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Pathological effects of cyanobacteria on sea fans in southeast Florida

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ABSTRACT

In early August 2008, observations by divers indicated that sea fans, particularly *Gorgonia ventalina*, *Gorgonia flabellum*, and *Iciligorgia schrammi*, were being covered by benthic filamentous cyanobacteria. From August 2008 through January 2009 and again in April 2009, tissue samples from a targeted *G. ventalina* colony affected by cyanobacteria and from a nearby, apparently healthy (without cyanobacteria) control colony, were collected monthly for histopathological examination. The primary cellular response of the sea fan to overgrowth by cyanobacteria was an increase in the number of acidophilic amoebocytes (with their granular contents dispersed) that were scattered throughout the coenenchyme tissue. Necrosis of scleroblasts and zooxanthellae and infiltration of degranulated amoebocytes were observed in the sea fan surface tissues at sites overgrown with cyanobacteria. Fungal hyphae in the axial skeleton were qualitatively more prominent in cyanobacteria-affected sea fans than in controls.

1. Introduction

Since 2002, benthic cyanobacterial blooms dominated by filamentous Oscillatoriales have caused major ecological problems on the Gulf Stream Reef and other inner reefs in Broward and Palm Beach counties, southeast Florida. These blooms have been associated with mortalities of soft corals and have severely affected sea fans (Cnidaria, Anthozoa, Octocorallia, Alcyonacea) (Tichenor, 2003, 2004a,b,c, 2005; Paul et al., 2005; Ritson-Williams et al., 2005; Banks et al., 2008; Sharp et al., 2009).

Benthic filamentous cyanobacteria that have been dominantly associated or implicated with coral mortalities or disease (mostly black band disease [BBD], red band disease [RBD] or cyanobacterial patches inducing lesions [CP]) include diverse taxa in the Oscillatoriales (Gantar et al., 2009; Rasoulouniriana et al., 2009; Glas et al., 2010; Casamatta et al., 2012; Kramarsky-Winter et al., 2014). Cyanobacterial overgrowths on *Pseudopterogorgia acerosa* and occasionally on *P. americana* were originally identified as *Phormidium corallyticum* in Sand Key, in north Florida (Feingold, 1988) and considered to be black band disease (BBD), but these findings were never confirmed (Cooney et al., 2002; Frias-Lopez et al., 2002). Klaus et al. (2011) reported BBD in *Gorgonia ventalina* for the Netherlands Antilles, but noted that the associated cyanobacteria were different from those reported from BBD in stony corals. A report of gorgonian sea fans dying from the rapid growth of suspected filamentous *Lyngbya* spp. on North Key Largo Dry Rocks in the Florida Keys was first documented in the 1980s (J. Hudson, pers. comm. in Peters, 1993). Working in Costa Rica, Guzmán and Cortés (1984) hypothesized that BBD caused the disappearance of tissue in *Gorgonia flabellum*, as only the axial skeleton remained (Peters, 1993), but it now appears that this pathological change was caused by aspergillosis (Smith et al., 1996; Gil-Agudelo et al., 2006). The earlier reports of BBD in sea fans, therefore, have not been substantiated. Santavy and Peters (1997) discussed reports of red band disease (RBD) in sea fans and stony corals in which an annular lesion developed, with a reddish microbial mat spreading over the surface of the coral and tissue dying, exposing the axial skeleton. Different cyanobacteria (*Schizothrix* spp., *Oscillatoria* spp.) were identified in these mats than those in BBD, and the microbial consortium of the RBD behaved differently than that of most BBD bands (Richardson, 1992). To our knowledge no one has examined the histopathology of cyanobacteria on the sea fan in RBD.

Sea fan mortalities from cyanobacteria-associated blooms, colonization by cyanobacteria, or penetration of cyanobacteria into tissues are well documented (Rützler and Santavy, 1983; Guzmán and Cortés, 1984; Feingold, 1988; Peters, 1993; Santavy and Peters, 1997; Goreau et al., 1998; Harvell et al., 2001; Paul et al.,

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2005; Sharp et al., 2009; Klaus et al., 2011; Carella et al., 2014; Yamashiro et al., 2014). In some cases, microcystins originating from filamentous cyanobacteria have a proposed role in coral disease and pathogenesis (Richardson et al., 2007, 2009; Gantar et al., 2009; Stanić et al., 2011; Casamatta et al., 2012; Miller and Richardson, 2012).

In early August 2008, diving observations by one of us (ET) indicated that sea fans, particularly *G. ventalina*, *G. flabellum* (Holaxonia, Gorgoniidae), and *Iciligorgia schrammi* (Scleraxonia, Anthothelidae), were being covered by cyanobacteria. Although cyanobacteria can induce fatal bleaching and necrosis in sea fans (Brinkhuis et al., 2008), little is known about the pathologic mechanisms involved. To investigate this aspect of cyanobacterial colonization, we developed sampling methodologies for sea fans and attached cyanobacteria *in situ* and documented whether any detectable pathologies were associated with chronic exposure to topical cyanobacteria mats. We describe here the pathological responses of sea fans naturally exposed to a complex of potentially toxic Oscillatoriales cyanobacteria at the gross and microscopic level.

2. Materials and methods

2.1. Field collections

Following approval by the Florida Fish and Wildlife Conservation Commission (FWC) of a special activity license (#08SRP-1098) for collecting samples, we revisited the sea fan area that was heavily colonized by cyanobacteria (Fig. 1a). We collected parallel monthly tissue samples from the same filamentous cyanobacteria-affected *G. ventalina* colony and from a nearby apparently healthy control without any attached cyanobacteria (Fig. 1a and b).

Sample collections, locations, and field protocols followed Tichenor (2003). Surveys were conducted at the Gulf Stream Reef, a reef system within the continental southeast Florida reef tract that extends from Biscayne Bay in Miami–Dade County, northward to West Palm Beach in northern Palm Beach County (Banks et al., 2008). The *G. ventalina* colony affected by cyanobacteria was located on hard bottom at 26°30.464′N, 80°01.969′W, approximately 2 km offshore from Briny Breezes, Palm Beach County, at a depth of approximately 21 m. The control *G. ventalina* colony was located approximately 3 m from the cyanobacteria-affected sea fan. The dive boat operator located the colonies each time by GPS.

Each month during August 2008 through January 2009 (except for November) and again in April 2009 for a total of 6 times, the Gulf Stream Reef location was revisited during daytime (Table 1). Dive logs and field observations were recorded, documenting the presence or absence of the cyanobacteria, gross condition of the sea fans, and water temperature was measured at the specific reef sampling locations. The targeted and control *G. ventalina* specimens were photographed for assessment of cyanobacterial coverage and progression. Tissue samples (measuring approximately 3–6 × 3–6 cm) were clipped from each of the two sea fans (referred to as the control [C]) and the Oscillatoriales cyanobacteria-affected sea fan [O; Oscillatoriales]). From O (affected individual), two representative tissue samples, either without (unaffected = [U]; Fig. 1b) or with attached cyanobacteria (affected = [A]; Figs. 1a and 2a) were taken every month in year 2008 ($n = 8$), but only A's were taken in year 2009 ($n = 5$) (Table 1). From the control individual (C), basically one representative tissue sample was taken initially, but they were mixed with affected samples ($n = 8$) (Table 1). Although the control sea fan was free of cyanobacteria and healthy in gross appearance during the 16 August and 13 September 2008 sampling periods, by 18 October 2008, it too was grossly observed to have been colonized by cyanobacteria. Therefore, from that time onward, an apparently healthy (unaffected) and a cyanobacteria-affected tissue sample were collected from the control sea fan.

Additionally, representative samples of cyanobacteria on the surface of O were clipped off and preserved in Lugol's iodine solution in a brown container for subsequent identification. Tissue samples were placed in screw-top jars and fixed in 125 ml of 5% buffered paraformaldehyde (PFMA) as soon as possible following collection at ambient temperature, and then were shipped to the FWC's Fish and Wildlife Research Institute (FWRI) at St. Petersburg, Florida.

Opportunistically, a separate benthic cyanobacterial mat was collected on 9 January 2009 about 1.5 km offshore of West Palm Beach, 26°42.812′N, 80°00.977′W, some 20 km north of the targeted sea fans, but in the same area affected by the cyanobacterial blooms. This sample was split and either fixed in Lugol's iodine solution for microscopic observation or placed in a resealable bag, kept on ice in a cooler, and then transported to FWRI. The fresh sample on ice was frozen and stored at −80 °C prior to processing for microcystins.

Deepwater sea fans, *I. schrammi,* adjacent to the *G. ventalina* specimens that were also affected by cyanobacterial overgrowth were collected in October 2008 (Fig. 1c), December 2008, January 2009, and April 2009. Each month tissue samples were clipped from a different colony that appeared to be moribund. No control specimens were available. Samples were preserved in PFMA as above and processed for histological examination.

2.2. Identification of cyanobacteria

Cyanobacteria from preserved samples, some associated with sea fans and others collected separately, were identified to the lowest feasible taxon morphologically by light microscopy, using a Zeiss Axiovert 100s inverted microscope (Carl Zeiss AG) equipped with an Olympus DP72 digital camera (Olympus) using standard taxonomic criteria (Komárek and Anagnostidis, 2005). We were unable to process these samples for molecular identifications.

2.3. Histological processing

Field-collected *G. ventalina* and *I. schrammi* samples were processed by routine embedding in paraffin (Paraplast Plus, Fisher Scientific) and glycol methacrylate plastic resin (JB-4; Electron Microscopy Sciences). Fixed samples were rinsed with tap water and decalcified overnight with formic acid–sodium citrate (Luna, 1968). Tissues sectioned at 4.0 μm were stained routinely with Mayer's hematoxylin and eosin (H&E) for paraffin-embedded samples (Luna, 1968) and with Wright's H&E, periodic acid Schiff/metanil yellow (PAS-MY; Quintero-Hunter et al., 1991), and thionin procedures for JB-4–embedded samples. The paraffin-embedded *I. schrammi* samples were also stained with Gram's procedure for bacteria and Fontana-Masson's silver method for melanin (Luna, 1968). Photomicrographs were captured with an Olympus BX51 light microscope equipped with an Olympus DP71 Digital Camera. Note that histopathological changes were purely descriptive and qualitative and were based on evaluations of one affected and one control sea fan colony. Clearly, no real-time movements of cells were observed in this type of study.

2.4. Cyanotoxin testing

A subsample of the field-collected benthic filamentous cyanobacterial mat was lyophilized for approximately 16 h using a FreeZone 6-l Freeze Dry System (Labconco). In a clean 50-ml plastic centrifuge tube, 0.5 g of dried cyanobacteria sample was