Biomonitoring of Mimachlamys varia transplanted to areas impacted by human activities (La Rochelle Marina, France)

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Research paper

Title Biomonitoring of *Mimachlamys varia* transplanted to areas impacted by human activities (La Rochelle Marina, France).

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Abstract

The development of human activities on French Atlantic coastlines (La Rochelle) lead to chronic pollution of the environment by organic (pesticides, hydrocarbons, agrochemicals) and inorganic (heavy metals) contaminants. These past years, several regulations have been implemented to preserve coastal environments. The purpose of this study was to perform biomonitoring of bivalve species using an outdoor caging technique. The goal of our work was to assess the impact of harbour's trace elements on the state of health of the marine bivalve *Mimachlamys varia.* First, various molecular defence biomarkers were measured: SOD (oxidative stress), GST (detoxification process), MDA (lipid peroxidation), and Laccase (immune reaction). Thus, in April 2016, scallops were collected at three caging sites, which differ by their levels of pollution, after transplantation into port areas (fairing, rainwater) and a control site (marsh). Bivalve samples were taken at three sampling dates (D0, D07, D21). Biomarker assays were performed in the digestive glands due to their bioaccumulation properties. The second aim was to explore the impacts of inorganic pollutants placed in environmental harbour's sites. After 21 days, the biomarker response of transplanted bivalves revealed a SOD decrease, Laccase and GST stimulations, higher concentrations in Cu, Fe, As, Co, Mn, Zn, Sn and no significant variation of MDA concentration. Our ecological relevance of biomarker approaches opens interesting perspectives to identify *M. varia* such as a pertinent marine sentinel species. The several selected biomarkers determined could confirm their ability to appraise the water quality of hydro-systems located in French coastlines, such as port areas.

Keywords: biomarkers, environmental quality, heavy metals, port area

1. Introduction

Over the years, concerns have been raised about heavy metal contaminations, particularly in port areas and their surroundings. The human activities and downtown locations, which have direct access to the sea, could generate different types of wastes released in the environment as dissolved particles, macro-wastes and liquid effluent. These sea wastewaters reveal the presence of a mixture of chemical contaminants in significant amount, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorobiphenyl (PCBs) and other chemicals (Dorris et al., 1972). Most of them are toxic, even at low concentrations (Long et al., 1995). Therefore, environmental contamination is a major threat to marine ecosystems and human health (Fasulo et al., 2012a; Sureda et al., 2011), particularly near harbours and cities.

One of the largest harbour sites in Europe is the marina of La Rochelle. The harbour stretches from the marina to 15 km of docks along the coastline of La Rochelle (France). This harbour, settled in the 1970s, has today 5,500 spaces and welcomes 160 companies of the nautical sector, as a result of urban and economic developments.

Previous reports published by CREOCEAN (2016) and recent studies performed in Bay of Biscay (Milinkovitch et al., 2015; Breitwieser et al., 2017) revealed that Cu, Cd, As and trace elements are found in high concentrations, exceeding the standard limit reported by national and international sediment quality guidelines (Low n° 1881/2006). Moreover, different projects documented the key role played by cities and harbours in the contamination of the coastline. The Charente-Maritime area has acknowledged the importance of environmental state of health and was recognised as an outstanding functional set on the Atlantic coast by the French Environmental Protection Ministry in 2006. Indeed, this marine region is a mudflat ecosystem full of various commercialised organisms such as bivalve species. Bivalve molluscs – in particular mussels, oysters and variegated scallops – are widely used as bio-indicators in environmental monitoring programmes (Mussel Watch Program, IFREMER networks) due to their large geographical distribution. In addition, these filter-feeding organisms have a sedentary lifestyle, which enables them to bioaccumulate and tolerate great amounts of pollutants in their tissues (Lacroix et al., 2015; Sureda et al., 2011; Luna-Acosta et al. 2010; Bustamante et al., 2005). In addition, as mussels, black scallops *Mimachlamys varia* are suitable to conduct caging experiments in port areas (active biomonitoring). *Mimachlamys varia* is located in water/sediment interface, which is a highly relevant area to assess sediment quality. Our approach allows a reduction of physiological variables by using variegated scallops from a single hatchery population, more controlled over the experiment, and an accurate evaluation of the effects of polymetallic contamination in natural environments (Cappello et al., 2013a, 2013b, 2015; Fasulo et al., 2012b; Lacroix et al., 2015; Marigomez et al., 2013; Jimeno-Romero et al., 2019).

This work presents some of the results from the DYPOMAR research project. A multi-biomarker approach is performed on the digestive glands of scallops to assess the potential adverse effects of polymetallic contamination on black scallops caged in the La Rochelle marina. The bivalve digestive glands are involved in digestion, bioaccumulation and are one of the first organs to be affected by environmental stressors. Therefore, they are frequently used as a model system in ecotoxicological studies (Bustamante et al., 2005; Milinkovitch et al., 2015; Frantzen et al., 2016; Breitwieser et al., 2016; Luna-Acosta et al., 2017). Further studies describe that after pollutants exposure, bivalve's digestive tissues are involved in immune defence, detoxification processes and in homeostatic regulation (Marigomez et al., 2002; Moore and Allen, 2002), and histopathological alterations (Garmendia et al., 2011); so, digestive gland could correspond to a standardised tool for monitoring the general state of health of organisms (Amiard-Triquet et al., 2013) is assessed.

Several biomarkers (defence and damage) were used during the study to highlight the different reactions that can be induced after contamination. The defence biomarkers used are superoxide dismutase (SOD), a marker of oxidative stress, glutathione S-transferase (GST), involved in the process of detoxification of xenobiotics, and Laccase with a role in the modulation of immune response. The biomarker of damage, the malondialdehyde (MDA), is involved in lipid peroxidation process. Indeed, aquatic invertebrates may suffer from impairment in the enzymatic systems after being exposed to toxic compounds (Amiard-Triquet et al., 2013). The concentrations of heavy metals in the digestive glands are also measured to carry out a qualitative correlative approach. The quality brings together several biometric parameters (length, thickness and width), using the following equation:

$$Quality = \frac{length + thickness}{width}$$

This measure of quality is found in particular within the framework of the Red Label program (quality index) or biomonitoring measures to evaluate the health status of bivalves.

In addition, metallothionein concentrations were measured in other organisms at similar sampling dates to collect additional information on metal contamination at port sites (fairing, rainwater). Metallothionein is a defence biomarker that can be overexpressed in tissues in the presence of high metal concentrations (Damiens et al., 2006).

2. Material and methods

2.1 Sampling sites and caging transplantation procedure

Two sampling sites located in the marina of La Rochelle (Nouvelle-Aquitaine region) and exposed to diffuse trace elements contamination are chosen. Sampling sites are named as follow: the careening area, exposed to contamination due to boat handling (antifouling paint residues, sacrificial anodes), and the rainwaters site, located near one of the biggest rainwaters outlets, which is characterised by a lack of filtration system (Figure 1). Previous studies revealed that the marina of La Rochelle contained high levels of heavy metals due to the port area being semi-closed (Breitwieser et al., 2016; Breitwieser et al., 2017). The third study site is a closed marsh of the laboratory, considered as the reference site, located a few kilometres away from the marina. In spring 2016, a batch of variegated intertidal scallop (*Mimachlamys varia*) from the Tinduff hatchery (Brittany Region) was caged in the marsh area for fifteen days in order to acclimate. Scallops were maintained one week in aerated seawater and then transplanted in selected sites in the two intra-harbour sites and the marsh. At each experiment day (D0, D07, D21), surviving species in the caging are counted and compared to the initial batch number (Fasulo et al., 2012b, Cappello et al. 2013a). A high mortality rate is observed during the acclimation period, likely due to the stress of transportation from Brittany to La Rochelle

(Maguire et al., 1999, Sarkis et al. 2005). In contrast, a low mortality rate (23%) is recorded after transplantation in the harbour sites. Ten scallops per site at two experiment dates (D07 and D21) are sampled and prepared on the spot for biochemical analysis and heavy metals quantification. Ten scallops are sampled as representative of D0 at the marsh site (Table 1). The dissections and the conservation in the fridge on site prevent from biological degradation.

2.2 Water physicochemical assessments

From 17 June 2016 to 14 July 2017, temperature, salinity, turbidity, chlorophyll and dissolved oxygen are measured at 5min intervals using a multi-parameter probe (YSI 6600V2). Three monitoring stations, composed of cages settled on floating structures moored to the dock, are set up at three locations: the rainwaters site, the fairway area and the marsh. The rainwaters and careening sites floated around 0.8 meters under water surface. The marsh probe is set on a pole at 1 meter under the water surface. These three stations are set up at less than 1 meter from the scallops (Figure 2).

Salinity and turbidity are laboratory calibrated using 12880$\mu s.cm^{-1}$ and 100 and 1000 NTU standard. Calibration constants are checked to be within the acceptable range mentioned in the user manual. Laboratory calibrations of turbidity sensors for SSC (Suspend Sediment Concentration) are performed in a 40L black bucket using wet sediments collected under the instruments. SSC are validated by filtration, using GF/C filters on field samples. Times-series data are processed using Matlab routines. Inter-calibration of probes are checked. No significant differences are noticed (paired T-student test with simultaneous field-measurements, $p<0.01$).

The ROX Optical Dissolved Oxygen sensors (6150 ROX probe) are calibrated using the one point « saturated water » method, mentioned in the « Calibration, Maintenance and Troubleshooting Tips » manual.

We carry out a post-deployment calibration on chlorophyll measurement using field samplings. Filters are analysed using a fluorometric method with acetone 90% (Fluorometer Turner TD 700), as proposed by Lorenz (1967).

2.3 Trace element assessments

Trace elements analyses are carried out according to previous studies (Milinkovitch et al., 2015; Breitwieser et al., 2017). Analyses of 14 trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, V, Zn) are performed using two measuring instruments: a Varian Vista-Pro ICP-OES and a Thermofisher Scientific XSeries 2 ICP-MS.

2.4 Biomarker assessments

All the biochemical assays (GST, Laccase, SOD, MDA) are performed using the same instrument: SAFAS Flx-xenius at the specific absorbance for each biomarker kind. The assays were carried out on the digestive glands of *M. varia* at the reference site (marsh) and the contaminated sites (outlet rainwaters and careening areas) at D0, 7 and 21 (D0, D7, D21, n = 10 per site and per date). Before statistical analysis, row data for GST, SOD and Laccase are expressed in U/mf of proteins. MDA row data concentrations are expressed in µM/mg of protein. Total protein concentrations are determined using the BCA Kit methodology (Bicinchononique Acid Kit, Sigma Aldrich). The BCA kit contains bovine serum albumin (BSA) as a standard and involves the reduction of alkaline Cu^{2+} by proteins (Smith et al., 1985) at absorbance 562 nm.

Moreover, another method to assess metallic pollution is used as additional data (Table S1). According to Mouneyrac (1998), metallothionein concentrations are determined in the digestive glands of scallops sampled at the same dates (D0, D07 and D21).

Glutathion S-transferase (GST) assay

Glutathion S-transferase (GST) specific activity plays an important role in xenobiotic detoxification. Total activity of GST (GST being a group of enzymes) is determined according to the kit method of Sigma (CS0410-1KT) (Breitwieser et al., 2017).

Superoxide Dismutase (SOD) assay

Superoxide dismutase (SOD) activity plays a key role in antioxidant response (Paoletti et al. 1986).

Malondialdehyde (MDA) assay

Oxidative stress is assessed through lipid peroxidation by quantifying malondialdehyde (MDA), a chemical metabolite of cell lipid degradation. MDA concentration is measured using a commercially available MDA assay kit (Oxis International) (Milinkovitch et al. 2015).

Laccase assay

The phenoloxidase (PO) activity (Laccase) reveals bivalve's immune system alteration. Its enzymatic activity is contaminant sensitive (Luna-Acosta et al., 2010) and plays a crucial role in the immune defence mechanism of marine invertebrates.

2.5 Statistical analysis

Statistical analysis is performed using R software (R core team, 3.2.2 version). The survey is structured according to the dates (3 dates), the sites (3 sites), and the size of the samples for each sub-group (10 specimens only).

For all variables, homogeneity of variances is assessed using Fligner-Killeen tests. Then, the multivariate response of individuals at our three sampling sites and D0, D07, D21 is measured using a Multivariate Factorial Analysis. Indeed, the sample group is of 10 specimens. Each sample distribution reveals great heterogeneity for each variable. Therefore, it is unlikely to conduct a MANOVA, considering the number of trace elements (14), the number of morphological traits (3) and the number of biomarkers (4). In order to overcome this issue, all variables are divided into two sections: low and high, whether the value of the sample is under or above the median value of the 70 individuals. This should also prevent from further issues due to abnormal values and non-normal distribution of variables inside each group of individuals. Normality is tested using a Shapiro-Wilk test. The normality hypothesis inside sub-groups (of 10 specimens) is rejected most of the time when examining the 21 variables. At D0 only 10 specimens are sampled, whereas 30 specimens are collected both at D7 and D21. Therefore, values of DO individuals are weighted by 3 to balance the results between the 3 periods. Thus, all variables are recorded as being high (1) or low (0) for each individual. Then, a qualitative multivariate analysis is conducted to underline relationships between variables (site, date, heavy metals, biomarkers and biometry parameters).

3. Results

3.1 Water physical parameters

The water physical parameters are presented in Table 2. We perform daily averages to understand the general trends of the environmental conditions. The temperature trends range from 16.8°C to 19.7°C and are representative of current weather conditions. In contrast, salinity and turbidity vary depending on the climatic conditions ranging from 27.61 to 33.19 PSU, and 0.004 to 0.037 g/L. These measures are mainly due to rainfall bringing fresh water and sediment. This phenomenon is observed at the rainwater station at D21. Chlorophyll trends also differ very slightly at sites and dates, likely due to sunstroke. Dissolved oxygen rarely drops below 80% in surface water. 100% saturations at the surface water occur during renewal of water bodies and in presence of wind.

3.2 Levels of trace elements

Table 3 presents the results of the analyses on heavy metals performed in the digestive glands of the black scallop *M. varia.* These results are presented in µg/g of dry weight.

By comparing these 14 heavy metal values with the ones from previous studies in pectinids (Metian et al., 2008; Milinkovitch et al., 2015; Viarengo et al., 2000; Breitwieser et al., 2017), results reveal a lower contamination (in italic in the table 3) by Pb at D7 (-30%) at all sampling sites in comparison with the marsh (2.3 ± 0.1 µg/g of dry weight). In addition, there is a lower contamination (in italic in the Table 3) by Cd (-18%), Co (-28%), Mn (-30%), Pb (26.2%), Se (-23%), Zn (-14%), and Ag (-20%) at the rainwaters and careening sites at D21, in comparison to the reference site. In contrast, there is a higher contamination (in bold in the Table 3) by As (+7.5%), Cd (+14%), Cr (+125%), Cu (+423%), Fe (+ 18%), Mn (+18%), V (+62%) and Zn (+117%) at the rainwater or/and careening sites at D7 as well as a higher contamination (in bold in the table 3) by As (+12%), Cr, Cu (+54%)and Fe (+52%) at the same sites at D21.

3.3 Biomarker responses

The enzymatic assays presented in Figure 3 underline the state of health of marine bivalves. These are performed in order to target the capacities of detoxification of xenobiotics (GST) and to assess the struggle (SOD) against reactive oxygen species (ROS), the integrity of cell membranes, the involvement of lipid peroxidation (MDA), and the modulations of the immune system (Laccase).

The scatter plot in Figure 3 displays an easy-to-read, descriptive and multi-criteria analysis that compares results depending on the sampling dates and the caging sites of variegated scallops. All pollution biomarkers show significant modulations of their responses at various dates and the two intra-harbour sites.

The plot in Figure 3 presents GST distributions. There are no significant differences. Only trends of mean specific activities seem to increase over time and are higher at the marsh site (0.75 ± 0.2 UI/mg of proteins at D07, 1 ± 0.2 UI/mg of proteins at D21) than at the rainwater (0.66 ± 0.1 UI/mg of proteins at D07, 0.85 ± 0.1 UI/mg of proteins at D21), or the careening areas (0.7 ± 0.1 UI/mg of proteins at D07, 0.94 ± 0.1 UI/mg of proteins at D21). The non-parametric test of Fligner-Killeen for homogeneity of variance with a p-value of 0.5817 confirms that we can compare both dates and sites for GST.

In contrast, MDA homogeneity of variance test fails either for date (p-value = 0.0388), site (p-value = 0.0013), and both date and site (p-value = 0.0002, < 0.05). In fact, at the rainwater site at D21, the large distribution is characterised by a high heterogeneity of MDA concentration (16.1 ± 13.4 µM/mg of proteins), which is not influenced by dates or sites.

The analysis of the Laccase activity at the three sites and sampling dates reveals discrimination at all sites at D21. The non-parametric test of Fligner-Killeen for homogeneity of variance confirms (p-value = 0.4522) the analysis. Whereas the behaviour of the Laccase enzyme is similar at D0 and D7, and stable for all sites (average of 4.5 ± 1 UI/mg of proteins), there is an overall increase at D21. However, at D21, Laccase is lower at the careening site (7.5 ± 1.1 UI/mg of proteins) than at the other sites (10.3 ± 2 UI/mg of proteins).

Superoxide Dismutase activity is compared for both sites and dates, as shown by the non-parametric test of Fligner-Killeen for homogeneity of variance (p-value = 0.2022). The rainwaters and careening sites have similar patterns: they increase slowly between D7 and D21, although they remain lower than the levels at the marsh site at D0 (57 ± 8 UI/mg of proteins) and D7 (72 ± 12 UI/mg of proteins). The marsh site highlights an irregular pattern: starting with high levels at the beginning (D0 and D7), they increase drastically before dropping at D21 (23 ± 11 UI/mg of proteins). The activity levels at D21 are lower than the ones of the rainwaters (40 ± 21 UI/mg of proteins) and careening sites (30 ± 11 UI/mg of proteins).

Moreover, metallothionein concentrations presented in Table S1 show a significantly higher response and a specific inorganic contamination at the careening and rainwaters areas, in comparison to the marsh site at D7.

3.4 Factor analysis to underline relationships between biomarker responses and trace element assessments

Figure 4 displays an easy-to-read, descriptive and multi-criteria analysis that compares results of the qualitative analysis used ("low signal" or "high signal"), the sampling dates and the various sites. The analysis focuses on the two first components, since the others do not bring further details (Figure S1). The first two axes explain 36.92% of the variance, which is enough to perform a qualitative factor analysis.

Looking at axis 1 (horizontal dimension), variables above the 0.5 contribution value (Figure S2 in supplementary data) indicate a high concentration of Pb and Ag, and a low concentration of Cu, Sn and As linked to strong SOD and low Laccase activities. Variables under the -0.5-contribution value show a high concentration of Cu, Cr, Mn, Sn, and As linked to a strong Laccase and GST activities, but a low SOD activity.

Looking at axis 2 (vertical dimension), variables above the 0.5 value shows the antagonistic effect of low concentration (for *high.0*) of Ni, Co, Pb, Mn elements against the effect of high concentration (for *high.1*) of Pb, Fe, Se, Ni, Zn, Co, and Mn.

Thus, individuals at the right of axis 1 have a strong SOD activity but a low Laccase activity. They seem to be less exposed to heavy metal elements than those on the left side of axis 1. Axis 1 reveals the effect of Cu, Sn and As on enzymatic activities (SOD, Laccase and GST), as a major interaction of the state of health of scallops. Furthermore, GST and SOD seem to have antagonist effects. Axis 2 underlines a separation between high concentrations of heavy metals such as Mn, Co, Zn, Ni, Se, Fe, and Pb and low concentrations of Mn, Ni, Co, Pb. It highlights the preponderant concentration of Mn, Ni and Pb in scallops (individuals having a high concentration in heavy metals are shown at the bottom of the Figure 4).

In order to check whether the site and date factors (temporal dimension) influence the specimen's repartition on the plan defined by axes 1 and 2, we have drawn confidence ellipses on the plot of Figure 4. A label and a distinct colour are assigned for each group or individual. Groups are divided as followed: D0, D7 and D21 at the marsh, D0, D7 and D21 at the careening area, and D0, D7 and D21 at the rainwaters site. Harbour sites (careening and rainwaters) have similar evolution for each date (D7 and D21), in comparison to the marsh groups, which are merely located at the right of axis 1. Over time (from D0 to D21), a movement towards the bottom of the axis 2 is common to all groups, which is symptomatic of an increasing concentration of Pb, Fe, Se, Ni, Zn, Co, and Mn trace elements. Equally, a movement of all groups towards the left of the axis 1 can be observed, due to a higher concentration of Fe, As, Sn, Mn, Cr, Cu trace elements conjointly with stronger GST and Laccase activities, and a lower SOD signal.

4. Discussion

4.1 Study method

So far, few caging multi-biomarker studies (biomarkers of effect and exposure) have been carried out in France using other molluscs such as the freshwater mussel *Unio tumidus* (Charissou et al., 2004), and the marine mussel *Mytilus* spp. (Lacroix et al., 2017). Caged bivalves could be successfully used as sentinel animals of contamination events in marine ecosystems. Some data show that the uptake and elimination of metals were evaluated in different tissues of the mussel *Pinna* *nobilis* L. transplanted to harbour's area of Tunisia (Jebali et al., 2014). Moreover, mussels of the specie *Mytilus galloprovincialis* could also reveal high levels of contaminants in bioactive monitoring experiments conducted in Spain (De los Rios et al., 2012; Marigomez et al., 2013; De los Rios et al., 2018).

The black scallop *M. varia* is located in the subtidal zone, unlike oysters and mussels that live higher in the intertidal zone. Unlike the last two species, the black scallop and its digestive glands are used as tools revealing the environmental quality of the port area (Breitwieser et al., 2017).

One of the goals of this study is to assess the feasibility of a caging experiment on marine bivalves of similar backgrounds in order to compare the environmental quality between intra-harbour sites (potentially contaminated environments by various chemical compounds) and a less contaminated area that had been rarely done before on variegated scallop (*M. varia*).

To this end, various precautions are taken: the choice of a calm and safe zone for the installation of the cages, the types of cages (dimensions and meshes adapted to the species) and the choice of the species, adapted to the condition's environmental aspects of the study areas. The shellfish from the Tinduff Hatchery are 18 months old and already acclimated to caging experiment conditions. In addition, the dissection of living bivalves is carried out on site to prevent from disruptions of the enzymatic activities.

Moreover, even if each individual is considered as a replicate of the study, we duplicate cages, fixed to a floating structure on each site, to accentuate the contact with trapped pollutants in the sediment. The cages are risen to the surface at each sampling date – D0, D07, D21 – (as exposure time is necessary for the bioaccumulation of bivalves). Our experimental design inspired of the study of Charissou (2004) is agreeing with the recent work which aimed to study impacts of dredging operations on biomarkers responses of caged bivalves (Moreira et al., 2019).

4.2 Sensitivity and relevance of parameters

The chemical composition of the water samples does not enable to classify the sites as mentioned by Charissou (2004). All the parameters suggest normal water quality based on values measured at each harbour site at D0.

Biomonitoring of variegated scallops allows putting forward a global classification of the three sites. In decreasing order of the biological quality, the classification is: careening<rainwaters<marsh. The marsh is considered as the best site, as only a few heavy metals are significantly higher, when compared to the other sites.

Many scientific studies underline that higher heavy metals values in bivalve organisms reveal an exposition to metal compounds and, thus, a risk of having toxic effects (Bouchard, 2018). At D21, our results show higher concentrations of As, Cr, Cu, Fe and Sn at the careening area, when compared to the marsh. Moreover, As, Cu, Fe concentrations are higher at the rainwaters area than at the marsh. Our results support the hypothesis of the World Health Organization and the potential ecological risk of environmental pollutants. It states that ambient environmental contamination is caused by a combination of agents from public road, roofing panels, metal adhesives, pigments, metal industry, and antifouling paint (Pourabadehei and Mulligan, 2016; Ranjbar Jafarabadi et al., 2017; Chen et al., 2017; Jahan and Strezov, 2018; Gargouri et al., 2018; Kim et al., 2018; Tunca et al., 2018).

After 21 days, concentrations of bioaccumulation in *in situ* scallops are similar to previous studies performed at the same location. Moreover, the biomarker response of transplanted bivalves matched the results of *in situ* bivalves obtained from a previous study (Breitwieser et al., 2017). Overall, this research revealed a SOD decrease, Laccase and GST stimulations, and higher concentrations in Cu, Fe, As, Co, Mn, Zn, Sn in the digestive glands. This is an interesting criterion to consider for future studies using the specie *M. varia*. Nonetheless, this finding does not explain the toxicology of heavy metals, which depends on molecular shapes. New methods for the analysis of toxicology induced by heavy metals could be used, such as the quantum dot method used for cadmium

(Jimeno-Romero et al., 2019) where the solubility of particles would be partly the cause toxicity generated in bivalves.

In addition to heavy metal assessments, it will be relevant to write down all the contaminants of the harbour of La Rochelle. This list could include pesticides already identified in coastal waters, as biocides (diuron, triazine) used in antifouling paint of ship and small boat, pharmaceutical residues and other micro-pollutants (micro-plastics, phtalates), which are all potentially dangerous for the health of organisms, and, thus, to consumers at the end of the food chain.

Multi-criteria analysis (Figure 4) is carried out. We compare the inorganic contaminant, molecular biomarker and quality qualitative analyses ("low signal=high.0" or "high signal=high.1") as well as the sampling dates and the various sites. Pollutant qualitative results (Pb, Fe, Se, Ni, Zn, Co, Mn, Cr, Sn, As) in the digestive gland of this species are positively correlated with biomarker of responses (GST and Laccase) and negatively correlated with the SOD biomarker. The stability of the MDA results reveals the efficacy of anti-oxidants and lipid peroxidation in variegated scallops, as already found in juvenile bivalves (Roméo et al., 2008). Moreover, several natural abiotic factors (turbidity, chlorophyll a, oxygen level, temperature, salinity) could also explain the modulation of oxidative stress, although no significant modulation is observed. Thus, the digestive glands of the variegated scallop are relevant organs to assess the environmental quality of the harbour (Triebskorn et al., 2001) as biomarker results reveal similar modulations to previous studies using this specie (Milinkovitch et al., 2015; Breitwieser et al., 2016; Breitwieser et al., 2017). Indeed, three biomarkers of exposure (Laccase, SOD and metallothioneins) show significant responses and the GST biomarker highlight an activity trend that displays environmental stress. These results suggest that the oxidative stress and reactive oxygen species neutralisation could be due to a mixture of contaminants (e.g pesticides, hydrocarbons, heavy metals) from wastes of watershed and urban activities (Luschak, 2011).

These molecular biomarkers of exposure are relevant and efficient tools to use for the harbour authorities. Overall the study demonstrated that differences in chemical bioaccumulation and biomarker responses, and confirm results of Brooks et al., 2015 which explain the importance of identifying the pertinent species when using bivalve in biological effects studies. In addition, new easy, rapid and inexpensive methods could be implemented. For instance, energetic metabolism with ATP assay could be linked to behavioural parameters (reproduction, growth). The study of energy allocation mechanisms at the biomolecular level up to the cellular level could bring a better knowledge of the responses of *M. varia*. Indeed, the energy cost of environmental stress may decrease the amount of energy available to maintain homeostasis and, therefore, reduce the fitness of individuals as highlighted in sea fish and mussel species (Lauwrence et al., 2003; Donaghy et al., 2016). The majority of energy would be allocated to the development of defences, which could lead to the increase in tolerance of marine species to environmental chemical contamination. Energy metabolism therefore remains a relevant pathway to investigate in order to understand how populations maintain themselves in the environment, particularly with abundance fluctuations between both species. It could be complementary to plan also to consider current perspectives and future directions of three main omics (transcriptomics, proteomics and metabolomics) with regards to identify, characterize and determine functional aspects across biological processes to better understand bivalve physiology, especially with regards to immune defences to environmental stresses (Fasulo et al., 2012b; Gouveia et al., 2019; Nguyen et al., 2019). Finally, it seems to be urge (1) to compare the concentration of trace elements found in bivalve *M. varia* collected from the La Rochelle Marina with the xenobiotics data determined with those reported in other French port's areas and (2) to participate to the enrichment of the data available on the potential risks to human health associated with the consumption of marine bivalve (Marengo et al., 2018).

Conclusion

This field study attests that scallops, particularly the digestive glands, are effective tools to assess environmental port’s quality. Biochemical parameters could represent reliable biomarkers for the assessment of environmental pollution on *M. varia* as biological biomonitoring model. In addition, for the first time, the research reveals a rapid bioaccumulation of heavy metals in this specie as well as a similar toxicity to previous studies performed *in situ* in the marina of La Rochelle. Further investigations are needed to evaluate the environmental impacts of environmental (biotic and abiotic) factors using multi-biomarker approaches to confirm such point of view.

Coastal waters collect storm waters containing substances, such as heavy metals and pesticides, which have negative impacts on the water quality. It could be interesting to explore some differences in histological microbiological and biomarker responses of scallops were site-dependent. In addition, dilution caused by tides, currents and rainfall have adverse effects on water. Thus, it is essential to develop low-cost and transposable approaches for monitoring the water quality in similar areas. Finally, we propose the use of scallops as indicator species for monitoring of diffuse pollution in the marine environment. We could consider the formation of European workgroup of marine chemical contamination in bivalve to develop an internationally accepted protocol to conduct harbour's active biomonitoring and collect preliminary data comparing coastal scallops from around the European port's area.

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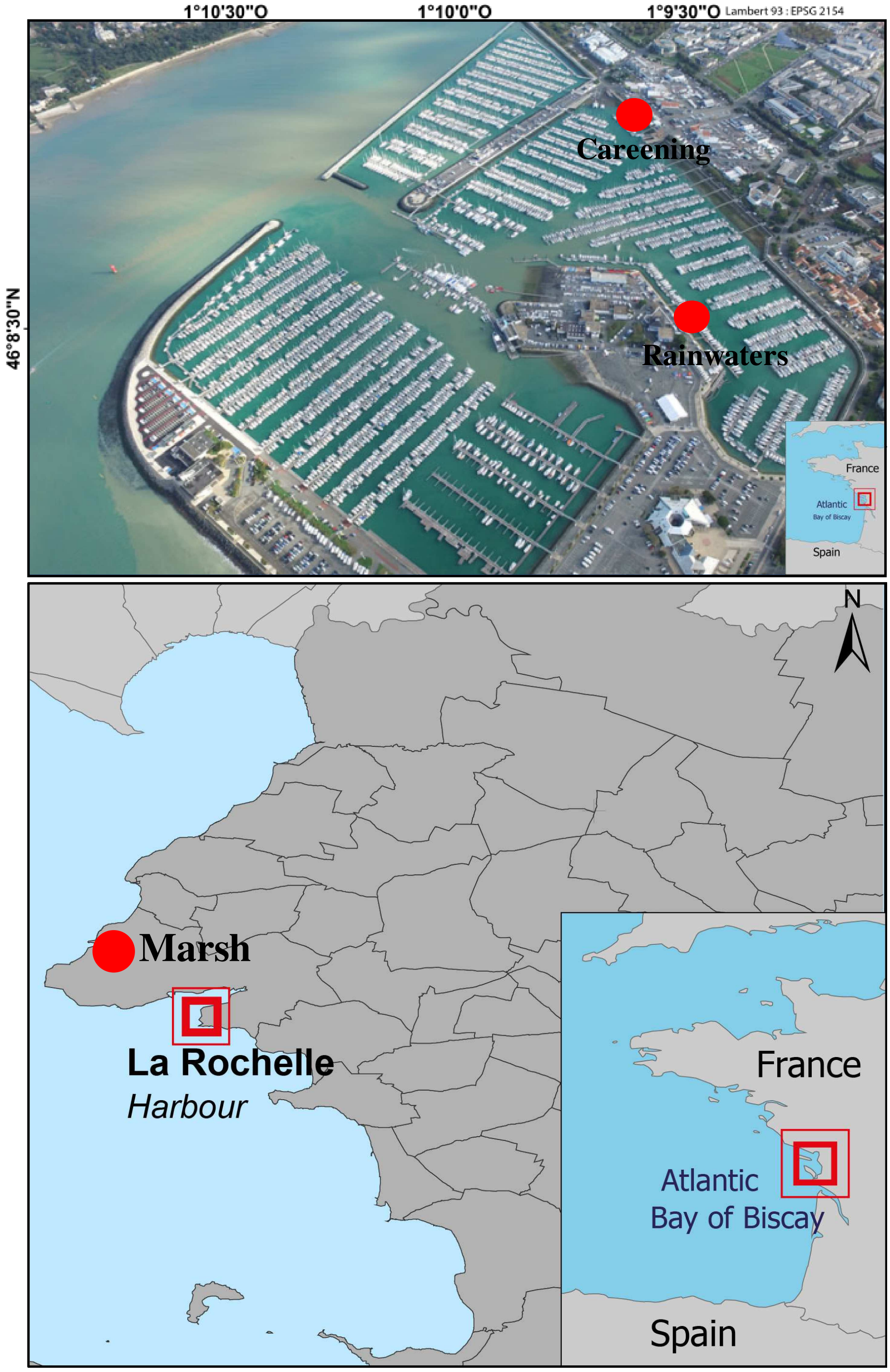

Figure 1: Map of France including the locations of the study sites. The marsh is the reference site (less-contaminated). The two impacted areas are the careening and the rainwaters sites, located in the harbour of La Rochelle.

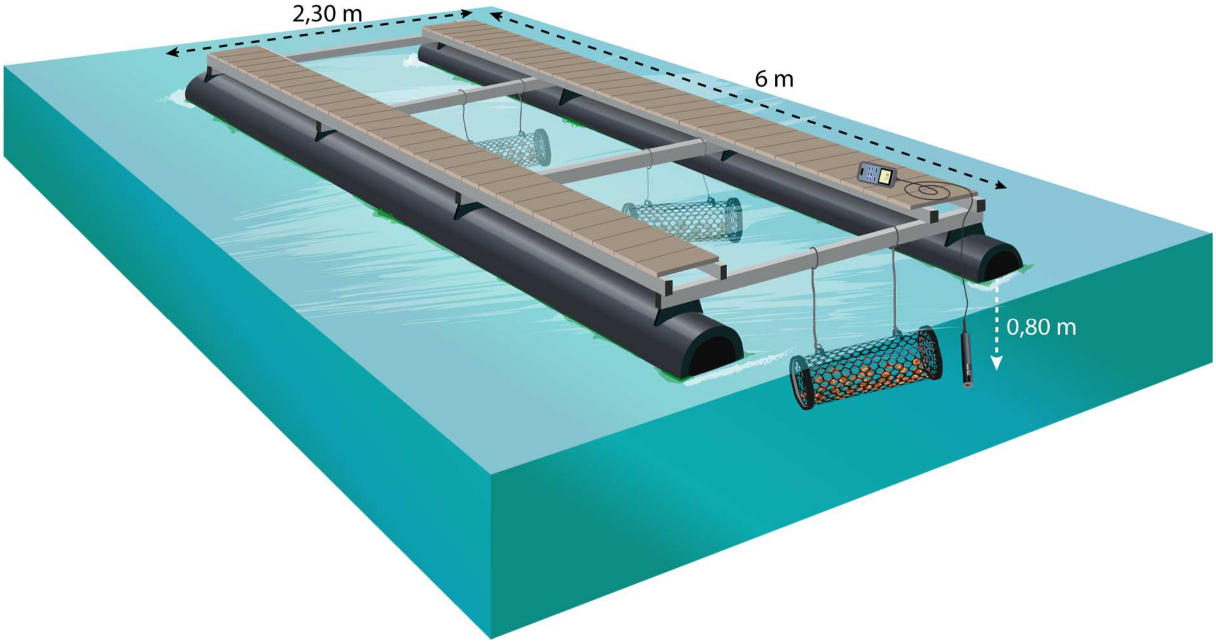

Figure 2: Illustration of the monitoring station with floating structures for the three locations: the rainwaters site, the fairway area and the marsh. (Source: Thierry GUYOT (LIENSs)).

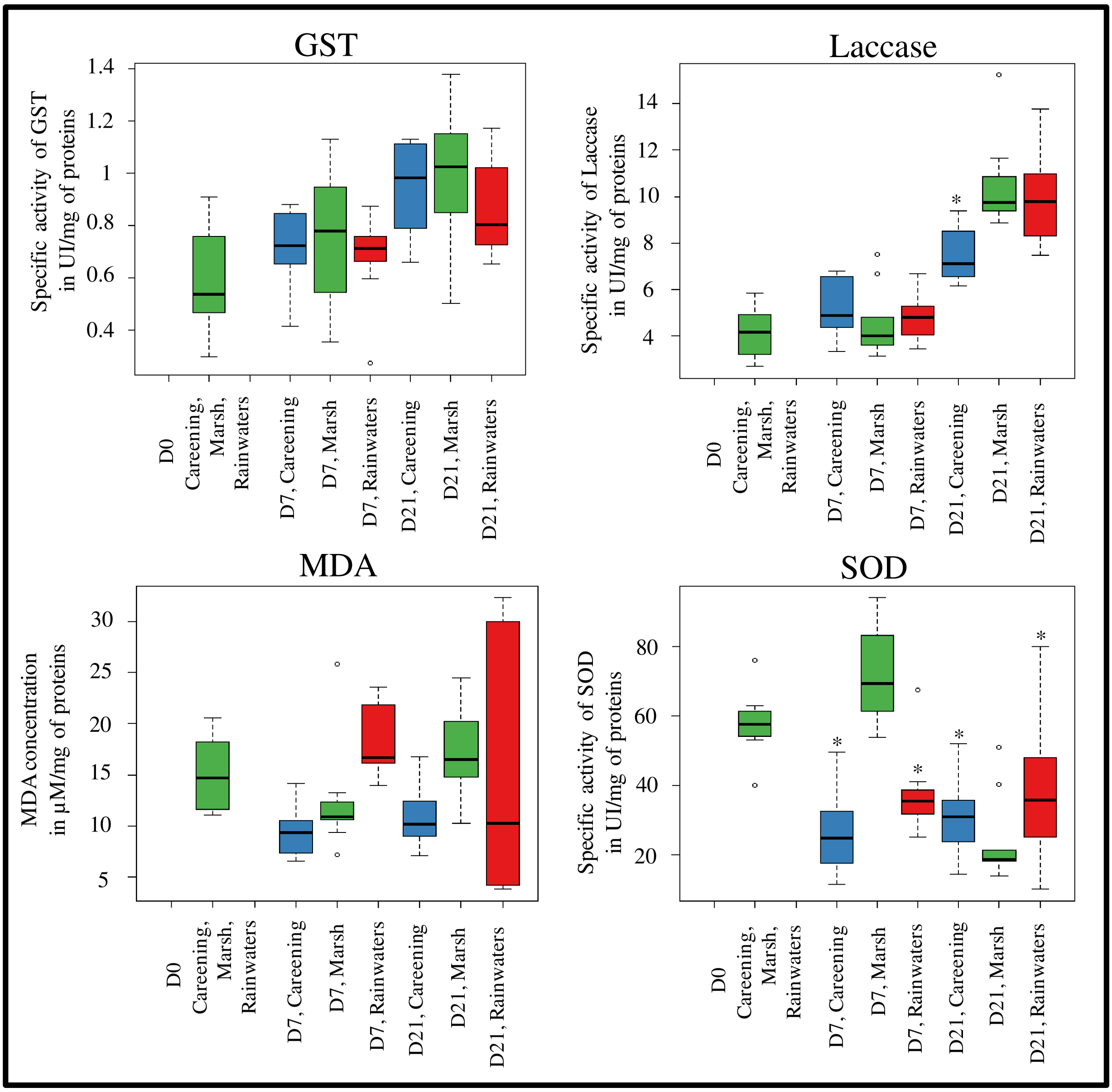

Figure 3: Boxplots showing biomarker results in the digestive glands of *M. varia* at the reference site (marsh) and the contaminated sites (outlet rainwaters and careening areas) at D0, 07 and 21 (D0, D07, D21, n = 10 per site and per date).
Significant differences between impacted sites and the marsh (control site) for each date are indicated using asterisks: *p<0.05; ***p<0.001.

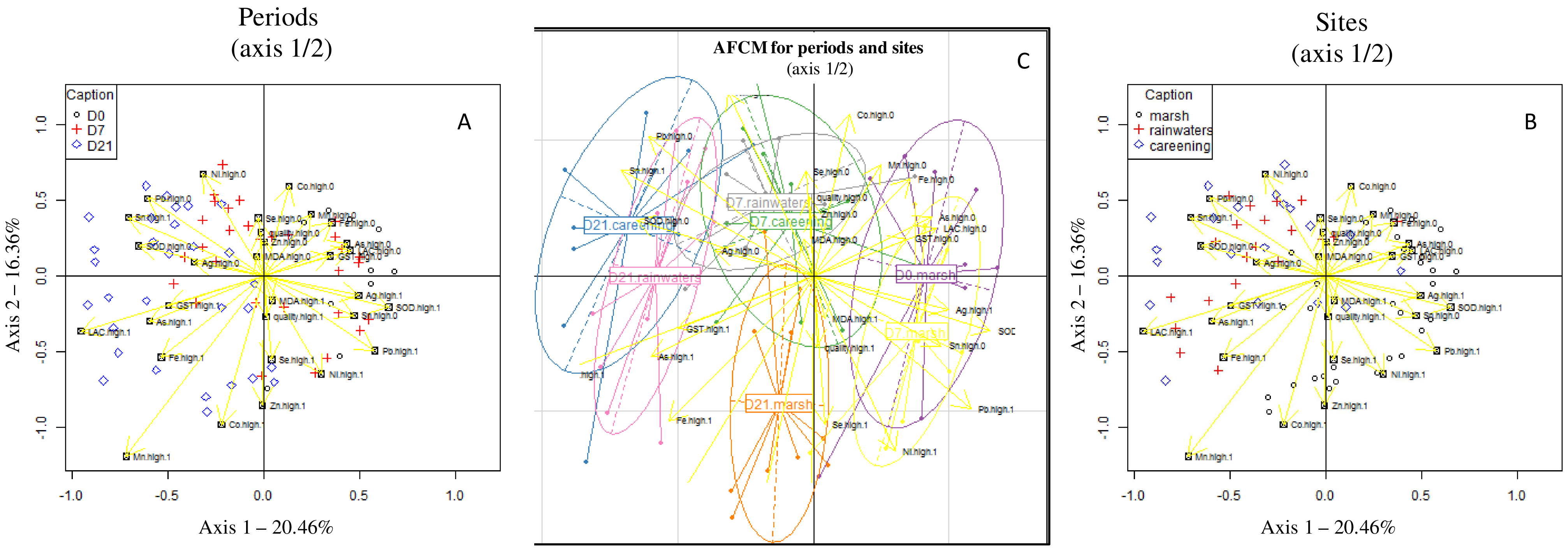

Figure 4: Multiple factor analysis plots indicating individual responses at the three sampling sites (represented in different colours) at D0, D07 and D21 as well as the qualitative trace metal variables explaining variations in the individuals x biomarkers matrix depending on periods or sites. (A: results by period, B: results by site, C: results by period and sites).

The first two axes reveal a 36.92% of variance in data (20.46% and 16.36%, respectively). MDA: MDA contents, SOD: SOD activity, GST: GST activity, LAC: Laccase activity.

Table 1: Number, length, width and thickness of scallops and weight of digestive gland sampled at the three sites (marsh, careening and rainwaters) at D0, D07 and D21.

	Marsh			Careening			Rainwaters		
	D0	**D07**	**D21**	**D0**	**D07**	**D21**	**D0**	**D07**	**D21**
Number	10	-	-	10	10	10	10	10	10
Weight (mg)	432.62	396.90	413.70	-	383.60	461.20	-	365.20	421.40
Length (mm)	41.70	41.20	44.20	-	44.80	43.60	-	39.94	42.40
Width (mm)	37.70	35.30	38.00	-	39.60	37.90	-	34.11	39.80
Thickness (mm)	14.50	14.15	15.80	-	15.30	14.00	-	12.78	16.00

Table 2: Physicochemical parameters at the three sampling sites: careening, rainwaters and marsh (reference site) areas.
Temperature (Temp) is in degrees Celsius, salinity (Sal) in Practical Salinity Unit (PSU), turbidity of sediments in Sed (g/L), chlorophyll a (Chla) in µg/L, dissolved oxygen (O^2) in percentage of saturation, and N.d indicates No data.

	Rainwaters			Careening			Marsh		
	D0	**D07**	**D21**	**D0**	**D07**	**D21**	**D0**	**D07**	**D21**
Temp (°C)	16.80	16.95	18.92	17.00	16.82	19.01	N.d	N.d	19.70
Sal (PSU)	30.54	31.61	27.61	31.96	31.94	32.00	N.d	N.d	33.19
Sed (g/L)	0.004	0.008	0.037	0.005	0.015	0.009	N.d	N.d	0.012
Chla (µg/L)	3.62	2.49	3.67	2.68	2.58	3.21	N.d	N.d	N.d
O^2 (%)	87.70	81.82	100	100	94.74	100	N.d	N.d	N.d

Table 3: Trace element concentrations (µg/g of dry weight) in the digestive glands of *M. varia* (n = 10 per site for each date) at the three sampling areas: the marsh (reference site), the careening and rainwaters areas.
Stars (*) above values in **bold indicate significantly higher difference** where p < 0.05 between the marsh and the harbour sites at each date and for each heavy metal. Stars (*) above values in *italic indicate significantly lower difference* where p < 0.05 between the marsh and the harbour sites at each date and for each heavy metal.

Dates	Sites	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	Ag	Sn
D0	**Marsh**	18.32 ± 0.9	32.32 ± 2	0.7 ± 0.04	1.3 ± 0.09	21.28 ± 2.5	566 ± 71	14.28 ± 2	2.08 ± 0.1	2.5 ± 0.2	9.1 ± 0.6	5.5 ± 0.6	157 ± 53	4.7 ± 0.7	0,02
D0	**Careening**	18.32 ± 0.9	32.32 ± 2	0.7 ± 0.04	1.3 ± 0.09	21.28 ± 2.5	566 ± 71	14.28 ± 2	2.08 ± 0.1	2.5 ± 0.2	9.1 ± 0.6	5.5 ± 0.6	157 ± 53	4.7 ± 0.7	0,02
D0	**Rainwaters**	18.32 ± 0.9	32.32 ± 2	0.7 ± 0.04	1.3 ± 0.09	21.28 ± 2.5	566 ± 71	14.28 ± 2	2.08 ± 0.1	2.5 ± 0.2	9.1 ± 0.6	5.5 ± 0.6	157 ± 53	4.7 ± 0.7	0,02
D07	**Marsh**	18.77 ± 0.8	29.95 ± 2.1	0.80 ± 0.05	1.2 ± 0.09	21.30 ± 2.1	488 ± 57	22.37 ± 3.4	2.3 ± 0.1	2.3 ± 0.1	11.35 ± 1	4.5 ± 0.6	157 ± 33	4.3 ± 0.8	0,01
D07	**Careening**	__20.15 ± 1.5*__	__33.46 ± 2.9*__	0.78 ± 0.05	1.5 ± 0.08	__109 ± 11*__	__570 ± 68*__	__26.15 ± 8.2*__	2 ± 0.1	_1.7 ± 0.1*_	9.6 ± 0.9	__7.3 ± 0.4*__	__342 ± 157*__	4.2 ± 0.7	0,06
D07	**Rainwaters**	19 ± 0.8	29.10 ± 1.2	0.76 ± 0.01	__2.7 ± 0.7*__	__112 ± 18*__	__580 ± 64*__	19.81 ± 2.1	2 ± 0.1	_1.5 ± 0.07*_	9.1 ± 0.8	__6.8 ± 0.8*__	159 ± 63	__5.2 ± 1.7*__	0,07
D21	**Marsh**	18.76 ± 1.1	33.63 ± 3.2	1.1 ± 0.05	1.3 ± 0.08	18.77 ± 1.6	506 ± 68	40.26 ± 6.1	2.3 ± 0.1	2.1 ± 0.2	10.75 ± 0.5	5.5 ± 0.9	248 ± 100	4.4 ± 0.6	0,01
D21	**Careening**	__21.17 ± 0.4*__	32.70 ± 3.2	_0,83 ± 0.07*_	__2 ± 0.3*__	__287 ± 48*__	__879 ± 229*__	_28.32 ± 4.6*_	1.8 ± 0.2	_1.7 ± 0.3*_	_8.3 ± 0.3*_	5,95 ± 0.6	_213 ± 48*_	_3.5 ± 0.4*_	__0,14*__
D21	**Rainwaters**	__22.02 ± 0.9*__	_27.47 ± 2.4*_	_0,75 ± 0.05*_	1.6 ± 0.1	__353 ± 38*__	__666 ± 90*__	_28 ± 4.5*_	1.4 ± 0.1	_1.4 ± 0.2*_	9.7 ± 0.5	5.2 ± 0.5	235 ± 74	_3.5 ± 0.8*_	0,07