Lessons from photo analyses of Autonomous Reef Monitoring Structures as tools to detect (bio-)geographical, spatial, and environmental effects

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ABSTRACT

We investigated the validity of Autonomous Reef Monitoring Structures (ARMS) as monitoring tools for hard bottoms across a wide geographic and environmental range. We deployed 36 ARMS in the northeast Atlantic, northwest Mediterranean, Adriatic and Red Sea at 7–17 m depth. After 12–16 months, community composition was inferred from photographs, in six plate-faces for each ARMS. Overall, we found a highly significant effect of sea region, site (within seas), and plate-face on community composition. Plate-faces thus represent distinct micro-habitats and provide pseudo-replicates, increasing statistical power. Within each sea region taken in-dividually, there was also a highly significant effect of site and plate-face. Because strong effects were obtained despite the fusion of taxonomic categories at high taxonomic ranks (to ensure comparability among biogeo-graphic provinces), ARMS photo-analysis appears a promising monitoring tool for each sea region. We re-commend keeping three ARMS per site and analyzing more numerous sites within a sea region to investigate environmental effects.

1. Introduction

In an era in which anthropogenic activities cause significant impacts on the marine environment, there is a requirement to minimize these impacts and to improve the environmental status of marine habitats. To achieve this, several European directives such as the Water Framework Directive and the Habitats Directive have been implemented over the last decades. More recently, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) has incorporated new monitoring requirements to assess environmental quality with the aim of achieving good environmental status (GES) of all European seas by 2020. This requires the monitoring and status assessment of a variety of descriptors, including some representing biological components, such as biodiversity or the presence of non-indigenous species (Danovaro et al., 2016). However, the assessment of biodiversity at any spatial scale is a challenge (Borja et al., 2016; Selig et al., 2013) and thus innovative methods and approaches are required (Danovaro et al., 2016).

The availability of well-established and standardized sampling methods, which do not require scuba-diving (Borja et al., 2000, 2015; Patrício et al., 2016), for soft bottoms habitats means that they have benefitted from more monitoring studies than hard bottoms and their biodiversity patterns and dynamics are thus better understood. Additionally, a variety of sensors allow the automated collection of data from sediments (e.g. redox potential, organic matter, contaminants) as well as on water quality, which provides valuable information in order to relate changes in community composition with these variables (Birchenough et al., 2012).

In order to standardize the monitoring of benthic hard bottoms, ecologists often use settlement plates or other artificial sampling units. Once these units are colonized by marine organisms, they can be used

to monitor or experimentally manipulate benthic communities (Altman and Whitlatch, 2007; Bowden et al., 2006; Féral et al., 2016; Judge and Craig, 1997; Marraffini et al., 2017; Moura et al., 2008; Piola and Johnston, 2008; Sorte et al., 2010). Although artificial structures have already been used to compare biodiversity of marine hard bottoms from distinct geographical regions, the artificial substrates varied in size and material while colonization time, processing and analytical protocols were also different, making large-scale comparisons difficult to establish.

To further standardize the sampling of benthic habitats, the Coral Reef Division (CRED) of the United States' National Oceanic and Atmospheric Administration (NOAA) developed Autonomous Reef Monitoring Structures (ARMS) (Zimmerman and Martin, 2004). The ARMS are composed of stacked PVC settlement plates and are designed to mimic the 3D structural complexity of coral reef habitats (Brainard et al., 2012; Knowlton et al., 2010; Plaisance et al., 2011). While ARMS were originally designed for coral reef habitats and have been used to assess diversity in the Caribbean and Indo-Pacific (Knowlton et al., 2010; Ransome et al., 2017) and in the Red Sea (Al-Rshaidat et al., 2016; Pearman et al., 2016a, 2018) they have also been deployed in other hard bottomed habitats on the Atlantic coast of the US (Leray and Knowlton, 2015) and in the Adriatic Sea (Pennesi and Danovaro, 2017). These PVC structures, unlike other artificial substrates (e.g. limestone), can be manufactured according to the same specifications globally, and thus enabled a standardized ARMS to be constructed (e.g. no geo-graphical variations in construction materials). While other artificial construction materials (e.g. limestone) better mimic some natural en-vironments, the use of a single material allowed for comparisons to be undertaken across a wide range of hard bottomed substrates in geo-graphically separated regions.

One of the main issues associated with marine environmental monitoring is time and cost constraints. While Leray and Knowlton (2015) assessed ARMS between temperate and subtropical regions they used a relatively expensive metabarcoding approach. In this study, we aim to test the potential of a photographic assessment of the sessile components of ARMS as a fast community screening tool across a range of environmental conditions. Towards this end, we analyzed the communities colonizing a subset of plates after more than one year of immersion under different environmental conditions across two regional European seas (Northeast Atlantic Ocean and Mediterranean Sea) as well as the Red Sea. Our general goal was to assess the relevance of photo analysis as a fast and efficient screening tool for biodiversity monitoring. Towards this aim, we first tested whether community composition, inferred from photographs, was significantly different among the distinct plate surfaces of the ARMS, between sites (within seas) and seas. Secondly, since monitoring protocols must be as simple and cost-effective as possible, we performed some analyses considering (i) each taxonomic group (e.g. Annelida, Bryozoa, Cnidaria…) alone in order to explore the possibility of using these taxa as surrogates of the entire ARMS colonizing community and (ii) each plate-face separately to see if these partial analyses of community composition had similar significant effects as did analyses combining all plate faces. Lastly, we investigated whether we could detect an effect of various environmental factors (reflecting both the level of anthropic pressure and the local habitat diversity) on biodiversity patterns. However, our experimental design was not optimized for this goal and so these results are presented as tentative.

2. Material and methods

2.1. Monitored sites

The sites sampled in this study were the southern part of the Bay of Biscay, which corresponds to the northeast Atlantic, the northwest Mediterranean coast of France, the Adriatic Sea, and the Red Sea (Fig. 1). Three replicate ARMS units were installed at three sites in each

sea region for a total of nine units per sea, at a depth of between 7 and 17 m (Table 1, Fig. 1). Information about times of deployment and sites is given in Table 1. The sites were chosen taking into account information on natural and human pressures (acknowledging the limitations inherent to this approach) and the a priori most anthropized sites of each sea region were Pasaia (for the Bay of Biscay), Elvine (for the Northeast Mediterranean region), Grotta Azzurra (for the Adriatic Sea) and JSR (for the Red sea) (cf Section 2.4 and Supplementary file S1).

2.2. ARMS implementation and recovery

Each ARMS unit is composed of nine 22.5 cm × 22.5 cm PVC plates and spacers stacked in an alternating series of open and closed formats, attached to a 35 cm × 45 cm base plate (Fig. 2). Further details on the standard assembly, deployment and recovery of the ARMS are available on NOAA's website[1] and in (González-Goñi et al., 2017).

The ARMS units were installed by divers and submerged for 12 to 16 months, depending on the sea region (Table 1). Subsequently, ARMS were recovered and returned to the laboratory, where they were dismantled and processed. Each plate surface was gently brushed to remove mobile fauna without detaching sessile organisms. Plates were kept in seawater aerated with bubblers until photographs were taken (Fig. 3).

2.3. Photo analyses

Three plates (P) (plates 1, 4, and 8) (Fig. 2) were selected for analysis and for each one, the top (T) and bottom (B) surfaces were analyzed individually (i.e., 6 plate-faces analyzed per ARMS). These faces were selected as representative of the different habitats found within an ARMS, which may represent different conditions experienced by the organisms in situ. The top surface of plate 1 is exposed to direct light and without any protection from predators, while the other five faces are not. Among these shaded plate-faces, P1B, P4T, P8T are open to the current, while faces P4B and P8B represent less hydrodynamic niches due to compartmentalization, which does not allow the current to flow through the space in between the plates (Figs. 2 and 3).

Photographs were analyzed using Photoquad® software (Trygonis and Sini, 2012). Each photograph was divided in 64 squares and one point was randomly selected within each square. The organism present at each point was identified to the lowest possible taxonomic level by scientists from the four sea regions (scientists analyzed ARMS from their own region). Prior to the analysis, some taxonomic categories were merged in order to be compatible among the four sea regions and to minimize possible observer effects. The initial taxonomic categories are provided in Supplementary material (File S2). The final (merged) categories were: Annelida, Bryozoa, Mollusca, Cnidaria, Porifera, Crustacea, colonial Tunicata, Tunicata, crustose coralline algae (hereafter CCA), other Rhodophyta, Chlorophyta, Phaeophyta, other Algae, Foraminifera, "undetermined", and "not alive". Points that fell on un-colonizable parts of the plate due to the presence of the compartmentalizing cross or screws (Fig. 3) were considered as "not alive" (since not all partners had created a category "uncolonizable" while analyzing their photos). The statistical community analyses presented in the paper were performed without including the categories "not alive" and "undetermined" in the dataset (the results were similar when including these categories).

2.4. Environmental factors

On the European coasts, the three sites were chosen by local scientists (from each sea region) to reflect contrasting environmental

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[1] https://www.pifsc.noaa.gov/cred/survey_methods/arms/overview.php.

Fig. 1. Geographic position of the DEVOTES ARMS sites. Their precise geographic positions are given Table 1.

Table 1
Information about ARMS' deployment and sites monitored by four partners: AZTI (Bay of Biscay sites), CNRS-IMBE (northwest Mediterranean sites), CoNISMa (Adriatic Sea sites), and KAUST (Red Sea sites).

Sea region (code)	SITE (code)	Deployment date	Recovery date	Site ID [replicates]	Latitude	Longitude	Depth (m)
Adriatic Sea (AdS)	Grotta Azzurra (Azz)	Jul-14	Jul-15	CONI_S1	N43 37.313	E13 31.691	7
	Due Sorelle (Sor)	Jun-14	Jul-15	CONI_S2	N43 32.953	E13 37.699	8.7
	La Scalaccia (Sca)	Jun-14	Jul-15	CONI_S3	N43 36.291	E13 33.102	8.8
NW Mediterranean (NWM)	Ile de l'Erevine (ELV)	Jun-13	Dec-14	CNRS_S1	N43 19.780	E05 14.210	17
	Ile Riou (RRS)	Jun-13	Dec-14	CNRS_S2	N43 10.370	E05 23.420	17
	Phare de Cassidaigne (CCA)	Jun-13	Dec-14	CNRS_S3	N43 08.740	E05 32.740	17
Bay of Biscay (BoB)	Lekeitio (Lek)	Jun-13	Jul-14	AZTI_S1	N43 22.311	W2 30.258	12.5
	Zumaia (Zum)	May-13	Jul-14	AZTI_S2	N43 18.748	W2 13.641	11
	Pasaia (Pas)	May-13	May-14	AZTI_S3	N43 20.230	W1 55.639	11
Red Sea_Jeddah (ReS)	Janib Sa'ara reef (JSR)	Apr-13	Jun-14	KAUS_S1	N21 27.253	E39 06.661	10
	South of Jeddah (SOJ)	Apr-13	Jun-14	KAUS_S2	N21 13.508	E39 07.237	10
	Qaham reef (QAR)	Apr-13	Jun-14	KAUS_S3	N21 04.921	E39 12.063	10

situations with respect to human pressure while being on hard bottoms and at a reasonable distance apart (to ensure that the potential pool of colonizer species was shared among sites within a sea). Nine binary environmental factors were assessed and coded as 0 (absence) and 1 (presence): a protected area for at least three years (such as national park or marine protected area), marine debris (i.e. reported presence of visible plastic objects, litter, and, abandoned, lost and otherwise discarded fishing gears), wastewater discharges, chemical pollution (assessed by pollutant dosages and/or imposex), urbanization, harbor, nearby seagrass meadows, nearby sand, and nearby mud (at < 15 m,

and with a probable influence on ARMS (scientific diver opinion)). Not all factors varied within each sea region. One site in the Northwest Mediterranean Sea region (RRS) has only been within a National Park since 2012; it was thus not considered protected in the analysis (although results do not change when it is considered as a protected area).

2.5. Statistical analyses

We used the PRIMER package (version 7) (Clarke et al., 2014; Clarke and Gorley, 2015) for all community analyses that were
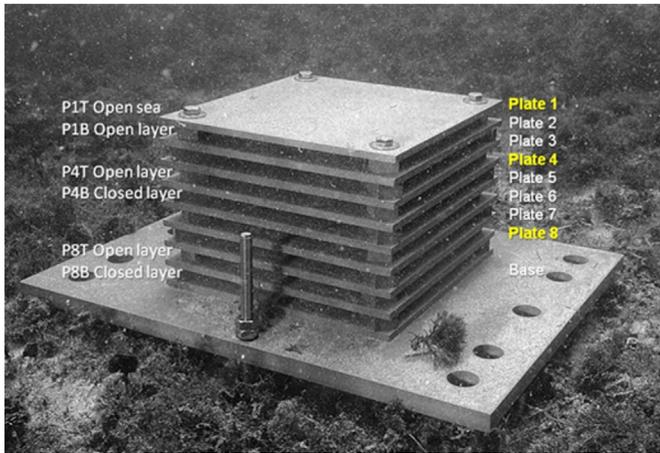
Fig. 2. A newly deployed ARMS (Ile de l'Erevine/Elevine, NW Mediterranean, site ELV). The alternative use of long and short PVC cross spacers give a tower of four open and four closed layers. © photo CNRS/F. Zuberer.

factors sea and site (within sea) but without the factor plate-face to show the consequences of not distinguishing plate-faces, as was undertaken in a recent metabarcoding study (Pearman et al., 2016a).

We compared the dispersion of the data on community compositions between seas, sites and plate-faces (and tested the null hypothesis that it was not varying) using PERMDISP.

Additional analyses were also carried out on partial datasets in order to estimate the power of simpler monitoring protocols either based on single taxonomic groups (eight analyses), or based on single plate-faces (six analyses). Taxon abundances were fourth-root transformed before performing PERMANOVA for testing whether effects of sea and site were significant on the abundances of each taxonomic category. For single-taxon analyses, the Euclidian distance was used instead of the Bray-Curtis coefficient (not defined when abundances are null). Similarly, we tested whether the effects of sea and site on community composition were significant for each plate-face individually using a nested PERMANOVA. We also tested separately for each sea and for each of two plate-faces (P1T, P4B) whether the effect of site on community composition was significant (thus eight separate analyses
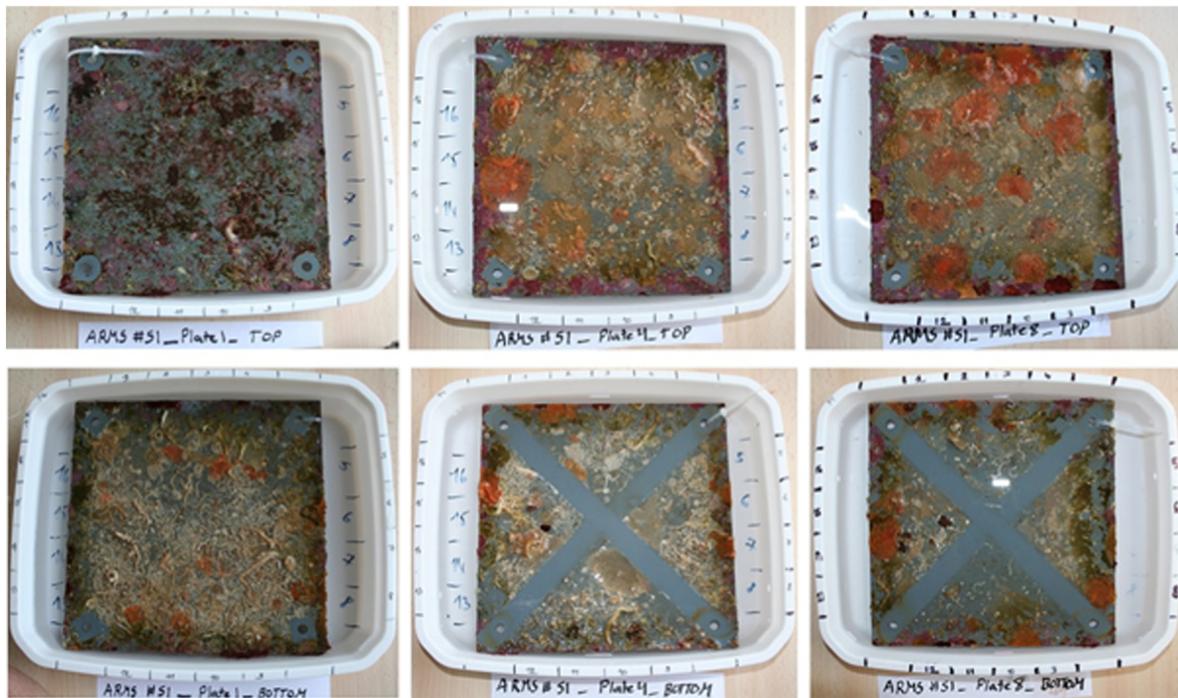

Fig. 3. Sampled ARMS face plates (from left to right: 1, 4 and 8; upper pictures: top faces; lower pictures: bottom faces) after being recovered from the Sea and having collected the mobile fraction [NW Mediterranean, Ile Riou, site RSS]. Cross markings in two of the bottom plates result from the cross spacers that alternate between some plates.

performed on the whole data set (with samples from all faces, sites and seas). For all the analyses we used the Bray-Curtis resemblance coefficient. All analyses were performed with fourth-root transformation of abundance data as recommended (results were similar with non-transformed abundances, not shown).

Multivariate analysis was performed using PERMANOVA (fixed effects, type III sums of squares except for nested designs where type I was used). We ran 9999 permutations for each test. We first tested the effect of sea, sites (nested within sea), and plate-face. We carried out pairwise sea comparisons in two-way PERMANOVAs with the following factors: sea and plate-face. We then tested the effect of each environmental factor in a three-way crossed design containing also the factors sea and plate-face (in these cases, we used partial data sets including only the sea regions for which the environmental factor was varying among sites). We also performed a nested PERMANOVA with the

were performed on small data sets, one for each selected plate-face in each of the four seas). Within seas, the three sites were chosen to have contrasting environmental conditions so the site effects on community composition may be due to environmental factors when there is some variation among sites (some Yes and some No for the corresponding Boolean). In an attempt to check this, we examined, for each sea, whether the site effect was maximized in the pairwise comparisons (of 1 site versus 2 sites) that separated the sites according to their status at an environmental factor (e.g. one 'Yes' site versus two 'No' sites, or one 'No' versus two 'Yes') more often than expected. We computed, for each sea separately, the effect of site in each of the three possible contrasted designs opposing one site versus the two other sites in a two-way crossed PERMANOVA with factors site (that therefore had two modalities) and plate-face. Since there are three possible designs of one site versus two sites, at random, one expects that the design with highest

Table 2

Anthropogenic and environmental context of the study sites. Each site (column) is designated by two three-letter codes separated by an underscore, for Sea region and site (full names in Table 1). Environmental factors are Boolean: presence is indicated by Y, absence by N. In Row 2: for each sea region, we tested whether the community composition of the site (the column head) was significantly different from the other two sites (in a crossed design considering also the effect of plate-face); there are three possible contrasts opposing one site with the other two: rank numbers (1, 2 and 3) refer respectively to the most, second and least significant contrasts (NS: not significant, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$). For each environmental factor (rows 3 to 12) we highlighted in green where the most contrasted site configuration singled out the site which differed from the other two for the environmental factor (rank 1 in row 2), in red when it did not. If environmental factors did not influence community composition, all 3 contrasts within a sea should be as probable; our results perfectly match random expectations with 6 green, 12 red.

Sea region	BoB			NWM			AdS			ReS		
Site	Lek	Zum	Pas	ELV	RRS	CCA	Azz	Sor	Sca	JSR	SOJ	QAR
Rank, from the most (1) to least (3) contrasted site for each Sea region (PERMANOVAs: Face x Site)	1****	3***	2***	1***	3**	2***	1****	2**	3 NS	3 NS	1****	2**
Protection status	N	N	N	Y	N	N	N	Y	Y	N	N	N
Marine debris	N	N	N	N	N	N	Y	Y	Y	Y	N	Y
Sewage output	Y	Y	Y	N	N	N	Y	N	Y	Y	N	N
Chemical pollution	N	N	Y	Y	Y	Y	Y	N	N	Y	N	N
Urbanization	N	N	N	Y	N	N	Y	N	Y	Y	N	N
Harbor	Y	Y	Y	Y	N	N	Y	N	N	Y	N	N
Nearby Seagrass meadows	N	N	N	Y	N	Y	N	N	N	N	N	N
Nearby sand	Y	N	N	Y	N	Y	N	N	N	Y	Y	Y
Nearby mud	N	N	N	Y	N	N	N	N	N	N	N	N

site effect matches in one third of the cases with environmental contrasts (Booleans). There were 20 such possible tests so the expected number of matches at random is 6.7, and non-matches 13.3 (Table 2).

Non metric multidimensional scaling analyses were performed based on Bray-Curtis resemblance coefficients between samples to vi-sualize community from distinct plate-faces for each sea region. Identification of coherence species groups (i.e. groups of taxa that tend to be significantly and positively associated among samples and distinct from one another) were performed using the "coherence plots" wizard in Primer7, which consists of computing the index of association among taxa and performing SIMPROF type 3 tests at the 5% P-value level, without removing any taxon.

3. Results

Two plate-face photographs out of 216 were lost (P8T from one ARMS in site BoB_Lek and BoB_Zum). The proportion of taxa which were identified at the species level was generally low except for Mollusca (Table 3). The list of all taxa initially identified in each sea (i.e. prior to merging) is available in the Supplementary file S2. The raw-data used for statistical community analyses correspond to the merged taxonomical category (cf above: Annelida, Bryozoa, Mollusca …) from the six plate-faces of all ARMS (File S3).

3.1. Taxon abundances and univariate (single taxon) PERMANOVAs

On average, the percentage of the ARMS area colonized by an

Table 3

Number of taxa identified at the species level.

Number of identified taxa	Species level	Other levels	Total
Annelida	5	15	20
Bryozoa	5	14	19
Cnidaria	1	6	7
Crustacea	2	2	4
Mollusca	8	2	10
Colonial Tunicata	2	0	2
Non-colonial Tunicata	1	3	4
CCA	1	3	4
Rhodophyta	2	5	7
Chlorophyta	1	1	2

identifiable taxonomic category ranged from 50% in the Adriatic Sea and 60% in the Red Sea to over 70–75% in the Bay of Biscay and the Mediterranean Sea. There was no consistent pattern for the proportion of colonized area between top and bottom faces across seas for plate 1 (the comparison was not relevant for plates 4 and 8 for which the bottom faces were compartmentalized contrary to top ones): in the Adriatic and Red Sea, bottom faces (P1B) were more colonized than top ones (P1T), with 72% and 52% for the Adriatic Sea and 84% and 71% for the Red Sea, but differences were small (and reversed) in the two other seas. These are underestimates of biological colonization because of undetermined points (on average 2% of points were undetermined in AdS, 4% in ReS and BoB, and 7% in NWM).

The most abundant groups represented on the plates (Fig. 4) were Annelida, Bryozoa, Porifera and Mollusca for metazoans, CCA and other Rhodophyta for algae. Groups like Tunicata (colonial and non-colonial Tunicata) and Cnidaria for metazoans and Chlorophyta and Phaeophyta for algae were much less abundant or widespread. Metazoans represented the largest part of the colonization with up to 54% for the northwest Mediterranean Sea, 48% for the Bay of Biscay, 38% for the Adriatic Sea and 32% for the Red Sea, whereas algae represented > 20% of the total colonization in northwest Mediterranean Sea and Bay of Biscay, 11% for the Adriatic Sea and 27% for the Red Sea. Across all seas, the relative abundances of the most abundant groups differed between the top and bottom faces of plates: Annelida and Bryozoa were more frequent on bottom faces whereas CCA and other Rhodophyta preferred top faces (Fig. 4). Annelida and Bryozoa were more abundant in the Bay of Biscay and northwest Mediterranean compared to the Adriatic Sea and the Red Sea. Mollusca were more abundant in the Adriatic Sea and Porifera were especially abundant in the Adriatic and Red Sea (particularly on the bottom faces of the plates), while CCA were more abundant in the northwest Mediterranean Sea and in the Red Sea (Fig. 4, Table 3).

For most taxa, the three-factor PERMANOVAs based on single taxon abundances revealed significant effects of plate faces (except for Foraminifera) and of sites (except for Tunicata, colonial Tunicata and Other Rhodophyta); a significant effect of sea was detected in seven out of twelve single taxon PERMANOVAs (Table 4). However, when performed within a single sea, PERMANOVAs based on a single taxon, except for Bryozoa, did not consistently detect a site effect (that is, a site effect in each of the four sea regions).
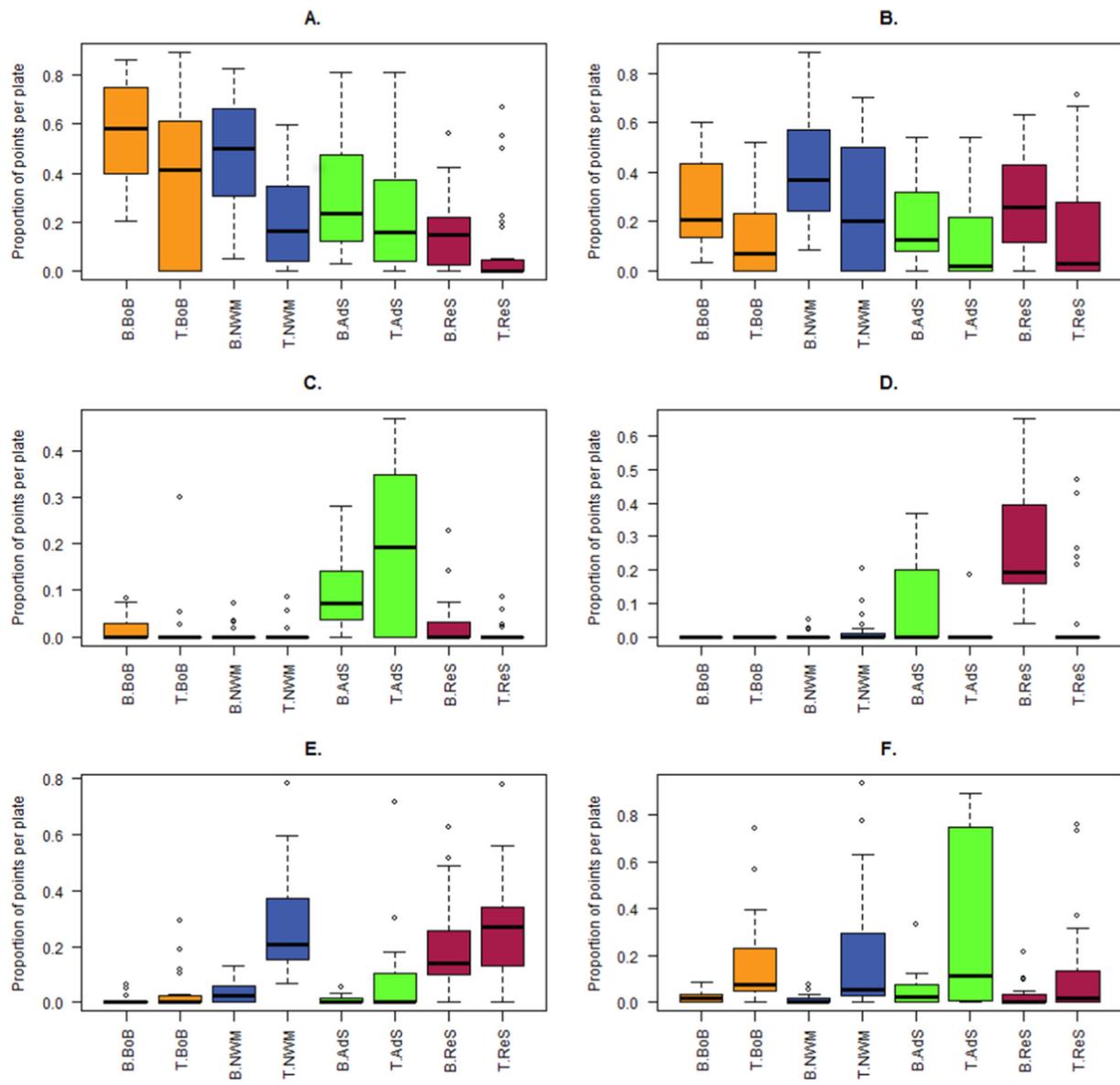
Fig. 4. Boxplots showing median [and quartiles 1 and 3] abundances of main animal and algal groups on ARMS from the four Sea regions. Annelida (A), Bryozoa (B), Mollusca (C), Porifera (D), Crustose Coralline Algae (E), other Rhodophyta (F), on ARMS from four Seas. Seas are ordered from west to east and named as in Table 1. Top (T) and bottom (B) plates were analyzed separately.

3.2. Analyses based on assemblage data (multivariate PERMANOVAs)

The PERMANOVA performed on the whole dataset revealed highly significant effects of sea, site (nested within sea), and plate-face on community composition (see S4 File for detailed PERMANOVA results, Table 4 for a summary). There was also a highly significant effect of the interaction between sea and plate-face, and a very significant effect of the interaction between site (nested within sea) and plate-face (File S4 part 1). In all PERMANOVAs, a similar proportion of the variation was explained by sea and by plate-face, between 17% and 20% for each factor according to the analyses, which is about twice that explained by the factor site, or by the interaction between sea and plate-face (File S4, parts 1, 10 and 11). All pairwise comparisons among seas (two-way crossed design of plate-face and sea) were highly significant (File S4, part 2). PERMDISP did not show significant differences in data dispersion between seas, nor between sites.

Within all sea regions, two-way PERMANOVAs (site and plate-face as factors) displayed significant effects of both factors (Table 4; File S4, part 11). All pairwise plate-face comparisons displayed highly

significant differences in community composition, except between faces P4T and P8T (not significant) or P4B-P8B (P = 0.048) (File S4, part 3). The dispersion of the assemblage data was highly differentiated among plate-faces (PERMDISP P-values = 0.0001) and pairwise PERMDISP tests indicated three levels of dispersion: highly dispersed faces (P1T, P8T), moderately dispersed faces (P4T, P1B), and less dispersed faces (P4B, P8B) (detailed values not shown). Non-metric multidimensional scaling (nMDS) (Fig. 5) illustrates the uniqueness of plate P1T communities with respect to the other ones within each sea and, although less clearly and not for all seas, differences between top and bottom plate-faces. Algal taxa (Chlorophyta, Phaeophyta, other Rhodophyta, and other algae, but not CCA) tend to be more abundant on the exposed faces (P1T) in all four sea regions as reflected by the position of these variables on the nMDS plot (Fig. S1). By contrast, all animal taxa are distant from P1T samples in the nMDS plot.

The coherence analyses revealed two groups of coherent taxa among photo samples: Annelida and Bryozoa, on the one hand, and Porifera, Colonial Tunicata, Cnidaria and Mollusca, on the other hand (File S5).

Table 4

Summary of the main effects from PERMANOVA for assemblage data and Single taxa abundances (fourth-root transformed). The design for global PERMAN-OVAs (column 2) included three factors, factor site nested within factor sea, crossed with factor plate face. Single sea PERMANOVAs included two crossed factors: site and plate face. See text and appendices for more detailed results. CCA: Crustose Coralline Algae. Significant effects are in bold. Only assemblage data and Bryozoa abundances recover an effect of site in each sea region.

Taxon	Global	Bob	NWM	AdS	ReS
Assemblage	**Sea*** **Site*** **Face***	**Site*** **Face***	**Site*** **Face***	**Site*** **Face***	**Site*** **Face***
Annelida	**Sea** **Site*** **Face***	**Site*** **Face***	Site NS **Face***	Site NS **Face***	**Site*** **Face***
Bryozoa	Sea NS **Site*** **Face***	**Site*** **Face***	**Site*** **Face***	**Site*** **Face***	**Site*** **Face***
Cnidaria	Sea NS **Site*** **Face***	Absent taxon	**Site** Face NS	**Site*** **Face**	Site NS Face NS
Foraminifera	Sea NS **Site*** Face NS	Absent taxon	**Site*** **Face***	Absent taxon	Site NS Face NS
Mollusca	**Sea*** **Site** **Face***	Site NS Face NS	**Site*** Face NS	Site NS **Face***	**Site*** **Face**
Porifera	**Sea*** **Site*** **Face***	Absent taxon	**Site*** **Face**	**Site*** **Face***	**Site*** **Face***
Tunicata	**Sea*** Site NS **Face**	**Site*** **Face**	Site NS Face NS	Absent taxon	Site NS Face NS
Colonial Tunicata	**Sea*** Site NS **Face***	Absent taxon	Absent taxon	Site NS **Face***	Site NS **Face***
CCA	**Sea** **Site** **Face***	**Site*** **Face***	Site NS **Face***	Site NS Face NS	**Site*** Face NS
Other Rhodophyta	Sea NS Site NS **Face***	**Site*** **Face***	Site NS **Face***	**Site*** **Face***	Site NS **Face***
Chlorophyta	Sea NS **Site*** **Face***	**Site*** **Face***	**Site*** Face NS	Site NS Face NS	Site NS Face NS
Phaeophyta	**Sea*** **Site*** **Face**	**Site*** **Face***	Site NS Face NS	**Site** **Face**	Absent taxon

NS: not significant, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$.

For each of the six plate-faces analyzed separately, there was a highly significant effect of both sea and site (Table 5) with the single exception of P8T for which site was not significant, but this is probably explained by missing data since P8T pictures were missing for two ARMS in the Bay of Biscay whereas photographs were available for all the other plate-faces of these ARMS. When analyzed within each sea separately, both faces P1T and P4B were (individually) able to detect

significant effects of sites except in the Red Sea (Table 6) but the number of distinct permutations was reduced in these small data sets (S4 File) consisting of at most 9 samples (three ARMS in each of three sites).

Among the six Boolean environmental factors which could be tested in multiple seas (i.e. whose status varied within at least two seas), five appeared significant, generally between the 0.1%–5% probability level but there was an even more significant effect of the interaction of the environmental factor with the sea (most $P < 0.01$ or 0.001) (and the interaction with sea explained a higher proportion of the variance than the Boolean environmental factor itself), suggesting that the observed effect of the environmental factor indeed reflected an effect of site (remember that the effect of site within sea was highly significant with the lowest P-value possible for the 9999 permutations of the data: 0.0001) (File S4, parts 4–9). Significant factors (but collinear with sites) were protection status, urbanization, chemical pollution, presence of a harbor and nearby sand. Presence of sewage output was not significant. For the environmental factors which varied within a single sea, the only way to address their potential effect on community composition was to check whether the site (among the 3 sites) which was singled out with respect to the Boolean environmental factor (e.g. the "Yes" site when the other sites were "No", or conversely) was also the one singled out in the bipartition (1 site versus 2 sites) that maximized the site effect on community composition. There are only three possible contrasts (bi-partitions) per sea thus, by chance, 1/3 of the cases are expected to match (underlined in green, Table 2). Globally we obtained 9 matches out of 18, which is not significantly higher than the expected proportion (18/3 = 6) under the null hypothesis that there is no effect of environmental factors on community composition (Table 2). Thus we have no reliable evidence of an influence of any tested environmental factor on community composition.

In the nested PERMANOVA with the factors sea and site (within sea), but without the factor plate-face, both sea and sites remained highly significant (S4 File, part 10).

4. Discussion

Despite the fact that we used high taxonomic categories, ARMS photo-analyses appeared to be a powerful way to compare marine benthic communities. We detected significant effects at all the levels of our experimental design: sea, site (within sea), and plate-face and within each sea region, site and plate-face effects remained highly significant.

The ability of ARMS photo analysis to discriminate among seas is not surprising because the sea regions correspond to well-differentiated biogeographical units (Spalding et al., 2007) with substantial differences in a variety of environmental parameters (such as salinity, light or nutrient availability). Differences among sea regions may be partly caused by the fact that the ARMS were not installed for identical durations (and in the case of NWM, also depth) in the four sea regions. Obvious seasonal effects are observable in temperate seas, including successions of organisms, as opposed to more equatorial latitudes (where little variation in day-length or temperature occurs over the year) (Mellin et al., 2016; van Hoytema et al., 2016) therefore the deployment and removal date discrepancies between seas probably contribute to the difference in community composition imputed to the "sea" factor in our statistical analyses. Nevertheless, our study is to our knowledge the first one encompassing such a variety of non-tropical regions.

What is particularly relevant for a pilot study aimed at assessing the potential usefulness of monitoring devices is their ability to discriminate among sites within a sea region, because distinct sites are likely to correspond to distinct environmental conditions. Indeed, the distinct sites in a given sea region share a common pool of potentially colonizing species; the role of natural selection in explaining differences in community composition is thus expected to be higher at this scale
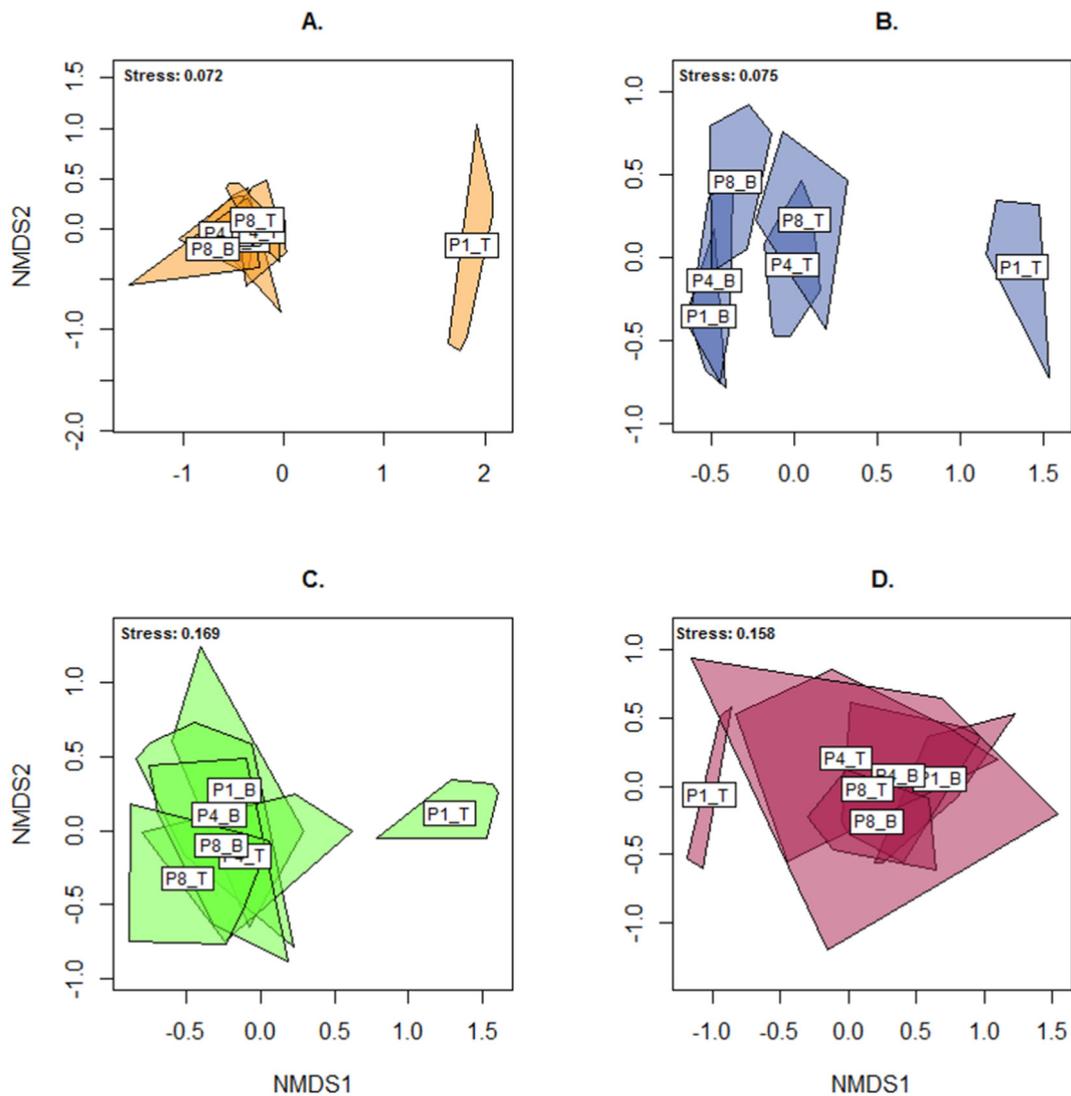
Fig. 5. Non-metric multidimensional scaling (nMDS) representation of community for each plate-face within each Sea region. A: Bay of Biscay, B: Northwest Mediterranean Sea; C: Adriatic Sea, D: Red Sea. Each dot represents one ARMS for a given plate-face, the stress value is indicated.

Table 5
Summary of PERMANOVA results for each plate-face: Plate-faces are denominated by PiT or PiB, with i for the plate number, and T for top face, or B for bottom face. P-values for both regions and sites (within regions). Plate peculiarities are indicated in parenthesis (plate 1 top is exposed to the exterior environment, and plates 4 bottom and 8 bottom are compartmentalized by a central cross). All P-values are highly significant except for P8T (there are two missing pictures, for P8T, both in BoB).

Plate-face	Sea region (P-value)	Site (P-value)
P1T (exposed)	< 0.001	< 0.001
P4T	< 0.001	0.004
P8T	< 0.001	0.749
P1B	< 0.001	< 0.001
P4B (comp.)	< 0.001	< 0.001
P8B (comp.)	< 0.001	0.002

Table 6
Results of one-way PERMANOVA testing the effect of sites for two plate-faces (in columns): P-values corresponding to the site effect are reported, written in bold when significant (P < 0.05). The nomenclature for plate-faces is explained in the legend of Table 5.

Sea region	P1T (P-value)	P4B (P-value)
Bay of Biscay	**0.047**	**0.047**
North Western Mediterranean	**0.007**	**0.047**
Adriatic Sea	**0.035**	**0.024**
Red Sea	0.670	0.051

than between distant areas. Since within each sea, ARMS communities significantly differed among sites and among plate-faces, ARMS photography analysis appears a promising monitoring tool in each of these four sea regions. A more appropriate design to investigate regional environmental effects would use substantially more sites with varying environmental conditions within a region in order to separate pure spatial effects (differences explained by low physical connectivity) from local environmental effects (differences explained by natural selection). For instance, with at least 6–12 sites linearly placed parallel to the coast line, and variations in environmental factors interspersed along this transect, it should be possible to isolate a purely spatial effect (expected to lead to a correlation between the community composition distances and the spatial distances separating sites) from the filtering effect of the environmental context. By focusing on a given region (for instance the Bay of Biscay, or the French Mediterranean Coast), it would also be possible to use finer taxonomic categories, which would probably

provide more power to detect environmental effects on species composition. For illustration, in the Bay of Biscay, North West Mediterranean, Adriatic Sea and Red Sea, we initially had reported 31, 36, 33 and 34 distinct taxa respectively, although our global analysis used only 12 categories (due to merging).

Indeed, we found no clear-cut evidence of an effect of the environmental factors investigated. We cannot conclude whether the significant differences found among sites (within sea) are due to typical spatial effects (the fact not all species could colonize ARMS in distinct sites within a year, due to limited connectivity) or to some purely environmental effects (that is, involving the filter of natural selection) that differed among sites. For the North West Mediterranean region, we have evidence from population genetics data of numerous species that gene flow and dispersal are limited between the sites of the present study, despite their proximity (Cahill et al., 2017; D e Jode, 2018). In 20 locations from the same area, a metabarcoding study of established coralligenous communities revealed a strong effect of the location but also strong effects of the physical environment such as depth, or slope, which were varying within locations) on community composition (indeed the same proportion of the variance, respectively 20% and 19%, was explained by location and by depth) (De Jode, 2018). This sea region is also the single one where most environmental factors (4 out of 6) matched the highest contrasts among sites (Table 1).

Our results support the hypothesis that photo identification of ARMS plates can be utilized as a fast-screening tool to detect changes in the community composition at relatively small spatial scales (tens of km). This result is expected to be conservative because we used very broad taxonomic categories, limiting the statistical power. Discriminatory power could also be increased in future studies focusing on a single sea by including more numerous sites. Some taxa, when considered alone, proved more powerful than others to discriminate among seas. However, single taxon abundances, contrary to community composition, were unable to discriminate sites within all seas, with the exception of Bryozoa. By contrast, the community analyses (nested PERMANOVA of sites within seas) found significant effects for both sea and site (even when the face factor was not considered). In addition, for five of the six plate-faces analyzed (the exception, for P8T, being ex-plained by two missing pictures), when taken individually, community composition significantly differed both between sea regions and sites (within seas). This is an illustration of the limit inherent to approaches based on single or few indicator taxa: community composition data are more powerful than single taxon abundance data to detect changes and thus to monitor ecological status (Borja et al., 2015). The fact that the sole plate-face (P8T) that was unable to differentiate sites within seas was the one for which two sites had missing data (and were therefore represented by duplicates rather than triplicates) suggests that tripli-cating ARMS in each monitored site is useful. When an ARMS unit is lost accidentally, the fact of having multiple faces in other ARMS units may partially compensate the lack of information, since single faces within a sea were able to differentiate sites in three out of four seas (Table 6). Indeed, one striking feature of our study is the strong dif-ferences in community composition among plate-faces of the ARMS: this establishes that the faces represent distinct micro-habitats. They display obvious differences in terms of light exposure, predation ex-posure, sedimentation and water flow, for instance. The fact that sev-eral algal taxa appear to contribute strongly to the uniqueness of plate P1T is a clear illustration, probably explained by light exposure. A re-cent study conducted on microalgae colonizing ARMS provided similar conclusions (Pennesi and Danovaro, 2017). For compartmentalized faces (P4B and P8B) each such face represents 4 independent coloni-zation units and/or is less susceptible to be affected by a given random event such as predation by a grazer (indeed we observed one sea urchin scouring a top face of an ARMS). This interpretation is supported by the fact that these faces display the lowest levels of data dispersion, and significantly less than the other ones (PERMDISP analyses). The sand-wich-like structure of ARMS thus appears a positive feature of these

systems, as compared with single layer colonization plates, because it allows sampling of distinct micro-habitats and guarantees a balanced, thus more powerful design (each plate-face being present in each sampling unit). Classic settlement plates are more or less equivalent to the top plate P1T of this study. Hard bottom benthic communities often form very complex 3D structures and some faunal, but also algal (non-green) organisms are typically not found on the surface. Other plate-faces that have less predation, less irradiance, less hydrodynamism or more sedimentation thus provide a higher diversity of habitats, making them more representative of the benthos established on natural substrate. A rich community may eventually establish on a surface layer of a classic settlement plate, but it would probably require more time than in sandwich-like structures like ARMS that readily provide shadowy and current-protected habitats.

Considering plate-faces separately is likely to improve statistical power but analyzing all 16 faces of each ARMS is probably not necessary for routine monitoring programs. We recommend however that at least one future pilot studies compares all 16 faces in order to provide a complete picture of the pairwise differences and dispersion of an ARMS. Based on the results, a subset of faces could be selected for repeated temporal monitoring according to their ability to reveal differences associated with particular environmental parameters.

Benthic substrata, when monitored with photographic approaches, only reveal a superficial part of the local biodiversity (Sini et al., 2015). Photo-inferred communities may not accurately represent the full diversity in highly complex 3D-habitats, such as coralligenous reefs. This is especially true as they only focus on the sessile component of the community. Molecular techniques, including barcoding and meta-barcoding (Leray and Knowlton, 2015), although with their own lim-itations (Carugati et al., 2015) could provide new opportunities to make the analysis of ARMS more efficient and standardized (Ransome et al., 2017). Indeed, (Pearman et al., 2016b) showed that for ARMS in the Red Sea, a higher diversity, encompassing a broader range of taxa, was observed when using molecular techniques compared with morpholo-gical approaches. In their analyses, metabarcoding was able to differ-entiate sites while morphological approaches did not show a significant difference in the composition between sites (despite the sessile fraction of all plate-faces having been pooled for metabarcoding). A higher di-versity by metabarcoding than traditional approaches was also found in Northwest Mediterranean hard bottoms, both in artificial and natural substrata (Cahill et al., 2018; D e Jode, 2018). An ongoing project will present the results of metabarcoding analyses carried out on the ARMS studied here (and including additional European regions).

This study established that ARMS photo-analyses provide an effi-cient tool to reveal the effect of seas and sites about 10–30 km distant one another with a protocol simple enough to be generalized for monitoring applications.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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