RESEARCH ARTICLE

REVISED Experimental insights on biofouling growth in marine renewable structures [version 2; peer review: 2 approved]

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Abstract

Background

Marine biofouling is a threat to industries working in the marine environment, representing significant 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 inspections and biofouling removal procedures.

Methods

This study investigated important parameters of macrofouling (*e.g.* composition, including the presence of non-indigenous species, thickness, and weight) from communities growing on samples that emulate tubular components of marine renewable devices. 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 during the scraping of biofouling from those components were investigated.

Results

Overall, results provide insights on the growth rates and removal requirements of biofouling in marine components. The results show that, while biofouling growth in early colonization stages might not present great detrimental effects to wave energy components, the consequent marine corrosion (fostered by biofouling) and the

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

settlement of non-indigenous species (NIS) should be factors of concern.

Conclusions

Performing biofouling-related maintenance activities after the peak of maximum growth and reproduction (during the warmer seasons in temperate to cold environments) is suggested to reduce the number and frequency of activities. NIS can be detected at very early stages 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.

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

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REVISED **Amendments from Version 1**

The manuscript Version 2 addresses all the comments made by the two reviewers. An overall improvement to the methods, results, and discussion sections has been made. Particularly:

Introduction

- Added a definition of marine biofouling

- Improved the text on the contribution of climate change to marine biofouling

- Improved the text about mechanical cleaning of biofouling (negative aspects/caveats)

- Improved description of the WEC sealing system and added Figure 1

Methods

- Improved explanation of the biofouling sampling and processing methodologies

- Clarified "subsequential scraping" of some cylinders to measure the friction forces generated from scraping decreasing levels of biofouling (macrofouling – biofilm – no biofouling)

- Improved explanation of friction resistance forces data acquisition and standardization per sample/submersion period/season

- Added data analysis: relations/correlations between frictional resistance forces and biofouling parameters (using RELATE and Pearson correlations; added in the results as Table 4)

- Applied the same type of data transformation (square root) to data used in PERMANOVA and SIMPER analyses

- Added PERMANOVA analysis based on BIOM (biofoulers biomass) and DENS (biofoulers number of individuals) descriptors

- Added PERMANOVA analysis based on the friction resistance forces data

Results

- Restructured the PERMANOVA and SIMPER section to better highlight the patterns among seasons and submersion periods

- Improved the section about biofouling multivariate patterns; Added key organisms' biomass and density (Table 5) and their contribution within submersion periods in each season (Figure 6, Figure 7, Figure 8)

- Improved Figure 5 (now shown as Figure 9), which now shows all the samples which were scraped more than once (previously only some examples were presented)

Discussion

- Improved text about biofouling weight and thickness measurements (comparison of measurements in water and outside of water)

- Improved suggestion for biofouling cleaning interventions

Any further responses from the reviewers can be found at the end of the article

Introduction

Marine biofouling, i.e. the growth of microorganisms such as bacteria and microalgae (microfouling) and macroorganisms such as barnacles, mussels and macroalgae (macrofouling) on artificial substrates, 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, biofouling (namely macrofouling) adds substantial weight to the equipment and structures (thus modifying their dynamic properties), 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: for example, larger organisms (macrofouling) facilitate microbiologically induced/influenced corrosion (MIC; *e.g.* Jia *et al.,* 2019; Videla & Herrera, 2005) which is initiated or exacerbated 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 of MRE structures (and others installed at sea) 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, including mechanical removal systems, paints, and coatings, among others (*e.g.* Hellio & Yebra, 2009; Vinagre *et al.*, 2020). However, biofouling structure and growth varies greatly depending on the geographical location, season, depth, and substrate composition and roughness, among many other factors. Hence, to date, no AF solution is simultaneously applicable worldwide and efficient against all biofouling organisms.

Biofouling management could become even more challenging in the near future due to climate change. First, ocean warming and acidification can contribute to changes in the expected biofouling communities' structure and abundance (Dobretsov *et al.,* 2019). For example, it could be detrimental to calcifying organisms (*e.g.* barnacles, mussels, tubeworms) which often make the bulk of biofouling, and which might be replaced by soft macrofouling species such as ascidians. On the other hand, marine growth is generally more extensive and rapidly-developing in warmer regions, meaning that biofouling in temperate and polar seas could become more severe with ocean warming. Second, the increased temperature and acidification may lead to changes in the durability and efficacy of some AF solutions (Dobretsov, 2009; Dobretsov *et al.,* 2019). Hence, at present, mechanical techniques such as biofouling removal,

brushing, or scraping appear the most efficient against biofouling. Nonetheless, mechanical methods also present limitations. For example, their effectiveness is maximised on flat (or relatively flat) surfaces and is very much reduced on moving parts and surfaces with complex geometries. Furthermore, biofouling removal using mechanical techniques may damage or remove existing antifouling or anticorrosive coatings.

In the MRE sector, the 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 predetermined periods. Those generally extended periods (several continuous months) allow the biofouling communities to grow and become more complex, thus reaching great abundance and thickness, which for maintenance activities represent several constraints, for example covering the assets whose integrity and functionality need to be assessed (*e.g.* Loxton *et al.*, 2017). On the other hand, understanding the structure and magnitude of biofouling 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 evaluate the best periods to deploy equipment at sea. It also allows to detect early the presence of NIS populations in the area and initiate mitigation measures to stop their proliferation.

The activities that led 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 (WECs) and were encompassed in the work package dedicated to performance assessment and improvement. The WEC tested under this project was developed by CorPower Ocean. It is of the point absorber energy converter type with an oscillating part consisting of a heaving buoy which moves with the motion of incoming ocean waves and a stationary part consisting of the anchoring system, mooring line, ocean rod, and PTO system (Figure 1). Particularly relevant for this research is the sealing system, the combination of the ocean rod surface and a seal gland, which acts as one of the interfaces between the oscillating and stationary parts. The sealing system is critical to the WEC's functioning as it must allow low friction between the two parts to deliver maximum efficiency in the conversion of motion to electricity and prevent ingress of seawater inside the buoy hull (Linden *et al.*, 2022). Thus, CorPower Ocean devised a mechanical cleaning system (scraping system) to prevent biofouling growth which could damage the sealing system and compromise the WEC integrity and functioning.

The present work uses small-scale samples of CorPower Ocean's device ocean rod with two objectives: (i) assess the biofouling growth, namely biofouling composition (including the presence of NIS), thickness, richness, biomass, and density, in the samples during short submersion periods, and (ii) assess the frictional resistance forces generated from scraping the biofouling from the samples using a prototype of CorPower Ocean mechanical cleaning system.

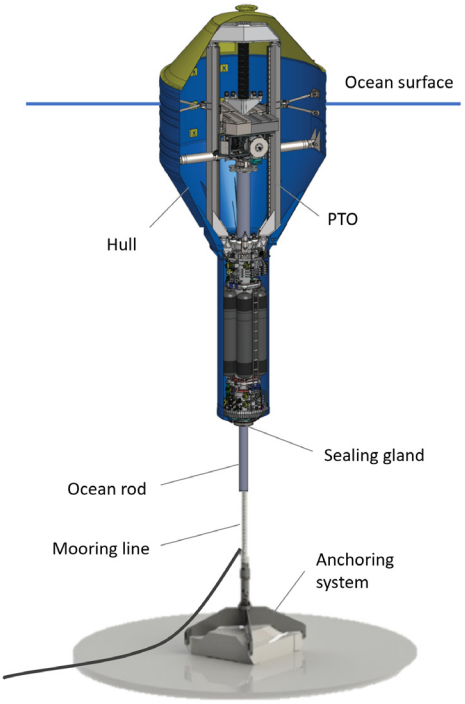

Figure 1. Schematic of CorPower Ocean wave energy device (source: CorPower Ocean).

The overall aim is 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) for coated steel-based tubular components of MRE devices 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), where the experimental investigation of this research was produced, is located in a temperate climate region on the south-western Atlantic coast of Europe, at about 6 km upstream of the mouth of Tagus estuary in Lisbon, Portugal (Figure 2). 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.

The study involved deploying and re-deploying eight cylindrical samples (230 mm × 80 mm; colonizable area: 180 mm × 80 mm) representing the rods of CorPower Ocean's PTO system. The cylinders were made of S355 steel and were coated with two different anti-corrosion treatments (for industry/research-based reasons):

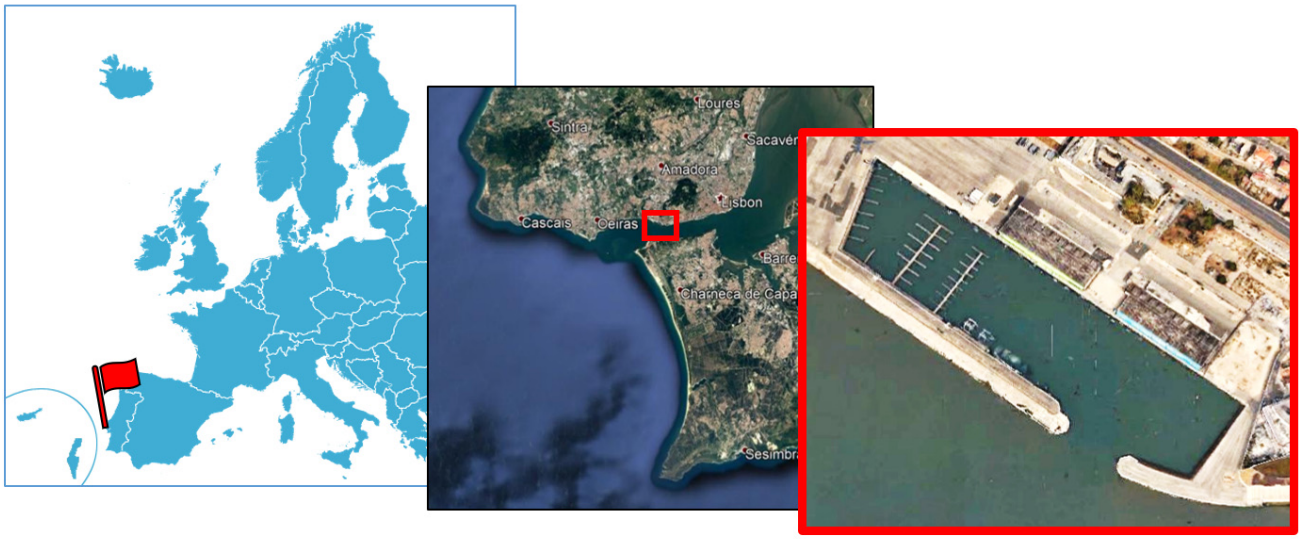

Figure 2. Test site location in south-west Europe.

- 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 electro-plated nickel-chromium; these two samples are hereafter named NC1 and NC2.

The cylinders were suspended in a floating rig and submerged at ~3 m depth for different periods – one, two, three, four, five, six and eight weeks (henceforth designated as 1–8W) – between May and November of 2019 (Table 1; Figure 3A.).

The deployment and processing of the samples followed the stepwise methodology presented below, using LC1 as an example:

1) The cylinders were first deployed for a period; in the case of LC1, this cylinder was first deployed for 1W in May 2019;
2) Then, the cylinders were retrieved from the field for processing in the laboratory;
3) In the laboratory, biofouling thickness (mm) was measured as the highest point from the cylinder surface, associated with the presence of barnacles, bryozoans, or other organisms, using a watertight digital calliper;

Table 1. Biofouling and frictional resistance sampling events. Each coloured box corresponds to a continuous submersion period of samples (numbers identify the number of submersion weeks). Light grey corresponds to samples without frictional resistance data available; Dark grey corresponds to samples scraped once; Black corresponds to samples that were scraped more than once.

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				

4) After, each cylinder was placed in the test rig conceived by WavEC and CorPower Ocean (Figure 3B.) submerged in water and biofouling was scraped with a circular plastic scraper: (i) The frictional resistance data were acquired at a 50 Hz frequency by force

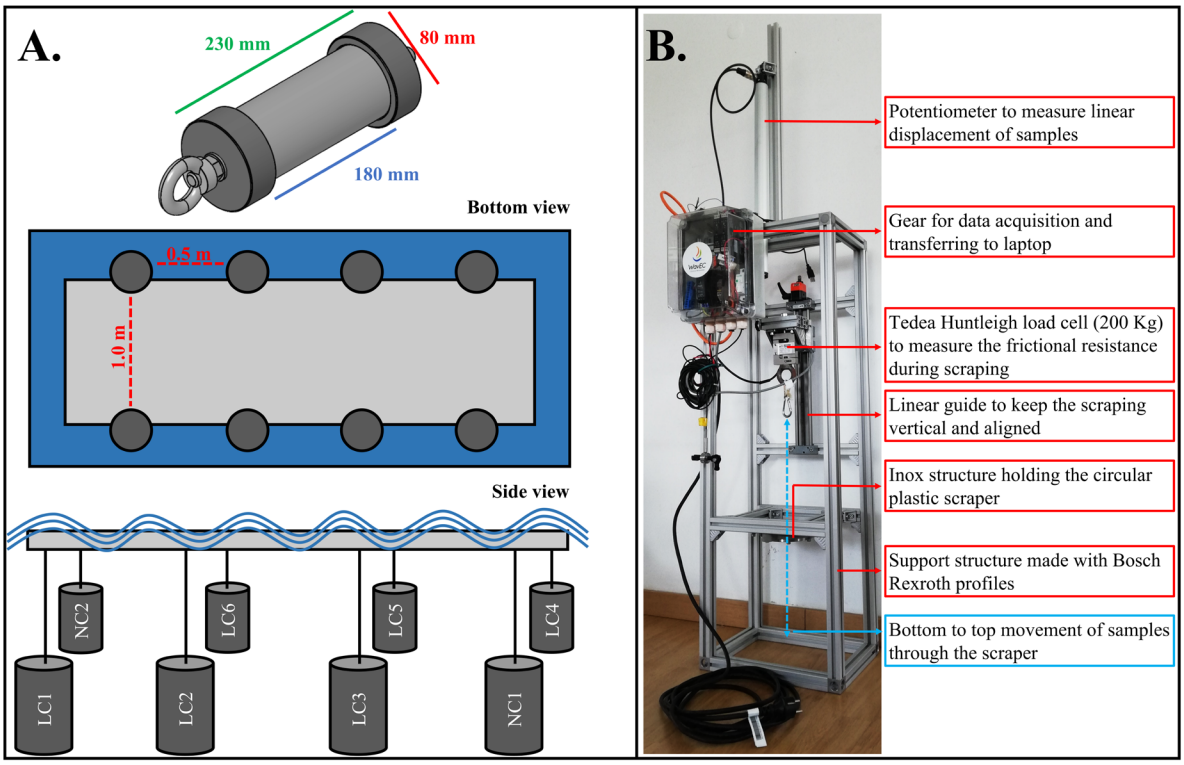

Figure 3. A. Cylinders and deployment design. B. The setup used for cylinders scraping.

and displacement measurements using a load cell and a potentiometer, respectively. These sensors were connected to the cylinder shaft that was pulled along a motorized linear guide (Figure 3B); (ii) Biofouling was kept for posterior processing. In some summer and autumn samples (Table 1), subsequent scrapings were done until complete cleaning was achieved, to measure the frictional resistance forces generated from scraping decreasing levels of biofouling (macrofouling – biofilm – no biofouling);

5) After being scraped, the cylinders were gently cleaned using a sponge and liquid detergent. This allowed to totally clean the cylinder while avoiding abrasion and scratches. The plastic scraper was replaced by a new one to avoid any indentations which could scratch the next cylinder to be scraped;

6) The cylinders were then re-deployed in the field for another submersion period; in the case of LC1 it was for 2W in June 2019.

It should be noted that the NC1 and NC2 cylinders showed signs of corrosion after re-deployments in September 2019, possibly owed to inefficient waterproofing of the untreated portion by the end caps. Corrosion can influence biofouling composition and growth, for example increasing the substrates roughness and physical-chemical properties. Therefore, data of biofouling growing in those samples in September 2019 were discarded from the analyses to avoid biased results and the NC samples were not used for further deployments.

The biofouling processing involved sieving the samples gently through a 0.5 mm mesh sieve. The organisms retained were then identified, counted, and weighed (fresh weight). 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). A PRIMER trial version can be downloaded from the PRIMER website. PERMANOVA is a non-parametric method based on permutation tests that can be used both for univariate and multivariate data, being especially useful when sample sizes are small or there are unequal groups sizes, which is the case of this research.

Seawater parameters. For each seawater parameter (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: individual organisms' biomass (*BIOM*) and density (*DENS*).

For statistical analysis of biofouling data, it was first assessed the feasibility of using the data of both cylinder treatments – LC and NC – together in subsequent analyses. Statistically significant differences between the two treatments were tested using PERMANOVA applied individually to *Richness*, *TBiom*, *BIOM, TDens, DENS*, and *Thickness*. 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. Before calculating the resemblance matrices, *TBiom, TDens, BIOM* and *DENS* data were square root-transformed to reduce the influence of naturally abundant organisms (*e.g.* barnacles) in the analyses. 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.

Afterwards, 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 each season were assessed individually for *Richness*, *TBiom*, *BIOM, TDens, DENS,* 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 each season were assessed selecting each season data and using a one-way design with the factor 'Submersion'. For all the SIMPER analyses a 90% cut-off was used, with square-root transformation of data.

Relationship 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).

Relationship between frictional resistance forces and biofouling data. The number of frictional resistance measurements along a cylinder depended on the velocity of scraping (faster scraping resulting in fewer measurements). As this was a first version of the scraping system, the measurement of scrapings velocity was not possible.

Before assessing the relationship between frictional resistance and biofouling data, the prior was pre-processed. Firstly, outliers were removed, for example, associated with the acceleration at the start or deceleration at the stop of the scraping event owed to the tightness of the plastic scraper to the samples. Then, mean values and standard deviation were calculated using all the samples within a submersion period within a season. For example, the mean ± standard deviation of the sample "summer 1W" was calculated using the measurements of the three "1W" samples of "summer". Using this frictional resistance data**, s**tatistical differences among seasons and among submersion periods within season were assessed with PERMANOVA. The same options as for the previous PERMANOVA of univariate data were used.

The relationships between the frictional resistance forces data and biofouling parameters (*Richness, TBiom, BIOM, TDens, DENS,* and *Thickness*), as well as among biofouling parameters, were calculated with RELATE (comparative Mantel-type tests on similarity matrices). The resemblance matrices for the friction forces and the biofouling parameters (all of which are univariate data) were calculated as previously for PERMANOVA. To match all resemblances matrices, samples without frictional resistance data were removed from the biofouling data matrices prior to calculating the resemblance

matrices. After, Pearson correlations between the frictional resistance forces data and biofouling parameters (*Richness*, *TBiom*, *TDens*, and *Thickness*) were calculated.

Results

Mean water temperature was higher in summer (18.5 ± 1.2 ºC), whereas mean salinity, DO, and Chl. were higher in spring (40.6 ± 0.1, 7.23 ± 0.29 mg L^{-1}, and 2.63 ± 0.73 µg L^{-1} respectively (Table 2A., Figure 4A.). Statistically significant differences were found among seasons for all parameters except DO (*Extended data*).

Biofouling parameters

The biofouling growth was noticeable between seasons and in each season with increasing submersion time of samples (Figure 4, Figure 5, Table 2B., Table 3). Strong statistically significant relationships (RELATE Rho≥0.70) (Table 4A.) were found among all the biofouling parameters, as well as high positive correlations among *Richness, TBiom* (total biomass), and *TDens* (total density) (≥0.70) (Table 4B.).

Richness, TBiom and *TDens* registered higher mean values in each season at the longest submersion period, with higher values calculated for autumn at 8W. Mean *Thickness* increased with increasing submersion in spring and summer (Table 2B.). Statistically significant differences were found among seasons for *Richness* (all seasons), *TBiom* (except between spring-summer), *BIOM* (all seasons), *TDens* (all seasons), *DENS* (all seasons), and *Thickness* (except between spring-autumn). Simultaneously, for all the parameters above, statistical differences were found among submersion periods in spring and summer; in autumn, statistical differences between submersion periods were found for *TDens* and *DENS* (*Extended data*).

In total 24 taxa were found, 9 macroalgal taxa and 15 macroinvertebrate taxa (Table 3). Summer and autumn registered more taxa (21 and 20 taxa, respectively), which in each season generally increased with the submersion period (maximum of 20 taxa in summer 4W, summer 5W, and autumn 8W) (Table 2B., Table 3).

Table 2. Seasonal values for the seawater parameters (A.) and the biofouling parameters (B.). Mean ± standard deviation are presented, except for Richness. Greater mean values among seasons are highlighted in black (white font). Greater mean values among submersion periods within season are presented in bold.

A.	**Temperature (°C)**	**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* (total no. taxa)**	***TBiom* (g FW m^{-2})**	***TDens* (ind m^{-2})**	***Thickness* (mm)**
Spring	16	23.9 ± 19.5	352.0 ± 280.9	0.78 ± 0.45
1W	3	0.03 ± 0.00	15.3 ± 0.0	0.20 ± 0.00
2W	7	0.71 ± 0.40	38.3 ± 54.1	0.25 ± 0.21
4W	**16**	**35.6 ± 10.6**	**512.6 ± 180.7**	**1.05 ± 0.22**
Summer	21	10.4 ± 10.3	1330.4 ± 1225.0	1.06 ± 0.87
1W	5	0.09 ± 0.16	12.1 ± 20.9	0.11 ± 0.18
2W	9	0.58 ± 0.14	63.3 ± 12.8	0.10 ± 0.00
3W	17	5.7 ± 3.3	482.3 ± 297.4	0.76 ± 0.55
4W	**20**	13.1± 2.5	2224.6 ± 478.0	1.65 ± 0.66
5W	**20**	**23.8 ± 8.8**	**2694.8 ± 373.6**	**1.90 ± 0.26**
Autumn	20	33.9 ± 13.5	2966.1 ± 1759.6	0.43 ± 0.28
4W	16	21.6 ± 0.31	1039.9 ± 217.4	**0.49 ± 0.01**
6W	15	35.6 ± 15.1	2929.9 ± 511.5	0.44 ± 0.47
8W	**20**	**44.4 ± 12.6**	**4928.4 ± 217.4**	0.37 ± 0.37

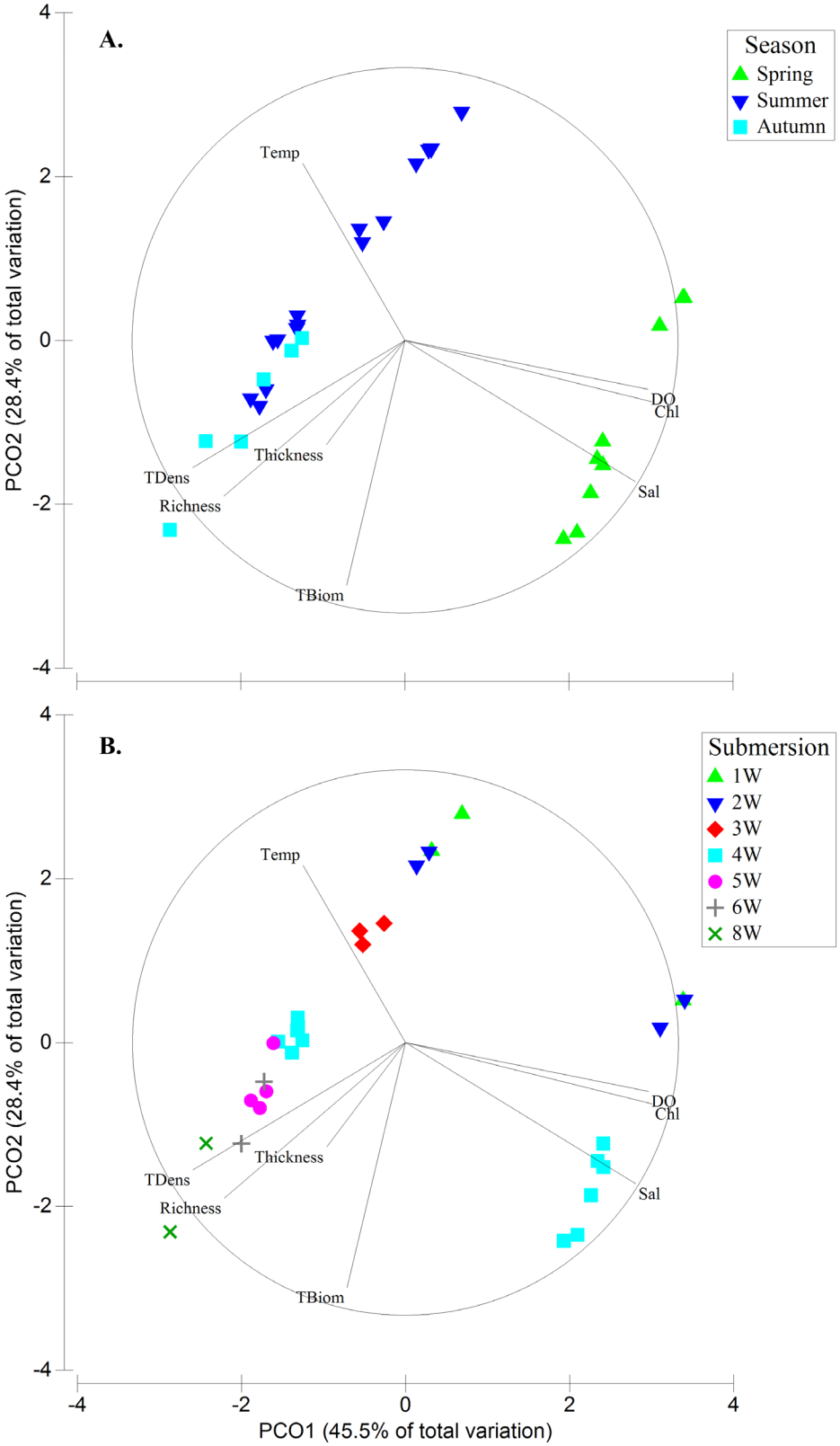

Figure 4. Principal Coordinates analysis (PCO) plot showing trends of seawater parameters (temperature, salinity, dissolved oxygen [DO] and total chlorophyll [Chl.]) and biofouling parameters (*Richness, TBiom, TDens,* and *Thickness*) among seasons (A.) and submersion periods (B.).

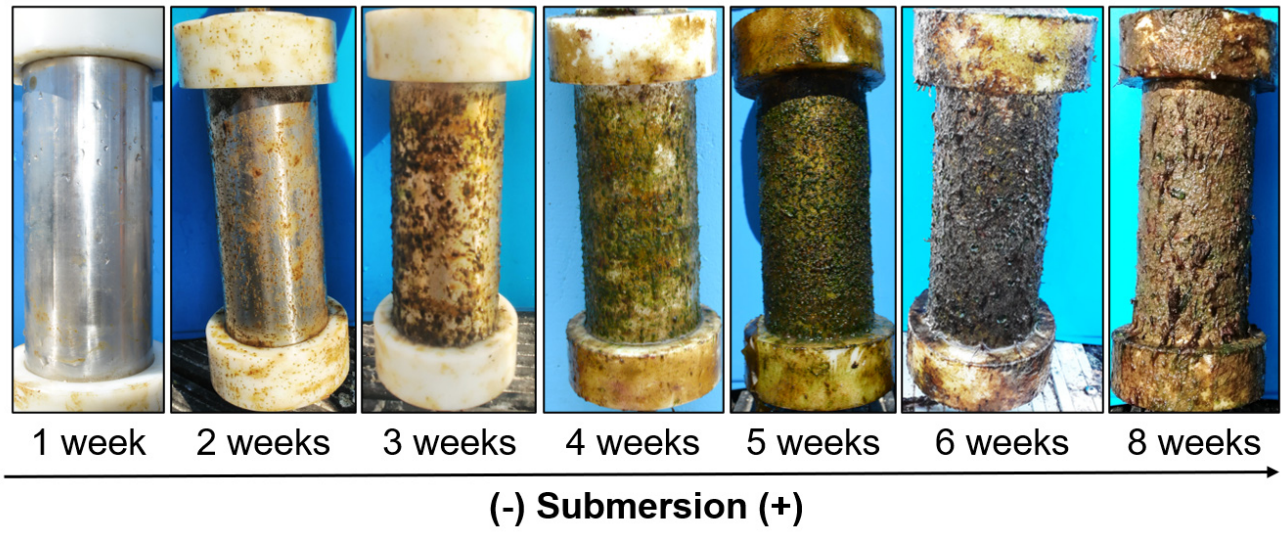

Figure 5. Biofouling growth after one, two, three, four, five, six, and eight weeks of samples submersion.

All macroalgal taxa in this study were recorded in every season, whereas more macroinvertebrate taxa (12 taxa) were found during summer. The most frequent taxa were the green algae *Ulva* sp. (both tubular-like and leaf-like forms) and barnacles (including *Perforatus perforatus, Amphibalanus amphitrite,* and the NIS *Austrominius modestus*) (10 occurrences each), followed by the brown algae *Hincksia* sp./*Sphacelaria* sp. and Amphipoda N.I. (9 occurrences each), the red algae *Polysiphonia* sp. and Rhodophyta N.I., and Bryozoa N.I. (8 occurrences each). Some taxa were found only in summer (Anomura/Brachyura N.I., Gnathiidae N.I., Syllidae N.I., and *Mytillus galloprovincialis*) or in autumn (*Ammothella longipes*, cf. *Crisilla semistriata*, and Nereididae N.I.) (Table 3). Overall, some species succession in the colonization process was observed. For example, after one week of submersion, only the opportunistic green algae (*Ulva* spp.), barnacles (cf. *P. perforatus/A. amphitrite* and *A. modestus*), bryozoans (Bryozoa N.I.; in summer) and other crustaceans (Anomura/Brachyura N.I.; in summer) were recorded; after two weeks, filamentous brown algae (*Hincksia* sp./*Sphacelaria* sp.), red algae (*e.g.* from the order Ceramiales; in summer) and many amphipod individuals were observed; after three or more weeks, several other macroalgal and macroinvertebrate taxa joined the biofouling communities (Table 3).

Not only the structure of the biofouling communities changed between seasons and between submersion periods in each season, so did the proportion of biofoulers biomass and density to the total (Figure 6):

Regarding the biofoulers biomass (Figure 6A.), in all seasons the green and brown algae accounted for the greatest share of the total (51–79% and 14–35%, respectively), followed by red algae (1% in summer, 8% in autumn), amphipods (3% in summer, 2% in autumn), bryozoans (3% in summer, 6% in autumn), and barnacles (4% in summer). Changes in the taxa and their proportions between submersion periods in each season were evident, and also between seasons at the same submersion period. Below are provided details for the submersion periods assessed in more than one season:

• **1W (spring vs. summer):** In spring, only green algae and barnacles were found, each accounting for 50% of the total biomass. In summer, more taxa (bryozoans, Anomura/Brachyura N.I.) were part of the assemblages compared to spring; green algae and barnacles presented much lower proportions (6% each) and bryozoans accounted for 83%.

• **2W (spring vs. summer):** In spring, brown algae and amphipods joined the assemblages; the green algae accounted for 96% of the total biomass. In summer, brown algae, red algae, and amphipods were also part of the assemblages; bryozoans had a very much reduced proportion (1%), whereas the proportion of green algae, brown algae, and amphipods increased (to 56%, 26%, and 11%, respectively).

• **4W (spring vs. summer vs. autumn:** The assemblages were composed of most of the taxa found in this study, especially in summer and autumn (*e.g.* bryozoans were not registered in spring). In all seasons the green algae accounted for greater proportion (66–68% in summer and autumn to 79% in spring), followed by brown algae (19% in spring to 28% in autumn). Other taxa accounted for very low proportions, with the higher values being observed in summer (amphipods and barnacles: 4% each, bryozoans: 3%, red algae: 1%).

Concerning to other submersion periods (*i.e.* summer 3W and 5W, and autumn 6W and 8W), the distribution of taxa

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. N.I.: Not identified.

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	Anomura / Brachyura N.I.												3
			cf. *Pasiphaea sivado*												5
		Or. Isopoda	Gnathiidae N.I.												2
			Tanais dulongii												7
		Or. Sessilia	Barnacles (*Perforatus perforatus*, *Amphibalanus amphitrite*, *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	F. Nereididae	Nereididae N.I.												1
		Cl. Bivalvia	*Mytillus galloprovincialis*												2
		Cl. Gastropoda	cf. *Crisilla semistriata*												1
Macroinvertebrate taxa per submersion period				2	3	**7**	4	2	9	**11**	**11**	7	8	**11**	
Macroinvertebrate taxa per season				7			**12**					11			
Total taxa per submersion period				3	7	**16**	5	9	17	**20**	**20**	16	15	**20**	
Total taxa per season				16			**21**					20			

Table 4. Relationships between the frictional resistance forces and biofouling parameters. A. RELATE analysis; stronger relations (Rho≥0.70) are presented in bold. B. Pearson correlations; higher correlations (≥0.50) are presented in bold.

A.	*Richness*	*TBiom*	*BIOM*	*TDens*	*DENS*	*Thickness*
Friction forces	0.396	0.237	0.382	0.248	0.380	0.094*
Richness		**0.696**	**0.824**	0.674	**0.845**	0.179
TBiom			**0.742**	**0.790**	**0.784**	0.118*
BIOM				0.668	**0.898**	0.104*
TDens					**0.803**	0.201
DENS						0.255
B.	***Richness***	***TBiom***	***TDens***	***Thickness***		
Friction forces	**-0.722**	-0.401	**-0.525**	**-0.532**		
Richness		**0.698**	**0.715**	**0.640**		
TBiom			**0.857**	0.192		
TDens				0.368		

* Not statistically significant (significance level of α = 0.05)

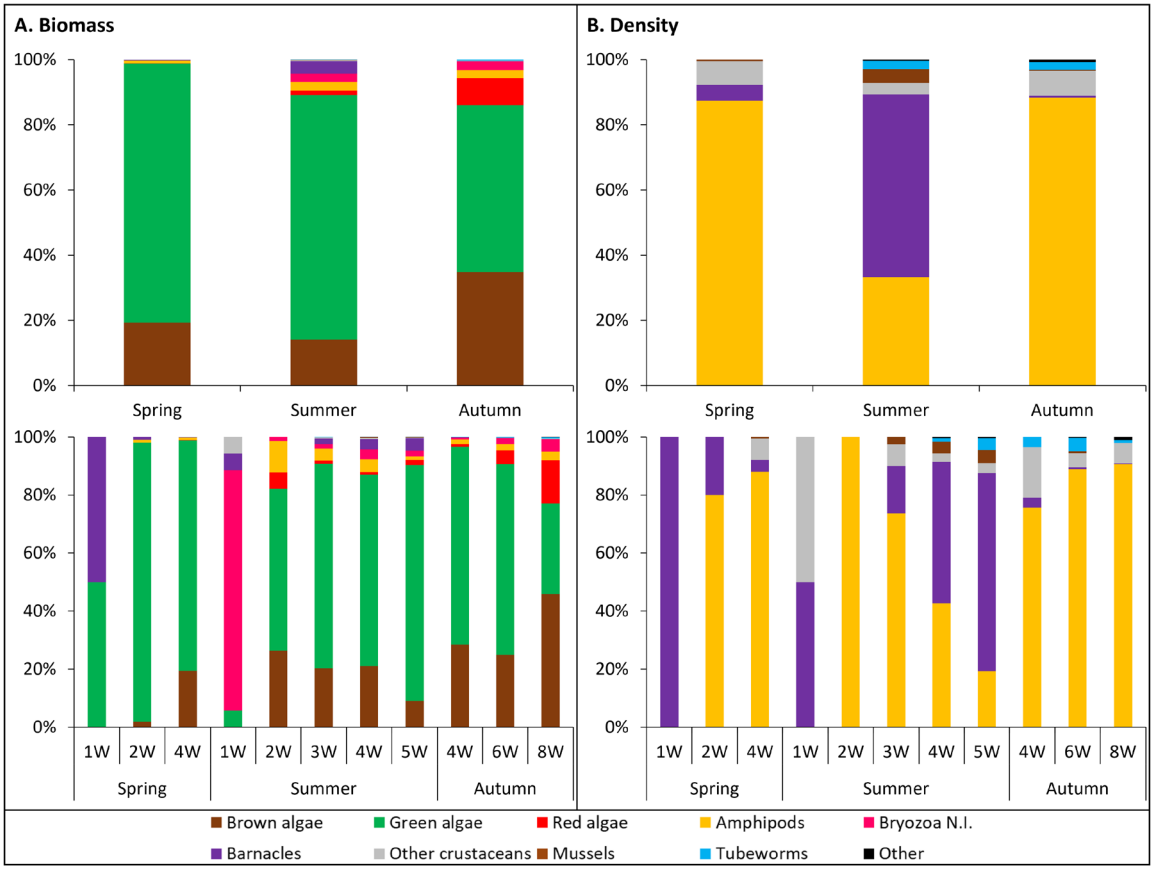

Figure 6. Contribution of major biofouling groups to the communities' biomass (A.) and density (B.) among seasons (top) and submersion periods (bottom).

proportion generally followed a similar order compared to the 4W, with green algae accounting for the greatest proportion, followed by brown algae, amphipods, barnacles in summer, bryozoans, and red algae.

Regarding the biofoulers density (Figure 6B.), in all seasons amphipods (33% in summer to 87–88% in spring-autumn), barnacles (1% in autumn to 56% in summer), and other crustaceans (3% in spring to 7–8% in spring-autumn) contributed the most to the total. Like for the biomass, the taxa and their proportions to the total density changed between submersion periods in each season and between seasons at the same submersion period. Below are provided details for the submersion periods assessed in more than one season:

• **1W (spring vs. summer):** As mentioned above for the biomass, in spring only green algae and barnacles were found. Thus, barnacles accounted for 100% of the density. In summer, barnacles and Anomura/Brachyura N.I. each accounted for 50%.

• **2W (spring vs. summer):** At 2W the amphipods achieved much greater expression. In spring, they accounted for 80% of the total density and barnacles accounted for the remaining 20%. In summer, amphipods accounted for 100% of the total density.

• **4W (spring vs. summer vs. autumn:** The amphipods generally accounted for the greatest proportions in all seasons (43% in summer to 88% in spring), followed by barnacles (3–4% in spring-autumn to 49% in summer), motile crustaceans (3% in summer to 17% in autumn), mussels (4% in summer), and tubeworms (1% in summer to 3% in autumn).

Like for the biomass, at other submersion periods (*i.e.* summer 3W and 5W, and autumn 6W and 8W) the distribution of taxa proportion generally follows a similar order compared to the 4W, with barnacles and amphipods accounting for the greatest proportions, followed by mussels, tubeworms, and motile crustaceans, in summer, and amphipods accounting for the greatest proportions, followed by motile crustaceans and tubeworms, in autumn.

The SIMPER analyses (cut-off 90%) provided further insights about the communities' structure, with similar patterns being found using *BIOM* or *DENS* (*Extended data*). The similarities within the seasons were high, ranging between 68.59% (*BIOM*) / 67.53% (*DENS)* in summer and 85.66% (*BIOM*) / 80.44% (DENS) in autumn. The greatest dissimilarities between seasons were found between spring-summer, 44.47% using *BIOM* and 55.1% using *DENS*.

In each season, similarities within the submersion periods were also high. Using *BIOM*, similarities ranged between 60.20% for spring 2W and 94.41% for autumn 4W. Using *DENS*, similarities ranged between 54.6% for summer 3W and 92.92% for summer 2W. Dissimilarities between submersion periods were also high, generally greater between the submersion periods farther from each other, *i.e.* in spring between 1W-4W (95.47% using *BIOM* and 79.85% using *DENS*), in summer between 1W-5W (95.61% using *BIOM* and 97.70% using *DENS*; second to 1W-2W: 96.82% and 100%, respectively) and in autumn between 4–8W (34.58% using *BIOM* and 43.38% using *DENS*). The lowest dissimilarities were observed between adjacent submersion periods, *i.e.* in spring between 1–2W (74.50%) using *BIOM* and between 2W–4W (74.01%) using *DENS*, in summer between 4–5W (25.93% using *BIOM* and 23.15% using *DENS*) and in autumn between 6–8W (21.69% using *BIOM* and 24.72% using *DENS*) (*Extended data*).

Sixteen taxa (7 macroalgal + 9 macroinvertebrate taxa) were responsible for the dissimilarities regarding *BIOM*, while 11 macroinvertebrate taxa were main contributors to the dissimilarities regarding *DENS*. Most of the taxa, and especially the Amphipoda N.I., registered biomass or density increase with increasing submersion period in each season and registered higher mean values in autumn (Figure 7, Figure 8; Table 5). Some exceptions were the barnacles and mussels (*M. galloprovincialis*) which registered much greater biomass and density in summer, and the opportunistic green algae *Ulva* spp. which were the only taxa with greater biomass in spring.

Frictional resistance forces and its relationship with biofouling data

Mean frictional resistance forces ranged between 240 N at 1W in spring and 100 N at 4W and 5W in summer (Figure 9A.). Also, mean frictional resistance forces generally increased with subsequent scrapings of the same sample, *i.e.* with decreasing levels of biofouling (Figure 9B.). Significant relationships between the frictional resistance data and the biofouling parameters *Richness, TBiom, BIOM, TDens,* and *DENS* were found (RELATE Rho between 0.24 and 0.40) (Table 4A.). In addition, the frictional resistance data and the biofouling parameters *Richness, TBiom, TDens,* and *Thickness* were negatively correlated (Pearson correlations between -0.40 and -0.72) (Table 4B.).

Discussion

In this study, as could be expected for this region, variations in seawater parameters and biofouling characteristics, such as composition, richness, and abundance (measured as biomass and density), were observed across the three surveyed seasons: spring, summer, and autumn. Although some succession in biofouling colonization was observed in each season, 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'. Among the early colonisers is a non-indigenous species (NIS), the Australasian barnacle *A. modestus*, which was found with great frequency and density after one to three weeks of samples submersion. It should be highlighted that colonisation of artificial structures by NIS offshore doesn't necessarily occur after such a short period. For example, in coastal areas such as the study site, colonisation can be enhanced by the many artificial substrates provided by ports and harbours. On the other hand, offshore conditions can be more challenging for larvae due to lower food availability and the need for larvae to travel greater distances to find suitable settlement sites.

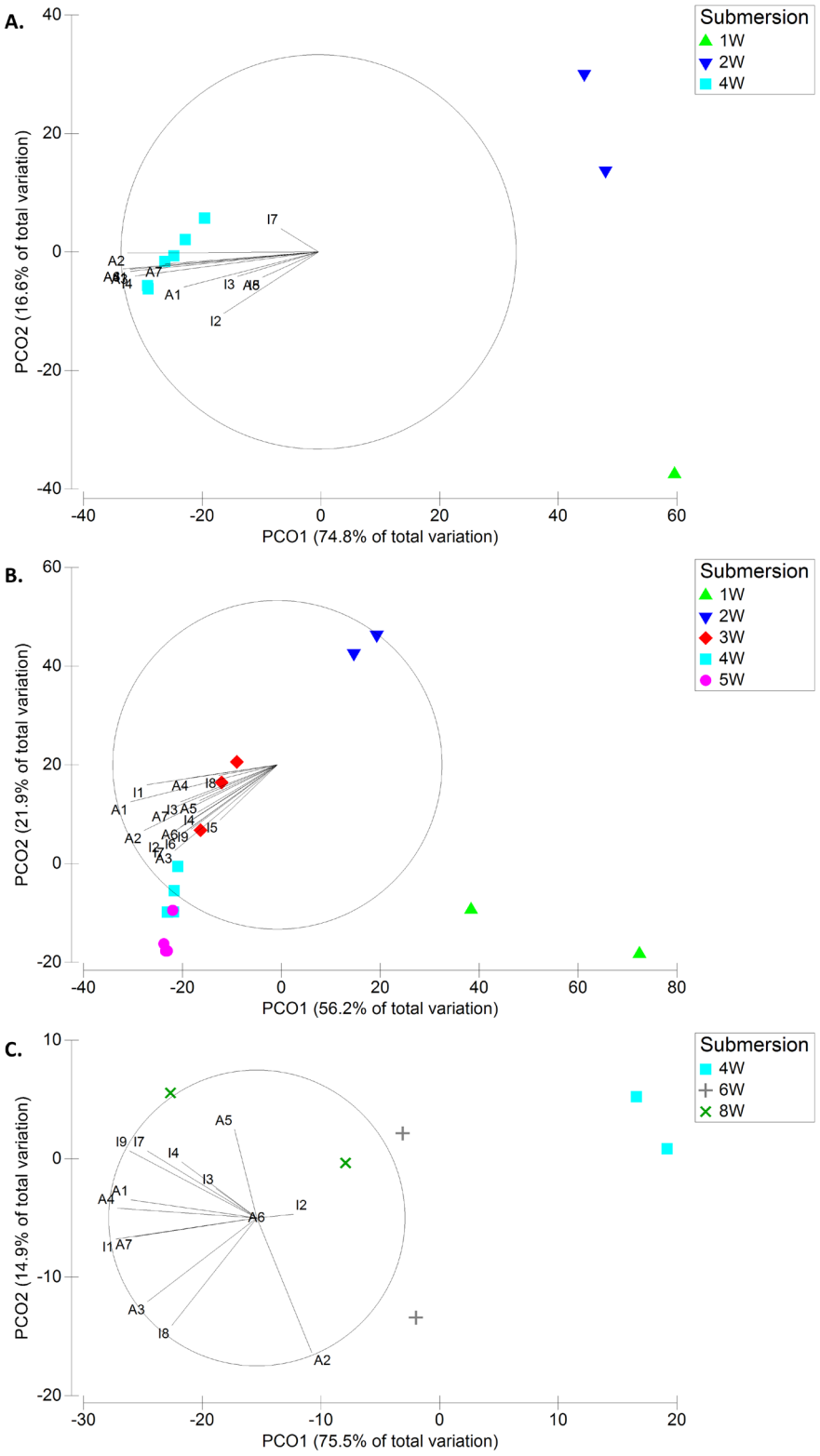

Figure 7. Principal Coordinates Ordination (PCO) plots of the taxa contributing the most to dissimilarities (from SIMPER) using biomass data (BIOM) in Spring (A.), Summer (B.), and Autumn (C.). Taxa key – Algae: A1-*Hincksia* sp./*Sphacelaria* sp.; A2-*Ulva* sp. (tubular-like form); A3-*Ulva* sp. (leaf-like form); A4-*Polysiphonia* sp.; A5-cf. *T. capitata;* A6-cf. *H. flosculosus/B. secundiflora;* A7-Rhodophyta N.I.. Invertebrates: I1-Amphipoda N.I.; I2-Barnacles; I3-*C. equilibra*; I4-*T. dulongii*; I5-Anomura/Brachyura N.I.; I6-*M. galloprovincialis*; I7-*Spirobranchus* sp.; I8-cf. *P. sivado*; I9-Bryozoa N.I.

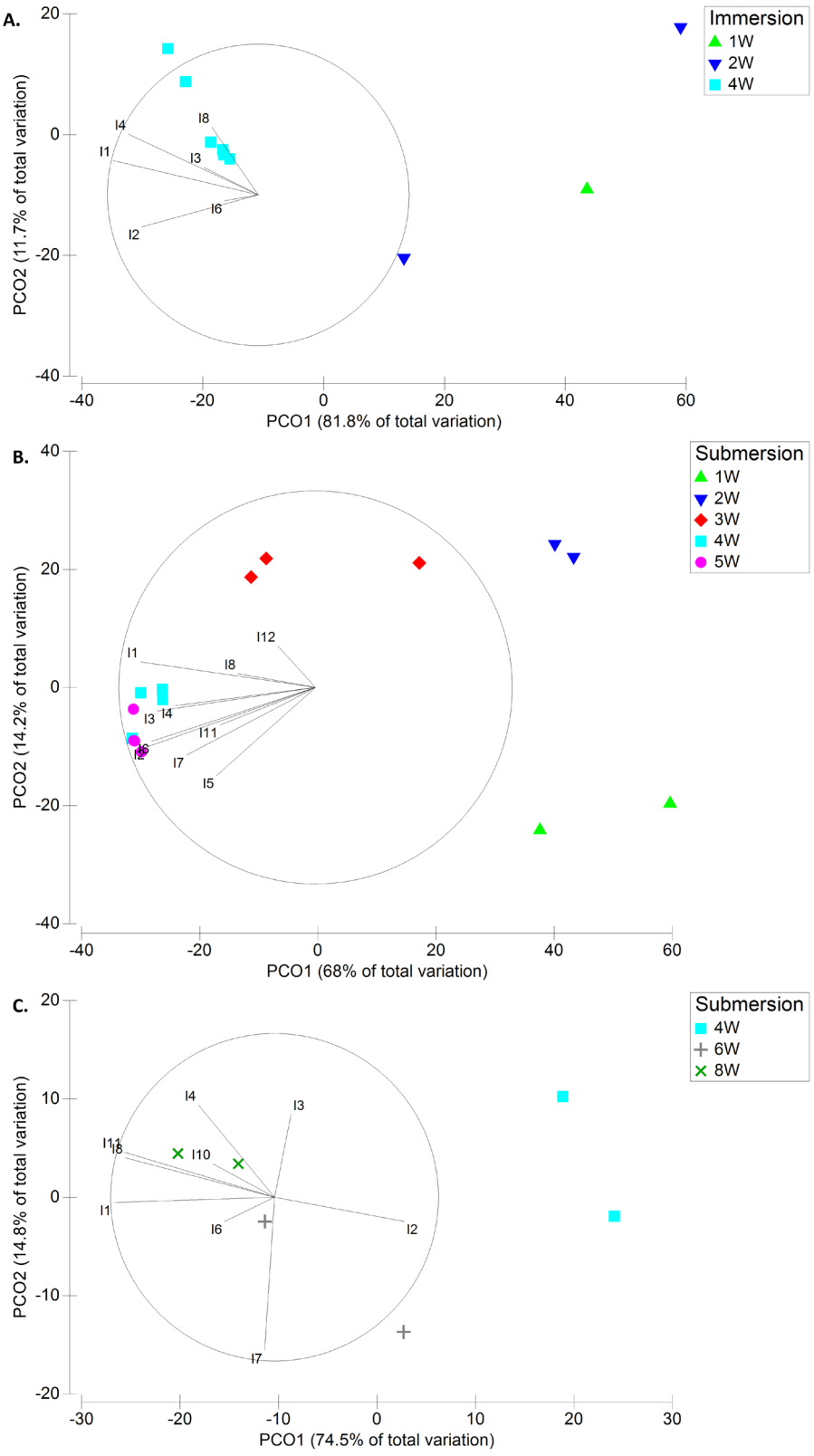

Figure 8. Principal Coordinates Ordination (PCO) plots of the taxa contributing the most to dissimilarities (from SIMPER) using density data (DENS) in Spring (A.), Summer (B.), and Autumn (C.). Taxa key – Invertebrates: I1-Amphipoda N.I.; I2-Barnacles; I3-*C. equilibra*; I4-*T. dulongii*; I5-Anomura/Brachyura N.I.; I6-*M. galloprovincialis*; I7-*Spirobranchus* sp.; I8-cf. *P. sivado*; I10-*A. semistriata*; I11-Syllidae N.I.; I12-Gnathiidae N.I..

Table 5. Biomass (g FW m^{-2}) (A.) and density (ind m^{-2}) (B.) of the main taxa contributing to similarities within and dissimilarities among seasons and among submersion periods within the seasons (from SIMPER analysis). Mean ± standard deviation values are presented. Grey colour identifies increasing values with increasing submersion period in each season.

A1. Macroalgae biomass		***Hincksia* sp. /*Sphacelaria* sp.**	***Ulva* sp. (tubular-like form)**	***Ulva* sp. (leaf-like form)**	**cf. *Polysiphonia* sp.**	**cf. *T. capitata***	**cf. *H. flosculosus* / B. secundiflora**	**Rhodophyta N.I.**			
Spring		4.62 ± 5.43	**15.55 ± 11.86**	**3.45 ± 2.91**	0.009 ± 0.006	0.002 ± 0.004	0.009±0.007	0.008 ± 0.007			
Summer		1.46 ± 1.19	6.58 ± 6.97	1.20 ± 1.79	0.019 ± 0.024	**0.069 ± 0.138**	0.012±0.018	0.044 ± 0.067			
Autumn		**11.79 ± 8.28**	14.61 ± 5.64	2.71 ± 2.10	**2.63 ± 2.74**	0.005 ± 0.008	**0.016±0**	**0.162 ± 0.126**			
Spring	**1W**	0 ± 0	0.014 ± 0	0±0	0 ± 0	0 ± 0	0±0	0 ± 0			
	2W	0.014 ± 0	0.65 ± 0.33	0.031 ± 0.031	0 ± 0	0 ± 0	0±0	0 ± 0			
	4W	6.92 ± 5.32	23.11 ± 6.30	5.17 ± 1.96	0.014 ± 0	0.002 ± 0.005	0.014±0	0.011 ± 0.005			
Summer	**1W**	0 ± 0	0 ± 0	0.005 ± 0.008	0 ± 0	0 ± 0	0±0	0 ± 0			
	2W	0.15 ± 0.03	0.32 ± 0.05	0.008 ± 0.008	0.008 ± 0.008	0.016 ± 0	0±0	0.008 ± 0.008			
	3W	1.16 ± 0.67	3.95 ± 2.93	0.066 ± 0.035	0.030 ± 0.031	0.016 ± 0	0.005±0.009	0.016 ± 0			
	4W	2.75 ± 0.67	8.58 ± 2.69	0.500 ± 0.579	0.005 ± 0.030	0.035 ± 0.027	0.026±0.032	0.035 ± 0.024			
	5W	2.15 ± 0.40	15.37 ± 6.65	3.92 ± 1.46	0.026 ± 0.016	0.23 ± 0.20	0.016±0	0.13 ± 0.08			
Autumn	**4W**	6.14 ± 0.53	14.59 ± 0.79	0.154 ± 0.009	0.14 ± 0.03	0.008 ±0.008	0.016±0	0.016 ± 0			
	6W	8.87 ± 2.43	18.29 ± 7.75	5.09 ± 0.93	1.38 ± 0.10	0 ± 0	0.016±0	0.27 ± 0.09			
	8W	20.37 ± 9.25	10.94 ± 2.80	2.89 ± 0.18	6.37 ± 0.87	0.008 ± 0.008	0.016±0	0.20 ± 0.07			
A2. Macroinvertebrates biomass		**Amphipoda N.I.**	**Barnacles**	***C. equilibra***	***T. dulongii***	**Anomura / Brachyura N.I.**	***M. galloprovincialis***	***Spirobranchus* sp.**	**cf. *P. sivado***	**Bryozoa N.I.**	
Spring		0.20 ± 0.16	0.033 ± 0.045	0.003±0.006	0.013±0.014	0±0	0±0	0.002±0.005	0.007±0.020	0 ± 0	
Summer		0.26 ± 0.25	**0.401 ± 0.469**	0.012±0.014	0.014±0.027	**0.013±0.027**	**0.007±0.008**	0.008±0.008	0.004±0.007	0.26 ± 0.34	
Autumn		**0.80 ± 0.42**	0.014 ± 0.006	**0.024±0.034**	**0.042±0.061**	0±0	0±0	**0.092±0.139**	**0.014±0.021**	**0.93 ± 1.02**	
Spring	**1W**	0 ± 0	0.014 ± 0	0±0	0±0	0±0	0±0	0±0	0±0	0 ± 0	
	2W	0.007 ± 0.007	0.007 ± 0.007	0±0	0±0	0±0	0±0	0±0	0±0	0 ± 0	
	4W	0.30 ± 0.09	0.045 ± 0.051	0.005±0.008	0.020±0.013	0±0	0±0	0.002±0.006	0.010±0.025	0 ± 0	
Summer	**1W**	0 ± 0	0.005 ± 0.008	0±0	0±0	0.005±0.009	0±0	0±0	0±0	0.08 ± 0.11	
	2W	0.063 ± 0.009	0 ± 0	0±0	0±0	0±0	0±0	0±0	0±0	0.008 ± 0.008	
	3W	0.22 ± 0.17	0.11 ± 0.12	0.011±0.009	0.011±0.009	0±0	0±0	0.005±0.009	0.005±0.009	0.09 ± 0.07	
	4W	0.50 ± 0.25	0.61 ± 0.36	0.017±0.015	0.008±0.009	0.035±0.049	0.016±0	0.012±0.008	0.012±0.008	0.33 ± 0.38	
	5W	0.27 ± 0.07	1.04 ± 0.26	0.030±0.010	0.054±0.049	0.011±0.009	0.011±0.009	0.016±0	0±0	0.46 ± 0.40	

Open Research Europe 2024, 2:108 Last updated: 16 AUG 2024

A1. Macroalgae biomass		***Hincksia* sp. /*Sphacelaria* sp.**	***Ulva* sp. (tubular-like form)**	***Ulva* sp. (leaf-like form)**	**cf. *Polysiphonia* sp.**	**cf. *T. capitata***	**cf. *H. flosculosus* / B. secundiflora**	**Rhodophyta N.I.**				
Autumn	**4W**	0.32 ± 0.05	0.016 ± 0	0.018±0.001	0.008±0.012	0±0	0±0	0.016±0	0±0	0.16 ± 0		
	6W	0.78 ± 0.13	0.016 ± 0	0.008±0.012	0.018±0.026	0±0	0±0	0.071±0.078	0.027±0.038	0.73 ± 0.55		
	8W	1.31 ± 0.06	0.008 ± 0.008	0.045±0.064	0.099±0.090	0±0	0±0	0.189±0.244	0.016±0	1.88 ± 1.12		
B. Macroinvertebrates density		**Amphipoda N.I**	**Barnacles**	***C. equilibra***	***T. dulongii***	**Anomura / Brachyura N.I.**	***M. galloprovincialis***	***Spirobranchus* sp.**	**cf. *P. sivado***	***A. semistriata***	**Syllidae N.I.**	**Gnathiidae N.I.**
Spring		297.6 ± 228.6	17.0 ± 8.7	10.2 ± 20.4	23.8 ± 24.0	0 ± 0	1.7 ± 4.8	0 ± 0	1.7±5.1	0±0	0±0	0±0
Summer		410.3 ± 387.3	**747.2 ± 833.8**	**31.7 ± 39.2**	26.0 ± 43.4	**12.4 ± 18.9**	**55.4 ± 71.9**	35.0 ± 63.8	4.5±8.1	0±0	4.5±8.1	**3.4±9.8**
Autumn		2592.3 ± 1512.0	21.1 ± 16.2	27.1 ± 30.9	**217.0 ± 170.0**	0 ± 0	6.0 ± 13.5	**72.3 ± 66.0**	**9.0±9.9**	**3.0±7.4**	**12.1±14.8**	0±0
Spring	**1W**	0 ± 0	15.3 ± 0	0 ± 0	0±0	0 ± 0	0 ± 0	0 ± 0	0±0	0±0	0±0	0±0
	2W	30.6 ± 30.6	7.7 ± 7.7	0 ± 0	0±0	0 ± 0	0 ± 0	0 ± 0	0±0	0±0	0±0	0±0
	4W	436.1 ± 142.7	20.4 ± 7.2	15.3 ± 23.4	35.7 ± 21.0	0 ± 0	2.6 ± 5.7	0 ± 0	2.6±6.2	0±0	0±0	0±0
Summer	**1W**	0 ± 0	6.0 ± 8.5	0 ± 0	0±0	6.0 ± 8.5	0 ± 0	0 ± 0	0±0	0±0	0±0	0±0
	2W	63.3 ± 9.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0±0	0±0	0±0	0±0
	3W	343.6 ± 227.3	78.4 ± 30.7	12.1 ± 8.5	18.1 ± 14.8	0 ± 0	12.1 ± 17.1	0 ± 0	6.0±10.4	0±0	0±0	12.1±20.9
	4W	729.5 ± 343.4	1386.6 ± 559.3	30.1 ± 27.1	12.1 ± 12.8	36.2 ± 26.7	102.5 ± 44.3	18.1 ± 20.2	13.6±9.0	0±0	9.0±10.4	0±0
	5W	447.6 ± 89.9	1840.2 ± 362.7	72.3 ± 44.3	72.3 ± 63.9	13.6 ± 7.8	122.1 ± 87.1	113.0 ± 85.2	0±0	0±0	9.0±10.4	4.5±9.0
Autumn	**4W**	759.6 ± 18.1	36.2 ± 0	27.1 ± 9.0	180.9 ± 144.7	0 ± 0	0 ± 0	36.2 ± 18.1	0±0	0±0	0±0	0±0
	6W	2595.3 ± 388.8	18.1 ± 18.1	9.0 ± 9.0	135.6 ± 27.1	0 ± 0	18.1 ± 18.1	135.6 ± 81.4	9.0±12.8	0±0	9.0±12.8	0±0
	8W	4422.0 ± 27.1	9.0 ± 9.0	45.2 ± 45.2	334.6 ± 208.0	0 ± 0	0 ± 0	45.2 ± 9.0	18.1±0	9.0±12.8	27.1±12.8	0±0

Open Research Europe 2024, 2:108 Last updated: 16 AUG 2024

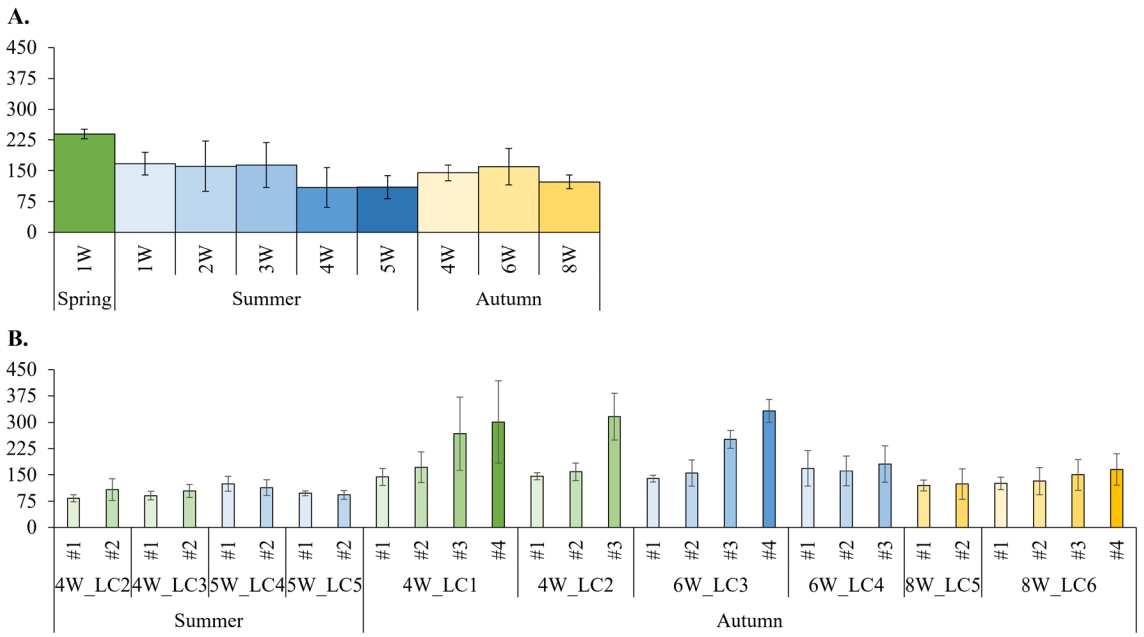

Figure 9. A. Frictional resistance forces in different submersion periods (1–8W) within each season; increasing colours darkness reflect increasing submersion periods. B. Frictional resistance forces from subsequent scrapings (#1 to #4) of some summer and autumn samples; increasing colours darkness reflect increasing scraping #. Mean ± standard deviation values are presented.

It is well documented that higher temperatures typically found in spring and summer favour the reproductive and growth rates of marine organisms (*e.g.* Gili & Petraitis, 2009; Newell & Branch, 1980). In this study, the highest biofouling biomass and abundance were registered in autumn, associated with longer submersion periods (six and eight weeks of submersion) that were not evaluated in spring and summer (maximum four and five weeks of submersion, respectively). Given longer submersion times, biofouling growth would most probably be more substantial in spring and summer. Therefore, in temperate to cold regions we recommend scheduling biofouling-related maintenance activities after the warmer seasons, namely in summer, to minimize the number of inspection and biofouling removal activities until the next season (*e.g.* 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). Additionally, summer generally offers longer and more frequent good weather windows, allowing for operators to conduct maintenance activities with lower costs and risks.

Biofouling biomass and thickness, both of which are mostly associated with sessile macrofoulers (*e.g.* macroalgae, bryozoans, barnacles, mussels, calcareous tubeworms), are critical biofouling parameters affecting several marine industries (*e.g.* Jusoh & Wolfram, 1996; Miller & Macleod, 2016; Tiron *et al.,* 2012; Titah-Benbouzid & Benbouzid, 2017; Yang *et al.,* 2017). In the present study, biofouling biomass and thickness were quite lower than those registered in more hydrodynamic locations offshore (*e.g.* European Biofouling Database; Vinagre *et al.,* 2020) and at a nearby harbour after a similarly short submersion period (Vinagre, 2023). This suggests that biofouling growing for short periods under sheltered conditions would have minimal impact on the loading, drag, or surface diameter of structures/components, compared to more hydrodynamic sites. It must be noted that biofouling biomass and thickness measurements taken out of water can be representative of underwater conditions but may not accurately reflect it. For example, organisms such as algae, tunicates and mussels incorporate water and have natural buoyancy which will reduce their effective weight underwater. These organisms, along with others such as arborescent bryozoans, can also exhibit increased volume underwater, thus representing greater thickness than that observed in dry conditions.

Besides contributing largely to biofouling weight and thickness, sessile macrofoulers can also cause physical damage to structures/components, for example damaging the substrates or their protective coatings by boring into them, when pulled by currents and waves, or during removal activities. Consequently, detrimental issues may arise 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 in the experimental setup after one week of submersion in components untreated against marine-induced corrosion (for example, on stainless steel nuts and washers 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 on metallic substrates used in marine conditions even if for short periods. Cathodic protection has been extensively used on steel structures, and new corrosion monitoring techniques have been

demonstrated and deployed. Recent anti-corrosion techniques proposed include 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 which in this study showed good anti-corrosive efficiency.

The results of the frictional resistance tests suggest that during these early colonization stages the slippery nature of biofouling could be acting as a 'lubricant' leading to a general trend of increasing forces being generated with decreasing biofouling levels. If early colonization stages can be accepted as a safe time interval to perform biofouling-related inspection and maintenance activities, then physical control, for example grooming, 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, because biofouling composition and growth are very variable and influenced by many factors (*e.g.* Hellio & Yebra, 2009; Vinagre *et al.,* 2020), the definition of 'acceptable biofouling growth' will be project-specific, for example depending on the type of structure/component and its functional requirements (*e.g.* free-moving versus static), the site location (*e.g.* latitude, seawater temperature, distance to shore) and hydrodynamic conditions (*e.g.* current velocity and wave exposure), and the bathymetry (*e.g.* shallow versus deep waters) and depth (*e.g.* surface versus mid water column) at which the structure/component is positioned.

The definition of "acceptable biofouling growth" may also be dependent on legislation (*e.g.* EU Directive 2008/56/EC, EU Regulation 1143/2014) that aims to prevent or manage the introduction and spread of NIS. 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. Because MRE equipment can act as stepping stones facilitating the dispersion of NIS between artificial and natural habitats (*e.g.* Adams *et al.*, 2014; De Mesel *et al.*, 2015), such effects can occur far away from the MRE deployment site. Also, it is very much possible that the dispersion of NIS is further enhanced if the organisms that reproduce sexually (*e.g.* barnacles, mussels) or propagules of organisms that reproduce asexually through budding or fragmentation (*e.g.* bryozoans, hydrozoans) are not recovered after using physical control strategies such as scraping or grooming.

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 equipment at sea (*e.g.* Cook *et al.,* 2014; Payne *et al.,* 2014), for example, through the development and installation of biofouling monitoring techniques. 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 findings of this study underscore the significance of managing biofouling during the early stages of colonisation. By mitigating the early attachment of biofoulers, there is a potential to curtail and delay subsequent biofouling, not only enhancing the preservation of materials and prolonging the interval between necessary biofouling-related maintenance operations but also reducing the possibility of NIS settlement and propagation, which can have profound ecological impacts.

The recommendation to schedule biofouling-related maintenance activities post-peak growth and reproduction periods, typically observed in warmer seasons within temperate to cold environments, emerges as a practical strategy. Adopting such a temporal approach could lead to a reduction in the frequency of cleaning operations, particularly before the subsequent growing season conducive to the breeding, spawning, and settlement of various key biofouling organisms.

It is necessary to acknowledge the limitations of this study, namely the very different environmental conditions of the study site compared to offshore and the fact that seasonal variations could not be assessed in different years. While providing valuable insights into early biofouling occurrences, the experimental design may not fully capture the dynamics of biofouling development. Therefore, it is crucial to interpret the present findings with caution. In light of these considerations, this research serves as a foundation, unveiling the need for more comprehensive investigations to refine our understanding of biofouling patterns and their implications for material preservation. Future studies should be conducted in offshore environments, with extended duration of experimental tests and larger sample sizes to ensure robust and representative results. In doing so, knowledge of biofouling dynamics can be advanced and preventive measures for the sustainable operation of MRE projects optimised.

Data availability

Underlying data

Zenodo: Experimental insights on biofouling growth in marine renewable structures, http://www.doi.org/10.5281/zenodo.6974716 (Vinagre *et al*., 2024a)

This project contains the following underlying data:

- Open Research Europe_Biological data.xlsx (Biofouling data)

Zenodo: Experimental insights on biofouling growth in marine renewable structures, http://www.doi.org/10.5281/zenodo.10966268 (Vinagre *et al*., 2024b)

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: Experimental insights on biofouling growth in marine renewable structures, http://www.doi.org/10.5281/zenodo.10966299 (Vinagre *et al*., 2024c)

Acknowledgements

The authors gratefully acknowledge Pedro Pires, Erica Cruz (both former WavEC Offshore Renewables, Portugal) and Antoine Bonel (CorPower Ocean, Sweden) for their help in designing and building support structures and in sampling. The authors express their thanks to the two reviewers for their constructive feedback and valuable comments and suggestions which helped to improve the quality of the manuscript.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 16 August 2024

https://doi.org/10.21956/openreseurope.19083.r41545

✔ **Andrew J Guerin**
Independent Researcher, Toronto, Canada

The article is substantially improved from the previous version. Additional details on the methodologies and analyses, along with a much deeper exploration of the data make this paper much more informative than it was. I thank the authors for the work that has gone into this revision. I have no additional comments at this stage; it is now acceptable for publication.

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 confirm that it is of an acceptable scientific standard.

Reviewer Report 27 June 2024

https://doi.org/10.21956/openreseurope.19083.r41546

✔ **Lenaïg Hemery**
Pacific Northwest National Laboratory, Washington, USA

The revised manuscript by Vinagre et al. is a substantial improvement from its earlier version a year ago. With all the changes made, I approve this new version, with the minor caveat that two relevant papers have been published since that the authors should consider discussing in theirs:

Portas et al. and Want et al. Ref [1,2]

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2. Reference Source

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.

Version 1

Reviewer Report 28 July 2023

https://doi.org/10.21956/openreseurope.16047.r32426

?

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.

Author Response 12 Apr 2024

Pedro Vinagre

The authors would like to kindly thank the Reviewer for the time spent reading and reviewing our research paper. The extensive comments provided were extremely constructive and the article has been fully reviewed according to the feedback provided below.

- Page 4 – "What were your controls? Did you have any samples without treatment?"
The study didn't include controls, i.e., samples without treatment. The authors recognise the importance of having controls vs. treatments in any study. However, the research conducted in the project aimed to infer the implications of biofouling growth in MRE structures that are treated against corrosion to be used in ocean environments. Thus, the project only tested samples that were treated against corrosion.

- Page 5 – "This is unnecessary information, you can delete."
While the authors understand that this information can be considered unnecessary for the scope of the present research, its inclusion was demanded by the Editorial team of the journal.

- Page 6 – "You said earlier that NC samples were omitted! Did you then redo the analyses without these samples?"
Data was discarded only when corrosion was found in the NC samples . The data from the first deployments, when corrosion was not present, were used for analysis. This was further clarified earlier in the paper: "The NC1 and NC2 cylinders showed signs of corrosion during trials in September 2019, possibly owed to inefficient waterproofing of the untreated portion by the end caps. Corrosion can influence biofouling composition and growth, for example increasing the substrates roughness and physical-chemical properties. Therefore, data of biofouling growing in those samples in September 2019 were discarded from the analyses to avoid biased results and the NC samples were not used for further deployments (see deployment strategy below)".

- Page 7 – “Any data analysis you could do to correlate the frictional resistance forces with BIOM, DENS, TBiom, or TDens? As is, these results leave a taste of "so what", try to dig a little deeper if you can.”
The RELATE routine and Pearson correlations were used to analyse relations/correlations between frictional resistance forces and biofouling parameters (Richness, TBiom, BIOM, TDens, DENS and Thickness) and among the biofouling parameters.

- Page 11 – “Don't you think that the biofouling control measures that you suggest earlier would help disperse NIS? Could you list other measures that would not and be good for developers to implement?”
We agree. It was added earlier in that section “Also, it is very much possible that the dispersion of NIS is further enhanced if the organisms that reproduce sexually (e.g. barnacles, mussels) or propagules of organisms that reproduce asexually through budding or fragmentation (e.g. bryozoans, hydrozoans) are not recovered after using physical control strategies such as scraping or grooming.” Other biofouling control measures were not listed because the feasibility of using them will depend on many factors such as the specific MRE technology and the type of equipment (e.g. static vs free moving), deployment location, environmental conditions, and regulatory requirements. The authors would like to kindly thank, once again, for the thoroughly made revision which has represented an asset for our research. We will be available for any questions/suggestions/corrections that you might raise. Best regards, The Authors

Competing Interests: No competing interests were disclosed.

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.

Author Response 12 Apr 2024

Pedro Vinagre

The authors would like to kindly thank the Reviewer for the time spent reading and reviewing our research paper. The extensive comments provided were extremely constructive and the article has been fully reviewed according to the feedback provided below.

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.
The original aim of this research is to understand and preliminarily evaluate the forces generated from removing different biofouling organisms in the samples (e.g. barnacles vs. algae). However, it was difficult to relate the forces measured with the specific occurrence of organisms in the samples. Nonetheless, the study provided interesting results:

1. **It allowed to characterise the biofouling communities growing during increasing periods in three different seasons.**
2. **It highlights the importance of using adequate biofouling management and anticorrosive strategies.**

Following your comments, we have: (i) updated the title of the paper; (ii) included several modifications throughout the paper to make its scope and impact better perceived by the reader; (iii) included a final comment on the conclusions section detailing the further work that is needed for a more comprehensive understanding of biofouling in metallic structures.

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. **The authors agree. Although the biofouling communities were characterised during three different seasons, no replication across years was possible. Thus, the discussion on temporal effects now takes these considerations into account.**

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. **All the comments below have been fully addressed.**

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. **Following your comment, some of the data previously provided as *Extended data* has now been incorporated into the paper.**

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. **A description of biofouling was added in the first sentence of the introduction.**

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. **These aspects were incorporated in the text.**

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. **The sentence was rephrased accordingly.**

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. **These aspects are**

now mentioned in the Discussion section.

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. **For each cylinder, it was done one scraping event (except for the cylinders scraped more than once, as mentioned in the text) along the area available for colonisation (presented in the text). During each scraping, frictional resistance forces were acquired at a 50 Hz frequency (this has been included in the paper). The number of measurements along a cylinder depended on the velocity of scraping, i.e., faster scraping resulted in fewer measurements. Unfortunately, this was a first version of the scraping system, and we didn't have a way to measure the velocity of scrapings (this has been added to the text in the Methods section). Additionally, differences in the number of measurements may also have resulted from removing outliers from the data (e.g., associated with the acceleration at the start or deceleration at the stop of the scraping event owed to the tightness of the plastic scraper to the samples) (this has also been added to the text).**

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? **PERMANOVA was used for different reasons, for example (i) it is a non-parametric method that can be used both for multivariate and univariate data; and (ii) being based on permutation tests, PERMANOVA is especially useful when sample sizes are small or there are unequal groups sizes, which is the case of this specific research. A comment on this has been included in the paper.**
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. **Only one sample was taken as the highest point (associated with barnacles, bryozoans, or other organisms) from the cylinders surface. This has been clarified in the text.**

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. **The square-root transformation was done to reduce the influence of naturally abundant species (such as barnacles) in the analyses. This dominance was observed both for biomass and abundance and was observed using either the total biofouling data**

(univariate data, TBiom and TDens) or the individual taxa data (multivariate data, BIOM and DENS). This explanation was added to the text. The SIMPER analysis was repeated using the same transformation (square-root), and the methods and results sections are now reflecting these changes.

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. **Following your suggestion, PERMANOVA analyses based on BIOM and DENS have now been conducted and their results have been added to the paper.**

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. **While the authors agree with the reviewer, we highlight that the aim was not to present the statistical differences but to show how the values changed among seasons. The text and the title of Table 2 were changed to reflect this accordingly.**

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. **As mentioned for the previous comment, in Table 2 we meant to highlight changes among seasons and increasing submersion periods, and not the statistical differences. Hence, the higher values were kept in bold. The text "mean ± standard deviation" was removed from the Table 2 title according to your suggestion.**

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. **The section on community structure and PERMANOVA and SIMPER results was restructured to better highlight patterns among seasons and submersion periods. SIMPER data was previously made available as *Extended data*. Part of that data have been included in the paper as Table 5.**

m) More description of the multivariate patterns would be worthwhile. The PCO plots are useful but some discussion of these would be useful. **Multivariate patterns are now better described in the Results section and discussed in the Discussion section.**

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. **As mentioned earlier, and following your suggestions, the section on SIMPER results was restructured to better highlight patterns among seasons and submersion periods. Also, part of the SIMPER data which was previously made available as *Extended data* is now included in the paper as Table 5.**

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. **Following your suggestion, PERMANOVA analyses based on the frictional resistance forces (univariate data) were conducted and their results were added to the paper.**

p) More information is needed to make sense of Figure 5, particularly sample sizes. **The mean values and standard deviation were calculated using all the samples within a submersion period within a season. For example, the mean + standard deviation of the sample "summer 1W" was calculated using the frictional resistance measurements of the three "1W" samples of "summer". A clarification on this has been added to the text in the Methods section.**

q) Does 5A show the mean ± sd of all 6 (or 8) samples? Are these differences statistically significant?
As mentioned for the previous comment, the mean + sd was calculated using all the samples within a submersion period within a season. PERMANOVA analyses based on the frictional resistance forces (univariate data) were conducted and their results were added to the paper.
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? **As it was now clarified in the Methods section, some of the cylinders were scraped more than once until complete cleaning was achieved to measure the friction forces generated from scraping decreasing levels of biofouling (macrofouling – biofilm – no biofouling). This was done only for some summer and autumn samples which stayed in water for longer submersion periods (4-8W). The previous version of the paper presented only some examples, and now it presents all the samples which were scraped more than once. Those samples are now identified in Table 1. PERMANOVA analyses were now conducted, and their results were added to the paper.**

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. **The reason for the subsequent scraping of some cylinders was presented for the previous comment. This information was added to the text in the Methods section.**

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. **The authors agree, and that notion was incorporated into the discussion. The authors would like to note that "density" refers to the number of individuals per square meter and not the mass per unit of volume.**
u) Similarly, the actual thickness of biofouling in water will differ from what is measured using calipers at the surface. **The authors agree. This is especially true when the thickness is related mostly to, for example, soft fouling like algae and ascidians, or arborescent bryozoans. This was incorporated into the discussion.**
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. **The intention was not to relate frictional resistance/scraping data with hydrodynamic load, and the authors agree that such data is not a good proxy for hydrodynamic load in operational conditions. This was clarified in the discussion. Also, differences between measurements underwater and outside of water are now highlighted.**

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. **The inference comes from the results presented in the paper, as shown in Figures 9A and 9B (previously Figures 5A and 5B) and mentioned in the text. This has been clarified in the text.**

Minor/Typographical:

Abstract: Instead of "small-scale wave energy components" perhaps 'small-scale wave energy device components' or something similar? **The sentence in the abstract was changed accordingly.**
"forces generated to scrape" - something like 'frictional resistance forces generated during scraping' (as used elsewhere in the paper) would be better. **The sentence in the abstract was changed accordingly.**

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? **Yes, according to the literature (for example the references used in the paper) biofouling (namely microfouling) can initiate MIC.**

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…". **The process occurs in many different sectors. The sentence was changed to "Another concern related to biofouling of MRE structures (and others installed at sea)".**
Results: "several crustaceans fauna amphipods were observed". I'm not sure what this means. **It has been corrected.**
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. **The sentence was changed accordingly. The authors would like to kindly thank, once again, for the thoroughly made revision which has represented an asset for our research. We will be available for any questions/suggestions/corrections that you shall raise. Best regards, The Authors**

Competing Interests: No competing interests were disclosed.

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.