Received: 30 March 2023 | Accepted: 3 September 2023

DOI: 10.1111/1462-2920.16505

RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

# Seasonal dynamics of a glycan-degrading flavobacterial genus in a tidally mixed coastal temperate habitat

**Maéva Brunet**[1] | **Nolwen Le Duff**[1] | **Fabienne Rigaut-Jalabert**[2] | **Sarah Romac**[3] | **Tristan Barbeyron**[1] | **François Thomas**[1]

[1]Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), Roscoff, France

[2]Sorbonne Université, CNRS, Station Biologique de Roscoff, FR2424, Roscoff, France

[3]Sorbonne Université, CNRS, Adaptation et Diversité en Milieu Marin (AD2M)-UMR7144, Station Biologique de Roscoff (SBR), Roscoff, France

**Correspondence**
François Thomas, Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France.
Email: francois.thomas@sb-roscoff.fr

**Funding information**
Agence Nationale de la Recherche, Grant/Award Numbers: ANR-10-BTBR-04, ANR-18-CE02-0001-01

## Abstract

Coastal marine habitats constitute hotspots of primary productivity. In temperate regions, this is due both to massive phytoplankton blooms and dense colonisation by macroalgae that mostly store carbon as glycans, contributing substantially to local and global carbon sequestration. Because they control carbon and energy fluxes, algae-degrading microorganisms are crucial for coastal ecosystem functions. Environmental surveys revealed consistent seasonal dynamics of alga-associated bacterial assemblages, yet resolving what factors regulate the in situ abundance, growth rate and ecological functions of individual taxa remains a challenge. Here, we specifically investigated the seasonal dynamics of abundance and activity for a well-known alga-degrading marine flavobacterial genus in a tidally mixed coastal habitat of the Western English Channel. We show that members of the genus *Zobellia* are a stable, low-abundance component of healthy macroalgal microbiota and can also colonise particles in the water column. This genus undergoes recurring seasonal variations with higher abundances in winter, significantly associated to biotic and abiotic variables. *Zobellia* can become a dominant part of bacterial communities on decaying macroalgae, showing a strong activity and high estimated in situ growth rates. These results provide insights into the seasonal dynamics and environmental constraints driving natural populations of alga-degrading bacteria that influence coastal carbon cycling.

## INTRODUCTION

Coastal marine habitats are unique and dynamic ecosystems at the interface between continents and seas. They constitute hotspots of primary productivity, accounting for ca. 20% of the global primary production on Earth (Gattuso et al., 1998). In temperate regions, this is largely due both to massive phytoplankton blooms and dense colonisation by macroalgae that mostly store carbon in the form of glycans, contributing substantially to local and global carbon sequestration (Santos et al., 2022). Microbial breakdown represents a crucial bottleneck to re-inject the large pool of algal organic matter in the marine carbon cycle (Buchan et al., 2014), by (i) making it accessible for higher trophic levels, (ii) liberating dissolved or particulate matter for local recycling or further export, (iii) remineralizing it back to atmospheric $CO_2$ and (iv) eventually influencing how much carbon is sequestered. Because they control the fluxes of carbon and energy both locally and between adjacent zones, algae-degrading micro-organisms are of critical importance for coastal ecosystem functions. The role of microorganisms as recyclers of algal biomass is even more relevant in the context of global change, since their action can influence the carbon balance in coastal ecosystems already threatened by human activities and pollution.

Surveys of natural coastal microbial communities have revealed the major role of heterotrophic bacteria in processing algae-derived compounds. Metagenomic analyses of long-term bacterioplankton series notably show predictable substrate-controlled successions of distinct bacterial taxa following phytoplankton blooms, mainly belonging to *Flavobacteriia*, *Gammaproteobacteria* classes and to the genus *Roseobacter* within *Alphaproteobacteria* (Gilbert et al., 2009; Teeling et al., 2012, 2016). Macroalgae also host abundant and diverse epibiotic bacterial communities (Egan et al., 2013; Martin et al., 2014; Singh & Reddy, 2016), the composition of which can greatly vary depending on algal species, thallus part, seasons and locations. Similar to phytoplankton bloom demise, breakdown of soluble macroalgal glycans and intact tissues is also accompanied by a succession of distinct heterotrophic bacterial clades (Brunet, de Bettignies, et al., 2021; Bunse et al., 2021; Enke et al., 2019). Environmental surveys revealed consistent seasonal dynamics of alga-associated bacterial assemblages, yet resolving what factors regulate the in situ abundance, growth rate and ecological functions of individual taxa remains a challenge (Bunse & Pinhassi, 2017). Studies on cultivated strains provided detailed insights into the metabolic pathways marine heterotrophic bacteria use to degrade algal biomass (Ficko-Blean et al., 2017; Kabisch et al., 2014; Koch et al., 2019; Reisky et al., 2019; Sichert et al., 2020; Thomas et al., 2012), but do not inform on the influence of biotic interactions with their host and abiotic constraints, hindering our understanding of their ecological impact.

Here, we specifically investigated the seasonal dynamics of abundance and activity for an alga-degrading marine flavobacterial genus in a tidally mixed coastal habitat of the Western English Channel, an epicontinental sea with strong seasonal and interannual variations of climatic, hydrological and biological conditions (Goberville et al., 2010; Lefran et al., 2021). To this aim, we targeted the genus *Zobellia*, which has received much attention in the last two decades as a specialist of macroalgal degradation among *Flavobacteriia* (Lami et al., 2021). Seven out of the nine validly described *Zobellia* species were previously isolated from algal surfaces and the others from beach sand and seawater (Barbeyron et al., 2001, 2021, 2023; Nedashkovskaya et al., 2004, 2021). In addition, *Zobellia* genes were reported in molecular studies of macroalgal microbiomes (Dogs et al., 2017; Miranda et al., 2013) and coastal seawater (Alonso et al., 2007). *Zobellia* members are known to be efficient degraders of algal purified polysaccharides (Thomas et al., 2017) as well as fresh algal tissues (Brunet et al., 2022). Hence, the genus *Zobellia* was reported to play a key role in algal biomass recycling but there is currently no data regarding its natural distribution. In the present study, we analysed two complementary time-series, including (i) a yearlong monthly epibiota sampling on five different healthy macroalgal species and decaying tissues and (ii) bi-weekly water samples over an 8-year period. Using (RT-)qPCR we quantified the number of *Zobellia* rRNA 16S genes and transcripts, aiming to assess its seasonal dynamics of abundance and activity in different ecological niches. To explained the observed patterns, we searched for significant correlations of *Zobellia* with abiotic hydrological parameters. We further questioned the presence of *Zobellia*, known mostly as macroalga epiphytes, in water column samples by screening for potential associations with eukaryotic plankton species, using publicly available protist abundance data from the same sampling site.

## EXPERIMENTAL PROCEDURES

### Sampling

Surface microbiota from macroalgae were sampled each month during low tides with a high tidal coefficient (>80) between February 2020 and January 2021. Due to the COVID-19 pandemic, no sampling was done in April and May 2020. Microbiota were collected using sterile swabs (Zymobiomics) on healthy adult specimens of the brown algae *Laminaria digitata* (Ldig, blade length 0.5–1 m), *Fucus serratus* (Fser) and *Ascophyllum nodosum* (Anod), the red alga *Palmaria palmata* (Ppal) and the green alga *Ulva* sp. (Ulva) at the Bloscon site (48°43′29.982″ N, 03°58′8.27″ W) in Roscoff (Brittany, France). Three individuals of each species were sampled. Additionally, samples from stranded algae (Std) were retrieved: within the mixture of diverse decomposing algae, microbiota from one *L. digitata*, one *F. serratus* and one *P. palmata* specimens were collected each month. Swabbed surface was standardised to 50 $cm^2$ using a 5-cm square template on both sides of the algal thallus when possible. For *Ascophyllum nodosum*, a 1 cm-width frond was sampled on 25 cm on both sides. Three different blade areas of the kelp *L. digitata* were sampled: the basal part (young tissue, hereafter LdigB and 'base'), the medium frond (ca. 20 cm away from the stipe/blade junction, hereafter LdigM and 'medium') and the old frond (the blade tip, hereafter LdigO and 'old'). The different algal surfaces sampled (different species and different thallus parts) are referred to as 'compartments' in the analyses. Swabs were immersed in DNA/RNA Shield reagent (ZymoBiomics), kept on ice during transport and stored at −20°C until DNA and RNA extraction.

Natural tidally mixed coastal surface seawater (1 m depth) was collected every 2 weeks, during high neap tides (tidal coefficient < 60), from 2009 to 2016 at the SOMLIT-Astan station in the western English Channel, 3.5 km off Roscoff (France, 48°46′40″ N, 3°56′15″ W) along with samples collected in the frame of the

SOMLIT monitoring programme (Service d'Observation en Milieu Littoral; http://www.somlit.fr). Seawater was collected using 5-L Niskin bottles and transported to the laboratory in 10-L Nalgene bottles. Seawater samples (5 L) were filtered onto successive 3 μm and 0.2 μm polycarbonate membranes (47 mm, Whatman) that were preserved in 1.5 mL of lysis buffer (sucrose 256 g $L^{-1}$, Tris-HCl 50 mM pH 8, EDTA 40 mM) and stored at −80°C until further processing (Caracciolo et al., 2022).

## Nucleic acids extraction

Environmental DNA and RNA from swabs on macroalgae were extracted simultaneously using the DNA/RNA Miniprep kit (ZymoBiomics) following the manufacturer's instructions. All samples were processed between December 2020/January 2021, except samples from February 2020 that were processed 2 weeks after collection. DNAse treatment was performed on RNA samples. DNA and RNA were eluted in 50 μL RNase-free water and stored at −20 and −80°C, respectively.

For coastal seawater samples, DNA was extracted from filters as described previously (Caracciolo et al., 2022) with cell lysis for 45 min at 37°C using 100 μL lysozyme (20 mg $mL^{-1}$) and 1 h at 56°C with 20 μL proteinase K (20 mg $mL^{-1}$) and 100 μL SDS 20%, followed by purification with phenol-chloroform methods and silica membranes from the NucleoSpin PlantII kit (Macherey-Nagel).

DNA concentration was assessed with the Quanti-Fluor dsDNA System (Promega) kit and samples were normalised at 0.5 ng $\mu L^{-1}$ before qPCR assays. RNA concentration was measured using a Qubit instrument (Thermofisher) with the RNA HS Assay Kit and samples were normalised at 1 ng $\mu L^{-1}$ before reverse transcription.

## qPCR and RT-qPCR assays

PCRs were carried out in 384-multiwell plates on a LightCycler 480 Instrument II (Roche) as described in Brunet, Le Duff, et al. (2021), using both universal bacterial primers 926F/1062R (Klindworth et al., 2013) and *Zobellia*-specific primers 142F/289R (Brunet, Le Duff, et al., 2021) to assess total and *Zobellia* 16S rRNA gene copies, respectively. Each 5 μL reaction contained 2.5 μL of LightCycler 480 SYBR Green I Master mix 2X (Roche Applied Science), 0.5 μL of each primer (300 nM final) and 1.5 μL of template DNA. Each reaction was prepared in technical triplicates. The amplification programme consisted of an initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 10 s, 20 s at the chosen annealing temperature (60 and 64°C for the universal and the *Zobellia*-specific primers respectively), and polymerisation at 72°C for 10 s. After the amplification step, dissociation curves were generated by increasing the temperature from 65 to 97°C. A dilution series of purified *Z. galactanivorans* Dsij$^T$ gDNA (prepared as in Gobet et al., 2018) representing from 10 to $10^8$ 16S rRNA gene copies was used as a standard curve and were amplified in triplicate in the same run as the environmental samples. Non-Template Controls were included in the run. The LightCycler 480 Software v1.5 (Roche) was used to determine the threshold cycle (Ct) for each sample. Linear standard curves were obtained by plotting Ct as a function of the logarithm of the initial number of 16S rRNA gene copies. PCR efficiency was calculated as $10^{-1/slope} - 1$. Samples with Ct outside the linear portion of the curve were considered as null. RT-qPCR was carried out for samples from *L. digitata* and from stranded alga. Before RT-qPCR, PCR reactions were carried out with the universal primers to ensure the absence of DNA contamination. Reverse transcription was conducted on the normalised RNA samples using the ImProm-II Reverse Transcription System (Promega). After addition of 1 μL of random primers to 9.5 μL of sample, RNA was denaturated and reverse transcripted by adding 10.5 μL of the reaction mix (4 μL of 5X Reaction Buffer, 3 μL of 25 mM $MgCl_2$, 1 μL of 10 mM [of each dNTP] dNTP Mix, 0.5 μL of 40 U $\mu L^{-1}$ Ribonuclease Inhibitor and 1 μL of Reverse Transcriptase) and incubating the mix for 5 min at 25°C, followed by 1 h at 42°C and 15 min at 70°C. qPCR was conducted immediately after reverse transcription with 1 μL of 10X diluted cDNA as described above (0.5 μL of molecular-grade water was added per reaction to have a final volume of 5 μL). MIQE information related to (RT-)qPCR experiments is available in Table S1.

## Hydrological data from the water column

All hydrological data used in this study were retrieved from the SOMLIT database (Service d'Observation en Milieu Littoral; http://www.somlit.fr, Cocquempot et al., 2019) on 7 April 2022, from the SOMLIT-Astan station (period 2009–2016) and the SOMLIT-Estacade station (period 2020–2021, 1.3 km from the macroalgae sampling site, 48°43′56″ N, 3°58′58″ W). Datasets include 15 hydrological parameters, namely surface seawater temperature (T, °C), salinity (S), dissolved oxygen (O, mL $L^{-1}$), pH, concentration of ammonium ($NH_4$, μM), nitrate ($NO_3$, μM), nitrite ($NO_2$, μM), phosphate ($PO_4$, μM), silicate ($SiOH_4$, μM), particulate organic carbon (COP, μg $L^{-1}$) and nitrogen (NOP, μg $L^{-1}$), suspended matter (MES, mg $L^{-1}$), $^{15}N$ (DN15, °/₀₀) and $^{13}C$ isotopes (DC13, °/₀₀) and Chlorophyll a (CHLA, μg $L^{-1}$). Protocols are available on the SOMLIT website and described in Gac et al. (2020).

## rRNA metabarcoding data for protists in the water column

The Operational Taxonomic Units (OTUs) table of publicly available 18S V4 rRNA metabarcoding data for protists in the size fraction >3 μm at the SOMLIT-Astan station (period 2009–2016) was retrieved on 9 December 2022 (https://zenodo.org/record/5032451#.Y5Maw-zMJTY). Pipelines for the generation of OTUs from raw reads and the OTU table were described previously (Caracciolo et al., 2022) and available at https://doi.org/10.5281/zenodo.5791089. The dataset comprised 21,418 protist OTUs with over 30 million sequence reads.

## Statistical analysis

For each sample, the number of *Zobellia* 16S rRNA gene copies was divided by that obtained with universal bacterial primers to assess the relative abundance of *Zobellia*. Statistical analyses were performed with R v3.5.0 (R Core Team, 2022) with significance threshold $\alpha = 0.05$. For macroalgal surface samples, 2-way ANOVA with interaction effect were performed to test the effect of macroalgal species and sampling months on the abundance of *Zobellia*, followed by pairwise post-hoc Tukey HSD when significant results were found. Data were log-transformed (we applied the formula log2(value + 1) for the number of copies and log2 (value + 0.0001) for the relative proportion) prior to analysis and ANOVA residuals were checked for normal distribution. Additionally, one-way ANOVA analyses were conducted to test the effect of macroalgal status ('healthy' vs. 'stranded') on the abundance of *Zobellia*. Pearson correlation coefficients were calculated between averaged triplicates of *Zobellia* absolute and relative abundance on macroalgal surfaces and the different parameters using the corrplot package (v0.90). Correlations were considered significant when Benjamini–Hochberg adjusted *p*-value was <0.05.

For water column samples, the difference between the abundance of *Zobellia* in free-living versus particle-associated fractions was tested using the Mann–Whitney test. The seasonal effect was tested using Kruskal–Wallis tests followed by post-hoc Dunn test with Benjamini–Hochberg corrections of *p*-values. Time-dependent associations between the relative abundance of *Zobellia* in the fraction >3 μm and hydrological parameters or relative abundance of eukaryotic OTUs were searched using extended Local Similarity Analysis (eLSA v1.0.2) (Xia et al., 2011). A prevalence filter of 50% was applied to keep only eukaryotic OTUs found in at least half of the 188 samples for analysis (Röttjers & Faust, 2018). eLSA was run with a maximum delay of 3 successive sampling events, 1000 permutations, the 'nearest' method to fill missing values and 'percentileZ' normalisation. Local Similarity scores were standardised on a [−1;1] scale (Xia et al., 2011) and are referred to as stdLS. Associations were considered significant when covering more than 75% of the time series (length > 141 successive sampling events) and $|\text{stdLS}| \geq 0.6$ and $p < 0.001$ and $Q < 0.05$.

# RESULTS

## Seasonal variations of *Zobellia* abundance and activity on different macroalgal surfaces

Quantitative PCR was carried out for the 238 DNA samples from algal microbiota to assess the seasonal prevalence and abundance of the genus *Zobellia* on healthy and stranded macroalgae with distinct chemical composition (Table S2; Figure 1). The sample LdigB_1 in June 2020 displayed 52,100 *Zobellia* 16S rRNA gene copies $cm^{-2}$, which is 3.2 times more than in the sample with the second highest abundance (16,300 *Zobellia* copies $cm^{-2}$). After boxplot analysis of all results, it was considered an outlier and was then removed from further analyses.

The total number of bacterial 16S rRNA gene copies varied significantly along the year (2-way ANOVA, 'sampling month' effect $F_{9,158} = 5.0$, $p < 0.001$) but not across macroalgal species ($F_{4,158} = 0.8$, $p = 0.531$) and with no interactions between the two factors ($F_{36,158} = 1.1$, $p = 0.3$). Overall, it was lowest in January–February (Figure S1). 16S rRNA genes from *Zobellia* were detected on the surface of the five tested healthy macroalgae. It accounted on average 2000–4500 copies $cm^{-2}$ (Figure 2A) and 0.04%–0.06% relative abundance (Figure 2B) but with a large variability between samples. Two-way ANOVA revealed a strong effect of the season both on the absolute abundance and relative proportion of *Zobellia* ($F_{9,158} = 53.5$, $p < 0.001$ and $F_{9,158} = 79.9$, $p < 0.001$, respectively). Pairwise comparisons revealed a lower abundance of *Zobellia* during summer, from June to September (Figure 3). The lowest number of *Zobellia* 16S rRNA gene copies was obtained in August for *L. digitata* (ca. 0–20 copies $cm^{-2}$ representing 0%–0.0005% of the total copies) and in July for the other species (ca. 0–600 copies $cm^{-2}$ representing 0–0.002% of the total copies). The abundance of *Zobellia* peaked twice, in February–March and October–November, with ca. $10^3$–$10^4$ *Zobellia* 16S rRNA gene copies $cm^{-2}$, representing 0.03%–0.5% of the total amount (Figure 3). By contrast, the algal species only had a weaker effect (2-way ANOVA, $F_{4,158} = 6.9$, $p < 0.001$ and $F_{4,158} = 4.3$, $p < 0.001$, respectively) with interactions between the two factors ($F_{36,158} = 3.6$, $p < 0.001$ and $F_{36,158} = 3.6$, $p < 0.001$, respectively). Separate comparisons at each month indeed showed that

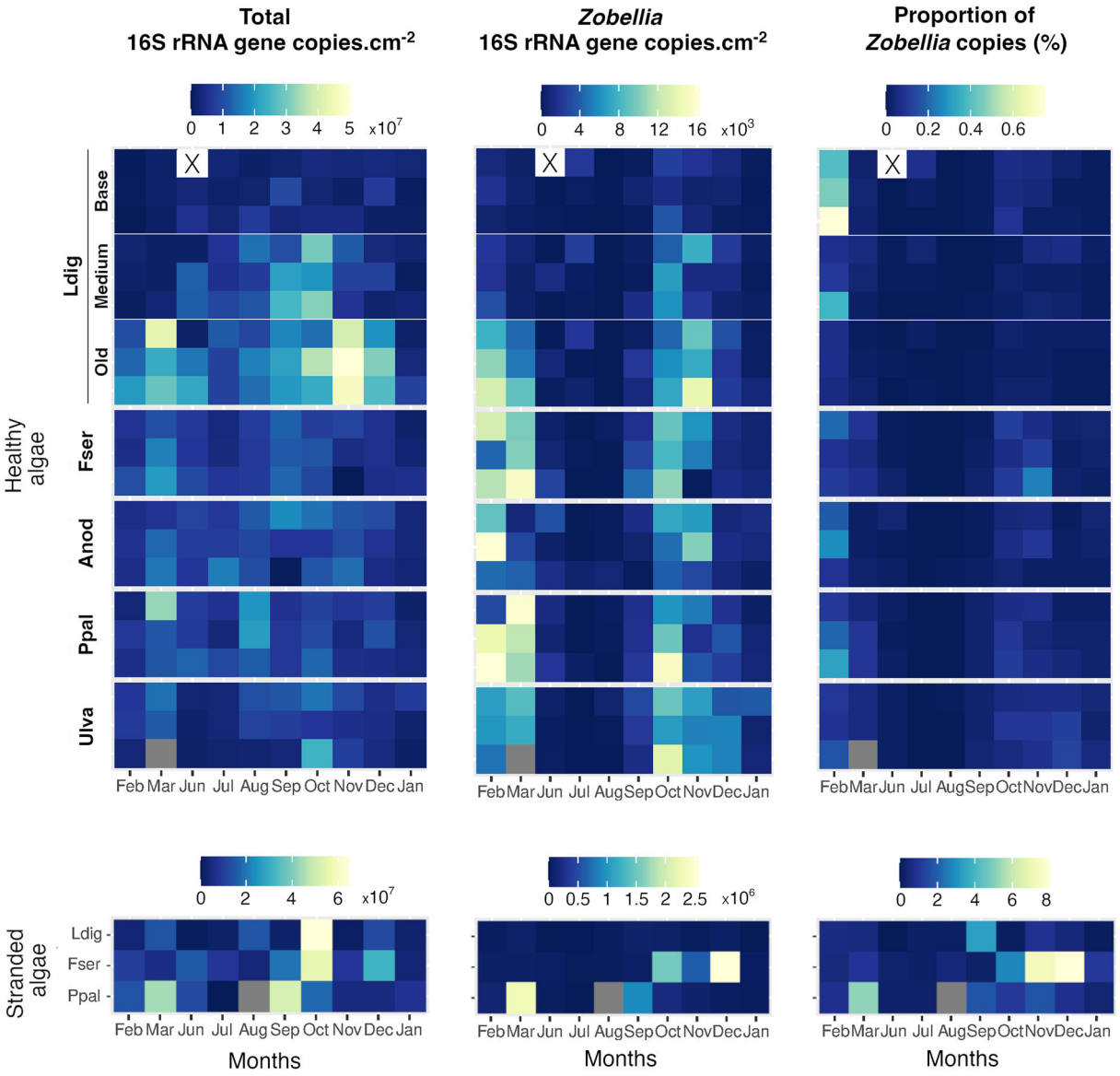


**FIGURE 1** Abundance of total and *Zobellia* 16S rRNA gene copies at the surface of healthy (top) and stranded (bottom) macroalgae. The number of total (left) and *Zobellia* (middle) 16S rRNA gene copies per $cm^2$ is shown, as well as the proportion of *Zobellia* copies (right). No data are available for *Ulva*_3 in March as the sample was lost. The sample LdigB_1 is considered as an outlier as 52,100 *Zobellia* copies were measured, and it was not considered for statistical analyses.

*Zobellia* absolute abundance (Figure S2) and relative proportion (Figure S3) were relatively stable across algal species. When detected, most differences were found between *Laminaria digitata* and other algae. This was mainly due to the lower abundance of *Zobellia* on the basal thallus of this kelp species (see below).

*Zobellia* was quantified on different parts of the thallus of *L. digitata* (Figure 2C,D), revealing variations of the absolute amount of 16S rRNA gene copies ($F_{2,59} = 8.6$, $p < 0.001$). *Zobellia* abundance increased from the base to the tip and represented up to $10^4$ copies $cm^{-2}$ at the extremity of the blade—characteristic of old tissues. The proportion of *Zobellia* followed the opposite pattern (although not significant, $F_{2,59} = 1.5$, $p = 0.2$), as the proportion on the base—where the tissues are renewed—tended to be higher (0.08% on average and up to 0.75%) than on the medium (0.04% on average and up to 0.37%) and old (0.02% on average and up to 0.08%) frond.

Analyses were also performed on stranded individuals in decomposition on the shore line (Figure 2E,F). The absolute and relative amounts of *Zobellia* 16S rRNA gene copies were highly enriched on the surface of stranded algae compared to the healthy ones ($F_{1,158} = 72.3$, $p < 0.001$ and $F_{1,158} = 72.7$, $p < 0.001$, respectively). *Zobellia* accounted on average for 1.5% of the total number of 16S rRNA gene copies on

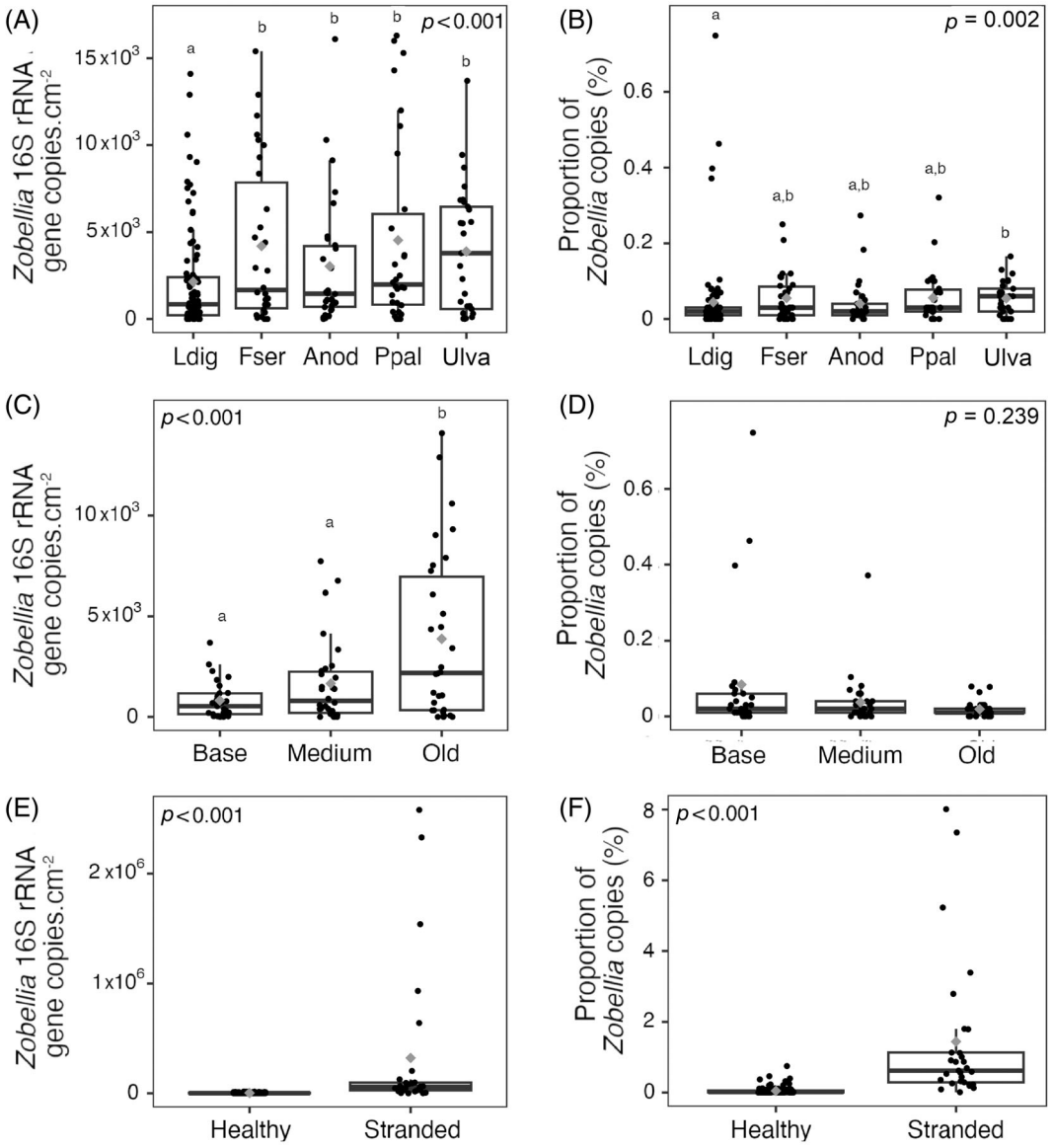


**FIGURE 2** Absolute abundance (A, C, E) and relative proportion (B, D, F) of *Zobellia* 16S rRNA gene copies per $cm^2$ depending on algal species (A, B), *Laminaria* blade parts (C, D) and algal status (E, F). *p*-Values results of two-way ANOVA test followed by post-hoc Tukey HSD test are displayed, with different letters denoting significant difference ($p < 0.05$) across species, blade part or status. Mean values are shown for each group as grey diamonds.

stranded algae (30-fold more than the average on healthy tissues) and more than 5% for three samples (*F. serratus* in November and December, *P. palmata* in March).

Among the 15 measured environmental parameters, the proportion of *Zobellia* 16S rRNA gene copies showed strongest positive correlation with concentrations of $NO_3$, $PO_4$, $SiOH_4$ and MES and strongest negative correlation with seawater temperature (T), salinity (S), pH, and concentrations of COP, NOP and Chl a, independently of the studied algae (Pearson correlation; Figure 4). Similar correlation patterns were observed for the absolute number of *Zobellia* copies (Figure S4).

The number of 16S rRNA transcripts from total bacteria and *Zobellia* cells were estimated using RT-qPCR for *L. digitata* and stranded macroalgal samples (Table S2). The ratio of 16S rRNA transcript copies over 16S rRNA gene copies (16S rRNA:rDNA ratio) was calculated as an index of activity (Campbell et al., 2011). When *Zobellia* 16S rRNA transcripts were detected, this *Zobellia*-specific 16S rRNA:rDNA ratio was significantly higher on stranded algae (123 ± 30, mean ± s.e.m.) compared to healthy *L. digitata* tissues (27 ± 3) (Wilcoxon test, $W = 281$, $p < 0.001$). Futhermore, the *Zobellia*-specific 16S rRNA:rDNA ratio was higher to the one calculated for the total bacterial community in the same sample on *L. digitata* tissues

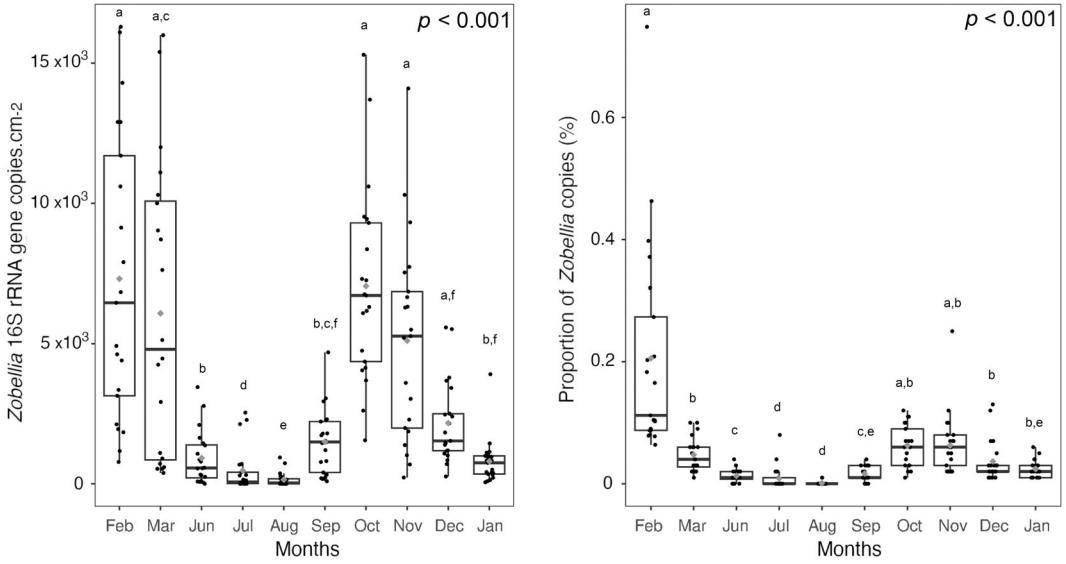


**FIGURE 3** Number of *Zobellia* 16S rRNA gene copies per $cm^2$ (left) and proportion of *Zobellia* copies (right) detected at different sampling months between February 2020 and January 2021 on macroalgal tissues. *p*-Values results of two-way ANOVA test followed by post-hoc Tukey HSD test are displayed, with different letters denoting significant difference ($p < 0.05$) across months. Mean values are shown for each group as grey diamonds.

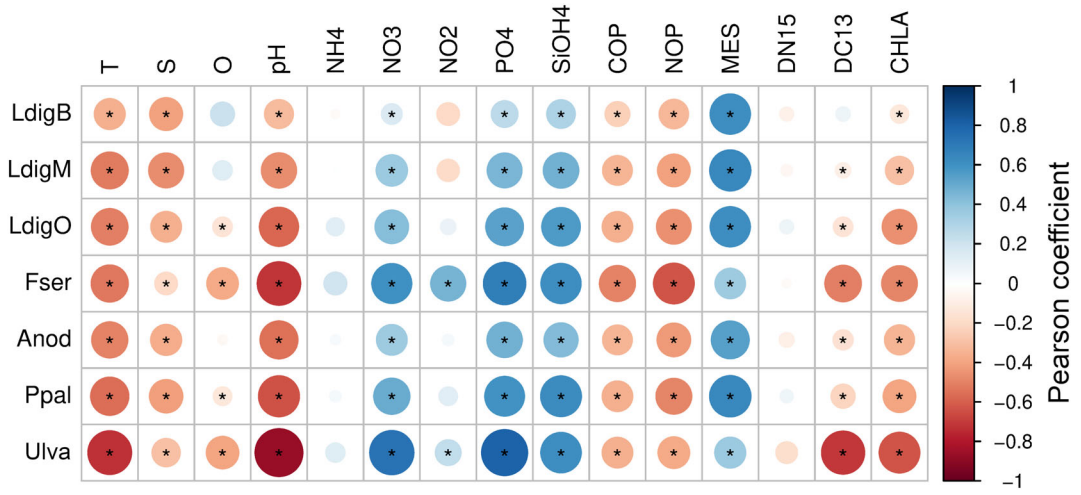


**FIGURE 4** Correlation matrix (Pearson coefficients) between the proportion of 16S rRNA *Zobellia* copies on the different algal tissues and environmental parameter measurements. Asterisks denote significant correlations (Benjamini–Hochberg adjusted *p*-values <0.05).

(2.6-fold higher, Wilcoxon paired test, $W = 1558$, $p < 0.001$), but not on stranded samples ($W = 130$, $p = 0.16$).

## Detection of *Zobellia* in tidally mixed coastal waters

The presence of the genus *Zobellia* was further monitored in coastal seawater samples on a bi-weekly time series from 2009 to 2016, using qPCR both on the free-living (0.2–3 μm) and particle-associated (>3 μm) fractions. 16S rRNA genes from *Zobellia* were detected in 35% (50/141) and 59% (98/166) of the free-living and particle-associated fractions, respectively (Figure S5). The maximum absolute abundance of *Zobellia* was respectively 5890 and 17,629 16S rRNA gene copies $L^{-1}$ in the free-living fraction (January 2011) and on particles (March 2009). Overall, *Zobellia* was 4-fold more abundant on particles (1608 ± 257 copies $L^{-1}$, mean ± s.e.m, $n = 166$) than on the free-living fraction (473 ± 94 copies $L^{-1}$, mean ± s.e.m, $n = 141$) (Mann–Whitney test, $W = 8302$, $p < 0.001$). The same trend was observed for the relative abundance of *Zobellia* within the total bacterial community (Mann–Whitney test, $W = 7783$, $p < 0.001$). On particles, *Zobellia*

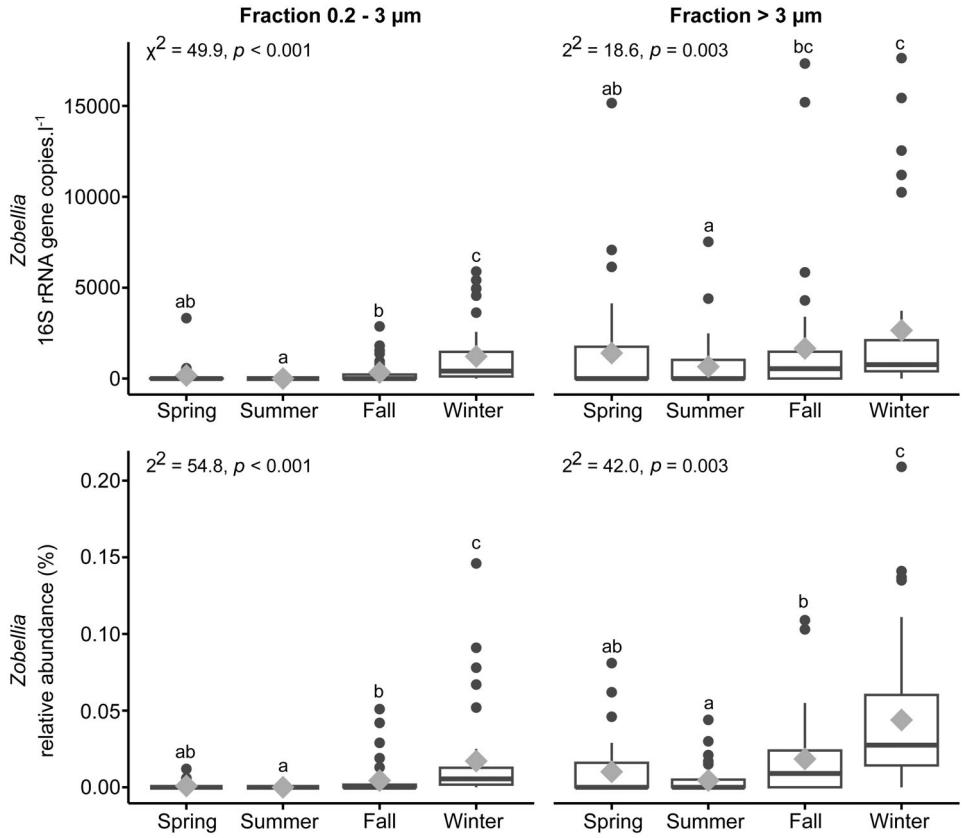


**FIGURE 5** Seasonal effect on the absolute (top row) and relative (bottom row) abundance of *Zobellia* in free-living (0.2–3 µm, left lane) and particle-associated (>3 µm, right lane) fractions. Seasons were defined as follows: Spring, ordinal days 81–172; Summer, ordinal days 173–264; Fall, ordinal days 265–355; Winter, ordinal days <81 and ≥356. $\chi^2$ and *p*-values results of Kruskal–Wallis test followed by post-hoc Dunn test of the season effect are displayed, with different letters denoting significant difference between seasons. Mean values are shown for each group as grey diamonds.

accounted for up to 0.21% of the total bacterial community. Both the absolute and relative abundance of *Zobellia* showed a recurring seasonal trend, with significantly higher values during the winter (Figure 5). To explain this seasonal pattern, we used eLSA to detect associations between the relative abundance of *Zobellia* and hydrological parameters, focusing only on the >3 µm particles were the presence of *Zobellia* was more consistent. The strongest positive and negative associations were found for nitrate concentration (stdLS = 0.952) and seawater temperature (stdLS = −1), respectively (Figure 6A). In addition, the relative abundance of *Zobellia* was positively associated to silicate, oxygen and phosphate concentrations, while it was negatively associated to salinity, chlorophyll a and ammonium concentrations (Table S3). eLSA also revealed positive associations between the relative abundance of *Zobellia* and that of 114 individual eukaryotic OTUs (Table S3). Most positive associations were found with Stramenopiles (37 OTUs, 32%) and Alveolata (35 OTUs, 31%). The relative abundance of *Zobellia* was notably associated positively without delay to three brown macroalgal OTUs within the Phaeophyceae and one red macroalgal Corallinales OTU (Figure 6B). Furthermore, strong positive associations were detected with protist OTUs (Figure 6C), including Ventrifissuridae (highest stdLS = 1), Parmales (highest stdLS = 0.951), Dinophyceae (highest stdLS = 0.945), *Chloropicon* (highest stdLS = 0.921) and Syndiniales (highest stdLS = 0.897).

## DISCUSSION

### *Zobellia* is a stable component of healthy macroalgal epibiota

Our study shows that the flavobacterial genus *Zobellia* is consistently part of the epibiota of macroalgae in a temperate coastal habitat. This agrees with the fact that most of the described *Zobellia* species have been isolated from macroalgae (Barbeyron et al., 2001, 2021; Nedashkovskaya et al., 2004, 2021) and that *Zobellia* sequences were found in metagenomic studies on macroalgae (Dogs et al., 2017; Miranda et al., 2013). Here, *Zobellia* was detected year-round on the five algal species tested, which cover all three macroalgal phyla and whose chemical composition largely differs

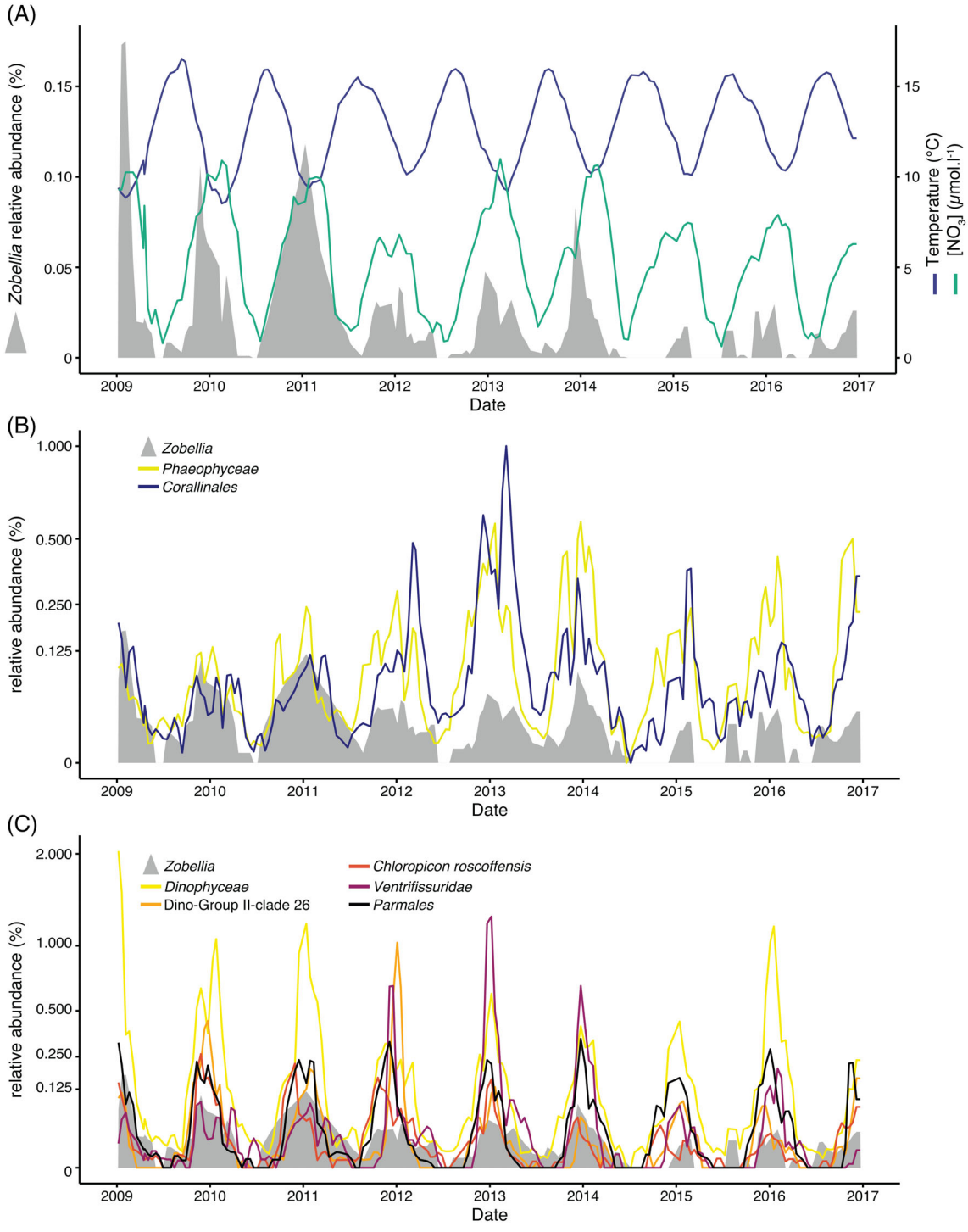


**FIGURE 6** Selected significant associations of *Zobellia* with physico-chemical parameters and eukaryotic taxa in the >3 µm fraction. (A) Relative abundance of *Zobellia* (left axis, grey area), seawater temperature (standardised LS = −1, length 185, delay = −2, right axis) and nitrate concentration (std LS = 0.952, length = 176, delay = −1, right axis). (B) Relative abundance of *Zobellia* (grey area) and two selected macroalgal OTUs with significant positive association, affiliated respectively to *Phaeophyceae* (std LS = 0.687, length 187, delay = 0) and *Corallinales* (std LS = 0.681, length = 187, delay = 0). (C) Relative abundance of *Zobellia* (grey area) and five selected protist OTUs with significant positive association, affiliated respectively to *Dinophyceae* (std LS = 0.945, length = 187, delay = 0), Dino Group II—clade 26 (std LS = 0.897, length = 187, delay = 0), *Chloropicon roscoffensis* (std LS = 0.921, length = 187, delay = 1), *Ventrifissuridae* (std LS = 1, length = 182, delay = 0) and *Parmales* (std LS = 0.951, length = 187, delay = 1). Values are rolling means (window width = 2 consecutive sampling events). For each year, the tick mark corresponds to January 1st. Full results are available in Table S3.

between one another (Kloareg et al., 2021). Given its stable presence on the diverse algal species, *Zobellia* might therefore display neutral or beneficial interactions with healthy hosts. Indeed, *Zobellia* representatives are known to possess multiple adaptive traits that may favour stable association with macroalgal hosts (Barbeyron et al., 2016). It includes resistance mechanisms against oxidative stress and halogenated compounds (Fournier et al., 2014; Grigorian et al., 2021) and the secretion of thallusin, a macroalgal morphogenetic compound (Ulrich et al., 2022). Furthermore, described *Zobellia* species can assimilate several of the main glycans produced by brown (e.g., alginate, fucan-containing sulfated polysaccharides, laminarin, mannitol), red (e.g., agar, porphyran, carrageenans) and green macroalgae (e.g., ulvans) (Barbeyron et al., 2001, 2021; Groisillier et al., 2015; Nedashkovskaya et al., 2004). This metabolic versatility might be an advantage to colonise diverse macroalgal tissues offering different substrate niches.

Despite this stable association with healthy macroalgae, *Zobellia* was not a dominant genus compared to other taxa such as *Granulosicoccus* spp. (*Gammaproteobacteria, Chromatiales*) or *Litorimonas* spp. and *Hellea* spp. (*Alphaproteobacteria, Maricaulales*) that can represent more than 10% of the biofilm community on kelps (Brunet, Le Duff, et al., 2021; Paix et al., 2019; Ramirez-Puebla et al., 2022). Here, we show that *Zobellia* accounted on average only for 0.05% of the total bacterial community, but reached up to 0.7% depending on the algal species and season (see below). Although small, this proportion appears ecologically relevant regarding the hundreds of different taxa reported to live on macroalgal surfaces (Egan et al., 2013). 16S rRNA:rDNA ratios also suggest *Zobellia* cells might be more transcriptionally active than the average bacterial epibionts on healthy macroalgae. In addition, living macroalgae might actively control the development of such potent degraders as *Zobellia*, known for their large CAZyme repertoires (Barbeyron et al., 2016; Chernysheva et al., 2019) and efficiency to utilise algal biomass (Brunet et al., 2022).

The abundance of *Zobellia* also varied along the thallus of the kelp *L. digitata*. As observed for the total bacterial community, the absolute amount of *Zobellia* 16S rRNA genes increased with the distance from the stipe/blade junction, being maximum at the distal ends. Such a pattern in bacterial abundance was already reported for several kelp species (Ihua et al., 2020; Staufenberger et al., 2008; Weigel & Pfister, 2019). Kelps grow from the meristematic region between the perennial stipe and the blade. Therefore, the base of the blade represents the youngest tissues that might not yet host as many bacterial cells as older tissues towards the apex. On the other hand, physical shedding of distal blades likely eases the access to algal compounds, favouring bacterial growth. By contrast, the relative abundance of *Zobellia* tended to follow an inverse trend, being higher on the basal blade of *L. digitata*. Previous studies have shown that younger tissues of several kelp species release more reactive oxygen species upon stress (Küpper et al., 2001) and contain higher concentrations of phlorotannins (Van Alstyne et al., 1999), which can both control the growth of epiphytic bacteria. Our results therefore suggest that *Zobellia* cells might better tolerate these algal defence reactions than other bacterial epibionts on young kelp tissues.

## The abundance and activity of *Zobellia* increase on stranded algae

Throughout the year, both the absolute and relative abundance of *Zobellia* were higher on stranded macroalgae compared to healthy ones. This suggests that *Zobellia* representatives become a dominant component of the bacterial community associated with decaying algal tissues, showing how competitive they can be to exploit this substrate-rich ecological niche. Together with their large genomes (ca. 5 Mb) and extended CAZyme repertoires, this illustrates their copiotrophic lifestyle, as reported for other flavobacteria (Lauro et al., 2009). Members of the genus *Zobellia* also feature additional traits that might partly help them compete with other members of the bacterial community, namely the synthesis of the dialkylresorcinol antimicrobial zobelliphol active against Gram-positive bacteria (Harms et al., 2018) and the predicted production of a homoserine lactone lyase putatively interfering with quorum sensing mechanisms (Barbeyron et al., 2016). We further showed that the 16S rRNA:rDNA ratio was high for *Zobellia* on stranded macroalgae, illustrating a strong activity. Since ribosome content correlates positively with growth rate for many bacteria, rRNA:rDNA ratio is a common proxy to estimate growth rates of specific bacterial taxa in natural communities (Campbell et al., 2011). Based on linear regression analysis of rRNA:rDNA versus growth rate for various marine and non-marine copiotrophic bacteria (Lankiewicz et al., 2016), we can extrapolate that the measured rRNA:rDNA ratio for *Zobellia* on stranded macroalgae would correspond to growth rates from 0.001 to 0.1 $h^{-1}$. This matches maximum growth rates previously measured on batch cultures of *Z. galactanivorans* Dsij$^T$ during the exponential phase in rich medium (Thomas et al., 2011). Hence, our estimate of high growth rates for natural populations of *Zobellia* on stranded macroalgae could further explain how they become dominant. We recently showed that *Zobellia* isolates actively degrade kelp tissues in laboratory microcosms (Brunet et al., 2022). The present data further indicates that *Zobellia* could significantly influence the fate of carbon sequestered by

macroalgae in the studied coastal area, since fast-growing bacteria might inject more organic carbon into the microbial loop and produce more $CO_2$ through respiration than slow growers (Del Giorgio & Cole, 1998; Pedler et al., 2014).

## *Zobellia* is also part of coastal bacterioplankton

Although the genus *Zobellia* is hitherto mostly known for its association with macroalgal surfaces, our results show that it is also recurrently found in coastal bacterioplankton of the Western English Channel. The abundance of *Zobellia* was higher on particles than in the free-living fraction, in agreement with its ability to adhere to, glide and form dense biofilms on surfaces (Nedashkovskaya & Suzuki, 2015; Salaün et al., 2012). The relative abundance of *Zobellia* was in the same order of magnitude on healthy macroalgae and on particles, suggesting the coastal water column might be a yet overlooked habitat for this genus. Indeed, one of the eight validly described species, *Z. amurskyensis* KMM 3526$^T$, was isolated from seawater in Amursky Bay, Sea of Japan (Nedashkovskaya et al., 2004). *Zobellia* representatives were also previously grown from seawater and particles larger than 10 μm during a phytoplankton spring bloom off Helgoland (Heins & Harder, 2023). Our analysis of long-term observation data revealed recurring associations of *Zobellia* with individual eukaryotic OTUs in the particle-associated fraction. This contrasts with previous studies of free-living bacterioplankton communities during coastal spring phytoplankton blooms over 4 years, which did not evidence clear correlations between distinct bacterial and diatom taxa (Teeling et al., 2016). Specific one-to-one interactions might therefore have a stronger influence on bacterial community composition for particle-associated clades, while different bacterial taxa sharing similar functional traits might at least partially substitute each other in the free-living fraction (Galand et al., 2018; Teeling et al., 2016). Here, we notably detected significant associations of *Zobellia* with individual OTUs affiliated to brown and red macroalgae in the particle-associated fractions. This indicates that *Zobellia* could thrive on macroalgal debris released in the water column. However, stronger associations were found with phytoplankton OTUs, including dinoflagellates, green microalgae, diatoms and Parmales (Stramenopiles, Bolidophyceae). Therefore, *Zobellia* might also be part of phytoplankton microbiomes in coastal waters, confirming previous cultivation attempts that retrieved *Zobellia* isolates from North Sea phytoplankton (Hahnke & Harder, 2013). Phytoplankton-derived polysaccharides, accounting for up to 90% of microalgal exudates (Myklestad, 1995), could be substrates for *Zobellia* growth. Recent analyses of diatom exopolysaccharides notably revealed the presence of a complex cocktail of glycans, including epitopes recognised by antibodies that target fucose-containing sulfated polysaccharide (FCSP), β-1,4-mannan, β-1,-3-glucan, xyloglucan, β-1,4-xylan, and alginate (Huang et al., 2021). Other detected associations of *Zobellia* with non-photosynthetic protists could indicate grazing interactions, for example for heterotrophic Stramenopiles flagellates known to graze on flavobacteria (Massana et al., 2009), or sharing of the same habitat, for example for heterotrophic Cercozoa that can feed on diatoms (Drebes et al., 1996) or Syndiniales that parasite photosynthetic dinoflagellates (Guillou et al., 2008).

## Seasonal variations

Although always representing <1% relative abundance, *Zobellia* showed similar seasonal variations both on macroalgal surfaces and in coastal seawater from Roscoff, with maximum and minimum abundance in fall/winter and summer, respectively. This illustrates the growth and loss dynamicity of rare microbial taxa, as previously shown for coastal marine bacterioplankton in the Bay of Biscay (Alonso-Sáez et al., 2015). This is consistent with previous reports of the higher winter abundance of *Bacteroidota* on the kelp *Laminaria hyperborea* from Norway (Bengtsson et al., 2010) and on *Fucus vesiculosus* from the Baltic Sea (Mensch et al., 2020). Yet, seasonal changes can vary depending on site, as already shown for *Macrocystis pyrifera* epibiota (Florez et al., 2019). Therefore, conclusions on *Zobellia* seasonality based on sampling in Roscoff might not be directly applicable to other locations. The highest abundance of *Zobellia* in winter contrasts with previous reports of recurring growth of planktonic *Flavobacteriia* following spring phytoplankton blooms. This highlights the importance of niche specialisation within marine flavobacteria (Díez-Vives et al., 2019) and supports the idea that the abundance of individual taxa can be more influenced by specific interactions rather than only based on substrate availability. Two types of parameters could explain the observed seasonality of *Zobellia*: (i) abiotic hydrological variables and (ii) fluctuations of biotic factors related to the hosts. Among abiotic factors, we notably found strong negative association of *Zobellia* with seawater temperature, which ranged from 8°C in winter to 18°C in summer. Temperature is well-known as a crucial driver of bacterial community structure on macroalgae (Paix et al., 2021; Takemura et al., 2014) and in coastal bacterioplankton (Teeling et al., 2016). Recent meta-analyses of ocean microbiome datasets showed that higher seawater temperatures universally favour slow-

growing taxa (Abreu et al., 2023), which could lead to higher competition for resources in summer. Together with potential stochastic effects, a deterministic effect of temperature could explain the observed lower peak abundance of *Zobellia* in coastal bacterioplankton in 2012 and after 2015, when winter seawater temperature stayed >10°C. Positive associations of *Zobellia* with nitrate and other nutrients likely reflect its copiotrophic lifestyle, thriving when resources are abundant. Furthermore, cultured *Zobellia* representatives possess a nitrate assimilation pathway and can use nitrate as a sole nitrogen source (Barbeyron et al., 2016). Similarly, Teeling et al. (2016) found that the strongest abiotic predictors of coastal bacterioplankton were temperature, salinity, silicate and nitrate concentrations. Seasonal variations in water column samples might also result from higher hydrodynamic forces during the winter in Roscoff, when more intense tidal mixing and winds produce macroalgal debris and resuspend benthic phytoplankton from sediments (Caracciolo et al., 2022). In addition, fluctuations of biotic factors might partly explain the observed seasonality of *Zobellia*. Algal defence chemicals such as halogenated compounds are strong selective factors for epiphytic bacterial colonisers (Goecke et al., 2010). The five macroalgal species studied here display maximum and minimum iodine content in winter and summer, respectively (Ar Gall et al., 2004; Nitschke et al., 2018). *Z. galactanivorans* Dsij$^T$ notably accumulates high intracellular iodine concentrations and tolerates haloacids, thanks to three vanadium-iodoperoxidases, an iodotyrosine dehalogenase and a haloacid dehalogenase that have homologues in all described *Zobellia* strains (Barbeyron et al., 2016; Fournier et al., 2014; Grigorian et al., 2021; Grigorian et al., 2023). This halogen metabolism likely constitutes a selective advantage over other less-equipped bacteria in winter, when halogen concentration is highest. The growth cycle of macroalgae might also influence their epibionts. For example, kelp blade renewal and expansion occur in spring at the meristem part. In consequence, in May, the whole blade consists of fresh tissue. Hence, the low proportion of *Zobellia* in summer suggests it might not be an early coloniser of macroalgae. Moreover, algal exudation is higher in summer (Abdullah & Fredriksen, 2004) and consumers of labile exudates might dominate the biofilm (Bengtsson et al., 2010). By contrast, the aging tissues in autumn-winter might select for specialist bacteria able to break down the complex extracellular matrix. Although this cannot be assessed with the present data, viral lysis and protist grazing also potentially control the abundance of *Zobellia*. Both factors are known to follow seasonal dynamics (Bunse & Pinhassi, 2017) and can apply selective pressure on flavobacterial taxa (Bartlau et al., 2022; Kirchman, 2002; Massana et al., 2009).

## CONCLUSION

Taking the well-studied genus *Zobellia* as a test case, our study highlights the seasonal dynamics and environmental constraints driving natural populations of algae-degrading bacteria. While not dominant, the genus *Zobellia* is a stable component of healthy macroalgal microbiota and can also be found mostly associated to particles in the water column. It undergoes recurring seasonal variations with higher abundances in winter, significantly associated to biotic and abiotic variables. Furthermore, it can become a dominant and strongly active member of bacterial communities on decaying macroalgae, likely contributing to algal biomass degradation and remineralization. These findings connect mechanistic knowledge obtained on laboratory models to potential ecological impacts on coastal carbon cycling.

### AUTHOR CONTRIBUTIONS

**Maéva Brunet:** Formal analysis (equal); investigation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Nolwen Le Duff:** Investigation (equal); writing – review and editing (equal). **Fabienne Rigaut-Jalabert:** Investigation (equal); writing – review and editing (equal). **Sarah Romac:** Investigation (equal); writing – review and editing (equal). **Tristan Barbeyron:** Investigation (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Francois Thomas:** Conceptualization (lead); formal analysis (equal); funding acquisition (lead); investigation (equal); project administration (lead); supervision (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

### ACKNOWLEDGEMENTS

The authors thank Manon Choulot (UMR8227, Roscoff) for her help during sample collection, Nathalie Simon (UMR7144, Roscoff) for scientific supervision of the SOMLIT monitoring, Christian Jeanthon (UMR7144, Roscoff) for useful discussions on the project, Nicolas Henry (FR2424, Roscoff) for the production and publication of eukaryotic metabarcoding datasets, Thierry Cariou (FR2424, Roscoff) for integration of hydrological parameters to SOMLIT, as well as the captains and crew of the Neomysis research ship for their help during sampling at the SOMLIT-Astan and SOMLIT-Estacade stations. This work has benefited from the facilities of the Genomer platform and from the computational resources of the ABiMS bioinformatics platform (FR 2424, CNRS-Sorbonne Université, Roscoff), which are part of the Biogenouest core facility network. This work was funded by the French Government via the National Research Agency programs ALGAVOR (ANR-18-CE02-0001-01) and IDEALG (ANR-10-BTBR-04).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are either available in the supplementary material of this article or openly available on the SOMLIT database (http://www.somlit.fr) and in zenodo at https://zenodo.org/record/5032451#.Y5Maw-zMJTY.

## ORCID

*François Thomas* https://orcid.org/0000-0003-1896-0774

## REFERENCES


Abdullah, M.I. & Fredriksen, S. (2004) Production, respiration and exudation of dissolved organic matter by the kelp *Laminaria hyperborea* along the west coast of Norway. *Journal of the Marine Biological Association of the UK*, 84, 887–894.

Abreu, C.I., Dal Bello, M., Bunse, C. Pinhassi, J. & Gore, J. (2023) Warmer temperatures favor slower-growing bacteria in natural marine communities. *Science Advances*, 9, eade8352.

Alonso, C., Warnecke, F., Amann, R. & Pernthaler, J. (2007) High local and global diversity of Flavobacteria in marine plankton. *Environmental Microbiology*, 9, 1253–1266.

Alonso-Sáez, L., Díaz-Pérez, L. & Morán, X.A.G. (2015) The hidden seasonality of the rare biosphere in coastal marine bacterioplankton. *Environmental Microbiology*, 17, 3766–3780.

Ar Gall, E., Kupper, F.C. & Kloareg, B. (2004) A survey of iodine content in *Laminaria digitata*. *Botanica Marina*, 47, 30–37.

Barbeyron, T., Le Duff, N., Duchaud, E. & Thomas, F. (2023) *Zobellia alginiliquefaciens* sp. nov., a novel member of the flavobacteria isolated from the epibiota of the brown alga *Ericaria zosteroides* (C. Agardh) Molinari & Guiry 2020. *International Journal of Systematic and Evolutionary Microbiology*, 73, 005924.

Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B. & Potin, P. (2001) *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 51, 985–997.

Barbeyron, T., Thiébaud, M., Le Duff, N., Martin, M., Corre, E., Tanguy, G. et al. (2021) *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*. *International Journal of Systematic and Evolutionary Microbiology*, 71, 004913.

Barbeyron, T., Thomas, F., Barbe, V., Teeling, H., Schenowitz, C., Dossat, C. et al. (2016) Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij$^{T}$. *Environmental Microbiology*, 18, 4610–4627.

Bartlau, N., Wichels, A., Krohne, G., Adriaenssens, E.M., Heins, A., Fuchs, B.M. et al. (2022) Highly diverse flavobacterial phages isolated from North Sea spring blooms. *The ISME Journal*, 16, 555–568.

Bengtsson, M.M., Sjøtun, K. & Øvreås, L. (2010) Seasonal dynamics of bacterial biofilms on the kelp *Laminaria hyperborea*. *Aquatic Microbial Ecology*, 60, 71–83.

Brunet, M., de Bettignies, F., Le Duff, N., Tanguy, G., Davoult, D., Leblanc, C. et al. (2021) Accumulation of detached kelp biomass in a subtidal temperate coastal ecosystem induces succession of epiphytic and sediment bacterial communities. *Environmental Microbiology*, 23, 1638–1655.

Brunet, M., Le Duff, N., Barbeyron, T. & Thomas, F. (2022) Consuming fresh macroalgae induces specific catabolic pathways, stress reactions and Type IX secretion in marine flavobacterial pioneer degraders. *The ISME Journal*, 16, 2027–2039.

Brunet, M., Le Duff, N., Fuchs, B.M., Amann, R., Barbeyron, T. & Thomas, F. (2021) Specific detection and quantification of the marine flavobacterial genus *Zobellia* on macroalgae using novel qPCR and CARD-FISH assays. *Systematic and Applied Microbiology*, 44, 126269.

Buchan, A., LeCleir, G.R., Gulvik, C.A. & González, J.M. (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nature Reviews. Microbiology*, 12, 686–698.

Bunse, C., Koch, H., Breider, S., Simon, M. & Wietz, M. (2021) Sweet spheres: succession and CAZyme expression of marine bacterial communities colonising a mix of alginate and pectin particles. *Environmental Microbiology*, 23, 3130–3148.

Bunse, C. & Pinhassi, J. (2017) Marine Bacterioplankton seasonal succession dynamics. *Trends in Microbiology*, 25, 494–505.

Campbell, B.J., Yu, L., Heidelberg, J.F. & Kirchman, D.L. (2011) Activity of abundant and rare bacteria in a coastal ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 108(31), 12776–12781.

Caracciolo, M., Rigaut-Jalabert, F., Romac, S., Mahé, F., Forsans, S., Gac, J.P. et al. (2022) Seasonal dynamics of marine protist communities in tidally mixed coastal waters. *Molecular Ecology*, 31, 3761–3783.

Chernysheva, N., Bystritskaya, E., Stenkova, A., Golovkin, I., Nedashkovskaya, O. & Isaeva, M. (2019) Comparative genomics and CAZyme genome repertoires of marine *Zobellia amurskyensis* KMM 3526$^{T}$ and *Zobellia laminariae* KMM 3676$^{T}$. *Marine Drugs*, 17, 661.

Cocquempot, L., Delacourt, C., Paillet, J., Riou, P., Aucan, J., Castelle, B. et al. (2019) Coastal Ocean and nearshore observation: a French case study. *Frontiers in Marine Science*, 6, 1–17.

Del Giorgio, P.A. & Cole, J.J. (1998) Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics*, 29, 503–541.

Díez-Vives, C., Nielsen, S., Sánchez, P., Palenzuela, O., Ferrera, I., Sebastián, M. et al. (2019) Delineation of ecologically distinct units of marine *Bacteroidetes* in the Northwestern Mediterranean Sea. *Molecular Ecology*, 28, 2846–2859.

Dogs, M., Wemheuer, B., Wolter, L., Bergen, N., Daniel, R., Simon, M. et al. (2017) *Rhodobacteraceae* on the marine brown alga *Fucus spiralis* are abundant and show physiological adaptation to an epiphytic lifestyle. *Systematic and Applied Microbiology*, 40, 370–382.

Drebes, G., Kühn, S.F., Gmelch, A. & Schnepf, E. (1996) *Cryothecomonas aestivalis* sp. nov., a colourless nanoflagellate feeding on the marine centric diatom *Guinardia delicatula* (Cleve) Hasle. *Helgolander Meeresuntersuchungen*, 50, 497–515.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S. & Thomas, T. (2013) The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews*, 37, 462–476.

Enke, T.N., Datta, M.S., Schwartzman, J., Cermak, N., Schmitz, D., Barrere, J. et al. (2019) Modular assembly of polysaccharide-degrading marine microbial communities. *Current Biology*, 29, 1528–1535.

Ficko-Blean, E., Préchoux, A., Thomas, F., Rochat, T., Larocque, R., Zhu, Y. et al. (2017) Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria. *Nature Communications*, 8, 1685.

Florez, J.Z., Camus, C., Hengst, M.B., Marchant, F. & Buschmann, A.H. (2019) Structure of the epiphytic bacterial communities of *Macrocystis pyrifera* in localities with contrasting

nitrogen concentrations and temperature. *Algal Research*, 44, 101706.

Fournier, J.B., Rebuffet, E., Delage, L., Grijol, R., Meslet-Cladière, L., Rzonca, J. et al. (2014) The vanadium iodoperoxidase from the marine *Flavobacteriaceae* species *Zobellia galactanivorans* reveals novel molecular and evolutionary features of halide specificity in the vanadium haloperoxidase enzyme family. *Applied and Environmental Microbiology*, 80, 7561–7573.

Gac, J.P., Marrec, P., Cariou, T., Guillerm, C., Macé, É., Vernet, M. et al. (2020) Cardinal buoys: an opportunity for the study of air-sea $CO_2$ fluxes in coastal ecosystems. *Frontiers in Marine Science*, 7, 1–21.

Galand, P.E., Pereira, O., Hochart, C., Auguet, J.C. & Debroas, D. (2018) A strong link between marine microbial community composition and function challenges the idea of functional redundancy. *The ISME Journal*, 12, 2470–2478.

Gattuso, J.-P., Frankignoulle, M. & Wollast, R. (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics*, 29, 405–434.

Gilbert, J.A., Field, D., Swift, P., Newbold, L., Oliver, A., Smyth, T. et al. (2009) The seasonal structure of microbial communities in the Western English Channel. *Environmental Microbiology*, 11, 3132–3139.

Goberville, E., Beaugrand, G., Sautour, B. & Tréguer, P. (2010) Climate-driven changes in coastal marine systems of western Europe. *Marine Ecology Progress Series*, 408, 129–147.

Gobet, A., Barbeyron, T., Matard-Mann, M., Magdelenat, G., Vallenet, D., Duchaud, E. et al. (2018) Evolutionary evidence of algal polysaccharide degradation acquisition by *Pseudoalteromonas carrageenovora* $9^T$ to adapt to macroalgal niches. *Frontiers in Microbiology*, 9, 1–16.

Goecke, F.R., Labes, A., Wiese, J. & Imhoff, J.F. (2010) Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, 409, 267–299.

Grigorian, E., Roret, T., Czjzek, M., Leblanc, C. & Delage, L. (2023) X-ray structure and mechanism of ZgHAD, a L −2-haloacid dehalogenase from the marine flavobacterium *Zobellia galactanivorans*. *Protein Science*, 32, 1–12.

Grigorian, E., Groisillier, A., Thomas, F., Leblanc, C. & Walsh, D.A. (2021) Functional characterization of a L-2-Haloacid dehalogenase from *Zobellia galactanivorans* Dsij$^T$ suggests a role in haloacetic acid catabolism and a wide distribution in marine environments. *Frontiers in Microbiology*, 12, 1–15.

Groisillier, A., Labourel, A., Michel, G. & Tonon, T. (2015) The mannitol utilization system of the marine bacterium *Zobellia galactanivorans*. *Applied and Environmental Microbiology*, 81, 1799–1812.

Guillou, L., Viprey, M., Chambouvet, A., Welsh, R.M., Kirkham, A.R., Massana, R. et al. (2008) Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environmental Microbiology*, 10, 3349–3365.

Hahnke, R.L. & Harder, J. (2013) Phylogenetic diversity of Flavobacteria isolated from the North Sea on solid media. *Systematic and Applied Microbiology*, 36, 497–504.

Harms, H., Klöckner, A., Schrör, J., Josten, M., Kehraus, S., Crüsemann, M. et al. (2018) Antimicrobial dialkylresorcins from marine-derived microorganisms: insights into their mode of action and putative ecological relevance. *Planta Med*, 84(18), 1363–1371.

Heins, A. & Harder, J. (2023) Particle-associated bacteria in seawater dominate the colony-forming microbiome on ZoBell marine agar. *FEMS Microbiology Ecology*, 99, 1–11.

Huang, G., Vidal-Melgosa, S., Sichert, A., Becker, S., Fang, Y., Niggemann, J. et al. (2021) Secretion of sulfated fucans by diatoms may contribute to marine aggregate formation. *Limnology and Oceanography*, 66, 3768–3782.

Ihua, M.W., FitzGerald, J.A., Guiheneuf, F., Jackson, S.A., Claesson, M.J., Stengel, D.B. et al. (2020) Diversity of bacteria populations associated with different thallus regions of the brown alga *Laminaria digitata*. *PLoS One*, 15, 1–15.

Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M. et al. (2014) Functional characterization of polysaccharide utilization loci in the marine *Bacteroidetes* "*Gramella forsetii*" KT0803. *The ISME Journal*, 8, 1492–1502.

Kirchman, D.L. (2002) The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiology Ecology*, 39, 91–100.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. et al. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41, 1–11.

Kloareg, B., Badis, Y., Cock, J.M. & Michel, G. (2021) Role and evolution of the extracellular matrix in the acquisition of complex multicellularity in eukaryotes: a macroalgal perspective. *Genes (Basel)*, 12, 1059.

Koch, H., Dürwald, A., Schweder, T., Noriega-Ortega, B., Vidal-Melgosa, S., Hehemann, J.H. et al. (2019) Biphasic cellular adaptations and ecological implications of *Alteromonas macleodii* degrading a mixture of algal polysaccharides. *The ISME Journal*, 13, 92–103.

Küpper, F.C., Kloareg, B., Guern, J. & Potin, P. (2001) Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiology*, 125, 278–291.

Lami, R., Grimaud, R., Sanchez-Brosseau, S., Six, C., Thomas, F., West, N.J. et al. (2021) Marine bacterial models for experimental biology. In: Boutet, A., & Schierwater, B. (Eds.) *Handbook of Marine Model Organisms in Experimental Biology*. London: Taylor & Francis Ltd.

Lankiewicz, T.S., Cottrell, M.T. & Kirchman, D.L. (2016) Growth rates and rRNA content of four marine bacteria in pure cultures and in the Delaware estuary. *The ISME Journal*, 10, 823–832.

Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S. et al. (2009) The genomic basis of trophic strategy in marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 15527–15533.

Lefran, A., Hernández-Fariñas, T., Gohin, F. & Claquin, P. (2021) Decadal trajectories of phytoplankton communities in contrasted estuarine systems in an epicontinental sea. *Estuarine, Coastal and Shelf Science*, 258, 107409.

Martin, M., Portetelle, D. & Michel, G. (2014) Microorganisms living on macroalgae: diversity, interactions, and biotechnological applications. *Applied Microbiology and Biotechnology*, 98, 2917–2935.

Massana, R., Unrein, F., Rodríguez-Martínez, R., Forn, I., Lefort, T., Pinhassi, J. et al. (2009) Grazing rates and functional diversity of uncultured heterotrophic flagellates. *The ISME Journal*, 3, 588–595.

Mensch, B., Neulinger, S.C., Künzel, S., Wahl, M. & Schmitz, R.A. (2020) Warming, but not acidification, restructures epibacterial communities of the Baltic macroalga *Fucus vesiculosus* with seasonal variability. *Frontiers in Microbiology*, 11, 1–18.

Miranda, L.N., Hutchison, K., Grossman, A.R. & Brawley, S.H. (2013) Diversity and abundance of the bacterial community of the red macroalga *Porphyra umbilicalis*: did bacterial farmers produce macroalgae? *PLoS One*, 8, e58269.

Myklestad, S.M. (1995) Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Sci Total Environ*, 165, 155–164.

Nedashkovskaya, O., Otstavnykh, N., Zhukova, N., Guzev, K., Chausova, V., Tekutyeva, L. et al. (2021) *Zobellia barbeyronii* sp. nov., a new member of the family *Flavobacteriaceae*, isolated from seaweed, and emended description of the species *Z. amurskyensis*, *Z. laminariae*, *Z. russellii* and *Z. uliginosa*. *Diversity*, 13, 520.

Nedashkovskaya, O., Suzuki, M., Vancanneyt, M., Cleenwerck, I., Lysenko, A., Mikhailov, V. et al. (2004) *Zobellia amurskyensis*

sp nov., *Zobellia laminariae* sp nov and *Zobellia russellii* sp nov., novel marine bacteria of the family *Flavobacteriaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 54, 1643–1648.

Nedashkovskaya, O.I. & Suzuki, M. (2015) *Zobellia*. In: Trujillo, M.E., Dedysh, S., DeVos, P., Hedlund, B., Kämpfer, P., Rainey, F. et al. (Eds.) *Bergey's manual of systematics of archaea and bacteria*. Hoboken, NJ: John Wiley & Sons, Inc.

Nitschke, U., Walsh, P., McDaid, J. & Stengel, D.B. (2018) Variability in iodine in temperate seaweeds and iodine accumulation kinetics of *Fucus vesiculosus* and *Laminaria digitata* (Phaeophyceae, Ochrophyta). *Journal of Phycology*, 54, 114–125.

Paix, B., Layglon, N., Le Poupon, C., Onofrio, S.D., Misson, B. & Garnier, C. (2021) Integration of spatio-temporal variations of surface metabolomes and epibacterial communities highlights the importance of copper stress as a major factor shaping host-microbiota interactions within a Mediterranean seaweed holobiont. *Microbiome*, 9, 201.

Paix, B., Othmani, A., Debroas, D., Culioli, G. & Briand, J.F. (2019) Temporal covariation of epibacterial community and surface metabolome in the Mediterranean seaweed holobiont *Taonia atomaria*. *Environmental Microbiology*, 21, 3346–3363.

Pedler, B.E., Aluwihare, L.I. & Azam, F. (2014) Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 7202–7207.

R Core Team. (2022) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ramirez-Puebla, S.T., Weigel, B.L., Jack, L., Schlundt, C., Pfister, C.A. & Welch, J.L.M. (2022) Spatial organization of the kelp microbiome at micron scales. *Microbiome*, 10, 52.

Reisky, L., Préchoux, A., Zühlke, M.-K., Bäumgen, M., Robb, C.S., Gerlach, N. et al. (2019) A complex enzyme cascade degrades the polysaccharide ulvan from green algae. *Nature Chemical Biology*, 15, 803–812.

Röttjers, L. & Faust, K. (2018) From hairballs to hypotheses–biological insights from microbial networks. *FEMS Microbiology Reviews*, 42, 761–780.

Salaün, S., La Barre, S., Dos Santos-Goncalvez, M., Potin, P., Haras, D. & Bazire, A. (2012) Influence of exudates of the kelp *Laminaria digitata* on biofilm formation of associated and exogenous bacterial epiphytes. *Microbial Ecology*, 64, 359–369.

Santos, I.R., Hatje, V., Serrano, O., Bastviken, D. & Krause-Jensen, D. (2022) Carbon sequestration in aquatic ecosystems: recent advances and challenges. *Limnology and Oceanography*, 67, S1–S5.

Sichert, A., Corzett, C.H., Schechter, M.S., Unfried, F., Markert, S., Becher, D. et al. (2020) Verrucomicrobia use hundreds of enzymes to digest the algal polysaccharide fucoidan. *Nature Microbiology*, 5(8), 1026–1039.

Singh, R.P. & Reddy, C.R.K. (2016) Unraveling the functions of the macroalgal microbiome. *Frontiers in Microbiology*, 6, 1–8.

Staufenberger, T., Thiel, V., Wiese, J. & Imhoff, J.F. (2008) Phylogenetic analysis of bacteria associated with *Laminaria saccharina*. *FEMS Microbiology Ecology*, 64, 65–77.

Takemura, A.F., Chien, D.M. & Polz, M.F. (2014) Associations and dynamics of *Vibrionaceae* in the environment, from the genus to the population level. *Frontiers in Microbiology*, 5, 1–26.

Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M. et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science*, 336, 608–611.

Teeling, H., Fuchs, B.M., Bennke, C.M., Krüger, K., Chafee, M., Kappelmann, L. et al. (2016) Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *eLife*, 5, 1–29.

Thomas, F., Barbeyron, T. & Michel, G. (2011) Evaluation of reference genes for real-time quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *Journal of Microbiological Methods*, 84, 61–66.

Thomas, F., Barbeyron, T., Tonon, T., Génicot, S., Czjzek, M. & Michel, G. (2012) Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine *Flavobacteriia* to their independent transfers to marine *Proteobacteria* and human gut *Bacteroides*. *Environmental Microbiology*, 14, 2379–2394.

Thomas, F., Bordron, P., Eveillard, D. & Michel, G. (2017) Gene expression analysis of *Zobellia galactanivorans* during the degradation of algal polysaccharides reveals both substrate-specific and shared transcriptome-wide responses. *Frontiers in Microbiology*, 8, 1808.

Ulrich, J.F., Gräfe, M.S., Dhiman, S., Wienecke, P., Arndt, H. & Wichard, T. (2022) Thallusin quantification in marine bacteria and algae cultures. *Marine Drugs*, 20, 690.

Van Alstyne, K.L., McCarthy, J.J., Hustead, C.L. & Kearns, L.J. (1999) Phlorotannin allocation among tissues of northeastern Pacific kelps and rockweeds. *Journal of Phycology*, 35, 483–492.

Weigel, B.L. & Pfister, C.A. (2019) Successional dynamics and seascape-level patterns of microbial communities on the canopy-forming kelps *Nereocystis luetkeana* and *Macrocystis pyrifera*. *Frontiers in Microbiology*, 10, 1–17.

Xia, L.C., Steele, J.A., Cram, J.A., Cardon, Z.G., Simmons, S.L., Vallino, J.J. et al. (2011) Extended local similarity analysis (eLSA) of microbial community and other time series data with replicates. *BMC Systems Biology*, 5, S15.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Brunet, M., Le Duff, N., Rigaut-Jalabert, F., Romac, S., Barbeyron, T. & Thomas, F. (2023) Seasonal dynamics of a glycan-degrading flavobacterial genus in a tidally mixed coastal temperate habitat. *Environmental Microbiology*, 25(12), 3192–3206. Available from: https://doi.org/10.1111/1462-2920.16505