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Seasonal changes in nutrient limitation and nitrate sources in the green macroalga *Ulva lactuca* at sites with and without green tides in a northeastern Pacific embayment

Kathryn L. Van Alstyne

Shannon Point Marine Center, Western Washington University, Anacortes, WA, USA

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A B S T R A C T

In Penn Cove, ulvoid green algal mats occur annually. To examine seasonal variation in their causes, nitrogen and carbon were measured in *Ulva lactuca* in May, July, and September and stable nitrogen and oxygen isotope ratios were quantified in *U. lactuca*, Penn Cove seawater, upwelled water from Saratoga Passage, water near the Skagit River outflow, and effluents from wastewater treatment facilities. Ulvoid growth was nitrogen limited and the sources of nitrogen used by the algae changed during the growing season. Algal nitrogen concentrations were 0.85–4.55% and were highest in September and at sites where algae were abundant. Upwelled waters were the primary nitrogen source for the algae, but anthropogenic sources also contributed to algal growth towards the end of the growing season. This study suggests that small nitrogen inputs can result in crossing a "tipping point", causing the release of nutrient limitation and localized increases in algal growth.

1. Introduction

Accumulations or blooms of seaweeds are becoming increasingly abundant and problematic worldwide (Smetacek and Zingone, 2013; Ye et al., 2011). These blooms occur over a wide range of latitudes, from tropical habitats to cold temperate environments and can be detrimental to other organisms as well as having negative economic impacts on commercial fisheries and recreational and tourist industries (Frankenstein, 2000; Liu et al., 2009; McGlathery, 2001; Teichberg et al., 2010, 2012; Williams and Feagin, 2007). The availability of nutrients plays a role in the development and persistence of seaweed blooms at all latitudes (Teichberg et al., 2012; Valiela et al., 1997); however, at higher latitudes, the importance of seasonal variation in environmental factors, including nutrients, light and temperature, may be more important to the regulation of bloom dynamics than it is at lower latitudes (Thom and Albright, 1990).

In the cold temperate waters of the Salish Sea (the inland marine waters of Washington State USA and British Columbia Canada comprised of Puget Sound, the Strait of Juan de Fuca, and Georgia Strait), the most commonly occurring seaweed blooms consist of large growths or accumulations of ulvoid green algae, which are often referred to as "green tides" (Nelson et al., 2003a). Blooms in this region frequently consist of mixed species assemblages of seaweeds belonging to the genera *Ulva* (including species previously described as *Enteromorpha*), *Monostroma*, and *Ulvaria* (Nelson et al., 2003b). They are problematic because they overgrow ecologically important eelgrasses (*Zostera marina*) (Nelson and Lee, 2001), alter abundances of infaunal organisms (Price and Hylleberg, 1982), introduce toxic natural products and reactive oxygen species into the environment (Van Alstyne et al., 2011a, 2013, 2014; van Hees and Van Alstyne, 2013), and cause malodorous piles on beaches (Frankenstein, 2000).

Unlike many macroalgal blooms in lower latitudes, the blooms in the Salish Sea have a distinct and relatively short growing season. Typically, these algae are absent or in low densities during the winter months, begin increasing in abundance in mid-spring as water temperatures warm, peak in abundance in late summer and early autumn, and experience large reductions in biomass following mid-autumn storms, a time of year when light levels become reduced (Nelson et al., 2003b; Thom and Albright, 1990). In the San Juan Islands, ulvoid algal biomasses tend to be positively correlated with day length and water temperature. Dissolved inorganic nitrogen (DIN) concentrations are also strongly and positively correlated with biomass among topographically-similar sites (Nelson et al., 2003b).

The relationship between DIN and ulvoid algal biomasses suggests that anthropogenic inputs of nitrogen could be an important contributor to the development of ulvoid algal mats in the Salish Sea. The fact that blooms tend to be more common in urbanized areas of central and south Puget Sound than in more northern, less urbanized sites (Nelson et al., 2009), supports this possibility. It is assumed that urbanized areas have higher inputs of nitrogen from anthropogenically-generated sources, such as fertilizer runoff and wastewater discharges. However, nutrient levels in the Salish Sea are also strongly impacted by freshwater inputs (Banas et al., 2015) and tidal inputs of upwelled

E-mail address: kathy.vanalstyne@wwu.edu.

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nutrients from the coast (Hickey and Banas, 2003), which vary seasonally and inter-annually (Babson et al., 2006). Because the region as a whole has relatively high seawater nitrogen concentrations (typically 2–20 μM), and because of the high rates of water movement due to tidal exchanges and currents, it is unlikely that anthropogenic nitrogen inputs produce large-scale eutrophication in the system (Mackas and Harrison, 1997); however, the region is topographically complex and anthropogenic inputs may be important in shallow embayments where mixing and water exchanges are limited.

The purpose of this study was to examine the importance of nitrogen limitation and nitrogen sources to ulvoid algae throughout a growing season in a small embayment, Penn Cove, Whidbey Island, in the Salish Sea. In Penn Cove (Fig. 1a), thick accumulations or blooms composed of *Ulva* spp. and *Ulvaria obscura* occur annually in some areas of the embayment, but are absent in others. In the northwest corner of Penn Cove (hereafter referred to as NW Penn Cove; Fig. 1b), summertime accumulations cover over 70% of the surface and do so consistently from one year to the next, unlike accumulations in many areas in Puget Sound that undergo substantial inter-annual fluctuations in size (Nelson et al., 2009). In 2006, the only year for which sufficient data are available for comparison, the ulvoid algal cover at a site in NW Penn Cove was 610% higher than the average cover in the Saratoga Passage and eastern Whidbey Island region, and 113% higher than the average for Central Puget Sound, the region most heavily impacted by algal accumulations (Nelson et al., 2009). Published summertime concentrations of seawater nitrogen taken from surface waters near the NW Penn Cove sampling site (Fig. 1) are 23 μM nitrate, 0.3 μM nitrite, and 11 μM ammonium (Van Alstyne et al., 2011b).

The causes of the ulvoid algal blooms in Penn Cove have not been directly examined but may be due to the high concentrations of nitrogen in Penn Cove's waters, which could result, in part, from anthropogenic inputs. Inorganic and organic nitrogen and *ortho*-phosphate can promote the growth of ulvoid algae (Fong et al., 2003; Valiela et al., 1997); however, nitrogen is the nutrient that most limits the growth of *Ulva* in Penn Cove. The molar ratio of nitrogen to phosphorus in *Ulva lactuca* tissues in Aug. 2007 at NW Penn Cove was approximately 12 (Van Alstyne et al., 2011b), which is within the range expected for nitrogen-limited seaweeds (Björnsäter and Wheeler, 1990). In lab experiments, both *Ulva* and *Ulvaria* from a nearby site grew better when nitrogen concentrations in the media were increased (Nelson et al., 2008; Van Alstyne et al., submitted), but growth did not change when media were supplemented with phosphorus (Van Alstyne et al., submitted).

There are several potential sources of nutrients that could be affecting algal abundance. In the east end of the embayment, there are two wastewater outflows that discharge nutrient-rich effluents: 1) the Coupeville wastewater treatment facility (CVTP in Fig. 1c) on the southern shore and 2) the smaller Penn Cove treatment facility (PCTV in Fig. 1c) on the northern shore. The Coupeville facility is estimated to discharge 45 lb of nitrogen daily. However, there are alternative sources of nutrients that could promote algal growth. These include upwelled waters transiting from Rosario Strait through Deception Pass between Fidalgo and Whidbey Islands into Saratoga Passage (Fig. 1) and freshwater from the Skagit River (SR in Fig. 1b), which drains a watershed that includes agricultural lands. Furthermore, ulvoid growth in Penn Cove can be promoted by remineralized nutrients that have accumulated in intertidal and shallow subtidal sediments and are later resuspended into the water column when the sediments are disturbed (Van Alstyne et al., 2011b).

Because marine algae take up nutrients in the same isotopic ratios that are present in the surrounding seawater, the proportions of isotopes that are present in the algae can provide information about the sources of the elements used by the algae (Michener and Lajtha, 2007; Fry, 2008). The ratio of the dominant form of nitrogen, ^{14}N, to its stable

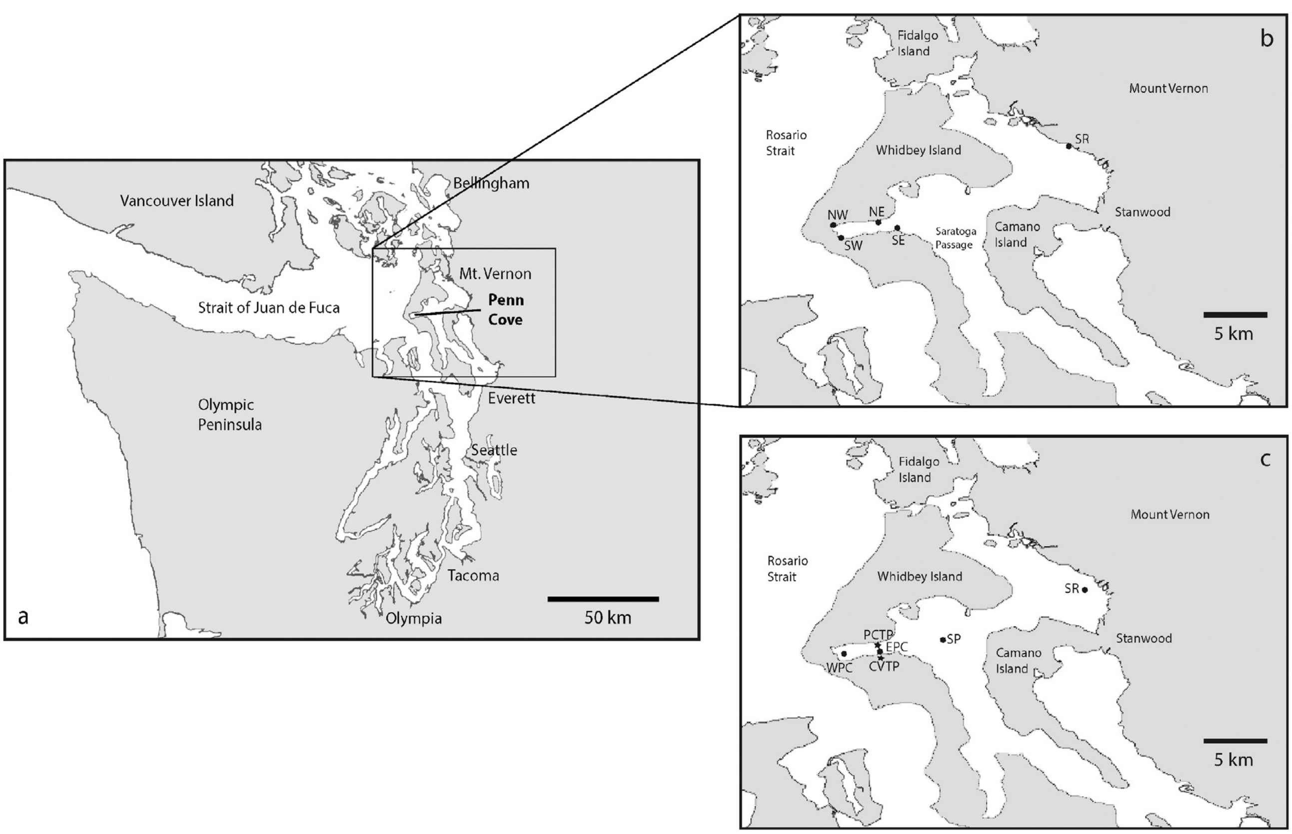

Fig. 1. a) Map of western Washington USA showing the location of Penn Cove. b) Sites where *Ulva lactuca* was sampled. NE: northeast Penn Cove (Monroe Landing), NW: northwest Penn Cove (Grasser's Lagoon), SE: southeast Penn Cove (Long Point), SR: Skagit River, SW: Southwest Penn Cove. c) Sites where seawater and wastewater were collected. CVTP: Coupeville Wastewater Treatment Plant, EPC: East Penn Cove, PCTP: Penn Cove Wastewater Treatment Plant, SP: Saratoga Passage, SR: Skagit River, WPC: West Penn Cove.