Comparison of biofouling communities among European regions

Pedro A. Vinagre, Enara Mardaras, Emiliano Pinori, Johan Svenson, Erica Cruz, Teresa Simas

Abstract—**Marine biofouling is a well-known problem across maritime sectors (e.g., shipping, oil and gas, ocean energy) worldwide. Among other negative impacts, biofouling may obstruct cooling pipeline systems, decrease marine sensor efficiency and increase biocorrosion, weight and drag. Marine biofouling should be highly regarded especially concerning the ocean energy devices which must be operational offshore for 10-20 years, where in situ maintenance is extraordinarily costly and risky due to the ocean conditions, and which are not designed to have short dry-dock intervals.**

Furthermore, offshore structures act as artificial reefs creating new surfaces on which the organisms attach, settle and grow, which may contribute to the propagation of non-native species in the marine environment, serving as 'stepping stones' for the organisms, often impacting biodiversity, habitats or ecological processes, and posing great ecological and economic threats.

Our work characterizes assemblages from different biogeographical regions (Portugal, Basque Country, Scotland) settled on different metallic substrates by different biological parameters (e.g., weight, abundance, size) of the organisms most problematic to the marine renewable energy sector: mussels (and other relevant bivalves), barnacles, calcareous tubeworms and bryozoans. This will increase the knowledge on Atlantic biofouling assemblages, where less information is available when compared to the North Sea. Information on biofouling assemblages as well as non-native species occurring in those regions is going to be presented and compared to understand their features and contribute to more effective and eco-friendly anti-fouling strategies for marine renewable energy projects.

Keywords—**Biofouling, Hard-fouling, Marine renewable energy, OCEANIC project.**

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I. INTRODUCTION

MARINE biofouling (a.k.a. marine growth) has been a worldwide problem during the last centuries and is considered a major threat for industries developing technologies and working in the marine environment. Regarding the marine renewable energy (MRE) technologies (offshore wind and ocean energy herein including wave and tidal technologies), hard-fouling organisms such as mussels, barnacles, calcareous tubeworms and bryozoans have been recognized worldwide as those causing the greatest economic impact [e.g., 1; 2; 3; 4]. For example, barnacles mainly impact structural integrity by adding weight and especially due to their strong adhesive and biocorrosive action. Mussels can exert substantial weight on devices and increase its surface diameter and roughness [4]. Furthermore, there might be an influence on corrosion, if the coating (e.g., of devices) is damaged by the attached organisms or during their removal from surfaces.

As a result, maritime industries have been seeking novel solutions to avoid technology and material malfunction to enhance their economic competitiveness and technology/material lifetime. The OCEANIC project (http://oceanic-project.eu) aimed to achieve a long-lasting (>10 years), "one-fit all", protection system against both corrosion and biofouling for the MRE sector, through combining two different techniques: the Low Emission Anti-fouling (FP7 Project; [5; 6]) and the Thermally Sprayed Aluminium. During the project different coatings were sequentially developed and tested, differing in their base materials (e.g., bronze, aluminium,

Fig. 1. Study sites location in Portugal (blue), Basque Country (green) and Scotland (pink). (base map: Google Earth).

TABLE I
SUMMARY OF TRIALS AND SUBSTRATES ANALYSED.

Site	Period	Substrates[a] (x no. samples)	Depth
Almagreira	Trial 1: Aug 2016-Jul 2017	Al, Al/Mg, Br, Br/Al/Mg, Mon (x1 each)	10 m
		Al, Al/Mg, Br, Br/Al/Mg, Mon (x2 each)	5 m
	Trial 2: Sep 2017-Jul 2018	Al (x3), Al/Mg (x2)	10 m
Lisbon	Trial 1: Apr-Nov 2018	Al, Al/Mg (x4 each)	Intertidal (0-3.5 m)
BiMEP	Trial 1: Jul-Nov 2016	Al, Al/Mg, Br, Br/Al/Mg, Mon (x2 each)	15 m
	Trial 2: Aug 2017-Apr 2018	Al (x2), Al/Mg (x4)	15 m
EMEC	Trial 1: Oct 2017-Aug 2018	Al, Al/Mg (x6 each)	22 m

[a]Al: Aluminium; Mg: Magnesium; Br: Bronze; Mon: Monel

polyester, polyethylene) and in the addition (or not) of a biocide molecule in the coatings' matrix. Different test trials were undertaken in three biogeographic regions (Portugal, Basque Country and UK) to assess the coatings efficiency against corrosion and biofouling in different environmental conditions (e.g., cold versus warm water).

This paper uses OCEANIC biofouling data gathered from panels without the biocide added, aiming to compare the biofouling communities settled on different metallic substrates between and within the different bioregions. The work includes the analysis of biological parameters of the assemblages such as the weight and thickness of biofouling on the panels, and the biomass, abundance and size of (the most problematic) hard-fouling organisms. Furthermore, the occurrence of non-native species is identified.

II. MATERIALS AND METHODS

A. *Test sites and substrates*

Almagreira test site: located at about 400 m of the Almagreira beach (Peniche, Portugal; 39°23'24.48''N, 9°18'25.08''W), in the WaveRoller test site (Fig. 1).

Lisbon test site: marina located near the Paço De Arcos municipality (Portugal; 38°41'26.85''N, 9°17'37.78''W) (Fig. 1).

BiMEP (Biscay Marine Energy Platform) test site: located at about 1,700 m of the Armintza village (Basque country; 43°26'13''N, 2°53'50''W) (Fig. 1).

EMEC (European Marine Energy Centre): located in the Scapa Flow at about 950 m from St. Mary's (Orkney, Scotland; 58°53'49.74"N, 2°57'00.06"W) (Fig. 1).

Table 1 summarizes the trials undertaken and the base materials tested at present. At Almagreira and BiMEP, structures holding test panels were affixed to the moorings of marking buoys. At EMEC, the coated panels were affixed to a metallic structure constituent of a Point Absorber buoy (property of CorPower Ocean). At Lisbon, structures with panels were affixed to metallic stairs (used by personnel to move from the pier to water (Fig. 2).

B. *Data collection*

In each trial the panels (20 cm x 15 cm) were analyzed individually to estimate:

i) Samples: Biofouling fresh biomass (gm^{-2}), thickness (mm) and coverage percentage (%). Owing to the

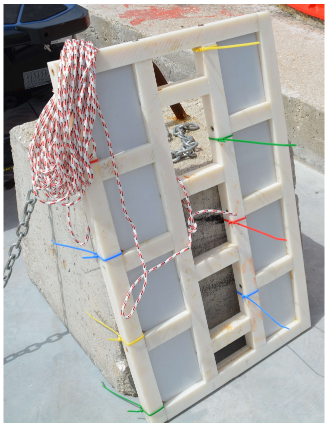

Fig. 2. Example of a structure holding metallic and plastic panels. The coloured zip ties are used to better identify samples during monitoring routines.

available data, for comparison was used either the total biomass (TBiomass) or the biomass estimated after sieving the samples through a 0.5 mm mesh (0.5mmBiomass). Coverage percentage (% of panels' area covered with biofouling) was estimated for BiMEP Trial 2 and EMEC samples only, using the open source software ImageJ (https://imagej.nih.gov/ij);

ii) Organisms: Fresh biomass (gm^{-2}), density (number of individuals m^{-2}; indm^{-2}) and size (e.g., tubeworm length, barnacle height; mm) of hard-fouling organisms. Measurement of individuals of a given species was made depending on the total individuals (e.g., if 20 individuals, all were measured; if 100 individuals, a portion was measured accounting for different size classes). Only the fraction of organisms retained in the 1 mm mesh sieve was analyzed.

Taxonomy was done to the lowest level (e.g., species level) whenever possible (e.g., [7]; Marine Species Identification Portal, http://species-identification.org), and was standardized in accordance to the World Register of Marine species (http://www.marinespecies.org).

C. Data analysis

The biofouling was assessed using six parameters: biomass, thickness, and coverage % of biofouling on the panels, and biomass, density and size of hard-fouling species.

Permutational multivariate analysis of variance (PERMANOVA; [8]) was applied individually to each parameter to analyze statistical differences in substrates within and among sites. Two designs were tested and included two fixed factors, Site and Substrate (nested/not nested in Site) (the number of levels varied with the number of sites and substrates in each analysis). For the univariate analyses was used the Euclidean distance in the calculation of similarity matrices, after square root transformation of data for biofouling biomass and thickness. For the multivariate analyses was used the Bray Curtis similarity, after square root transformation data for hard-fouling biomass and abundance. The statistical significance of variance components was tested using 999 permutations with unrestricted permutation of raw data and a significance level of α = 0.05. For tests with possible permutations <100 was selected the Monte Carlo α [p(MC)].

III. Results

D. Almagreira Trial 1 Vs Trial 2

Al Vs Al/Mg

The Almagreira Trial 1 and Trial 2 were undertaken virtually for the same period (11-12 months) and covered the same seasons (summer to summer). During Trial 1, mean water temperature ranged between 12.9 °C in January 2017 and 20.2 °C in August 2017. Mean wave height ranged between 1.6 m in April 2018 and 3.1 m in February 2018. During Trial 2, mean water temperature ranged between 12.0 °C in February 2018 and 19.7 °C in October 2017. Mean wave height ranged between 1.4 m in July 2018 and 3.8 m in March 2018 (Fig. 3).

In Trial 1 were registered 15 taxa and in Trial 2 24 taxa, in both trials mostly distributed among Phylum Mollusca (4 and 8 taxa, respectively) and Annelida (2 and 6 taxa,

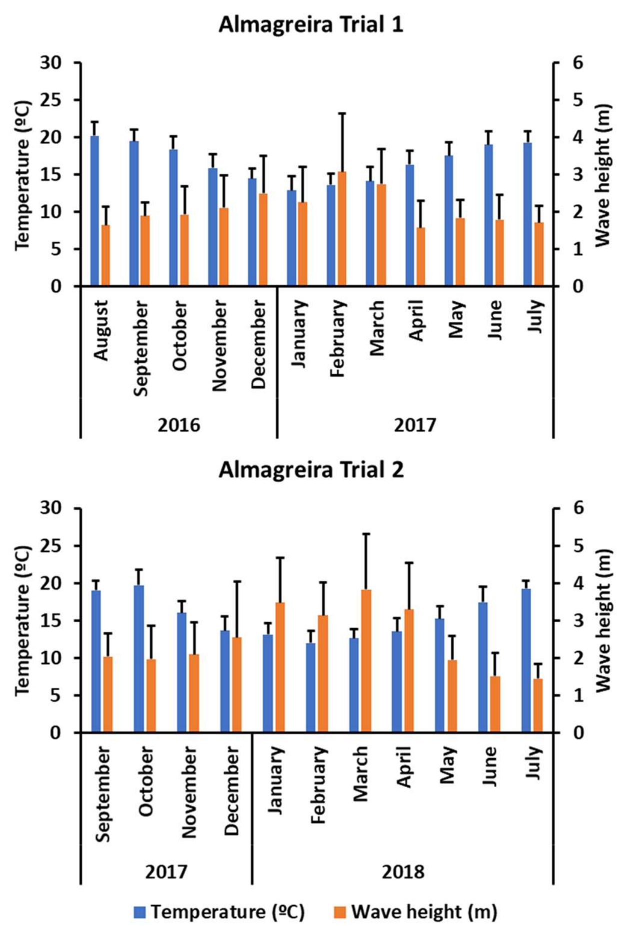

Fig. 3. Temperature (°C) and wave height (m) (mean + sd) in Almagreira Trial 1 and Trial 2.

respectively) and Sub-Phylum Crustacea (5 taxa in both trials) (Appendix I). In Trial 1 were registered more taxa on Al/Mg (15 taxa) and less on Al (3 taxa), whereas in Trial 2 were registered more taxa in Al (21) and less on Al/Mg (16).

Biofouling TBiomass and thickness were much greater in Trial 1 compared to Trial 2, and in both trials were greater on Al/Mg (Table II).

TABLE II
BIOFOULING TBIOMASS AND THICKNESS (MEAN ± SD) IN ALMAGREIRA TRIAL 1 AND TRIAL 2. HIGHER VALUES BETWEEN SUBSTRATES HIGHLIGHTED IN GREY.

		TBiomass (gm^{-2})	Thickness (mm)
Trial 1	Al	6,255.6 ± 2,625.6	37.3 ± 11.0
	Al/Mg	9,755.6 ± 8,498.4	43.7 ±34.5
Trial 2	Al	120.8 ± 83.5	5.7 ± 0.4
	Al/Mg	206.1 ± 197.8	6.5 ± 0.6

Regarding the hard-fouling organisms, *M. galloprovincialis*, *P. perforatus* and Bryozoa had comparable data between trials (Table III). Results varied with the trial: the three taxa registered greater biomass and size (except Bryozoa which are not measured) in Trial 1. *Mytilus galloprovincialis* density was much greater in Trial 2, whereas *P. perforatus* density was much greater in Trial 1. *Mytilus galloprovincialis* generally registered greater biomass, density and size on Al/Mg, *P. perforatus* showed different trends between trials and Bryozoa registered greater biomass on Al.

TABLE III
HARD-FOULING BIOMASS, DENSITY AND SIZE (MEAN ± SD) IN ALMAGREIRA TRIAL 1 AND TRIAL 2. HIGHER VALUES BETWEEN SUBSTRATES HIGHLIGHTED IN GREY.

Biomass (gm^{-2})		*M. galloprovincialis*	*P. perforatus*	Bryozoa
Trial 1	Al	1,732.5 ±1,982.7	2,481.5 ± 2,300.9	233.7 ± 398.9
	Al/Mg	4,570.5 ± 7,314.5	2,197.3 ± 1,777.4	103.2 ± 177.6
Trial 2	Al	6.5 ± 7.2	39.7 ± 24.6	0.66 ± 0.35
	Al/Mg	6.9 ± 8.6	157.1 ± 166.9	0.35 ± 0.49
Density ($indm^{-2}$)				
Trial 1	Al	366.7 ± 405.5	7,422.2 ± 3,893.2	
	Al/Mg	466.7 ± 300.0	5,866.7 ± 2,602.8	
Trial 2	Al	4,388.9 ± 4,894.3	444.4 ± 96.2	
	Al/Mg	2,333.3 ± 2,121.3	1,766.7 ± 1,979.9	
Size (mm)				
Trial 1	Al	29.26 ± 10.58	7.38 ± 2.55	
	Al/Mg	31.61 ± 33.03	6.89 ± 1.75	
Trial 2	Al	2.14 ± 0.39	4.08 ± 0.30	
	Al/Mg	2.43 ± 0.31	4.26 ± 0.35	

For Almagreira Trial 2 was available biomass, density and size data for three additional hard-foulers, the calcareous tubeworm *Spirobranchus* sp., and the bivalves *Hiatella arctica* and *Anomia ephippium*. Biomass, density and size of *Spirobranchus* sp. and *H. arctica* were greater on Al, whereas for *A. ephippium* that was observed on Al/Mg (Table IV).

TABLE IV
SIZE (MEAN ± SD) OF ADDITIONAL HARD-FOULING IN ALMAGREIRA TRIAL 2. HIGHER VALUES BETWEEN SUBSTRATES HIGHLIGHTED IN GREY.

Biomass (gm^{-2})		*Spirobranchus* sp.	*H. arctica*	*A. ephippium*
Trial 2	Al	31.5 ± 18.4	0.09 ± 0.10	0.01 ± 0.02
	Al/Mg	21.3 ± 5.4	0.02 ± 0.02	0.10 ± 0.10
Density ($indm^{-2}$)				
Trial 2	Al	1011.1 ± 401.8	122.2 ± 154.0	11.1 ± 19.2
	Al/Mg	766.7 ± 0.0	50.0 ± 70.7	33.3 ± 0.0
Size (mm)				
Trial 2	Al	15.81 ± 1.27	1.66 ± 0.23	1.08 ± 1.88
	Al/Mg	14.84 ± 2.45	0.86 ± 1.21	2.86 ± 1.05

Statistically significant differences were found between Almagreira Trial 1 and Trial 2 using the biofouling biomass and thickness and hard-fouling biomass and density (using all species and using each species individually; no differences between trials were found in Bryozoa biomass) but no differences were observed in substrates between sites. For all parameters (including the size of hard-foulers, using all species and using each species individually) no differences were found among substrates within site (Appendix II).

E. Almagreira Trial 1 Vs BiMEP Trial 1

Br Vs Br/Al/Mg Vs Monel

The BiMEP Trial 1 was undertaken for 5 months covering summer to autumn 2016. During the trial, mean water temperature ranged between 12.2 °C in November 2016 and 20.9 °C in August 2016. Mean wave height ranged between 1.1 m in July 2016 and 1.9 m in November 2016 (Fig. 4).

In Almagreira were registered 20 macroinvertebrate taxa, mainly distributed among Phyla Annelida (5 taxa) and Mollusca (6 taxa) and Sub-Phylum Crustacea (5 taxa). In BiMEP were found 7 taxa, mostly mollusks (4 taxa). In Almagreira were registered more taxa on Monel (16 taxa) and less on Br (8 taxa), whereas in BiMEP similar number of taxa were found on Br (7 taxa), Br/Al/Mg and Monel (both with 6 taxa) (Appendix I).

Biofouling TBiomass and thickness were much greater at Almagreira compared to BiMEP, and generally greater on Br and lower on Br/Al/Mg (Table V).

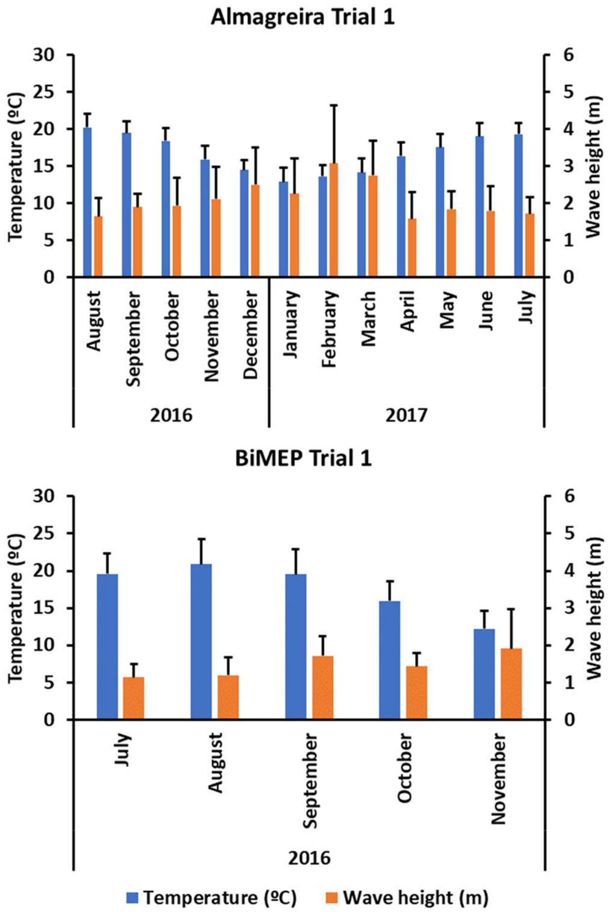

Fig. 4. Temperature (°C) and wave height (m) (mean + sd) in Almagreira Trial 1 and BiMEP Trial 1.

Regarding the hard-fouling organisms, comparable data between sites existed for *M. galloprovincialis* and *P. perforatus* only. Both species registered much greater biomass and density in Almagreira (Table V). There, *M. galloprovincialis* generally reached much greater biomass than the barnacle, with the converse observed for density. In BiMEP *P. perforatus* reached greater biomass than the mussel, with the converse observed for density. In both sites, both species reached greater biomass and density on Br. Lower *M. galloprovincialis* biomass and density was registered on Br/Al/Mg, whereas for *P. perforatus* that was observed on Monel. The size of hard-foulers was measured only in Almagreira. *Mytilus galloprovincialis* registered greater size than *P. perforatus*, and greater size of both species (*M. galloprovincialis* length and *P. perforatus* height) was found on Br, while Monel registered lower values (Table V).

Statistically significant differences were found between Almagreira and BiMEP using the biofouling biomass and thickness and hard-fouling biomass and density (using all species and using each species individually) but no differences were observed in substrates between sites. For all parameters (including the size of hard-foulers, using all species and using each species individually) no differences were found among substrates within site (Appendix II).

TABLE V

BIOFOULING TBIOMASS AND THICKNESS, AND HARD-FOULING BIOMASS, DENSITY AND SIZE (MEAN ± SD) IN ALMAGREIRA TRIAL 1 AND BIMEP TRIAL 1. HIGHER VALUES AMONG SUBSTRATES HIGHLIGHTED IN GREY.

Biofouling		**TBiomass (gm^{-2})**	**Thickness (mm)**
Almagreira	Br	25,588.9 ± 6,826.9	64.7 ± 7.7
	Br/Al/Mg	8,966.7 ± 6,285.5	46.7 ± 21.0
	Monel	12,755.6 ± 3,397.1	59.5 ± 1.3
BiMEP	Br	137.2 ± 45.0	10.3 ± 2.4
	Br/Al/Mg	116.5 ± 112.0	8.1 ± 0.5
	Monel	144.8 ± 27.1	6.6 ± 4.5
Hard-fouling			
Biomass (gm^{-2})		***M. galloprovincialis***	***P. perforatus***
Almagreira	Br	17,831.1 ± 5,650.5	2,172.4 ± 1,702.5
	Br/Al/Mg	4,176.0 ± 3,271.1	1,919.7 ± 2,338.8
	Monel	6,749.9 ± 4,983.0	687.1 ±395.9
BiMEP	Br	11.3 ± 3.3	71.5 ± 34.6
	Br/Al/Mg	2.0 ± 0.9	47.0 ± 35.4
	Monel	2.5 ± 3.5	36.7 ± 51.9
Density (indm^{-2})			
Almagreira	Br	2,444.4 ± 818.1	7,033.3 ± 5,616.7
	Br/Al/Mg	411.1 ± 203.7	5,177.8 ± 4,568.0
	Monel	1,900.0 ± 1,332.1	2,877.8 ± 1,828.3
BiMEP	Br	183.3 ± 70.7	83.3 ± 70.7
	Br/Al/Mg	66.7 ± 41.1	50.0 ± 23.6
	Monel	66.7 ± 94.3	33.3 ± 47.1
Size (mm)			
Almagreira	Br	50.75 ± 3.81	7.48 ± 2.95
	Br/Al/Mg	47.22 ±24.28	6.61 ± 3.70
	Monel	47.14 ± 24.11	5.90 ± 0.85

F. Almagreira Trial 2 Vs BiMEP Trial 2 Vs EMEC Vs Lisbon

Al Vs Al/Mg

These four trials were undertaken for similar periods of time but covered different seasons: in Almagreira, 11 months covering summer 2017 to summer 2018; in BiMEP, 9 months covering from summer 2017 to spring 2018; in EMEC, 11 months covering autumn 2017 to summer 2018; and in Lisbon, 8 months covering spring to autumn 2018.

During Almagreira Trial 2, mean water temperature ranged between 12.0 °C in February 2018 and 19.7 °C in October 2017. Mean wave height ranged between 1.4 m in July 2018 and 3.8 m in March 2018. During BiMEP Trial 2, mean water temperature ranged between 7.7 °C in February 2018 and 20.2 °C in August 2017. Mean wave height ranged between 1.1 m in July 2018 and 3.5 m in January 2018. During EMEC trial, mean water temperature ranged between 4.8 °C in March 2018 and 14.3 °C in July 2018. Mean wave height ranged between 1.2 m in July 2018 and 3.4 m in November 2017. During Lisbon trial, mean water temperature ranged between 14.4 °C in April 2018 and 23.3 °C in August 2018. Wave height data were not available (Fig. 5).

Using Al and Al/Mg panels, in Almagreira and BiMEP Trial 2 was registered similar number of taxa (24 and 8, respectively) compared with Almagreira and BiMEP

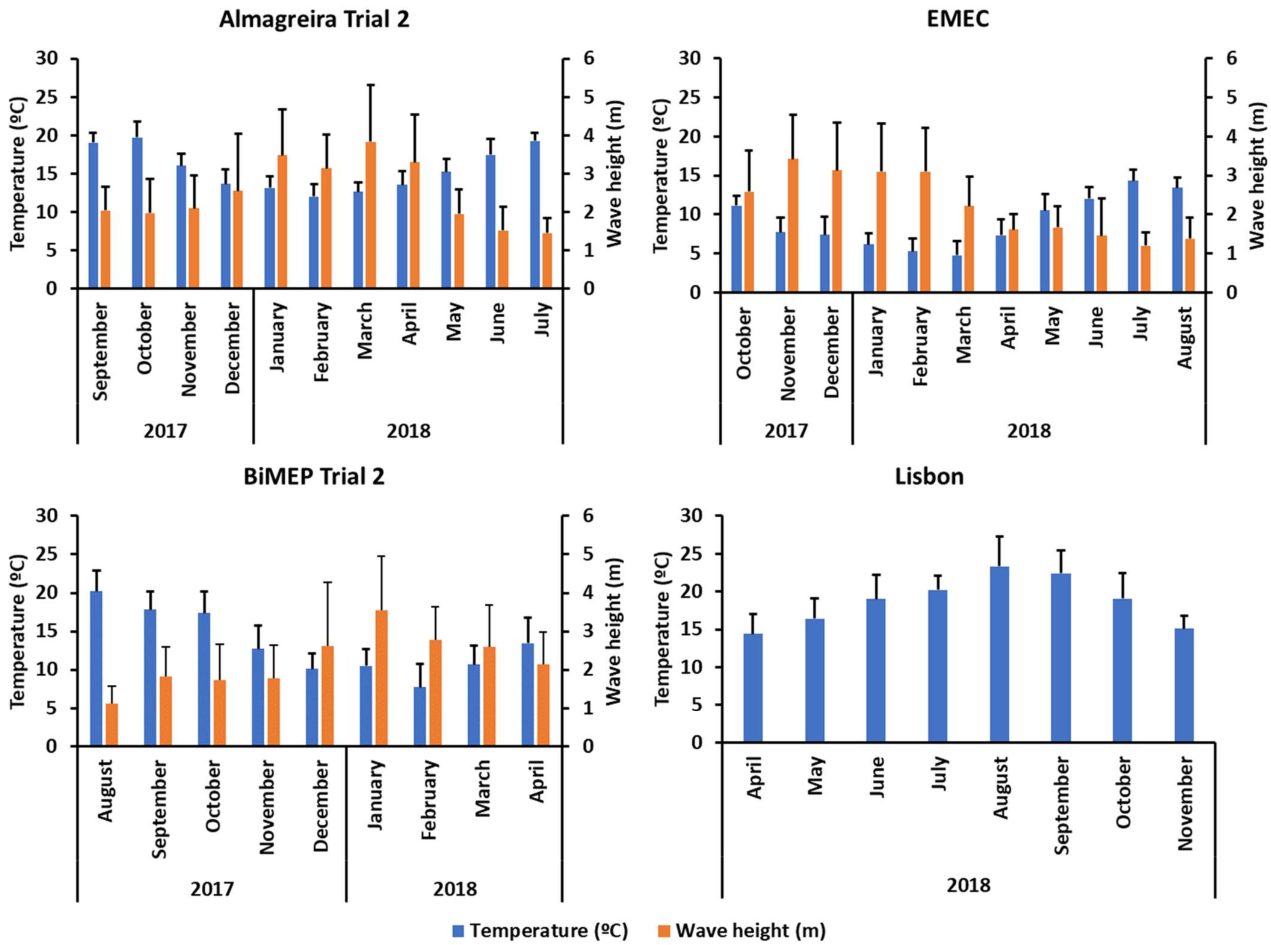

Fig. 5. Temperature (°C) and wave height (m) (mean + sd) in Almagreira Trial 2, BiMEP Trial 2, EMEC and Lisbon.

using Br, Br/Al/Mg and Monel. In EMEC was registered intermediate number of taxa (13) and in Lisbon the greatest number of taxa (35) of all trials. In Almagreira and EMEC were registered more taxa on Al (21 and 11 taxa, against 17 and 10 taxa on Al/Mg, respectively), whereas in BiMEP and Lisbon were registered more taxa on Al/Mg (8 and 28 taxa, against 6 and 25 taxa on Al, respectively) (Appendix I).

Biofouling 0.5mmBiomass and thickness were generally greater at BiMEP and Lisbon, and in all trials (except BiMEP) were greater on Al/Mg. Coverage % (estimated only for BiMEP and EMEC) was greater at BiMEP and in both sites was slightly greater on Al (Table VI).

Six hard-fouling organisms had comparable data between sites, *M. galloprovincialis* (not observed in EMEC), Barnacles (including *P. perforatus*, found in Almagreira, BiMEP and Lisbon; *Austrominius modestus*, non-native species found in Lisbon and EMEC; and *Balanus balanus* and *B. crenatus*, found in EMEC), *Spirobranchus* sp., *H. arctica, A. ephippium* and Bryozoa. Regarding hard-fouling biomass (Table VII-A.), density (Table VII-B.) and size (Table VII-C.), higher values were more frequently observed on Al/Mg. Regarding biomass,

TABLE VI

BIOFOULING 0.5MMBIOMASS, THICKNESS AND COVERAGE (MEAN ± SD) IN ALMAGREIRA TRIAL 2, BIMEP TRIAL 2, LISBON AND EMEC. HIGHER VALUES BETWEEN SUBSTRATES HIGHLIGHTED IN GREY.

		0.5mmBiomass (gm^{-2})	Thickness (mm)	Coverage (%)
Almagreira Trial 2	Al	101.1 ± 64.8	5.7 ± 0.4	
	Al/Mg	204.4 ± 195.4	6.5 ± 0.6	
BiMEP Trial 2	Al	487.9 ± 73.9	7.0 ± 1.9	56.5 ± 0.4
	Al/Mg	447.8 ± 201.8	5.4 ± 7.5	55.5 ± 3.0
EMEC	Al	90.3 ± 58.6	2.7 ± 0.6	26.1 ± 8.8
	Al/Mg	93.3 ± 58.8	3.0 ± 0.9	25.9 ± 12.8
Lisbon	Al	395.2 ± 177.2	5.9 ± 0.9	
	Al/Mg	473.6 ± 276.5	7.9 ± 2.0	

it was especially observed for *M. galloprovincialis* and Barnacles (Bryozoa showed higher values more frequently on Al), regarding density it was not observed only for *H. arctica*, and regarding size it was especially observed for *M. galloprovincialis* and *Spirobranchus* sp. (*H. arctica* showed higher values more frequently on Al). Overall, greater biomass and size were more frequently observed in Lisbon and greater density in Almagreira. On the other hand, lower biomass and size were more frequently observed in EMEC and lower density in BiMEP.

TABLE VII
HARD-FOULING BIOMASS (A.), DENSITY (B.) AND SIZE (C.) (MEAN ± SD) IN ALMAGREIRA TRIAL 2, BIMEP TRIAL 2, LISBON AND EMEC. HIGHER VALUES BETWEEN SUBSTRATES HIGHLIGHTED IN GREY.

A. Biomass (gm^{-2})		*M. galloprovincialis*	Barnacles	*Spirobranchus* sp.	*H. arctica*	*A. ephippium*	Bryozoa
Almagreira Trial 2	Al	6.5 ± 7.2	39.7 ± 24.6	31.5 ± 18.4	0.09 ± 0.10	0.01 ± 0.02	0.66 ± 0.35
	Al/Mg	6.9 ± 8.6	157.1 ± 166.9	21.3 ± 5.4	0.02 ± 0.02	0.10 ± 0.10	0.35 ± 0.49
BiMEP Trial 2	Al	0.0 ± 0.0	11.2 ± 5.0	2.2 ± 0.14	0.37 ± 0.52	0.76 ± 0.96	226.1 ± 165.3
	Al/Mg	0.01 ± 0.02	40.7 ± 78.7	2.1 ± 1.8	0.01 ± 0.02	0.32 ± 0.63	142.2 ± 41.7
Lisbon	Al	8.5 ± 7.1	60.0 ± 46.6	66.3 ± 113.3	0.02 ± 0.02	6.7 ± 12.6	58.1 ± 35.4
	Al/Mg	34.3 ± 36.1	134.6 ± 83.3	90.5 ± 118.1	0.1 ± 0.07	6.8 ± 7.6	42.8 ± 27.6
EMEC	Al	0.0 ± 0.0	86.1 ± 57.3	0.56 ± 0.46	0.16 ± 0.24	0.11 ± 0.11	3.3 ± 1.8
	Al/Mg	0.0 ± 0.0	87.4 ± 55.6	0.90 ± 0.47	0.45 ± 0.96	0.10 ± 0.09	4.4 ± 3.1
B. Density (indm^{-2})							
Almagreira Trial 2	Al	4,388.9 ± 4,894.3	444.4 ± 96.2	1,011.1 ± 401.8	122.2 ± 154.0	11.1 ± 19.2	
	Al/Mg	2,333.3 ± 2,121.3	1,766.7 ± 1,979.9	766.7 ± 0.0	50.0 ± 70.7	33.3 ± 0.0	
BiMEP Trial 2	Al	0.0 ± 0.0	33.3 ± 0.0	83.3 ± 23.6	33.3 ± 47.1	16.7 ± 23.6	
	Al/Mg	8.3 ± 16.7	58.3 ± 50.0	100.0 ± 112.2	8.3 ± 16.7	8.3 ± 16.7	
Lisbon	Al	500.0 ± 362.1	3,900.0 ± 3,209.1	1,483.3 ± 2,084.4	25.0 ± 16.7	108.3 ± 113.4	
	Al/Mg	700.0 ± 547.7	3,191.7 ± 1,128.2	1,625.0 ± 1,891.4	75.0 ± 56.9	333.3 ± 309.1	
EMEC	Al	0.0 ± 0.0	32,466.7 ± 21,336.2	333.3 ± 298.1	72.2± 71.2	138.9 ± 170.5	
	Al/Mg	0.0 ± 0.0	33,033.3 ± 30,096.7	588.9 ± 472.2	105.6 ± 112.4	177.8 ± 91.1	
C. Size (mm)							
Almagreira Trial 2	Al	2.14 ± 0.39	4.08 ± 0.30	15.81 ± 1.27	1.66 ± 0.23	1.08 ± 1.88	
	Al/Mg	2.43 ± 0.31	4.26 ± 0.35	14.84 ± 2.45	0.86 ± 1.21	2.86 ± 1.05	
BiMEP Trial 2	Al	0.0 ± 0.0	7.29 ± 2.36	4.79 ± 0.10	2.19 ± 3.10	3.79 ± 5.35	
	Al/Mg	0.72 ± 1.43	4.48 ± 5.39	4.72 ± 0.19	0.56 ± 1.11	1.40 ± 2.80	
Lisbon	Al	4.38 ± 1.50	2.02 ± 0.51	17.58 ± 8.83	0.86 ± 1.01	4.03 ± 3.22	
	Al/Mg	5.00 ± 2.96	2.02 ± 0.78	25.07 ± 14.11	1.43 ± 0.96	4.84 ± 1.83	
EMEC	Al	0.0 ± 0.0	2.01 ± 0.26	3.94 ± 2.47	2.15 ± 2.19	1.86 ± 0.61	
	Al/Mg	0.0 ± 0.0	2.05 ± 0.29	5.21 ± 2.66	2.05 ± 1.72	1.78 ± 0.92	

Statistically significant differences were observed among sites regarding biomass, with both Almagreira and EMEC different from BiMEP and Lisbon, regarding thickness, with EMEC different from BiMEP and Lisbon, and regarding coverage %, with BiMEP different from EMEC (Appendix III). Regarding the hard-fouling, all sites were different regarding biomass, density and size when using all taxa. When using individual taxa, results were species-dependent: *Mytilus galloprovincialis* showed fewer differences between BiMEP and EMEC (especially considering density and size); Barnacles showed fewer differences between Almagreira and BiMEP and between Lisbon and EMEC (no differences between sites were found using biomass, but all sites were different using density); *Spirobranchus* sp. showed more differences between Almagreira and BiMEP (especially considering biomass and density) and between Lisbon and the other sites (especially considering size); *H. arctica* did not show differences among sites; *A. ephippium* showed differences between Lisbon and EMEC regarding biomass and size but for density those sites were not different; and Bryozoa showed differences (regarding biomass) between all sites (Appendix III).

IV. DISCUSSION

The MRE sector offers a more sustainable energy supply and is therefore expected to grow during the current century. A great number of structures and equipment is deployed at sea, and more are expected in the next decades, especially in the North Sea where the MRE sector is well developed with a great number and variety of equipment functioning for years ([9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20]), and which have allowed for a good amount of information on biofouling communities (e.g., occurrence, structure, abundance) to be available (see OCEANIC Biofouling Database, http://oceanic-project.eu/biofouling-database).

In the present work the biofouling communities changed with latitude and seem to be more dependent on local characteristics of the sites (more than on the period of immersion). Overall, the communities were mostly represented by two groups of filter-feeding species –

mussels and barnacles – which are well adapted to such harsh conditions. Mussels dominated in biomass and were the major component of biofouling in lower latitudes (e.g., Almagreira), whereas barnacles dominated in density and were the dominant group at higher latitude (EMEC). More specifically:

- The seawater temperatures overall decreased from lower to higher altitude (Portugal sites > BiMEP > EMEC), and the same was generally observed for the number of taxa, and biofouling biomass and thickness;
- The Lisbon site registered higher seawater temperatures (minimum and maximum) and, consisting of a marina, should register the lowest wave heights. These conditions, together with increased nutrient load (possibly coming from the Tagus Estuary), may have allowed for increased diversity (Lisbon registered the greatest number of taxa of all trials) and high abundance of organisms, especially the calcareous tubeworm *Spirobranchus* sp. which showed in Lisbon the greatest biomass, density and size;
- The Almagreira site registered the highest wave heights (minimum and maximum). The strong hydrodynamics of the area, together with higher temperatures, may explain the increased abundance and size of filter feeders, namely *M. galloprovincialis* and *P. perforatus*, compared to the other sites. The differences found between Almagreira Trial 1 and Trial 2 (namely the much greater biomass and thickness and hard-fouling abundance and size registered in Trial 1) could be owed to storms occurring during Trial 2 possibly displacing organisms from the test panels, resulting in lower numbers;
- The EMEC site, contrary to the Lisbon site, registered the lowest minimum and maximum seawater temperatures. The EMEC presented the lowest biofouling biomass, thickness and coverage, and the second lowest number of taxa. In EMEC, the cold water typical of the North Sea may have caused fewer organisms to occur in the area and consequently on the test panels (evidenced by the simpler, less mature communities) promoting greater possibility for small barnacles (which registered the greatest density in EMEC) to adhere to the panels and settle;
- The BiMEP site generally registered intermediate wave heights and seawater temperature comparing to the Almagreira and EMEC sites (temperatures were closer to those at Almagreira). At BiMEP the trends were more species-specific and seem intermediate to those found at Almagreira and EMEC sites: BiMEP presented the lowest number of species (followed by EMEC) but the greatest coverage (compared only to EMEC); registered *M. galloprovincialis* second lowest biomass, density and size (all after EMEC); registered *P. perforatus* lowest density (followed by Almagreira) but the second greatest size (after Almagreira); registered Bryozoa second lowest biomass (after Almagreira); and registered *Spirobranchus* sp. (followed by EMEC), *A. ephippium* (followed by Almagreira) and *H. arctica* (followed by Lisbon) lowest densities.

Regarding the materials used for the panels, considering all trials the number of taxa and biofouling biomass and thickness were more frequently greater on the Al/Mg substrate compared to the Al. The Al/Mg also presented more frequently greater *M. galloprovincialis* biomass and density, *P. perforatus* biomass, density and size, *Spirobranchus* sp. density and *A. ephippium* density, whereas Al registered greater Bryozoa biomass and *H. arctica* size. These results suggest a greater resistance to biofouling in general for Al compared to Al/Mg.

In earlier trials (Almagreira Trial 1 and BiMEP Trial 1) were tested the substrates Br, Br/Al/Mg and Monel. These three substrates generally presented great biofouling biomass and thickness and *M. galloprovincialis* and barnacles biomass, density and size (especially in Almagreira Trial 1) and, since these substrates showed signs of corrosion (particularly the Monel, which in cases the panels ruptured), they were excluded from further trials (Almagreira Trial 2, BiMEP Trial 2, EMEC and Lisbon).

Regarding non-native species occurrence, only the New-Zealand/Australasian barnacle *A. modestus* was registered in the EMEC and Lisbon sites. This species had already been observed in the UK (England: [9; 12; 20]) and in other areas of the North Sea (Belgium: [10; 13; 14; 15; 19]; Netherlands: [11; 16; 17; 18]). Curiously, it was not found in the Almagreira site which is located between the EMEC and Lisbon sites. The presence of *A. modestus* in Lisbon could be related to the great amount of ships (commercial and industrial) in the Tagus Estuary, which could have promoted the introduction of the non-native species larvae in the area in turn carried in the currents to nearby areas including the marina. On the other hand, the man-made structures where the test panels were placed in the Almagreira site (moorings of marking buoys) are recent and may have not been colonized by that barnacle species to date.

Besides the geographical attributes (e.g., seawater temperature), other factors may have contributed to the differences in the biofouling communities among sites, such as the different periods of immersion and deployment depths. Longer periods of immersion should allow the communities to reach greater maturity and complexity. But probably more important (at least in the present trials) is the season after which the panels are removed. For example, if biofouling is allowed to grow during spring-summer (which was the most often case in the present trials) the organisms may achieve greater growth and reproductive rates, resulting in more complex communities, whereas recovering the panels (e.g.) after winter could bias the results owed to the diminished growth and reproduction and greater displacement of organisms, resulting in poorer communities. The position at which the test panels are deployed is also of importance. At present, deployment depths ranged from intertidal in Lisbon to 22 m depth in EMEC, and in both sites the panels were closer to the seabed than in Almagreira and BiMEP. On one hand, deeper sections in the water column are expected to present different biofouling composition than at surface (e.g., shifting from barnacle- to mussel-dominant communities from the surface across a few tenths of meters, depending on site-specific conditions and the type of equipment). Greater proximity to the seabed is expected to increase the organisms' susceptibility to chafing, often determining the lower limit of biofouling occurrence. Simultaneously, proximity may promote the recruitment of larvae especially to the assemblages settled nearby. The quality of data used at present could be improved, e.g., more homogeneous sampling of each substrate in each trial (e.g., instead of using two replicates of one substrate and 6 replicates of another). This was the aim in the beginning of each trial; however, it was not possible owed to severe storms during winter which affected some of the trials (i.e., samples were lost).

APPENDIX

Appendices I, II and III are available for download at https://www.researchgate.net/profile/Pedro_Vinagre.

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