Journal of Experimental Marine Biology and Ecology 449 (2013) 221–229

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Assessing the relationship between seagrass health and habitat quality with wasting disease prevalence in the Florida Keys

Stacey M. Trevathan-Tackett [1], Nathan Lauer, Kyle Loucks, Anthony M. Rossi, Cliff Ross *

Department of Biological Sciences, University of North Florida, 1 UNF Drive, Jacksonville, FL 32224, USA

ARTICLE INFO

Article history:
Received 28 April 2013
Received in revised form 4 October 2013
Accepted 5 October 2013
Available online 26 October 2013

Keywords:
Anthropogenic effects
Labyrinthula sp.
Thalassia testudinum
Wasting disease

ABSTRACT

Marine pathogens of the genus *Labyrinthula* have been identified as the cause of wasting disease in seagrass systems in both temperate and subtropical regions. An understanding of the association between environmental factors and the prevalence of wasting disease in seagrass meadows is important for elucidating plant–pathogen interactions in coastal environments. We conducted a survey of 7 turtle grass-dominated beds within the Florida Keys National Marine Sanctuary to assess the relationship between environmental and biological parameters on seagrass health. All sites contained *Labyrinthula* spp.; the most pathogenic strain was obtained from an anthropogenically impacted site. Leaf and total biomass, in addition to root/rhizome carbon content, canopy light and % light transmitted, all displayed strong negative correlations with a wasting index (WI). It was noted that many of the same environmental measurements that showed negative correlations with WI also displayed strong positive correlations with canopy light levels. These data suggest that light availability may be an important factor that has previously been understated in the seagrass disease literature yet warrants more attention with respect to turtle grass susceptibility to infection. Studies such as this are important because they identify gaps in our understanding of plant–pathogen interactions in subtropical marine ecosystems. Furthermore, the relationships identified in this study may offer insight into which factors are most useful in identifying "at-risk" meadows prior to the onset of larger scale die-off events.

1. Introduction

As one of the most significant sources of coastal primary production as well as critical habitat for juvenile reef fish and commercial fisheries, seagrass meadows are essential both ecologically and socioeconomically (Heck et al., 2008; Waycott et al., 2009). Regrettably, seagrass populations have experienced significant declines in recent decades due to persistent threats from both local and global scale sources (Orth et al., 2006). In conjunction with rising atmospheric [CO_2], surface temperatures are projected to increase by approximately 3–4 °C (Meehl et al., 2007). This climatic shift could feasibly elevate average tropical sea surface temperatures to 35–40 °C, thus pushing many seagrass species beyond their physiological optimum or tolerance (Koch et al., 2012). In addition, local stressors including salinity fluctuations, light availability, pollutant exposure and physical uprooting can contribute to drastic population losses (Waycott et al., 2009). It is becoming clear that the additive pressures associated with multiple stressors, at various temporal and spatial scales, may have large negative impacts on seagrass bed communities (Orth et al., 2006; Waycott et al., 2009).

The Florida Keys National Marine Sanctuary (FKNMS) contains one of the largest areas of seagrass beds in the state with an approximate coverage of 183.3 and 4.45 ha in the Lower Keys (including the Marquesas Keys) and Dry Tortugas, respectively (Yarbro and Carlson, 2011). *Thalassia testudinum* Banks ex König (turtle grass) is a dominant species of the sanctuary and is considered to be a biological sentinel whose loss could represent a significant decline in the overall health of this region (Fourqurean and Robblee, 1999; Muehlstein, 1989; Orth et al., 2006). Since the 1980s, controlled studies and seagrass monitoring programs in FKNMS have been used to assess the short- and long-term impacts of anthropogenic nutrient input on water quality (Boyer and Briceño, 2009; Lapointe and Clark, 1992; Lapointe et al., 2004; Odgen et al., 1994). Data from the Sustained Ecological Research Related to the Management of the Florida Keys (SEAKEYS) monitoring program reported elevated nutrient loading in nearshore water columns and sediments compared to offshore areas (Ogden et al., 1994). While natural nutrient gradients exist, precipitation and hydrologic exchange serve as efficient transport mechanisms allowing runoff from the Everglades to readily reach the Lower Keys, thus facilitating eutrophication (Lapointe et al., 2004). Ensuing eutrophic and hypereutrophic conditions have a negative impact on ecosystem health by promoting decreased seagrass

Abbreviations: %LT, percent light transmitted; BBTT, density of *Thalassia testudinum* using Braun-Blanquet method; Chl, chlorophyll; DT, Dry Tortugas; F_o, initial fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence; I_o, surface irradiance; I_z, irradiance at depth; k_d, light attenuation coefficient; KW, Key West; MQY, maximum quantum yield; SSA, serum seawater agar; WI, wasting index; WK, Woman Key.

* Corresponding author. Tel.: +1 904 620 1853.
E-mail address: cliff.ross@unf.edu (C. Ross).
[1] Present address: University of Technology, Sydney, Broadway, NSW 2007, Australia.

0022-0981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jembe.2013.10.004

shoot densities, rhizome growth rates and increased epiphyte loading, the latter leading to increased light attenuation for seagrasses (Lapointe et al., 1994).

Aside from excessive nutrient loading, it has become evident that other environmental stressors (e.g., high salinity, high temperature, sulfide toxicity) weaken seagrasses making them vulnerable to die-off events (Koch et al., 2007b; Robblee et al., 1991). In particular, between 1987 and 1991 approximately 23,000 ha of *T. testudinum* habitat was either decimated or negatively affected causing a decline in local shrimp harvest and in canopy dwelling fish populations (Robblee et al., 1991). It was speculated that virulent forms of the pathogenic slime mold *Labyrinthula* spp. (Division Stramenopila) were a major contributor towards this die-off event (Durako and Kuss, 1994; Koch et al., 2007b; Robblee et al., 1991).

Labyrinthula are protists that act primarily as saprobes of dead marine plant material and are found on both living and dead *T. testudinum* leaves (Muehlstein et al., 1988; Raghukumar, 2002). Pathogenic *Labyrinthula* infect live tissue creating characteristic black lesions at the infection site and later spread via blade-to-blade contact (Muehlstein, 1992; Muehlstein et al., 1988; Raghukumar, 2002). The pathogen *Labyrinthula zosterae* had been previously identified as the causative agent in multiple and sometimes devastating die-offs of the temperate seagrass *Zostera marina* (eelgrass) along North Atlantic coasts (Muehlstein et al., 1991; Short et al., 1987).

Wasting disease outbreaks have had long-lasting effects on seagrass populations; for example, many eelgrass populations affected by the disease have not fully recovered, and some populations were reported as driven to local extinction (Muehlstein, 1989 and Refs. therein). Such a potential outcome for turtle grass in Florida has made it essential to determine the distribution of *Labyrinthula* both between and within *T. testudinum* populations. To date, little information exists regarding the distribution of this pathogen in Florida, and few surveys have included the presence of wasting disease in *T. testudinum* as a quantifiable parameter (Bowles and Bell, 2004; Burdick et al., 1993; Short et al., 1993). Thus, little information is available correlating environmental conditions to wasting disease severity and prevalence (Short et al., 1993). It seems reasonable that environmental stressors (anthropogenic or natural) can cause healthy seagrass population to become weak and prone to pathogenic invasion and deterioration. The surveys conducted in our study were intended to determine if selected environmental parameters and indicators of seagrass health along an anthropogenic gradient correlated with wasting disease prevalence in *T. testudinum* within the FKNMS.

2. Materials and methods

2.1. Study sites

Seven seagrass beds dominated by *T. testudinum* were sampled 6–12 July 2011 between Key West, Florida, USA and Dry Tortugas National Park (Fig. 1). Two survey sites were located near the coast of Key West: Pearl Basin (KW1; 24°35.825′N, 81°50.387′W) and Fleming Key (KW2; 24°34.716′N, 81°52.055′W). Both were near heavy boating areas and the latter was adjacent to an active landfill. In the Dry Tortugas National Park, DT1 (24°37.612′N, 82°52.055′W) was located next to Bush Key and contained a large, residential sooty tern population. DT2 (24°37.652′N, 82°52.53′W) was located next to the embankment of Fort Jefferson and was characterized by a rocky substrate and coral rubble. Along the Marquesas island chain, one survey was taken outside Mooney Harbor (MH1; 24°32.459′N, 82°07.474′W) south of Gull Key within the intertidal zone. Another survey was taken inside Mooney Harbor (MH2; 24°33.324′N, 82°07.397′W) and was characterized by extremely fine sediment and protection from wave action. A final survey was taken at a near-shore site along Woman Key (WK; 24°31.345′N, 81°58.192′W).

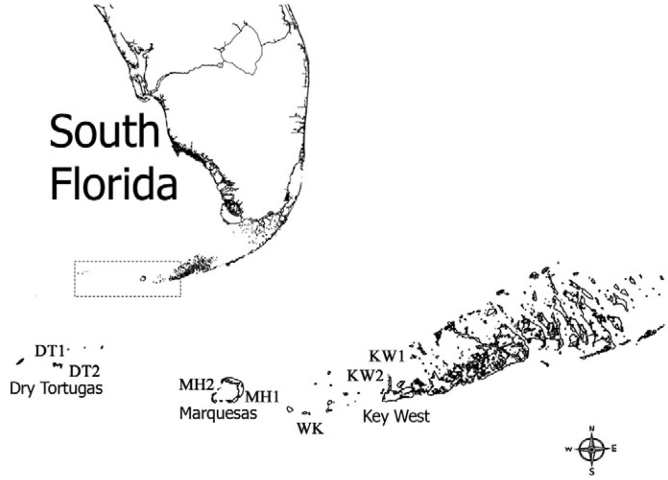

Fig. 1. Map of study area.

2.2. Field surveys

Site surveys were conducted along a 50-m transect with sampling at 10-m intervals. Quadrats (0.25 m^2) were centered along each transect at 10-m intervals, and within each quadrat, *T. testudinum* abundance, wasting index (WI), and photosynthetic efficiency were measured. Above- and belowground biomass of *T. testudinum* was collected at each quadrat using a 15-cm diameter corer. Plant material was cleaned of sediment and stored at −80 °C until further analysis. No belowground biomass was collected from DT2 due to the rocky substrate. At each site, overall depth (i.e. distance from the surface to substrate) and canopy depth (i.e. distance from the surface to top of plants) were recorded. Temperature, salinity and dissolved oxygen were sampled 10 cm below the water surface at similar times of the day (Fourqurean et al., 2003) using a YSI Model 85 Multiparameter probe (YSI, Inc., Yellow Springs, OH, USA). Incident light was measured using a 4π PAR sensor (LI-COR, Lincoln, NE, USA) at the surface (I_o) and at the canopy depth (I_z). The light attenuation coefficient (k_d) was then calculated using the Lambert–Beer Law with $k_d = [-\ln (I_z / I_o)] / z$, where z is the canopy depth. k_d was used to calculate percent light transmission, % light transmission $= 100 * e^{(-k_d * Z)}$ (Dennison et al., 1993).

Turtle grass cover was estimated using a modified Braun-Blanquet method with overall cover given a score between 0 and 5 (Fourqurean et al., 2002). The scoring was as follows: 0 = Absent; 0.1 = Solitary individual ramet; 0.5 = Few individual ramets; 1.0 = Many individual ramets; 2.0 = 5–25% cover; 3.0 = 25–50% cover; 4.0 = 50–75% cover; and 5.0 = 75–100% cover. Based on these scores, *T. testudinum* density was calculated from the equations given in Fourqurean et al. (2002).

Presence of wasting disease on *T. testudinum* blades was assessed using the wasting index (WI) method which is expressed as percent lesion coverage (Burdick et al., 1993). Haphazardly selected blades symptomatic of wasting disease were collected from each transect (3 sub-samples per quadrat) and plated on SSA agar plates in order to isolate *Labyrinthula* and determine if necrotic tissue was associated with its presence (Trevathan et al., 2011).

Photosynthetic efficiency was assessed in each quadrat using a Diving Pulse Amplitude Modulated (PAM) fluorometer (Heinz-Walz GmbH©, Effeltrich, Germany). Maximum quantum yield (MQY) was measured with 3 haphazardly chosen *T. testudinum* short shoots in each quadrat. The second rank blade, i.e., the second youngest blade, was gently cleaned of epiphytes before dark adapting for 5 min (Durako and Kunzelman, 2002). All measurements were taken at the middle of the blade and were conducted ~5 cm away from any lesion to avoid the influence of blackened leaf tissue as noted by Ralph and Short (2002). A dark leaf clip (DIVING-LC) was used to hold the fiber