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ORIGINAL ARTICLE

Mats of *Beggiatoa* bacteria reveal that organic pollution from lumber mills inhibits growth of *Zostera marina*

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Abstract

The objectives of this study were to determine the distribution and abundance of *Zostera marina* (eelgrass) in relation to the distribution of the mat forming bacteria *Beggiatoa* spp., and the levels of sulfide and organic material (wood waste) in the sediment. Underwater videography and intertidal surveys were used to map the distribution and abundance of *Z. marina* beds and *Beggiatoa* in the nearshore area of Commencement Bay, WA (USA), a location that has a long history of sawmill activity. *Zostera marina* occurred from the intertidal to −6 m mean lower low water (MLLW) on sandy substrates in areas with low levels of sulfide (<50 μM) and organic material (<5 % total volatile solids). Areas with high sulfide levels (>200 μM) occurred where there were significant amounts of organic material in the sediments, which was found to be wood waste that had been discarded from sawmills. *Zostera marina* was absent from the intertidal and occurred at lower densities in areas with high sulfide levels. In contrast, mats of *Beggiatoa* were only found in areas where the sulfide levels were >1000 μM and there were significant deposits of wood. Thus, the negative correlation between the distribution and abundance of *Z. marina* and *Beggiatoa* suggests that the presence of *Beggiatoa* mats could be used as a biological indicator of inhibiting levels of hydrogen sulfide in the marine environment.

Problem

Seagrass loss results from a variety of anthropogenic disturbances, such as dredging and filling, coastal development, pollution, nutrient and sediment loading from runoff, oil spills, and certain fishing practices (Short & Wyllie-Echeverria 1996). Consequently, because seagrass resources are critical contributors to nearshore productivity and biodiversity (reviewed in Kenworthy *et al.* 2005), several coastal nations have ratified legislation that reduces or prohibits human activity that negatively impacts seagrass distribution and abundance (Coles & Fortes 2001). While the protection of natural resources from the detrimental effect of present or future human alteration of natural processes has been somewhat controlled, the ongoing impact of what took place in the past is not commonly the focus of natural resource protection policies (*e.g.*, Barrett *et al.* 2001). Whereas the impacts of coastal deforestation and wood processing plants are known to impact the seagrass biome (Short & Wyllie-Echeverria 1996; Hemminga & Duarte 2000), the effect of the historic dumping of wood waste in the marine environment has not been investigated. Historically, lumber mills have dumped large amounts of wood waste into the environment: 'In some cases, wood waste has been used to fill along shorelines or otherwise deposited into intertidal and subtidal areas' (Kendall & Michelsen 1997). Over time the wood waste typically becomes buried within the sediment where it acts as organic enrichment. Increases in total organic carbon enhances the production of the toxic metabolite H_2S during bacterial reduction of sulfate (Hyland *et al.* 2005). Sulfides are phytotoxins and high concentrations are known to negatively affect seagrass photosynthesis, growth, and survival (Carlson *et al.* 1994; Goodman *et al.* 1995; Terrados *et al.* 1999; Holmer & Bondgaard 2001; Koch & Erskine 2001; Holmer *et al.* 2005). Sulfide toxicity has been linked to seagrass die-offs, such as those observed in Florida Bay (*e.g.*, Lee & Dunton 2000; Borum *et al.* 2005). Thus, the increased organic loading from dumping wood waste into the marine environment may create sediment conditions that are harmful to seagrasses.

In this study we evaluated the impact of an increase in sediment sulfide, in most part due to the bacterial breakdown of wood waste in the sediment, on the seagrass *Zostera marina* L. *Zostera marina* is the most commonly occurring seagrass in northeast Pacific waters (Wyllie-Echeverria & Ackerman 2003) and as such grows in small embayments and along fringing coastline throughout Puget Sound (Berry *et al.* 2003). To identify areas of high sediment sulfide levels that are detrimental to *Z. marina*, we explored the use of a mat forming, sulfide-oxidizing bacterium *Beggiatoa* spp. Trevisan 1842 as an indicator species. Visible mats of *Beggiatoa* in shallow water have previously been regarded as an indicator of organic enrichment (Fenchel & Bernard 1995) from either natural or anthropogenic sources. They have been specifically cited as indicators of organic pollution from pulp mills (Pearson 1975), and high organic enrichment to sediments below salmon farms (Crawford *et al.* 2001). To determine the reliability of this potential indicator species for toxic levels of hydrogen sulfide produced from the decomposition of wood waste, we examined the distribution and abundance of *Z. marina* in relation to the distribution of *Beggiatoa*.

Methods

Study area

The Ruston Way shoreline is located on the southern shore of Commencement Bay, WA (USA) (47.2838 °N, 122.4830 °W). Over 30 lumber mills operated on the shoreline of Ruston Way from 1869 to 1977 (Nerheim 2004), and their locations are indicated on the map in Fig. 1.

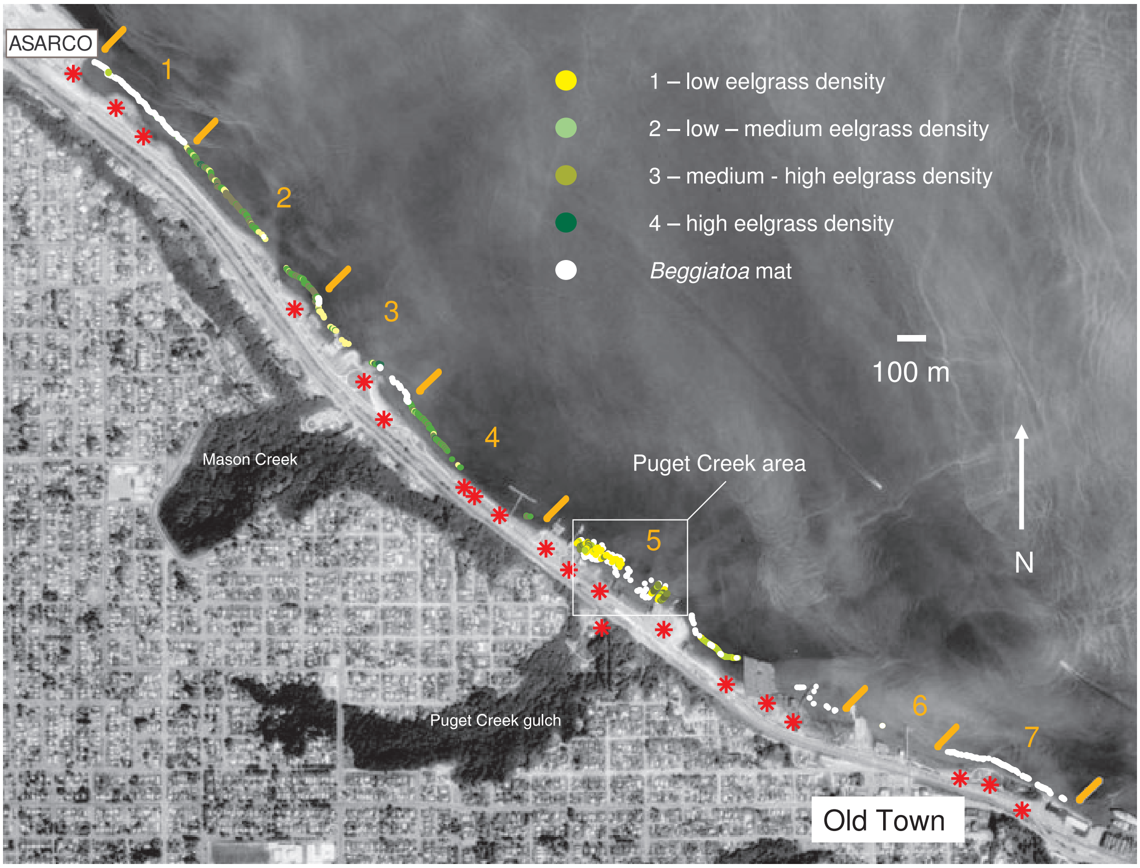

Fig. 1. Map of the Ruston Way shoreline in Commencement Bay, WA, showing distribution and abundance of *Zostera marina* (eelgrass) and mats of *Beggiatoa* observed during subtidal videography surveys. Red asterisks indicate locations of former sawmills. The seven sites evaluated are differentiated by orange lines at right angles to the shore with the label for each individual site also in orange appearing off shore.

A broad-scale survey of the distribution and abundance of *Z. marina* and *Beggiatoa* was conducted along the full length of the Ruston Way waterfront. Detailed analyses were conducted on the nearshore zone at the mouth of Puget Creek, an area where habitat restoration efforts are being conducted in both the nearshore and upland areas.

Subtidal video surveys

Underwater video survey methods followed the procedure described in Norris *et al.* (1997) and Berry *et al.* (2003). An underwater video system was deployed from a 6-m boat and the camera was towed at a slow speed by using a bow-mounted electric trolling motor. The geographic location of the video images was determined by using a Trimble GeoXT GPS system, and the GPS coordinates were overlain on video images. For all of Ruston Way (including Puget Creek), the video images were analyzed at 1-s intervals to determine the presence (and relative density) or absence of *Zostera marina*, and the presence or absence of *Beggiatoa*. *Zostera marina* was considered to be 'present' if a single shoot was visible within the video frame and the density was estimated by scoring the frame with values ranging from low (1 = a few shoots visible in frame) to high (4 = 100 % cover of *Z. marina*). Similarly, *Beggiatoa* was considered 'present' if any was visible on the sediment within the video frame. No attempt was made to determine *Beggiatoa* density.

In the nearshore zone at the mouth of Puget Creek, video transects were also conducted with spatial data collected for the areas with and without *Z. marina* using the video images and a line feature on the GPS unit (start of line = beginning of *Z. marina* presence, end of line = end of *Z. marina* presence). The depth of each location was determined using a depth sounder (Humminbird, Model 535).

Intertidal survey

Depth contours for each site were determined by walking the tide flat with a Trimble GeoXT GPS system during summer maximum low tides. At the same time, the intertidal distribution of *Z. marina* from the upper limit to approximately −1.0 m MLLW was recorded.

Input of data into GIS

For both the subtidal and the intertidal surveys, the video and GPS data were incorporated into a Geographic Information System (ArcView 9.1). Maps of *Z. marina* distribution and abundance, *Beggiatoa* distribution, and the bathymetry of the nearshore zone were generated for the Ruston Way waterfront and in greater detail for the nearshore zone at the mouth of Puget Creek. For the depth data, the depth initially recorded was corrected to meters below the MLLW based on the tidal height for the site at the time of surveying.

Sediment analyses

A 5-cm diameter coring apparatus (Wildco Ogeechee Corer) was used to sample sandy sediments at sites 1, 2, 4, and 5 (Fig. 1). For all four sites, cores were taken from areas representing *Z. marina* patches and bare sediment within the seagrass zone and for sites 1 and 5 cores were also taken from areas with *Beggiatoa* mats. The cores were taken to the maximum depths at which the corer could penetrate the sediment (35–90 cm). For each core, the sulfide values, wood debris content, and wood waste depth were determined. Each day the cores were taken back to the lab and analyzed for total sulfides within 2–3 h. Sulfide levels were measured at 5, 10, 15, and 20 cm below the sediment surface by extracting 0.1–2 ml of pore water with a syringe fitted with a 16 gauge needle. The samples were then mixed with nitrogen bubbled distilled water to a volume of 15 ml in a sealed Falcon brand centrifuge tube. Following this, the sample was centrifuged at 1200 *g* in an IEC Clinical centrifuge for 5 min to remove sand debris. Finally, the supernatant was mixed with an additional 10 ml of nitrogen bubbled distilled water and analyzed for total sulfide using the Hach Sulfide Methylene Blue Method (DR/4000 Procedure). To examine wood waste within the cores, the depth of the wood waste was measured and sediment subsamples were taken from the surface, middle (15 cm), and deep (~35–45 cm) sections of the cores. Total volatile solids (%) were then determined for each subsample (Tetra Tech 1986). For cores taken within *Z. marina* patches, leaf, root and rhizome material were removed, and for all cores surface debris and algae were removed.

Analysis of bacterial mats

In order to determine the composition of the bacterial mats observed, samples were collected from the sediment surface in small vials and brought back to the laboratory. The samples were then examined using a compound microscope at 400× magnification and filamentous bacteria were identified (Holt *et al.* 1994).

Statistical analyses

The statistical program SPSS was used for all comparisons. ANOVA was used to compare sulfide and total volatile solid (TVS) levels at different depths in cores and between sample types (*Z. marina*, sand, and *Beggiatoa*).

Data were transformed to meet assumptions of normality and equal variances when appropriate. *Post-hoc* paired comparisons were conducted using PLSD tests. Mann–Whitney *U*-tests were used to compare sulfide and TVS levels among locations because of unequal variances among samples. *T*-tests were used to compare *Z. marina* patch density between individual locations.

Results

Subtidal video surveys

On the broad scale of Ruston Way, *Beggiatoa* mats typically occurred in the vicinity of former lumber mills at sites 1, 3, 5, and 7 (Fig. 1). In contrast, *Zostera marina* occurred at highest densities in areas away from former lumber mills where no *Beggiatoa* was observed (sites 2, 4).

In the subtidal region of the Puget Creek site (5), *Z. marina* beds existed on the northwest and southeast sides of the outlet from the creek (Fig. 2). Average density of these patches was significantly lower in the northwest area (mean density = 1.4 density units) in comparison to the southeast area (mean density = 2.7 density units, $P < 0.0001$, *t*-test). *Zostera marina* was absent or occurred in low density patches near the mouth of Puget Creek (Fig. 2). The lower limit of *Z. marina* growth ranged from a minimum of −1 m MLLW to a maximum of −6 m MLLW (range = −5 m MLLW, mean depth = −2 m MLLW). Low-density *Z. marina* patches were most commonly observed between −1 m and −2 m MLLW, while medium-density eelgrass was most abundant from −2 m to −4 m MLLW. High-density *Z. marina* patches were most abundant from −2 m to −3 m MLLW. High-density *Z. marina* patches were not observed in the shallower depths (less than −2 m MLLW) during this survey.

Beggiatoa was most abundant, often occurring in extensive mats, at the northwest end of site 5 (Fig. 2). *Beggiatoa* was observed from the intertidal to −7 m MLLW, but it was most abundant at approximately −2 m MLLW. *Beggiatoa* was primarily observed in areas where *Z. marina* was either absent or was present in low densities. *Beggiatoa* co-occurred with *Z. marina* in 19.6% of the video frames analyzed. Most of these co-occurrences were with low-density *Z. marina* (15.9 %), with a small

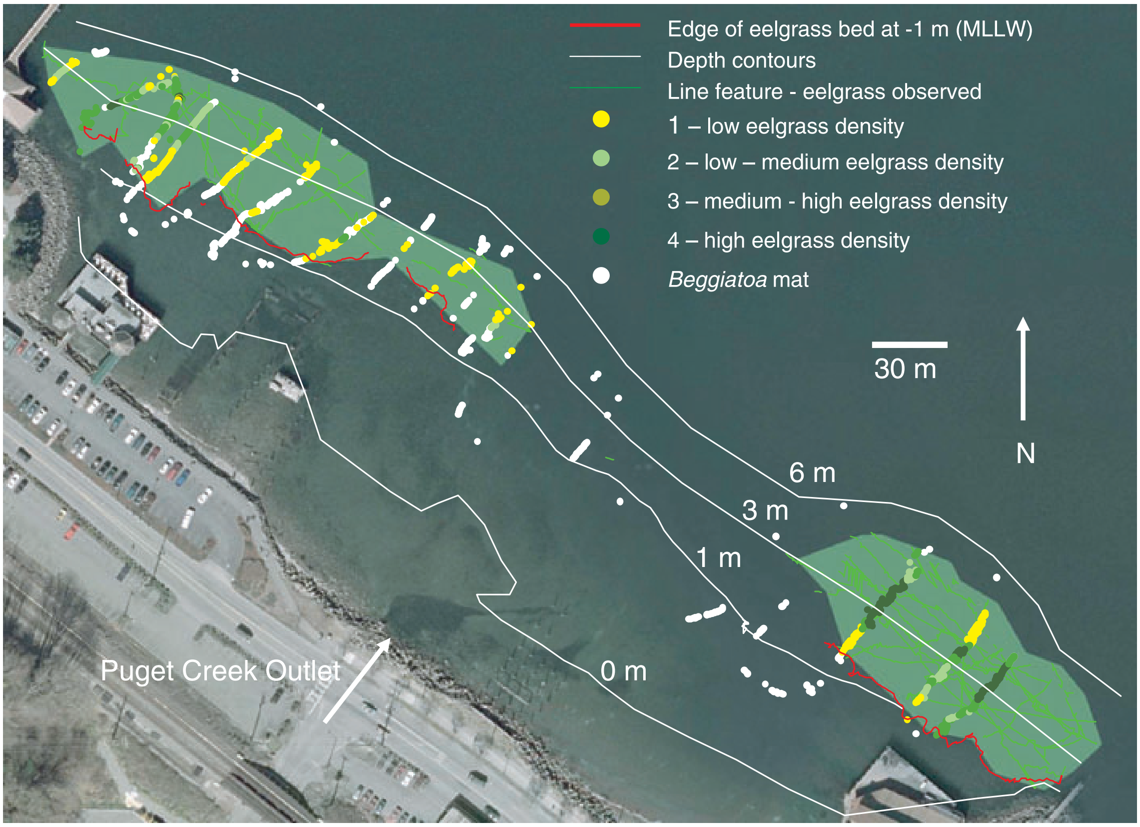

Fig. 2. Map of the Puget Creek area (site 5) showing detailed data for distribution and abundance of *Zostera marina* (eelgrass) and mats of *Beggiatoa* observed during subtidal videography surveys and intertidal surveys. The shaded green areas indicate the estimated boundaries of the eelgrass beds.

percentage of observations occurring in classes 2 and 3 of *Z. marina* density. *Beggiatoa* was not observed in the high-density patches of *Z. marina* that were most common on the southeast section of the Puget Creek area.

Intertidal surveys

At sites 2 and 4 (Fig. 1), *Z. marina* beds extended up into the intertidal and significant portions of the beds were completely exposed during the lowest tides of the year (−1.2 m MLLW). In contrast, at sites 1, 3, and 5, *Z. marina* was rarely exposed during maximum low tide events (Fig. 2). At site 1, the beds were entirely subtidal (−2 m MLLW). At site 5, the northwest beds were exclusively subtidal except for a small portion of the southeast section which was exposed during the extreme low tides (Fig. 2).

Beggiatoa was observed during early morning low tide surveys in shallow water (0 to −2 m MLLW). However, *Beggiatoa* was absent from the same areas during midday surveys when the tide flat was exposed. In addition, wave action appeared to cause the mats to disperse and become less visible on the sediment surface.

Sediment analyses

Cores taken from site 1 and the northwest section of site 5 typically had sawdust and wood chips in the bottom sections of the core, whereas wood debris was not observed in cores taken from sites 2 and 4. Based on these observations, sites 1 and 5 were considered 'impacted' and sites 2 and 4 were 'unimpacted' by wood waste. Cores with wood waste had higher TVS levels. The TVS levels were significantly higher (5–10 times) at impacted sites (mean = 7% for all depths, 16.2 % at 35–45 cm) than unimpacted sites (mean = 1.3 %, P < 0.0001, Mann–Whitney *U*-test). At the impacted sites TVS increased significantly with depth (Tables 1 and 2), but not at the unimpacted site (Tables 1 and 3). In addition, TVS levels were almost twice as high at the deeper sediment depths in areas of *Beggiatoa* mats (22.3 %) than in *Z. marina* beds (9.5%) (Table 1). The TVS levels in the bare sediment areas were lower than in *Beggiatoa* areas but this trend was not statistically significant (Table 2).

Table 2. ANOVA for comparisons of total volatile solids (% of total dry weight of sediment) levels among depths and sediment types in impacted areas (*Beggiatoa*, *Zostera marina*, sand). Mean values for each group appear in Table 1. Analysis conducted with arcsin (square root) transformed % data. The assumption of equal variances could not be met by transforming data.

source	df	F	P
sediment	2, 99	3.291	0.0413
depth	2, 99	34.239	<0.0001
sediment × depth	4, 99	2.108	0.0854

Table 3. ANOVA for comparisons of total volatile solids (% of total dry weight of sediment) levels among depths and sediment types in unimpacted areas (*Zostera marina*, sand). Mean values for each group appear in Table 1. Analysis conducted with arcsin (square root) transformed % data.

source	df	F	P
sediment	1, 24	0.418	0.5240
depth	2, 24	1.015	0.3773
depth × sediment	2, 24	0.461	0.6364

Cores taken from impacted sites typically contained dark black sediment and smelled strongly of hydrogen sulfide. These cores also had significantly higher pore water sulfide levels than cores from unimpacted sites (Table 4, P < 0.0001, Mann–Whitney *U*-test). In contrast, cores taken from unimpacted areas were composed of light gray sand and did not have a hydrogen sulfide smell. The pore water sulfide levels in sediments from unimpacted sites (mean = 10 μM) were more than 150 times lower than in the sediments from impacted sites (mean = 1538 μM).

At the impacted sites (1, 5), cores taken from within mats of *Beggiatoa* had significantly higher sulfide levels

Table 1. Mean values for total volatile solids (% of total dry weight of sediment) among depths in different sediment types (*Beggiatoa*, *Zostera marina*, sand). Twelve replicate measurements were made at each depth interval in impacted areas (n = 36 for each sediment type) and five replicate measurements were made at each depth interval in unimpacted areas (n = 15 for each sediment type).

	impacted sites						unimpacted sites			
	Z. marina		sand		*Beggiatoa*		*Z. marina*		*sand*	
depth [cm]	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
surface	1.7	0.2	1.7	0.1	1.8	0.4	1.10	0.2	1.17	0.02
15	2.2	0.4	2.4	0.4	4.9	1.4	1.36	0.2	1.99	0.69
35–45	9.5	3.5	16.8	4.5	22.3	3.9	1.47	0.3	1.20	0.06

Table 4. Mean values for sulfide levels (μM) among different depths in different sediment types (*Beggiatoa*, *Zostera marina*, sand). Seven replicate sulfide measurements were made at each depth interval in impacted areas (n = 28 for each sediment type) and five replicate sulfide measurements were made at each depth interval in unimpacted areas (n = 20 for each sediment type).

	impacted sites						unimpacted sites			
	Z. marina		sand		*Beggiatoa*		*Z. marina*		*sand*	
depth [cm]	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
5	279	86	321	126	3229	1652	16	7	12	4
10	406	117	346	134	3511	1336	26	10	7	2
15	448	245	289	97	3944	1251	3	2	5	2
20	604	475	790	327	3761	969	6	4	5	2

than sand and *Z. marina* sites (Tables 4 and 5, $P < 0.0001$). The sulfide levels of cores taken from within *Beggiatoa* mats (mean = 3600 μM) were approximately 10 times greater than those taken from *Z. marina* patches (mean = 400 μM) and six times greater than those taken from sand (mean = 600 μM). Sulfide levels reached a maximum of 12 348 μM in the top 20 cm of sediment in areas of *Beggiatoa*. Sulfide levels appeared to increase with depth in areas populated with *Z. marina* and sand was dominant, but this was not statistically significant (Table 5).

Within unimpacted sites, the sulfide levels were twice as high in the top 10 cm of sediment in *Z. marina* patches (mean = 21 μM) than in sand (mean = 9.5 μM), but this trend was not statistically significant (Table 6). In addition, sulfide levels were three to four times higher near the surface (5–10 cm) than at the depth (15–20 cm) in both *Z. marina* patches and sand, but this trend was not statistically significant (Tables 4 and 6).

Table 5. ANOVA for comparisons of sulfide levels among depths and sediment types in impacted areas (*Beggiatoa*, *Zostera marina*, sand). Mean values for each group appear in Table 1. Analysis conducted with log transformed data.

source	df	F	P
sediment	2, 72	25.935	<0.0001
depth	3, 72	1.225	0.3067
sediment × depth	6, 72	0.190	0.9787

Table 6. ANOVA for comparisons of sulfide levels among depths and sediment types in unimpacted areas (*Zostera marina*, sand). Mean values for each group appear in Table 1. Analysis conducted with log transformed data.

source	df	F	P
sediment	1, 32	1.171	0.2872
depth	3, 32	2.399	0.0861
sediment × depth	3, 32	0.6942	0.6942

Discussion

The results of this study demonstrate that visible mats of *Beggiatoa* in shallow water indicate high levels of sulfide in marine sediments. For Commencement Bay, the source of this sulfide is the decomposition of buried wood waste by anaerobic sulfate-reducing bacteria. This is similar to Watson Bayou, Florida, where significant amounts of pulpmill-derived organic matter (pine wood) was released into the sediments and concentrations of dissolved sulfide reached maximum concentrations of 2000 μM at 6 cm depth because of enhanced rates of bacterial sulfate reduction (Brüchert & Pratt 1999). In Commencement Bay, sulfide levels were much higher, reaching a maximum recorded concentration of 12 348 μM in the top 20 cm of sediment. *Beggiatoa* is a sulfide-oxidizing bacterium, and the presence of these high sediment sulfide levels (>1000 μM) likely enhanced its mat formation at the sediment–water interface in Commencement Bay. According to Mussmann *et al.* (2003), 'filamentous sulfide oxidizing *Beggiatoa* spp. often occur in large numbers in the coastal seabed without forming visible mats on the sediment surface.' Thus, in sediments with lower sulfide levels or other characteristics, *Beggiatoa* was likely present but did not form visible mats at the surface.

Beggiatoa mats in shallow water have been described as indicators of anthropogenic organic enrichment (*e.g.*, Pearson 1975; Crawford *et al.* 2001), and our study has shown that their presence indicates areas of increased organic enrichment from wood waste. The wood was discarded as a waste product by former logging mills that operated along the shoreline of Commencement Bay from 1869 to 1977 (Nerheim 2004). Studies conducted by the Washington Department of Ecology (Science Applications International Corporation 1999; Norton *et al.* 2000) to examine wood waste in Port Angeles Harbor and Shelton Harbor, WA, have also reported bacterial mats in areas with wood waste in the sediment. Given that sawmills and wood processing plants have operated in estuaries at a variety of geographic locations, it appears that mats of

Beggiatoa may be generally useful as biological indicators of areas where there are significant levels of wood debris buried in the sediments.

If *Beggiatoa* is to be used as an indicator of high organic enrichment levels in shallow water, it should be noted that it is not always present on the sediment surface. It may only be visible on the sediment surface during periods when there are low oxygen levels in the overlying water column (Jørgensen 1977). *Beggiatoa* is also known to perform diurnal migrations, being more visible on the surface at night or during periods with low light levels (Fenchel & Bernard 1995). This suggests that surveys for its presence would most effectively be conducted during the winter months or at night or early morning during the summer. Another factor that appeared to influence the presence of *Beggiatoa* on the sediment surface was wave action, which caused the mat to become less visible at the surface. Once the boundary layer above the sediment is disturbed by waves, oxygen is transported down into the sediment–water interface. This allows *Beggiatoa* to move deeper into the sediments where it can obtain higher levels of sulfide and maintain an adequate supply of oxygen. Wave action may also disturb the *Beggiatoa* mat, dispersing filaments of the bacterium into the water column (Grant & Bathman 1987).

As mats of *Beggiatoa* occur in areas of high sulfide, the presence of these mats may also be useful in identifying sediment conditions that are harmful to seagrasses. Studies show that seagrass growth, survival, photosynthesis, aerobic metabolism, and nutrient uptake are negatively affected by poor oxygen conditions and the presence of sulfides (Holmer & Bondgaard 2001; Borum *et al.* 2005; Holmer *et al.* 2005). Goodman *et al.* (1995) suggested that even modest increases in pore water sulfide can affect the photosynthetic capacity of *Zostera marina*. In a controlled experiment examining the effects of low oxygen and high sulfide conditions on seagrass growth, Holmer & Bondgaard (2001) found that sulfide levels of 100–1000 μM negatively influenced growth. Under these conditions the seagrass exhibited damage to the meristematic regions, 'soft spots in the tissue,' several shoots were released from the rhizomes, and 'shoot density was reduced by 31% at harvest in one of the transplants, whereas no plants survived the second transplant.' The aboveground biomass was also negatively affected by exposure to low oxygen and high sulfides. The extremely high sulfide levels observed in our study suggest that the growth, survival, and reproduction of *Z. marina* could be strongly affected by the sediment conditions in the impacted areas.

In seagrass beds, the phytotoxic sulfide may be 're-oxidized to harmless sulfate' by the oxygen microzone around the roots and rhizomes (Schaub & Gemerden 1996). However, if the below ground transport of oxygen, a by-product of photosynthesis, is limited by unsuitable submarine conditions (especially temperature, salinity and light; *e.g.*, Smith *et al.* 1988; Zimmerman *et al.* 1991), the oxic microshield cannot be maintained and sulfide may invade the plant tissues (Pedersen *et al.* 2004). Poor oxygen supply typically occurs at night, but it may also result from increased turbidity, hypersalinity, increased water temperatures, or pathogen infection (Pedersen *et al.* 2004). Our observation that *Z. marina* beds in impacted areas were primarily subtidal suggests that intertidal conditions may also be stressful for *Z. marina* in areas of high sulfide. When seagrasses are exposed during low tide, photosynthesis decreases, so the oxic layer surrounding the rhizomes could decrease and there could be an increased risk of sulfide toxicity.

Although the presence of *Beggiatoa* indicates harmful conditions for *Z. marina*, the bacterium is not directly harmful, and its presence may actually be beneficial. By removing toxic sulfide, *Beggiatoa* and other sulfide oxidizing bacteria may allow 'sulphide sensitive organisms [to] extend their habitat range' (Mussmann *et al.* 2003). Filamentous sulfide oxidizing bacteria were observed within *Z. marina* rhizomes collected in areas where wood waste was present, suggesting that sulfide oxidizing bacteria may be facilitating *Z. marina* growth in sediments with high levels of wood waste.

In conclusion, our results show that the bacterium *Beggiatoa* can be used as a biological indicator of high sediment sulfide conditions (resulting from the buried wood waste of historic lumber mills) which may significantly impact the distribution and abundance of *Z. marina*. We suggest that future research in this area examine whether *Z. marina* in the areas of high sulfide levels have specific adaptations for these conditions and whether the *Beggiatoa* found in the rhizomes provides benefits to the plant under stressful sulfide levels.

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us submit this paper to this special issue honoring her work.

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