Harmful Algae 19 (2012) 39–45

Contents lists available at SciVerse ScienceDirect

Harmful Algae

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

Remote sampling of harmful algal blooms: A case study on the Washington State coast☆

Bich-Thuy L. Eberhart *, Brian D. Bill, Vera L. Trainer

Marine Biotoxins Program, Northwest Fisheries Science Center, NOAA Fisheries, 2725 Montlake Blvd E., Seattle, WA 98112, USA

ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 22 May 2012
Accepted 22 May 2012
Available online 9 June 2012

Keywords:
Domoic acid
Enzyme-linked immunosorbent assay (ELISA)
Harmful algal bloom (HAB)
Integrated ocean observing system
Remote sampler
Willapa Bay
Washington State

ABSTRACT

Strategic placement of moorings as an integrated element of ocean observing systems will be essential in the effective monitoring of harmful algal blooms that impact the sustainability of seafood harvest as well as human and marine animal health. Recent efforts have focused on *in situ* collection and analysis of biological samples, an arguably more difficult task than the measurement of chemical and physical parameters that has been automated for many years. Remote sampling and preservation of samples for later analysis can fill a gap that will allow analysis of time-series data that are essential for establishing interannual trends in coastal regions and provide timely warning of approaching harmful algal blooms. In addition, stored samples for subsequent laboratory analysis will provide important control samples needed to validate *in situ*, robotic analysis of biological samples. This monitoring for harmful algae and their toxins on moorings, gliders and other autonomous platforms as part of ocean observing systems requires consideration of sampling locations as well other factors such as preservative type used for sample collection and storage combined with a compatible method for toxin analysis. To that end, *Pseudo-nitzschia* abundance and domoic acid concentrations in seawater were measured from samples collected with a remote sampler moored in Willapa Bay, Washington, during the spring and summer from 2002 through 2006, and compared to data from two adjacent beach sites, Twin Harbors Beach and Long Beach, by Olympic Region Harmful Algal Bloom (ORHAB) personnel. Using enzyme-linked immunosorbent assay (ELISA), total toxin measurements in formalin-preserved whole water samples from Willapa Bay were shown to correlate well with changes in particulate domoic acid concentrations in filtered (particulate) seawater samples from adjacent beaches. A series of experiments confirm, for the first time, that formalin, but not Lugol's iodine or glutaraldehyde, is an effective preservative for phytoplankton samples that are stored for later analysis of domoic acid by ELISA. Together, these data confirm that placement of moorings for *in situ* sampling of biological and environmental parameters in the sheltered environment of Willapa Bay can accurately detect the arrival of harmful algal blooms that originate from offshore hotspots to shellfish harvesting beaches.

Published by Elsevier B.V.

1. Introduction

Prevention, control, and mitigation of the impacts of harmful algal blooms (HABs) require the development of permanent, operational ocean observing systems that continuously provide the data and information necessary for rapid detection and timely forecasts of HABs. Such systems are developing regionally in U.S. coastal and Great Lakes waters (http://www.ioos.gov). The regional components of the U.S. Integrated Ocean Observing System (IOOS®) are working with many stakeholders, from government agencies to shellfish harvesters and growers to the general public, to establish the necessary observing networks for the HABs that occur in their waters. Recent proposals for ocean observing systems that will be used to obtain temporal data on HAB occurrence with the ultimate goal to provide early warning of HABs, have resulted in widespread interest in the deployment of automated sampling systems that can either analyze samples *in situ* or collect samples for later time-series analysis in the laboratory (Greenfield et al., 2008; Scholin et al., 2008). These remote sampling systems can be programmed to sample more intensively once preset thresholds are reached in order to supplement shore-based monitoring programs. One such monitoring program with targeted sampling on the Washington State coast is the Olympic Region Harmful Algal Bloom (ORHAB) partnership.

The ORHAB partnership was initiated collaboratively among federal, state and local governments, coastal tribes, academic institutions and other interest groups to develop a monitoring program that promotes cooperation to provide effective and rapid

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* Corresponding author. Tel.: +1 206 860 3324; fax: +1 206 860 3335.
E-mail address: bich-thuy.le.eberhart@noaa.gov (B.L. Eberhart).

1568-9883/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.hal.2012.05.005

response to the occurrence of HABs in coastal waters of Washington State (Cox et al., 2004). The ORHAB partnership has been instrumental in providing detailed information about the timing of coastal HAB events, the presence of HAB species, and the accumulation of HAB toxins in razor clams that result in closures of this coastal fishery, and the spatial and temporal variability of these toxic events on coastal beaches (Trainer, 2002). Building on the initial project that instituted sampling on the coastal beaches in 2000, Willapa Bay was added as a sampling site to the ORHAB program in 2002. HAB events at representative north-central (Kalaloch) and southern (Long Beach) coastal beaches sampled by the ORHAB monitoring program previously have been described, including detailed scanning electron microscope (SEM) analysis of *Pseudo-nitzschia* species compositions (Trainer and Suddleson, 2005). Here we build upon those descriptions, by focusing on data from the central Washington State outer coast beaches and Willapa Bay.

Willapa Bay, one of two large estuaries located on the outer Washington State coast, was selected as the site for placement of an autosampler for its accessibility by boat and protection from extreme weather. This bay, nestled between Twin Harbors Beach and Long Beach, is noted for its abundant commercial seafood harvest including crabs, mussels and oysters (Dumbauld et al., 2009). Willapa Bay is a coastal plain estuary connected to a highly active eastern boundary ocean upwelling system, the northern reach of the California Current System (Hickey, 1989). Event-scale dynamical coupling between ocean and estuary is of particular interest in Willapa Bay, because upwelling, not river flow, is the primary source of estuarine nutrients and primary production (Hickey and Banas, 2003). Phytoplankton assemblages with their origins in coastal waters can be transported to Willapa Bay (Sayce and Horner, 1996; Roegner et al., 2002).

On the Pacific Northwest coast, blooms of the diatom genus *Pseudo-nitzschia* have the potential to cause amnesic shellfish poisoning (ASP) which can cause confusion, memory loss, seizures, and coma in humans and marine animals (Todd, 1990; Fritz et al., 1992; Scholin et al., 2000). The neurotoxin domoic acid (DA), responsible for ASP, accumulates in shellfish and planktivorous fish through ingestion of the toxin by filter feeding. High concentrations of DA can be retained for over 1 year by the Pacific razor clam (*Siliqua patula*), a shellfish species of significant recreational, commercial and tribal value in Washington State (Adams et al., 2000). Collectively, DA and a suite of other toxins produced by marine protists contribute to events known as harmful algal blooms (HABs). A single Washington coast HAB event can cost an estimated $22 million in lost revenue (Dyson and Huppert, 2010).

In this paper, we present DA concentrations and *Pseudo-nitzschia* cell abundance in formalin preserved whole seawater samples collected by an autosampler moored in Willapa Bay, WA from 2002 through the end of 2006. These data are compared to samples collected from the surf zone of two adjacent outer coast sites (Long Beach and Twin Harbors Beach) to determine if the more sheltered Willapa Bay might serve as an effective warning site for HABs that impact these neighboring coastal beaches. Finally, we tested the effectiveness of an antibody-based method (ELISA) for the determination of DA in phytoplankton samples stored using three common preservatives, Lugol's iodine, glutaraldehyde and formalin, including an evaluation of potential matrix effects from these preservatives by use of seawater samples spiked with known DA concentrations.

2. Methods

2.1. Sample collection

Willapa Bay – Whole seawater samples were collected from April through November 2002 to 2006, at the Bay Center mooring in Willapa Bay using an automated portable sampling unit (ISCO model 6712, Teledyne Isco Inc., Lincoln, NE) deployed by the Pacific Shellfish Institute and the Washington Department of Ecology as a collaboration with the ORHAB partnership. The unit was secured to a mooring positioned at the mouth of the Bay Center Channel (Fig. 1), slightly northwest of Goose Point (123°59′15″W, 46°38′45″N). The sampling unit, housing twelve 1-L polypropylene sample bottles, was programmed to collect seawater within the top meter of the surface at 2-day intervals. A pump inside the unit aspirated and delivered 950 mL of seawater into a 1-L sample bottle (Teledyne Isco, Inc., Lincoln, NE) containing 50 mL of formalin (1% final concentration). Every 2 days, the collection arm rotated to the next bottle, flushed and purged the line with seawater before delivering another sample to the next bottle. All sample bottles were removed from the autosampler every 2–3 weeks. After removal, sample bottles were mixed gently to evenly distribute cells; one aliquot (100 mL) was taken for cell counts and another aliquot (10 mL) was stored at room temperature in the dark for up to 2 years until analyzed for total DA using the ELISA method described below.

At the two coastal sites, Twin Harbors Beach and Long Beach, surface seawater samples were collected on a weekly basis from the surf zone as part of the routine ORHAB sampling program (Trainer and Suddleson, 2005); 100 mL of whole seawater was preserved with formalin (1% final concentration) for phytoplankton species identification and enumeration.

2.2. Enumeration of Pseudo-nitzschia

Preserved seawater samples were mixed gently and phytoplankton were counted neat or concentrated 10-fold by allowing them to settle overnight. After settling, the top 90 mL was carefully removed from the surface without agitation of the settled cells. The preserved, concentrated sample (10 mL) was stored in a 12-mL glass vial at room temperature. For total cell counts, the sample vial

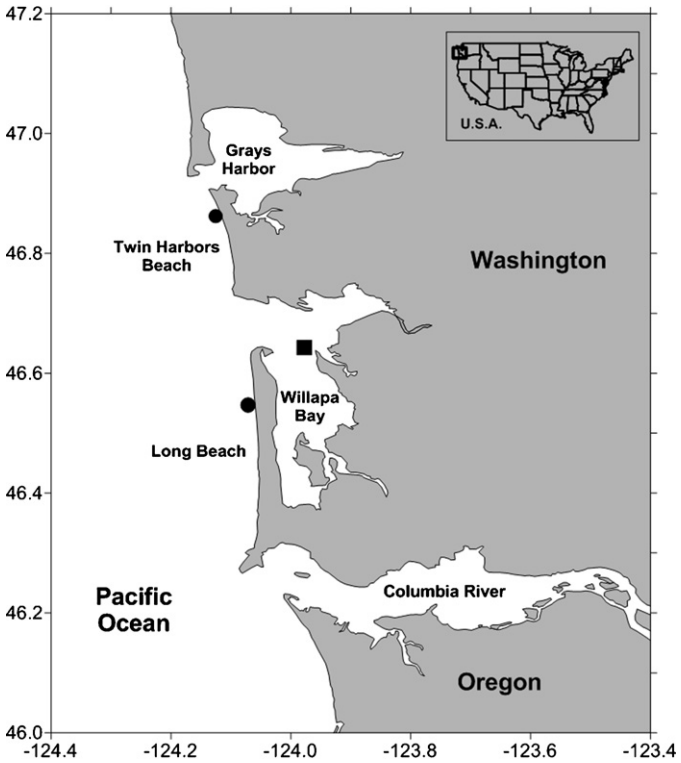

Fig. 1. Map of sampling sites on the Washington State outer coast at Twin Harbors Beach and Long Beach (●) and the autosampler location in Willapa Bay (■).