Harmful Algae 78 (2018) 27–35

Contents lists available at ScienceDirect

Harmful Algae

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

Seawater nitrogen concentration and light independently alter performance, growth, and resource allocation in the bloom-forming seaweeds *Ulva lactuca* and *Ulvaria obscura* (Chlorophyta)

Kathryn L. Van Alstyne

Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, WA 98221, USA

ARTICLE INFO

Keywords:
Dimethylsulfoniopropionate
Dopamine
Green tides
Growth
Photosynthetic yield

ABSTRACT

Ulva lactuca and *Ulvaria obscura* are seaweeds that form green tides on Salish Sea shores. They have similar macroscopic morphologies but differ in their biochemistries and physiological responses. To understand how they are affected by changes in environmental conditions, a factorial experiment was conducted in which algae were grown in artificial seawater with either low (10 μM) or high (160 μM) nitrate (NO_3^-) concentrations at high (29 mol photons·m^{-2}·day^{-1}) and low (4 mol photons·m^{-2}·day^{-1}) light levels. Light and NO_3^- affected algal responses, but always independently. After two weeks, *U. lactuca* grown in high light were larger, had lower maximum quantum yields (MQYs), and lower nitrogen (N), carbon (C), pigment, and dimethylsulfoniopropionate (DMSP) concentrations, respectively, relative to algae in low light. In contrast, *U. obscura* growth was unaffected by light. Like *U. lactuca*, *U. obscura* grown in high light had lower MQYs, and N, pigment, and DMSP concentrations. In high light, *U. obscura* also had 89% higher dopamine concentrations and a tendency to fragment. Both *U. lactuca* and *U. obscura* grown in 160 μM NO_3^- were larger, had higher MQYs, and higher N, pigment, and DMSP concentrations, respectively, than algae in 10 μM NO_3^-. Also, when *U. obscura* was grown in the 160 μM NO_3^- medium, it significantly increased its surface area/mass ratio. Although both species grew faster in high NO_3^-, high light only promoted the growth of *Ulva*, which may explain the dominance of *Ulva* in summer months. High light was physiologically stressful to both species and caused increases in photoprotective mechanisms, such as the production of dopamine, a melanin precursor, in *Ulvaria*, and DMSP lysis in *Ulva* to generate antioxidants. Growing in 10 μM NO_3- produced responses that were consistent with nitrogen limitation and had greater impacts on *Ulvaria* than *Ulva*, suggesting that *Ulvaria* responds more strongly to eutrophication.

1. Introduction

Green tides formed from blooms or accumulations of ulvoid green seaweeds are occurring with increasing frequency worldwide and can create both ecological and economic problems (Smetacek and Zingone, 2013; Ye et al., 2011). They interfere with recreational uses of marine waters and impact local economies (LaPointe et al., 2018). They also alter marine ecosystems by fragmenting seagrass beds (McGlathery, 2001; Nelson and Lee, 2001), altering the composition of communities of benthic invertebrates (Lyons et al., 2014), and affecting the chemistry of local waters by causing hypoxia, altering seawater pH, and releasing toxins (Van Alstyne et al., 2015b). In addition, they produce noxious odors (Frankenstein, 2000) and affect atmospheric chemistry through the release of volatile sulfur compounds (Jorgensen and Okholm-Hansen, 1985; Van Alstyne et al., 2015a), some of which form cloud condensation nuclei (CCN) and may impact climate (Charlson et al., 1987).

The occurrence of green tides is frequently associated with anthropogenic nutrient inputs (Teichberg et al., 2010; Valiela et al., 1997). Ulvoid growth rates and tissue nitrogen (N) concentrations are often correlated with the seawater dissolved inorganic nitrogen (DIN) concentrations (Björnsäter and Wheeler, 1990; Fan et al., 2014; Fujita et al., 1989; Sun et al., 2015; Teichberg et al., 2010); however, in areas with very high DIN concentrations, phosphorus and iron may limit macroalgal growth (Teichberg et al., 2010; Viaroli et al., 2005). At higher latitudes, solar irradiance, particularly during winter months, may also limit the growth of macroalgae in intertidal and shallow subtidal habitats (Thom and Albright, 1990), affecting the seasonality of blooms. In addition to altering the growth of bloom-forming ulvoid algae, and hence the formation of blooms, light and nitrogen

Abbreviations: C, carbon; chl, chlorophyll; DIN, dissolved inorganic nitrogen; DM, dry mass; DMS, dimethyl sulfide; DMSP, dimethylsulfoniopropionate; E_k, saturation irradiance; LLW, lower low-water; MQY, maximum quantum yield; N, nitrogen; P_{max}, maximum photosynthetic rate; SA, surface area; TC, total carotenoid

https://doi.org/10.1016/j.hal.2018.07.005
Received 1 March 2018; Received in revised form 15 July 2018; Accepted 16 July 2018

availability can affect other physiological or biochemical properties of seaweeds by changing the allocation of carbon and nitrogen to chemicals associated with resource acquisition (Buapet et al., 2008; Lapointe and Tenore, 1981), nutritional content (Cruz Rivera and Hay, 2003), and chemical defense (Cronin and Hay, 1996; Cruz Rivera and Hay, 2003; Yates and Peckol, 1993).

Like many areas of the world, the coast of the Salish Sea (the waters composed of Puget Sound, the Strait of Juan de Fuca, and the Strait of Georgia in Washington State, USA and British Columbia, Canada) frequently experiences green tides (Nelson et al., 2003a, b; Nelson et al., 2009). Because the Salish Sea is at a relatively high latitude (47–50 °N) compared to other areas where green tides occur, both light and nutrient availability may play a role in the growth and physiology of green tide seaweeds, and ultimately affect the formation of blooms. Although DIN concentrations in the Salish Sea tend to be high due to nitrogen-rich freshwater inputs and coastal upwelling (Davis et al., 2014), macroalgal growth in the area can be nitrogen-limited in the spring and summer (Thom and Albright, 1990; Van Alstyne, 2016). Furthermore, ulvoid abundances on central Salish Sea shores correlate with seawater nitrogen concentrations in topographically similar areas (Nelson et al., 2003b).

Seasonal changes in solar irradiance also can limit the growth of macroalgae, sediment microalgae, and eelgrasses in the region, especially in the winter and early spring. Irradiances at the water surface in the winter can be one-tenth of those occurring in mid-summer (Thom and Albright, 1990). Furthermore, the central Salish Sea has mixed semidiurnal tides with lower-low water (LLW) spring tides occurring in mid-morning to midday in the summer. In the winter, LLW spring tides occur at night (NOAA, 2017), further limiting light availability to intertidal and subtidal algae during the winter.

The purpose of this study was to examine the combined effects of light and nitrogen limitation on two species of ulvoid algae that commonly form blooms in the Salish Sea, *Ulva lactuca* L. (formerly *Ulva fenestrata* and hereafter referred to as *Ulva*) and *Ulvaria obscura* (Kützing) Gayral var. *blytii* (Areschoug) Bliding (formerly *Monostroma fuscum* and hereafter referred to as *Ulvaria*). Although the distributions of these species overlap, *Ulva* tends to be zoned higher than *Ulvaria*, extending up into the mid-intertidal zone, whereas *Ulvaria* is generally found in the low intertidal and shallow subtidal zones (Nelson et al., 2003b). Macroscopically, these algae are similar in appearance. Both can form large sheets; however, *Ulvaria* is monostromatic, and *Ulva* is distromatic. In blooms, these algae can completely cover the substratum and be multiple layers thick (Nelson et al., 2003a), limiting light availability and lowering temperatures in the lower layers on sunny days (Van Alstyne and Olson, 2014).

Differences in the vertical distributions of *Ulva* and *Ulvaria* may be related to differences in their physiologies and biochemistries. *Ulva* is more tolerant of desiccation than *Ulvaria* (Nelson et al., 2010) and grows faster than *Ulvaria* in intertidal, but not subtidal habitats (Nelson et al., 2008). However, *Ulvaria* is more resistant to grazing than *Ulva* (Nelson et al., 2008; Van Alstyne et al., 2006). Both species produce large and comparable quantities of dimethylsulfoniopropionate (DMSP) (Van Alstyne et al., 2007), a compatible solute that may also be a storage mechanism for excess energy, carbon, or sulfur (Stefels, 2000; Van Alstyne, 2008). DMSP can be enzymatically lysed to form dimethyl sulfide (DMS) and acrylic acid (Steinke et al., 1996), which function as feeding deterrents to herbivorous invertebrates (Van Alstyne and Houser, 2003; Van Alstyne et al., 2009, 2001) and antioxidants (Ross and Van Alstyne, 2007). Also, *U. obscura* produces large quantities of dopamine, a nitrogen-containing catecholamine that deters feeding by herbivorous invertebrates (Tocher and Craigie, 1966; Van Alstyne et al., 2006).

Previous studies have explored separately the effects of depth and nitrogen addition on the growth of *Ulva* and *Ulvaria* (Nelson et al., 2008) and the effects of enhanced nutrients and saturating light on DMSP concentrations in *Ulva* (Van Alstyne et al., 2007); however, light and nitrogen availability often affect algal growth, metabolism, and resource acquisition synergistically (e.g., Lapointe and Tenore, 1981; Shivji, 1985). Furthermore, these studies were not designed to examine the effects of light or nitrogen at levels corresponding to their seasonal lows. In this study, factorial experiments were conducted in which *Ulva* and *Ulvaria* were grown in nitrogen-limited and nitrogen-replete artificial seawater crossed with typical winter and summer light levels in order to determine both the main and interactive effects of the two factors on algal performance (maximum quantum yield [MQY]) of the algae, growth (increase in surface area [SA] and mass), resource allocation to light acquisition (chlorophyll [chl] and total carotenoid [TC] concentrations), elemental composition (carbon [C] and nitrogen [N] concentrations), and defensive natural products (DMSP and dopamine). It was expected that algal performance and growth would be higher under both high light and nitrogen replete conditions, and that the two factors would act synergistically. It was also expected that algae grown in higher light would have higher DMSP concentrations and that *Ulvaria* grown in the nitrogen replete medium would have higher concentrations of dopamine because it is a nitrogen-containing metabolite.

2. Materials and methods

2.1. Experimental design

Ulva and *Ulvaria* were collected by hand at low tide from an eelgrass (*Zostera marina*) bed at Ship Harbor, Washington, USA. They were immediately taken to the Shannon Point Marine Center (SPMC) where they were identified microscopically. Two 2.5 × 2.5 cm pieces were then cut from areas on each thallus that had no visible evidence of grazing or fouling (n = 40 per species). Each pair of pieces was placed in a separate 10 cm diameter jar containing 200 ml of one of two types of artificial seawater (n = 20 per medium). The first was an enriched seawater, artificial water (ESAW) medium (Anderson et al., 2005) that had been modified by reducing the sodium nitrate concentration ($NaNO_3$) to 10 μM (N-limited treatment). The second medium was the same, except that the $NaNO_3$ concentration was 160 μM (NO_3^-; N-replete treatment). The culture media were changed daily. The lower nitrogen concentration was similar to the lowest values measured near green tide blooms in 2006–2009 (Van Alstyne and Nelson, unpublished data) and at SPMC as part of its long-term monitoring program (Fig. 1).

Half of the jars containing each type of medium were covered with shade cloth, and half were left uncovered. All jars were placed in an incubator (12 °C, 506 μmol photons·m^{-2}·sec^{-1}, 16 h: 8 h light: dark, equivalent to 29 mol photons·m^{-2}·day^{-1}). The photon flux of photosynthetically active radiation (PAR) in the jars covered with shade cloth

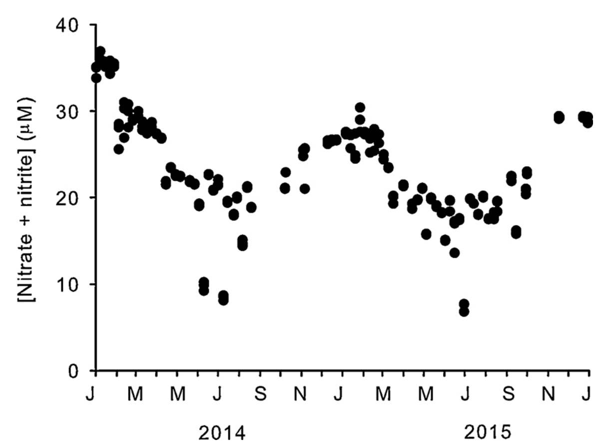

Fig. 1. Concentrations of seawater nitrate + nitrite (μM) in 2014 and 2015 from water collected off the Shannon Point Marine Center (SPMC) in Anacortes, Washington, USA showing seasonal variation in dissolved nitrogen concentrations. Data are courtesy of SPMC.