Combined effect of warming and infection by *Labyrinthula* sp. on the Mediterranean seagrass *Cymodocea nodosa*

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ABSTRACT: Global warming is predicted to alter host–pathogen relationships and increase disease outbreaks in terrestrial and marine environments. We evaluated the effect of warming on the susceptibility of *Cymodocea nodosa* to infection by *Labyrinthula* sp. (the causative agent of seagrass wasting disease) by monitoring disease symptoms and seagrass photobiology. Seagrass shoots were incubated at temperatures between 24 and 32°C, encompassing maximum summer seawater temperatures projected for the Mediterranean during the 21st century, and exposed to *Labyrinthula* sp. for 2 wk. The effect of temperature on pathogen growth was also tested by growing *Labyrinthula* sp. in liquid medium for 24 h. Disease severity, measured as lesion size, decreased with warming, but the presence of lesions had a negative effect on quantum yield, quantum efficiency, optimum irradiance and the maximum electron transport rate (ETR$_{\text{max}}$) in adjacent tissue across the full range of temperatures. The direct effect of increased temperature on biochemical efficiency was positive in terms of quantum yield, whereas compensation and optimum irradiances and ETR$_{\text{max}}$ decreased slightly with warming. Warming stimulated *Labyrinthula* sp. growth up to a threshold of around 26 to 28°C, beyond which cell division and elongation of the ectoplasmic network decreased. At 32°C almost no growth was observed. Our results indicate that warming does not make *C. nodosa* more susceptible to infection by *Labyrinthula* sp. and that the disease is unlikely to pose a serious threat to *C. nodosa*, but that the pathogen is able to persist during forecasted warm periods.

KEY WORDS: Wasting disease · Pathogen · Host · Seagrass · Photosynthetic performance · Temperature · Climate change

INTRODUCTION

Seagrasses are ecologically and economically important marine ecosystems that, in addition to being major habitat-builders and supporting high levels of biodiversity, play a major role in sediment stabilization, biogeochemical cycling and carbon sequestration and burial. Over the past century, seagrass meadows have declined, largely due to anthropogenic activities and coastal development causing degraded water quality and global climate change (Waycott et al. 2009). The increasing rate of global warming predicted for the coming decades may lead to shifts in distribution and phenology of seagrasses (Short & Neckles 1999) since temperature affects many aspects of seagrass performance, including photosynthesis, respiration, growth and reproduction (Short & Neckles 1999, Campbell et al. 2006). While some seagrass species may benefit from climate change, others are likely to suffer declines with
increasing temperatures, for example, as observed in Mediterranean *Posidonia oceanica* (Díaz-Almela et al. 2009, Marbà & Duarte 2010, Jordà et al. 2012).

Wasting disease caused by the marine protist *Labyrinthula zosterae* has been implicated as a possible cause for large-scale die-offs of eelgrass along the Atlantic coast of North America (Renn 1936, Short et al. 1987, Muehlstein et al. 1991) and in *Thalassa testudinum* in Florida Bay (Robblee et al. 1991). The symptoms of infection are black-brown dots or streaks on the leaves (Muehlstein et al. 1988, Short et al. 1988), which lead to reduced photosynthetic performance, ultimately resulting in mortality of the seagrass (Ralph & Short 2002). These lesions are produced as the pathogen uses extracellular enzymes to penetrate internal cell walls where it destroys the cytoplasm (Muehlstein et al. 1991). The pathogen is spread through direct contact between leaves and it can rapidly move through tissue along an ectoplasmic network (Muehlstein et al. 1991, Ralph & Short 2002). While *Labyrinthula* spp. have been identified as the causative agent of wasting disease, it is possible that pathogenic infection simply renders the plants more vulnerable and that, ultimately, environmental stressors like sulfide intrusion are responsible for the die-off (Durako & Kuss 1994). *Labyrinthula* spp. may be naturally occurring on most seagrasses (Vergeer & den Hartog 1996) and play a role in the breakdown of old leaf tissue, as suggested by patterns of increasing wasting index with leaf age (Hily et al. 2002). It is possible that environmental factors such as eutrophication, low light conditions, warming and herbivory play a role in wasting disease outbreaks, but the actual conditions that lead to large-scale disease outbreaks are yet to be understood. Previous research has indicated that environmental stressors regulate the relationship between seagrass hosts and *Labyrinthula* spp. by making the seagrass host more susceptible to opportunistic pathogen infection or by regulating the virulence and growth of the pathogen. For example, eelgrass growing in areas of low salinity (<20–25‰) may have escaped infection during the 1930s epidemic due to the inhibition of *L. zosterae* at lower salinities (Muehlstein et al. 1988, Burdick et al. 1993, Martín et al. 2009, McKone & Tanner 2009). Temperature may also have played a role; an examination of temperature data during the outbreaks of wasting disease in *Zostera marina* in the 1930s suggest that increased temperatures observed during this period may have contributed to the fatality of an already ongoing epidemic, although there was no clear relationship between warming and die-offs (Giesen et al. 1990, Robblee et al. 1991).

Mediterranean seagrasses are increasingly exposed to warm temperatures and heat waves. Sea-water temperatures in the Mediterranean have increased over the past few decades (Metaxas et al. 1991, Marbà & Duarte 2010) and are predicted to increase further in the coming century (Jordà et al. 2012). Warming in excess of 5°C of the current maximum summer temperature and an increase in the frequency of heat waves in the southwest Mediterranean area have been predicted (Sanchez et al. 2004, Jordà et al. 2012).

Optimal growth between 15 and 30°C has been recorded for *Labyrinthula* sp. isolated from *T. testudinum* (Sykes and Porter 1973) and between 15 and 24°C for *Labyrinthula zosterae* isolated from *Z. marina* (Tutin 1938). For *Labyrinthula* sp. isolated from Mediterranean *P. oceanica*, a threshold in growth was found at 28°C and temperatures above this dramatically inhibited cell division and growth, leading to a reduction in the severity of infection with warming (Olsen et al. 2014).

The pathogenicity of *Labyrinthula* spp. in the Mediterranean is largely unknown although no diebacks caused by the disease have been recorded. In a recent study, 70% of seagrass beds around the Balearic Islands were infected with *Labyrinthula* spp., including all 3 meadows of *Cymodocea nodosa* examined (Garcías-Bonet et al. 2011). *Labyrinthula* sp. has also been isolated from *C. nodosa* in Venice lagoon, but no detrimental effects on biomass and shoot dynamics were found (den Hartog et al. 1996). Yet, the impact of *Labyrinthula* sp. on *Cymodocea nodosa* has not yet been examined. *C. nodosa* typically occupies shallow, sheltered lagoons in the Mediterranean, where maximum temperatures often exceed those in the open coastlines dominated by *P. oceanica*. Hence, Mediterranean warming may lead to more extreme temperatures in *C. nodosa* habitats than those for *P. oceanica* meadows.

Here, we investigated whether warming affects the susceptibility of the Mediterranean seagrass *C. nodosa* to infection by *Labyrinthula* sp. We also examine the effect of infection on the photosynthetic performance of *C. nodosa* under the temperature regimes predicted in the Mediterranean over coming decades. Warming enhances seagrass metabolism, particularly respiration, thereby reducing the carbon balance of the seagrass, whereas pathogens are likely to suppress photosynthesis by causing lesions in the seagrass tissue. Warming may also lower plant resistance to pathogens. We therefore hypothesized that increased temperatures make seagrasses more vulnerable to pathogens and that the combined
effects of pathogenic infection and warming would be additive and cause stress and seagrass mortality at lower temperatures than is observed for healthy seagrasses.

MATERIALS AND METHODS

Shoots of *Cymodocea nodosa* were collected from 3 meadows around Mallorca, Spain and *Labyrinthula* sp. isolated from its leaves as described by Muehlstein et al. (1988). Briefly, seagrass leaves were cut into 1 to 2 cm length fragments which were surface-sterilized by dipping into 0.5% sodium hypochlorite solution for 2 min, rinsed in distilled water for 2 min and then in sterile seawater for 2 min. Fragments were placed on serum-seawater agar (SSA) medium (1.2% agar in 0.4 µm filtered and autoclaved seawater, 0.003 g l⁻¹ GeO₂, 25 ml l⁻¹ penicillin/streptomycin [10 000 units penicillin G ml⁻¹ and 10 mg streptomycin ml⁻¹], 1% (v/v) horse serum, 0.1 g l⁻¹ sucrose, 0.01 g l⁻¹ peptone and 0.01 g l⁻¹ yeast extract) in sterile petri dishes and maintained at 25 °C. *Labyrinthula* sp. were identified based on their characteristic growth patterns by light microscopy of the cultures using phase contrast optics and gross cell morphology and growth patterns under 400x magnification. Cultures were then tested for their ability to produce lesions in healthy shoots of *C. nodosa* (methods of infection described in detail below). To adhere to Koch’s postulates, *Labyrinthula* sp. were re-isolated from randomly selected lesions on the cultured and infected plants and grown on agar following the methodology described above. The culture that produced the highest frequency of disease lesions was selected for subsequent experiments. To maintain a growing culture, cells from the growing front were transferred onto new plates approximately every 2 wk.

To test the effect of temperature on susceptibility of *C. nodosa* to *Labyrinthula* sp., we incubated seagrass shoots in the presence of the pathogen at 5 temperatures (24, 26, 28, 30 and 32°C), encompassing maximum summer seawater temperatures projected for the Mediterranean Sea during the 21st century (IPCC 2007, Jordà et al. 2012). Healthy shoots of *C. nodosa* were collected from Illetas, Mallorca. Three shoots with >5 cm of rhizome attached were placed in each of 8 replicate 2 l plastic tanks per incubation temperature and maintained in aerated seawater at 22°C to allow them to acclimate to aquaria conditions for 3 d before the start of the experiments. Shoots were placed upright at the bottom of each tank and no sediment was added. Aquaria were aerated and to avoid cross-contamination, no water circulated between the tanks. The tanks were illuminated by fluorescent bulbs set to a photoperiod of 12:12 h and delivering PAR light levels ~200 µmol photons m⁻² s⁻¹ at the seagrass canopy level. Salinity was around 35–38‰. Temperatures in the aquaria were raised slowly (1–2°C per day) for 7 d using an Aqua Star temperature control system until the 5 incubation temperatures had been reached. Infection with *Labyrinthula* sp. was carried out according to the procedures described by Muehlstein et al. (1988). Autoclaved pieces of seagrass leaves were used as vectors and left in contact with *Labyrinthula* sp. cultures on petri dishes for 7 d at 25°C. Vectors carrying the *Labyrinthula* were subsequently clamped near the base of the second leaf of the seagrass shoots with the help of a piece of clear, flexible PVC tubing split open along one side. All shoots in 4 randomly assigned tanks per temperature were infected. Sterile leaf sections that had not been infected were clamped to the seagrass in the remaining 4 tanks of each temperature as controls. At the end of a 2 wk incubation, we recorded the presence or absence of lesions and measured lesion area to assess infection (Burdick et al. 1993). Each leaf was photographed and the lesion area measured using ImageJ.

We evaluated photosynthetic performance using a diving Pulse Amplitude Modulation (PAM) fluorometer (Waltz, Germany). All measurements were made around 5 cm above the meristem of the second leaf, immediately above the clipped-on inoculum or control vector. Maximum quantum yield, Fm (minimum fluorescence measured without actinic light) and Fm' (maximum fluorescence measured during a saturating pulse) were measured by applying a saturating pulse to leaves that had been dark-adapted in a leaf clip for 5 min. Leaf absorbance (AF) was measured according to Beer et al. (1998) by placing leaves in front of the instrument PAR sensor and recording the percentage light absorbed by the seagrass. Irradiance through 1 to 4 leaves placed on top of one another was recorded underwater in 5 replicates from each station and the ln of the values was then plotted against the number of leaf layers. Using linear correlation the slope (k) of the resulting line was determined and AF calculated as $1 - \exp(-k)$. Rapid light curves (RLCs) were recorded on light-adapted leaves. Samples were illuminated with a consecutive series of 8 increasing actinic light intensities (0–1930 µmol PAR m⁻² s⁻¹) at 10 s intervals. At the end of each interval a saturating light pulse was applied and the effective quantum yield recorded.
We calculated electron transport rates (ETR) as $ETR = \text{effective quantum yield} \times \text{irradiance} \times 0.5 \times \text{AF}$ (Beer et al. 2001). The maximum ETR ($ETR_{\text{max}}$), photosynthetic quantum efficiency ($\alpha$), saturation irradiance ($I_k$), and optimum irradiance ($I_{\text{opt}}$) were derived by fitting the RLCs to a model of a production curve described by Eilers & Peeters (1988) using Kaleidagraph (Synergy Software).

To examine the effect of temperature on growth rates of $Labyrinthula$ sp., cells were grown in liquid medium according to the methods of Martin et al. (2009). A small plug of agar (0.5 × 0.5 cm) was removed from a pure culture of $Labyrinthula$ sp. and placed upside down in the centre of a sterile petri dish containing liquid growth medium (prepared as above but without the agar). Five replicate petri dishes per temperature were incubated at between 22 and 32°C (at 2°C increments) for 24h. After incubation, the liquid was drained from the petri dishes, the cells stained with Hucker ammonium oxalate crystal violet, gently rinsed with water and the samples dried at 60°C. The growing edge of the culture was outlined with a marker on the bottom of each petri dish. The dried cultures were then photographed against a cm scale and the total surface area of growth estimated from the photographs using ImageJ. To estimate cell counts, 8 photographs were taken at one-third and two-thirds distance along the radius of the growth in each quadrant of the petri dish at 200× magnification using an inverted Zeiss microscope fitted with a digital camera. The cells were counted manually from the photographs and average cell density estimated. Total cell counts were estimated by multiplying the average cell density by the total growth area.

**Statistical analysis**

To evaluate area of diseased tissue and the response of photosynthesis to the experimental treatments we constructed a suite of general linear models (GLM) using the nlme library in R (R Core Development Team 2013). Models were constructed for the response variables: quantum yield, $F_0$, $F_m$, $ETR_{\text{max}}$, $\alpha$, $I_k$, $I_{\text{opt}}$ and lesion area, with temperature and treatment (control vs. $Labyrinthula$) as predictor variables. We compared and ranked models using weights of Akaike’s information criterion corrected for small sample size ($wAIC_c$) (Burnham & Anderson 2002). Growth responses of $Labyrinthula$ sp. to temperature were evaluated using 1-way ANOVA in R (R Core Development Team 2013).

**RESULTS**

Seagrass infected with $Labyrinthula$ sp. developed characteristic disease lesions on 100% of shoots measuring 3 to 184 mm², whereas uninfected shoots (controls) had few or no dark spots (average spot size of only 0.64 mm²) (Fig. 1). In the diseased seagrass, lesions were largest at the lower temperatures, but no effect of temperature was observed in the control shoots. This was corroborated by the GLM analysis, which indicated that the model including an interaction between treatment and temperature had majority support ($wAIC_c = 1.000$, deviance explained = 81.66%).

Quantum yield, $F_0$ and $F_m$ of $C. nodosa$ all increased with warming (Fig 2). There was no significant effect of $Labyrinthula$ sp. infection on $F_0$, but $F_m$ and yield were both significantly lower for infected shoots. $F_0$ was therefore best explained by temperature, whereas for $F_m$ and yield, the best models included both treatment and temperature (Table 1). The RLC parameters responded differently to temperature and treatment (Fig. 3). $\alpha$ was lower in diseased shoots, but unaffected by temperature (Table 2). $I_k$ and $I_{\text{opt}}$ both declined with warming ($t = −2.72$, $p < 0.01$ and $t = −3.91$, $p < 0.001$, respectively) (Fig. 3) and, whereas $I_k$ was similar for infected and control shoots, there was a small negative effect of disease on $I_{\text{opt}}$ ($t = −2.38$, $p < 0.05$). The best GLM for $I_{\text{opt}}$ included both temperature and treatment and explained approximately 36% of the deviance, and temperature alone explained around 16% of the deviance in $I_k$ (Table 2). $ETR_{\text{max}}$ decreased with warm-
whereas disease significantly reduced  

\[ F \]  

ters (minimum fluorescence \[ F_0 \], maximum fluorescence \[ F_{\Delta} \], and maximum quantum yield) measured on dark-adapted leaves of  *Cymodocea nodosa* shoots subjected to infection ( *Labyrinthula*-infected and uninfected controls) and temperature treatments after 2 wk incubations. Significant regressions are shown (\( p < 0.05 \)). GLM analysis indicated a positive effect of warming on all parameters. There was no difference in \( F_0 \) between diseased and control plants, whereas disease significantly reduced \( F_m \) and yield (statistical results given in Table 1).

Across the lower range of temperatures tested, between 22 and 26°C, the area of growth and the cell counts were fairly similar, but both measurements peaked at 28°C. Beyond this optimum temperature (between 28 and 32°C) we observed linear declines in area (from 570 to 3 mm\(^2\)), and in total cell count (from 880 000 to only 1000 cells). Cell densities peaked at approximately 2000 cells mm\(^{-2}\) at 26°C and declined as temperatures increased or decreased from this optimum (Fig. 4). The lowest densities (mean = 145 cells mm\(^{-2}\)) were recorded at 32°C.

### DISCUSSION

We found a clear influence of temperature on  *Labyrinthula* sp. infection in  *Cymodocea nodosa*. The extent of disease lesions on seagrass leaves significantly decreased at higher temperatures. A previous study on the impact of warming on  *Labyrinthula* sp. infection in the Mediterranean seagrass  *Posidonia oceanica* demonstrated a similar negative relationship between elevated temperatures and lesion size, and observed a threshold in infection severity at around 28°C (Olsen et al. 2014). Several underlying mechanisms may be responsible for this temperature-driven response in disease occurrence and severity. For example, temperature may regulate pathogen replication and spreading (\( t = -3.41, p < 0.01 \)) and there was a negative effect of disease (\( t = -2.69, p < 0.05 \)) (Fig. 3). For \( \text{ETR}_{\text{max}} \) the GLM including temperature and treatment had the lowest AIC\(_c\) and explained 34% of the total deviance (Table 2).

Growth of  *Labyrinthula* sp. cells in liquid medium measured as area of growth (\( F = 6.64, p < 0.05 \)), cell density (\( F = 6.70, p < 0.05 \)) and total cell number (\( F = 5.56, p < 0.05 \)) was affected by temperature (Fig. 3). There was a positive effect of warming on all parameters. There was no difference in \( F_0 \) between diseased and control plants, whereas disease significantly reduced \( F_m \) and yield (statistical results given in Table 1).

Table 1. General mixed models of the effects of temperature and treatment ( *Labyrinthula*-infected or control) on photosynthetic performance (\( F_0, F_m \) and maximum quantum yield) of  *Cymodocea nodosa* (\( n = 4 \)). Test results are shown for the 2 top ranked models and the intercept-only model (NULL model). AIC\(_c\) = Akaike's information criterion corrected for small samples, \( \Delta \)AIC\(_c\) = difference in AIC\(_c\) for each model compared to the top ranked model, wAIC\(_c\) = the model weight, LL = maximum log-likelihood, % dev = percent deviance explained.

<table>
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<th>Variable</th>
<th>Model</th>
<th>AIC(_c)</th>
<th>( \Delta )AIC(_c)</th>
<th>wAIC(_c)</th>
<th>LL</th>
<th>% dev</th>
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<tr>
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<td>( F_m \sim \text{Temperature + Treatment} )</td>
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<td>( F_m \sim \text{Temperature \times Treatment} )</td>
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<td>2.00</td>
<td>0.27</td>
<td>−268.43</td>
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<td>R(^2) = 0.61</td>
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transmission rates. The effect of temperature on the growth of *Labyrinthula* sp. produced a unimodal response, with steady growth until it reached a temperature threshold, beyond which growth declined. The positive effect of warming at the lower temperatures appeared to be linked to increased cell division as the cell density increased sharply between 22 and 26°C, while the area of the colonies did not change much. Above 28°C, cell density declined with increasing temperature, suggesting a negative effect on cell division. Culture area was largest at 28°C and only declined significantly at 32°C; therefore, the development of the ectoplasmic network may be compromised at these high temperatures. Similar observations were made for *Labyrinthula* sp. isolated from *P. oceanica* where growth declined dramatically above 28°C (Olsen et al. 2014). This threshold was more dramatic than in the present study, where a more gradual decline was observed and growth of *Labyrinthula* sp. isolated from *C. nodosa* maintained relatively high growth rates up to 30°C. *Labyrinthula* sp. associated with *C. nodosa* may therefore be more temperature tolerant and survive short-term exposure to elevated temperatures. This difference was also observed in the seagrass-pathogen incubations, where disease lesions were a considerable size in *C. nodosa* at temperatures up to 32°C, whereas lesions in *P. oceanica* were minute at temperatures above 26°C. Given the high tolerance of *Labyrinthula* sp. associated with *C. nodosa* to high temperature and the presence of lesions across the temperature range tested, *C. nodosa* is unlikely to experience lower rates of *Labyrinthula* sp. infection during summer heat waves, as was found for *P. oceanica* (Olsen et al. 2014).

Incidence of disease can also be regulated by temperature if warming alters the susceptibility of the host (Harvell et al. 1999). Stressed individuals may have lower resistance and therefore be more susceptible to infection. Effects of thermal stress in seagrasses can be rapidly detected with chl a fluorescence measurements (Ralph 1998). Fundamental characteristics of heat stress include a decrease in photosynthetic yield and reductions in minimum and maximum fluorescence (Campbell et al. 2006). The decline in maximum fluorescence indicates heat-induced photoinhibition including closing of PSII reaction centers and dysfunction of the chloroplast. The effect of warming on *C. nodosa* appeared to have the opposite effect: an increase in *F₀*, *Fₘ* and quantum yield. This species therefore appears to be relatively tolerant to elevated temperatures and may even be stimulated by warming across the range tested here. There was also no evidence of photoinhibition or damage to chloroplasts within the temperature range tested. In contrast, results from the RLCs showed a slight decrease in optimal and compensation irradiances and in the maximum electron transport rates

![Graphs and charts showing growth and temperature relationships for *Labyrinthula* sp. isolated from *P. oceanica* and *C. nodosa*.](image-url)
with increasing temperature. Within the plant’s optimum temperature range the slopes of photosynthesis-irradiance ($P-I$) curves tend not to change, however, as temperature exceeds the limit of this range, the photosynthetic capacity and the slope are reduced (Bulthuis 1987). While we cannot directly compare results from $P-I$ curves to those of RLCs, the overall patterns of the curves are comparable.

*Labyrinthula* sp. infection reduced photosynthetic performance in *C. nodosa*, a result that is consistent with observations made in several previous studies (e.g. Durako & Kuss 1994, Ralph & Short 2002). Measurements on the necrotic tissue in *Zostera marina* displayed reduced effective quantum yield, and green tissue up to 3 mm away from lesions also showed a reduction in photosynthetic efficiency by up to 50% (Ralph & Short 2002). Additionally, once a lesion bisected the leaf, photosynthetic activity in the acropetal tissue was negatively affected up to 5 cm away from the lesion. Durako & Kuss (1994) demonstrated reduced photosynthetic performance of *Labyrinthula*-infected turtle grass *Thalassia testudinum* using $P-I$ curves. Photosynthetic capacity and oxygen production decreased significantly with infection and the maximum photosynthetic rate was reduced to zero when 25% of the leaf tissue had lesions. In addition, leaf respiration rates of infected leaves were up to 3 times higher than in healthy leaves suggesting the plant oxygen budget is severely reduced. In contrast, little effect on photosynthetic performance was found in laboratory studies of *T. testudinum* and *P. oceanica* after incubations with *Labyrinthula* spp. (Trevathan et al. 2011, Olsen et al. 2014). In both of these studies, lesions were small (1−5% of total leaf) and did not bisect the leaves, which may explain the lack of an effect.

In diseased leaves of *C. nodosa* we observed a significant reduction in the maximum fluorescence signal and in the quantum yield. Quenching of the maximum fluorescence signal is thought to be a photoinhibitory response associated with closing of...
the PSII reaction centers and chloroplast dysfunction (Ralph 1998).

The main effect of disease on C. nodosa RLCs was a reduction in the initial slope, or quantum efficiency and a reduction in the ETR$_{\text{max}}$ in diseased tissue. Similarly, Ralph & Short (2002) observed limited photosynthetic activity in diseased tissue of Z. marina. Whereas ETR in healthy tissue increased with irradiance up to around 400–800 µmol photons m$^{-2}$ s$^{-1}$, ETR in intermediate (adjacent to the necrotic tissue) and blackened tissue increased up to 200 µmol photons m$^{-2}$ s$^{-1}$ above which it declined significantly. Labyrinthula spp. infection appears to impair the photosynthetic activity of seagrass by gradually destroying chloroplasts (Renn 1936). As the disease progresses, chloroplasts tend to clump and change colour and eventually fragment. This results in a reduction in the slopes of the light-limiting region of the light curves and the ETR$_{\text{max}}$ as observed in diseased Zostera marina (Ralph & Short 2002). Labyrinthula sp. infection in C. nodosa produced a similar response, suggesting the disease damaged the chloroplasts. There was, however, no significant interaction between temperature and disease on any of the photosynthetic responses measured, and the observed effect of disease on photobiology of C. nodosa did not appear to be linked to lesion size. Measurements made with a PAM fluorometer only indicate the status of the photosystem at the site of the measurement, so the lack of a relationship between lesion size and photosynthetic impairment show that the localized damage was independent of the size of the lesions. Since temperature was found to regulate the size of lesions, we expect that the overall photosynthetic capacity of the plants is influenced by temperature; shoots with larger lesions are likely to have a larger percentage of their chloroplasts damaged leading to reduced overall photosynthetic rates and increased respiration, as seen in other species of seagrass (Durako & Kuss 1994, Trevathan et al. 2011). Larger lesions will therefore ultimately have a more severe effect on the whole shoot and the interaction between temperature and disease is relevant to the fitness of the overall plant.

Whilst our results (being specific to C. nodosa in the Mediterranean) cannot be extrapolated to seagrass species elsewhere, this study illustrates that predicting the effect of warming on seagrass requires due consideration of community effects and not just those of the seagrass themselves. Here, we demonstrate the importance of mismatches between temperature thresholds of a seagrass species and a pathogen, yet similar mismatches between seagrass and other components of the ecosystem inducing losses (e.g. herbivores) or benefits (e.g. symbionts and mutualistic species) may lead to seagrass responses to warming deviating from those expected when considering the responses of the seagrass alone. Whilst the prevalence of some marine diseases may increase with warming (Harvell et al. 1999, 2002), that of Labyrinthula spp. may not, since our study suggests that this pathogen exhibits lower temperature limits than its host seagrass.

CONCLUSION

We hypothesized that thermal stress would make Mediterranean seagrass Cymodocea nodosa more vulnerable to infection by Labyrinthula sp. and that the combined effects of high temperature and disease would be additive and yield negative effects at lower temperatures than we would observe for healthy seagrasses. However, the results indicate that high temperature, in fact, reduces rates of Labyrinthula sp. infection in C. nodosa. Thus, while we observed some negative effects of elevated temperature on photosynthetic capacity in C. nodosa, warming did not appear to increase susceptibility to infection, thereby having an antagonistic interaction. Labyrinthula sp. was able to sustain growth even at temperatures as high as 30°C and may therefore remain active even during heat waves. This study suggests that Labyrinthula sp. will persist, but appears unlikely to pose a serious threat to C. nodosa, even under a scenario of warming.

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LITERATURE CITED


Renn CE (1936) The wasting disease of _Zostera marina_. Woods Hole Oceanographic Institution, Massachusetts

Olsen & Duarte: Warming and disease in _Cymodocea nodosa_