RESEARCH ARTICLE

Early biofouling colonization stages: Implications for operation and maintenance planning in marine renewable energy projects [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: Marine biofouling is a threat to industries working in the marine environment, representing enormous costs associated with equipment impairment and loss of performance. In the Marine Renewable Energy (MRE) and other maritime sectors which operate at sea for long periods, an important aspect of biofouling is related to the type and frequency of maintenance.

Methods: This study investigated important parameters of macrofouling (for example composition, including the presence of non-indigenous species, thickness, and weight) from communities growing on small-scale wave energy components in marine conditions. The trials were performed during short periods of submersion (one to eight weeks) in the seasons when the colonisation process should be most intensive (spring, summer, and autumn). Furthermore, the frictional resistance forces generated to scrape the biofouling from those artificial components were investigated.

Results: Overall, results show that while biofouling growth in early colonization stages might not present great detrimental effects to wave energy components, although marine corrosion and the settlement of non-indigenous species (NIS) should be factors of concern.

Conclusions: It is suggested to perform biofouling-related maintenance activities after the peak of maximum growth and reproduction (during the warmer seasons in temperate to cold environments) to reduce the number and frequency of activities. NIS can be detected very early in the colonization process, highlighting the importance of biofouling monitoring and the implementation of biosecurity risk assessment plans early in the operational stage of MRE projects.

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Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Biofouling, Colonization, Macrofouling, Marine Renewable Energy, Non-indigenous species, Operations and Maintenance

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Introduction

Marine biofouling is a natural process which poses great challenges to the maritime sectors (*e.g.* marine renewable energy, oil and gas, shipping, aquaculture), most often resulting in loss of structural integrity, performance and productivity representing enormous costs to the maritime sectors (*e.g.* Bannister *et al.,* 2019; Loxton *et al.,* 2017; Satpathy *et al.,* 2010; Schultz *et al.,* 2011; Titah-Benbouzid & Benbouzid, 2017).

With regards to the marine renewable energy (MRE) sector (including ocean energy and offshore wind), biofouling (namely macrofouling) adds substantial weight to the equipment and structures, and increases their surface diameter and roughness, resulting in increased drag of moving parts and loss of equipment functionality and performance (*e.g.* Blair *et al.,* 2014; Jusoh & Wolfram, 1996; Titah-Benbouzid & Benbouzid, 2017; Yang *et al.,* 2017). Moreover, biofouling may induce or accelerate corrosion in the equipment: larger organisms (macrofouling) facilitate microbiologically induced/influenced corrosion (*e.g.* Jia *et al.,* 2019; Videla & Herrera, 2005) which is initiated by microbial communities (microfouling) growing under the macrofoulers in oxygen-depleted conditions; corrosion may further be accelerated by some macrofoulers via mechanical or chemical actions used to adhere to (acorn barnacles) or perforate (boring bivalves) substrates (*e.g.* Blackwood *et al.,* 2017; Kleemann, 1996).

Another concern related to biofouling is that it creates opportunity for non-indigenous species (NIS) to settle and spread across geographical regions. This has been the case of several MRE structures and equipment deployed at sea in the last years (*e.g.* Adams *et al.*, 2014; De Mesel *et al.*, 2015; Kerckhof *et al.,* 2011; Langhamer, 2012; Nall *et al.*, 2017).

To overcome the biofouling challenge to the maritime sectors, several anti-fouling (AF) solutions have been developed over the last decades. However, biofouling structure and growth varies greatly depending on the geographical location, season, depth, and substrate composition and roughness, among many other factors (*e.g.* Hellio & Yebra, 2009; Vinagre *et al.*, 2020). Hence, to date, no AF solution is simultaneously applicable worldwide and efficient against all biofouling organisms. Furthermore, the AF industry may face a further challenge with the increase of seawater temperature and acidification associated with climate change (Dobretsov *et al.,* 2019): First, the increased temperature and acidification have detrimental effects to many marine organisms, especially calcifying organisms such as barnacles, mussels, and tubeworms, which often make the bulk of biofouling and which are generally targeted by AF solutions; second, increased temperature and acidification may lead to changes in the durability and efficacy of some AF solutions (Dobretsov, 2009; Dobretsov *et al.,* 2019). Hence, mechanical techniques (*e.g.* cleaning, brushing, scraping) appears at present the only method capable of being totally efficient against biofouling worldwide, ensuring that no organism (or part of it, for example barnacle shells), remains on the equipment.

With regards to the MRE sector, monitoring of biofouling (namely macrofouling) often analyses composition, abundance (as biomass, density, or coverage) and/or thickness parameters after the equipment has been deployed in marine conditions for several continuous months or years. This allows the biofouling communities to become more complex, capturing high values of those biofouling parameters which could represent worst-case scenarios. On the other hand, understanding the structure of biofouling composition and its magnitude in early colonization stages, especially during different seasons, is of utmost importance. This allows, for example, to estimate minimum/maximum time intervals to perform maintenance tasks and to understand which could be the best periods to deploy equipment at sea. In terms of conservation, it allows to early detect the presence of NIS populations in the area and initiate mitigation measures to their proliferation.

The activities that lead to the present work were developed under the Horizon 2020 project WaveBoost which designed and developed an advanced power take off (PTO) system for enhanced reliability and performance of Wave Energy Converters and were encompassed in the work package dedicated to performance assessment and improvement. The wave energy technology tested under this project was developed by CorPower Ocean, where the energy converter is of the point absorber type, with a heaving buoy on the surface absorbing energy from ocean waves. The activities included the assessment of (i) biofouling composition (including the presence of NIS) and key biofouling parameters (thickness, richness, biomass, and density), and (ii) the frictional resistance forces generated during the scraping of biofouling, from small-scale samples of the rods of the CorPower Ocean's PTO deployed in marine conditions.

The aim of this work was to increase understanding on biofouling community structure in early colonization stages (during short, increasing periods of one to eight weeks of submersion) across different seasons (spring, summer, and autumn) and, based on that, to delineate some recommendations on biofouling management which could aid the implementation and the planning of operations and maintenance activities of MRE projects.

Methods

Study site and sampling

The Pedrouços harbour (Lisbon, Portugal; 38º41'38''N, 9º13'31''W) is located in a temperate climate region on the south-western Atlantic coast of Europe, at about 6 km upstream the mouth of Tagus estuary in Lisbon, Portugal (Figure 1). The harbour serves a restricted number of small fishing vessels. Openings in the harbour walls allow for seawater to pass through creating light wave action (maximum 0.5 m) and water circulation.

At the harbour, depth in the area of sampling ranges between ~5 m at low tide and ~8 m at hight tide.

Eight cylindrical samples (230 mm × 80 mm; colonizable area: 180 mm × 80 mm) representing rods of a hydraulic PTO system were placed (suspended in a floating rig) submerged at ~3 m depth for different periods of time (one, two, three, four, five, six and eight weeks, henceforth designated as 1–8W) between May and November of 2019 (Table 1; Figure 2A.).

The cylinders were made of S355 steel and were coated with two different anti-corrosion treatments (for industry/research-based reasons):

- Six out of the eight samples were coated with a laser cladded alloy (similar to Stellite) based on corrosion-resistant metals (stainless steel, nickel, chrome, and cobalt; kept confidential to protect commercial interests); these six samples are hereafter named LC1, LC2, LC3, LC4, LC5 and LC6.
- Two out of the eight samples were coated with electroplated nickel-chromium; these two samples are hereafter named NC1 and NC2.

The NC1 and NC2 cylinders showed signs of corrosion during trials in September 2019 and were not used for further trials (data of biofouling growing in those conditions were discarded from the analyses to avoid biased results).

Figure 1. Test site location in south-west Europe.

Table 1. Biofouling and frictional resistance sampling events. Each grey box corresponds to a continuous submersion period of samples (numbers identify the number of submersion weeks). Light grey corresponds to frictional resistance data available for the analyses.

Month / Sample	Spring								Summer												Autumn							
	May				June				July				Aug				Sep				Oct				Nov			
LC1				1		2			3				1				1				4							
LC2				2					4								1				4							
LC3				4					4								2				6							
LC4				4					5								2				6							
LC5				4					5								3				8							
LC6				4					5								3				8							
NC1				4					4				4															
NC2				4					5																			

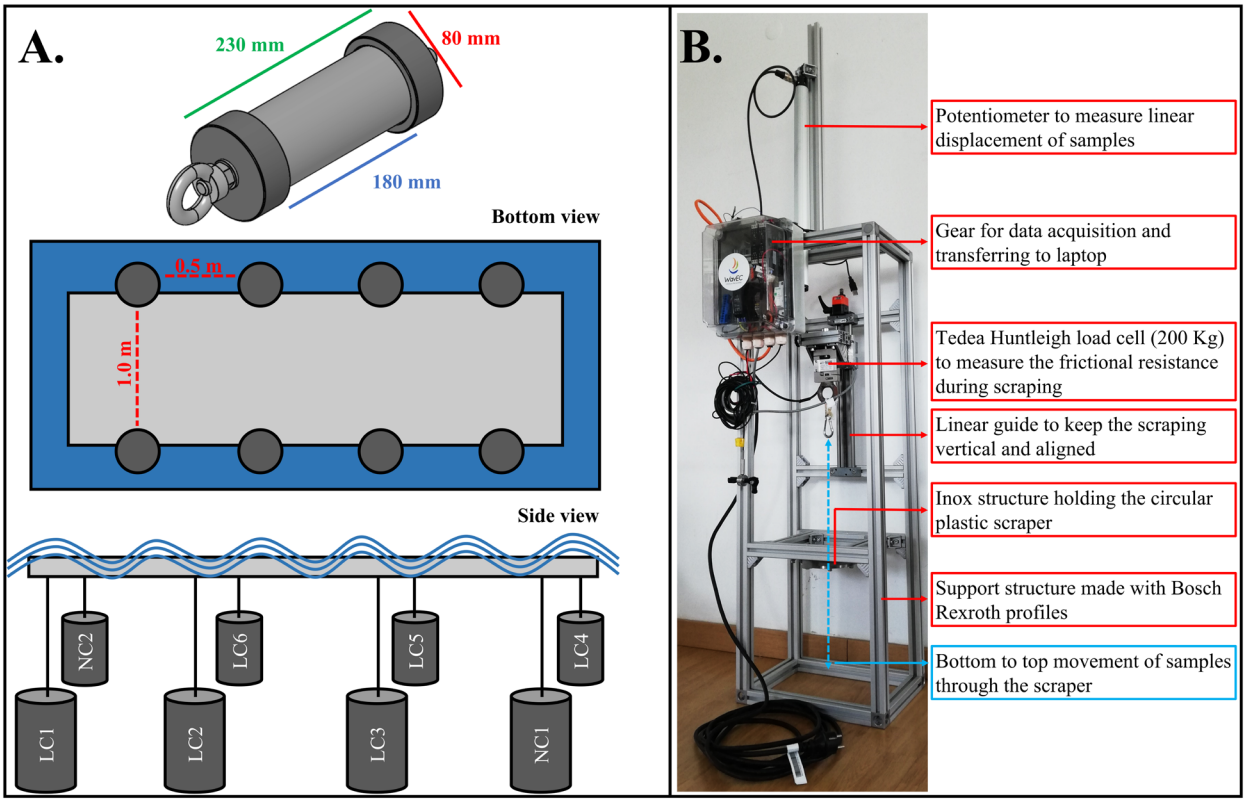

Figure 2. A. Cylinders and deployment design. B. The setup used for cylinders scraping.

The cylinders were processed following a stepwise methodology (example is given for sample LC1):

1. Each sample was retrieved from field after a first submersion period (shown in Table 1; in the case of LC1 it was after one week of submersion in May);

2. In the laboratory, each sample thickness (mm) was measured using a watertight digital calliper;

3. After, each sample was placed in the test rig conceived by WavEC and CorPower Ocean (Figure 2B.) submerged in water and was scraped with a circular plastic scraper. The aim was to recover the biofouling from the cylinders and to measure the frictional resistance forces created during the removal of biofouling. The frictional resistance data was acquired by force and displacement measurements using a loadcell and a potentiometer, respectively. These sensors were connected to the cylinder shaft that was pulled along a motorized linear guide;

4. After being scraped from the cylinders, the biofouling was sieved gently through a 0.5 mm mesh sieve and the organisms retained were processed. Each cylinder sample was then gently cleaned using a sponge and liquid detergent and was re-deployed in the field for another submersion period (in the case of LC1, for submersion during the second and third weeks of June 2019). The plastic scraper was replaced by a new one to avoid any indentations which could scratch the next sample;

5. Upon processing, all biofouling organisms were identified, counted, and weighed. Taxonomy for macroinvertebrates and macroalgae was done to the lowest taxonomic level possible and was standardized in accordance to the World Register of Marine Species (WoRMS) and the AlgaeBase, respectively.

In parallel to biofouling sampling, seawater temperature (°C), salinity, dissolved oxygen (DO; mg L^{-1}) and total chlorophyll (Chl.; µg L^{-1}) were measured at 3 m depth using a YSI ProDSS handheld multiparameter probe. With no particular reason, a greater number of measurements coincided with low tides (spring: two out of two sampling events; summer: three out of six sampling events; autumn: three out of five sampling events).

Data analysis

All statistical analyses were performed with PRIMER 6 + PERMANOVA software (Anderson *et al.*, 2008; Clarke & Gorley, 2006). The PERMANOVA, SIMPER, and PCO analyses can be performed using open-source software such as R (using the Vegan package in R) or PAST (except SIMPER;

PAST available from the University of Oslo Natural History Museum website). A PRIMER trial version can be downloaded from the PRIMER website.

Seawater parameters. For each seawater parameters (temperature, salinity, DO, and Chl.), statistically significant differences among seasons were tested using permutational multivariate analysis of variance (PERMANOVA). The design included one fixed factor, 'Season' (three levels: spring, summer, and autumn). The Euclidean distance was used in the calculation of the resemblance matrix. The statistical significance of variance components was tested using 999 permutations and unrestricted permutation of raw data, with a significance level of $\alpha = 0.05$.

Biofouling parameters. Prior to data analysis, macroinvertebrate density was standardized to number of individuals per square metre (ind m^{-2}), and invertebrate and algae biomass were standardized to grams of fresh weight per square metre (g FW m^{-2}).

Six biofouling parameters were used to describe the biofouling communities. Four were univariate parameters: number of taxa (*Richness*), total biofouling biomass (*TBiom*), total biofouling density (*TDens*) and *Thickness*, and two were multivariate parameters: organisms biomass (*BIOM*) and density (*DENS*).

For statistical analysis of biofouling data, the feasibility of using the data of both cylinder treatments – LC and NC – together in subsequent analyses was first assessed. Statistically significant differences between the two treatments were tested using PERMANOVA applied individually to *Richness*, *TBiom*, *TDens, Thickness, BIOM*, and *DENS*. The statistical design included the fixed factors 'Treatment' (two levels: LC and NC), 'Season' (three levels: spring, summer, and autumn) and 'Submersion' (seven levels: 1, 2, 3, 4, 5, 6 and 8W) nested in 'Season'. The Euclidean distance (univariate data) or Bray Curtis similarity (multivariate data) were used in the calculation of resemblance matrices, with addition of a dummy variable of the lowest value in the source data matrix. Prior to calculating the resemblance matrices, *TBiom, TDens, BIOM* and *DENS* data were square root-transformed. The statistical significance of variance components was tested using 999 permutations, with unrestricted permutation of raw data (univariate data) or permutation of residuals under a reduced model (multivariate data), with a significance level of $\alpha = 0.05$. When the possible permutations were <100 the Monte Carlo *p* value was selected.

After, using the LC and NC data combined (because no statistical differences were previously found; see *Extended data*), statistical differences among seasons and among submersion periods within season were assessed individually for *Richness*, *TBiom*, *TDens* and *Thickness*. The statistical design included the factors 'Season' and 'Submersion' nested in 'Season', and the same options were used as for the previous PERMANOVA.

Following this, analysis of similarity percentages (SIMPER) was applied individually to *BIOM* and *DENS* to identify the taxa which contributed mostly to the statistical differences. First, dissimilarities among seasons were assessed using two-way crossed designs with factors 'Season' and 'Submersion'. Then, dissimilarities among submersion periods within season were assessed selecting each season data and using a one-way design with the factor 'Submersion'. For all SIMPER analyses a 95% cut-off was used, without transformation of data.

Relation between the biofouling and seawater parameters. To visualize the seasonal relation between biofouling parameters (*Richness, TBiom, TDens,* and *Thickness*) and seawater parameters (temperature, salinity, DO, and Chl.) a principal coordinates analysis (PCO) was conducted. To do this, the seawater parameters data were averaged per season and that value was used for each biofouling sample in that season (*e.g.* spring water temperature was the same for the spring biofouling 1W, 2W, and 4W samples).

Frictional resistance forces data. The friction forces data obtained by scraping biofouling from the sample cylinders were pre-processed to remove outliers, *e.g.* associated with the acceleration at start or deceleration at stop of the scraping system owed to the tightness of the plastic scraper to the samples.

Results

Mean water temperature was greater in the summer, while spring registered greater salinity, DO, and Chl. (the latter two decreasing from spring to autumn) (Table 2A., Figure 3). Statistically significant differences were found among seasons for water temperature, salinity, and Chl. (*Extended data*).

The biofouling growth was noticeable with increasing submersion time of samples (Figure 3, Figure 4, Table 3). *Richness* (statistically different among all seasons; *Extended data*) and *Thickness* (statistically different between summer and the other seasons; *Extended data*) were greater in summer at the maximum submersion of 5W. *TBiom* (statistically different between autumn and the other seasons; *Extended data*) and *TDens* (statistically different among all seasons; *Extended data*) were greater in autumn at the maximum submersion of 8W (Table 2B.).

The above trends were reflected by some species succession in the colonization process (Table 3). For example, after one week of submersion, only the opportunistic green algae (*Ulva* sp.), barnacles (*Perforatus perforatus* and the NIS *Austrominius modestus*) and bryozoans were recorded; after two weeks, filamentous brown algae (*Hincksia* sp./*Sphacelaria* sp.), red algae (*e.g.* from the order Ceramiales) and several crustaceans fauna amphipods were observed; after three or more weeks, several other macroalgal and macroinvertebrate taxa joined the biofouling communities.

The SIMPER analyses using the organisms' individual biomass (*BIOM*) and density (*DENS*) were largely in agreement with the overall trends in total biomass (*TBiom*) and total density (*TDens*). Using *BIOM*, nine taxa were the main contributors (cut-off 95%) for the dissimilarities among seasons and among submersion periods within season (*Extended data*).

Table 2. Seasonal values (mean ± standard deviation, except for Richness) for the seawater parameters (A.) and the biofouling parameters (B.). Greater numbers are presented in bold.

A.		Temperature (ºC)	Salinity	Dissolved Oxygen (mg L^{-1})	Chlorophyll (µg L^{-1})
Spring		16.8 ± 0.2	**40.6 ± 0.1**	**7.23 ± 0.29**	**2.63 ± 0.73**
Summer		**18.5 ± 1.2**	38.6 ± 1.1	6.83 ± 0.68	1.50 ± 1.13
Autumn		16.7 ± 1.6	38.9 ± 1.2	6.84 ± 0.21	0.76 ± 0.28
B.		***Richness***	***Thickness**** **(mm)**	***TBiom**** **(g FW m^{-2})**	***TDens**** **(ind m^{-2})**
Spring	1W	2	0.20 ± 0.00	0.03 ± 0.00	15.3 ± 0.0
	2W	6	0.25 ± 0.21	0.71 ± 0.40	38.3 ± 54.1
	4W	**15**	**1.05 ± 0.22**	**35.6 ± 10.6**	**512.6 ± 180.7**
Summer	1W	4	0.11 ± 0.18	0.09 ± 0.16	12.1 ± 20.9
	2W	9	0.10 ± 0.00	0.58 ± 0.14	63.3 ± 12.8
	3W	16	0.76 ± 0.55	5.7 ± 3.3	482.3 ± 297.4
	4W	**19**	1.65 ± 0.66	13.1± 2.5	2224.6 ± 478.0
	5W	**19**	**1.90 ± 0.26**	**23.8 ± 8.8**	**2694.8 ± 373.6**
Autumn	4W	15	**0.49 ± 0.01**	21.6 ± 0.31	1039.9 ± 217.4
	6W	14	0.44 ± 0.47	35.6 ± 15.1	2929.9 ± 511.5
	8W	**18**	0.37 ± 0.37	**44.4 ± 12.6**	**4928.4 ± 217.4**

* Mean ± standard deviation

With few exceptions, the biomass of those taxa was greater in autumn, and in every season increased with increasing submersion period (*Extended data*). Using *DENS*, seven taxa were the main contributors to the dissimilarities (*Extended data*). Although most of these organisms, and especially barnacles, registered greater density in the summer, the greatest values were presented by Amphipoda in autumn. With few exceptions, those taxa showed increasing density with increasing submersion period within each season (*Extended data*).

With regards to the frictional resistance forces created when scraping the biofouling from the cylindrical samples, the trend was slightly contrary to that of the biofouling growth. Mean friction forces were about 250 N at 1W in spring and appeared to decrease to about 100 N with increasing submersion period until the 4W-5W submersion periods (Figure 5A.). Also, mean friction forces increased with subsequent scrapings of the same sample (Figure 5B.).

Discussion

In this study, and as expected for this region, seasonal patterns were observed in the seawater parameters and the biofouling composition, richness, and abundance virtually accompanied seasonal changes. It was also expected that the greatest biofouling weight and density would be registered in spring and/or summer when the higher temperatures would favour the reproductive and growth rates of organisms (*e.g.* Gili & Petraitis, 2009; Newell & Branch, 1980). Although both parameters were highest in autumn (at eight weeks of submersion), when considering the longest submersion period (four weeks) common among the three seasons, spring registered the greatest biomass (greatly associated with filamentous green and brown algae), while summer registered the greatest density (greatly associated with barnacles and amphipods). Thus, it could be expected that biofouling growth would be greater in these seasons instead of autumn if it was allowed for over four-five weeks. Accordingly, it is recommended that biofouling-related maintenance activities in temperate to cold regions are performed after warm seasons (for example summer) to avoid the elevated biofouling growth and, thus, to minimize the number of maintenance activities until the next season (for example the next spring) most suitable for the breeding, spawning, and settlement of numerous biofoulers (*e.g.* Anil *et al.,* 2012; Hellio & Yebra, 2009; Kupriyanova *et al.,* 2001).

Biofouling biomass and thickness, both associated with macrofouling, are key biofouling parameters affecting several industries working in the marine environment (*e.g.* Jusoh & Wolfram, 1996; Miller & Macleod, 2016; Tiron *et al.,* 2012; Titah-Benbouzid & Benbouzid, 2017; Yang *et al.,* 2017). In

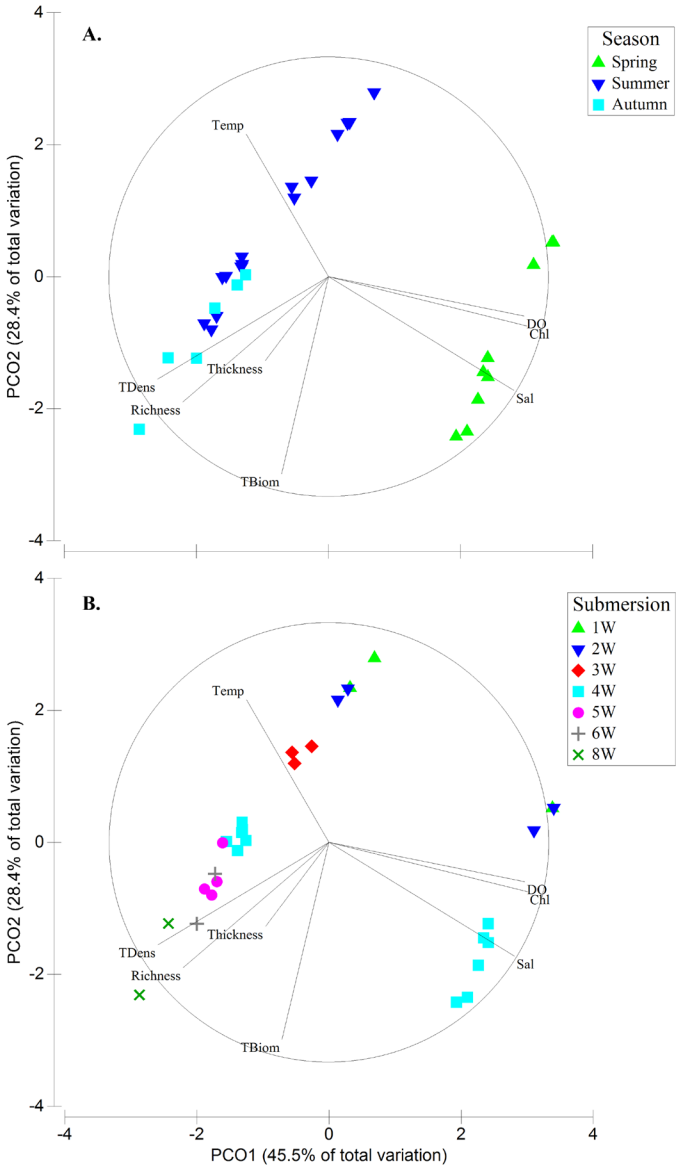

Figure 3. Principal Coordinates analysis (PCO) plot showing trends of seawater parameters (temperature, salinity, dissolved oxygen [DO] and total chlorophyll [Chl.]) and biological parameters (*Richness, Total biomass [TBiom], Total density [TDens]* and *Thickness*) among seasons (A.) and submersion periods (B.).

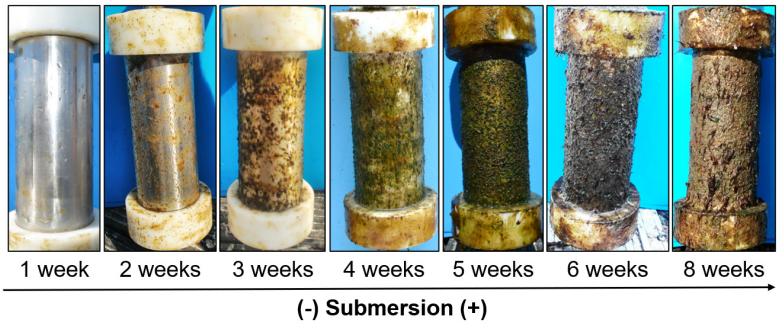

Figure 4. Biofouling growth after one, two, three, four, five, six and eight weeks of samples submersion.

Table 3. List of taxa found in this study, showing their presence (in grey) across submersion periods (1–8W) within each season. Total macroalgal and macroinvertebrate taxa are presented per submersion period and per season at the bottom. The number of occurrences (occ.) of each taxon in the study is shown on the right side. Greater numbers are presented in bold.

Group			Taxa	Spring			Summer					Autum			Occ.
				1W	2W	4W	1W	2W	3W	4W	5W	4W	6W	8W	
Macroalgae	Ph. Chlorophyta	Or. Ulvales	*Ulva* sp. (tubular-like form)	■	■	■		■	■	■	■	■	■	■	**10**
			Ulva sp. (leaf-like form)		■	■	■	■	■	■	■	■	■	■	**10**
	Ph. Rhodophyta	Or. Ceramiales	cf. *Tiffaniella capitata*			■		■	■	■	■	■		■	7
			cf. *Pterothamnion crispum*			■				■	■	■		■	5
			Polysiphonia sp.			■		■	■	■	■	■	■	■	8
			cf. *Halurus flosculosus* / *Bornetia secundiflora*			■			■	■	■	■	■	■	7
		Ph. Rhodophya	Rhodophyta N.I.			■		■	■	■	■	■	■	■	8
	Cl. Phaeophyceae	Or. Ectocarpales / Or. Sphacelariales	*Hincksia* sp. / *Sphacelaria* sp.		■	■		■	■	■	■	■	■	■	**9**
Macroalgal taxa per submersion period				1	4	**9**	1	7	8	**9**	**9**	**9**	7	**9**	
Macroalgal taxa per season					**9**				**9**				**9**		
Macroinvertebrates	Ph. Bryozoa	Ph. Bryozoa	Bryozoa N.I.				■	■	■	■	■	■	■	■	8
	S.Ph. Crustacea	Or. Amphipoda	Amphipoda N.I.		■	■		■	■	■	■	■	■	■	**9**
			Caprella equilibra			■			■	■	■	■	■	■	7
		Or. Decapoda	cf. Anomura N.I. / Brachyura N.I.				■			■	■				3
			cf. *Pasiphaea sivado*			■			■	■			■	■	5
		Or. Isopoda	Gnathiidae N.I.						■		■				2
			Tanais dulongii			■			■	■	■	■	■	■	7
		Or. Sessilia	Barnacles (*Perforatus perforatus, Austrominius modestus*)	■	■	■	■		■	■	■	■	■	■	**10**
	Cl. Pycnogonida	Or. Pantopoda	*Ammothella longipes*											■	1
	Cl. Polychaeta	F. Serpulidae	*Spirobranchus* sp.			■			■	■	■	■	■	■	7
		F. Syllidae	Syllidae N.I.							■	■				2
	Ph. Mollusca	Cl. Bivalvia	*Mytillus galloprovincialis*							■	■				2
		Cl. Gastropoda	cf. *Crisilla semistriata*											■	1
Macroinvertebrate taxa per submersion period				1	2	**6**	3	2	8	**10**	**10**	6	7	**9**	
Macroinvertebrate taxa per season					6				**11**				9		
Total taxa per submersion period				2	6	**15**	4	9	16	**19**	**19**	15	14	**18**	
Total taxa per season					15				**19**				18		

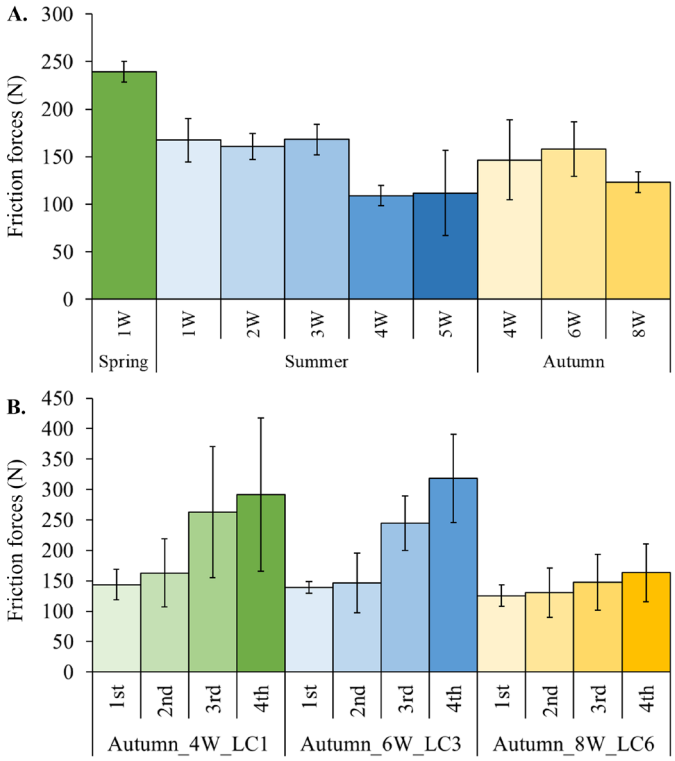

Figure 5. A. Frictional resistance forces (mean ± standard deviation) across submersion periods (1-8W) in different seasons. B. Frictional resistance forces (mean ± standard deviation) from four subsequent scrapings of three different samples.

the present study, the values observed for those two parameters were quite below the values registered in more hydrodynamic areas (*e.g.* nearshore/offshore) and longer submersion periods (*e.g.* Vinagre *et al.,* 2022), even at a nearby harbour (OCEANIC project European Biofouling Database, Vinagre *et al.,* 2020). Accordingly, the weight and size of biofouling organisms (algae, bryozoans, barnacles, mussels, and calcareous tubeworms) which usually cause greater direct physical damage to structures/components (e.g. damaging the substrates or their protective coatings by boring into them, or when pulled by currents and waves) were also low. Therefore, it seems unlikely that biofouling growing for six to eight weeks under the present conditions would greatly increase the loading, frictional resistance, or surface diameter of structures/components. In fact, with regards to frictional resistance, it was found that during these early colonization stages the slippery nature of biofouling could be acting as a 'lubricant' leading to lower forces generated from scraping the samples in areas with biofouling compared to areas without biofouling. If the eight weeks can be accepted as a safe time interval to perform biofouling-related maintenance actions, then physical control, for example using water jetting/cavitation or acoustic methods (*e.g.* Legg *et al.,* 2015), could be an option to maintain the components' integrity and equipment functionality and performance. However, that will depend, among many other factors, on the type of structure/component and its functional requirements (for example, free-moving versus static), the location (for example, latitude, seawater temperature, distance to shore) and hydrodynamic conditions (for example, current velocity and wave exposure) of the site, the depth at which the structure/component is positioned (for example, surface versus mid water column), and season (for example, warm seasons versus cold seasons) (*e.g.* Hellio & Yebra, 2009; Vinagre *et al.,* 2020).

Besides physical damage to structures/components by biofouling, detrimental issues may arise quickly concerning different types of corrosion (*e.g.* Blackwood *et al.,* 2017; Jia *et al.,* 2019; Kleemann, 1996; Videla & Herrera, 2005). In the present study, corrosion was observed after one week of deployment in components untreated against marine-induced corrosion (stainless steel nuts used to tighten the caps) as well as in sections of NC samples (possibly owed to inefficient waterproofing of the untreated portion by the end caps) after four to five weeks of submersion in summer. This reinforces the importance of employing adequate anti-corrosion techniques to metallic substrates used in marine conditions (even if for short periods of time), for example by applying thermally sprayed aluminium which has proven capability to protect steel substrates (*e.g.* Syrek-Gerstenkorn *et al.,* 2019; Syrek-Gerstenkorn *et al.,* 2020; Vinagre *et al.*, 2022) or laser-cladded materials (*e.g.* Stellite) which in the present study showed good anti-corrosive efficiency, depending on the specific needs and cost.

Another concern, environment-related, is the settlement and propagation of NIS using the biofouling assemblages growing on MRE devices/structures. This is because NIS may pose serious ecological threats by competing with, predating on, and/or excluding indigenous organisms, affecting community composition and structure, and potentially causing habitat modifications (*e.g.* Cook *et al.,* 2014; Crooks, 2002; Lengyel *et al.,* 2009), consequently affecting ecosystems functioning and ecosystem services provision.

In the present study, although some succession in biofouling colonization was observed, the presence of hard-fouling organisms such as barnacles after only one week of submersion is aligned with a more 'probabilistic model' of colonization (Clare *et al.,* 1992; Maki & Mitchell, 2002) rather than a 'successional model', meaning that other organisms in the area, including NIS, can also settle in the artificial substrates early. At present, one NIS – the Australasian barnacle *A. modestus* – was found with great frequency and density. It is highly possible that its introduction in the area was caused by the shipping industry, considering the great traffic of ships (commercial, industrial and leisure) into and out of the Tagus Estuary. Unfortunately, after one to two weeks of samples submersion, some individual barnacle were very small and fragile and could not be well distinguished between *A. modestus* and *P. perforatus*. Hence, it is only certain that the NIS was registered after three weeks of submersion. As a vector of NIS propagation biofouling is comprised in legislative frameworks (*e.g.* EU Directive 2008/56/EC, EU Regulation 1143/2014) that aim to prevent or manage the introduction and spread of NIS. Thus, it is valuable for the preservation of marine ecosystems and for MRE project developers to implement biosecurity risk management plans that can appropriately address biofouling and NIS propagation on their structures at sea (*e.g.* Cook *et al.,* 2014; Payne *et al.,* 2014). This should be especially considered for MRE projects undertaken in areas where numerous NIS are registered, such as those next to shipping lanes, commercial harbours, or nearshore/offshore, for example in the North Sea (*e.g.* De Mesel *et al.,* 2015; Kerckhof *et al.,* 2018; Vinagre *et al.,* 2020). An important outcome to the developers could be that such management plans support or complement environmental impact assessments, potentially increasing the acceptability of projects and speeding up the licensing process.

Conclusions

The results of this study indicate that avoiding the greater availability of hard-fouling colonizers attaching to the devices early in the submersion period may contribute to reduce and delay further hard-fouling attachment with effects on materials preservation, increasing the extension of the period until the next cleaning operation. It is recommended to conduct biofouling-related maintenance activities after the peak of maximum growth and reproduction, generally occurring during the warmer seasons in temperate to cold environments. This way, the number of cleaning activities until the next growing season suitable for the breeding, spawning and settlement of numerous biofoulers can be reduced. The detection of NIS in this study after submerging artificial substrates for a short period (maximum of three weeks) highlights the importance of biofouling monitoring and the implementation of biosecurity risk assessment plans early in the operational phase of MRE projects as a good practice to maximise the prevention of NIS settlement.

Data availability

Underlying data

Zenodo: Early biofouling colonization stages: Implications for operation and maintenance planning in Marine Renewable Energy projects, https://doi.org/10.5281/zenodo.6974716 (Vinagre & Fonseca, 2022a)

This project contains the following underlying data:

- Open Research Europe_Biological data.xlsx (Biofouling data)

Zenodo: Early biofouling colonization stages: Implications for operation and maintenance planning in Marine Renewable Energy projects, https://doi.org/10.5281/zenodo.6974740 (Vinagre & Fonseca, 2022b)

This project contains the following underlying data:

- Open Research Europe_Friction forces_all samples.xlsx
- Open Research Europe_Friction forces_subsequential scrapings.xlsx

Extended data

Zenodo: Early biofouling colonization stages: Implications for operation and maintenance planning in Marine Renewable Energy projects, https://doi.org/10.5281/zenodo.6962235 (Vinagre & Fonseca, 2022c)

Acknowledgements

The authors thank Pedro Pires, Erica Cruz (both former WavEC Offshore Renewables, Portugal) and Antoine Bonel (CorPower Ocean, Sweden) for the help in designing and building support structures and in sampling.

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Open Peer Review

Current Peer Review Status: ✗ ?

Version 1

Reviewer Report 28 July 2023

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?

Lenaïg Hemery
Pacific Northwest National Laboratory, Washington, USA

The article by Vinagre & Fonseca is interesting and describes results from a field study relevant to the development of the marine renewable energy industry. However, the manuscript would benefit from several edits to the text to clarify some parts, and from additional data analyses to identify any correlation between biofouling and frictional resistance. See comments in the PDF file provided here.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Benthic ecology, marine renewable energy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 13 July 2023

https://doi.org/10.21956/openreseurope.16047.r32422

Andrew J Guerin
Independent Researcher, Toronto, Canada

Vinagre and Fonseca study the early stages of the colonisation of surfaces in a site in Portugal, in the context of the development of a renewable energy device component. They record several biofouling metrics and assess community composition.

Overall issues:

1) It is not completely clear what the purpose of this study was, and no hypotheses are stated; “to increase understanding on biofouling community structure” is very vague. Since the study was part of a larger project developing a power take-off system for renewable energy devices, it was apparently designed around testing components for this device. The data generated were undoubtedly important for the device developers, but it is not clear what the study contributes to our broader understanding. The measurement of frictional resistance during scraping is relatively novel, but the authors do not really explain why this data is useful or interesting. Given the small sample sizes, and the fact that link between the test samples and operational devices is weak, the strength of the evidence supporting the recommendations is limited.

2) Since all data were collected in one year, ‘season’ is not replicated. It is therefore not strictly valid to claim that seasonal effects have been assessed. Biofouling assemblages change over time, and in the location studied, seasonal changes are very likely to be important drivers of the observed differences. However, multiple years data are needed to distinguish seasonal differences from those resulting from other short- and long-term changes. The authors should be more careful in their language when discussing these temporal effects.

3) Some aspects of the data have not been fully analysed. There are some technical details of the data analysis and presentation that need to be clarified (see below), and some places where more detail is required.

4) It is always good to see the data being made available, but there is little metadata to help the user understand what is actually provided.

Details:

Introduction:

a) “Marine biofouling is a natural process”. Especially since this is not a specialist journal, it is important to define what ‘marine biofouling’ is, here at the start of the paper.

b) The text on climate change is a bit tenuous. Not all research has to be tied to climate change to be of value. The current text is also a little confusing. It is mentioned that climate change may be detrimental to some organisms that often make up fouling communities, but that sounds like it would be a good thing! However, marine biofouling is often extensive and rapidly-developing in warmer regions, so an important point that is missed here is that biofouling in temperate and polar seas could become more severe with ocean warming.

c) To say that mechanical techniques are ‘capable of being totally efficient’ is not really correct. True, in optimal conditions, correctly applied, on a flat surface, mechanical techniques could remove most fouling. However, it seems unlikely that they would be able to remove everything, including all larvae and microfouling. Their negative sides are not mentioned. They will not be effective on moving parts, complex surfaces and niche areas (seawater intakes, vessel sea chests, heat exchangers, etc.) which are often problem areas for biofouling and are common on renewable energy devices. Mechanical techniques could also damage/remove existing anti-fouling and anti-corrosive coatings.

d) It is true that deployment/maintenance of equipment could be timed to minimise fouling, but is this really realistic? Are deployment/maintenance schedules flexible enough? Operators may select summer for maintenance because this is when ocean conditions (wind, wave) are best for offshore operations and therefore lower-risk.

Methods:

e) More information is needed on the measurement of friction forces. How many measurements for each sample and/or at what sampling frequency were they collected? There are a lot of numbers for each sample at each sampling time in the extended data, it would be useful to know why there are differences in the amount of data for each sampling event.

f) Why was PERMANOVA used for analysis of univariate measures? I do not have a problem with this method but no reason is given for why a conventional ANOVA (on raw or suitably-transformed data) was not acceptable. Were the data very heteroscedastic, for example?

g) For biofouling thickness, how many measurements were taken on each sample at each sampling event - just one, or are the data an average of several measurements? This should be made clear.

h) Why were the data for some univariate variables (TBiom, TDens) square-root transformed for analysis? This is often done to help data meet ANOVA assumptions, but since PERMANOVA was used, such transforms may not be necessary. For the multivariate analyses (BIOM, DENS) why was the same (square-root) transform used? The justification for transforming multivariate data is usually to prevent one or two abundant species from dominating the analysis, so it seems odd (at

first glance) that the same transform was used as for the univariate variables. Why were SIMPER analyses then run on untransformed data? It would seem more logical to use the same transforms as for the PERMANOVA. Whatever the reasons for the transforms that you used, it is important to justify these decisions.

i) Once the authors decided to combine the LC/NC data, why were no PERMANOVA analyses carried out on the multivariate data (BIOM, DENS)? This is very important to demonstrate the significance of any differences among 'seasons' and deployment durations. The SIMPER analyses are useful for examining patterns of resemblances and identifying important species, but they do not show whether or not these differences are statistically significant in the first place.

Results:

j) It seems that the differences among seasons in DO were not statistically significant. Therefore it is not appropriate to say that DO was greater in spring. Similarly in Table 2A the DO value for spring should not be highlighted as being greater if the difference was not significant.

k) Table 2 - generally only statistically significant differences should be highlighted. Then 'Significantly greater ($p < 0.05$) numbers are presented in bold' can be stated in the legend. Also, since the legend stated that the values are mean ± standard deviation, it is not necessary to repeat this with an * and a footnote under the table.

l) There is very little information on the fouling composition and the actual differences among samples taken at different times and after different lengths of deployment. This is particularly notable since the primary stated aim of the work was to increase understanding of community structure. The data are available for download, but something should be presented in the paper, even if just to highlight which were the key taxa identified by SIMPER. A plot giving the proportional contributions of different taxonomic groups (eg. % biomass for algae, crustacea, etc.) at different times/sampling durations would be more informative.

m) More description of the multivariate patterns would be worthwhile. The PCO plots are useful but some discussion of these would be useful.

n) What does it mean to say that the SIMPER agrees with the overall trends in TBiom/TDens? There should be more detail about the actual patterns.

o) The big problem with the friction data is that there does not appear to have been any statistical analysis, which limits what we can infer from the data.

p) More information is needed to make sense of Figure 5, particularly sample sizes.

q) Does 5A show the mean ± sd of all 6 (or 8) samples? Are these differences statistically significant?

r) For 5B, why these 3 samples from these three time periods? Are they generally representative of other samples at other times? Some information is needed on why these were selected. I assume that for each bar, the mean and SD are those of all the measurements collected during the

scraping of each sample. Again, are any of these differences statistically significant?

s) 5B it is not all clear what is meant by subsequent scrapings. There is nothing about subsequent scrapings in the methods. I have to admit that I find this confusing.

Discussion:

t) It is important to note that the weight added to a structure by biofouling is not the same as the fresh weight of those organisms in air. The density of the organisms is important - soft fouling species such as algae, tunicates, etc. will incorporate a lot of water and will have some natural buoyancy which will reduce their weight in the water. In these cases it is the increased roughness and hydrodynamic drag resulting from fouling by these species which increases structural loading.

u) Similarly, the actual thickness of biofouling in water will differ from what is measured using calipers at the surface.

v) The discussion seems to link frictional resistance/structural loading in water with the frictional resistance measurements obtained during scraping. It is very unlikely that frictional resistance during scraping is a good proxy for hydrodynamic load in operational conditions, and no evidence is provided (or cited) that the force required to remove fouling organisms is correlated with loading on fouled structures in this way. If such evidence exists, it should be mentioned here. If the authors were not intending to make this implied link, they should be clearer, and separate the discussion regarding scraping from that regarding structural loading.

w) “In fact, with regards to frictional resistance, it was found that during these early colonization stages the slippery nature of biofouling could be acting as a ‘lubricant’ leading to lower forces generated from scraping the samples in areas with biofouling compared to areas without biofouling.” It is not clear where this inference has come from. If it is from the results of this study, the authors need to be clearer about how they arrived at this conclusion, and what data support it. If it has come from literature, additional citations would be needed to support this claim.

Minor/Typographical:

Abstract:

- Instead of “small-scale wave energy components” perhaps ‘small-scale wave energy device components’ or something similar?

- “forces generated to scrape” - something like ‘frictional resistance forces generated during scraping’ (as used elsewhere in the paper) would be better.

Introduction:

- I am not familiar with standard terms in the MIC literature, but is it really correct to say that MIC is ‘initiated’ by micro-organisms, or is it just accelerated/enhanced?

- Another concern related to biofouling is that it creates opportunity for NIS…” perhaps the meaning would be clearer if this read: ‘Another concern related to biofouling of renewable

energy structures is that...".

Results:
- "several crustaceans fauna amphipods were observed". I'm not sure what this means.

Discussion:
- "...NIS using the biofouling..." Perhaps 'NIS within the biofouling assemblages' would be preferable, since NIS are part of the biofouling assemblages, not something separate.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Marine biofouling, aquatic ecology, non-native marine and freshwater organisms

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Comments on this article

Version 1

Author Response 29 May 2023

Pedro Vinagre

There is an issue in Table 3 as the information on the species present in each sample is not shown.

Competing Interests: No competing interests were disclosed.