



## SYMPOSIUM

### Environmental Chemistry and Chemical Ecology of “Green Tide” Seaweed Blooms

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**Synopsis** Green tides are large growths or accumulations of green seaweeds that have been increasing in magnitude and frequency around the world. Because green tides consist of vast biomasses of algae in a limited area and are often seasonal or episodic, they go through periods of rapid growth in which they take up large amounts of nutrients and dissolved gases and generate bioactive natural products that may be stored in the plants, released into the environment, or broken down during decomposition. As a result of the use and production of inorganic and organic compounds, the algae in these blooms can have detrimental impacts on other organisms. Here, we review some of the effects that green tides have on the chemistry of seawater and the effects of the natural products that they produce. As blooms are developing and expanding, algae in green tides take up inorganic nutrients, such as nitrate and ortho-phosphate, which can limit their availability to other photosynthetic organisms. Their uptake of dissolved inorganic carbon for use in photosynthesis can cause localized spikes in the pH of seawater during the day with concomitant drops in the pH at night when the algae are respiring. Many of the algae that form green-tide blooms produce allelopathic compounds, which are metabolites that affect other species. The best documented allelopathic compounds include dimethylsulfoniopropionate (DMSP), dopamine, and reactive oxygen species (ROS) and their breakdown products. DMSP and dopamine are involved in defenses against herbivores. Dopamine and ROS are released into seawater where they can be allelopathic or toxic to other organisms. Thus, these macroalgal blooms can have harmful effects on nearby organisms by altering concentrations of nutrients and dissolved gas in seawater and by producing and releasing allelopathic or toxic compounds.

#### Introduction

Green tides are large growths or accumulations of seaweeds in the Phylum Chlorophyta (Ye et al. 2011; Smetacek and Zingone 2013). Although these blooms occur in temperate and tropical habitats around the world, the areas most impacted are the eastern and western coasts of North America, Europe, and the Asia-Pacific region (Ye et al. 2011). In recent years, green tides have received increasing attention because of well-publicized blooms in China and France. In China, blooms of *Ulva prolifera* have been occurring in the Yellow Sea and accumulating along the coastline seasonally since 2007 (Ye et al. 2011; Liu et al. 2013; Zhao et al. 2013). They received international attention in 2008 when a

bloom of >1 million tons covered an area of 13,000–30,000 km<sup>2</sup> a few weeks before the Olympic sailboat competitions were due to occur there (Leliaert et al. 2009; Ye et al. 2011). Blooms of *Ulva* spp. have been occurring in Brittany, France since the 1980s where they accumulate to depths of up to a meter (Charlier et al. 2007). In 2009 and 2011, hydrogen-sulfide gas resulting from the blooms' decomposition was reported to cause the death of 1 horse and 30 wild boars (Smetacek and Zingone 2013).

One of the results of this increased attention is an increased interest in the causes and consequences of green tides. Although many factors affect the formation of blooms, inputs of nutrients are a common contributor and many studies have examined the

effects of nutrient inputs on their formation (reviewed by Valiela et al. 1997; Teichberg et al. 2010). Their consequences can include economic effects, such as deterring tourists from visiting beaches and interfering with aquacultural operations (Smetacek and Zingone 2013; Lyons et al. 2014); and ecological effects resulting from changes to the physical environment, such as altering the flow of seawater and decreasing availability of light for other algae and plants (Valiela 1997; McGlathery 2001; Ye et al. 2011).

Less well-known are the impacts that green tides have as a result of their use and production of inorganic and organic chemicals (Frankenstein 2000; Nelson et al. 2003a). By definition, algal blooms are large biomasses of photosynthetic organisms that are concentrated in a defined area. Many blooms are episodic or seasonal, especially in temperate areas. Thus, there is an early period when the algae are rapidly growing, followed by a later period of senescence and decomposition. During the periods of rapid growth, algae take up inorganic macronutrients and micronutrients, fix carbon via photosynthesis, and produce organic molecules that are used to generate structure, sustain physiological processes, and mediate ecological interactions. During senescence, organic molecules are released or are broken down by detritivores and decomposers. Both the removal of inorganic compounds from seawater and the release of metabolites affect the chemistry of the seawater surrounding the plants and other organisms inhabiting these areas.

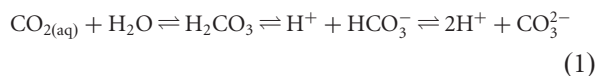
The purpose of this article is to review some of the consequences of green tides through their uptake and release of dissolved gases and nutrients and their production of allelochemicals and toxins. Specifically, we focus on three processes likely to have significant impacts on other organisms: (1) alterations in oxygen (O<sub>2</sub>) and dissolved inorganic carbon (DIC) resulting from photosynthesis and respiration, (2) reductions in dissolved nitrogen, the nutrient that most frequently limits the growth of marine macrophytes and phytoplankton, and (3) the production of allelochemicals and toxins, which potentially affect organisms feeding on, or living on or near, these algae. We also present new information on the synthesis of allelochemicals and the chemistry of seawater at sites with green tides. Although the term “green tides” can describe high densities of many macroalgae and even some microalgae, we will focus on the most commonly reported green tide algae, members of the Order Ulvales. Within this order, algae in the genera *Ulva*, *Ulvaria*, *Monostroma*, *Percursaria*, and *Blidingia* can form green tides.

## Alterations to the chemistry of seawater

### Dissolved gases

Anthropogenic inputs of nutrients into watersheds and oceans, and carbon dioxide (CO<sub>2</sub>) into the atmosphere, can alter the chemistry of oceans and affect the growth of marine macrophytes. Nutrient-loading often increases primary production by phytoplankton, macroalgae, and seagrasses (Valiela et al. 1992; Raven and Taylor 2003; Anderson et al. 2008), and the species that increase depend on the degree of eutrophication (Harlin 1993; Burkholder et al. 2007). While these organisms are net generators of O<sub>2</sub> when they are growing, they can cause hypoxia during periods of reduced photosynthesis, such as at night or when populations undergo senescence and decompose (Diaz and Rosenberg 1995; Diaz 2001).

Atmospheric CO<sub>2</sub> is absorbed by the oceans where it alters the chemistry of DIC. CO<sub>2</sub> in seawater undergoes a series of reversible reactions (1) that generate hydrogen ions (H<sup>+</sup>), causing a decrease in the pH of seawater as aqueous CO<sub>2</sub> concentrations increase (Doney et al. 2009):



At the current average oceanic pH of 8.1, 90% of DIC is bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) and 9% is carbonate ion (CO<sub>3</sub><sup>2-</sup>). If atmospheric CO<sub>2</sub> increases at predicted rates, by 2100 H<sup>+</sup> concentrations will increase 150%, causing the average oceanic pH to decrease by 0.3–0.4 units. CO<sub>3</sub><sup>2-</sup> will decrease by 50% and the relative proportions of HCO<sub>3</sub><sup>-</sup> and aqueous CO<sub>2</sub> will increase (Orr et al. 2005). It is important to note that these processes do not occur in isolation and other factors impact these reaction products on a variety of time scales. In coastal ecosystems, the pH of seawater is also affected by biological production, upwelling, freshwater inputs, and changes in land-use patterns that affect biogeochemical cycling (Duarte et al. 2013; Baumann et al. 2014). On local scales, the impacts of these processes on seawater pH and DIC concentrations may be larger than the impacts of atmospheric CO<sub>2</sub> (Duarte et al. 2013).

Large growths of primary producers, such as green tides, can alter concentrations of dissolved gases through the uptake and release of DIC and O<sub>2</sub> during photosynthesis and respiration. Marine plants, animals, and microbes take up O<sub>2</sub> and release CO<sub>2</sub> for cellular respiration during the day and at night. However, during the day, actively-growing plants, algae, and microbes that photosynthesize can generate O<sub>2</sub> and cause a net release of O<sub>2</sub> into seawater.

They also take up carbon, either directly as  $\text{CO}_2$  or as  $\text{HCO}_3^-$  if they possess carbon-concentrating mechanisms (CCMs; Raven and Beardall 2003; Raven et al. 2008). Thus, during the day, photosynthetic organisms can remove more carbon than they generate through respiration (although this may not be true during extreme physiological stress or senescence when photosynthesis is reduced [Quadir et al. 1979; Beer and Eshel 1983]). If the biomass of photosynthesizing organisms is sufficiently large, then diurnal cycles in dissolved gases will be generated in which  $\text{O}_2$  will be measurably higher during the day and DIC will be higher at night.

$\text{CO}_2$  released into seawater by marine organisms will have the same effects on DIC as aqueous  $\text{CO}_2$  that originates from anthropogenic sources; that is, it will increase  $\text{H}^+$  and  $\text{HCO}_3^-$  and decrease pH. Because the reactions in Equation (1) are reversible, net uptakes of  $\text{CO}_2$  or  $\text{HCO}_3^-$  that result from photosynthesis will decrease  $\text{H}^+$  and increase pH. Thus, where biomasses of seaweeds are high, such as sites with green tides, the pH of seawater should also increase during the day and decrease at night. Measurements at a wave-exposed area in Denmark with a mixed-species assemblage of macroalgae dominated by *Fucus* showed the expected pattern (Middelboe and Hansen 2007). The average pH was 8.6 during the day and 7.8 at night.  $\text{O}_2$  concentrations averaged 91% of saturation at night and were supersaturated during the day, averaging 125% of saturation. DIC was not measured at night but during the day, it tended to be lowest between 1200 and 1800. High midday pH values have also been documented at other wave-exposed, rocky shores (Wootton et al. 2008), in salt marshes (Baumann et al. 2014), seagrass beds (Buapet et al. 2013a; Hendriks et al. 2014), lagoons containing mats of the macroalgae *Chaetomorpha linum* and *Gracilaria verrucosa* (Menéndez et al. 2001), and in tidepools containing ulvoid algae (Larsson et al. 1997).

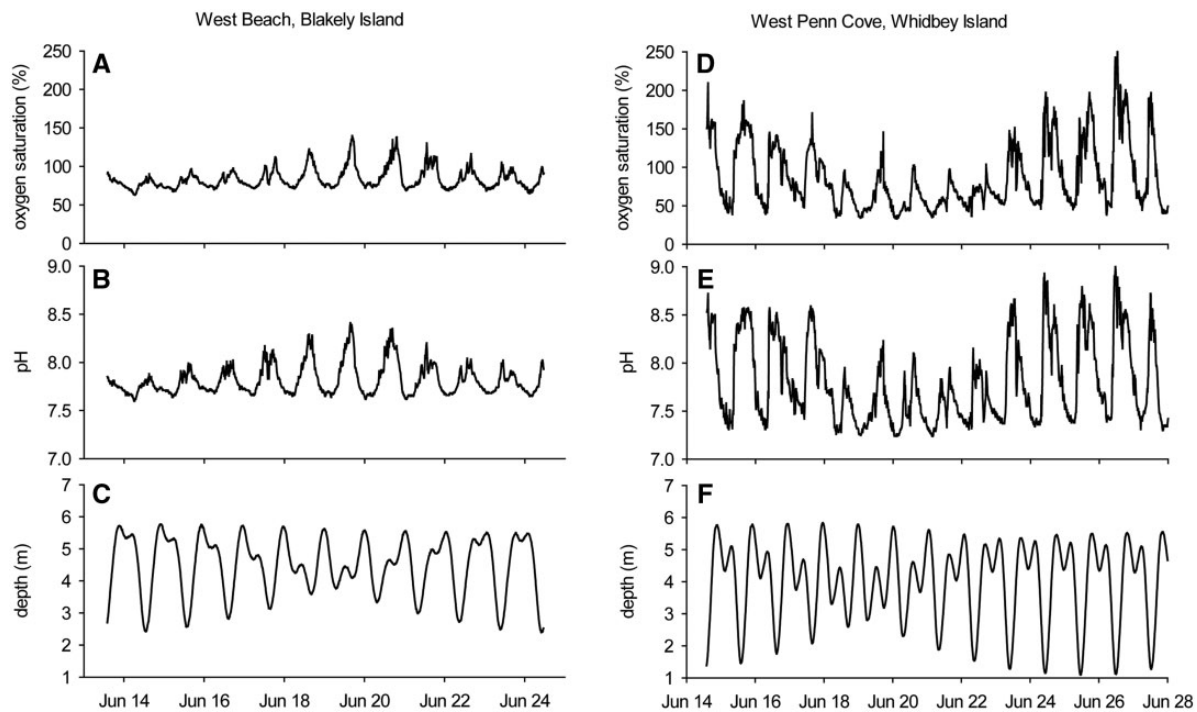
Because green tides are composed of high biomasses of algae that have multiple CCMs that allow them to take up  $\text{HCO}_3^-$  at rapid rates (Bjork et al. 1992; Axelsson et al. 1999), large diurnal fluctuations in pH and  $\text{O}_2$  concentrations should occur in the seawater surrounding them. At 2 sites where green tides occur annually in Washington State, West Beach on Blakely Island ( $48^\circ 33.9' \text{ N}$ ,  $122^\circ 49.8' \text{ W}$ ) and Penn Cove on Whidbey Island ( $48^\circ 13.9' \text{ N}$ ,  $122^\circ 43.9' \text{ W}$ ), we have measured daily fluctuations of pH up to 1.8 units during 2 weeks in the spring of 2006 when ulvoid algae were rapidly growing (Fig. 1).  $\text{O}_2$  concentrations were correlated with pH and ranged from about 40% of saturation at night to

supersaturation during the day. The magnitudes of the fluctuations were higher at Penn Cove, a sheltered embayment, than at West Beach, which is more strongly affected by tidal currents.

Changes in the magnitudes of these fluctuations were correlated with the sizes of the tides (Fig. 1C, F), which affect water depth and the volume of water near the blooms. Differences in the length of the path of light through the water column can decrease the attenuation of light at low tide and increase it at high tide (Nelson and Waaland 1997), causing the diurnal cycles of pH and  $\text{O}_2$  concentrations to be overlain by daily tidal cycles (Fig. 1). Changes in the magnitudes of the diurnal cycles were correlated with spring and neap tides, but also differed between the two sites. In Penn Cove, an embayment with little water motion, the daily variance in pH and  $\text{O}_2$  was higher during spring than during neap tides (Fig. 1D–F). However, at West Beach, which has strong tidal currents and slack currents that are not coincident with low and high tide, the rapid flushing of water around the bloom during the spring tide lowered the daily variance, whereas the reduced movement of water during neap tides increased it (Fig. 1A–C).

Changes in  $\text{O}_2$ , pH, and DIC caused by green tides are likely to affect organisms living in or near the blooms. Hypoxia (defined as an  $\text{O}_2$  concentration  $<2 \text{ mg O}_2$  per liter; Diaz 2001) associated with blooms can occur late at night and early in the morning and when plants are senescing or decomposing, especially during and after population crashes (Valiela 1997; Franz and Friedman 2002). Low  $\text{O}_2$  concentrations are known to cause mortality and sublethal stress to marine animals and can alter the structures of marine communities (e.g., Baden et al. 1990; Diaz and Rosenberg 1995; Diaz 2001; McAllen et al. 2009).

Changes in DIC and pH caused by ulvoid algae can affect other marine plants and algae through their impacts of photosynthesis. When the seaweed *Chondrus crispus* is grown in tidepools with *Ulva intestinalis*, the maximal quantum yields ( $F_v/F_m$ ) of *C. crispus* decreases and the alga becomes bleached within 3 days (Björk et al. 2004). High levels of pH produced by photosynthesizing *U. intestinalis* decrease photosynthetic rates in the seagrass *Zostera marina* (Mvungi et al. 2012). In laboratory experiments, changes in seawater chemistry resulting from the photosynthesis of *U. intestinalis* also lowers photosynthetic rates of *Z. marina* and *Ruppia maritima*, and these changes are negatively correlated with pH (Buapet et al. 2013b). Reducing  $\text{O}_2$  concentrations in water in which *Ulva* has been incubated increases the



**Fig. 1.** Oxygen saturation (A, D), pH (B, E), and water depth (C, F) data from Hydrolab DS5 data sondes that were calibrated according to the manufacturer's instructions and deployed in green-tide blooms at West Beach, Blakely Island, Washington (A, B, C) and in the northwest corner of Penn Cove, Whidbey Island, Washington (D, E, F). The sondes were attached to anchors on the substrate and measurements were taken at 5-min intervals over 2 weeks in 2006.

photosynthetic rates of the seagrasses and reduces the rate at which photosynthesis decreases in response to higher pH in *R. maritima*, but not in *Z. marina*. The decreases in photosynthesis with increasing pH were attributed to reductions in usable DIC (Buapet et al. 2013b). Lower photosynthetic rates under higher  $O_2$  conditions may result from photorespiration in which the ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) enzyme has a lower affinity for  $CO_2$  in the presence of  $O_2$ , thereby reducing the rates of carbon assimilation and decreasing the rate of photosynthesis (Mass et al. 2010). Reductions of flow caused by algal mats could also contribute to lower photosynthetic rates in neighboring plants and algae by reducing the rate at which  $O_2$  is effluxed from the area and by increasing photorespiration. High-pH waters associated with green tides may affect the species composition of the phytoplankton community and their production of toxins. Individual species of microalgae have different pH optima for growth and some species grow only in a narrow range of pH (Hansen 2002; Hinga 2002), suggesting that these species might grow poorly near green-tide blooms that create large fluctuations in pH. The high-pH water associated with green tides might also promote the formation of

dinoflagellate blooms. In a series of experimental manipulations of pH, Hinga (1992) found that several species of dinoflagellates (*Peridinium* spp., *Heterocapsa triquerta*, and *Exuviaella* sp.) were more likely to become abundant and bloom in mixed-species assemblages of phytoplankton grown at high pH (>8.5). However, other dinoflagellates, such as *Prorocentrum redfieldi*, *Prorocentrum gracile*, *Dinophysis acuminata* (Hinga 1992), and *Ceratium* spp. (Søderberg and Hansen 2007) did not grow well in water with a high pH. The growth of diatoms, including those that produce the neurotoxin domoic acid, is also affected by pH in a species-specific manner. While the growth of some *Pseudo-nitzschia* and *Nitzschia* strains were inhibited by high pH, *Pseudo-nitzschia multiseriis* grew until the pH of the culture reached 8.8–9.0 (Lundholm et al. 2004). The production of domoic acid by *P. multiseriis* increased with increasing pH, even when the algal populations were no longer growing. While these studies did not specifically address the types of fluctuations of pH associated with green tides, their results suggest that the changes in seawater chemistry caused by green tides could be altering the functioning and species composition of nearby plankton communities.

## Nutrients

In marine environments, the growth of plants and algae is often limited by the availability of dissolved inorganic nitrogen (DIN), although in some areas, limitation by other nutrients, such as phosphorus, can occur (Wheeler and Björnsäter 1992). Nitrogen-limitation is typically affected by the supply of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), as diatomic nitrogen ( $\text{N}_2$ ) cannot be used by these organisms and nitrite ( $\text{NO}_2^-$ ) usually occurs in low concentrations. Marine plants also can use dissolved organic nitrogen compounds (DON), such as urea, as sources of nitrogen (Tyler et al. 2001). Numerous correlative field studies and experimental manipulations of nitrogen concentrations have demonstrated that the growth of ulvoid algae frequently is limited by the availability of dissolved inorganic nitrogen (e.g., Taylor et al. 1995; Valiela et al. 1997; McGlathery 2001; Buapet et al. 2008; Teichberg et al. 2010).

Relative to other algae, ulvoid algae tend to have high surface-area-to-volume ratios and are efficient at taking up nitrogen; uptake rates ( $V_{\text{max}}$ ) ranging from 20–779  $\mu\text{mol/h/g}$  dry weight for nitrate and 5–996  $\mu\text{mol/h/g}$  dry weight for ammonium have been measured (reviewed by Raven and Taylor 2003). These high rates of uptake allow the algae to respond rapidly to pulses of nutrients and contribute to the high rates of growth that have been measured in these species. Reports of growth rates of 10–50% of the plants' wet mass/day are not unusual (Duke et al. 1986; Björnsäter and Wheeler 1990). Luxury uptake of nutrients, in which greater amounts of nitrogen are taken up than can be used for immediate metabolic needs and for growth also occurs, and stores of nitrogen in ulvoid algae can exceed 5% of the plants' dry mass (Björnsäter and Wheeler 1990; Nelson et al. 2008).

Because green-tide seaweeds rapidly take up nutrients and occur in high biomasses when they form blooms, they can deplete the usable nitrogen in the water column. As a result of nutrient uptake by *Ulva rigida*, DIN in the Po River Delta of Italy in the summer are one-third to one-half of the concentrations in winter (Naldi and Viaroli 2002). When *U. rigida* populations crash, DON in the water column increases as the plants decompose. Ulvoid algae can also intercept DON released from the sediments and reduce the movement of DIN from the water column to the sediments (Tyler et al. 2001).

Based on changes in ecosystems that have been observed over time, it has been hypothesized that when concentrations of nitrogen are low, seagrasses

dominate lagoonal systems, but as concentrations increase, angiosperms are replaced by macroalgae and ultimately by phytoplankton (e.g., Valiela et al. 1997). The situation is not so clear, however, based on experimental manipulations involving *Ulva* spp. In mesocosms, *Ulva lactuca* reduced the growth of a diatom-dominated assemblage of phytoplankton under nutrient-replete conditions (Smith and Horne 1988). Because this reduction of growth did not occur when phytoplankton were grown in nutrient-enriched water that had previously contained *U. lactuca*, the authors concluded that the reduction in phytoplankton growth was due to lack of nutrients rather than due to the presence of allelochemicals. Fong et al. (1993) demonstrated that attached macroalgae and mats of cyanobacteria were better competitors for high levels of nutrients than were phytoplankton in Southern California. Ulvoid biomass was stimulated regardless of the supply of phosphorus when nitrogen was supplied at natural rates. In the absence of macroalgae, the phytoplankton community was dominated by small flagellates; however, when macroalgae were present, the assemblage was dominated by small cyanobacteria in low-nutrient treatment and by a mix of diatoms, flagellates, and unicellular cyanobacteria when nutrient levels were high. Fong et al. (1994) then developed a model based upon Michaelis-Menten equations that adequately described the nitrogen dynamics of lagoonal algae. In a simplified mesocosm experiment, microalgal mats dominated when the addition of nitrogen was low, but were replaced by foliose algae at higher loads of nitrogen (0.068–0.090 mg/l/day).

Two explanations could reconcile the differences seen between the observational and experimental data. First, the observational changes typically occurred in situations of extreme anthropogenic eutrophication. A non-linear response to total availability of nitrogen may favor ulvoids as nitrogen increases up to a tipping point, beyond which further increase favors phytoplankton. Alternatively, chronic versus episodic eutrophication may determine which growth-form prevails, with the macroalgae presumably having greater capacity than microalgae for storing nutrients (Pedersen and Borum 1996) and thus thriving in areas with strong nutrient pulses.

Alterations in nutrient availability by ulvoid algae may affect vascular plants and invertebrates. In situations in which availability of nutrients is very low, seagrasses may compete for nutrients with algae (Aveytua-Alcázar et al. 2008), although the angiosperms usually have access to nutrients in the substratum that are unavailable to algae (Nelson 1997). Releases of nutrients by ulvoid algae may also be

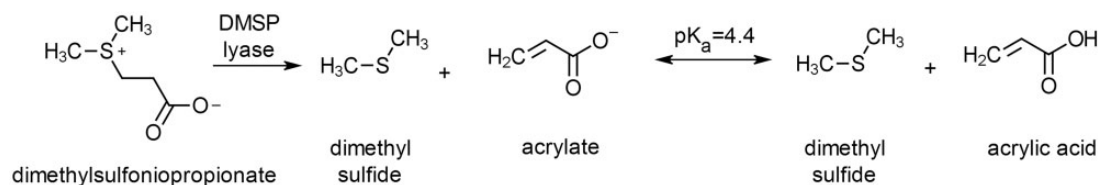


Fig. 2. Breakdown of DMSP by the enzyme DMSP lyase.

problematic. When ulvoid algae senesce in the middle of a dense mat, they may release ammonium at concentrations that are high enough to be toxic to eelgrass (*Zostera marina*) in light-limited conditions (McGlathery 2001) and to invertebrates at aquaculture facilities (Wang et al. 2011).

### Production and Release of Allelochemicals

Marine macroalgae, including those that form green tides, produce natural products that mediate interactions with other organisms which are also referred to as allelopathic compounds or allelochemicals (Paul 1992; Amsler 2008). A number of studies have shown that exudates and extracts from ulvoid algae have detrimental effects on other organisms. Ulvoid algae and water-soluble extracts from them have been associated with reducing settlement rates of barnacles (Magre 1974); increasing mortality rates of crab (Johnson and Welsh 1985) and oyster (Nelson and Gregg 2013) larvae; inhibiting the germination of seaweed zygotes (Nelson et al. 2003a); reducing growth rates of macroalgae (Nelson et al. 2003a; Xu et al. 2013) and benthic (Nelson et al. 2003a) and planktonic (Jin and Dong 2003; Wang et al. 2009; Tang and Gobler 2011) microalgae, including species that form harmful blooms; and, inhibiting the growth of marine bacteria (Sieburth 1964) and viruses (Lu and Liu 2008). Although some of these effects are likely due to alterations of the chemistry of seawater, there is increasing evidence that these algae are producing and releasing allelopathic compounds. The best-studied of these allelochemicals are dimethylsulfoniopropionate (DMSP), reactive oxygen species (ROS), and dopamine.

### DMSP

One of the most abundant metabolites in algae that form green tides is DMSP, which occurs in most species of *Ulva* (Van Alstyne 2008) and in some species of *Ulvaria* (Van Alstyne et al. 2006), *Monostroma* (Bischoff et al. 1994), and *Blidingia* (Karsten et al. 1991, 1992). DMSP is a compatible solute that is

involved in osmotic acclimation and cryoprotection (Karsten et al. 1992, 1996; Kirst 1996), and thus might allow green tides to form in areas that experience reduced or fluctuating salinities or freezing temperatures. It may also help ulvoid algae become abundant in areas where grazers are present as DMSP functions as a precursor for an activated anti-herbivore defense, a type of defense in which plants store non-toxic or mildly toxic precursors that are rapidly, and often enzymatically, converted to herbivore-deterrent compounds (Paul and Van Alstyne 1992). In ulvoid algae, the precursor, DMSP, is broken down (Fig. 2) following grazing, into dimethyl sulfide (DMS) and either acrylic acid or acrylate by an enzyme or enzymes that are designated as DMSP lyase (De Souza et al. 1996; Steinke and Kirst 1996). DMS and acrylic acid deter feeding by sea urchins (*Strongylocentrotus droebachiensis*) (Van Alstyne et al. 2001; Van Alstyne and Houser 2003; Lyons et al. 2007), and acrylic acid deters feeding by *Littorina sitkana* (Van Alstyne et al. 2009), herbivores that are common near green tides in the northeastern Pacific. Although grazing activates this defense, it does not appear to induce additional production of DMSP (Van Alstyne et al. 2007).

DMSP produced by green algae may also have other impacts on the environment and the organisms inhabiting it. Ulvoid algae tend to have few algal epiphytes on their surfaces. DMSP has been implicated as an antifouling compound in the intertidal alga, *Fucus vesiculosus* (Saha et al. 2012), and acrylic acid and acrylate have antibiotic properties (Sieburth 1960, 1961). Thus, DMSP or its breakdown products could prevent fouling by bacteria and algae, which would limit shading and could increase the productivity of the plants. DMSP also is cleaved during decomposition (Zinder et al. 1977; Jorgensen and Okholm-Hansen 1985) and when algae experience oxidative stresses, such as low salinities, desiccation, or the presence of ROS (Sunda 2002; Van Alstyne et al. 2003; Ross and Van Alstyne 2007). Cleaving DMSP in response to oxidative stresses enables the plants to rapidly generate DMS, acrylate, and acrylic acid, which are much stronger antioxidants than DMSP.

DMS may also be responsible for some of the economic effects of green tides as it is a volatile and odoriferous compound. At low concentrations, it is associated with the “smell of the sea” (Lovelock 1982); however, at high concentrations, it produces a noxious odor that may be the cause of complaints about the smell of algal accumulations by residents of beachfront properties (Frankenstein 2000). Other odoriferous and toxic sulfur compounds such as hydrogen sulfide, methyl mercaptan, and methyl sulfide, also are produced as ulvoid algal mats decompose (Zinder et al. 1977).

Ultimately, the release of DMS by ulvoid algae may affect other organisms via its effects on the formation of clouds. DMS released into the atmosphere oxidizes to form compounds that function as cloud-condensation nuclei (CCN) (Bates et al. 1987; Charlson et al. 1987; Malin and Kirst 1997). It has been hypothesized that the CCNs that result from the biological production of DMS increase cloud cover in areas with high amounts of DMS production, resulting in changes in the albedo of the atmosphere and lowering local temperatures. In coastal areas, anthropogenic production of CCNs from the combustion of fossil fuels, smelting of metal, and milling of pulp and paper can be large (Hobbs et al. 1970; Charlson et al. 1992; Spracklen et al. 2011) and may dwarf the contribution from marine seaweeds. Therefore, whether green tides can influence climate by way of their production of CCNs remains questionable.

## ROS

Another group of biologically-active molecules that are commonly produced by green tides are ROS. ROS, which include singlet oxygen, superoxide ( $\text{O}_2^{\cdot-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl radicals (OH), are formed during the reduction of diatomic oxygen ( $\text{O}_2$ ) in photosynthesis and respiration (Apel and Hirt 2004; Halliwell 2006). Singlet oxygen is generated when excitation energy is transferred from chlorophyll to ground-state  $\text{O}_2$  in photosystem II, whereas superoxide radicals are generated when an electron is transferred to  $\text{O}_2$  in respiration or photosystem I.  $\text{H}_2\text{O}_2$  is generated enzymatically as a result of the reduction of superoxide by superoxide dismutases. It subsequently can form hydroxyl radicals, which are the most reactive of the oxygen radicals. ROS can also be products of reactions like the oxidation of dopamine (see below).

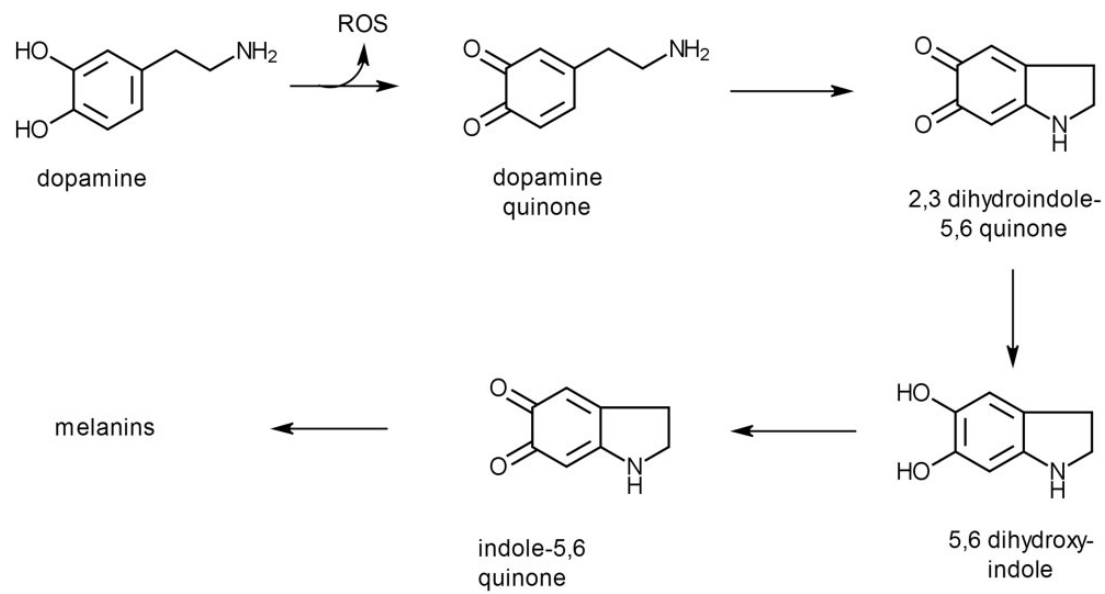
ROS have several functions in plants, most notably serving as anti-pathogen defenses (Lamb and Dixon 1997) and signaling molecules that regulate

intracellular processes (Mittler et al. 2011). In terrestrial plants, suites of genes control the production of ROS in chloroplasts, mitochondria, peroxisomes, and microbodies, as well as the uptake of ROS through the production of scavenging molecules (Shapiguzov et al. 2012). This, in turn, mediates the use of ROS as signals in processes such as stress-responses, development, growth, and programmed cell-death (Miller et al. 2008). Relatively little is known about ROS-signaling in seaweeds. In aquatic plants, ROS are released into cells that are infected by pathogens and into the extracellular environment during oxidative bursts (Potin 2008), as well as in response to physiological stress (e.g., Collén and Pedersen 1996; Choo et al. 2004; Lu et al. 2006) and wounding (Ross and Van Alstyne 2007). In laboratory experiments, when *Ulvaria obscura* and *U. lactuca* were desiccated for 2 h and then rehydrated, the algae released ROS at rates of 0.25 and 1  $\mu\text{mol/h/g}$  of algal fresh mass, respectively (Van Hees and Van Alstyne 2013).

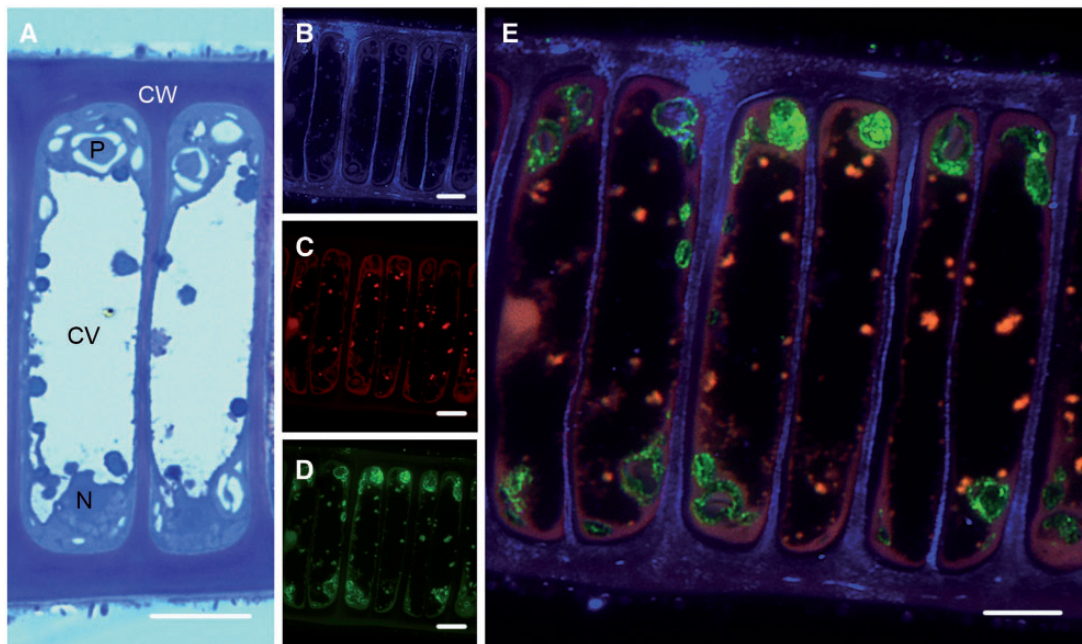
The effects of ROS released by green-tide algae on other organisms growing in the vicinity of green tides has not been well characterized but the combination of their general toxicity, their ability to affect physiological processes, their production rate, and the high biomasses of algae in blooms makes it likely that they have deleterious effects. The production of ROS associated with red-tide microalgal blooms has been implicated as the cause of fish-kills (Kim et al. 1999).

## Dopamine

Dopamine, a nitrogen-containing catecholamine (Fig. 3), is another bioactive metabolite produced by green-tide seaweeds (Tocher and Craigie 1966; Van Alstyne et al. 2006). Although dopamine is a well-known neurotransmitter and hormone in animals (Kandel et al. 2000) and is found in many terrestrial plants (Kuklin and Conger 1995), *U. obscura*, a prominent component of many green tides in the Salish Sea (Nelson 2001; Nelson et al. 2008), is the only seaweed known to produce it. Recent histochemical evidence suggests that, in *Ulvaria*, dopamine is produced in the stromal regions of chloroplasts (Fig. 4), and is then transported into the cytosol (R. L. Ridgway et al., unpublished data). It is subsequently taken up and stored in Golgi-derived vesicles, which migrate to the cortex of the cells (Fig. 5). The synthesis of dopamine in specific regions of the chloroplasts and its storage in intracellular vesicles may help isolate this reactive compound from the rest of the cell, thereby reducing

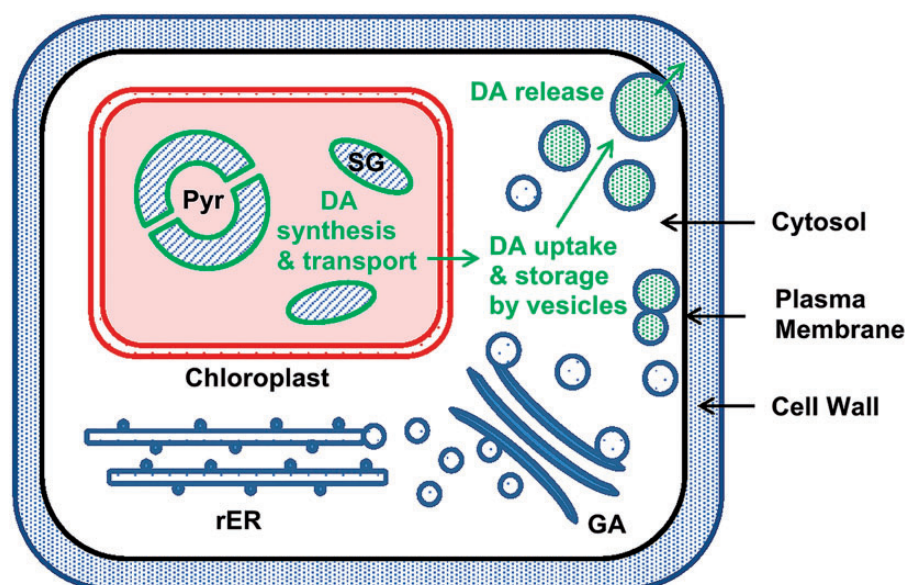


**Fig. 3.** Proposed pathway for the oxidation of dopamine into dopamine quinone and the subsequent reactions that lead to the formation of melanins.



**Fig. 4.** Cell structure and immunohistochemical localization of dopamine in the ulvoid macroalga, *Ulvaria obscura*. (A): Thallus cross-sectional view using brightfield microscopy showing key structural features of cells as stained by Stevenel's Blue. The thallus consists of a single layer of elongated vegetative cells with relatively thick cell walls (CW) at the surface. Chloroplasts are located within the cortical cytosol at the poles of the cell; these contain pyrenoids (P) that are readily identified by their shell-like starch sheathes, as well as by adjacent ellipsoidal starch grains (seen as white voids). A central vacuole (CV) occupies most of the volume of the cell, relegating the cytosol to a thin layer between the tonoplast and the cell membrane except in the region of the nucleus (N) where rough endoplasmic reticulum and Golgi bodies predominate. (B–E): A thallus' cross-sectional view using epifluorescence microscopy. The color channels shown include: (B; blue channel) CalcoFluor-White staining of cell-wall cellulose, (C; red channel) chlorophyll autofluorescence, (D; green channel) dopamine immunofluorescence staining via a commercial monoclonal primary antibody (Gemacbio S.A., Saint-Jean d'Illac., France) generated against dopamine conjugated to bovine serum albumen via glutaraldehyde, and (E) a merged image combining the three channels at higher magnification. Note the strong immunofluorescence signals associated with stromal regions around pyrenoids and starch grains. All measure bars = 10 μm.





**Fig. 5.** Proposed cellular pathway for synthesis, storage, and release of dopamine in *Ulvaria obscura*. Histochemical evidence suggests dopamine (indicated by green coloration) is produced in chloroplasts' stromal regions adjacent to starch grains (SG) and pyrenoidal (Pyr) starch sheaths. Dopamine then appears to be transported to the cytosol where it is taken up and stored in vesicles derived from the endomembrane system, which includes the rough endoplasmic reticulum (rER) and Golgi apparatus (GA). The dopamine-containing vesicles, which vary in size as a result of fusion events, migrate to cortical regions of the cytosol. This model infers that the release of dopamine could be either a regulated (exocytotic) process or, if the integrity of the plasma membrane is lost, a non-regulated process.

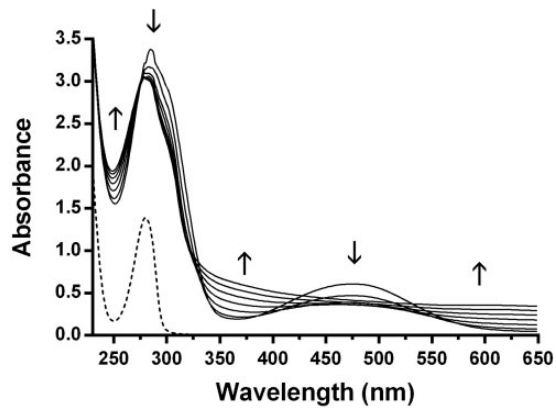
damage to the alga producing it. In *Ulvaria*, dopamine concentrations typically are about 1% of the alga's fresh mass (Van Alstyne et al. 2006). These concentrations vary seasonally and peak in mid-summer (Van Alstyne et al. 2011), when densities of *Ulvaria* tend to be declining.

In *U. obscura*, dopamine functions as a feeding deterrent against local herbivores, which may help these seaweeds persist and form blooms. When given a choice of *U. obscura* and either *U. lactuca* or *Ulva linza*, the isopod *Idotea wosnesenskii*, the snails *Lacuna vincta*, *Lacuna variegata*, and *L. sitkana*, and the sea urchin *S. droebachiensis*, consume more of the two *Ulva* species than they do of *Ulvaria* (Nelson et al. 2008). When dopamine is incorporated into diets, it deters feeding by *I. wosnesenskii*, *L. sitkana*, and *S. droebachiensis* (Van Alstyne et al. 2006).

The feeding-deterrent properties and toxicity of dopamine may result from its oxidation products, which are well known because of their importance to vertebrates. In aqueous solutions, dopamine oxidizes either via autoxidation or enzymatically to form ROS and dopamine quinone (Fig. 3) (Bindoli et al. 1992; Stokes et al. 1999). Dopamine quinone in solution produces a variety of other bioactive, red to bluish quinones (Palmer 1963; Stokes et al. 1999; Whitehead et al. 2001) and potentially neurotoxic

compounds such as 6-hydroxydopamine (Kostrzewa and Jacobowitz 1974). The quinones can then polymerize to form dark-colored melanins (Tan and Chen 1992). Quinones in general are reactive and have cytotoxic, immunotoxic, and cytogenetic effects (Bolton et al. 2000) and ROS are toxic and can cause the peroxidation of lipids, the oxidation of amino acids, and damage to nucleic acids (Fridovich 1978; Asada and Takahashi 1987).

Spectrophotometric evidence suggests that similar types of reactions are taking place in the marine environment when dopamine is released from *Ulvaria*. When dopamine is added to filtered seawater, the solution eventually becomes reddish-colored and then dark-colored precipitates form, consistent with the formation of colored quinones and melanins (Fig. 3). When an oxidizing agent, such as sodium meta-periodate ( $\text{NaIO}_4$ ), is added to a solution of dopamine-HCl in phosphate buffer (pH 7.4) (Graham 1978; Bisaglia et al. 2007), dopamine initially is seen as a single peak ( $\lambda_{\text{max}} = 280 \text{ nm}$ ) that gives rise to two peaks ( $\lambda_{\text{max}} = 300$  and  $475 \text{ nm}$ ) as intramolecular cyclization occurs and 2,3-dihydroindole-5,6 quinone is formed (Fig. 6). These two peaks gradually diminish as oxidation continues, giving rise to indole-5,6 quinone (a purple-gray chromophore) that is associated with an increase in absorbance throughout the visual



**Fig. 6.** UV-visible spectrum of the oxidation products of dopamine after the addition of an equimolar amount of sodium metaperiodate to a 2 mM dopamine-HCl sample in phosphate buffer (pH 7.4). The solid lines are overlaid spectra taken at different time-points (2, 5, 10, 15, 20, 25, and 30 min after addition of periodate). They reveal the time-evolution of the 2 peaks ( $\lambda_{\text{max}} = 300$  and 475 nm) associated with production of 2,3-dihydroindole-5,6 quinone (also known as aminochrome). These peaks gradually decay (downward arrows) as the oxidation continues, giving rise to products that increase in absorbance throughout the visible spectrum (upward arrows) as melanins are formed. For reference, a single spectrum of a 2 mM dopamine-HCl sample in distilled water (dashed line) shows the characteristic peak absorption at 280 nm.

spectrum. Similar spectra are also observed when dopamine is released by *U. obscura* in the presence of  $\text{NaIO}_4$  (R. L. Ridgway et al., unpublished data).

Dopamine can be released into the surrounding seawater by *Ulvaria* when the alga is stranded during a low tide, becomes desiccated, and then is rehydrated during the incoming tide (Van Alstyne et al. 2011, 2013). This can result in a reddish discoloration to seawater near blooms with high densities of *Ulvaria* (Nelson et al. 2003a), presumably from the reddish-colored quinones that result when dopamine is oxidized. Concentrations of dopamine in seawater have not been measured in the field; however, in laboratory experiments, when *Ulvaria* was placed in containers at densities comparable to those in the field and the release of dopamine was triggered by a simulated low tide, the resulting dopamine concentrations in the seawater surrounding the alga could exceed  $500 \mu\text{M}$  (Van Alstyne et al. 2011).

Because the oxidation products of dopamine are toxic, the release of dopamine into the environment near green-tide blooms is likely to have detrimental effects on nearby planktonic and benthic organisms. Dopamine or its oxidation products can reduce germination of zygotes of the rockweed *Fucus distichus* and are toxic to the zoeal stage of crab larvae

(*Metacarcinus magister*); however, they are not toxic to megalopae or recently metamorphosed juveniles of the crab *Cancer oregonensis* (Van Alstyne et al. 2014). Dopamine in seawater may also have sublethal effects as a result of its hormonal and physiological functions. For example, it inhibits the elongation of post-oral arms of *Strongylocentrotus purpuratus* larvae (Adams et al. 2011). In these urchins, the lengths of arms are positively correlated with the feeding rates of larvae, and the inhibition of the growth of arms is a phenotypic plasticity that allows larvae to conserve maternally-inherited triglycerides when the density of planktonic food is high. In the absence of dopamine, larvae have longer arms, which have a fitness-advantage when the density of food is low. However, if dopamine released by *Ulvaria* causes larvae to produce short arms when food densities are low, then the larvae would be mismatched with their environment causing their fitness to be reduced. Since urchins are consumers of benthic macroalgae, this may be a mechanism that allows *Ulvaria* to reduce future losses to grazers by interfering with developmental pathways that affect fitness at pre-settlement stages. Similar decreases in arm-lengths were seen in response to 2 h of exposure to 10 or  $100 \mu\text{M}$  dopamine in plutei of the sand dollar *Dendraster excentricus* (Rivera Vazquez 2014), suggesting that this response may be common among echinoderm larvae.

Dopamine also can have sublethal effects on potential competitors of *Ulvaria*. When *U. lactuca* was cultured in the presence of dopamine, significant reductions in growth occurred at concentrations above  $100 \mu\text{M}$  (Van Alstyne et al. 2014). Inhibiting the growth of *U. lactuca*, which commonly grows in blooms with *Ulvaria* (Nelson et al. 2003b), may be a mechanism that reduces competition for space, nutrients, or light.

#### Other secondary metabolites

While DMSP, dopamine, and ROS have been the best-studied allelopathic compounds released by ulvoid algae, these plants likely produce other natural products that have ecological activity. Ulvoid algae also host microorganisms that produce bioactive metabolites. For example, *Ulva reticulata* grows in association with bacteria (*Vibrio* sp.) that produce large polysaccharides and sulfate esters that inhibit the growth of fouling organisms (Harder et al. 2004). *Ulva lactuca* also hosts bacterial epiphytes, including some that inhibit the growth of bacteria and fungi (Egan et al. 2000).

## Summary

Green tides affect other organisms by altering the chemistry of the environment in which they occur and by producing allelochemicals that mediate ecological interactions. In addition to changing the physical environment by reducing light intensities and altering water-flows, the algae that form green tides can change concentrations of nutrients, DIC, and O<sub>2</sub>. They also produce and release allelopathic or toxic compounds. Many of these allelochemicals are used to mediate ecological interactions, such as deterring feeding by herbivores and reducing the growth of pathogens, competitors, and fouling organisms.

Our understanding of these effects is currently very limited. Most studies that examine the effects of alterations in seawater chemistry deal with changes in mean concentrations and do not take into account variation that occurs diurnally, over tidal cycles, or seasonally. This is in part because there are few studies that have examined fluctuations over longer time periods and across sites that differ in topography, tidal regimes, and the sizes and compositions of macrophyte blooms. Factors that affect the release of allelochemicals produced by green tides are also not well understood. It is known that concentrations of these compounds in the algae varies over space and time and are influenced by a variety of environmental factors (e.g., Karsten 1991, 1992; Van Alstyne and Puglisi 2007; Van Alstyne et al. 2007; Lyons et al. 2010), but measuring releases in the field can be logistically difficult and even laboratory studies are limited in number.

There are also likely to be interactions between alterations in seawater chemistry caused by green tides and the allelochemicals that the algae release. The effects of these compounds often are due to the products that they generate, and the breakdown of DMSP and the oxidation of dopamine are both affected by the temperature and pH of the ambient medium (Li and Christensen 1994; de Souza et al. 1996; Steinke and Kirst 1996). Changes in physical and chemical characteristics of seawater, whether they are mediated by the algae growing in the area or by anthropogenic activities unrelated to the blooms, are likely to affect the rate at which bioactive reaction products are generated and consequently how organisms are affected by these compounds. Synergistic effects in the physiological responses of organisms to allelochemicals and stresses caused by changes in seawater chemistry, such as hypoxia and/or reduced availability of usable carbon or nutrients, are also likely to be occurring.

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