ANALYSIS OF THE AMPHIPOD SYNTAXON ON HARD BOTTOMS ANTI-TRAWLING STRUCTURES

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Abstract: This work is part of an environmental monitoring project conducted by Ispra since 2008, with the installation of two marine cables between the Lazio coast and Sardinia. Following the deployment of anti-trawl tripods, since 2017, a new Ispra monitoring plan was established to assess the integrity of the marine environment. The anti-trawl tripods have been periodically analyzed to examine various aspects of the marine area where they have been installed, including any changes in faunal and algal biodiversity resulting from the introduction of artificial structures. This study involves observing recruitment on the anti-trawl barriers to assess the colonization of artificial substrates introduced into the marine environment.

Keywords: Crustacea Amphipoda; sea environmental monitoring; recruitment; hard-bottom; Sardinia Sea

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Introduction

The high demand for energy for domestic use has resulted in the increasing of human infrastructure such as electricity cables. These structures have been laid during last decades both on Italian onshore, both on Italian off-shore, on the seabed. Often it had been necessary to lay these cables between the Italian Continent and the major Italian Island such Sardinia. The realization of such structures could generate different impacts both on the water column and on the sea bottom. Up to now several studies have proved that offshore activities can induce changes in the characteristics of sediment regarding these off-shore structures. In particular, the presence of these constructions might have some sort of impact on benthic communities inhabiting the surrounding seabed. The variations in sediment physical features (e.g. sediment grain-size, sedimentation rates) might determine qualitative and quantitative changes in the structure of soft-bottom benthic communities living immediately around the installations. [1; 3; 4; 7; 8; 9; 10; 11; 12; 13].

Our work is a part of a broader environmental monitoring initiative conducted by the Institute for Environmental Protection and Research (ISPRA) within the SAPEI Project, implemented by Terna S.p.A. with the installation of two marine cables between the Lazio coast and Sardinia. During inspections conducted at various points along the cables, damage attributable to anthropogenic actions, likely illegal bottom trawling activities, was observed. Then, to safeguard the connection, protecting the cables from potential illicit activities while simultaneously promoting the protection of existing ecosystems, since 2017 tripods, artificial anti-trawling barriers, were introduced. Following the deployment of anti-trawl tripods, a sub-monitoring plan was established always by Ispra to assess the integrity of the marine environment. The anti-trawl tripods have been periodically analyzed to examine various aspects of the marine area where they have been installed, including any changes in faunal and algal biodiversity resulting from the introduction of artificial structures. This study involves observing recruitment on the anti-trawl barriers to assess the colonization of artificial substrates introduced into the marine environment. In general, the complete benthic community structure was investigated, in order to have a biological lecture of the marine ecosystem. These assemblages were studied since 2018, and then up to October 2022. In particular, here we present the amphipod species sampled in eight stations. univariate and multivariate analyses were conducted in order to assess both the qualitative and quantitative structure of the syntaxon amphipoda assemblages. the work has focused on amphipod syntaxon as a partial descriptor of the community and, therefore, a tool for assessing the current state of the ecosystem. it was very interesting from an ecological point of view to observe that from the very beginning (2018) to 2022 there had been an evolution of community, in term both of number and species richness, also looking at the differentiation of niches and ecology of sampled species. Many of them showed an ecology both linked to hard bottom and to sandy soft-bottom. Some speciesmen are more linked to bottom more exposed to the light, while others to the dark side of tripods. Furthermore, elaborating Amphipoda structure with multivariate statistics, we can see that a partial differentiation began to exist among upper stations and down stations, indicating then the preference of different species and then to different speciesmen just described above, in relation to the light along the water column. These submerged artificial structures were analyzed seasonally to assess potential changes in the marine area where they were installed. Over the years, anti-trawling tripods have proven effective in reducing the impact of bottom trawling and promoting marine biodiversity. However, their success largely depends on their ability to attract and support colonization by marine species. This colonization process, known as recruitment, is crucial for the restoration of marine habitats and the establishment of resilient marine communities. Our work then focuses on observing recruitment on anti-trawling barriers to evaluate the colonization of artificial substrates introduced into the marine environment. In particular, the research involved the identification of amphipod species collected by the means of scuba diving, in eight selected samples. Amphipods emerge as one of the most diversified and fascinating groups of crustaceans inhabiting marine waters and can serve as a partial descriptor of the community, providing a tool to assess the current state of the ecosystem.

Material and methods

Sampling strategy, field and laboratory procedures, data elaboration

During the whole project the colonization of the artificial substrates released into the marine environment was analyzed through two methods: direct and indirect, during the various phases of succession of the reef population as indicated by the FAO guidelines for the monitoring of artificial reefs in the Mediterranean Sea [5].

In consideration of the different types of substrates and environments present in the reefs, a sampling plan was designed which envisages the study of the population in two bathymetric bands: SUPERFICIAL BAND (UP photophilous and DOWN (DW) sciaphilous)) between 10 and 15 m; and DEEP BAND, between 20 and 30 m (UP photophilous and DOWN (DW) sciaphilous)).

For each band, an artificial barrier (tripod) was chosen, selected on the basis of its representativeness of the average environmental conditions of the area in which it was positioned: for the superficial band 1 tripod (station) in Punta Tramontana (PT_2_F), and 1 tripod (station) in Fiume Santo (FS_6_C); for the deep band 1 tripod (station) in Punta Tramontana (PT_18_F) , and 1 tripod (station) in Fiume Santo (FS_61_C), for a total of 2 stations for area. Then, for each band 2 samples were taken for each station: 1 (UP) in a horizontal position on the tripod, and 1 (DW) in a vertical position on the tripod. Totally, then, in the Punta Tramontana area 1 station x 2 samples at the superficial band and 1 station x 2 samples at the deep band were performed, as well as in Fiume Santo site, for a total of 4 stations x 2 areas = 8 samples. for the deep band (Table 1).

Table 1 – Sampling Stations and Samples in Punta Tramontana and Fiume Santo areas.

SAMPLING STATIONS	
SUPERFICIAL BAND	**DEEP BAND**
1 horizontal station:	**1 horizontal station:**
PT_2_F_UP_1_23	**PT_18_F_UP_3_23**
1 vertical station:	**1 vertical station:**
PT_2_F_DW_2_23	**PT_18_F_DW_4_23**
1 horizontal station:	**1 horizontal station:**
FS_6C_UP_5_23	**FS_61_C_UP_7_23**
1 vertical station:	**1 vertical station:**
FS_6C_DW_6_23	**FS_61_C_DW_8_23**

The underwater activities for the direct sampling were performed by an OTS (*Operatore Tecnico Subacqueo*, that is *Underwater Technical Operator*) Researcher from ISPRA supported by OTS from the Oceansub company contracted specifically for this activity. In each station, the ISPRA underwater researcher took steps to directly collect the biological samples by completely manual grating of all the material adhering to the surface of the reef, collected using an air operated suction suppler of a surface enclosed in a 20 x 20 cm square, operated by an OTS diver from Oceansub. All samples were stored in 80% denatured alcohol solution. Here we do not present materials and methods used for the indirect sampling, since we decide to analyze only the portion of benthic community sampled by the means of direct method.

Qualitative and quantitative analysis of macrozoobenthic communities has been carried out in the laboratory [6], and all individuals were classified and taxonomically recognized, down to the lowest possible taxonomic level, i.e. the species. Amphipoda were all separated from all other taxa. The structure of the Amphipoda community was assessed by considering both species and individual organisms to characterize the biocenotic traits of the study area. Subsequently, we subjected the collected data to statistical analysis to gain deeper insights into the population structure and to detect any spatial and temporal variations. Univariate analyses were performed: different structural parameters and ecological indices were employed: Total abundance N, Total species richness S, the Shannon-Wiener

Specific Diversity Index H'; Pielou index J. Abundance data underwent analysis using advanced multivariate statistical methods. A multivariate assessment was carried out based on quantitative matrices featuring "taxa x stations". This involved the application of non-metric Multidimensional Scaling (nMDS) and Cluster analysis utilizing the average linkage algorithm. These analyses relied on the Bray-Curtis similarity index and were conducted using Primer 6.1.6 software [2].

Results and Discussions

Here we present results regarding only Amphipoda sampled by the direct method.

Qualitative-quantitative analysis

The analysis of the qualitative and quantitative composition, including the number of species and their abundances, within the eight stations under consideration, reveals the following patterns: the number of species appears similar between the stations of *Punta Tramontana* and those of *Fiume Santo*, with peaks detected in station FS_61_C_DW_8_23 followed by station PT_18_F_DW_4_23, while the lowest values were observed in stations FS_6C_DW_6_23 and FS_6C_UP_5_23 (both sciaphilous and photophilous). As regards abundance, it is possible to observe a greater variation between the stations considered, with the largest peak being observed in the FS_61_C_DW_8_23 station while the smallest one was observed at the PT_18_F_UP_3_23 station (Fig. 1).

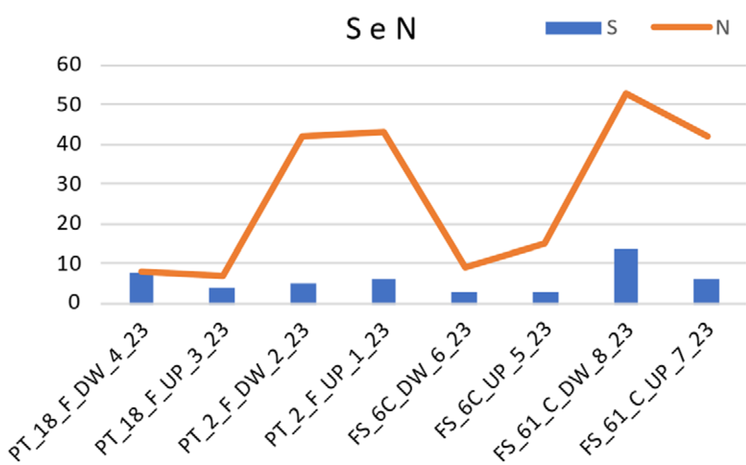

Figure 1 – Qualitative-quantitative amphipods composition in the 8 studied stations. S = Number of Species; N = Number of Individuals.

Structural indices

Regarding species richness, for the *Punta Tramontana* stations the highest value was found at station PT_18_F_DW_4_23, followed by station PT_2_F_UP_1_23, and then station PT_2_F_DW_2_23. The lowest value was identified at station PT_18_F_UP_3_23. For the Fiume Santo stations the species richness values are similar for stations FS_6C_UP_5_23 and FS_6C_DW_6_23, but increases slightly for station FS_61_C_UP_7_23. The highest value ever, among all the eight stations considered, was found in station FS_61_C_DW_8_23. The Evenness Index shows a similarity between the stations of the same reef. In particular, in the PT_2_F_UP_1_23 and PT_2_F_DW_2_23 stations (both sciaphilous and photophilous), a high Evenness Index is noticeable, with the maximum value reached at station PT_2_F_UP_1_23. The stations of reef 18_F (PT_18_F_UP_3_23 and PT_18_F_DW_4_23), on the other hand, exhibit significantly lower Evenness Index values, especially concerning station (PT_18_F_UP_3_23.

Regarding *Fiume Santo* stations the smallest values were detected in stations FS_6_C_DW_6_23 and FS_6_C_UP_5_23. The evenness index instead reaches high values in stations FS_61_C_DW_8_23 and FS_61_C_UP_7_23. The Margalef's species richness index is quite similar among the stations PT_18_F_UP_3_23, PT_2_F_DW_2_23, and PT_2_F_UP_1_23. A more diversified value for this index was instead found for station PT_18_F_DW_4_23, where the highest value of Margalef's species richness index was identified. The Margalef index is substantially very similar in the Fiume Santo stations, with a slightly higher value for the FS_61_C_DW_8_23 station. Regarding the Pielou index, the values are very similar between the counterparts (sciaphilous and photophilous) of the two reefs. The index shows similar values between the Punta Tramontana and Fiume Santo stations. There are no particular variations in this value even between the two 6_C (FS_6_C_DW_6_23 and FS_6_C_UP_5_23) stations and the two 61_C (FS_61_C_DW_8_23 and FS_61_C_UP_7_23) stations.

The Shannon-Wiener Specific Diversity Index in Punta Tramontana showed very similar values between the stations PT_2_F_UP_1_23 and PT_2_F_DW_2_23, although it is slightly higher in station PT_2_F_UP_1_23, while the highest Shannon-Wiener Specific Diversity Index in this area was found in station PT_18_F_DW_4_23. The lowest value among Fiume Santo stations was detected in the FS_6_C_UP_5_23 station, while the highest in the FS_61_C_DW_8_23 station. As for the Simpson's Diversity Index, in Punta Tramontana, its value is very similar between the shaded and well-lit stations of the same reef. Even for the Fiume Santo stations there are no particular differences in the value of this index (Fig. 2).

The nMDS analysis based on the abundance data showed the presence of 2 main groups. The dendrogram in Figure 3 reveals a similarity of approximately 62 % between the PT_18_F_UP_3_23 and FS_6_C_DW_6_23 stations. The station that shows the greatest dissimilarity with all the other stations is FS_6_C_UP_5_23.

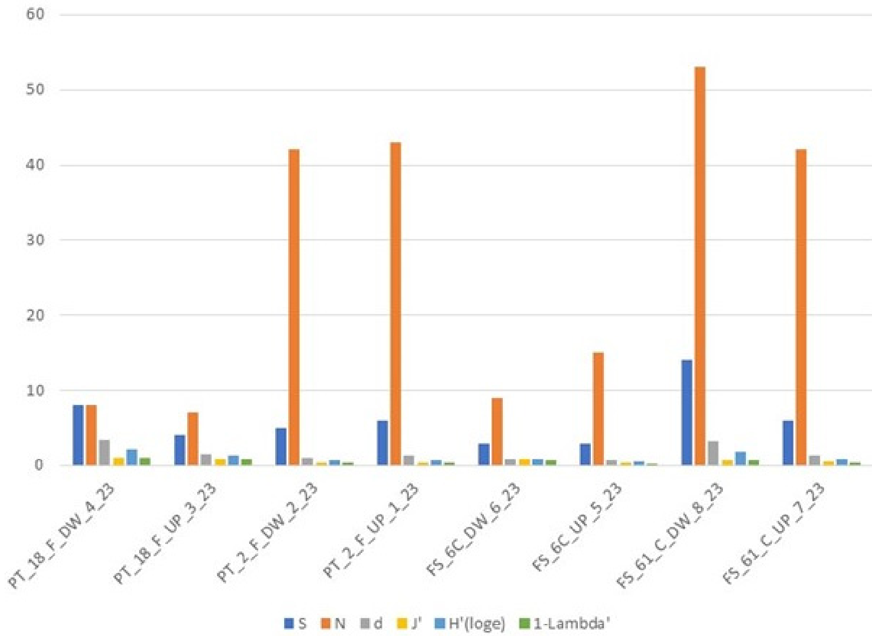

Figure 2 – Comparison of structural indices in the 8 studied stations. S = Number of Species; N = Number of Individuals; d = Margalef Specific Richness; J’ = Pielou Equitability; H’ = Shannon Diversity; 1 – lambda = Simpson Diversity.

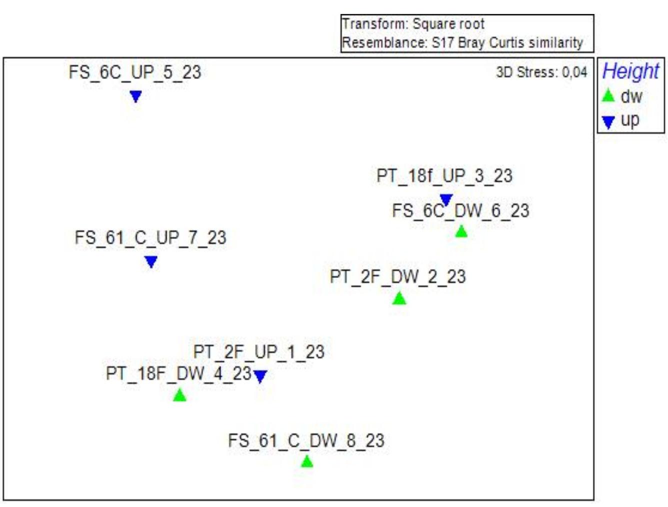

Figure 3 – nMDS *scaling* analysis in the 4 stations (8 samples). Stations were differentiated using the *factor* of *height* (depth).

Conclusions

Totally Amphipods emerge as one of the most diversified and fascinating groups of crustaceans inhabiting marine waters and can serve as a partial descriptor of the community, providing a tool to assess the current state of the ecosystem.

The data obtained by the sample analysed confirm findings from similar studies conducted in other coastal areas and highlight the challenges in predicting the processes and timing of colonization by macrobenthic organisms.

Moreover, the data obtained indicate a well-established state of colonization of the anti-trawl barriers, progressing towards a climax stage. The vagile fauna primarily consists of small-sized organisms that inhabit the algal mats. Changes in the abundance and composition of amphipod species over time on the artificial reef provide valuable and essential insights into the ecology of these species and their intra/interspecific relationships.

This work has confirmed what has already been demonstrated by similar studies regarding the utility of anti-trawl barriers as artificial habitats, attracting a wide range of organisms, from small invertebrates to fish, and therefore have positive effects on fish repopulation and biodiversity. Tripods were indeed introduced as invasive structures designed to prevent the passage of trawl fishing gear on the seafloor, thus protecting the habitats and species living there. Monitoring activities remain highly relevant because the evolution of the colonizing population needs to be studied and evaluated over time.

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