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## In situ observations and modelling revealed environmental factors favouring occurrence of *Vibrio* in microbiome of the pelagic *Sargassum* responsible for strandings

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### Abstract

Historically, pelagic *Sargassum* were only found in the Sargasso Sea. Since 2011, blooms were regularly observed in warmer water, further south. Their developments in Central Atlantic are associated with mass strandings on the coasts, causing important damages and potentially dispersion of new bacteria. Microbiomes associated with pelagic *Sargassum* were analysed at large scale in Central Atlantic and near Caribbean Islands with a focus on pathogenic bacteria. *Vibrio* appeared widely distributed among pelagic *Sargassum* microbiome of our samples with higher occurrence than previously found in Mexico Gulf. Six out the 16 *Vibrio*-OTUs (Operational Taxonomic Unit), representing  $81.2 \pm 13.1\%$  of the sequences, felt in cluster containing pathogens. Among the four different microbial profiles of pelagic *Sargassum* microbiome, *Vibrio* attained about 2% in two profiles whereas it peaked, in the two others, at 6.5 and 26.8 % respectively, largely above the concentrations found in seawater surrounding raft (0.5%). In addition to sampling and measurements, we performed backward Lagrangian modelling of trajectories of rafts, and rebuilt the sampled rafts environmental history allowing us to estimate *Sargassum* growth rates along raft displacements. We found that *Vibrio* was favoured by high *Sargassum* growth rate and *in situ* ammonium and nitrite, modelled phosphate and nitrate concentrations, whereas zooplankters, benthic copepods, and calm wind (proxy of raft buoyancy near the sea surface) were less favourable for them. Relations between *Vibrio* and other main bacterial groups identified a competition with *Alteromonas*. According to forward Lagrangian tracking, part of rafts containing *Vibrio* could strand on the Caribbean coasts, however the strong decreases of modelled *Sargassum* growth rates along this displacement suggest unfavourable environment for *Vibrio*. For the conditions and areas observed, the sanitary risk seemed in consequence minor, but in other areas or conditions where high *Sargassum* growth rate occurred near coasts, it could be more important.

**Keyword:** *Alteromonas*, copepod, microbiome, nutrient, *Sargassum* growth rate, wind

### 1. Introduction

Harmful macroalgal blooms are of global concern, generating ecological, economic and health damages (Smetacek and Zingone, 2013; Lapointe et al., 2018). Among the most widely proliferating seaweeds, several species of the genus *Sargassum* C. Agardh (Phaeophyceae, Fucales) are causing serious threats to coastal ecosystems (Bouchon et al., 1992; Stiger and Payri, 1999). Most of the

species are found in the tropical and subtropical regions and are benthic. In the North Atlantic Ocean, two species have been described as fully pelagic (i.e., holopelagic), *Sargassum natans* (Linnaeus) Gaillon and *Sargassum fluitans* (Børgesen) Børgesen. These holopelagic *Sargassum* are normally positively buoyant and hence close to the sea surface during light winds and calm (Johnson and Richardson, 1977). They float deeper in the water column with fewer leaves thrust through the surface during winds  $\geq 4 \text{ m}\cdot\text{s}^{-1}$  (Woodcock, 1950). They were commonly reported in the Sargasso Sea and in the Gulf of Mexico (Parr, 1939; Ardron et al., 2011). Winds, waves and currents aggregate these surface drifters into configurations that range from widely dispersed clumps to large neustonic rafts tens of meters wide and windrows that extend across the ocean surface for tens of kilometres (e.g. Marmorino et al., 2011; Ody et al., 2019). In the Sargasso Sea, the pelagic *Sargassum* taxa are part of the natural ecosystems in the mid-Atlantic. However, in the Gulf of Mexico, pelagic *Sargassum* recurrently strands in large quantities on the coasts (Gower et al., 2006; Gower and King, 2011; Lapointe et al., 2018) and since 2011, massive new strandings of pelagic *Sargassum* have been reported on the coasts of the Caribbean islands, Northern Brazil, Guiana and West Africa. They cause widespread economic and ecological damages (Smetacek and Zingone, 2013). These massive strandings are associated with the extensive occurrence of *Sargassum* aggregations in the Central Atlantic basin: the great Atlantic *Sargassum* belt reported by satellite imagery (Wang and Hu, 2016; Wang et al., 2019) is larger further south than the usual location of the Sargasso Sea.

Many *Sargassum* species have a rough texture and sticky mucus, which favours the colonization of microorganisms that can affect the development of their host. Pelagic *Sargassum* have been shown to release large amounts of organic compounds (Brylinsky, 1977; Powers et al., 2019) that have the potential to increase bacterial colonization. As early as in 1960s-70s, direct observations have noticed colonies of bacteria identified at that time as *Dichothrix* belonging to *Cyanobacteria*, on pelagic *Sargassum* in the Sargasso Sea and in the Gulf Stream with a latitudinal decline below South Bermuda (Carpenter and Cox, 1974). Since then, with the exception of a single work on the impact of oil on *Sargassum* microbial communities in the Gulf of Mexico (Torralba et al., 2017), very few information is available on the pelagic *Sargassum*-related microbiome.

From the few studies on algal microbiome, the composition of bacterial communities of macroalgal surfaces differs from that of surrounding water or even communities colonizing an inert surface (Dobretsov et al., 2006; Wahl et al., 2012). In the study of Torralba et al. (2017), microbial community associated with pelagic *Sargassum* appeared to be complex and dominated mostly by various species of *Rhodobacteraceae* ( $48.4 \pm 17 \%$ ) followed by the second most abundant taxa, *Saprospiraceae* ( $1.6 \pm 0.8 \%$ ). The new localization of the great Atlantic *Sargassum* belt in further south and warmer waters can favour the development of different microbial communities associated with pelagic *Sargassum*, some taxa being potentially pathogen. Among them, the genus *Vibrio*, with about 13 species described as causing several human diseases, is a putative candidate (Ramamurthy et al., 2014). Indeed, exudate of the benthic algae *Turbinaria ornata*, belonging to *Sargassaceae*, can favor *Vibrionaceae* development (Nelson et al., 2013). In addition, several reports showed an indisputable positive correlation between increasing environmental temperature and spread of *Vibrio* diseases (Ceccarelli et al., 2019). Furthermore, prevalence and severity of a wide range of diseases of marine organisms, e.g., corals, bivalves, and fish, are linked to elevated sea surface temperature and *Vibrio* infections (Vezzulli et al., 2013). Several characteristics of *Vibrio* could explain these observations: about half of the described species are pathogenic for animals and plants, many of them present an optimal temperature of 30-40°C and a metabolic versatility (Vezzulli et al., 2013). *Vibrio* spp. are naturally occurring bacteria in riverine, coastal, and estuarine ecosystems around the world. Potential pathogens such as *Vibrio* were not yet identified in pelagic *Sargassum*-related microbiome in the Gulf of Mexico (Torralba et al., 2017). The *Sargassum* rafts proliferation in warm tropical water of the Central Atlantic could be a vector of *Vibrio* dispersion by favouring their survival in open water. In these conditions, microbial threat could add to the harmful consequences of strandings.

In this context, investigating the role played by biotic and abiotic environmental factors in the occurrence of the *Sargassum* microbiomes and the dispersion of these bacteria, are crucial to assess their potential threat for living organisms. In this study, we performed a large-scale analysis of

prokaryotic assemblages associated with pelagic *Sargassum* using data from two transatlantic cruises spanning mostly Central Atlantic and near Caribbean islands. The occurrence of *Vibrio* among the different *Sargassum* microbiomes were analysed by Canonical Correspondence Analysis (CCA) taking into account the biotic and abiotic environmental conditions at the time of sampling but also the sampled rafts environmental history rebuild using backward Lagrangian modelling. The potential threats to human activities have been assessed by forward Lagrangian modelling of the routes of *Vibrio*-impacted rafts.

## 2. Material and methods

### 2.1 Field cruises and *Sargassum* rafts

Samples were collected during two cruises conducted in 2017 in the Central Atlantic, East of Caribbean Sea and South of Sargasso Sea. West Atlantic - *Sargassum* Expedition (<http://dx.doi.org/10.17600/17004300>) took place on board the N/O ANTEA from June 19<sup>th</sup> to July 13<sup>th</sup> 2017, and explored the new high *Sargassum* abundance region situated from off the Northeast coast of Brazil to the Caribbean arc, as well as the historical Sargasso Sea (25°N). The Transatlantic - *Sargassum* Expedition (<http://dx.doi.org/10.17600/17016900>) took place on board the M/V YERSIN from October 6<sup>th</sup> to 24<sup>th</sup> 2017 from Cape-Verde Islands, crossing the Atlantic between 8 and 12°N to the island of Martinique (Ody et al., 2019). The pelagic *Sargassum* rafts were composed with three morphotypes. Each fragment was identified as *S. natans I* Parr (herein morphotype #2), *S. fluitans III* Parr (herein morphotype #3), or *S. natans VIII* Parr (herein morphotype #1) (Schell et al., 2015). Benthic *Sargassum* (9 of *Sargassum hystrix* J. Agardh and 3 of *Sargassum furcatum* Kützing) were collected by scuba diving on the Caribbean coast (Martinique) the last days of the October cruise (Table S1).

### 2.2 Biotic and abiotic factors

#### 2.2.1 Microbial sampling and analysis

Nine stations were sampled during each cruise. Water was collected at 1 m depth with a Niskin bottle in triplicates. For each water sample, four liters were filtered on sterile 45 mm diameter - 0.22 µm porosity filters under sterile condition immediately after sampling. Filters were kept frozen at -20°C on board and at -80°C in lab until analysis. For community associated with *Sargassum*, an individual algal fragment was collected manually with nitrile gloves directly from water or from net and were identified according to their morphology. Whenever possible, triplicates of each morphotype were collected at each station. About 5 g wet weight fragment of *Sargassum* were placed into 100 cm<sup>3</sup> sterile container with 70 cm<sup>3</sup> sterile seawater and subjected to 30 sec of sonication (46 kHz, 30 W); the retrieved microbial cells were then collected by filtration of the water on 0.22 µm sterile filter. This treatment was performed 3 times on each fragment. Filters were kept frozen at -20°C on board and at -80°C in lab until analysis. Along the 2 cruises, 49 samples of water, 35 of morphotype #1 pelagic *Sargassum*, 34 of morphotype #2 pelagic *Sargassum*, 32 of morphotype #3 pelagic *Sargassum* 12 of the benthic *Sargassum* were collected. Each filter was treated with TE-Lysis buffer (20 mmol.L<sup>-1</sup> Tris, 25 mmol.L<sup>-1</sup> EDTA, 1 µg.mm<sup>-3</sup> Lysozyme) followed by 1% SDS treatment. The extractions were performed twice with an equal volume of phenol/chloroform/isoamyl alcohol pH8 (25/24/1), followed by a treatment with chloroform/isoamyl alcohol (24/1) and DNA precipitation by isopropanol. For ribosomal diversity analysis, the V4 region of the bacterial and archaeal 16S rDNA genes were amplified and analysed as described in Garel et al (2019) (see detail in Suppl Mat.). Raw sequence data are available at the NCBI Sequence Read Archive under bio project accession PRJNA597297. The OTUs (Operational Taxonomic Unit) classified into the genus *Vibrio* were investigated at the species level using blast similarity search against the specific 16S rRNA database at the NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The species identification proposed for some of these OTUs exhibited a minimum sequence identity of 98.70 % (Chun et al., 2018).

#### 2.2.2. Zooplankton sampling and analysis

Sampling was done with 1000  $\mu\text{m}$  mesh nets mounted with filtering cod ends. Hauls were done horizontally, just under the sea surface at a speed of  $1\text{m}\cdot\text{s}^{-1}$  for 5 minutes. Organisms were preserved in 4% buffered formaldehyde for later taxonomic identification and abundance determination. Samples were split using a Motoda box. Species/genus identification was done according to Rose (1933), and Razouls et al. (2018). The abundance of the various taxa (groups, genera, or species) was divided by the sample volume to determine the concentration of individuals per cubic meter ( $\text{ind. m}^{-3}$ ).

### 2.2.3 Nutrient sampling and analysis

Samples for nutrient determination were collected at 1 m depth with a Niskin bottle. Subsamples of water for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{PO}_4^{3-}$  were collected with a syringe and transferred after  $0.45\mu\text{m}$  filtration into polyethylene bottle. Samples for  $\text{NH}_4^+$  determination were stored at  $-20^\circ\text{C}$  whereas the others were stored at  $4^\circ\text{C}$  after the addition of  $\text{HgCl}_2$  ( $30\mu\text{g}\cdot\text{cm}^{-3}$ ).  $\text{NH}_4$  concentration was determined by the fluorescence method according to Holmes et al. (1999) on a trilogy fluorometer (Turner Design). The detection limit was  $0.01\mu\text{mol}\cdot\text{L}^{-1}$ .  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{PO}_4^{3-}$  concentrations were determined using a segmented flow analyser according to Aminot and Kerouel (2007). The detection limit was 0.01 and  $0.005\mu\text{mol}\cdot\text{L}^{-1}$  for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{PO}_4^{3-}$ , respectively.

### 2.2.4 Modelling of environmental factors and Sargassum growth by Lagrangian tracking of rafts trajectories

Trajectories of *Sargassum* rafts have been computed using the Lagrangian transport model Ichthyop (Lett et al., 2008), which simulates horizontal and vertical advection and dispersion. In our study, we considered our particles as passive surface tracers and only used horizontal advection in the movement equation.

Two sets of simulations were carried out: (1) backward simulations, where particles were advected backward in time during 7 or 30 days, to evaluate the environmental conditions during the raft trajectory before sampling (see details in Suppl. Mat.) (2) forward simulations where particles were advected forward in time for 30 days to determine the raft location after sampling and the *Sargassum* growth rate. Main characteristics of water masses (temperature, macro-nutrients) occurring at the location of rafts have been used to compute the growth rate of *Sargassum* in the raft (see details in Suppl. Mat.).

### 2.5 Data analysis

All statistics and factorial analyses were performed using Statistica v10, PAST v3.10 softwares.

## 3. Results

During the June-July cruise, three stations were sampled in surface of Central Atlantic, four in the Caribbean Sea and two in the Sargasso Sea. During the October cruise, six stations were located in Central Atlantic and one in the Caribbean Sea (Fig. 1). The most frequently encountered raft size has been the #2 (6/16), and in decreasing order raft #3 (4/16) raft #4 (3/16), raft #5 (2/16) and raft #1(1/16) (Fig. 1 and Table S1).

### 3.1 Analysis of surface microbial community and *Sargassum* microbiomes

The prokaryotic microbiome associated to pelagic *Sargassum* (16 stations) belonging to three morphotypes (*S. natans I* Parr, *S. fluitans III* Parr, or *S. natans VIII* Parr ) and located in rafts of different sizes has been analysed on global scale and compared with communities of free living prokaryotes in surrounding water (15 stations), or associated with benthic *Sargassum* (2 stations). The V4 16S rDNA metabarcoding of 161 samples yielded in average 22223 raw reads per sample ranging from 11973 to 41591 (Fig. S1). Overall 462 Operational Taxonomic Units (OTUs) were identified and disseminated across 21 bacterial and two archaeal phyla (Table S2). Out of 462 OTUs, 335 were found in water as well as in pelagic or benthic *Sargassum* samples. According to Bray-

Curtis dissimilarity and the Permanova analysis, pelagic *Sargassum*, benthic *Sargassum* and water were significantly different ( $P = 0.001$ ). From Shannon index, microbiomes of pelagic and benthic *Sargassum* appeared more diverse than prokaryotes found in water column (Fig.2 A).

The microbial composition of the water ( $n = 49$ ) consisted mostly of SAR 11 (25.5%), *Oceanospirillales* (13.3%), *Cyanobacteria* (10.7%) and *Flavobacteriales* (10.4%) (Fig.2 B, Table S2). In water samples, *Vibrionales* that contains *Vibrio* genus reached 0.5%. In contrast, the community structure associated to pelagic *Sargassum* ( $n = 100$ ) was different since *Rhodobacterales* (20.3%) were the most abundant order followed by *Alteromonadales* (15.8%), *Sphingobacteriales* (8.7%) and *Flavobacteriales* (8.7%). The proportion of *Vibrionales* associated with pelagic *Sargassum* represented overall 6.1% and *Cyanobacteria* 0.5%. The *Sargassum* morphotypes has no significant impact on the associated microbiomes ( $P = 0.294$ ). Therefore, pelagic *Sargassum* morphotype was not taken into account further on. For the two analysed benthic species of *Sargassum* ( $n = 12$ ), the most abundant taxa were in decreasing order *Rhodobacterales* (18.54%), *Flavobacteriales* (16.5%) and *Vibrionales* (10.2%) (Fig.2 B). At the genus level, highly significant difference in *Vibrio* abundances were observed between micro-organisms of water and pelagic *Sargassum* microbiome or between microbiomes associated with pelagic and benthic seaweeds ( $P \leq 0.001$ ).

*Vibrio* on *Sargassum* outnumbered significantly those in surrounding water ( $P \leq 0.001$ ) (Fig.3, Table S3) and could exceed more than 10% of the sequences for several pelagic *Sargassum* samples (stations S6, S8, S9, S10, S18, Y4 and Y6) with Y6 showing the highest abundance in average ( $3508 \pm 668$  out of 11973 sequences; i.e  $29.3 \pm 5.5$  %). Among the 16 *Vibrio* OTUs identified in our study (Table S3), six could be potential pathogen according to blast results and the literature. Indeed, although the species identification search can be “multispecies” (same identity score for different species), six OTUs showed above 98.87% identity with *V. parahaemolyticus*, *V. europaeus*, *V. tubiashii*, *V. campbellii*, *V. ichthyenteri* and *V. alginolyticus* all known to be pathogen. The abundance of these six OTUs represented  $81.2 \pm 13.1\%$  of the total *Vibrio* in pelagic *Sargassum* microbiome.

Among benthic *Sargassum*, there is a higher proportion *Vibrio* within *S. hystrix* microbiome (B-Y17 samples, location impacted by strandings) than that of *S. furcatum* (B-Y18 samples, few impacted by strandings) ( $P = 0.004$ ). If few *Vibrio* could be detected in *Sargassum* surrounding water, their concentrations did not correlate with those of seaweeds microbiomes ( $P = 0.023$ ).

## 3.2 Biotic and abiotic environmental factors

### 3.2.1 Identification of different microbial assemblages

Large *Sargassum* biofilm could favour microbial interaction. We have investigated the relations between *Vibrio* and the other bacterial members of pelagic *Sargassum* microbiome. Within the most abundant OTUs, Pearson distance and dendrogram of single linkage identified significant different prokaryotic assemblages, among our samples, referred as profiles hereafter ( $P = 0.001$ ) (Fig. S2). The pelagic *Sargassum* microbiomes were split in four counterparts: profiles P-Rho, P-Mex, P-Alt, P-Vib specific to pelagic *Sargassum* samples ( $n = 96$ ) whereas the four remaining samples presented similar assemblage to the community of the seawater. Principal Component Analysis (PCA) identified OTUs responsible for this classification (Fig.4). The first axis (PCA1 explained 46% of the variance, Fig4A) permitted to differentiate P-Mex at one extremity, P-Vib and P-Alt to the other one, with P-Rho in central position. This axis followed the relative abundance within microbiomes of OTUs belonging to Gammaproteobacteria versus Alphaproteobacteria. A positive coordinate on this first axis corresponds to the occurrence in high proportion of 3 Gammaproteobacteria : *Alteromonas* (OTU D-349, weighting 0.81 of PCA1), *Pseudoalteromonas* (OTU D5-360, weighting 0.25 of PCA1) and *Vibrio* (OTU D5-428, weighting 0.45 of PCA1). The second axis, (PCA 2 explained 32% of the variance, Fig. 4A) allowed us to differentiate profile P-Alt from P-Vib by the relative proportion of

abundance of *Vibrio* (weighting 0.82 of PCA2), meanwhile *Pseudoalteromonas* (D5-360) presented similar abundance in both profiles (Fig.4A). The third axis (PCA3 explained 8.2% of the variance, (Fig. 4B) allowed to differentiate P-Rho from P-Mex with the relative abundance of OTUs belonging Alphaproteobacteria such as *Rhodobacteracea* (OTU D5-261; weighting 0.81), and *Hyphomodaceae* (OTU D5-189, weighting 0.2) versus *Rickettsiales*-SAR11 (OTU D5-289, weighting 0.34). In profile P-Alt (n=10), the abundance of *Vibrio* (D5-428) attained  $6.5 \pm 4.1\%$ , i.e. tenfold the average percentage found in seawater. *Alteromonas* (D5-349) largely dominated and represented in average  $34.9 \pm 3.9\%$  and could peaked at 64% in sample S-S61ep with abundant epiflora. In contrast, in profile P-Vib, (n=15), the *Vibrio* peaked at  $26.8 \pm 8.7\%$  i.e. 56 fold the percentage found in seawater and the abundance of *Alteromonas* was lower (in average  $10.7 \pm 4.1\%$ ). The two other profiles, P-Mex (n = 24 samples) and P-Rho (n = 47 samples) were both characterized by low abundance of *Vibrio* (around 1- 2%), slightly above the proportion found in seawater surrounding rafts (0.5%).

### 3.2.2 *In situ* and modelled abiotic factors

The microbiome on pelagic *Sargassum* was organised in biofilms. Their microbial diversity that we observed through DNA analysis was the result of the history of rafts, taking into account global, regional hydrodynamic circulation and wind fields that could have aggregated parts from different geographic locations harbouring potentially different nutrient concentrations or physical conditions. To take into account the history of the rafts, the trajectories of their clumps were modelled by Lagrangian displacements. Mean nutrient concentrations, temperature, salinity and wind speed were determined, taking into account the parameters available monthly at global scale for the Atlantic Ocean (i.e. nitrate, phosphate, iron, temperature, salinity and wind). The chosen time scales were 7 days for short term and 30 days for long term analysis. In consequence, environmental factors were analysed when possible at the time of sampling, and at short term (7 days) and long term (30 days) by modelling. Ammonium, nitrate, nitrite and phosphate concentrations determined at the time of sampling varied respectively from 0.175 to 4.14  $\mu\text{mol.L}^{-1}$ , 0.052 to 1.75  $\mu\text{mol.L}^{-1}$ , 0.049 to 0.403  $\mu\text{mol.L}^{-1}$ , and 0.013 to 0.27  $\mu\text{mol.L}^{-1}$  (Table S1). The *in situ* measurements of nitrate and phosphate concentrations and the averages of these nutrients modelled over 7- days or 30-days were in the same range. Modelled nitrate concentration averaged over 7 or 30 days varied from 0.0008  $\mu\text{mol.L}^{-1}$  (Y12) to 0.3927  $\mu\text{mol.L}^{-1}$  (Y6) and from 0.009  $\mu\text{mol.L}^{-1}$  (Y12) to 0.1375  $\mu\text{mol.L}^{-1}$ (Y6), respectively (Table S4). Averaged nitrate concentration encountered by rafts over the 7- or 30-days prior sampling could vary largely, especially for stations impacted by the Amazon River (S6, S8) (Lagrangian displacement shown Fig.5). Decreased of 192 and 1366% between averages of these two periods were observed for S8 and S6, respectively. Modelled phosphate concentrations averaged over 7- or 30-days periods varied from 0.0056  $\mu\text{mol.L}^{-1}$  (S23) to 0.0958  $\mu\text{mol.L}^{-1}$  (Y6) and from 0.0102  $\mu\text{mol.L}^{-1}$  (S18) to 0.1267  $\mu\text{mol.L}^{-1}$ (S8), respectively (Table S4). The highest variations of phosphate mean concentration between 7 days and 30 days corresponded to stations S16 (decrease of 417%, from central Atlantic toward Caribbean Sea) and Y2 (decrease of 120%, from Central Atlantic toward African Coast). Modelling enabled also to take into account additional factors such as iron concentration, wind speed (affecting buoyancy and raft size), temperature and salinity (Table S4). Modelled iron concentration average over 7- or 30-days period varied from 0.0003  $\mu\text{mol.L}^{-1}$  (Y6) to 0.0009  $\mu\text{mol.L}^{-1}$  (S6) respectively. Variations of mean concentrations between the 7- and 30-days periods were lower than for other factors and reached 13% for station Y6 (Central Atlantic). Modelled wind speed averaged over 7- and 30- days period varied from 4 (S19) to 10.9  $\text{m.s}^{-1}$  (Y8), and to 4.6 (S16) to 11.1  $\text{m.s}^{-1}$  (Y11), respectively (Table S4). The maximal variation (increase) of mean wind speed between 7- and 30-days reached 48% for station Y2 (Central Atlantic toward African Coast). The 7-days modelled temperature and salinity encountered by rafts varied in average among stations between 27.7 and 29.1°C and 32.8 to 36.6, respectively. Their variations between 7 days and 30 days for a station were about 1 to 4%. Consequently, 30 days modelling of the latter parameters was no longer included in the analysis (Table S4).

### 3.2.3 Biotic factors: modelled *Sargassum* growth rates and *in situ* zooplankton abundance

*Vibrio*, as many main members of the microbiomes of pelagic *Sargassum* of this study, belongs to taxon harbouring heterotrophs. Consequently, their development relied on available organic carbon. As pelagic *Sargassum* have been shown to produce large amounts of Dissolved Organic Carbon (DOC) (Powers et al., 2019), we hypothesized that the algal physiological status depending on environmental factors could influence this DOC exudation. In consequence, the *Sargassum* growth rates were also modelled, taking into account the factors available monthly at global scale for the Atlantic Ocean (i.e. nitrate, phosphate, iron and temperature). The variations of the growth were shown on the backward Lagrangian trajectories of the rafts (Fig.5). Modelled *Sargassum* growth rate, averaged over 7 or 30 days, varied from -0.014 (Y11) to 0.048 d<sup>-1</sup> (Y6) and from -0.018 (Y12) to 0.32 d<sup>-1</sup> (Y6) respectively. For most sampled rafts, large variation of this parameter could be observed from 33% (Y6) to 689% (S16) (Table S4).

Zooplankton, and specially copepods, could potentially influence the distribution of bacterial taxon among *Sargassum* microbiomes, either directly as *Vibrio* reservoir or by grazing heterotrophic bacteria or indirectly by impacting nutrient cycle with their carcasses or faecal pellets (Frangoulis et al., 2011; Huq et al., 1983; Venkateswaran et al., 1989). Zooplankton ((80-1000 µm size range) abundance in water surrounding rafts varied between 72.97 (S19) to 2971.92 ind.m<sup>-3</sup> (Y15) (1082.55, 444.74, 1719.67, 1616.82, 1372.72, 799.90, 667.31, 1187.05, 2815.46, 2047.07, 480.75, 761.51, 1977.11, 1959.91 Ind.m<sup>-3</sup> for S6, S8, S9, S10, S12, S16, S18, S23, Y2, Y4, Y6, Y8, Y11, Y12 respectively). A focus was made on benthic copepods (*Harpacticoida*, *Cyclopoida*) because of their potential tight association with pelagic *Sargassum* (Table S5). Their concentration varied between 10.63 (S19) and 1500.38 ind.m<sup>-3</sup> (Y2).

### 3.3 Impact of biotic and abiotic environmental factors on *Vibrio* occurrence in pelagic *Sargassum* microbiomes

Canonical Correspondence Analysis (CCA) allowed to identify the environmental factors that influenced the occurrence of the different profiles of pelagic *Sargassum* microbiome (Fig.6). The two profiles containing the highest abundance of *Vibrio* (P-Vib and P-Alt) corresponded to the highest nutrient concentration ranges, either measured (ammonium, nitrite) or modelled (7-days-PO<sub>4</sub>, 30-days -PO<sub>4</sub>, 7-days -NO<sub>3</sub>, 30-days-NO<sub>3</sub>). Among these two profiles, P-Vib harbouring the highest concentration of *Vibrio* (26.8%) was favoured by the highest *Sargassum* growth rate observed in this study, *in situ* nitrite concentration and modelled nitrate concentrations (short and long term). The P-Alt profile harbouring, 6.5% of *Vibrio*, corresponded to short term high wind speed and higher iron concentration. In contrast, profiles containing low abundance of *Vibrio* (P-Mex and P-Rho, about 1-2%) were characterized by high abundance of zooplankton. Low wind speed and high raft size, benthic copepods and high temperature favoured profile P-Mex, whereas, iron favoured profile P-Rho. Salinity, and *in situ* nitrate and phosphate concentrations did not explain the different profiles and the *Vibrio* occurrence.

### 3.4 Forward modelling of the raft routes

The temporal stability of a microbial community is unknown as well as the changing dynamic from one profile to another. However, we modelled forward tracking of rafts containing *Vibrio* (S6, S8, S9, S10, S18, Y4 and Y6) during one month after sampling to test whether they could reach coasts (Fig. 7). The growth rate levels of the *Sargassum* originating from these stations were modelled along the trajectories. Our results indicated that rafts originating from stations S6, S8, S9 S10 in July and Y11 in October potentially strand on Caribbean islands. During their displacements, the growth of *Sargassum* within rafts dropped to very low rates. In contrast, rafts from other stations (Y4, Y6, S18) stayed offshore, and presented different growth rate tendencies. Whereas low growth rates were observed for clumps originating from Y4 station, moderate growths were found for that coming from station S18 and high rate for those coming from station Y6.

## 3 Discussion and conclusion

Analyses of microbial communities of several seaweeds have shown that the microorganisms present on the algae surface were different and more diverse than that in the surrounding waters (Dobretsov et al., 2006; Wahl et al., 2012; Torralba et al., 2017). Among seaweed microbiome studies, that of

pelagic *Sargassum* have a particular interest due to their peculiar lifestyle and to the actual context of stranding. Their proliferation in a new area, their long-distance movement, and stranding on coasts could lead to introductions of bacteria and among them potential pathogens.

Our results on microbiome associated with pelagic *Sargassum* from the Central Atlantic and the two analysed benthic ones from the Martinique Island, have confirmed previous findings on other seaweeds, i.e. they carried a greater diversity than the surrounding water. Furthermore, at higher taxonomic rank our results on microbiome of *Sargassum* were in agreement with the seasonally independent tendencies (predominance of Proteobacteria and Bacteroidetes) observed for the benthic *S. muticum* (Serebryakova et al., 2018). However, at lower taxonomic rank, we observed differences between the microbiomes of pelagic *Sargassum* and of benthic counterpart collected at the same period (*Sargassum hystrix*, *Sargassum furcatum*). These differences suggested that the larger range of variations of environmental factors encountered by the pelagic algae could trigger differences in microbiome composition.

For these reasons, we have focused our attention on the microbiome of the pelagic *Sargassum* and on the potential occurrence of pathogenic bacteria. We identified in the central Atlantic, Caribbean Sea and southern Sargasso Sea, four different microbiomes associated to pelagic *Sargassum*. These profiles were identified during both cruises (June-July, and October) and in all *Sargassum* morphotypes. One of the profiles (P-Mex) dominated by *Rhodobacteraceae* (alpha proteobacteria) and *Saprospiraceae* (Bacteroidetes) was similar to the pelagic *Sargassum* microbiome described in the Gulf of Mexico by Torralba et al. (2017). However, differences appeared between previous findings and our results. This profile that seemed shared in all samples in the Gulf of Mexico, was minor in our study since it corresponded to one quarter of our samples only (25 out of 100). Furthermore while no *Vibrio* was detected in the study in the Gulf of Mexico (Torralba et al., 2017), we found *Vibrio* at low concentrations (1-2%) within the P-Mex. Beside profile P-Mex, three extra microbiome profiles were also identified, all of them harbouring *Vibrio*, at low concentration (1-2%) for P-Rho (n=75), or at higher concentration for P-Alt (6.5%) and P-Vib (28%) (n=25). Among *Vibrio* OTUs found herein, six potential pathogens have been identified that fell in a cluster containing *V. parahaemolyticus*, *V. alginolyticus*, *V. campbellii* (Ruwandeeepika et al., 2012), *V. europaeus* (Dubert et al., 2016), *V. tubiashi* (Dubert et al., 2017), and *V. ichthyenteri* (Ishimaru et al. 1996).

Many studies have described the capacity of *Vibrio* to attach to various organisms, but data of their enrichment compared to the water column is often lacking (Takemura et al., 2014). The presence of *Vibrio*, sometimes at high concentration, herein in pelagic *Sargassum* microbiome questioned the potential links of *Vibrio* abundances between water column and *Sargassum* microbiome. From literature, *Vibrio* abundance reached generally 0-2% in the water column although they could be engaged punctually in massive blooms (Gilbert et al., 2012). The proportion of *Vibrio* sequences found in the water column in this study (around 0.5%) is in agreement with the usual observations (0-2% range). No correlation was observed between *Vibrio* abundance in *Sargassum* and in the water column suggesting tight relations between *Vibrio* and the seaweeds.

On the ecological and sanitary point of view, the determination of factors favouring or not the development of this genus seems important since rafts could strand on coasts. From a meta-analysis, of samples from water column of temperate and coastal areas, the strongest environmental factors that correlated with total *Vibrio* are temperature and salinity (Gilbert et al., 2012; Takemura et al., 2014; Vezzulli et al., 2013). In our study, this trend was not observed, and the modelled water temperature or salinity indicated that higher values favoured rather profiles P-Mex and P-Rho harbouring low abundance of *Vibrio*. The conditions herein were different since *Vibrio* were not free living but associated with *Sargassum*. Furthermore, the sampling zone and season of our study, in open sea near equator, and during summer and autumn seasons, had water temperatures and salinities close to the *Vibrio* optimal growth range (ranging from 30 to 40°C, 0.2 and 3% NaCl) (Olivier et al., 2013).

*Vibrios* have been also described to present high property for colonization of chitin surfaces and chitinous organisms, especially zooplankton, which are considered by some authors the main

environmental reservoir of these bacteria in aquatic environments (Vezzulli et al., 2010). In our study, the presence of zooplankters and copepods did not favour occurrence of *Vibrio* associated with pelagic *Sargassum*, and zones with the highest abundance of zooplankton and copepods were correlated with profiles harbouring *Vibrio* in low abundance. This finding suggested that copepods and zooplankton were not responsible for the seeding of *Vibrio* on pelagic *Sargassum*. Furthermore, copepods, as heterotrophic bacteria grazers (Kwok et al., 2015) could be an important factor for the control of fast-growing gamma proteobacteria found in profiles P-Alt and P-Vib biofilm on *Sargassum*. Other environmental factors appeared to disfavour *Vibrio*. Depending of the wind speed, *Sargassum* buoyancy and raft size differed. For lower wind, *Sargassum* aggregated in larger rafts that are located near the sea surface and received high solar radiations. These latter have been shown to have detrimental effects on bacteria by reducing DNA and protein synthesis and metabolisms alteration (Abboudi et al., 2008). For example, the marine bacterium *Vibrio angustum* is sensitive to UV-B that reduces its growth (Abboudi et al., 2008). This tendency was observed in pelagic *Sargassum* microbiome since profiles P-Mex that corresponded to rafts located near the surface presented low *Vibrio* abundance. In contrast, it contained the highest proportion of *Rhodobacteracea* (9-12% range) that is a taxon containing pigmented strains usually described to have capacity to resist to solar radiations and to the induced oxidative stress (Pujalte et al., 2014; Petit et al., 2015).

Trophic interactions were also reported to play a significant role in controlling *Vibrio* proliferation including bottom upregulation by the food resource, such as dissolved organic carbon and nutrients (Kirschner et al., 2008). These parameters appeared important in our study. Our results indicated that modelled nutrient factor (nitrate, phosphate), *in situ* nitrite and ammonium concentration favoured *Vibrio* occurrence. Profile P-Vib was strongly explained by *Sargassum* growth rate and modelled nitrate concentration (7-days and 30-days) had more impact than *in situ* counterpart did. Both observations suggested an indirect impact of nutrient on *Vibrio* whose abundance would rather rely on physiological conditions of *Sargassum* than on nutrient concentrations themselves. The impact of host condition on variation of bacterial community has been shown on another brown algae (Kelp) (Marzinelli et al., 2015). These findings were probably due to the heterotrophic nature of *Vibrio* and to its development upon *Sargassum* exudates composed of Dissolved Organic Carbon (DOC). Indeed, heterotrophic bacteria have been shown to consume DOC released by brown algae between 20 to 70% range (Brylinsky, 1977). Albeit, no DOC measurement has been performed in our study, previous studies have shown that holopelagic *Sargassum* could exudate DOC at rates ranging from 3 to 6  $\mu\text{g C}^{-1}_{\text{biomass}} \text{h}^{-1}$  (dry weight) (Brylinsky, 1977), and that different incubation conditions could induced rate variation (Powers et al., 2019). However, the bacterial companions of *Vibrio* identified in this study contained all mainly heterotrophs, and it is likely that they competed for *Sargassum* exudates. *Vibrio* presenting a high doubling rate in lab as well as in the field (Gilbert et al., 2012) could take advantage over the other main bacterial groups identified, particularly if the environmental factors disfavoured its competitors. This could be the case for profile V-Alt that differed by the balance between *Vibrio* and *Alteromonas*. *Vibrio* seemed less sensitive to iron concentration and to solar radiation (wind speed as proxy of buoyancy) than *Alteromonas*. In conditions where *Alteromonas* flourished, *Vibrio* abundance was maintained around 6.5% and when it declined, it could leave the field free to *Vibrio* that could reach up to 28%. Environmental factors seemed to impact *Vibrio* abundance by influencing physiology of *Sargassum* but also, that of its competitors. Burst of *Vibrio* appeared not to be the rule but to be the result of conjunction of several factors.

Our results have shown that an important parameter for the occurrence of *Vibrio* in high proportion was the *Sargassum* growth rate. Several spots in the Central Atlantic, favourable for *Vibrio* development in *Sargassum* microbiome were identified in our study. Our sampling was dedicated to study the risk of *Sargassum* stranding on Caribbean Islands. Forward modelling of raft trajectories containing *Vibrio* together with that of *Sargassum* growth rate have indicated that the physiological status of the seaweeds that could strand on these islands was most probably not suitable for *Vibrio* development. That took away the sanitary risk for this geographic zone with the environmental factors encountered herein. If these results are extended to other zones of the Atlantic and seasons, it

appeared that all the zones favouring *Sargassum* growth particularly due to the released of N inputs could be potential sources of *Vibrio*.

### Credit author statement

**Valérie Michotey:** conceptualization, methodology, visualization, validation, writing – original draft, writing - Review & Editing, supervision. **Aurélié Blanfuné:** formal analysis, investigation, visualization, editing. **Cristèle Chevalier:** conceptualization, methodology, software, visualization. **Marc Garel:** formal analysis, data curation, visualization. **Frédéric Diaz :** conceptualization, review. **Léo Berline:** conceptualization. **Louis Le Grand:** methodology. **Fabrice Armougom:** validation. **Sophie Guasco:** investigation, resources. **Sandrine Ruitton:** investigation. **Thomas Changeux:** investigation, funding acquisition, project administration, editing. **Bruno Belloni:** investigation. **Jean Blanchot:** investigation **Frédéric Ménard:** funding acquisition, review, project administration. **Thierry Thibaut:** original draft, investigation, funding acquisition, project administration

### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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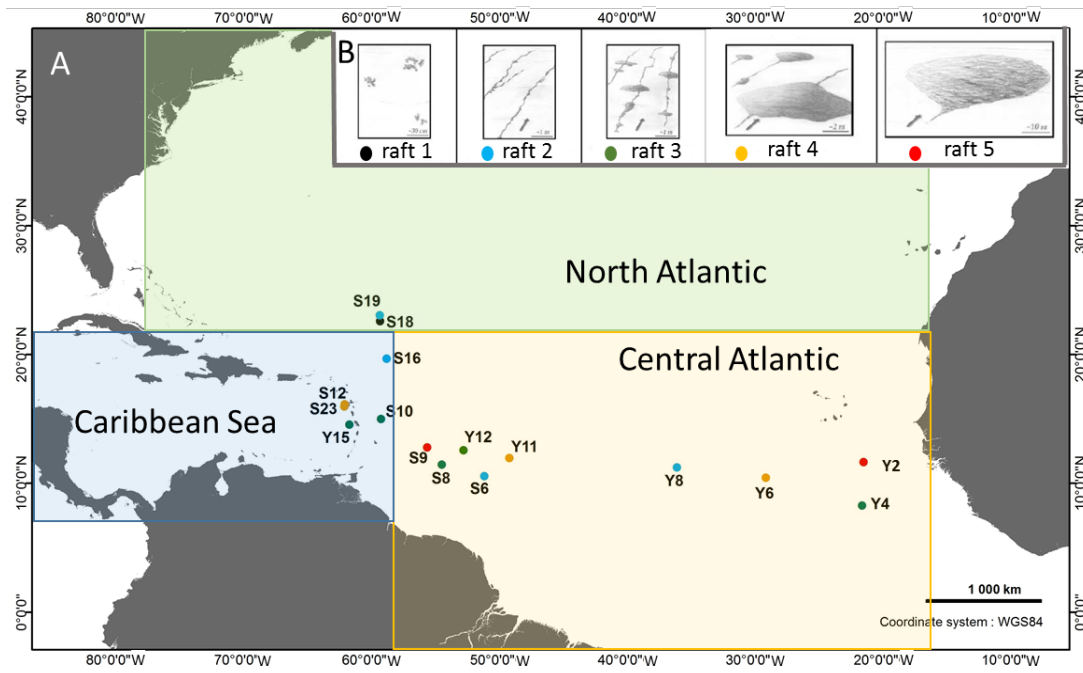
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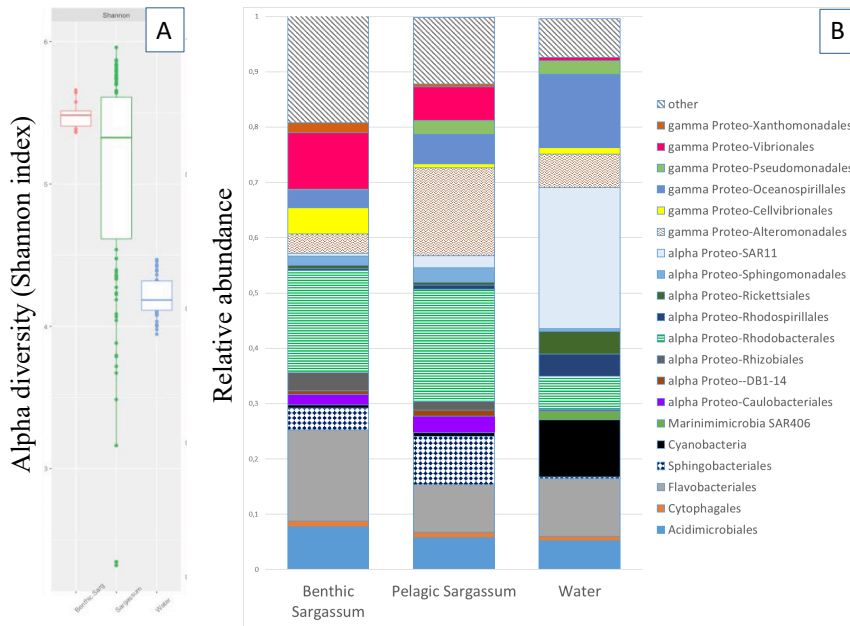
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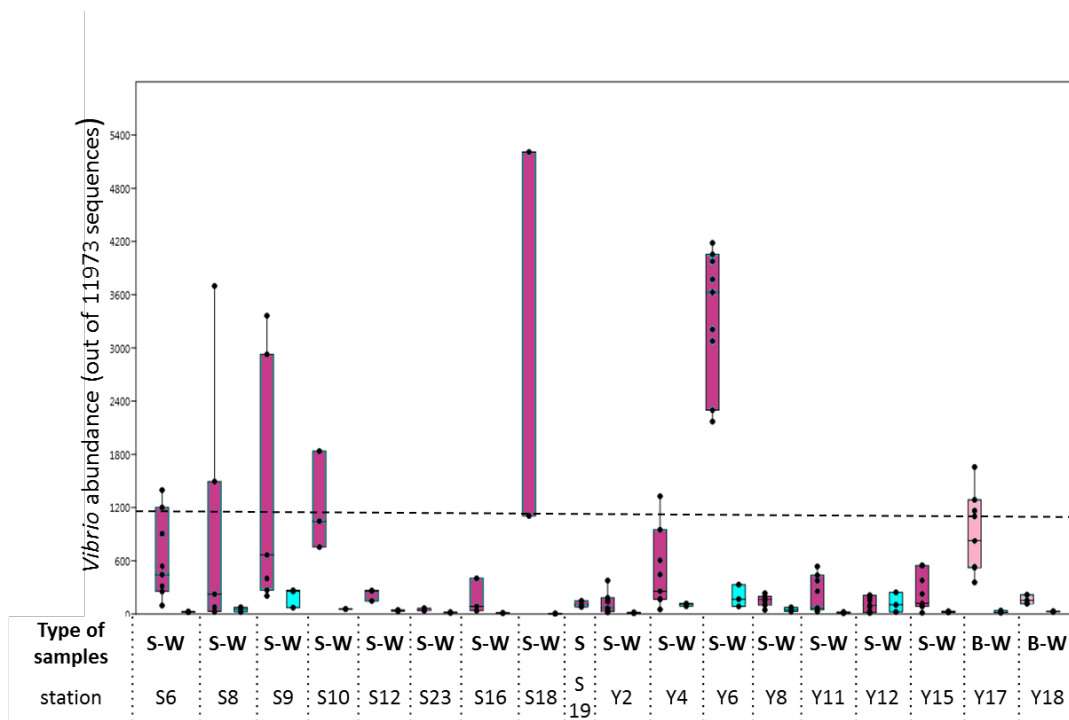


**Fig. 1. Collection locations of pelagic *Sargassum* (A) and raft size (A, B).**

Stations « S » and « Y » correspond to June 19<sup>th</sup>-July 13<sup>th</sup>, 2017 cruise and October 6<sup>th</sup> to 24<sup>th</sup> 2017 respectively. The color of dot corresponds to *Sargassum* raft type determined according to Ody et al. (2019). Geographic coverage has been indicated according to Wang et al. (2019) (the great Atlantic *Sargassum* belt). The 2 benthic stations on Martinique Island (Y17: 14°26.678'N 61°02.373'W ; Y18 :14°29.805'N 61°05.393'W) were not showed.

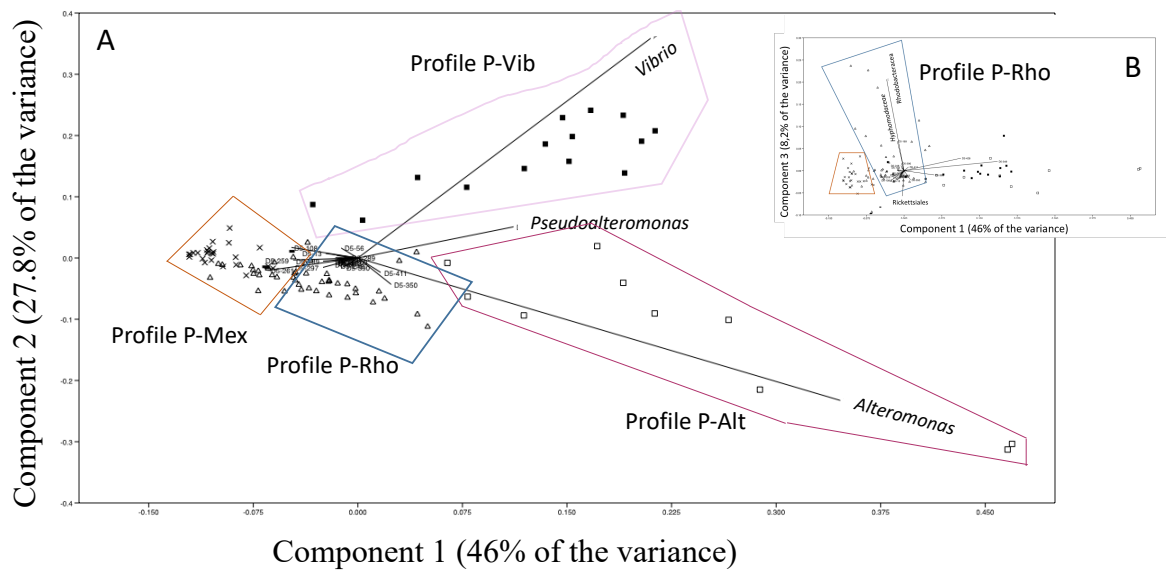


**Fig. 2. Alpha prokaryotic diversity (A) and main prokaryotic taxa (B) of water samples (n=49) or of benthic *Sargassum* (n=12) or pelagic *Sargassum* (n=100) microbiomes according to occurrence of ribosomal sequences of the different taxa (n=11973 sequences per sample).**



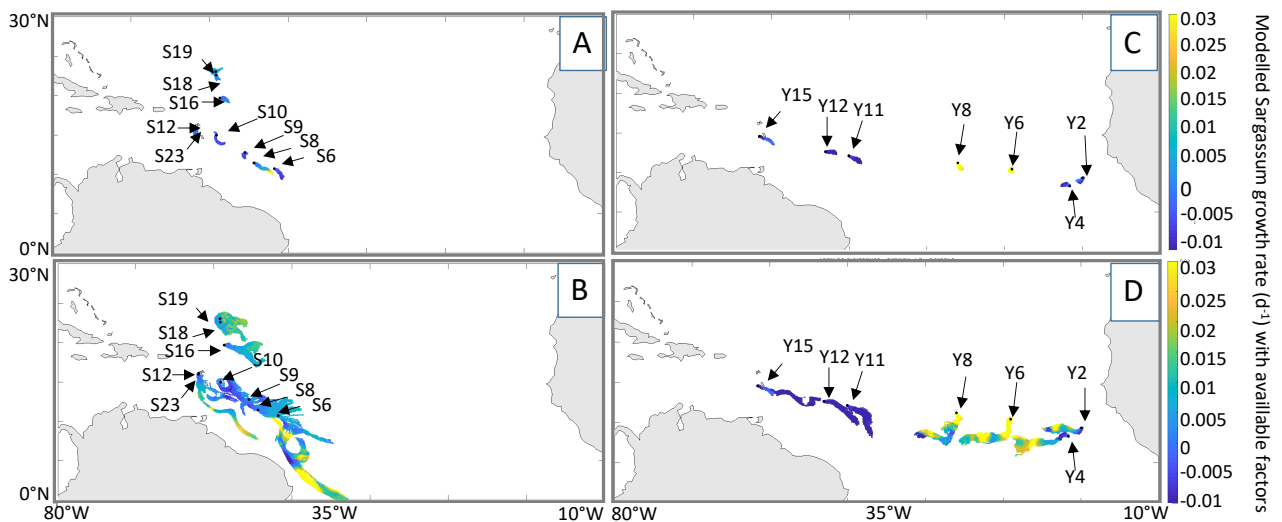
**Fig. 3. Abundance of *Vibrio* among pelagic, benthic *Sargassum* microbiome and in surrounding water .**

Occurrences of *Vibrio* were expressed as the number of their ribosomal sequences out of the total prokaryotic counterparts within each sample (normalized at 11973). Box- Jitter-plot, the box correspond to 25-75 percent quartiles, and the horizontal line inside the box to the median. S : Pelagic *Sargassum* (purple), W : water sample (blue) B: Benthic *Sargassum* (pink) dotted line corresponds to relative abundance of 10%. B-Y17 and B-Y18 benthic *Sargassum* correspond to *S. hystrix*, and *S. furcatum* respectively. S and Y stations were sampled in June and October respectively.



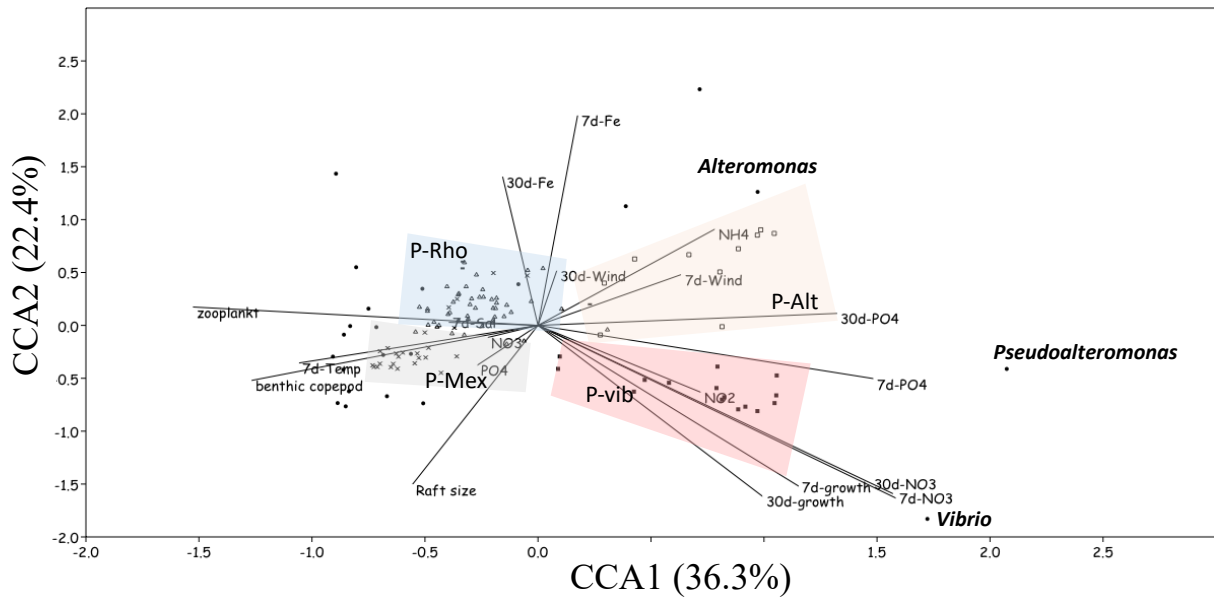
**Fig. 4. Repartition of the hundred microbiomes of pelagic *Sargassum* to the four different profiles by Principal component analysis**

The analysis was performed with 21 Operational Taxonomic Units (OTU) that weighted at least 1% in average among the 100 samples of pelagic *Sargassum* microbiomes. The groups were identified by Pearson distance and simple linkage classification. A: PCA1 and PCA2; B: PCA1 and PCA3. Each square, triangle cross is a different sample, empty square: Profile P-Vib, square: Profile P-Alt, cross: profile P-Mex, triangle: profile P-Rho. Biplot identified taxons characteristic of the different profiles. The name of the 3 relevant OTUs only are indicated for clarity of the figure.



**Fig. 5. Backward Lagrangian modelling of trajectories of *Sargassum* rafts for 7 days or 30 days before sampling and modelled *Sargassum* growth rates along trajectories taking into account temperature, salinity, nitrate, phosphate and iron.**

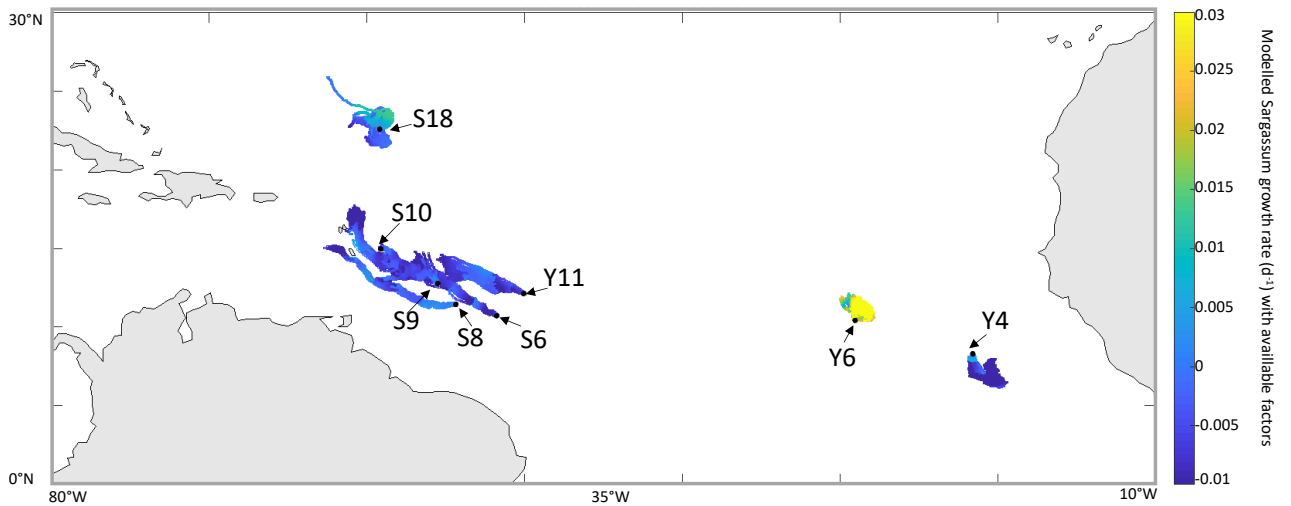
(A) 7 days backward modelling June-July cruise, (B) 30 days backward modelling June-July cruise, (C) 7 days backward modelling October cruise (D) 30 days backward modelling October cruise. The black dots and the harrows correspond to the sampling locations



**Fig. 6. Identification of the factors responsible for the occurrence of *Vibrio* in the four microbiome profiles specific of pelagic *Sargassum*, by Canonical Correspondence Analysis.**

The analysis was performed with 100 samples of pelagic *Sargassum* microbiomes, taking into account the Operational Taxonomic Units (OTUs) weighting at least 1% in average. Each square, triangle and cross is a different sample, affected to one of the four microbial profiles. Empty square: Profile P-Vib, Square: Profile P-Alt, Cross: profile P-Mex, Triangle: profile P-Rho. 19 factors were analysed: *In situ* measurements (benthic copepods, zooplankton abundances,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) but also modelled factors averaged for 7 days (7d-  $\text{NO}_3^-$ , 7d -  $\text{PO}_4^{3-}$ , 7d-Fe, 7d-temperature, 7d

salinity, 7d-Wind speed, 7d-*Sargassum* growth) or 30 days. Dots represent the position of the different OTUs. The name of the 3 relevant OTUs only are indicated for clarity of the figure.



**Fig. 7. Forward Lagrangian tracking for 30 days of raft harboring at the time of sampling highest abundance of *Vibrio* and modelled *Sargassum* growth rates ( $d^{-1}$ ) along trajectories** (S stations for June and Y stations for October 2017). The black dots and the arrows correspond to the position of the initial sampling. *Sargassum* growth rates have been modelled with available factors (temperature, salinity, nitrate, phosphate and iron)