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Algicidal and growth-inhibiting bacteria associated with seagrass and macroalgae beds in Puget Sound, WA, USA

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ABSTRACT

The algicidal and growth-inhibiting bacteria associated with seagrasses and macroalgae were characterized during the summer of 2012 and 2013 throughout Puget Sound, WA, USA. In 2012, *Heterosigma akashiwo*-killing bacteria were observed in concentrations of 2.8×10^6 CFU g^{-1} wet in the outer organic layer (biofilm) on the common eelgrass (*Zostera marina*) in north Padilla Bay. Bacteria that inhibited the growth of *Alexandrium tamarense* were detected within the biofilm formed on the eelgrass canopy at Dumas Bay and North Bay at densities of $\sim 10^8$ CFU g^{-1} wet weight. Additionally, up to 4100 CFU mL^{-1} of algicidal and growth-inhibiting bacteria affecting both *A. tamarense* and *H. akashiwo* were detected in seawater adjacent to seven different eelgrass beds. In 2013, *H. akashiwo*-killing bacteria were found on *Z. marina* and *Ulva lactuca* with the highest densities of $\sim 10^8$ CFU g^{-1} wet weight at Shallow Bay, Sucia Island. Bacteria that inhibited the growth of *H. akashiwo* and *A. tamarense* were also detected on *Z. marina* and *Z. japonica* at central Padilla Bay. *Heterosigma akashiwo* cysts were detected at a concentration of 3400 cysts g^{-1} wet weight in the sediment from Westcott Bay (northern San Juan Island), a location where eelgrass disappeared in 2002. These findings provide new insights on the ecology of algicidal and growth-inhibiting bacteria, and suggest that seagrass and macroalgae provide an environment that may influence the abundance of harmful algae in this region. This work highlights the importance of protection and restoration of native seagrasses and macroalgae in nearshore environments, in particular those regions where shellfish restoration initiatives are in place to satisfy a growing demand for seafood.

1. Introduction

Harmful algal blooms (HABs) caused by the proliferation of microalgae are a natural, global phenomenon, resulting in damage to aquaculture industries and wild fish populations while also presenting a risk to human health. The fish-killing raphidophyte, *Heterosigma akashiwo*, and species of the neurotoxin-producing dinoflagellate, *Alexandrium*, have been problematic for decades in Puget Sound, WA, USA and the Strait of Georgia, British Columbia, Canada, which comprise the Salish Sea (Lewitus et al., 2012). Blooms of *H. akashiwo* have caused economic damage up to two million US dollars annually to wild and net-penned farmed fish since the first reported bloom in north Puget Sound in 1976 (Rensel, 2007). Mass mortalities of juvenile sockeye salmon along their migration routes to the Bering Sea have been reported in the Salish Sea during years when *H. akashiwo* blooms were severe, suggesting that these blooms may play a role in salmon mortality (Rensel et al., 2010).

Species of the genus *Alexandrium* are known to produce potent neurotoxins that accumulate in filter-feeding shellfish. Consumption of these contaminated shellfish can cause paralytic shellfish poisoning (PSP) which has resulted in human deaths in the Salish Sea region (Trainer et al., 2003). Shellfish beds can be closed for

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several weeks at a time due to these biological hazards impacting subsistence, cultural, recreational and commercial shellfish harvesting (Trainer et al., 2003) and compromising the vitality of the $108 million per year shellfish industry in Washington State (based on 2008 and 2009 data compiled by the Pacific Coast Shellfish Growers Association). Therefore, there is an urgent need to establish mitigation strategies for these harmful algal events.

In the past two decades, the use of bacteria and viruses has been recognized as a potentially promising tool to control HABs (Nagasaki and Yamaguchi, 1998; Imai et al., 1998; Kim et al., 1998), however the field application of these microorganisms as mitigation strategies has not yet been realized. Imai et al. (2009) and Onishi et al. (2014) found high densities of algicidal and growth-inhibiting bacteria from the biofilm associated with eelgrass (*Zostera marina*) that affect several harmful algal species. Empirical data show that *Z. marina* beds have a lower density of phytoplankton, often populated with epiphytic diatoms closely associated with the *Z. marina* canopy (Jacobs and Noten, 1980; Coleman and Burkholder, 1995; Huh et al., 1998). No reasonable explanations have been given for these low abundances of phytoplankton, however the presence of high-density algicidal and growth-inhibiting bacteria on the biofilm of seagrass leaves may play a role. Preserving these bacterial assemblages through restoration of seagrass meadows may create ecosystems that can control HABs (Imai, 2015). The majority of studies on the effects of algicidal and growth-inhibiting bacteria have been performed on seagrass from the coastal waters of Japan (Imai et al., 2009; Onishi et al., 2014), but due to their potential as HAB mitigation, it is essential to investigate these bacterial phenomena in other coastal areas of the world.

In this study, sites in Puget Sound with seagrass or macroalgae containing bacteria having algicidal or growth inhibiting activities against *H. akashiwo* or *Alexandrium* are documented. Algicidal and growth-inhibiting bacteria associated with the eelgrass (*Z. marina*) and the green alga (*Ulva lactuca*) were investigated throughout Puget Sound, WA, USA, during the summer of 2012 and expanded to include other species of seagrass and dominant macroalgae in north Puget Sound in 2013. Enumeration of the cysts of *H. akashiwo* and *Alexandrium* sp. in sediments was carried out at several sites in 2013, including Westcott Bay where *Z. marina* recently has disappeared.

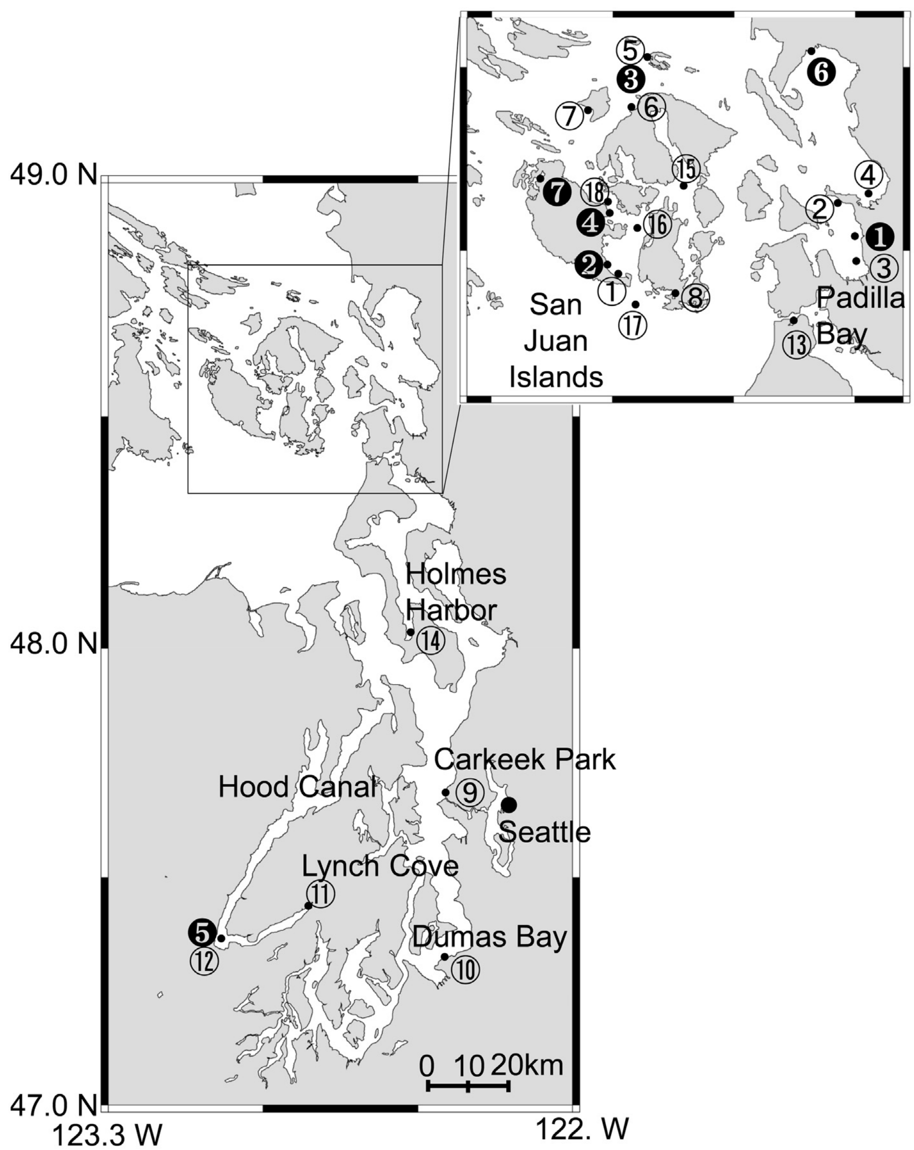

Fig. 1. Sampling locations in Puget Sound, WA, USA in 2012 (○; labeled 1–18) and 2013 (●; labeled 1–7). Site names, species sampled and dates of sampling are listed in Table 1 (2012) and Table 2 (2013).