Joint European Research Infrastructure network for Coastal Observatories

Report on JERICO Biofouling Monitoring Program (BMP)

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Involved Institutions: **CNR-ISMAR**, HCMR, AZTI, IFREMER, CEFAS, SMHI, SYKE

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History			
Revision	Date	Modification	Author
V1	april.2015	Document received,	M. Faimali
V1.1	Jan. 2016	Formatting + summary	I. Puillar

1. Executive Summary

The main aim for this document is to report after the Biofouling Monitoring Program (BMP) aiming at identifying major organisms responsible for biofouling in different geographical areas, as an input to the development and application of a more suitable approach to any specific region. This report is strongly linked to the JERICO deliverable D4.3 "Report on Biofouling Prevention Methods", available at http://www.jerico-fp7.eu/deliverables/d4-3-report-on-biofouling-prevention-methods.

2. Selected partners and monitoring sites

The following partners gave their available for participating to JERICO BMP:

N°	Partners	Reference person	e-mail	Coastal Water	Open Water
1	ISMAR	Marco Faimali	marco.faimali@ismar.cnr.it	x	x
2	HCMR	Manolis Ntoumas	mntou@hcmr.gr	x	x
3	AZTI	Carlos Hernandez	chernandez@azti.es	x	x
4	IFREMER	Laurent Delauney	laurent.delauney@ifremer.fr	x	x
5	CEFAS	Dave Sivyer	dave.sivyer@cefas.co.uk	x	x
6	SMHI	Bengt Karlson	Bengt.Karlson@smhi.se	x	
7	SYKE	Jukka Seppala	jukka.seppala@ymparisto.fi	x	

The following map (Figure 1) represents the distribution of 11 different monitoring sites (open water and coastal water) along a European geographical gradient.

Figure 1: Sampling map

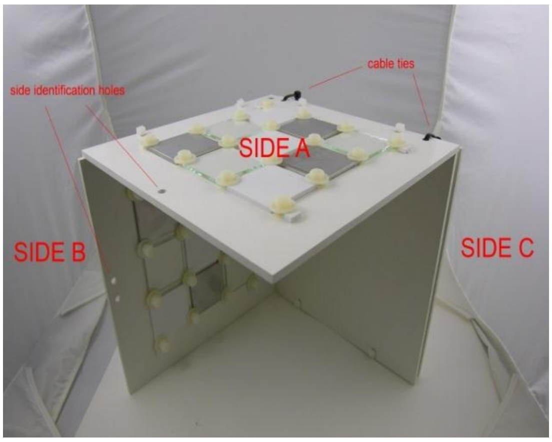
Biofouling Monitoring Box - BMB

ISMAR-CNR has developed a special sampling system (Biofouling Monitoring Box - BMB) for each partner and each sampling site. The sampling system has been delivered at the begin of June 2013 to each BMP partner.

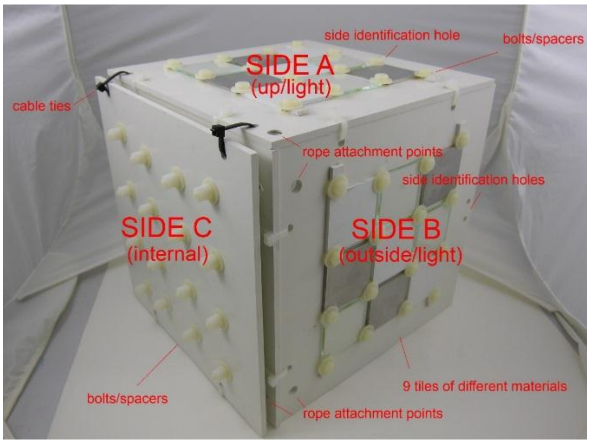
The BMB is a monitoring device specifically designed to provide substrates with spatial and structural heterogeneity that can simulate the complexity of the sensors and sensor housing/containers.

Each partner was asked to immerse the system (BMB) close to a particular sensor, selected as the reference sensor, for this long-term study.

Pictures describing the BMB (panels 25x25 cm and tiles 6x6 cm) are reported below (Figures 2-3).



a



B

Figure 2: Overview of the biofouling monitoring box (A-front); (B-back)

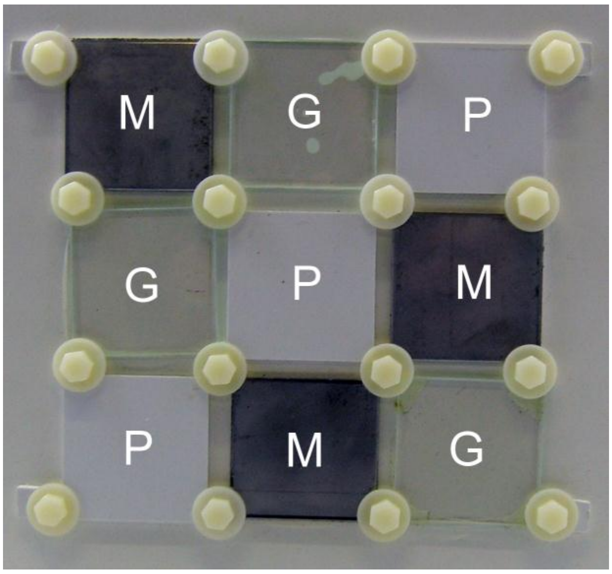


Figure 3: Latin square with 9 tiles of different materials (M = Metal; G = Glass; P = Plastic)

The BMB is composed of:

- a horizontal side (A) in which a series of 6 tiles of different materials (Plastic-Metal-Glass) are positioned on both sides (side exposed to light and to the dark).
- a vertical side (B) with the same series of 6 tiles of different materials on both sides (side exposed to light and to the dark)
- a second vertical side (C) composed of two panels held together by cable ties, where a series of 6 tiles of different materials is inserted (in order to simulate an interstitial environment in dark condition).

On each side, bolts are used as spacers to easily take pictures of all sides avoiding the potential damaging of the corresponding opposite side.

Each partner had to photograph the different parts of the BMB and, simultaneously, the different parts of reference sensor every month, if possible, or at least every 2 months for the coastal monitoring stations and whenever possible for open ocean sites, by following the instructions below. After 6-12 months, each partner was expected to ship the BMBs to ISMAR laboratory, after appropriate conservation treatment.

3. Materials and methods

Protocol for photographic sampling

Once collected the immersed structures from the dive site (BMB and/or reference sensor), some shots of the complete structure of the BMB and of the sensor were taken, in order to provide a general overview of them. Each partner was expected to photograph monthly (or when possible) all the components of the BMB. Photos had to be taken starting from SIDE A (Up-Down); SIDE B (Up-Down) and SIDE C. Pictures had to be taken in *macro* format with a quality high enough to obtain cropped images of each tile of 1000×1000 pixels minimum (the camera quality had to be 8MP minimum).

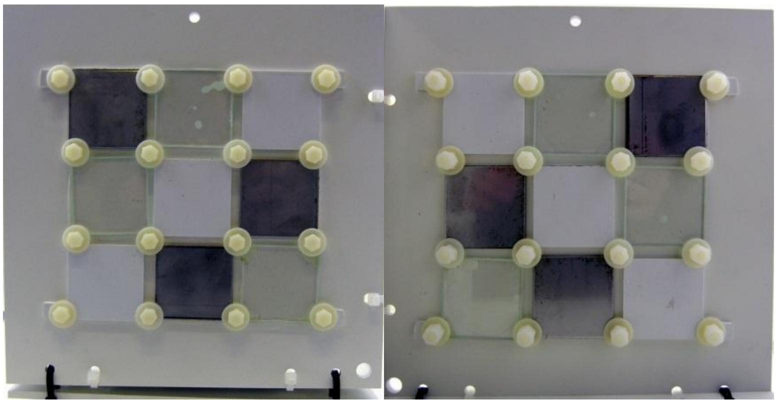


Figure 4: Left: Side A up/light (1 hole on the top, 2 holes on the right), right: side A down/dark (1 hole on the top, 2 holes on the left)

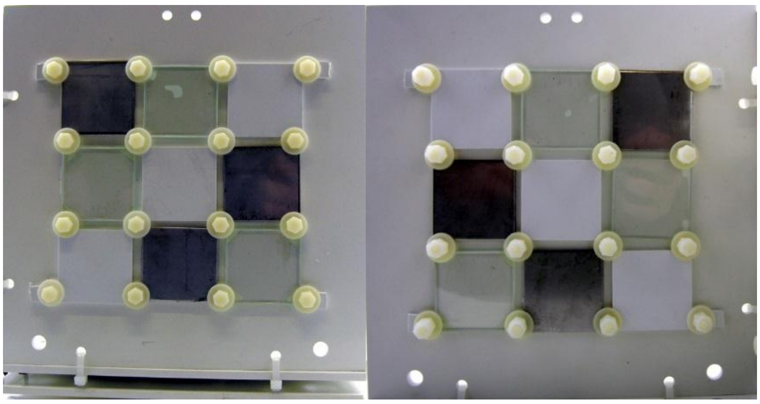


Figure 5: Left: side B outside/light (2 holes on the top, 2 holes on the left); right: side B inside/dark (2 holes on the top, 2 hole on the right)

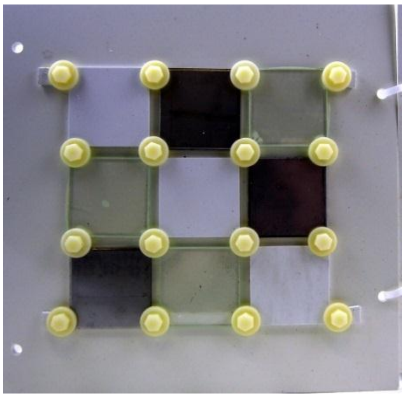


Figure 6: Side C internal

Photo analysis through Photogrid software

The fouling coverage had to be monitored monthly for one year.

Biofouling coverage of each tile and sides was estimated by Photogrid software (Bird, 2003).

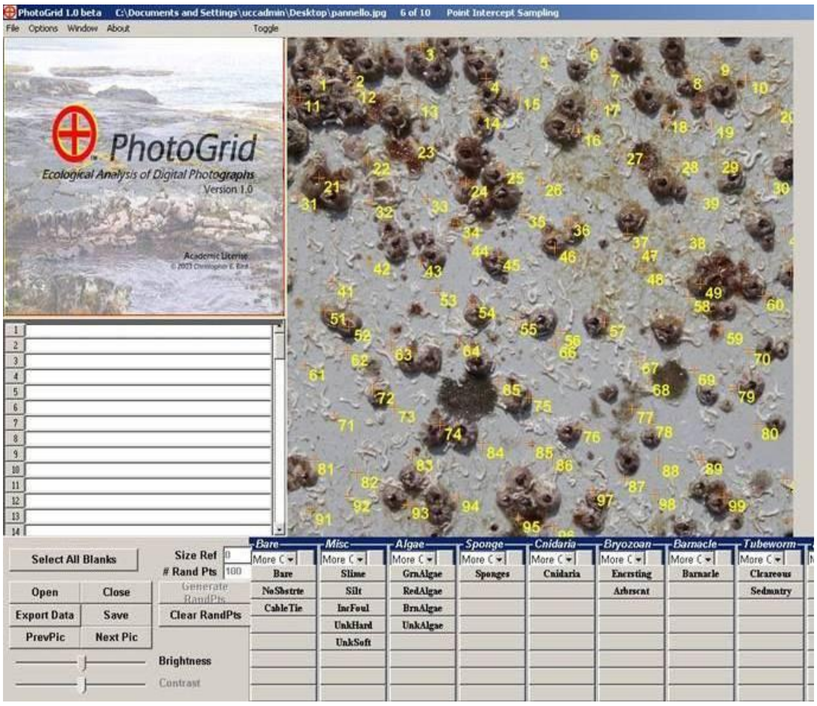


Figure 7: Estimation of biofouling coverage by Photogrid software

The software creates 100 random points on a digital picture and allows zooming in/out to recognize the main taxonomic groups of biofouling assemblages on a surface (Figure 7). The output of the software is "total coverage" and percentage coverage for each identified taxonomic group. The main taxonomic groups can be modified and adapted to the local representative species of the considered geographic location.

After 12 months, the BMB was expected to be collected and the fouled surface had to be analysed by standard microscopy techniques and a modified Dethier method (Dethier et al. 1993). The analysis was carried out placing a small grid (20×20 cm) on the sample estimating, in a fixed number of random squares inside the grid, the presence and abundance of each taxa (representative groups of biofouling assemblages). Each randomly selected square was scored for each identified taxonomic group as follows: 0 absence, 1 if the taxon is covering 1/4 of the square surface, 2 if the taxon is covering 1/2 of the square surface, 3 if the taxon is covering 3/4 of the square surface, 4 if the taxon is covering the whole square surface.

Figure 8: example of biofouling coverage on a tile (side A, light, metal, after 3 month of immersion in Genoa)

BMB photos available for Photogrid identification:

1. **Genoa** : every month
2. **IFREMER**: 3-6-12 month (Scuba photo: 3 month)
3. **HCMR** (Greece): 3-6 month
4. **Venice**: 2-3 month (no Side A: lost!)

BMB photos analysed

1. **Genoa:** 1-3-12 month
2. **IFREMER:** 3 month

3. **HMRC:** 3 month
4. **Venice:** 3 month

BMB system available for Dethier analyses at the end of experiment:

1. **Genoa**
2. **IFREMER**

Other partners:

AZTI: problem with BMBs deployment; after deployment, BMBs destroyed by storms.

CEFAS: BMB lost/destroyed by storms.

SMHI: problems with BMB deployment; after deployment, BMB completely covered by mussels.

SYKE: BMB deployed, but no data received.

4. Results

In the first section, results related to photo analysis with Photogrid software of the BMB system of Genoa are discussed. We report results relative to 1, 3, 12 month of exposure in coastal water.

<u>ISMAR-Ge</u>

<u>Genoa (Italy) – 1st month</u>

After 1 month of exposure, metal and plastic panels were mainly covered by hard fouling, both in side A and B, more at dark exposure than at light. Soft fouling was the main component of glass panels, except for dark condition, where hard fouling reached a 60 % of coverage. This evidence underlines that the condition of light plays a key role in the composition of the fouling community.

Hard fouling community was mainly represented by calcareous tubeworms in metal and plastic panels, while barnacles preferred glass panels. Such data confirm that the substrate material has a strong influence on the composition of the community.

Looking at side C, most of the surface area was bare. Metal panel confirmed to be the material with the highest percentage of hard fouling. Hard fouling was represented by calcareous tubeworms that find their ideal settlement location.

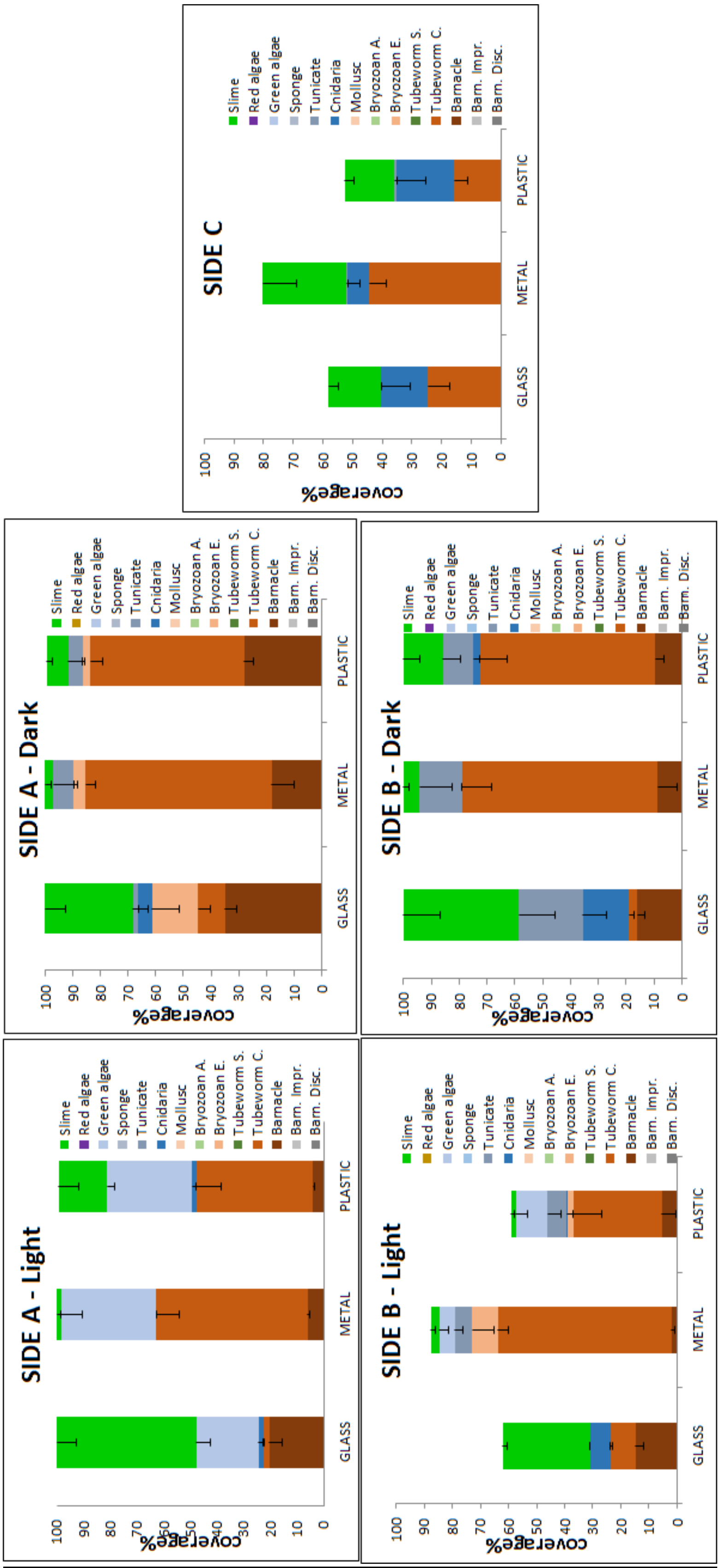


Figure 9: coverage percentages of fouling communities after 1 month of exposure in Genoa (M±es) (n=3)

Genoa (Italy) – 3rd month

Looking at Side A and B, for all the surfaces hard fouling represented the main component of the fouling community (metal > plastic > glass), in any condition of light/dark and type of exposure (side A-B).

Barnacles and calcareous tubeworms represented the main organisms of hard fouling.

Barnacles settled preferentially on the dark side, while calcareous tubeworms preferred light exposure.

Barnacles settled indifferently on metal, plastic and glass surfaces, while calcareous tubeworms preferred metal surfaces. In this case, both the light conditions and the materials had a key role in the composition of the fouling community.

A low percentage of space was occupied by slime, more on side B rather than on side A.

Side C was covered with higher percentages of slime on all the materials employed. Hard fouling was represented mainly by calcareous tubeworms, which were also observed to be abundant on metal surfaces.

At the 8th month of exposure, barnacles of Side A-Dark detached from the glass panels. At the 11 month of exposure (reported in Figure 11), barnacles of side A-dark detached from a large area of the frame.

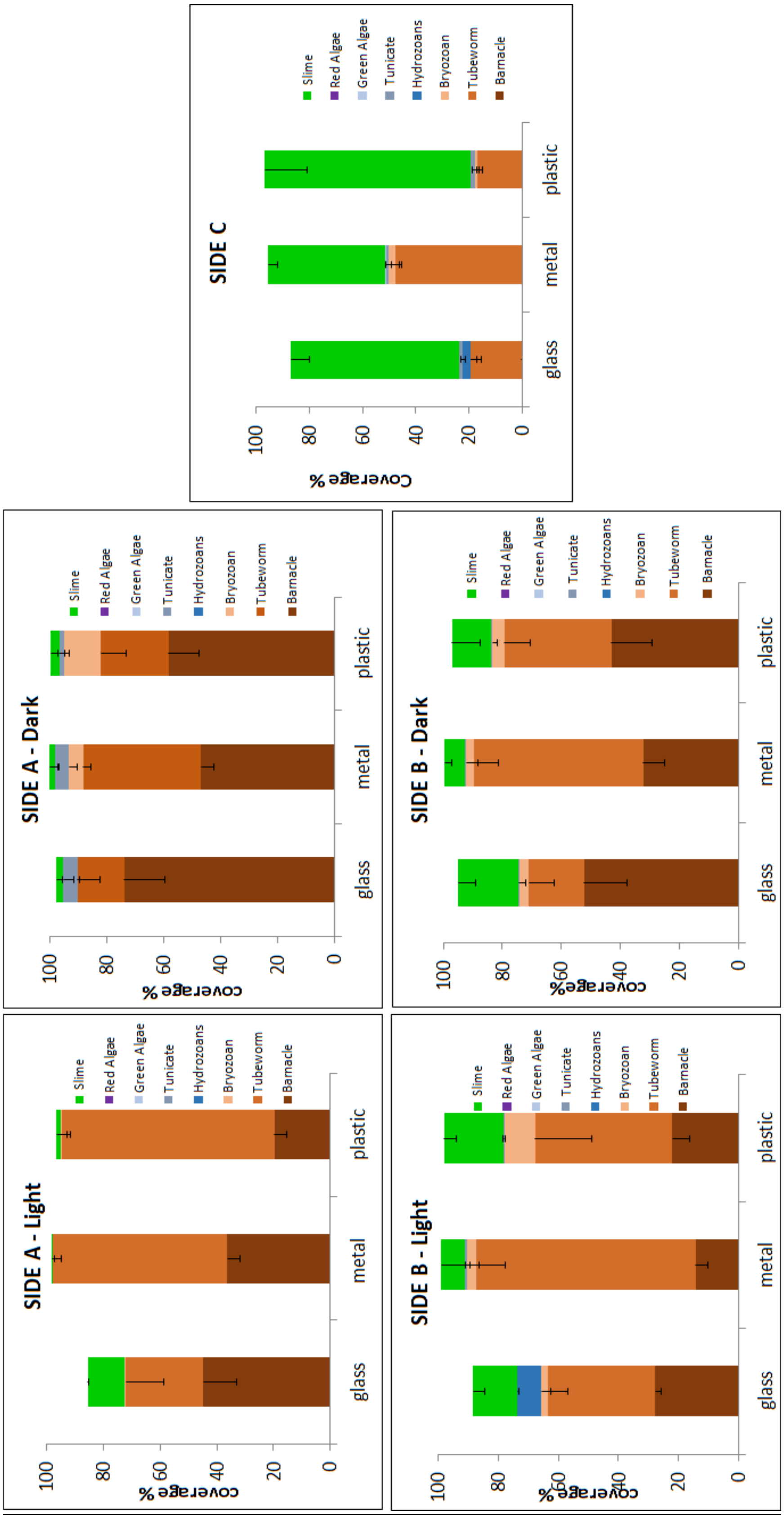

SIDE C
SIDE A - Dark
SIDE B - Dark
SIDE A - Light
SIDE B - Light
Coverage %
100
80
60
40
20
0
glass
metal
plastic
Slime
Red Algae
Green Algae
Tunicate
Hydrozoans
Bryozoan
Tubeworm
Barnacle

Figure 10: coverage percentages of fouling communities after 3 months of exposure in Genoa (M±es) (n=3)

Genoa (Italy) – 12th month

Looking at Side A and B, for all the surfaces employed hard fouling represented again the main component of the fouling community, especially on metal panels. Nevertheless, compared to previous months, the coverage percentage of hard fouler changed and decreased. The percentage of barnacles decreased especially on glass panels.

As barnacle detached from the surfaces, the percentage of bryozoans increased, especially on the dark side; in enlightened conditions, red and green algae grew.

A quite stable composition of the community of Side c was observed, with higher percentages of slime on all the materials employed. Hard fouling was represented mainly by calcareous tubeworms, which were also observed to be abundant on metal surfaces, as it happened in previous months.

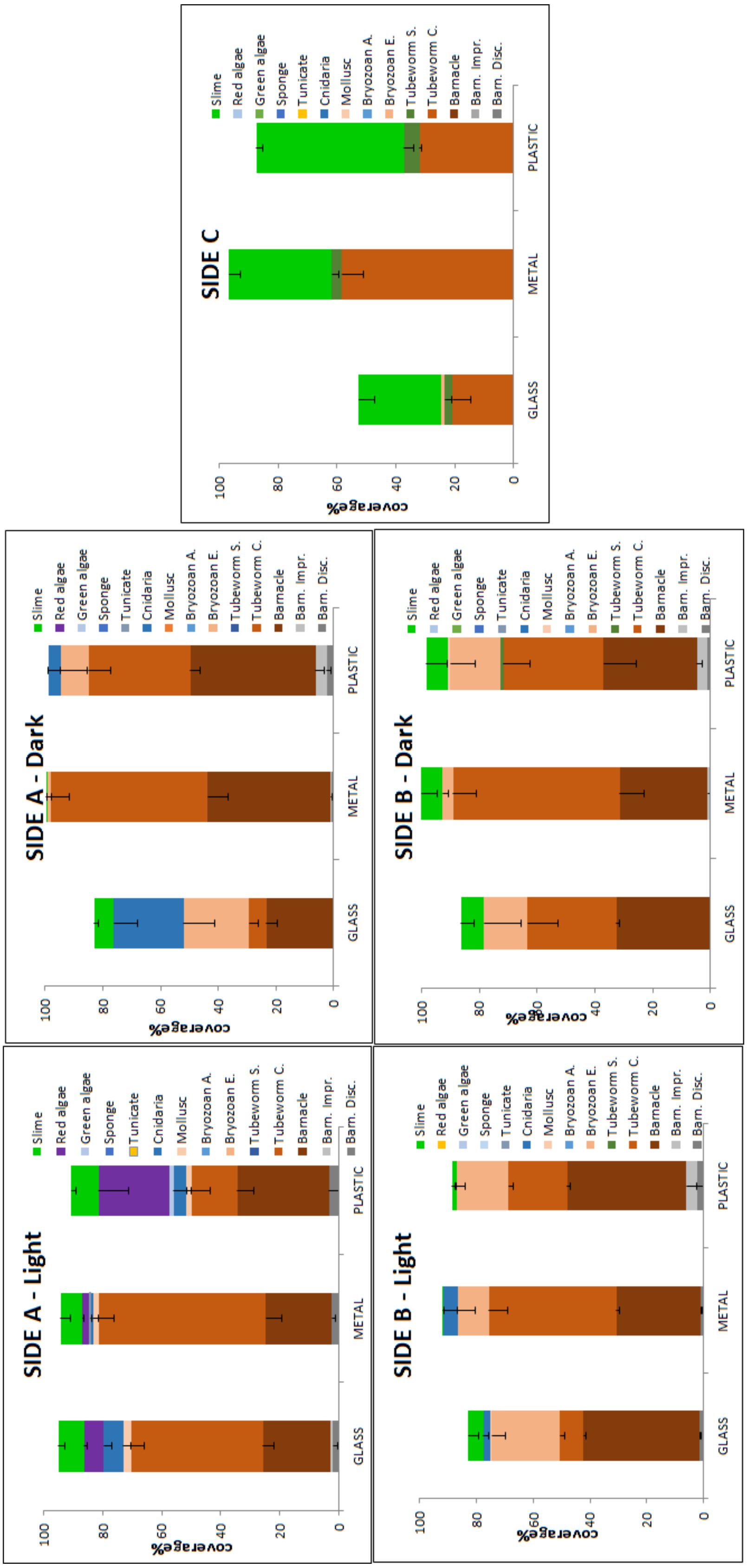


Figure 11: coverage percentages of fouling communities after 12 month of exposure in Genoa (M±es) (n=3)

In the following section the results related to photo analysis of the BMB system after 3 months of exposure are reported. Photos were available from the following partners: ISMAR (coastal and open water); IFREMER (coastal and open water) and HRMC (coastal water).

ISMAR-Ve

Venice (Italy) - open water

NOTE: NO SIDE A (detached, due to a storm event), in side B Light there is only 1 replicate for Metal and Plastic (photos not sharp)

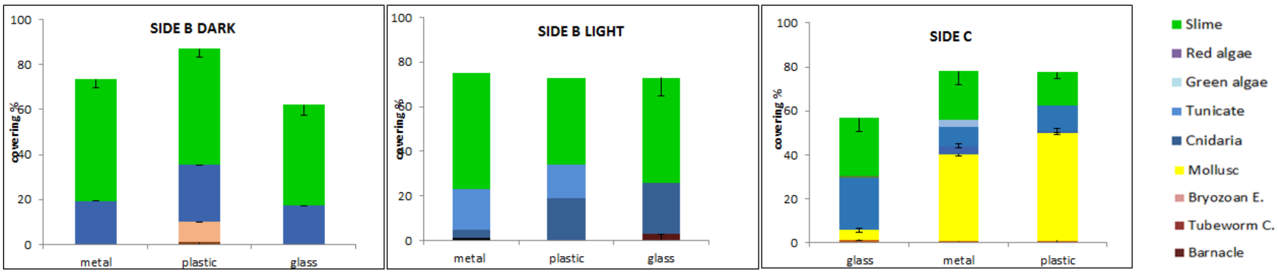


Figure 12: coverage percentages of fouling communities after 3 months of exposure in Venice (M±es) (n=3)

Hard fouling was less present on the substratum of side B, which was mainly covered by slime, while on plastic and glass surfaces of side C higher percentage of hard fouling were present (opposite conditions compared to Genoa); furthermore, all the materials tested in this site were largely bare (Fig. 12).

Hydrozoans were the main organisms of the soft fouling on side B, where hard fouling was absent; conversely, on metal and plastic surfaces of side C it was largely represented by limpets.

In all the conditions investigated (light /dark,side B- C), high percentages of surface area were completely bare.

HCMR (GREECE)- coastal water

The average values recorded in this sampling period (November 2013) were:

Salinity: 38.11 ppt

Temperature: 21.7 °C

Conductivity: 5.35 S/m

Pressure (depth) : 4.17

Data were recorded by the CTD sensor (http://www.seabird.com/sbe37im-microcat-CTD) that was installed next to the BMB at Souda bay

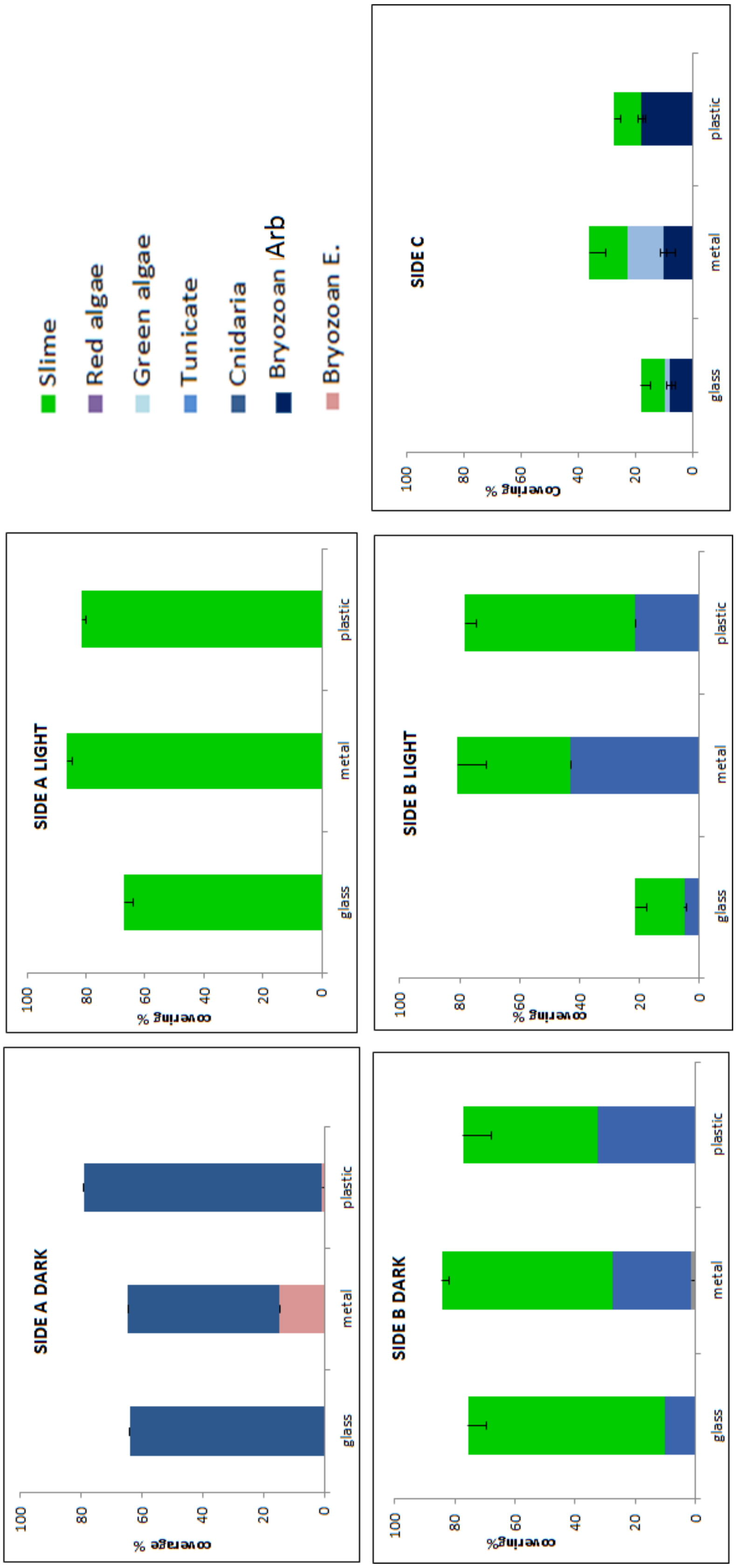


Figure 13: coverage percentages of fouling communities after 3 months of exposure (M±es) (n=3)

Hard fouling was less present on all the surfaces. Soft fouling was largely present on side A in dark conditions, it decreased on side B (both at light and dark condition) and it was not present at light exposure of side A, which was almost completely covered by slime. Side C was almost completely bare (>60%) (Fig. 13).
As stated above, hard fouling was absent, with the exception of metal (SIDE A-dark), where it was represented by encrusting bryozoans.
Soft fouling was mainly represented by hydrozoans. In dark and light conditions, plastic surfaces showed the highest percentages of hydrozoans, with respect to the other materials. Glass surfaces were the substratum with smallest coverage of hydrozoans.
Side C was largely bare, especially on glass surfaces, as previously observed in dark and light conditions for side A and side B. Furthermore, side C was poorly occupied by arborescent bryozoans.

IFREMER - coastal water

NOTE: photos not sharp.

Hard fouling was less represented, with the exception of a small percentage (<25%) on metal surfaces (side A) exposed to dark: here, all the surfaces were largely bare or partially covered by soft fouling; slime was present only at light exposure. Side C was largely bare.
On the panels of this BMB, a great variety of taxa (as detected for coastal water in Genoa) was represented, mainly belonging to soft fouling (differently from Genoa).
In **dark conditions,** barnacles were the main organisms of the hard fouling, which settled indifferently on all the surfaces (see Genoa); moreover, on metal and plastic surfaces there were encrusting bryozoans as hard foulers. The percentage of hard fouling was very small compared to Genoa, for both coastal sites.
Arborescent bryozoans, hydrozoans and tunicates (soft fouling) grew on all the surfaces.
In **light conditions,** the panels of side A were largely colonized by algae (red algae and green algae). Differently, on the panels exposed at light condition of side B soft fouling is mainly represented by cnidaria.
Both in light and in dark conditions, all the substrata presented high percentages of bare surface, as it happened on side C. The remaining area was covered by soft fouling (e.g. tunicates).

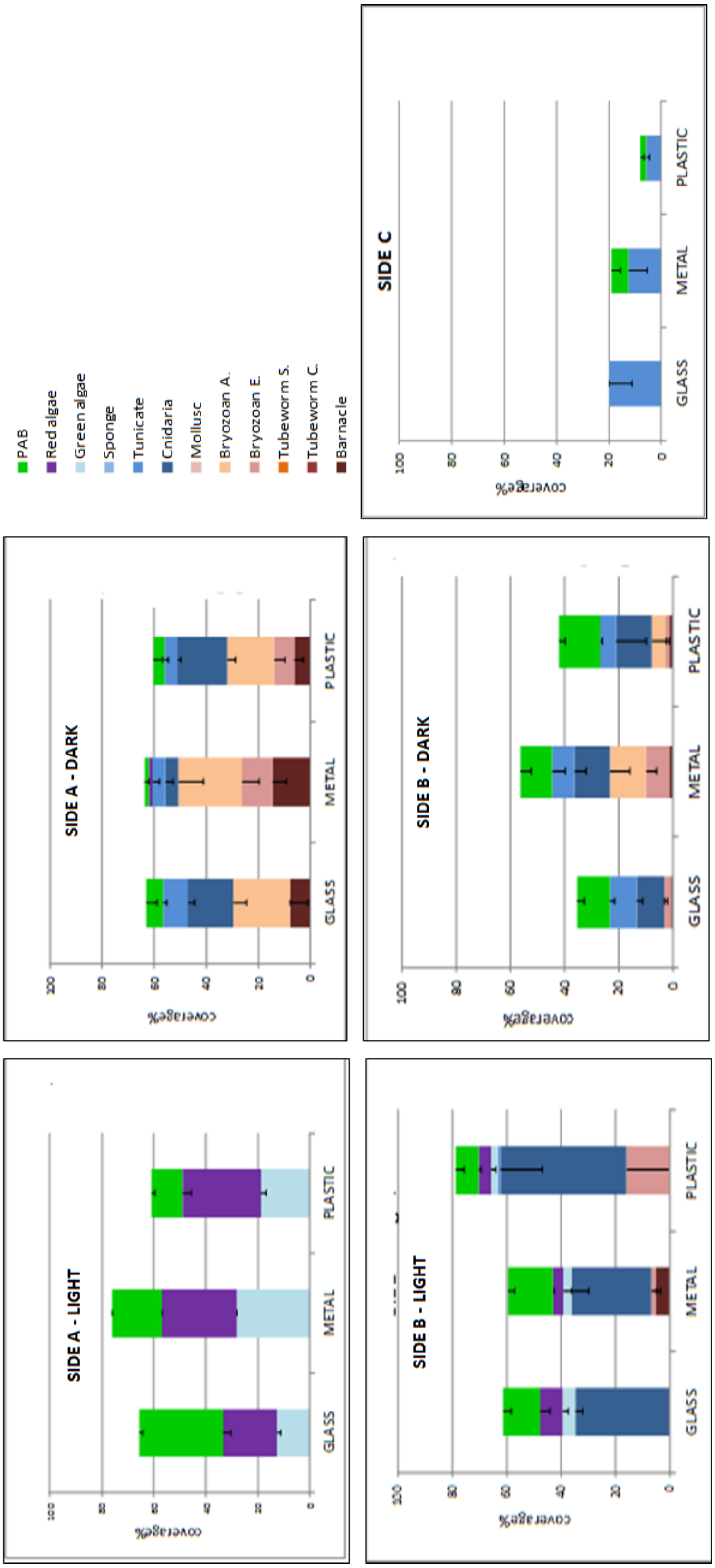


Figura 14: coverage percentages of fouling communities after 3 months of exposure (M±es) (n=3)

5. Discussion and Conclusion

Marine observing systems are essential to support the maritime economy, collect data about the marine environment and its changes. Along European coast, many centers for ocean observation follow a data processing chain involving sensors carried by fixed or mobile platforms for data collection. During the years, this allowed to get knowledge and details on the evolution of physical and biological parameters.

Data collected by BMB systems can contribute to detect and understand the most crucial biological process that affects marine technologies in different sites. Analyzing collected data, a significant heterogeneity in fouling community can be seen among the different sampling sites.

Indeed, as it was easily predictable, the different environmental parameters deeply influence larval settlement. Nevertheless, thanks to BMB preliminary experiment, we demonstrated the complexity and heterogeneity of fouling community, showing how it can be influenced by several parameters.

First, we considered which are the main factors that influence the fouling community in one site (Genoa) during 1 year of exposure, and how they influence such community. The factors that we focused on were: the materials employed (metal, plastic and glass), the spatial orientation of the panels (vertical plane, horizontal plane and interstitial plane), the exposure to light. Secondly, we compared the Genoa's results with the data obtained from other sites, in order to understand the differences and the similarities of all these interactions in the fouling processes.

From Genoa's experiment, after 1 month of exposure, it can be observed that the condition of light and the materials employed play a key role in the composition of the fouling community. Hard fouling was the main component of the panels exposed both in the horizontal and vertical planes. A different situation occurred in the interstitial space: here the panels presented lower percentage of hard fouling; most of the area was bare.

The metal panels were the substrate mainly colonized by hard foulers, while the glass panels the less colonized. Hard fouling were represented by barnacles and calcareous tubeworms. Barnacles attached preferably on glass panels and at dark exposure; calcareous tubeworms preferred metal surfaces and light condition.

Data collected after 3 months of exposure showed a similar trend to data collected after 1 month. It can be noted a higher percentage of hard fouling on all the substrata exposed in the horizontal and vertical plane. The panels exposed to dark condition were almost completely covered by hard fouling. At light condition, the glass panels were the materials with less percentage of area covered by hard fouling. The fouling composition of interstitial space remained stable.

At the end of the immersion period (12 months), the percentage of hard fouling decrease because of the detachment of barnacles that concluded their life cycle. The detachment was higher on glass panels. This allowed the growth of other organisms, such as bryozoans, red and green algae. As in the previous sampling time, the fouling community of the panels in the interstitial remained stable.

From Genoa's experiment, collected data highlights that the fouling community was strongly influenced by the exposure to light and substrate material.

The hard fouling community attached preferably on panels exposed to dark condition; the glass panels showed the best antifouling properties compared to other materials. Such result is in agreement with data from literature.

Furthermore, the stability of the community in the interstitial space within the months, in opposite with the heterogeneity of the community of side A and B, pointed out the importance of the orientation in the space of panels in the larval settlement.

With the second part of the experiments, the results allowed to underline differences in fouling community comparing different sites of exposure.

The results obtained from Venice's BMB showed that the panels positioned in the vertical plane were covered by soft fouling (cnidarians) and slime. Hard fouling settled only on the panels positioned in the interstitial space and it was represented by limpet, especially on plastic and metal surfaces.

After the same range of exposure, the fouling composition was completely different compared to Genoa's results: a high percentage of soft fouling was observed on Side B, while hard fouling settled only on Side C.

Looking at these results, the most important parameter that influenced fouling community was the orientation in the space and, as a consequence, the condition of light: the dark interstitial space attracted hard fouler, here represented by limpets.

Despite the differences in fouling composition, the light exposure confirmed to play a key role in the fouling community, as it happened in Genoa site. Hard fouler preferred dark exposition.

The results regarding HCMR's BMB showed that, after 3 month of immersion, most of the surface area was indifferently occupied by soft fouling and slime. Furthermore, a large part of the surface was bare. Hard fouling was scarce. This trend is very different from "ISMAR - coastal water" BMB, even if the BMB was immersed in coastal water; data are more similar to Venice (open water) than Genoa (coastal water). The dissimilarity in environmental conditions between greek and ligurian sites (see salinity, temperature) determined the fouling community.

IFREMER's experiment showed another different situation. After 3 month of immersion, hard fouling was scarce, except for a certain percentage that settled on panels exposed at dark condition, more on Side A than side B. Metal was the substrata preferred by hard foulers.

As it happened for HCMR and ISMAR-Ve, the fouling community that grew on this box was very different from the one which grew on Genoa BMB. Furthermore, on the panels of this box a great variety of taxa was represented, mainly belonging to soft fouling.

The experiments performed in different sampling areas showed, as might be expected, that the fouling community was very different among sites. After 3 months of exposure, Genoa was the site with the highest percentage of hard fouling, followed by ISMAR-Ve, IFREMER, HCMR.

The settlement of marine organisms is influenced by complex interaction between different variables, including biotic and abiotic factors. The choice of settlement substratum is modulated by a series of abiotic factors that included environmental parameters and the physical nature of surfaces; these parameters also affect the biofilm growth that represents in turn an important biotic factor that affected larval settlement. Biofilm formation is strictly related to surface characterization, and it is known to influence larval settlement as, for example, larvae of *B. amphitrite.*

As shown by our data, we investigated the role of some abiotic factors that deeply affected the settlement of a variety of macro- and microscopic marine organisms. Despite the differences in fouling composition among sampling sites, some factors played always a key role in the settlement of organisms. Such factors included the light availability, the materials employed and its physical nature.

Our results contribute to show that organisms' settlement is a process affected by many chemical, physical and biological factors. These field experiments highlight how complex and numerous are the interactions among factors, that can not mimicked in the laboratory. These mutual interactions play a key role during the settlement process, determining the variety and the heterogeneity in fouling composition, which characterize the different sampling sites.

Comparing the data from the different sampling sites, we can learn more about the reasons that determine the complex process of fouling colonization. Such studies are required and these preliminary experiments move a first step toward this direction. Further joint research have to be carried out in this field in order to understand all the interactions involved and to obtain a better characterization of settlement behavior and fouling process.