Marine Pollution Bulletin 77 (2013) 210–219

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Ecology of the ciguatera causing dinoflagellates from the Northern Great Barrier Reef: Changes in community distribution and coastal eutrophication

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ARTICLE INFO

Keywords:
Ciguatera
Dinoflagellates
Gambierdiscus
Prorocentrum
Ostreopsis
Nutrients

ABSTRACT

Ciguatera fish poisoning (CFP) is known to be caused by the ciguatoxins from the dinoflagellate genus *Gambierdiscus*, however, there is the potential for other toxins such as okadaic acid and dinophysistoxins from the genus *Prorocentrum*, and palytoxin from the genus *Ostreopsis*, to contaminate seafood. These genera may also be indicators of ecosystem health and potentially impact on coral reef ecosystems and the role they may play in the succession of coral to macroalgae dominated reefs has not been researched. Sixteen GBR field sites spanning inshore, mid-lagoon and outer lagoon (offshore) regions were studied. Samples were collected from September 2006 to December 2007 and abundance of benthic dinoflagellates on different host macroalgae and concentration of nutrients present in the water column were determined. The maximum abundance of *Prorocentrum*, *Ostreopsis* and *Gambierdiscus* found was 112, 793 and 50 cells per gram wet weight of host macroalgae, respectively. The average level of Dissolved Inorganic Nitrogen (DIN) in the water column across all sites (0.03 mg/L) was found to be more than double the threshold critical value (0.013 mg/L) for healthy coral reefs. Compared to a previous study 1984, there is evidence of a major shift in the distribution and abundance of these dinoflagellates. Inshore reefs have either of *Prorocentrum* (as at Green Island) or *Ostreopsis* (as at Magnetic Island) dominating the macroalgal surface niche which was once dominated by *Gambierdiscus*, whilst at offshore regions *Gambierdiscus* is still dominant. This succession may be linked to the ongoing eutrophication of the GBR lagoon and have consequences for the sources of toxins for ongoing cases of ciguatera.

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1. Introduction

The past few decades have seen a significant increase in coastal eutrophication globally, leading to widespread hypoxia and anoxia, habitat degradation, alteration of food web structures, loss of biodiversity, and the increased frequency, spatial extent and duration of harmful algal blooms, (Howarth, 2008). In this context, changing land-use practices in North Queensland have resulted in the clearing of extensive tracks of land for agricultural production in the last 150 years, resulting in dramatic increases in inputs of sediments, nutrients and pesticides into the Great Barrier Reef (GBR) lagoon, and the widespread decline in lagoon water quality (Smith et al., 2005). Furnas et al. (2005) further suggest that terrestrial runoff of sediment and nutrients to the GBR lagoon has increased 2–4-fold over the last century. In view of these inputs, the GBR lagoon should be considered a partially enclosed sea with the associated potential to accumulate nutrients discharged from ever-increasing anthropogenic activities (Bell, 1992). The evidence that nutrient enrichment, increased siltation and excess turbidity can lead to the local degradation of coral reefs is unequivocal (Fabricius et al., 2005). Elevated nutrient levels and higher suspended sediment loads have been cited as the cause of reductions in coral growth and a shift in the relative abundance and composition of coral and algae; particularly in coastal areas adjacent to catchments with intensive agricultural activities (Alongi and McKinnon, 2005). On some near shore reefs on the GBR, high nutrient availability, in conjunction with substrate availability (low coral cover) and insufficient grazing pressure, has also lead to altered benthic communities with high macroalgal cover (Schaffelke et al., 2005). Similarly, increased nutrient levels (and/or reduced herbivory) can also lead to more substrate for toxigenic dinoflagellates, exacerbating their impacts (Parsons and Preskitt, 2007). As noted by Parsons et al. (2010) nutrient enrichment and warming sea surface temperatures can stimulate *Gambierdiscus* growth and result in higher cell densities. By corollary, elevated sea surface

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0025-326X/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.marpolbul.2013.10.003

temperatures (SSTs) associated with global warming may further exacerbate the growing extent and range of ciguatera distribution being driven by eutrophication and other anthropogenic influences.

Ciguatera field studies have largely concentrated on dinoflagellates of the genus *Gambierdiscus*, well known to be the producer of ciguatoxin precursors, but have largely ignored the potential role of toxins from the genera *Prorocentrum* and *Ostreopsis* as causative agents in ciguatera fish poisoning (CFP). Little is known about the extent to which ciguatera related toxins (from benthic dinoflagellates) may be affecting trophic levels, or their potential to alter coral reef ecosystem function. This highlights the need to monitor changes in water quality and various indicators of ecosystem health to help determine if management plans on land are having a noticeable impact on the ecosystems and water quality of the GBR (Udy et al., 2005). Such monitoring might be augmented with assessments of the abundance of potentially toxic benthic HABs that have the potential to provide a useful bio-indicator of coral reef ecosystem health.

Toxic benthic dinoflagellates, like their macroalgal hosts, are at the bottom of the food chain. Whether the succession to algal dominated reefs, either initiated due to natural disturbances (cyclonic weather conditions, predation, etc.) or from anthropogenic causes (global warming, eutrophication, sedimentation) or a combination of such influences, the loss of coral cover results in more macroalgal surface for epiphytic benthic dinoflagellates. For example, phase shifts that involve coral bleaching water temperatures provide macroalgal habitat for *Gambierdiscus* at temperatures highly favorable for their growth (Tester et al., 2010). In fact, coral reef succession to algal dominance might even be reinforced by the effects of benthic dinoflagellate toxins on herbivore fecundity or feeding. Such effects have been little studied but may contribute to increased incidence of ciguatera as recently shown for Pacific Island nations (Skinner et al., 2011).

There has been only one previous published study of these three genera on the Northern Great Barrier Reef (Gillespie et al., 1985). The objective of this study is to determine the distribution and abundance of the ciguatera and other potential causative dinoflagellates across inshore island fringing reefs, middle GBR lagoon reefs and outer continental shelf reefs. We then correlated nutrient levels (ammonia, nitrites or nitrates) and benthic dinoflagellate abundance in relation to macroalgae sampled. Finally, a comparison to a previous study (Gillespie et al., 1985) of benthic dinoflagellates from similar field sites was undertaken.

2. Methods

2.1. Sampling regime

Sixteen Great Barrier Reef field sites were sampled: Inshore, mid-lagoon and outer lagoon (offshore) from Lizard, Snapper and Low Islands (offshore of Port Douglas) in the north, Double and Green Islands, Upolu and Michelmas Cays, Fitzroy and Normanby Island (Frankland Islands), centrally located (near to Cairns) to King reef, Dunk and Magnetic (next to Townsville) Islands in the south (see Fig. 1; Table 1). Sampling occurred at ten sites being sampled three or more times, between September 2006 and December 2007. Green Island (two sites on either side of the island, included 9 monthly collections) and Magnetic Island (Nelly, Geoffrey and Arthur Bays sampled at six, monthly collections) where the most intensively sampled of all the field sites. At all other multiply sampled sites at least 3 samplings occurred, including 3 separate sites at Dunk Island and two separate sites at Low, Normanby Islands and Upolu Cay, nearly all of which where 200 m apart, except at Upolu Cay which was 1 km apart. Sampling generally took place at a distance of 100 m from shore (or pontoon at reef sites), in a water depth of 1–2 m at all field sites.

2.2. Description of field sites

The sites covered a three main reef types, inshore GBR coral reefs, fringing continental islands: Magnetic Island at over 5000 ha of land surface (more than half of which is national park) is inhabited with approx. 2000 people and is the only permanently inhabited site and is located close to Townsville, Snapper Island at 56 ha is 4 km east of the Daintree river, Double Island similar to Snapper and offshore from Palm Cove, Fitzroy Island at 339 ha (mostly national park), King reef that is only 200 m off the mainland coast at Kurrimine, Cape Richards on Hinchenbrook Island and, Dunk Island at 970 ha is also mostly national park; Mid-lagoon platform coral cays and reefs: Green Island at 15 ha land surface and with 710 ha of reef that is mostly sea grass, Lizard Island at 1000 ha is 240 km north of Cairns, Michelmas Cay is only 2 ha and 30 km NE of Cairns, Low Isles has 22 ha of reef found 15 km NE of Port Douglas, Upolu Cay is 28 km NE of Cairns, and Normanby Island of the Frankland group which is 10 km east of Russell river; and outer barrier coral reefs included: Norman Reef has 430 ha of reef located 70 km NE of Cairns, Moore Reef which is 2650 ha and 45 km SE from Cairns, Flynn Reef with 420 ha, found 50 km SE of Cairns and Thetford Reef at 200 ha, is 60 km SE of Cairns. Please note that the benthic HAB abundance is not presented in this paper for each site, as only those with multiple samples or representative abundances are shown.

2.3. Protocol of microalgal sampling

Many similar methods for epiphytic microalgal collection and enumeration have been used following the original sampling of Yasumoto et al. (1980). Divers collected a broad diversity of reef macroalgae harvested using plastic bags. Three or more of the most common species of macroalgae; in this study *Sargussum flaricans*, *Padina australis*, *Halimedia opunita*, *Turbinaria ornata*, *Laurencia intricata*, *Dignea simplex*, *Dictyota bartayresi*, *Amphiroa foliacea*, *Hypnea saidana*, *Jania crassa* and seagrass if present, were collected at a site. The macroalgae sample bags were vigorously shaken for 1 min to dislodge the epiphytic flora. The resulting dislodged epiphytes were sieved and the 38 μm wash back, transferred to a 50 ml vials and fixed with 10% Lugol's solution. All fixed samples were stored at 12 °C in the dark until dinoflagellate abundance could be enumerated. The weight of each macrophyte sample was blotted dry and weighted for comparison.

2.4. Abundance and identification of ciguatera dinoflagellates

Ciguatera dinoflagellates were identified to genus level by morphology using the light microscope (a CE, XSZ-107BN Series). For abundance a 0.5 ml portion of the shaken wash back sample was pippetted onto a Sedgewick Rafter counting slide and diluted to 1 ml and viewed at 64×, for counting and 160×, for identification. Abundance was calculated by taking the number of each genus present and multiplying this by the amount of dilution and the size of the sample and dividing by the wet weight of the sample macroalgae to arrive at the number of cells per gram wet weight of macroalgae. Photographs of different species were taken with a digital camera (Nikon) focused down the tube of the microscope for further identification. Some samples were returned to the Centre for Microscopy and Microanalysis (CMM), University of Queensland for SEM analysis. Fixed samples were desalted using a 10% step gradient from seawater to freshwater on polycarbonate filter paper and dehydrated by using a step gradient of ethanol