Lower *Vibrio* spp. abundances in *Zostera marina* leaf canopies suggest a novel ecosystem function for temperate seagrass beds

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Abstract

Seagrasses, a polyphyletic group of about 60 marine angiosperm species, are the foundation of diverse and functionally important marine habitats along sheltered sedimentary coasts. As a novel ecological function with high societal relevance, a role of the seagrass leaf canopy for reducing potentially harmful bacteria has recently been hypothesized in tropical regions, but data for temperate regions are lacking. Here, we tested whether or not the abundance of general bacteria and more specifically, those belonging to the genus *Vibrio* were reduced within temperate *Zostera marina* (eelgrass) meadows compared to adjacent sand flats and sampled 5 sites in the south-western Baltic Sea using SCUBA. Compared to non-vegetated area, we found an average reduction of 39% for all *Vibrio* and 63% for the potentially harmful *V. vulnificus/cholerae* subtype based on robust plate counting data on *Vibrio* selective agar. The underlying mechanism of the reduction in bacterial load is currently elusive and clearly merits further study. Our results underline the critical importance of seagrasses in maintaining shallow water ecosystem functioning including water quality and provide further motivation for their protection and restoration.

Introduction

Marine angiosperms, or seagrasses, are the foundation of some of the most valuable coastal marine ecosystems, seagrass beds (Hemminga and Duarte 2000). As ecosystem engineers, their presence turns sedimentary bottoms with mostly infauna into a habitat featuring a rich diversity of

associated animals and plants, along with substantial ecosystem services such as nursery areas, coastal protection and local carbon sequestration (Fourqurean et al. 2012; Heckwolf et al. 2021). Among the many ecological services associated with seagrasses, the reduction of potentially harmful bacteria in the water column has recently been described for tropical seagrass beds (Lamb et al. 2017). Lamb et al. (2017) showed that both, the abundance of possible human pathogenic bacteria as well as bacterial strains infecting marine invertebrates, were reduced in coral reef areas of the Indo-Pacific region with neighboring intact seagrass beds.

We were interested whether this effect is of general nature, and would also apply to temperate seagrasses of the northern hemisphere (Boström et al. 2014). Hence, we studied the effects of a leaf canopy of the widespread temperate seagrass *Zostera marina* (eelgrass) on water column abundance of *Vibrio* spp. bacteria, an abundant and diverse bacterial group thriving in marine waters that are generally favored by global warming and seawater freshening (Baker-Austin et al. 2012; Oberbeckmann et al. 2012). This applies particularly to our study region, the Baltic Sea, where *Vibrio* spp. abundances have already increased in the past decades due to sea surface temperature increase (Baker-Austin et al. 2012; Gyraite et al. 2019), with a rate that is substantially higher than the predicted global ocean warming (Reusch

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et al. 2018). It is, therefore, concerning that environmental conditions are predicted to become even more conducive to further expansion of *Vibrio* due to the freshening of the brackish water body (Meier et al. 2006; Meier and Saraiva 2020). Alarming, the incidence of severe *Vibrio vulnificus* infections upon swimming or other contact with Baltic Sea waters has increased in recent years (Baker-Austin et al. 2016) which has sparked considerable media interest and potentially endangers touristic activities and revenues in the area (Gyraite et al. 2019).

Here, we present first pilot data on the abundance of bacterial groups belonging to the *Vibrio* spp. group in temperate seagrass beds of the south-western Baltic Sea based on a robust plate counting approach. We hypothesized that, analogous to tropical seagrass beds (Lamb et al. 2017), *Zostera marina* (eelgrass) meadows would reduce the bacterial abundance in seawater within the seagrass leaf canopy compared to adjacent sand flats. To this end, we designed a sampling program *in situ* by SCUBA at five locations in the south-western Baltic Sea and assessed the bacterial abundance in seawater from inside and outside the leaf canopy at each site with an unselective, and a *Vibrio*-specific culture medium.

Materials and methods

We sampled defined microhabitats associated at locations in the south-western Baltic Sea that harbor extensive eelgrass *Zostera marina* beds. These are typically interspersed with larger sandy areas. At each site, two un-vegetated and two vegetated areas were sampled in duplicates in September 2019 except for site “Gelting” where only one isolated patch of 20 m² survived a recent die-off event in 2015 due to as yet unidentified causes (Table 1). Sampling points were at least 5 m away from the vegetation edge, again with the exception of the within-vegetation sampling point in Gelting that was only 2.5 m inside the remaining patch. In general, shoot densities in the south-western Baltic vary between 240 and 310 leaf shoots m⁻², and shoot heights are 50–85 cm (Reusch et al. 1994; Munkes et al. 2015). Three sites (“Kiekut”,

“Möltenort” and “Falckenstein”) are in front of recreational beaches. During each sampling, water temperatures and salinities were measured with a handheld WTW instrument in 30–50 cm depth.

Water samples were taken by SCUBA at a water depth between 0.4 and 3.4 m. The depth was dictated by the presence of a dense seagrass canopy which varied at each chosen site. In the western Baltic Sea, no lunar tides exist while wind-driven tides did not occur at the time of sampling. Divers approached the dedicated sampling points against the prevailing current which was determined by slowly releasing seawater blobs stained with uranine (0.5 g L⁻¹) (Reusch and Williams 1999). Typical current speeds in the area were between 1–5 cm s⁻¹. Water samples were taken by gentle suction of 100 mL water using sterile syringes 20 cm above the sediment bottom. Great care was taken that during suction sampling, SCUBA divers were well in buoyancy and did not stir up sediment or material from the seagrass blades. Syringes were flushed three times underwater before the final sample suction.

Water samples were transferred to coolers at 4 °C within 6 h to the laboratory whereupon gentle mixing, for each sample, aliquots of 250 and 100 µL of seawater were plated onto *Vibrio* selective agar (CHROMagar_vibrio™, Chromagar Ltd. Paris, France; (Hara-Kudo et al. 2001)) and onto marine broth agar, respectively. Upon a 4-day incubation at 25 °C for the CHROMagar medium, and 30 °C for marine broth, colonies were counted. Colonies growing on CHROMagar can be assigned to the three major *Vibrio* spp. subtypes *V. parahaemolyticus*, *V. vulnificus/cholerae*, and *V. alginolyticus* via colony color (Hara-Kudo et al. 2001).

Count data of all four response variables (3 *Vibrio* subtypes/marine broth colonies) were square-root transformed to improve variance homogeneity (Sokal and Rohlf 1995), while approximate normality was checked using residual plots. To accommodate the inflation of type I-errors owing to multiple testing (Johnson and Field 1993), count data were first subjected to multivariate analysis of variance (MANOVA, mean effect test) to test for an overall effect of vegetation absence/presence (hereafter “vegetation”)

Table 1 Sampling sites in the south-western Baltic Sea in September 2019

Site	Region	Date	Geographic coordinates		Water depth (m)		Environmental conditions	
			Lat (N)	Long (E)	<i>Zostera</i>	sand	Salinity (psu)	Water temperature (°C)
Gelting	Flensburg Fjord	24 Sep	54.7617	9.8789	0.9	0.6	22.1	15
Kiekut	Eckenförde Bay	4 Sep	54.4485	9.8708	1.9–2.1	0.9–1.0	18	18.5
Falckenstein	Kiel Fjord	9 Sep	54.3936	10.1923	1.1–3.3	0.6–0.9	15.3	18
Möltenort	Kiel Fjord	19 Sep	54.3816	10.2007	1.1–3.1	0.8–1.1	16.6	15
Heiligenhafen	Kiel Bight	11 Sep	54.3805	11.0077	0.8–3.3	0.5–2.3	14.3	16.8

on bacterial and *Vibrio* spp. abundance. A calculation of a cross-correlation matrix explored the covariance among the 4 response variables. Subsequently, all 4 response variables were also tested in univariate analyses of variance (ANOVA) with type-III error estimation separately. At all sites, more than one vegetated or non-vegetated subarea, respectively, was sampled as seagrass grows typically in zones parallel to shore interspersed with sandy areas. In an initial step, we used nested analysis of variance to check for any influence of subarea as “subarea (vegetation vs. no vegetation)”-effect. If this was not the case, error terms can be pooled and all sampled sub-replicates would be treated as true replicates sampled within or outside vegetation at each site, respectively. For all univariate response variables (colony-forming units = cfu marine broth bacteria/all *Vibrio* subgroups) the nested factor was not significant (all $P > 0.11$). Based on recommendations in Underwood (1997), we therefore pooled error terms of the nested effect and the residuals to test for the main effects “vegetation” and “site” and their interaction. Since the habitat types were partially confounded with water depth, we also tested the effect of the covariate “depth” on any of the 4 bacterial abundances in an ANCOVA model. All statistical analyses were conducted in JMP v. 10.0 (Statsoft Inc. 2012).

Results and discussion

Overall *Vibrio* spp. counts (as colony-forming units, cfu $250 \mu\text{l}^{-1}$) varied between 32.3 and 66.1 among all sites, while general bacterial counts (marine broth) were substantially higher and ranged between 88.6 and 179.9 cfu $100 \mu\text{l}^{-1}$. Colonies indicative of *V. parahaemolyticus* were rare regardless of the presence of seagrass vegetation across sites (mean $\pm$ SD 1.97 ± 2.02 cfu $250 \mu\text{l}^{-1}$), followed in abundance by the *V. vulnificus* group and the most abundant *V. alginolyticus* group (15.0 ± 9.9 and 31.3 ± 18.2 cfu $250 \mu\text{l}^{-1}$, respectively). An overall MANOVA revealed that both main factors “site” and “vegetation presence/absence” had significant effects on the overall abundance of the 4 bacterial groups tested (3 *Vibrio* subtypes/general marine bacteria on marine broth; Pillai-trace test statistic, all $P < 0.01$; Supplementary Table 1). The abundance of bacteria on marine broth was only weakly correlated with overall and specific *Vibrio* group abundances (Supplementary Table S2). The abundance of the three *Vibrio* subtypes among themselves correlated only moderately with one another (all $|r| < 0.23$; Supplementary Table S2).

Upon the statistically significant MANOVA, we proceeded with univariate tests to examine the influence of “site” and “vegetation” on the abundance of general marine bacteria and the *Vibrio* subgroups separately. Abundances based on colony counts on marine broth revealed a

significant reduction within the vegetation (– 30%; main effect vegetation, Fig. 1A, Table 2). For bacteria counted on marine broth, there was also a strong “site * vegetation presence/absence”-interaction mainly driven by the site “Kiekut” where the bacterial abundance was slightly higher within the vegetation.

In accordance with the results of general bacterial abundance, we found that across all sites the presence of vegetation reduced the abundance of all *Vibrio* spp. by 39% compared to adjacent non-vegetated area, a reduction that was slightly higher compared to bacteria on marine broth. When broken down into subtypes, *Vibrio* spp. abundance was reduced by 63, 61 and 23% (*V. parahaemolyticus* /*vulnificus* /*alginolyticus* respectively), (Fig. 1B–D). While the main effect “vegetation” was significant for all *Vibrio* subtypes (all $P_{\text{veg}} < 0.007$; Table 2), we also found a marked effect of “site” on all bacterial abundances, suggesting

Fig. 1 Mean abundance (± 1 SD, $N=4$) of *Vibrio* spp. assessed as plate counts of colony forming units (cfu) at 5 sites in the southwestern Baltic Sea. For details on sampling sites see Table 1, for statistical analysis Table 2

Table 2 Results of two-way type-III ANOVAs on bacterial abundance as function of sampling site and vegetation presence/absence

Response variable	Effect	df	SQ	MS	F	P
Bacteria marine broth	Site	4	90.31	22.57	14.13	<.0001
	Vegetation	1	34.89	34.89	21.83	<.0001
	Site* veg	4	47.24	11.81	8.346	<.0001
	Error	28	44.75	1.598		
all <i>Vibrio</i> spp.	Site	4	25.74	6.434	10.83	<.0001
	Vegetation	1	28.32	28.32	47.68	<.0001
	Site* veg	4	19.57	4.892	8.236	0.0002
	Error	28	16.63	0.5940		
<i>V. parahaemolyticus</i>	Site	4	10.40	2.599	6.293	0.001
	Vegetation	1	3.680	3.680	8.902	<.0001
	Site* veg	4	0.8444	0.2111	0.5112	0.728
	Error	28	11.564	0.4130		
<i>V. vulnificus/cholerae</i>	Site	4	10.79	2.697	4.904	0.004
	Vegetation	1	25.38	25.38	46.15	<.0001
	Site* veg	4	4.776	1.194	2.171	0.0983
	Error	28	15.40	0.5499		
<i>V. alginolyticus</i>	Site	4	51.57	12.89	18.54	<.0001
	Vegetation	1	5.976	5.976	8.595	0.007
	Site* veg	4	14.47	3.618	5.203	0.0029
	Error	28	19.47	0.6953		

df degrees of freedom, SQ sums of squares, MS mean squares, F F-ratio

regional heterogeneities in overall bacterial abundances at the km-scale (Table 2). Because site “Gelting” possessed only one remaining *Z. marina* patch of limited size we also analyzed *Vibrio* spp. abundance without that site. This changed the statistical test results only marginally (2-factorial ANOVA, sqrt transformed *Vibrio* spp. counts, $F_{1,25} = 28.4$, $P_{\text{vegetation}} < 0.001$).

The reduction in bacterial abundance was consistent across most of the sites. Specifically, despite significant overall site-differences in abundance, there was no interaction detectable among both main factors for the *V. vulnificus/cholerae* subtype (Fig. 1C). For the 2 other *Vibrio* subtypes (*V. alginolyticus* and *parahaemolyticus*) there was a significant “site*vegetation”-interaction. For *V. parahaemolyticus*, above statistical interaction reflected a reduction for this *Vibrio* subtype that varied strongly among sites, while the general direction of bacterial reduction inside the leaf canopy was similar across all 5 sites (Fig. 1B). This was not the case in *V. alginolyticus*, where a closer inspection revealed that this effect was due to the site “Kiekut” where the direction of effect reversed and we observed a higher abundance of bacteria within the seagrass canopy (Fig. 1D). We tested and confirmed this by excluding that site from the data set which let the statistical interaction disappear (analysis not shown).

In an ANCOVA model, we found a negative effect of water depth only on the abundance of marine broth bacteria ($r = -0.77 \pm 0.26$ SE) on sqrt-transformed bacterial colony

counts ($P = 0.0081$). In contrast, all statistical models on overall and *Vibrio* subtype abundances did not reduce the unexplained error variance by including the predictor variable “water depth” into the ANCOVA (all $P > 0.13$, analysis not shown).

To the best of our knowledge, these are the first data on temperate seagrass beds that show a reduction in the abundance of potentially harmful bacteria of the *Vibrio* spp. group, consistent with earlier findings for tropical seagrasses (Lamb et al. 2017). Overall, the effects on all *Vibrio* spp. were consistent with the data on bacterial counts obtained on a general marine broth medium. An exception was the potentially harmful group of *V. vulnificus/cholerae*, where the mean reduction in cfu was with 63% the strongest in samples inside the leaf canopy compared to the abundance found in the water column above non-vegetated sandy areas outside. A similarly marked reduction was observed for *V. parahaemolyticus*, yet since the overall abundance of this *Vibrio* subtype was only ~ 2 cfu $250 \mu\text{l}^{-1}$, these data have to be interpreted with caution. While the assignment to *Vibrio* subtypes based on colony color was unambiguous, further studies characterize colonies via 16S rDNA sequencing to further examine their exact taxonomic composition.

Marked site effects as well as interactions of “site” with *Zostera* presence on bacterial abundance suggest heterogeneity of processes at a km-scale, hence our data should be taken as pilot data that would need confirmation in a more exhaustive spatio-temporal sampling design. Note that in the

south-western Baltic, salinity values (Table 1) vary strongly with actual wind conditions. Hence, to examine salinity effects on *Vibrio* spp. abundance, more frequent temporal sampling would be needed throughout the year. A denser temporal sampling would also be needed to better cover the temperature dependence. Nevertheless, owing to the growing importance of *Vibrio* spp. for tourism and human health during summer months in rapidly warming northern European seas (Bier et al. 2015; Gyraite et al. 2019) we consider this data set worthwhile for publication to motivate further studies.

Similar to the analogous role of tropical seagrass beds (Lamb et al. 2017), the underlying mechanism of seagrass on surrounding water column bacteria is currently elusive. Possible non-exclusive mechanisms include (i) a simple increase of sedimentation rate through hydrodynamic attenuation of the canopy that would reduce particle load (Fonseca et al. 1982; Nowell and Jumars 1984; Worcester 1995) and hence, bacteria associated with organic particles, (ii) filter feeding of fauna associated with *Z. marina* beds that removes plankton known as reservoirs for *Vibrio* or directly consumes bacteria (Bradley and Kenneth 2001; González-Ortiz et al. 2014), or (iii) allelopathic chemicals exuding from *Z. marina* leaves (Papazian et al. 2019). It is well established that the first two effects contribute to enhancement of water clarity in seagrass beds (Adams et al. 2016). The approximately similar magnitude of reduction of bacterial colonies growing on marine broth versus CHROMagar (indicative of *Vibrio* spp.) supports the hypothesis that the reduction is mainly non-selective with respect to the bacterial groups. On the other hand, for the subtype *V. vulnificus*, the reduction was even 63%. In conclusion, further study is required to verify mechanisms and possible selectivity towards certain bacterial groups. Regardless of the specific mechanism, it is clear that the seagrass holobiont alters the microbiological composition in its vicinity (Trevathan-Tackett et al. 2019), and our data are in line with these predictions.

Nature based solutions (Gattuso et al. 2018; Seddon et al. 2020), such as the manipulation of microbiomes (Trevathan-Tackett et al. 2019) or the large-scale restoration of macroalgae and seagrasses (Gattuso et al. 2018) have recently been proposed to combat various negative effects of global change and human perturbation on coastal ecosystems. Achievable goals include an improvement of water quality and the enhancement of carbon sequestration. Our study provides another example as to how the protection and possible restoration of seagrass beds (Tan et al. 2020) may help sustaining as yet understudied ecosystem services such as the suppression of bacterial load. Along with already known effects on biodiversity enhancement and improvement of water quality seagrasses may contribute to preserve the potential of coastal regions to provide tourism services without risking

the health of humans visiting the ocean (Bier et al. 2015) or consuming local seafood (Rippey 1994).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00227-021-03963-3>.

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Author's contributions TR, PS and RK conceived the study, PS along with TR organized and conducted the sampling, DG and SMM conducted the laboratory analysis with advice from KB and UH, TR wrote the manuscript, all authors discussed the results, and commented and edited the manuscript.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of organisms were followed.

Data availability Original data are available in Supplementary Table S3.

Code availability NA.

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