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THE ABUNDANCE OF POTENTIALLY TOXIC EPIPHYTIC DINOFLAGELLATES AND NUTRIENTS FROM BALI AND GILI TRAWANGAN, INDONESIA

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Abstract

There are strong indicators that the degradation of coral reefs may increase the intensity and frequency of ciguatera fish poisoning (CFP) outbreaks when denuded reef surfaces are colonised by macro algae that are the preferred hosts of toxic dinoflagellates that can cause the disease. Ciguatera field studies have concentrated on the dinoflagellate genus *Gambierdiscus*, well known to be the producer of ciguatoxin precursors. Moreover the potential of toxins from other dinoflagellate genera such as *Prorocentrum* and *Ostreopsis* is less often considered. Whilst it is known that toxic compounds produced by *Ostreopsis* species include ostreotoxins (OTX) and ostreocins, the potential harmful impacts of *Ostreopsis, Prorocentrum* and *Gambierdiscus* species on coral reef organisms (and ecological impacts) are largely unknown. In addition, CFP has historically largely occurred on the oceanic islands of the world's tropical seas, but has only recently been reported in the major archipelagoes of Indonesia and the Philippines. In view of their degraded coral reef ecosystems and the potential link between reef health and CFP, this study examined three sites in Bali and Gili Trawangan over one year, to assess the prevalence and distribution of CFP related dinoflagellate species. Beside finding *Gambierdiscus, Ostreopsis* and *Prorocentrum* at most sites, water column nitrogen nutrient levels were also found on the average to be 3−4 times higher than that recommended for a healthy coral reef (0.013mg/L). However the maximum abundance of *Prorocentrum, Ostreopsis* and *Gambierdiscus* found were low (103, 2,860 and 28 cells per gram of wet weight of host macroalgae, respectively). A factor that may play a part in the potential for toxicity at these sites is the abundance of sea grass (and associated micro flora and fauna) which are often present within and adjacent to coral reef ecosystems across the Indonesian archipelago.

Keywords: Ciguatera**,** *Gambierdiscus*, *Prorocentrum, Ostreopsis,* Bali, nutrients.

INTRODUCTION

Harmful algal blooms are increasing worldwide as well as coral reef degradation with increased potential for the incidence of ciguatera fish poisoning (CFP) (Lewis, 2001). The destruction of reefs (eg. bleaching or damage from global warming, crown of thorns starfish, ocean acidification, over fishing, maritime installations, chemical and biological pollution, eutrophication, sedimentation) is expected to create condition that would contribute to CFP outbreaks. This can occur following an expansion of denuded reef surfaces that become colonized by macro algae, which are the preferred hosts of the dinoflagellate that causes the disease (Kaly and Jones, 1994). Other niches where toxic dinoflagellates may be found include sand, seagrass, mangrove flats and turf algae (Faust and Gulledge, 2002). Such diverse sources of CFP toxins contribute to the ~400 species of fish implicated as potentially ciguateric (Glaziou and Legrand, 1994).

In 1967 the neurotoxin primarily responsible for the symptoms of ciguatera was first isolated from the flesh of a moray eel and named cigua-

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toxin. It is lipid soluble, odorless and tasteless and is bioaccumulated in the flesh and viscera of fishes via the marine food web. The disc shaped benthic dinoflagellate *Gambierdiscus toxicus* was discovered in the Gambier Islands, French Polynesia in 1977. However an earlier reference does exist for this species, referred to as *Goniodoma* sp. and found at Boa Vista, of the Cape Verde Islands (Sousa de Silva 1956). A structural relationship was found to exist between the ciguatoxin extracted from fish and the toxin extracted from biodetritus containing a wild population of *G. toxicus*. Ciguatoxin has been identified from cultured *G. toxicus*, confirming that it is this organism that responsible for ciguatera (Holmes 1998). *Gambierdiscus* has a broad distribution from the Indian Ocean to numerous locations in the Pacific Ocean, the Caribbean Sea as well as in a number of Southeast Asian countries. Ten species have now been described (Litaker *et al.* 2009). *Gambierdiscus* is generally an epiphyte of macro algae of coral reefs or unattached benthic algae. The importance of the algal host to the growth and toxicity of wild *Gambierdiscus* is unclear other than providing obvious support and shelter. *Gambierdiscus* is a phototropic opportunistic species that can potentially live in temperatures of 20−34° C, salinity of 25 to 40 ‰ and at depths from 0.1 to 30 m. However, most ciguatera endemic areas are characterized by tropical oceanic saltwater (Holmes, 1998). Temperature, light, bacteria, nutrients, growth phase and strain have been found to influence gross cell toxicity (Lewis and Holmes, 1993).

In a review article dated by Lehane and Lewis (2000) state "ecological studies on *G. toxicus* are needed to determine which factors stimulate its proliferation and, more importantly its production of toxin". Ciguatera is a prevalent disease with an increasing incidence worldwide, then it is suggested to allocate more attention to this area of research. The instances of association of upsurges of ciguatera with reef degradation may indicate that anthropogenic impacts can exasperate the problem of ciguatera (Tester *et al.,* 2010). Lewis and Holmes (1993) commented that it may be possible to reduce ciguatera through the appropriate control or modification of environmental factors impacting on ciguatera prone areas. They added that the factors that cause the proliferation of the ciguatoxins must be better understood. Therefore, if we can obtain a better understanding of those factors, we would increase our knowledge and our ability to deal with the management and control of ciguatera outbreaks and the impact of the disease on human health as well as the impact of the dinoflagellate toxins on the coral reef ecosystem.

Of the many diseases impacting upon the inhabitants of Indonesia, CFP is generally unrecognised and unreported. As a disease of any major consequence, some symptoms could go unrecognised by health care workers as there are many water born diseases that have more far reaching consequences in terms of human health. It is customary in Southeast Asia to eat small fish whole and as the head and viscera can have elevated levels of toxins, then CFP could be even more prevalent. However some reported cases of ciguatera have occurred, an outbreak of clupeoid poisoning of 191 cases and another of 95 cases in 1983, where it was unknown what toxic dinoflagellate was responsible (Landsberg, 2002). Fish exported from Indonesian/Philippine waters have also been responsible for CFP in Hong Kong. In this study, we sought to locate and identify the specific epiphytic dinoflagellates (genus *Gambierdiscus*, *Ostreopsis* and *Prorocentrum*) known to be associated with degraded coral/sea grass ecosystems and possible sites of ciguatera fish poisoning, determine the abundance of ciguatera dinoflagellates on the different macro-algae substrates, and correlate abundance with nutrient water quality analysis. These data are compared to other field sites to gain an insight into the influence of anthropogenic environmental factors (nutrients) on the abundance of toxic benthic dinoflagellates.

MATERIALS AND METHODS

Sampling regime

Pilot fieldwork at Gili Trawangan took place in January 2005 and then samplings took place in April, June and November 2005 and April 2006. Sampling took place in Bali from November 2004 to March 2006 (13, monthly samples) at Kuta, from January 2005 to April 2006 (13, monthly samples) at Sanur and from August 2005 to May 2006 (7, monthly samples) at Geger beach, Nusa Dua. Study sites, see figure 1, can be identified

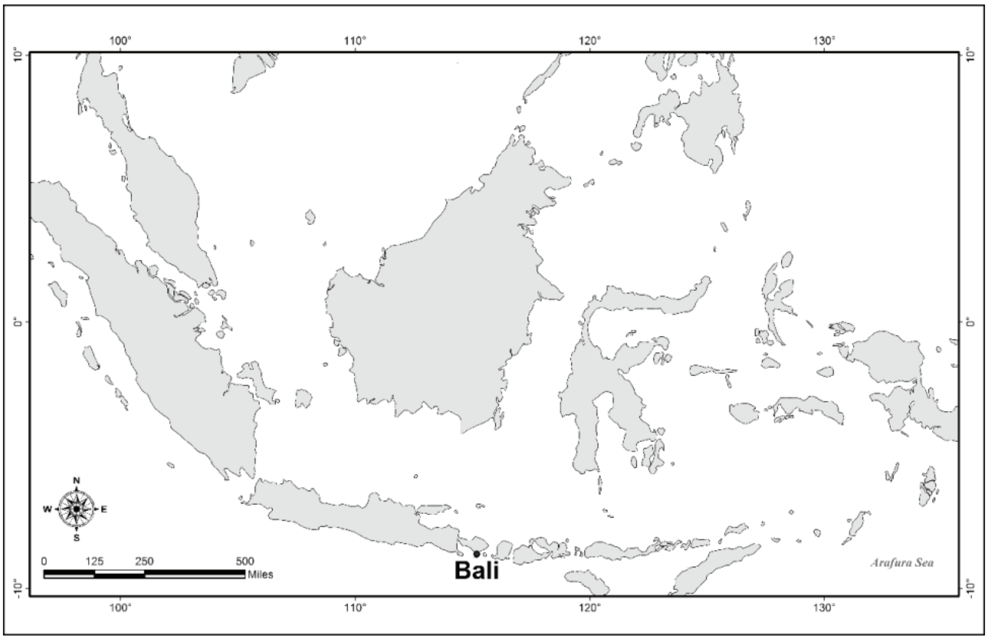

Figure 1. Map of Bali and Lombok showing the field sites, Sanur, Kuta and Nusa Dua and the island of Gili Trawangan is located to the north west where 3 field sites are to the north, the east and to the south of the island.

as may produce ciguateric fish from well-defined geographic locations, associated with a degraded coral reef ecosystem, as examples in this study at Gili Trawangan, Sanur, Kuta and Nusa Dua (Southern Bali) reefs from the Indonesian archipelago. Sampling took place in several sites. Due to remoteness of Gili Island, sampling process done every 3−4 months while at Bali sites, sampling process done monthly for more than a year. Sites at Bali and Gili Trawangan were chosen because they cover different ecosystems caused by the water movement at the sites. These sites have been impacted upon by coral bleaching (as 90% of hard corals were bleached around this island group in the 1996 to 1998 global bleaching event); and destructive fishing techniques and the corals are now in various states of degradation, showing little return to good coral cover. There has certainly been a phase shift at the eastern site of Gili Trawangan next to where the only village (population approx. 1,000) on the island is located, to mostly macroalgal cover and if destructive fishing techniques were stopped, recovery of these reefs from algal to coral dominance may depend upon whether pollution (nutrients from sewerage contaminated groundwater) from the associated tourist and resident population, and a Lombok river (5 kilometres to the east) plume, is minimal.

The sampling regime has allowed for wet/dry seasonal changes, the wet season is normally from November to March. It has included the sampling of macroalgae (including benthic/epiphytic dinoflagellate species), sea grass and coral rubble, as well as the monitoring of water column nutrient and water quality at each of the six sites.

Protocol of Microalgal sampling

Many similar methods for epiphytic micro-algal 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. At the surface, the bags were vigorously shaken for 1 minute to dislodge the epiphytic flora. The resulting dislodged epiphytes were sieved and the 38µm washback, 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 epiphytic biomass could be enumerated. The weight of each macrophyte sample was blotted dried and weighted for comparison.

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 0.5ml portion of the shaken wash back sample was pipetted onto a Sedgewick Rafter counting slide and diluted to 1ml and viewed at 64X for counting and 160X for identification. Photographs of differrent 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, University of Queensland for SEM analysis so that the genera *Gambierdiscus* could be identified to species level.

Scanning Electron Microscope Sample preparation

Fixed samples will be desalted using a 10% step gradient from seawater to freshwater on polycarbonate filter paper and dehydrated by using a step gradient of ethanol (50%-100%), sputter coated with 1.5 nm of platinum.

Water column nutrient sample analysis

Water column samples were collected with a 60 ml syringe (rinsed 3 times with site water), about 30 cm above the lagoon floor, whilst snorkeling slowly for 10 m. Sample was filtered (using a 0.45 µm membrane millipore sterile disposable filter unit) into a sterile falcon tube and stored frozen (for no more than 6 months at - 4ºC) until analysis at the Nutrient Laboratory at the Queensland Health and Scientific Services (QHSS), Brisbane, Australia; a National Association of Testing Authorities (NATA) accredited laboratory. Analyses for FRP, NO2, NO3, and NH3 are performed simultaneously using an automated LACHAT 8000QC flow injection system using methodology based on: (a) ascorbic acid reduction of phosphomolybdate for FRP, (b) cadmium reduction of nitrate to nitrite by diazotizing the nitrite with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride for NOx, (c) production of the indophenols blue colour complex for NH3 (see APHA 2005).

Statistical analysis

Statistical analysis was undertaken using SPSS 19 (2010, SPSS Inc., Chicago, USA). *Prorocentrum*, *Ostreopsis* and *Gambierdiscus* (abundance) data were tested for significant correlation (Pearson) and non parametric correlation (Spearman) with nutrient water column variables (dissolved inorganic nitrogen, ammonium and nitrates).

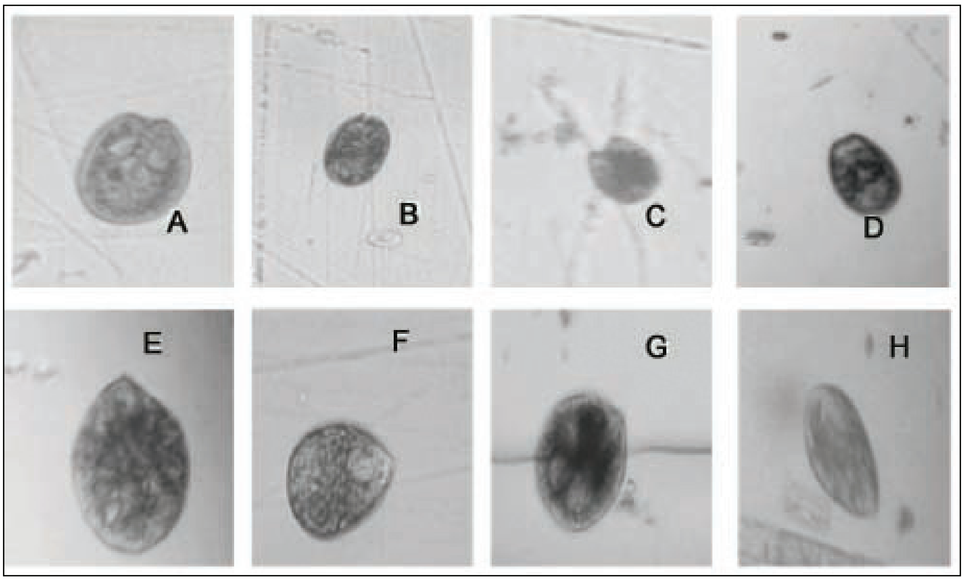

Figure 2. *Prorocentrum* species found on Bali reefs. LM of *P. concavum* (A), *P. mexicanum* (B), *P. emarginatium* (C) and *P. lima* (D). *Ostreopsis* species found on Bali reefs: *O. lenticularis* (E), *O. siamensis* (F), *O. labens* (G) and *O. ovata* (H).

RESULTS

Dinoflagellate species present

Figure 2 displays the morphological identifications of the benthic dinoflagellates present in the samples and whilst not exhaustive of the species present give an indication of the possible toxic specimens in the samples. It is impossible to manually count each species separately whilst enumerating them as some species need identification by SEM and so counting of cells was also done at the genera level (Fig. 3).

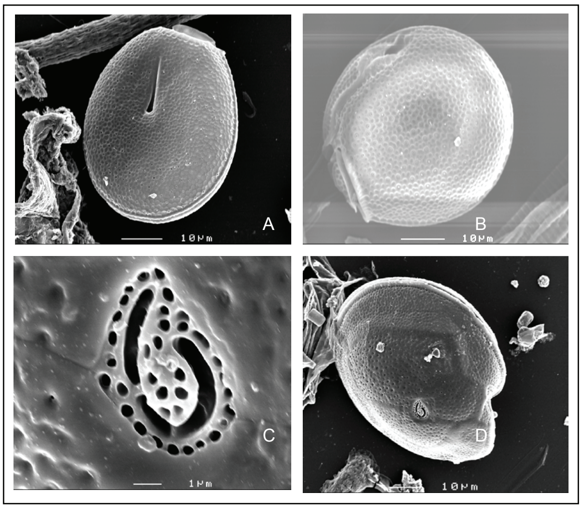

Figure 3. SEM: *S. canaliculata*, left lateal plate (A) and right lateral plate (B); *G. polynesiensis*, apical pore plate (C), apical view (D); collected from Bali reefs.

Dinoflagellate abundance and substrate

Seagrass and coral rubble were always available for sampling where at times the availability of macroalgae would change, depending upon the site. At Gili Trawangan the brown alga *Turbinaria*, *Padina* (not available at the Northern site) and the red alga *Laurencia* carried dinoflagellates whilst the green alga *Halimeda* (not available at the Southern site) did not. At the northern site of Gili Trawangan (see Fig. 4) there was a small bloom of *Ostreopsis* at the first sampling but then few occurred at later sampling dates. *Prorocentrum* occurred in low abundances but only when the *Ostreopsis* was not blooming, on all substrates. *Gambierdiscus* was only found to be present on two macroalgal samples, in low counts.

At the southern site (see Fig. 5), *Ostreopsis* was found to only occur in the two earlier samplings (April and July 2005), at low abundances. *Prorocentrum* conversely was only to be found in the later two samplings (November 2005 and April 2006), across all substrates at low to moderate abundances.

Gambierdiscus was only found in a single sample of *Padina*. Both similar north and south site ecosystems have a similar pattern to dinoflagellate presence, either the *Ostreopsis* or *Prorocentrum* occurring, generally without the other at different times.

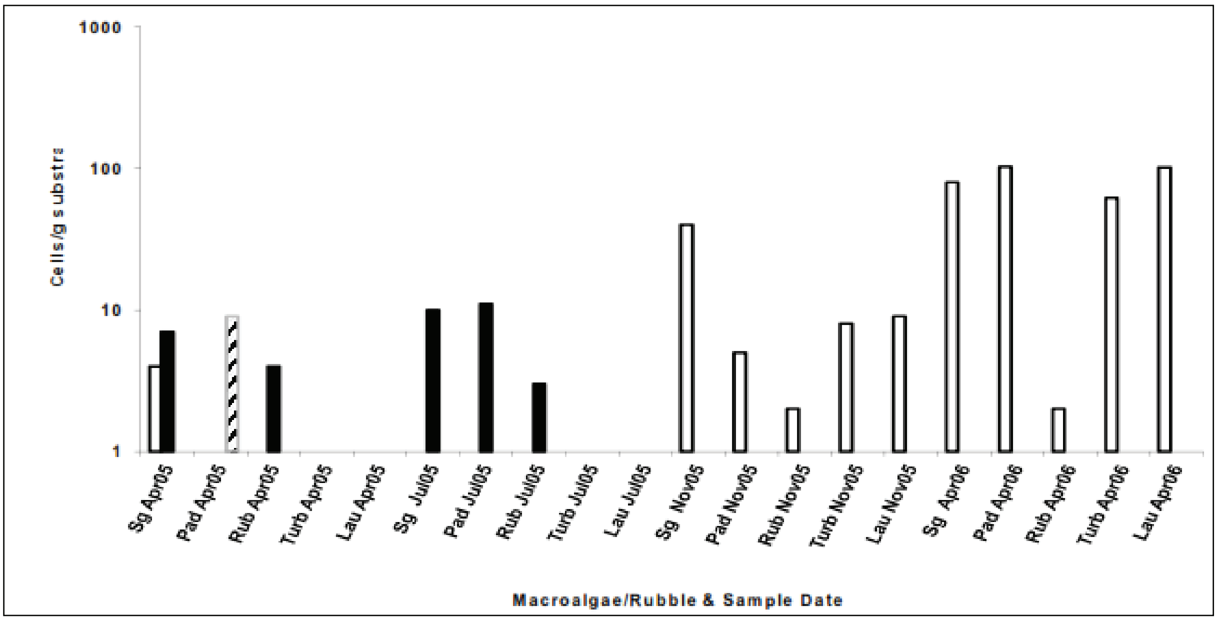

Figure 4. Abundance of the genera *Gambierdiscus* (Strip Bar), *Prorocentrum* (white bar) and Ostreopsis (black Bar) sampled from different macroalgal substrates (*Halimeda*, *Turbinaria*, and *Laurencia*), seagrass and rubble at the northern field site on Gili Trawangan in April, July, November 2005 and then April 2006.

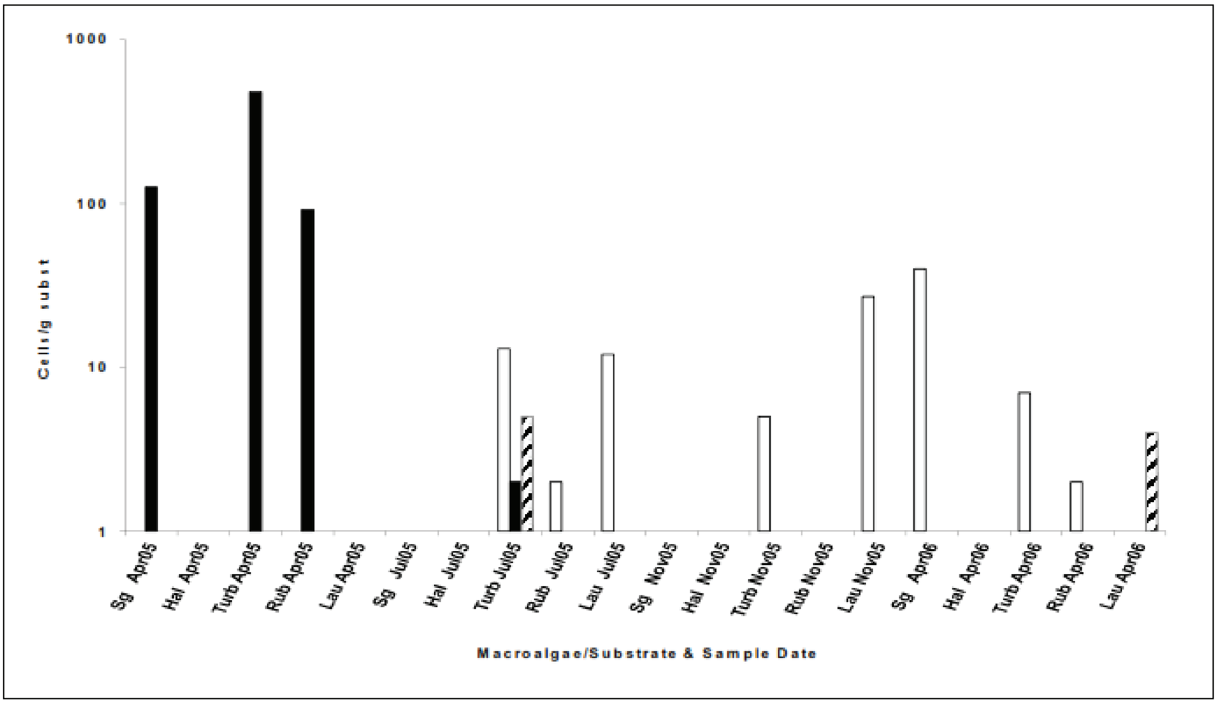

Figure 5. Abundance of the genera *Gambierdiscus* (Strip Bar), *Prorocentrum* (White Bar) and *Ostreopsis* (Black Bar) sampled from different substrates, sea grass, rubble and macroalgae (*Padina*, *Turbinaria* and *Laurencia*) in April, July, November 2005 and then April 2006 at the southern field site of Gili Trawangan.

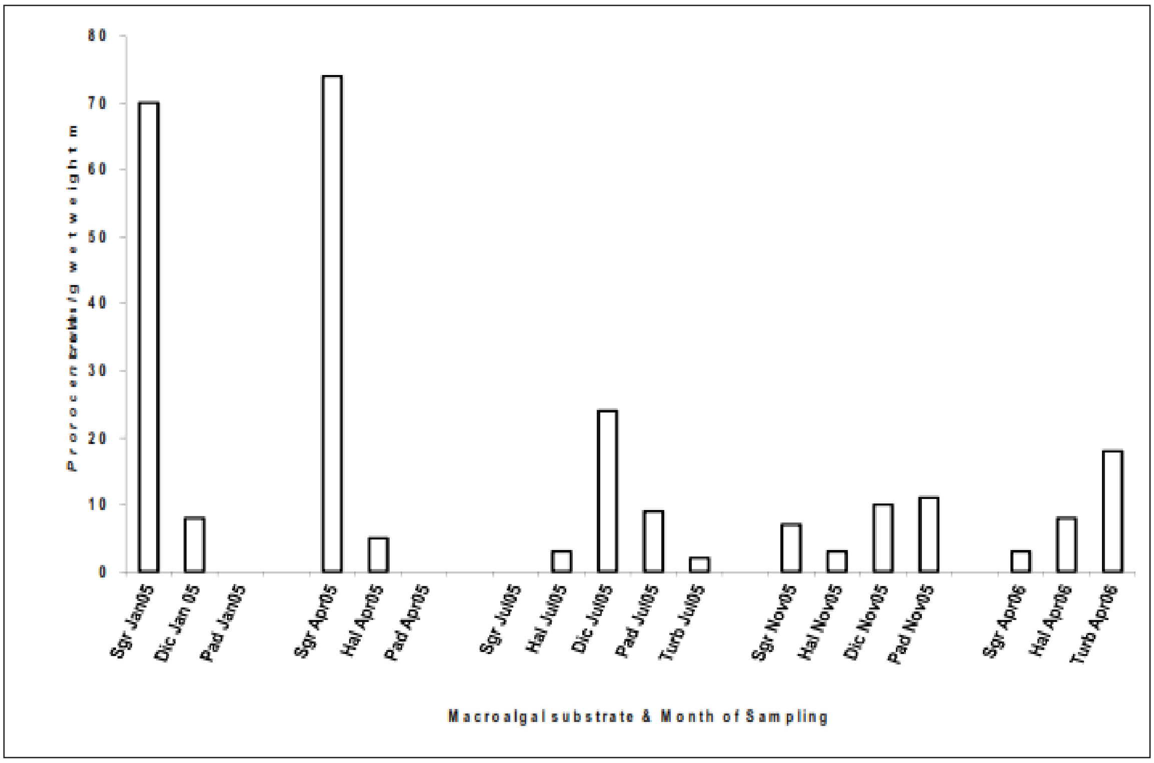

Figure 6. *Prorocentrum* abundance only (no other dinoflagellates present) from differrent substrates at the Eastern field site of Gili Trawangan in January, April, July, November 2005 and April 2006.

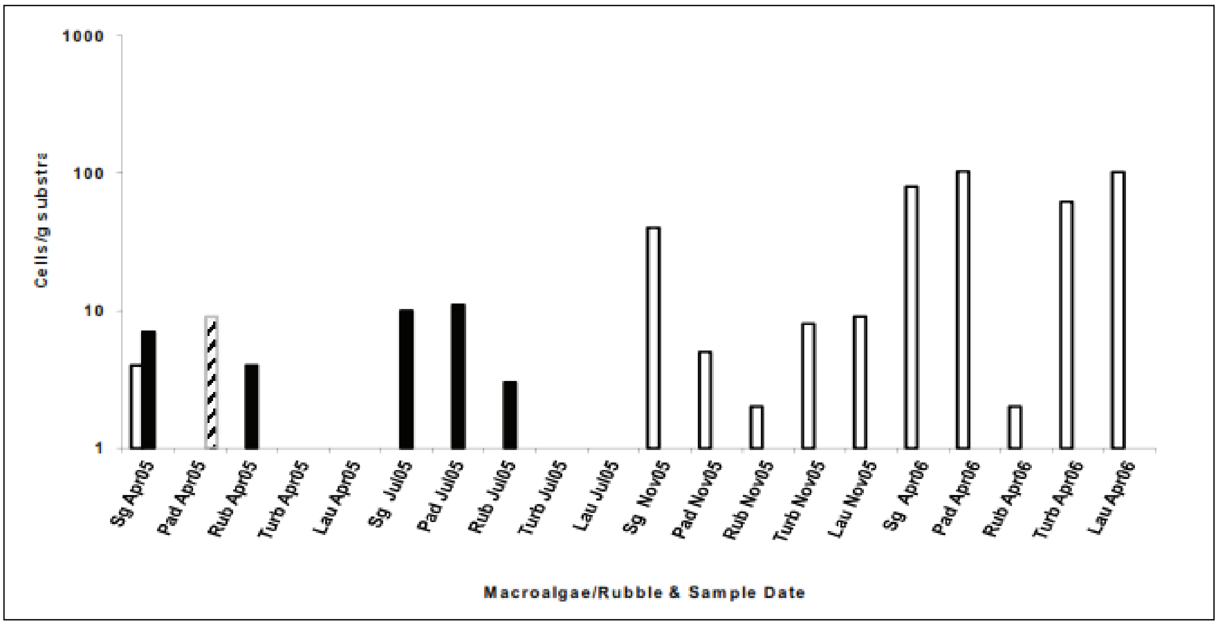

Figure 7. Dinoflagellate abundance amongst macroalgae *Padina* and *Halimeda*, and coral rubble at Kuta reef, Bali. Sampled monthly on 13 occasions, from November 2004 to March 2006. *Gambierdiscus* (strip bar), *Prorocentrum* (white bar) and *Ostreopsis* (black bar).

At the eastern site (see Fig. 6), in front of the village, on the southern side of the wharf, seagrass and a variety of macroalgae was found including *Dictyota*, *Halimeda*, *Turbinaria* and *Padina* but not all were found to be available at every sampling.

Ostreopsis and *Gambierdiscus* was not found at any sampling, yet *Prorocentrum* was found at moderate abundances amongst seagrass at the first two samplings and then at low abundances amongst most substrates on the later two samplings.

At Kuta reef, Bali (see Fig. 7), generally only the macroalgae *Halimeda* or *Padina*, was found at the site for sampling, all counts of dinoflagellates were low. *Gambierdiscus* was only found on 3 occasions, *Ostreopsis* twice and *Prorocentrum* on 7 of the 13 samplings at low abundances.

At the Sanur site (see Fig. 8), sea grass and Sargassum grow abundantly, whilst Padina and Turbinaria was found growing on most occasions and sampled when available. *Gambierdiscus* was only found on the first 2 samplings at low abundance, *Prorocentrum* was found from low to moderate abundances sporadically earlier in the sampling and then mostly at moderate abundances amongst all substrates, towards the latter half of sampling. *Ostreopsis* was found to bloom in October with a peak of 2,860 cells/g of *Sargassum*, and was found intermittently on other occasions at low to moderate abundance.

At the Nusa Dua (Geger beach) field site (see Fig. 9), seagrass and a variety of macroalgae were found to be growing at various times. No *Gambierdiscus* was found, *Prorocentrum* was found at low abundances, on most occasions and the only time *Ostreopsis* appeared, was as a bloom (600 cells/g *Sargassum*) amongst all the macroalgae samples in April 2006.

Nutrient concentrations

At Gili Trawangan there was a high degree of variability in the dissolved inorganic nitrogen, DIN, (ammonia & nitrogen oxides combined) levels in the water column within sites and between sites, sampled at the same time, ranging from 0.004 to 0.087 mg/L. Seven of the 12 samples exceeded the recommended (see Lapointe 1997) Nitrogen level (0.013 mg/L) for the growth of healthy coral reefs. Phosphorus levels were always at or below the levels of detection (0.003 mg/L).

At Kuta the water column DIN (ammonia and nitrogen oxides) readings, show a large range (from 0.002 to 0.07 mg/L), with the highest monthly readings at the end of the wet seasons and on 9 out of 13 samplings these readings were

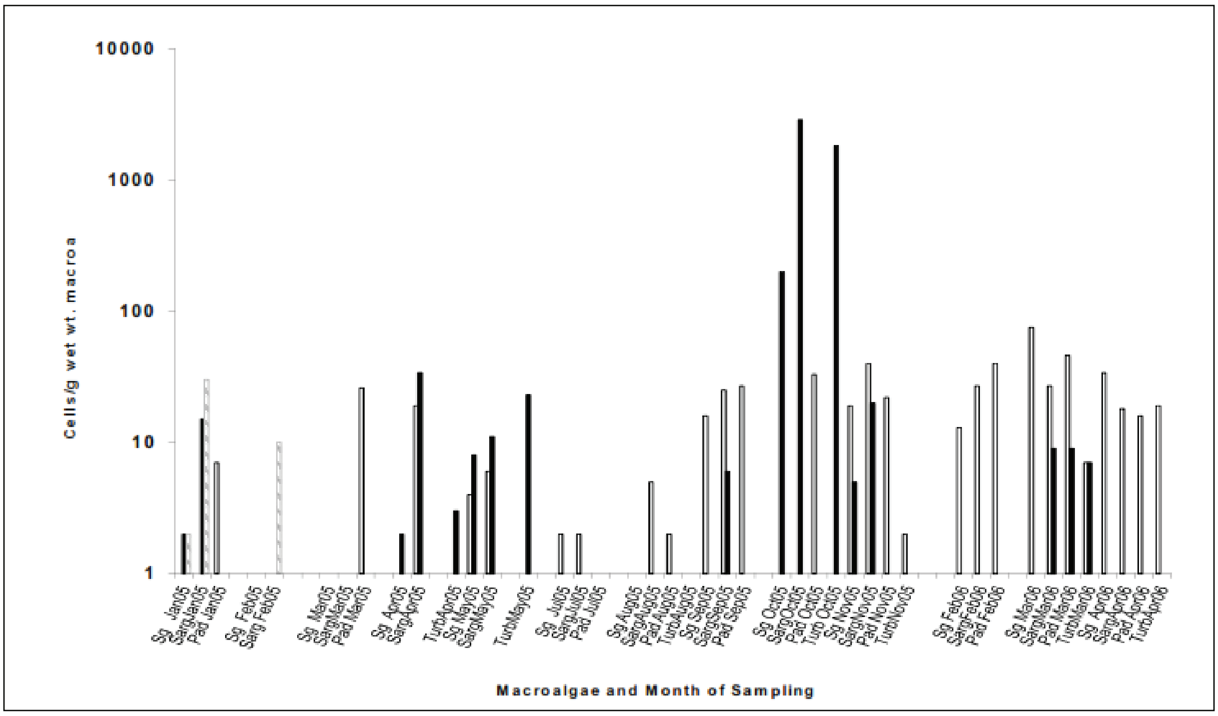

Figure 8. Dinoflagellate abundance at Sanur, amongst seagrass and the macroalgae (*Sargassum*, *Padina* and *Turbinaria*), sampled on 13 occasions from January 2005 to April 2006 (not including June, December 2005 and January 2006). *Gambierdiscus* (strip bar), *Prorocentrum* (white bar) and *Ostreopsis* (black bar).

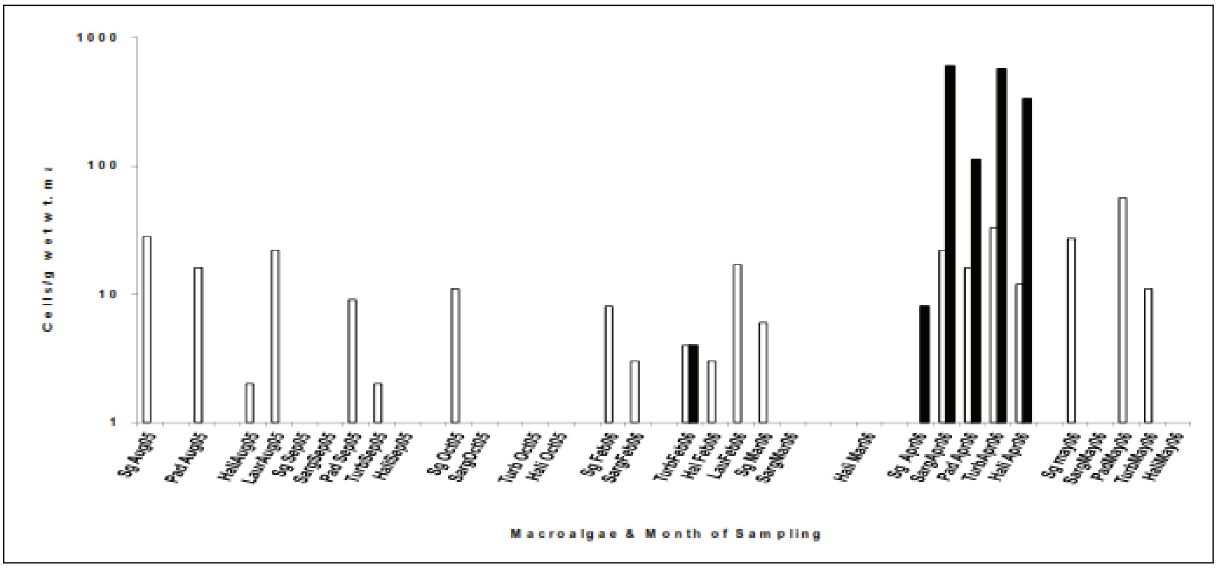

Figure 9. Dinoflagellate abundance sampled from seagrass and a variety of macroalgae at Geger lagoon, Nusa Dua, Bali. Sampled from August 2005 to May 2006 (not including December, January and February).

higher than what may be considered the critical concentration (0.013 mg/L) for healthy coral reefs. Total phosphorus readings ranged from 0.002 to 0.007 mg/L and on five occasions were over the critical level for phosphates. At Sanur, the water column, DIN ranged from 0.006 to 0.056 mg/L, and 7 out of 12 samples were over the critical level for healthy coral reefs. Phosphorus results rarely exceeded the levels of detection and on only one occasion was the level over the critical point. At Geger beach, Nusa Dua, water column DIN levels, ranged from 0.002 to 0.027 mg/L and 3 out of 7 samplings exceeded the critical level for healthy coral reefs. Phosphorus levels rarely exceeded detection levels and were only higher than the critical level on one sampling.

When DIN concentrations are averaged (see figure 10), for each site at Gili Trawangan and Bali, we can see that each of the sites has a higher level than the critical nitrogen level for healthy coral reefs, and at Gili Trawangan more than double for 2 out of the 3 sites. When averaged for the date of sampling on 3 out of 4 occasions the critical nitrogen level was exceeded by more than double the concentration.

Statistical analysis

At Geger Beach lagoon (Nusa Dua) there was a significant correlation (Pearson's, n = 7, p = 0.018) with *Prorocentrum* abundance from sea grass, *Halimeda* and *Padina*, with the concentrations of DIN in the water column. At Sanur there was a significant correlation with *Ostreopsis* abundance (from seagrass and *Sargassum*) and ammonia in the water column (Spearman's non parametric, n = 9, p = 0.033) and *Ostreopsis* and DIN in the water column (Spearman's, n = 9, p = 0.026). No correlations (n =4) were found between dinoflagellate abundance and water column nutrients on Gili Trawangan or at Kuta, Bali.

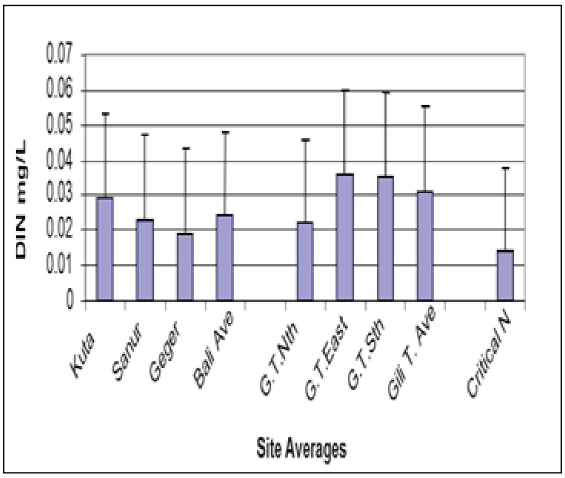

Figure 10. Averages for the DIN water column concentrations at the three sites in Bali: Kuta (n=13), Sanur (n=12) and Geger (n=7); and the three sites North, East & South at Gili Trawangan (n=4). The critical threshold for healthycoral reefs, 0.014mg/L for Nitrogen is shown at the right. Sampled between February and May 2006.

DISCUSSION

Increasing linkages between nutrient loading and coastal marine HABs have more recently been recognized (Heisler *et al.,* 2008). The evidence for marine eutrophication is unambiguous and direct; coastal waters globally, are becoming enriched with anthropogenic nutrients, termed "cultural eutrophication" (Smayda, 2008). A statistical correlation may not establish a causal link in the HAB-eutrophication hypothesis but the relationship suggests that coastal eutrophication may be a causative agent of change (Heisler *et al.,* 2008). This study has found significant correlations between the dinoflagellate genera *Prorocentrum*, and *Ostreopsis* with the DIN in the water column, where critical levels for nutrient thresholds were also exceeded. Thus nutrient thresholds, as set out for coral reefs, could be employed to establish a nutrophication level that might be expected to alter phytoplankton assemblage and to assist to create HABs.

Coral reef ecosystems have nutrient threshold concentrations set by the eutrophication threshold model (Lapointe, 1997), where eutrophication refers to situations where nutrient enrichment increased macroalgal growth rates to the extent that changes in the benthic community (coral to macroalgal phase shift) structure begin. The large changes in macroalgae growth rates upon coral reefs can be caused by very small variations in nutrient concentrations, around the nutrient threshold concentrations (Bell *et al.,* 1992). These same thresholds should be used for epiphytic (benthic) ciguatera related HABs, in coral reef ecosystems, due to their close mutualistic association with the macroalgae. When one considers the potential of toxic benthic HABs to impact on the "top down" herbivory trophic level, by way of toxins impacting sub lethally upon physiological functioning (like fecundity of fishes), the greater the impact of the 'bottom-up" eutrophication on the coral to macroalgal phase shift, as herbivory of macroalgae falls. Over time, less palatable macroalgae, combined with their epiphytic microalgae, tend to dominate inshore eutrophic reefs (Bell *et al.,* 1992). However a second phase shift needs to be considered in semienclosed water bodies such as island fringing reef lagoons with long retention times, and that is a further shift from macroalgal reefs to HAB reefs, where toxic cyanobacteria, toxic dinoflagellates and toxic diatoms, can induce conditions through ecotoxicological interactions, that establish a dominating influence over the ecosystem, for their own survival. The two sites, Geger beach at Nusa Dua and Sanur in this study are both fringing reef lagoons with high retentions times and with the episodic input of nutrients, there have been blooms of *Ostreopsis*. Chronic or episodic exogenous nutrients are often necessary for such high biomass HABs to be sustained or they can be sustained on nutrients that are regenerated and recycled (Heisler *et al.,* 2008). In this study we found correlations existed between dinoflagellate abundances (*Prorocentrum* and *Ostreopsis*) and water column nutrients (nitrates and ammonia), which could also be partly responsible for the presence of a bloom of *Ostreopsis*.

It is hypothesised that on coral reefs the expansion of macroalgae over the coral cover, attributed to major impacts such as coral bleaching (global climate change), crown of thorns, eutrophication or cyclones, etc., could also be attributed to the presence of epiphytic dinoflagellates and their toxins, that can be responsible for the decline of not only herbivory by fish and invertebrates but also impact upon biodiversity of the coral reef ecosystem as a whole. Dinoflagellate toxins are a stressor to coral reef ecosystems that has been ignored by coral reef scientists for far too long, with the blame of decreased herbivory mainly attributed to over fishing and destructive fishing practices. Both ciguatoxins produced by *Gambierdiscus* and okadaic acid produced by *Prorocentrum* produced toxins are known to have sublethal impacts upon fish, eggs and hatchlings and fish larval development (Capra *et al.,* 1988, Edmunds *et al*., 1999, Escoffier *et al.,* 2007, Ajuzie 2008). The post ingestive release of toxic compounds from dinoflagellate prey cells in fish can result in physical incapacitation, reduction in fecundity, depressed viability of gametes or mortality of the predator (Cembella, 2003). If there are enough toxin pulses coming from continuous moderate to high densities of epiphytic dinoflagellates and combined with a continuous slow trickle of similar toxins, from other genera, who are often present but in low quantities that pass through the food chain of the coral reef ecosystem, then it is probable that the organisms within the related food webs are being impacted upon. Whenever and whatever the major cause of phase shift from coral to macroalgal dominance occurs, it is certain that the toxic CFP suite of dinoflagellates are present. Tropical benthic dinoflagellate communities, that can inhabit a variety of substrates, include numerous toxic epiphytic genera, which are inadvertently consumed by benthic browsers and filter feeders, so that reef dwelling organisms have the potential to be exposed to numerous toxins and the combinations of toxins that are potentially available to higher trophic levels is considerable (Landsberg, 2002). This is apparent when one considers the numerous combinations of symptoms that may occur with ciguatera fish poisoning.

Benthic dinoflagellates of the genus *Ostreopsis* are often found as epiphytic on sea grasses, turf algae and macroalgae and amongst coral debris and sediments from coral reefs. Nine species of *Ostreopsis* have been described (Fukuyo 1981, Faust *et al.,* 1996, Faust, 1999). Five species are known to produce toxic compounds and all *Ostreopsis* species are potentially toxic as the other four species have not been examined for toxins. Toxic compounds produced by *Ostreopsis* species include ostreotoxins (OTX), described as neurotoxic compounds, which were isolated from a Caribbean strain of O. lenticularis (Tindall *et al.,* 1989, Mercado *et al.,* 1994, Meunier *et al.,* 1997) and Ostreocins, palytoxin analogues, isolated from *O. siamensis* (Usami *et al.,* 1995, Ukena *et al.,* 2002, Rhodes *et al.,* 2000). Extracts of clonal cultures of *O. heptagona* were toxic to mice (Norris *et al.,* 1985). *O. ovata* analysed for toxic activity showed the presence of palytoxin. (Penna *et al.,* 2005, Ciminiello *et al.,* 2006). The production of new analogues of palytoxin (McTX) was also recorded from a benthic bloom of the dinoflagellate *O. mascarenensis* which has the same habit as *O. siamensis* and could be grazed by fishes and its toxins accumulated throughout the food webs, including humans (Lenoir *et al.,* 2004).

Palytoxin was confirmed as the causative agent in human seafood poisoning through the consumption of mackerel (Kodama *et al.,* 1989), parrotfish (Taniyama *et al.,* 2003), triggerfish (Fukui *et al.,* 1988) and benthic feeding sardines (Onuma *et al.,* 1999). Toxic *Ostreopsis* genrea are suspected to be a source of palytoxin in seafood poisoning, just as clupeotoxism, a potentially fatal human intoxication is caused by ingestion of clupeoid fish (Onuma *et al.,* 1999). Further studies are necessary to confirm the relation between toxic *Ostreopsis* species and palytoxin seafood poisonings (Lenoir *et al.,* 2004). The involvement of benthic dinoflagellates of the genus *Gambierdiscus* in CFP is confirmed, the role of the genus *Ostreopsis* in palytoxin poisoning remains to be fully elucidated (Lenoir *et al*., 2004).

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