Quantitative PCR Reveals Strong Spatial and Temporal Variation of the Wasting Disease Pathogen, *Labyrinthula zosterae* in Northern European Eelgrass (*Zostera marina*) Beds

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**Abstract**

Seagrass beds are the foundation species of functionally important coastal ecosystems worldwide. The world’s largest losses of the widespread seagrass *Zostera marina* (eelgrass) have been reported as a consequence of wasting disease, an infection with the endophytic protist *Labyrinthula zosterae*. During one of the most extended epidemics in the marine realm, ~90% of East and Western Atlantic eelgrass beds died-off between 1932 and 1934. Today, small outbreaks continue to be reported, but the current extent of *L. zosterae* in European meadows is completely unknown. In this study we quantify the abundance and prevalence of the wasting disease pathogen among 19 *Z. marina* populations in northern European coastal waters, using quantitative PCR (qPCR) with primers targeting a species specific portion of the internally transcribed spacer (ITS1) of *L. zosterae*. Spatially, we found marked variation among sites with abundances varying between 0 and 126 cells mg⁻¹ *Z. marina* dry weight (mean: 5.7 *Z. zosterae* cells mg⁻¹ *Z. marina* dry weight ± 1.9 SE) and prevalences ranged from 0–88.9%. Temporally, abundances varied between 0 and 271 cells mg⁻¹ *Z. marina* dry weight (mean: 8.5±2.6 SE), while prevalences ranged from zero in winter and early spring to 96% in summer. Field concentrations accessed via bulk DNA extraction and subsequent qPCR correlated well with prevalence data estimated via isolation and cultivation from live plant tissue. *L. zosterae* was not only detectable in black lesions, a sign of *Labyrinthula*-induced necrosis, but also occurred in green, apparently healthy tissue. We conclude that *L. zosterae* infection is common (84% infected populations) in (northern) European eelgrass populations with highest abundances during the summer months. In the light of global climate change and increasing rate of marine diseases our data provide a baseline for further studies on the causes of pathogenic outbreaks of *L. zosterae*.

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**Introduction**

Seagrass beds are among the most threatened coastal ecosystems worldwide [1] while at the same time, they provide very important ecological functions as nursery habitat, sediment stabilizer, and via carbon and nutrient fixation [2]. We are now witnessing a century of accelerated seagrass decline driven by growing human populations, coastal development, ecological degradation and climate change [1], [3], [4]. However, the world’s largest and fastest losses of *Zostera marina* occurred in the 1930’s and were attributed to eelgrass wasting disease, caused by the net-slime mold *Labyrinthula zosterae* (Straminopiles, an endophytic protist reviewed by [5]). Among the many other known factors causing eelgrass decline, the role of pathogens has so far largely been neglected, although diseases are already noticeably on the increase not only in marine ecosystems [6], [7]. The main objective of this study was to obtain first quantitative data on the prevalence and abundance of the wasting disease pathogen *Labyrinthula zosterae* in contemporary *Z. marina* populations of Northern Europe.

Although detailed data are scarce, it is generally accepted that *Z. marina* beds were very common before the disease struck throughout the North Atlantic (see e.g. [8] for the Wadden Sea, [9] for the Netherlands, [10] for Danmark, [11] for the German Baltic and [12] 2008 for France). Historical records of a large eelgrass industry producing insulation and mattresses suggest high abundances of extended eelgrass beds in France, The Netherlands and Canada [13], [14]. This changed dramatically when in the 1930’s, a pandemic caused by the net-slime mold *L. zosterae* struck eelgrass beds on both sides of the North Atlantic. Beginning in 1930, eelgrass beds disappeared from large areas ranging from New Brunswick to north-west Carolina at the Atlantic West Coast within only two years [15], [16]. In 1931, similar die-offs were reported from Brittany and the Norman-Breton Gulf in France [17], and in the subsequent year from sublittoral eelgrass beds in the Dutch Wadden Sea [18]. In 1933, the epidemic reached...
Materials and Methods

Sampling

In total, we sampled 19 coastal sites in a water depth of 0.5–3 m (Fig. 1). Eighteen of the 19 sites were situated within the affected region of the 1930’s wasting disease epidemic, while they presently show no signs of decline due to wasting disease (Fig. 1). We were particularly interested to analyze the few remnant permanently submerged Zostera marina populations in Wadden Sea tidal creeks, because they are the only subtidal sites that recovered after the wasting disease. These subtidal populations consist of vegetation patches of 0.5–5 m width, distributed along creek banks (33% cover, ± 5.5 SE). The intertidal populations sampled in the Wadden Sea are continuous but show sparse eelgrass coverage (mean of all sample sites: 13.4% ± 0.3 SE) with low shoot densities (71 shoots m⁻² ± 1.8 SE). Although intertidal plants are phenotypically distinct from subtidal Z. marina (e.g. shoot lengths in intertidal_1930s: 24.7 cm ± 0.9 SE, shoot length Sylt_subtidal_september_2012: 65.3 cm ± 2.9 SE). Microsatellite analysis confirmed low but significant genetic differentiation (Fst = 0.009, P = 0.067) between Wadden Sea populations, resulting from divergent selection detected on genes linked with three of 25 microsatellite loci tested [36]. All other populations in this study were continuous eelgrass beds in 0.5–3 m water depth, extending over several 100 m² (Table 1).

At each site, fresh leaves of at least twenty Z. marina-shoots were collected between May and August of the years 2010 (1 site), 2011 (8 sites) and 2012 (10 sites, Table 1), separately stored in Zip-lock bags with ambient sea water and kept cool until return to the lab 1–3 days later. Sampling at Ellenbogen Creek was permitted by nature conservation authority and Mr. Diedrichsen, the owner of this private property. We took care that by picking a leaf piece the entire plant was kept alive in situ and/or sampled outside areas not open to public. Therefore no special permission was necessary at all other sites.

Before starting the spatial survey, we wanted to address within-plant variation in Labyrinthula zosterae abundance. To this end DNA was extracted from all leaves of eight individual plants of two sites (Lemvig and Wackerballig), dividing each leaf in three sections (top, middle, basis). Initial QPCR-assay results revealed that the highest L. zosterae prevalences and/or abundances were found in the middle part of the 3rd oldest leaf (for means and statistical tests see Tables 2 and 3); therefore, we analyzed the 3rd leaf in all subsequent samples.

After sampling, leaves from all populations were air dried. Leaves from five of these populations (Table 1) were additionally examined for black lesions on the leaves. Then all leaves were cut in half, longitudinally. One half was dried for later DNA extraction, the other half served as inoculum for cultivation of Labyrinthula zosterae on seawater-agar medium.

To assess temporal variation in L. zosterae prevalence and abundance, the same population was sampled 1x in Falkensteins (7.4, 21.4, 3.5, 19.5, 9.6, 23.6, 7.7, 15.7, 5.8, 28.9, 1.11 and 28.12.2011, 23.2, 25.5 and 23.12.2012) and 6x at Ellenbogen creek (18.5, 9.6, 4.7, 4.8, 5.9 and 10.11.2011).

DNA Extraction

Ca. 2–4 mg of dried leaf material was first ground in a ball mill (Retsch, Germany) at maximal speed setting for 5 min. DNA extractions of L. zosterae were performed with an Invitrogen spin tissue mini kit (Invitrogen, Berlin, Germany) following the manufacturer’s instructions. To enhance extraction efficiency and to ensure that even low amounts of target DNA were carried through the filter absorption steps, 1 μL (containing ~500 ng) of UltraPure™ salmon sperm DNA solution (Invitrogen, life technologies, USA) was added to each extraction to saturate silica columns with DNA. Target DNA was purified using a one-step PCR inhibitor removal kit (Zymo Research, USA).

Quantitative PCR (QPCR)- assay

Following on the original assay protocol of Bergmann [33] we modified the method to enhance specificity and sensitivity by developing a novel, TaqMan based assay with the consensus sequence of Labyrinthula zosterae [35]; accession numbers
Using the software PrimerXpress (Applied Biosystems) the forward primer Laby ITS_Taq_f: TTGAACGTAA-CATTCACTTTGCT and the reverse primer Laby_IT-S_Taq_r: ACACATGAAGCGGTCTTCTT were identified, along with the probe Laby_ITS_Taq_pr: TGGAC-GAGTGTGTTTTG that carried the fluorescence label 6-Fam at the 5’ end and the dark quencher BHQ-1 at the 3’ end. Reactions were carried out using standard conditions recommended by the manufacturer using the 10 μL TaqMan universal Master Mix (Applied Biosystems, now Life Technologies) in a 20 μL reaction volume: 2 μL 1:10 diluted template DNA, 2.4 μL (40.8 nM) of the two primers, 2.4 μL Milli-Q H2O and 0.8 μL probe (50 nM), respectively. The thermo-cycling program on a Step-One QPCR machine was 2 min at 50°C and 10 min at 95°C, followed by 48 cycles at 95°C for 15 s and 1 min at 60°C. All samples were tested in triplicate and the standard deviation of triplicates never exceeded 0.3 units of cycle threshold (Ct). CT values ≤39 were considered. Standard curves using preparations of Labyrinthula zosterae with known cell numbers attained correlation coefficients between r^2 = 0.97 and 0.99 and a detection limit of ~0.01 cells. Abundance as the number of L. zosterae cells in each milligram (dry weight) Zostera marina sample was calculated from the linear regression of the standard curve (standard cell number against mean standard Ct calculated from all QPCR reactions; 150 cells 22.493 Ct ±0.060 SE, 15 cells = 27.080 Ct ±0.080 SE, 0.5cells = 32.215 Ct ±0.125 SE).

Cell number = (−a + b * (de log (Ct))/w * 10

where a = intercept, b = slope and w = sample dry weight. Cell numbers were multiplied by 10 because the samples were diluted 1:10 prior QPCR.

Prevalence was calculated as the percentage of samples of each site with a Ct<39.

Cultures

Seawater-agar medium. For one liter of seawater-agar medium (for 50 Petri-dishes 10 cm in diameter): 12 g agar (bacteriological grade, Roth, Germany), 1 g glucose, 0.1 g yeast extract (Roth, Germany), 0.1 g peptone (Fluka, Germany) in 1 L.
Table 1. Sampling locations, salinity and sample size for assessing spatial variance in abundance and prevalence.

| Area                | Location          | Geograph. coordinates | Sampling date | Salinity (psu) | N  | % leaves with lesions | % pos. cult. from leaves with lesions |
|---------------------|-------------------|-----------------------|---------------|----------------|----|----------------------|---------------------------------------|
| Langeness, Wadden Sea, Germany | Langeness North | N 54.6396 E 08.5781 | 22.07.12 | >30 | 20 | 0 | No data |
|                     | Langeness Creek* | N 54.6320 E 08.5440 | 28.06.11 | >30 | 20 | 13 | No data |
| Hooge, Wadden Sea, Germany | Hooge-NW         | N 54.5700 E 08.5180 | 23.07.12 | >30 | 20 | 0 | No data |
|                     | Hooge Creek      | N 54.5723 E 08.5240 | 23.07.12 | >30 | 20 | 0 | No data |
| Amrum, Wadden Sea Germany | Amrum-NW        | N 54.6960 E 08.3400 | 19.07.12 | >30 | 20 | 0 | No data |
| Pellworm, Wadden Sea, Germany | Pellworm       | N 54.5904 E 08.5990 | 26.07.12 | >30 | 20 | 0 | No data |
| Dagebüll, Wadden Sea, Germany | Dagebüll      | N 54.7212 E 08.7051 | 24.07.12 | >30 | 20 | 0 | No data |
| Sylt, Wadden Sea, Germany | Puan Klent      | N 54.0798 E 08.2960 | 18.07.12 | >30 | 20 | 2 | No data |
|                     | Tønnesfjæring Bay | N 55.0258 E 08.4323 | 17.07.12 | >30 | 20 | 0 | No data |
| Limfjord, Denmark | Lemvik*           | N 56.6300 E 08.2961 | 28.05.11 | >30 | 19 | 58 | 100.00 |
| Skagern, Norway | Sandspollen*       | N 59.6657 E 10.5869 | 10.05.10 | 20–25 | 21 | 57 | 100.00 |
| Åbyfjord, Sweden | Fiskebäckvik*     | N 58.3822 E 11.4078 | 01.06.11 | 20–25 | 18 | 0 | No data |
| Gullmarsfjord, Sweden | Bredvik-Snäckebacke-bukten | N 58.1987 E 11.3244 | 03.07.11 | 20–30 | 20 | 0 | No data |
|                     | Kungsälv         | N 57.5405 E 11.4083 | 04.07.11 | 6–14 | 20 | 0 | No data |
| Flensburg Fjord, Germany | Wackerballig*   | N 54.7557 E 09.8668 | 12.07.11 | 15–17 | 19 | 80 | 100.00 |
| Kiel Fjord, Germany | Falkenstein       | N 54.3954 E 10.1935 | 15.07.11 | 15–17 | 20 | 95 | 100.00 |
| Faro lagoon, Portugal | Ilha Culatra     | N 37.0005 W 07.4921 | 05.08.11 | 36 | 20 | 0 | No data |

The percentage of Zostera marina plants with lesions and successful Labyrinthula isolations are shown where available.

* = subset of 5 populations chosen for methods comparison.

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Table 2. Mean *Labyrinthula zosterae* abundance and prevalence in different leaf parts.

| Leaf part | N  | *L. zosterae* cells x mg plant DW$^{-1}$ | Std. Err | Prevalence (%) |
|-----------|----|----------------------------------------|----------|----------------|
| Top       | 27 | 0.30                                   | 0.14     | 18.92          |
| Middle    | 22 | 71.31                                  | 67.54    | 38.71          |
| Basis     | 19 | 9.37                                   | 3.08     | 31.03          |

Abundance per g *Zostera marina* dry weight (DW) with standard errors: Wilcoxon-Kruskal-Wallis $\chi^2$ for leaf part: $df = 2$, $X^2 = 6.05$, $p = 0.03$, planned comparison$abundance$: top = middle*, basis = middle**. Prevalence (%): Nominal logistic regression$leaf$ parts: $df = 2$, deviance $= 14.47$, $p = 0.001$, planned comparison$prevalence$: top = middle*, * = significantly different at $p > 0.05$, ** = $p < 0.02$.

Table 3. Mean *Labyrinthula zosterae* abundance and prevalence among different *Zostera marina* leaves.

| Leaf number | N  | *L. zosterae* cells x mg plant DW$^{-1}$ | Std. Err | Prevalence (%) |
|-------------|----|----------------------------------------|----------|----------------|
| 1           | 16 | 6.00                                   | 2.52     | 12.50          |
| 2           | 19 | 5.22                                   | 2.01     | 10.53          |
| 3           | 18 | 6.00                                   | 80.84    | 50.00          |
| 4           | 12 | 0.33                                   | 0.09     | 33.33          |
| 5           | 3  | 2.48                                   | 0.00     | One data point only |

Abundance per g *Zostera marina* weight (DW) with standard errors: Wilcoxon-Kruskal-Wallis $\chi^2$ for leaf number: $df = 4$, $X^2 = 5.37$, $p = 0.25$. Prevalence (%): Nominal logistic regression$leaf$ number: $df = 4$, deviance $= 9.71$, $p = 0.05$, planned comparison$prevalence$: leaf 2 $= 3**$, ** = significantly different at $p > 0.02$.

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Results

Prevalence and Abundance of *Labyrinthula Zosterae*

Using the QPCR assay, *L. zosterae* was present in 16 of 19 populations tested, with a statistically significant variation of prevalence among sites (Nominal logistic regression$site$: $df = 18$, deviance $= 116.06$, $p = 0.0001$). Because we had no *a priori* expectations about site-specific abundances, we did not perform any post-hoc tests. The highest prevalence of 88.9% was found in Falckenstein, the population in Kiel Fjord. Lemvig plants were ranked second in terms of prevalence (58%, Fig. 1). The Swedish Kungscavag population showed the lowest prevalence (5%). No *L. zosterae* was found in Tonnenleger Bay, Amrum NW, Dagshuul and Langeness North (intertidal populations, Table 1).

The abundance of *L. zosterae* was standardized relative to eelgrass dry weight (DW) and revealed high variation within and among sites (minimum: 0.01 *L. zosterae* cells mg$^{-1}$ plant DW, maximum: 504 *L. zosterae* cells mg$^{-1}$ plant DW, Fig. 2). Note that cell numbers <1 are possible because the amplified ITS-region belongs to the multi-copy rDNA gene and the detection limit per PCR-reaction was 0.01 cells. Similar to prevalence, abundance was highest in Falckenstein (16.40 cells mg$^{-1}$ plant DW ± 6.84 SE), followed by Fiskebacksvik (6.17 cells mg$^{-1}$ plant DW ± 4.03 SE) as shown in Fig. 3. The lowest abundances were found in the positive samples from Hooge NW and Pellworm Creek (0.01 cells mg$^{-1}$ plant DW). Site differences were significant (Wilcoxon/Kruskal-Wallis-test$\chi^2$ $= 25.27$, $df = 14$, $p = 0.032$; note that only positive values were included into the analysis resulting in an exclusion of sites without *L. zosterae*).

Lesion, Isolation and Prevalence of *Labyrinthula Zosterae*

For a subset of five sites, we investigated the presence of lesions and the isolation success of *L. zosterae* in addition to QPCR-assay analysis. Prevalences of *L. zosterae* assessed as isolation success via cultivation did not differ significantly from obtained via the QPCR assay. We analyzed the method applied together with site differences in prevalence in one model. Differences were only found for site and for the method used (Fig. 4, Nominal logistic regression$method$ and $site$: method: $df = 1$, deviance $= 0.04$, $p = 0.850$, site: $df = 2$, deviance $= 20.20$, $p = 0.0004$, method$\times$site: $df = 4$, deviance $= 3.245$, $p = 0.5177$, ns). The mean prevalence across all sites was 26% for the QPCR-approach and 30% for the isolation approach.

Table 3. Mean *Labyrinthula zosterae* abundance and prevalence among different *Zostera marina* leaves.
The percentage of leaves with lesions (small black or brown spots, between 1 mm and 2 cm in diameter) differed markedly among populations ranging from 11% in Fiskebäckvik to 80% in Wackerballig (Table 1, Nominal logistic regression; lesion for site: df = 4, deviance = 17.81, p = 0.0013). Across all sites, the probability of obtaining a positive L. zosterae culture or a positive QPCR result was significantly higher in leaves with lesions that without, although the protist was also found in plants without lesions. 48.8% of the leaves where L. zosterae has been detected by QPCR showed lesions, whereas the protist was only found in 10.4% leaves without lesions (Nominal logistic regression; lesion: df = 1, deviance = 15.87, p = 0.001, log odds ratio = 1.39, SE = 0.585). Using isolation, L. zosterae could be detected in 56.5% leaves with lesions but only in 8.3% without (Nominal logistic regression; lesion: df = 1, deviance = 32.37, p = 0.0001, log odds ratio = 2.88, SE = 0.616).

Interestingly, isolates of L. zosterae were easily obtained from lesions on the leaves at Sandspollen, Fiskebäckvik, Wackerballig and Lemvig, whereas this was not the case with the leaves from sublittoral eelgrass plants in Ellenbogen Creek. Here, 57% of the Labyrinthula isolated came from green leaves without any lesions.

Temporal Variation in Abundance and Prevalence of Labyrinthula zosterae

At two selected sites, prevalence and abundance of L. zosterae were monitored throughout one year. Overall the temporal patterns were congruent. Prevalence data varied strongly and ranged between 0 and 25% between April and June, 67–95% between the end of June and September. At the western Baltic Sea site of Falckenstein (Table 1 and Fig. 3) L. zosterae occurred at very low abundances between April and June (0.01–0.09 cells mg⁻¹ Z. marina dry weight), increasing from the end of June and September (4.4–24.3 cells mg⁻¹ Z. marina dry weight) and declining from October until March (ca. 1 cell/mg Z. marina dry weight (Wilkoxon/Kruskal-Wallis-test, sampling date: df = 12, χ² = 141.40, p<0.0001). The Wadden Sea site at Ellenbogen Creek (Table 1 and Fig. 3) revealed much lower prevalences and abundances than the Baltic Sea Falckenstein location. Here, only about 20% of plants were infected during the July-August period and abundances also remained low (0.6–0.9 cells mg⁻¹ Z. marina dry weight, Wilkoxon/Kruskal-Wallis-test, sampling date: df = 4, χ² = 28.256, p<0.0001).

Discussion

After nearly a century of investigations on Labyrinthula zosterae as putative agent of eelgrass wasting disease there is still no conclusive picture of what triggers pathogenic outbreaks. We show here that background prevalence is extremely high in contemporary eelgrass beds in northern Europe, with up to 89% of the plants carrying L. zosterae. Using a specific QPCR assay, we show that Labyrinthula zosterae is present in almost all populations assessed even though most plants showed few lesions, let alone signs of an epidemic outbreak. The QPCR assay thus provides a valuable tool to assess background levels (~0.01 cells mg⁻¹ DW) of L. zosterae independent of lesions. Prevalence, as determined by either QPCR data or isolation and culture were comparable. Since the latter is far more laborious and slow, preference should be given to a QPCR assay.
which also works with dried samples. A direct comparison of QPCR values with the “wasting disease index” [33] has to be interpreted with caution, as the QPCR and the wasting index measure different processes. The wasting disease index reflects the cumulative pathogenic effects of a L. zosterae infection (including e.g. defense reactions of the plant), whereas the QPCR value reflects abundance only. The two should be seen as complementary.

Currently we do not know whether the very low background concentrations of the L. zosterae endophyte in winter and spring are the only inoculum that gives rise to high abundances during summer, or whether eelgrass leaves are secondarily infected every year from L. zosterae spores the environment. Although a number of life history studies on L. zosterae have been conducted earlier [37–40], the details of zoospore formation as well as the emergence and location of resting stages (cysts) in the environment remain unknown. While have not yet searched for resting stages in the sediments and/or water column our QPCR approach may be the suitable tool to do so. Equally unknown is how the endophyte disperses which could take place via the drift of decaying infected leaves. L. zosterae can be transmitted rapidly by direct contact of leaves (AC Bockelmann, personal observation).

With a mean value of 5.7 L. zosterae cells mg^-1 Z. marina dry weight (±1.9 SE), abundances of L. zosterae seem low on an absolute scale but are consistent with a scenario of chronic, non-pathogenic infection, while the variation across and among sites is very high. Z. marina plants from four intertidal sites in the Wadden Sea were completely uninfected, even in summer. High intra- as well as inter-population variability may be due to stochastic infection dynamics [41, 42], genotypic resistance effects of the host, as shown for other pathogen-host associations [43, 44] or due to differential physiological activity among leaves and among individuals. For example, a single eelgrass shoot from one individual can harbor 20,000 times as many L. zosterae cells as a

Figure 3. Temporal variation in the abundance and prevalence of Labyrinthula zosterae in infected Zostera marina plants. Means with standard error bars, N = 10–25, Falckenstein = Baltic Sea, Ellenbogen Creek = Wadden Sea (sublittoral). doi:10.1371/journal.pone.0062169.g003
shoot from another individual just a few meters away (this study). Rapid changes in abundance of *Labyrinthula* spp. have been shown in culture where cells can spread 10 mm hr$^{-1}$ [30]; spread has also been shown to correlate with reduced photosynthetic capacity across an infected area of the leaf at a velocity of 0.8 mm hr$^{-1}$ [45]. Thus infection of the physiologically most active parts of the plants undoubtedly contributes to high intra-individual variation. The extremely low abundance of *L. zosterae* in subtidal Wadden Sea populations may be a result of high resistance to infection, resulting from the 1930s epidemic which destroyed almost all subtidal eelgrass beds.

Experimental investigations of *L. zosterae* and lesion development revealed that neither high temperatures, nor high salinity or low light availability could be identified as variables that satisfactorily explain the 1930's pandemic [5], [33], [46–48]. Next to environmental factors, interactions with biotic effects such as herbivory [49] and competition with epiphytes and bacteria on the leaf surface [50] are likely to impact infection dynamics. Our QPCR assay also provides the opportunity to study historical museum material (AC Bockelmann unpublished) in order to determine whether the *L. zosterae* present in today eelgrass meadows is the same strain that caused the 1930’s wasting disease epidemic and thus provide a clue about the endophyte’s possible origins.

A commensalistic or even mutualistic relationship [43], [44] for *Labyrinthula* species is also worthy of further investigation, as has been shown for many terrestrial plant-endophyte associations [43], [44], [51–53]. Several other *Labyrinthula* species have now been identified in the Baltic [32], suggesting that a commensally associated species may be more likely than previously supposed. It is conceivable that the presence of the endophyte in low concentration confers some sort of chemical protection against other infections like known from bacteria or fungi [53], [54]. Schmoller [55] found that in culture *Labyrinthula coenocystis* can actually be nourished by a bacterial film. Furthermore, the rapid decay and mineralization of senescent leaves [50] could alleviate nutrient limitation for eelgrass plants. Switches between pathogenic and mutualistic relationships are common in plant-endophyte symbiosis [56], [57], which could also be the case here. There is thus a pressing need to experimentally disentangle the role of different environmental and biotic factors as well as the mechanism of host defense [58].

In culture, morphological differences in colony growth form, cell morphology, and in pathogenicity and infectiousness have been observed, which suggests different genetic backgrounds [59], [60], (AC Bockelmann, personal observation). However, there is currently no genetic or definitive experimental data available. Whereas species differences have been documented using 18S ribosomal rDNA sequence analysis [32], there are currently no genetic markers to distinguish among specific strains that are of commensalistic vs. pathogenic nature.

With climate change resulting in a multitude of altered environmental conditions, for example warmer temperatures and ocean acidification, marine diseases in several taxonomic groups are already noticeably increasing [6], [61–63]. Given that endophytes such as *Labyrinthula* species are diverse and that only very few have been studied thus far (as *L. zosterae* for *Z. marina*), it may be useful to other endophytes in addition to *Labyrinthula zosterae* in future studies on eelgrass health and performance [64].

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Author Contributions
Conceived and designed the experiments: ACB. Performed the experiments: VT JP PRS. Analyzed the data: ACB. Contributed reagents/materials/methods/analysis tools: VT JP PRS. Wrote the paper: ACB TBHR.

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