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Green Tide-Causing Species in Northern Mindanao, Philippines: Taxonomic Profiling and Morphological Descriptions

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ABSTRACT.– "Green tide", or green macroalgal bloom, is increasingly prevalent in many localities around the world where it could contribute to ecological imbalance in coastal ecosystems. An outbreak was reported in the coastal waters along Macajalar Bay at El Salvador, Misamis Oriental, Philippines. Thus, examination and determination of the composition of the green benthic macroalgae species associated with "green tide" events in the area were conducted to better understand the origin and persistence of "green tide" blooms there and to provide initial information concerning the dynamics of the blooms. Four species of green algae, composing the "green tide" species, were identified from the study area, namely: *Cladophora vagabunda*, *Ulva clathrata, U. intestinalis*, and *U. reticulata*. Members of the genus *Ulva* often dominate the mass accumulation of fast-growing ephemeral algae frequently reported worldwide; this is one of the few reports of *Cladophora* species causing noxious marine blooms.

KEY WORDS: **Algal blooms, *Cladophora*, eutrophication, marine pollution, *Ulva***

INTRODUCTION

The last two decades have seen numerous reports of "green tides" from many countries around the world. A few of these reports have captured international attention and concern like in the case of the algal bloom in China which threatened to disrupt the yachting competition of the 2008 Summer Olympic Games. Leliaert *et al.* (2009) attributed the bloom in Qingdao Bay to a single yet polymorphic strain of the green alga *Ulva* by means of molecular tools. Regardless of scale and geography, "green tides" often occur as vast accumulations of monospecific green macroalgal biomass that multiply uncontrollably under environmental conditions suitable to them (Blomster *et al.*, 2002) and become dominant and overwhelming with biomass accumulation amounting to as high as 27 kg wet weight/m^2 (Fletcher, 1996). "Green tide" outbreaks have been most often associated with marine and estuarine areas with high concentration of nutrients (Calumpong & Meñez, 1997; Goshorn *et al.*, 2001) and which could promote diel hypoxia especially in estuaries adversely affecting aquatic life there (Valiela *et al.*, 1997). Increasing or altered availability of inorganic nutrients (especially N and P) due to anthropogenic activities particularly in coastal areas is known to stimulate the abundance of green macroalgae in shallow coastal waters (Sand-Jensen & Borum, 1991; Duarte, 1995; Valiela *et al.*, 1997) creating conditions that interfere with the health and diversity of indigenous fish, plant, and animal populations (MAIA, 2003). Mass accumulation is most often due to the fast-growing ephemeral foliose or uniseriate green filamentous algae belonging to the genera *Ulva*, *Enteromorpha*, or *Cladophora* (Fletcher, 1996; Raffaelli *et al.*, 1998).

In the Philippines, "green tide" was probably first reported to occur in the shallow waters of Macajalar Bay in El Salvador, ca. 18 km west of Cagayan de Oro City (Reyes, 1999). Another outbreak of "green tide" was investigated by Largo *et al.* (2004) from the central Philippines. More reports of "green tide" outbreaks will be likely be seen in various parts of the Philippines as increasing and more frequent occurrences are documented in many countries around the world.

Macajalar Bay off the northern coast of Mindanao is a large marine ecosystem that is subjected to anthropogenic threats derived mainly from unregulated agro-industrial development along its shoreline and where episodes of algal blooms have been reported more frequently. Although rapid industrialization in the area is a boon to economic progress, it is subjecting the Bay to increasing and adverse environmental pressures. As the human population near the coasts grows, potential threats to human health and productivity of coastal waters increase. Energy generation practices, transportation, water treatment, all have the possibility to transport toxic compounds to local waterways, either directly or indirectly, which consequently increase nutrient load, fertilizer and pesticide run-offs and atmospheric deposition along coastal waters. Although "green tide" is of lesser notoriety than the red tide caused by toxic dinoflagellates, it is yet another item in the growing list of human-induced threats to the integrity and sustainability of coastal marine ecosystems (Reyes, 1999).

Little is known about the "green tide" phenomena along Macajalar Bay, specifically at El Salvador, Misamis Oriental. It is obviously desirable to have a better understanding of the origin and persistence of "green tide" blooms in order to address the problems they cause (Blomster *et al.*, 2002). For one, taxonomic identification of the causative algae can provide information concerning the ecological dynamics of the blooms (Coat *et al.*, 1998; Blomster *et al.*, 2002). According to Karez *et al.* (2004), the stimulation of algae with increasing nutrient richness is related to eco-physiological traits (such as growth rates, nutrient requirements and uptake rates). This study, therefore, aimed to examine and determine the species composition of the green benthic macroalgae associated with "green tide" events in the intertidal zone of Macajalar Bay, particularly in El Salvador, Misamis Oriental which was first reported by Reyes (1999). Such information will be useful to come up with informed management decisions. In this regard, the data could help in economic and social policy-making decisions regarding the management and protection of the marine resources of the Bay by predicting bloom dynamics involving particular green algal species.

MATERIALS AND METHODS

Study area

The study was conducted in El Salvador, Misamis Oriental (between 124°30'40" and 124°31'00" E longitude and between 8°34'15" and 8°34'40" N latitude) on the western portion of Macajalar Bay (Fig. 1) on the northern coast of Mindanao Island. It has an intertidal area of about 10-15 km^2, which is predominantly composed of dead

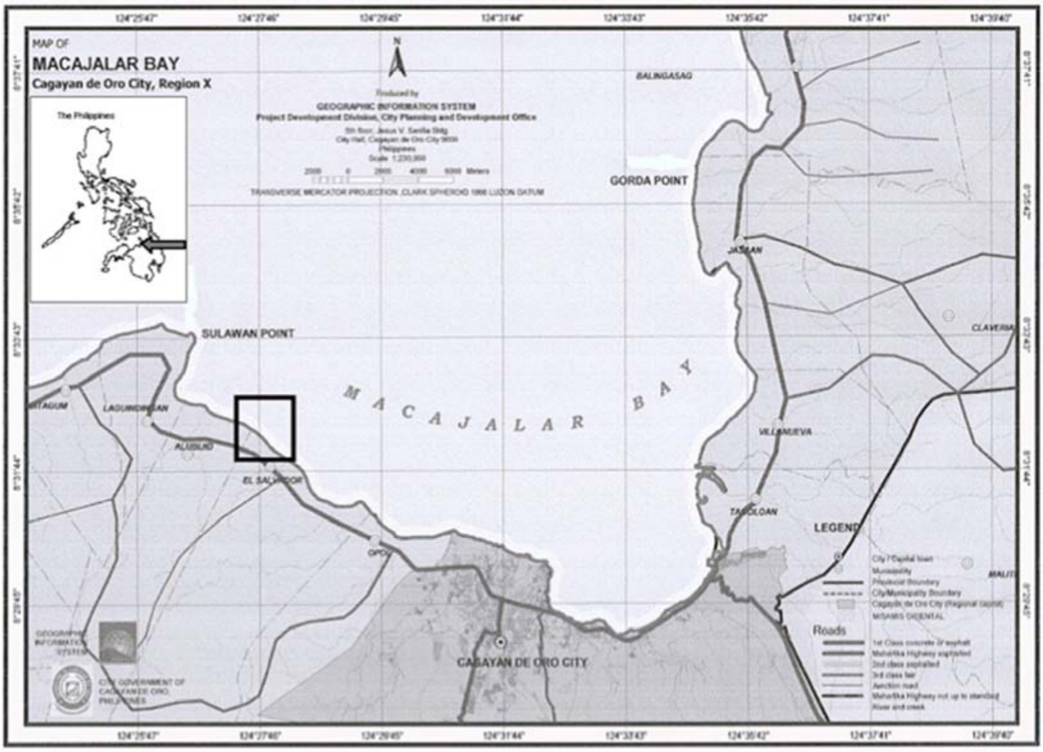

FIGURE 1. Macajalar Bay showing the study site (square box) at El Salvador, Misamis Oriental province (Source: KVR Satellite 1992, City Assessment, Land Management Division, Department of Environment and Natural Resources and the City Engineering Office, 2000)

corals distributed over patchy sandy, sandy-muddy, sandy-rocky and muddy substrates. Residential houses and medium-sized industries can be found adjacent to the study site. In addition, the area is <50-100 m away from the major highway. A very large industrial establishment can be found towards the uphill portion several meters from the survey site.

Collection and identification of green tide-causing species

Fresh samples of green tide-causing species were collected by hand during low tide and immediately placed in self-sealing plastic bags, labeled and brought to the laboratory for identification. Specimens were identified down to the species level based on external morphology, color, size, and anatomical structures such as shape and arrangement of surface cells with the help of taxonomic books and comparison with authentic specimens. Photo-documentation of fresh samples and anatomical sections of samples were taken using a digital camera. Vouchers were prepared as herbarium sheets and deposited in the Department of Biology, Xavier University-Ateneo de Cagayan.

RESULTS

Four species of green algae have been identified from the study area as comprising

FIGURE 2. *Cladophora vagabunda* (A1-3): habit (1), branches with fasciculate filaments (2), detail of filaments with cross-walls (3); *Ulva clathrata* (B4, 5): habit (B4), detail of thallus showing multiseriate main axis and uniseriate branchlets (5); *Ulva intestinalis* (C6-8): habit (c6), close-up of unbranched tubular thalli with constriction at certain points (7), surface view of polygonal cells filled with chloroplasts (8); *Ulva reticulata* (D9-11): habit (D9), surface view of blade showing smooth margin (10), closer view of roundish surface cells with chloroplasts (11)

the bulk of "green tide" biomass, namely: *Cladophora vagabunda*, *Ulva clathrata*, *Ulva intestinalis,* and *Ulva reticulata*. The detailed descriptions of each species together with some remarks follow below.

Order Cladophorales
Family Cladophoraceae

Cladophora vagabunda (Linnaeus) van den Hoek **(Fig 2. A1-3)**

Nizamuddin & Begum 1973: 7, Fig. 4 (as *Cladophora fascicularis*); Tseng 1983: 258, pl. 128, Fig. 4 (as *Cladophora fascicularis*); van den Hoek & Chihara 2000: 183, Fig. 76, 187, Fig. 79E.

This species is a grass-green seaweed with a bushy appearance produced by many long filaments that produce clusters of short branches (0.5-5 cm) that tend to be produced unilaterally in one direction. Branching filaments are often spaced distantly and sparingly. Main axis stem-like; filaments erect, sparsely branched at short intervals. Branches short, di-, tri-, or polychotomous, opposite or alternate. Branchlets short, di-, or polychotomous (2-6 branches), straight or upper part slightly curved, densely fasciculate. Ramuli single or paired from each articulation, 1-2 segmented, straight; apices obtuse. Upper branchlets 1-3 segmented.

Order Ulvales
Family Ulvaceae
Ulva clathrata Roth **(Fig 2. B4, 5)**
Saifullah & Nizamuddin 1977: 530, pl. IV D (as *Enteromorpha clathrata*); Koeman & van den Hoek 1984: 54, Fig. 146 (as *Enteromorpha clathrata*); Trono 1997: 6, Fig. 1 (as *Enteromorpha clathrata*)

Thallus grass green, forming dense, soft, delicate and fleece-like mats, composed of fine, hollow, cylindrical filaments to about 40 cm in height, up to 2 mm in diameter; branching profusely. Rectangular or polygonal surface cells are arranged in longitudinal rows. Determinate branchlets common toward the tips, multiseriate below but becoming uniseriate at or near their tips. Found frequently entangled among each other or with other algae.

Ulva intestinalis Linnaeus **(Fig 2. C6-8)**
Saifullah & Nizamuddin 1977: 527, pl. III C, 529, #25 (as *Enteromorpha intestinalis*); Koeman 1985: 85, Fig. 78 (as *Enteromorpha intestinalis*); Trono 1997: 8, Fig. 2 (as *Enteromorpha intestinalis*)

Thallus is bright to yellowish green in color. Its several unbranched tubular branches arise from a common short stipe with discoid holdfast. Branches are erect, tapered below, inflated above, constricted or contorted at certain points, 6 to 20 cm long, up to 10 mm wide. Cells are polyhedral from surface view, irregularly arranged and filled with chloroplasts.

Ulva reticulata Forsskål **(Fig 2. D9-11)**
Segawa 1974: 3, pl. 2.10; Saifullah & Nizamuddin 1977: 523, pl. I A, 524, #2; Calumpong & Meñez 1997: 101

Thallus is light to dark green characterized by reticulate blades with holes of various sizes and shapes, blade margins smooth. Cross-section of the blade shows two-cell layers (distromatic) with cells containing many chloroplasts most of which are concentrated on the side close to the surface of blade.

DISCUSSION

The occurrence of algal blooms has been documented for at least a century. In what was one of the earliest reports, Williamson (1907) noted a species of *Ulva* as thriving at the sewage system discharge outlet in Belfast and emitting pungent odors upon decomposition. He demonstrated a remarkable understanding of the link between the proliferation of the seaweed and the nutrients that came with the sewage discharge when he said “the effluent, whilst clear to the eye, contains the food for the *Ulva latissima* Linnaeus in the form of nitrates, and so long as the effluent contains these it will, when mixed with sea water, nourish the *U. latissima*, and therefore will have no tendency to reduce the objectionable deposit on the foreshore.” His early views of this 20th century environmental menace are supported to this day.

Typically, problematic “green tides” and other macroalgal blooms are monospecific (Valiela *et al.*, 1997), although species composition may vary depending upon prevailing environmental conditions (Lotze & Schramm, 2000). In this study, four species belonging to two genera (*Cladophora* and *Ulva*) have been identified to compose the bulk of the “green tide” in the study area in approximately mixed and equal proportion. These green macroalgal genera are known most often to dominate the mass accumulation of fast-growing ephemeral algae (Fong *et al.*, 2004; Karez *et al.*, 2004; Fletcher, 1996; Sanderson, 1997) in widespread locales such as southern California (Kamer & Fong, 2001), New England, Western Australia, Scotland, Italy (Fong *et al.*, 1996), Portugal (Martins *et al.*, 1999) and more.

In addition, monospecific "green tides" have been documented in similarly widespread areas worldwide. For example, species of *Ulva* have been reported to be responsible for blooms on the west coast of Finland (Blomster *et al.* 2002, as *Enteromorpha*), in several bays around Japan (Shimada *et al.*, 2003; Kawai *et al.*, 2007), and recently in central Philippines (Largo *et al.*, 2004). Furthermore, species of *Cladophora* have been increasingly blamed for blooms in New England (Fong *et al.*, 1996), Russia (Vershinin & Kamnev, 2000), Mediterranean Spain (Menéndez & Comín, 2000) among other localities. Other green algae such as species of *Bryopsis* and *Chaetomorpha* have been recently reported to occur as blooms and should require further monitoring (Liu *et al.* 2007; Teichberg *et al.* 2012; Deng *et al.* 2014).

Much has been written about the negative impacts of macroalgal blooms, specifically "green tides" ranging from altering biogeochemical cycles in coastal environments (Valiela *et al.*, 1997) to inhibiting photosynthesis and growth on shaded seagrass beds (Brun *et al.*, 2003) and disrupting the feeding of wading birds due to dense nuisance algal biomass (Raffaelli *et al.*, 1998). The world's largest macroalgal bloom off the coast of eastern China was triggered ironically by weedy *Ulva prolifera* O.F. Müller [as *Enteromorpha prolifera* (O.F. Müller) J. Agardh] growing on near-shore seaweed aquaculture nets which are discarded during the harvesting of the green laver, *Pyropia yezoensis* (Ueda) M.S. Hwang & H.G. Choi [as *Porphyra yezoensis* Ueda]. According to Liu *et al.* (2009), large biomass of these discarded green algae formed small patches visible in satellite photos and drifted northwards where at one time, it threatened the 2008 Summer Olympics yachting competitions as initially reported by Leliaert *et al.* (2008). Using more powerful molecular tools and better sampling, Duan *et al.* (2012) identified the main bloom forming species to belong to the *Ulva linza-procera-prolifera* clade. But despite the notoriety of these "green tide" blooms, some little-known positive ecological outcomes have been reported. For example, the abundance of nuisance algal biomass exerted a marked influence on water flow over marine sediments including particulate material flux across the sediment-water interface. In a laboratory study of the interaction of *Ulva* biomass with sediment dynamics conducted by Romano *et al.* (2003), it was found that at 10% algal cover on bare sediments, there was a 60% reduction in sediment erosion. This value increased to 90% reduction with 60% algal cover.

Species of *Ulva* have been studied for various potential applications with important implications for environmental remediation. Alamsjah *et al.* (2008) have isolated polyunsaturated fatty acids from two *Ulva* species and tested the same on harmful blooms of microalgae with positive algicidal results. Tang & Gobler (2011) tested some extracts from *Ulva* against some common harmful algal bloom species and reported rapid lysis in three notorious red tide-forming dinoflagellate species namely, *Prorocentrum micans* Ehrenberg, *P. donghaiense* D. Lu ex Lu & Gooebel and *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara & Chihara. The growth of two other red tide species, the dinoflagellate *Alexandrium tamarense*

(Lebour) Balech and the diatom *Chaetoceros gracilis* Pantocsek were inhibited when added with *Ulva* extracts (Nan *et al.*, 2004). This mode of action has been attributed to allelopathy caused by heat-stable allelochemicals in *Ulva* (Tang & Gobler, 2011). Desiccated forms of two water-soluble extracts from ulvoid species caused the inhibition of growth of some bivalve larvae (Nelson *et al.*, 2003).

The biosorption of heavy metal pollutants dissolved in sea water is well studied using brown algae because of the binding affinity of their cell wall alginates with these elements. However, recent studies have shown that non-living biomass of *Ulva* in the form of powders can adsorp Cu, Zn and Cd in aqueous solution as a function of pH, contact time, initial concentrations and adsorbent size (Kumar *et al.*, 2006, 2007). In a double-edged move, Suzuki *et al.* (2005) proposed the harvesting of *Ulva* biomass from coastal areas infested with algal blooms and use it as heavy metal biosorbent. By so doing, the removal of seaweed biomass does not lead to another environmental problem of waste disposal as initially feared.

In an attempt to harness the eco-physiological preference for eutrophicated conditions among *Ulva* species, living biomass has been tapped as biofilters in Integrated Multi-trophic Aquaculture (IMTA). While most IMTA set-ups require high-tech and costly investment, an example from Tanzania used indigenous gravity generated water flow systems instead of electrical pumps with *Ulva reticulata* Forsskål as fishpond effluent biofilter. The growth and yield of *Ulva* in the IMTA experiment nearly doubled while the effluents improved considerably as shown in the increase of dissolved oxygen concentration from 5.6 mg L^{-1} to 13.4 mg L^{-1} and pH from 7.8 to 8.4 (Msuya *et al.*, 2006).

Good taxonomy of bloom forming species certainly has important implications towards the understanding of “green tide” dynamics. Blomster *et al.* (2002) documented some changes in gross morphology of such species under eutrophicated conditions. Morphological changes can also be induced by altered nutrient supply when seaweed becomes nutrient sinks (Valiela *et al.*, 1997) and by fluctuations in salinity (Reed & Russell, 1978).

The growing use of powerful molecular tools can contribute towards a stable and reliable taxonomic approach in discriminating “green tide” species (e.g., Blomster *et al.*, 2002). Molecular approaches are clearly superior over morphological methods especially when dealing with morphologically plastic species of *Ulva*. In a survey of *Ulva* species in a medium-sized bay (Mikawa Bay) in Japan, Kawai *et al.* (2007) found nine species using ITS2 rDNA sequencing including two new records for Japan. The recent finding of two new records in a country that has one of the best known marine flora brings to the fore serious issues on intercontinental species introductions using such avenues as ballast waters of ships (Flagella *et al.*, 2007) and hitchhiking species on ships’ hulls (Blomster *et al.*, 2000).

It is the goal of the researchers to employ molecular methods alongside morphological approaches for “green tide” species found in the Philippines. For now, the species reported herein are sufficiently different and can be discriminated satisfactorily using morphological methods. However, the prospects of cryptic species among them cannot be totally discounted.

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