different stages of progression. Simpson's diversity index, reflecting the number and abundance of taxa, was significantly higher on Um el Faroud than the nearby natural surface, suggesting a developmental stage rather than a terminal assemblage. Functional group analysis revealed distinctions between wrecks and rocks, although turfs were dominant on both. Despite taxonomic dissimilarity, there was slight overlap in functional groups between MV Karwela and P29 as shown by the Non-metric Multidimensional Scaling analyses. However, Um el Faroud was clearly distinct from the other two wrecks, in terms of functional groups. Overall, there was a higher taxonomic dissimilarity than functional group dissimilarity among wrecks. These results suggest a truncated ecological succession on wreck surfaces, with each wreck exhibiting unique characteristics and developmental patterns.

Conclusions

The presence of uncolonized areas on wreck surfaces, even after prolonged immersion, demonstrates repeated surface sloughing and subsequent re-colonization. Mosaic colonization patterns were evident on all three wrecks, distinct from those on natural substrata, indicating a disclimax community system on each wreck. Variations in epibiotic functional group coverage between wrecks may result from geographic location, hydrodynamics, depth, and other factors, requiring further exploration. Notably, the Um el Faroud exhibited higher short arborescent epibiota cover, the MV Karwela featured greater encrusting epibiont presence, and only the

P29 hosted tall arborescent species. Succession on wrecks is a dynamic process and there is no long-term stability.

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