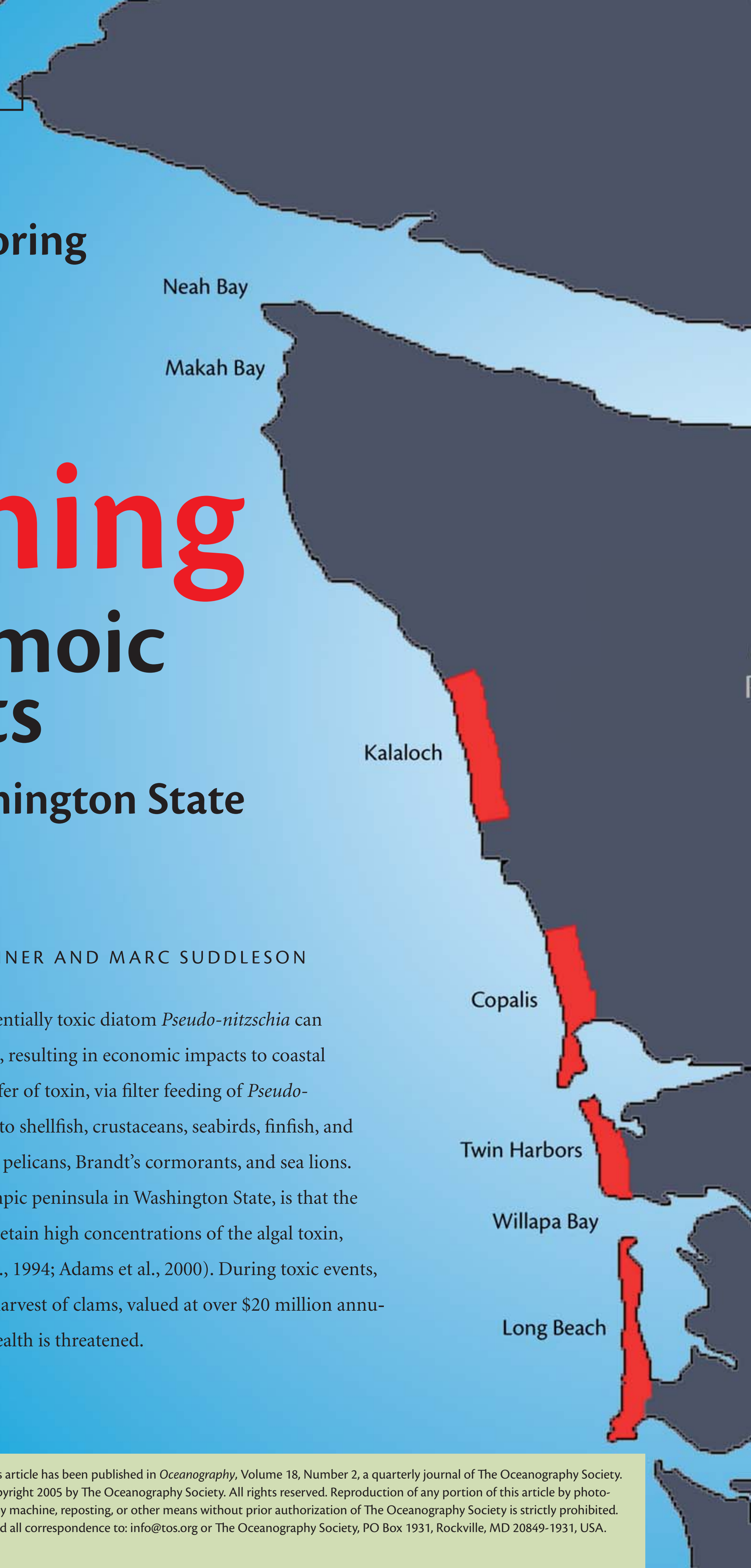
Monitoring Approaches for Early Warning of Domoic Acid Events in Washington State



BY VERA L. TRAINER AND MARC SUDDLESON

On the U.S. west coast, blooms of the potentially toxic diatom *Pseudo-nitzschia* can cause amnesic shellfish poisoning (ASP), resulting in economic impacts to coastal economies and public health concerns. The transfer of toxin, via filter feeding of *Pseudo-nitzschia* or ingestion of filter feeding organisms, to shellfish, crustaceans, seabirds, finfish, and marine mammals has led to mortalities of brown pelicans, Brandt's cormorants, and sea lions. A unique problem on the outer coast of the Olympic peninsula in Washington State, is that the Pacific razor clam, *Siliqua patula* (Figure 1), can retain high concentrations of the algal toxin, domoic acid (DA), for over one year (Wekell et al., 1994; Adams et al., 2000). During toxic events, recreational, commercial, and tribal subsistence harvest of clams, valued at over $20 million annually (Anderson, 1995), is suspended and public health is threatened.

Figure 1. Olympic Region Harmful Algal Bloom (ORHAB) sampling locations include major areas of razor clam harvest (shown in red). Razor clams (inset) are harvested year round for recreational, commercial, and tribal subsistence purposes. Throughout the text, Kalaloch and Copalis are the central beaches, whereas Twin Harbors and Long Beach are the southern beaches.

Data collected on ships of opportunity in recent years show that the Juan de Fuca eddy region often contains much higher concentrations of DA than elsewhere off the Washington coast. This counterclockwise cold eddy off the Strait of Juan de Fuca, which is a dominant feature of circulation patterns off the northern Washington coast, forms in spring and declines in fall, a result of the interaction between effluent from the Strait, southward wind-driven currents along the continental slope, and the underlying topography. Extensive offshore surveys and beach sampling support the possibility that during some years, DA from the vicinity of this eddy moves southward in prolonged upwelling events and then onshore during the first major storm of the fall season, resulting in high levels of DA in razor clams on coastal beaches.

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INCEPTION OF THE ORHAB PROGRAM

In 1999, academic, federal, tribal, and state researchers and managers in Washington State formed the Olympic Region Harmful Algal Bloom (ORHAB) partnership (for a list of collaborators, see the acknowledgements and www.orhab.org), which has established a monitoring program for harmful algal blooms (HABs). The objectives of ORHAB are to investigate the origins of toxic algae blooms, monitor where and when the blooms occur, assess the environmental conditions conducive to blooms and toxification of intertidal shellfish, and to explore methods that can be used to reduce HAB impacts on humans and the environment. This collaboration, funded for five years by the National Oceanic and Atmospheric Administration (NOAA) Monitoring and Event Response to Harmful Algal Blooms program has resulted in reduced costs and faster analysis of shellfish samples, thereby leading to more beach openings for commercial, recreational, and subsistence harvest of shellfish.

COMPONENTS OF THE ORHAB MONITORING PROGRAM

Patterns are emerging regarding the seasonality, duration, and magnitude of *Pseudo-nitzschia* blooms that impact coastal shellfish. *Pseudo-nitzschia* species are very difficult to monitor in natural populations where several species of that same genus occur together. Precise identification often requires extensive electron microscopy. It is not viable to identify every sample collected as part of a monitoring program using such an approach. To overcome this problem, the ORHAB program currently uses a simple combination of analytical techniques, including twice-weekly determinations of total *Pseudo-nitzschia* cells using light microscopy and DA levels in seawater to give an effective early warning of shellfish toxicity. This approach has assisted shellfish resource managers on the outer coast of Washington State by providing them with an early warning of DA accumulation by shellfish.

The success of the ORHAB program is a result of intensive monitoring over the past five years. ORHAB technicians sample at seven locations on the Washington coast that include major areas of razor clam harvest (Figure 1). Twice weekly, technicians identify phytoplankton using light microscopy (Adams et al., 2000) and quantify total numbers of *Pseudo-nitzschia*. Because many species of *Pseudo-nitzschia* are observed in Washington State's outer coastal waters, these species are grouped according to size and morphological similarities including: (1) *pungens, multiseries* (long and narrow), (2) *heimii, fraudulenta, australis* (short and broad), and (3) *delicatissima, pseudodelicatissima* (small and narrow). These size groups include both toxic and weakly or nontoxic species (e.g., *P. pungens* is weakly toxic and *P. multiseries*

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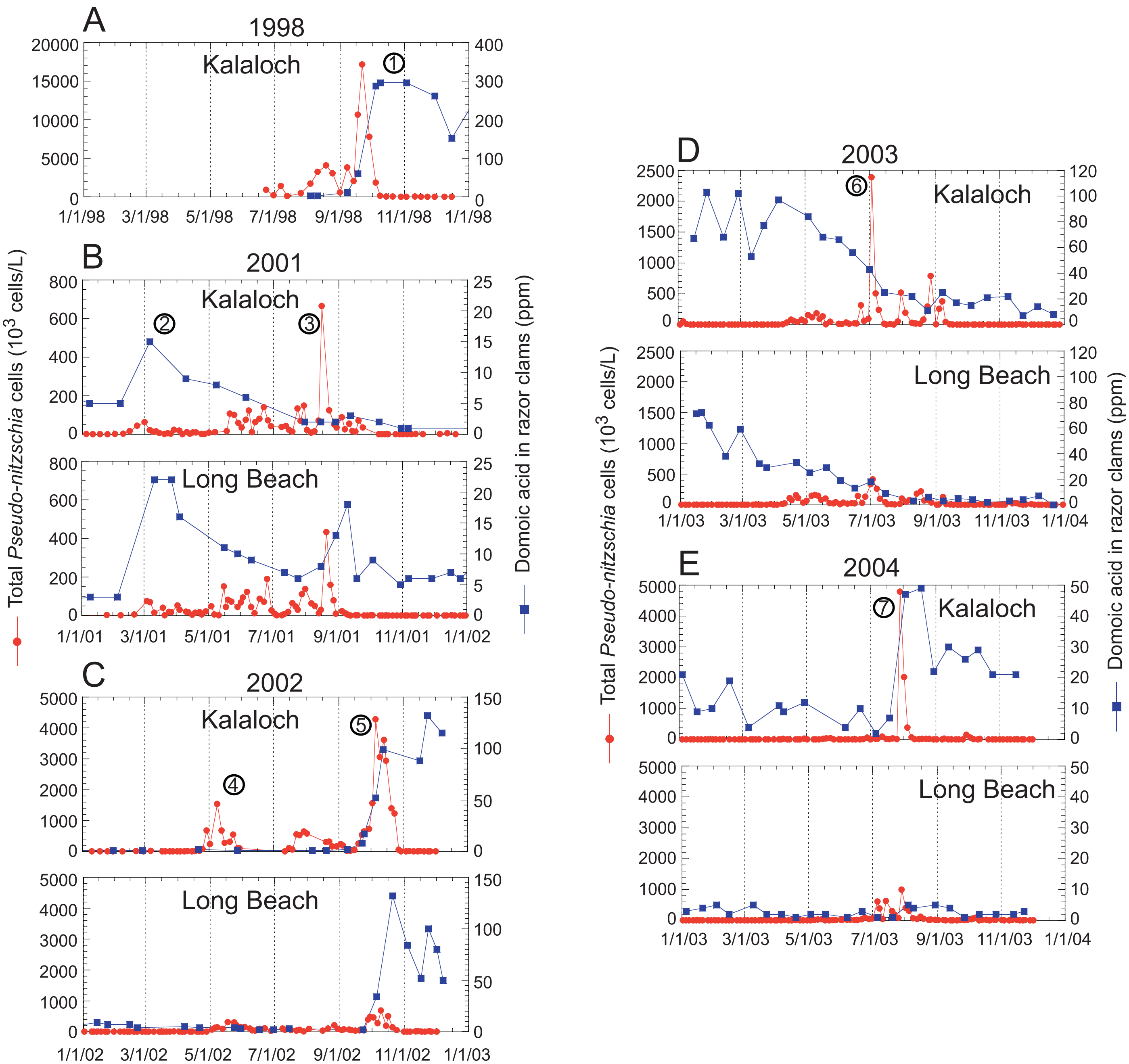

Figure 2. *Pseudo-nitzschia* cell numbers and domoic acid (DA) in razor clams at Kalaloch (1998, 2001 to 2004) and Long Beach (2001 to 2004) in Washington State. Identification and enumeration of Pseudo-nitzschia species present during each event were used to determine threshold levels of each size group that will trigger toxin testing in future samples. These events were used to determine threshold levels of *Pseudo-nitzschia* size groups that will trigger toxin testing: at least 3 x 10^4 cells/L for *P. australis/heimii/fraudulenta* (short and broad), at least 1 x 10^6 cells/L of *P.* cf. *pseudodelicatissima/delicatissima* (small and narrow) for a minimum of one week, and 1 x 10^5 cells/L for *P. multiseries/pungens* (long and narrow). The rapid testing of domoic acid in seawater will allow managers to assess the total toxicity of a *Pseudo-nitzschia* assemblage and thereby determine the potential for toxin transfer to shellfish.

Table 1. Bloom Events

Event[1]	Closure	Max. Pn[2] (cells/L)	Dominant Pn Species[3]	SEM (%)[4]	Max. DA in Clams (ppm)[5]
1. Summer 1998	coastwide	17 x 10^6	cf. psdeli	cf. psdeli (90) heimii (~10)	300
2. Spring 2001	southern beaches	7.4 x 10^4	aus	aus (48) pun (33) cf. psdeli (2) deli (2)	22
3. Summer 2001	none	6.6 x 10^5 4.3 x 10^5	cf. psdeli (Kal) cf. psdeli (LB)	cf. psdeli (100) cf. psdeli (55) aus (45)	3 18
4. Spring 2002	none	1.5 x 10^6	deli	deli (80) cf. psdeli (15) heimii (5)	2
5. Summer 2002	coastwide	4.3 x 10^6	aus	aus (100)	99
6. Summer 2003	Kalaloch Beach only	2.4 x 10^6	cf. psdeli	cf. psdeli (80) aus (20)	25
7. Summer 2004	Kalaloch Beach only	4.8 x 10^6	cf. psdeli	cf. psdeli (90) aus (10)	49

[1]Events correspond to numbers in Figure 2.

[2]Pn = *Pseudo-nitzschia*. Indicates maximum number of all Pn species.

[3]Abbreviations are as follows: cf. psdeli; *P.* cf. *pseudodelicassima*; aus, *P. australis*; pun, *P. pungens*; heimii, *P. heimii*; deli, *P. delicatissima*; Kal, Kalaloch Beach; LB, Long Beach.

[4]Percentage estimates of all Pn species present when maximum total Pn were observed. Estimates are derived using scanning electron microscopy (SEM). The major toxin-producing species in Washington State outer coastal waters (based on culture and field observations) are, to date, *P. australis*, *P.* cf. *pseudodelicatissima*, *P. delicatissima*, and *P. multiseries*, although the latter two species have not yet been observed in high numbers.

[5]The regulatory limit for DA in shellfish is 20 ppm.

produces substantial amounts of toxin), therefore, it is important to know the total cellular DA in a sample in order to determine the potential for toxin transfer to shellfish. Selected samples of *Pseudo-nizschia* are also identified to the species level using scanning electron microscopy (Miller and Scholin, 1998). In addition, 1 liter of seawater is filtered to test for cellular DA using the receptor binding assay (Adams et al., 2000) (results expressed as "particulate DA" and defined as all material captured on a 0.45 μm filter, including all *Pseudo-nitzschia* cells) and razor clams are sampled about twice a month and tested for DA using high-performance liquid chromatography (Hatfield et al., 1994). When numbers of *Pseudo-nitzschia* reach a threshold (see Figure 2 caption for threshold levels now in place for each *Pseudo-nitzschia* size group), a rapid toxin test (Jellett Rapid Test, Jellett Rapid Testing Ltd., Nova Scotia, Canada) is performed on the filtered seawater particulate sample. Because observations at the central and southern beaches were similar, only results from Kalaloch Beach and Long Beach are shown.

Seven instances of elevated *Pseudo-nitzschia* cell numbers along the Olym-

[1]cf. = *Latin* confer, meaning to compare. The phylogeny of *P. pseudodelicatissima* has recently changed and this species has been divided into at least three separate species (see Lundholm et al., 2003), therefore we use the notation *P.* cf. *pseudodelicatissima* to describe these species with morphology indistinguishable from *P. pseudodelicatissima* by scanning electron microscopy.

pic coast, used to determine threshold levels of cells that motivate further testing of toxin in seawater and clams as part of ORHAB's monitoring program for DA, are listed in Table 1. Not all of these events resulted in razor clam toxicity above the regulatory level of 20 ppm. In the first event that occurred in 1998, prior to the beginning of ORHAB (Adams et al., 2000), record levels of DA in razor clams resulted in a coast-wide closure of the shellfish harvest that persisted for more than one year. During this event, total *Pseudo-nitzschia* cell numbers reached 17 x 10^6 cells/L (Figure 2a, and Table 1, Event 1). Numbers of *P.* cf. *pseudodelicatissima*[1] reached 15 x 10^6 cells/L (Table 1) and remained above 1 x 10^6 cells/L for at least 21 days. In spring 2001, only the southern beaches were closed, due primarily to clam exposure to *P. australis* reaching 3.5 x 10^4 cells/L for at least four days (Table 1, Event 2, and Figure 2b). The central beaches were not closed although DA in razor clams increased to 15 ppm and maximum *P. australis* cell numbers were similar to those seen in the south. During late summer 2001 (Table 1, Event 3 and Figure 2b), a short-lived bloom of *P.* cf. *pseudodelicatissima* at Kalaloch Beach in numbers up to 6.6 x 10^5 cells/L was observed. DA in razor clams remained low at this site. However, at Long Beach, elevated numbers of both *P.* cf. *pseudodelicatissima* and *P. australis* were observed on a single sampling date (Table 1). No beach closures occurred on Long Beach, but DA levels in razor clams were close to the regulatory limit. On the same day that DA levels of 18 ppm were measured in clams at Long Beach, *Pseudo-nitzschia* cell numbers were low, giving managers confidence that levels of DA in razor clams would not continue to increase, thereby allowing the clamming season to open. In May 2002 (Table 1, Event 4), *P.* cf. *pseudodelicatissima* and *P. delicatissima* cell numbers totaled 1.4 x 10^6 cells/L on a single sampling date, however, levels of DA in razor clams never exceeded 2 ppm (Figure 2c). In September and October 2002, a coast-wide closure occurred due to sustained numbers of *P. australis* of at least 2.5 x 10^6 cells/L for about a 10-day period (Figure 2c, and Table 1, Event 5). In July 2003 (Table 1, Event 6), over 1 x 10^6 cells/L of

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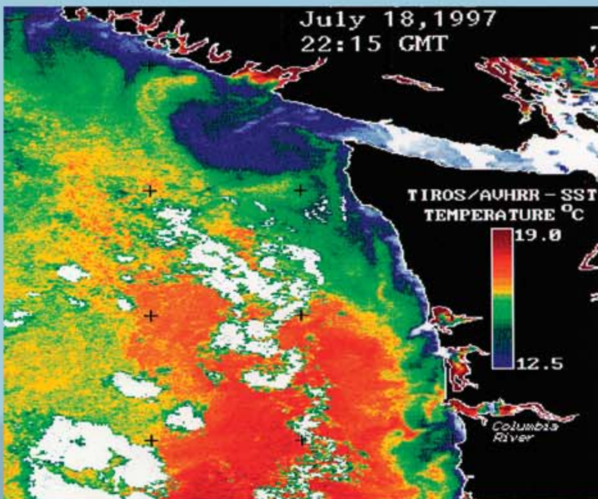
Table 2. Domoic acid levels and *Pseudo-nitzschia* cell numbers preceding and during the late summer 2002 bloom event at Kalaloch Beach, Washington.

Date	*Pseudo-nitzschia* (cells/L x 10^5)	Domoic Acid	
		Seawater (nM)	Razor Clam (ppm)
Aug 30	1.6	0	
Sept 3	2.3	0	
Sept 5	1.9	0.1	2
Sept 9	0.1	0.1	2
Sept 12	0.2	0.1	
Sept 16	0.4	0.7	
Sept 18			2
Sept 20	2.5	2.0	
Sept 23	5.3	7.3	8
Sept 25	6.4	10.5	17
Sept 30	7.2	1.0	26
Oct 3	15.6	9.8	
Oct 6	42.7	10.9	52
Oct 10	30.5	13.0	
Oct 13			99



The Juan de Fuca eddy region is a site of persistent upwelling (nutrient enrichment) throughout the summer months. Blooms of toxic *Pseudo-nitzschia* are thought to initiate in this zone. The duration of upwelling and the timing of fall storms are factors believed to influence the levels of toxin that reach coastal razor clams.

The ORHAB partnership monitors seawater at several coastal sites for a rapid increase in the numbres of *Pseudo-nitzschia* and for toxin in seawater. The combination of microscopic monitoring of the algae and assessment of cellular toxicity using test strips (Jellet Rapid Test, see inset) has given managers an early warning of dangerous levels of toxins in razor clams.

Figure 3. Olympic Region Harmful Algal Bloom (ORHAB) monitoring gives coastal managers early warning of harmful algal blooms.

P. cf. *pseudodelicatissima* and 0.5 x 10^6 cells/L of *P. australis* were observed on a single sampling date at Kalaloch Beach. The closure at Kalaloch Beach in summer 2003 was possibly due to the retention of DA by clams resulting from the summer 2002 event, and not a new toxic episode. The event in July 2004 (Table 1, Event 7) featured at least 1.8 x 10^6 cells/L of *P.* cf. *pseudodelicatissima* for about five days (together with numbers of *P. australis* reaching 0.5 x 10^6 cells/L), resulting in clam DA levels of over 40 ppm. In summary, total numbers of *Pseudo-nitzschia* cells, *Pseudo-nitzschia* species composition, and duration of clam exposure to toxic cells together dictate the level of toxicity attained by clams during any given event.

Data from the late-summer bloom event in 2002 are detailed in Table 2. On September 16, particulate DA in seawater rose to 0.7 nM at Kalaloch Beach. On September 20, *Pseudo-nitzschia* numbers rose to 2.5 x 10^5 cells/L and particulate DA measured 2 nM, indicating that a rise in DA levels in razor clams was likely. Indeed, on September 30, DA in razor clams increased to 26 ppm, resulting in a harvest closure. Although the level of DA in razor clams from Copalis Beach (Figure 1) was only 17 ppm, the continued increase in *Pseudo-nitzschia* cell numbers at all monitoring sites gave Washington State managers the confidence to invoke a coast-wide closure in early October 2002. The rising DA in shellfish was also observed along beaches in the neighboring state of Oregon. Unfortunately, because Oregon does not monitor phytoplankton, the sudden rise of DA in razor clams there resulted in a costly recall of shellfish harvested in early October. These data demonstrate that if elevated *Pseudo-nitzschia* cell numbers are used to initiate the measurement of particulate DA, a timely warning of dangerous levels of DA in shellfish is possible. The measurement of 0.7 nM particulate DA on September 16 was sufficient to provide an approximately two-week warning of the 26 ppm DA in razor clams on September 30 (Table 2). Because toxin tests are simple and inexpensive to perform, frequent use of Jellett Rapid Tests during times of elevated *Pseudo-nitzschia* cell numbers will reduce the risk associated with potential toxicity of razor clams.

In summary, *P. australis* and *P.* cf. *pseudodelicatissima* are, to date, the major "problem" species of *Pseudo-nitzschia* on the coast of Washington State. *P. delicatissima* is less frequently seen in high numbers, but has also been shown in culture to produce similar levels of DA as *P.* cf. *pseudodelicatissma* (Baugh et al., in press). The central and southern razor clam beaches are exposed to different numbers and, at times, different species of toxigenic *Pseudo-nitzschia*, occasionally resulting in selective beach closures. The environmental factors responsible for these differences are the focus of regional cruises for the Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB PNW), which began in the summer of 2003. Measurements of increasing levels of *Pseudo-nitzschia* and particulate DA in seawater that supplement razor clam DA data give extra confidence to managers when establishing beach closures. Using the results of the last five years of monitoring as a guide, the suggested "warning" levels of *Pseudo-nitzschia* on the Washington coast are when the *P. australis/heimii/fraudulenta* (short and broad) size group reaches 3 x 10^4 cells/L (see, for example, Table 1, event 2) or when the *P.*cf. *pseudodelicatissima/delicatissima* (small and narrow) group exceeds 1 x 10^6 cells/L for over one week (see, for example, Table 1, event 6). To date, *P. multiseries* has not been a problem species on the outer Washington coast, however, we suggest a warning level of 1 x 10^5 cells/L for the *P. multiseries/pungens* (long and narrow) group. If coincident toxin testing of seawater particulates can be done, warnings should be released to managers only when the level of particulate DA in seawater exceeds 0.7 nM.

The ORHAB partnership has enhanced our knowledge of the seasonality, duration, and magnitude of *Pseudo-nitzschia* blooms associated with shellfish harvest closures on the Washington coast. Prior to ORHAB, shellfish toxin data alone were used as a decision-making tool to close clamming beaches, resulting in emergency closures and loss of public trust in management. The results of the ORHAB partnership show that a simple combination of analytical techniques, including twice-weekly determination of total *Pseudo-nitzschia* cells using light microscopy and levels of DA in seawater using Jellett Rapid Tests, gives an effective early warning of shellfish toxification events (Figure 3). Because *Pseudo-nitzschia* are not identified to the species level when enumerating size groups by light microscope, the estimation of particulate DA in seawater gives further information regarding the relative toxicity of the *Pseudo-nitzschia* assemblage present at the time of sampling. This information provides a greater degree of certainty to managers by minimizing the false warnings that might occur if rising numbers of *Pseudo-nitzschia* cells are weakly or non-toxic and therefore not a danger to the clam population.

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MOVING TOWARD INTEGRATED HARMFUL ALGAL BLOOM FORECASTING

To sustain a monitoring program such as ORHAB, it must be integrated into the state's management plan for harmful algal blooms (HABs); Washington State must also be responsible for funding the program. By rapidly assisting managers during toxic bloom events, ORHAB partners have effectively demonstrated to state legislators how integral the monitoring program is to effective and timely management of shellfish resources. Resulting legislation has instated a surcharge on shellfish license fees that will provide enough funding to sustain a state-run program beyond the summer of 2005 when the federally funded program ends.

During toxic events, recreational, commercial, and tribal subsistence harvest of clams, valued at over $20 million annually (Anderson, 1995), is suspended and public health is threatened.

This basic monitoring program, which employs coastal technicians to measure cells and toxins, provides a foundation for a more integrated HAB forecasting system in Washington State. Such an integrated forecasting system will include an understanding of how physical, biological, and chemical processes interact to favor harmful *Pseudo-nitzschia* blooms, with subsequent development of reliable regional biophysical models to predict bloom development, persistence, toxicity, and movement—all goals of the current ECOHAB PNW research program.

Comparisons of data from an ORHAB mooring off Kalaloch Beach with information from ECOHAB PNW moorings farther offshore have illustrated that the nearshore site is an ideal location for observation of well-developed HAB events that are likely to impact the coast. Such information should prove valuable for integration of HAB efforts with regional ocean observation systems, such as the Northwest Association of Networked Ocean Observing Systems (NaNOOS) and the Neptune ocean observing system off the west coast of North America. These observing networks should consider using new HAB detection technology and model results in planning their deployments. Combining these components into an integrated forecasting system will transform the existing, labor-intensive ORHAB early warning system into a forecasting system with longer lead time for prediction of incoming bloom events.

SPECIFIC FORECASTING COMPONENTS FOR THE FUTURE

Developing a U.S. west coast HAB forecasting capability for the transport and impact of toxic *Pseudo-nitzschia* blooms will require sustained monitoring as well as additional efforts in the critical areas of basic research and model development. The ultimate goal of a HAB forecasting system is to provide an early warning network for the detection and transport of toxic *Pseudo-nitzschia* blooms using an integrated suite of sensors. Sensors to be used include satellites and stationary platforms that together measure ocean water properties, currents, *Pseudo-nitzschia* cell numbers, and DA, all of which will add real-time elements to shore-based lab testing.

A remote-sensing technology that could be used in forecasting is automated molecular detection and quantification of cells (Scholin et al., 1999), an adaptation of current microscopic methods for enumerating *Pseudo-nitzschia* species groups. The suite of real-time data collected from moorings will be used to initialize, calibrate, and validate physical and biological models and associated forecasts. Such models will allow state monitoring and health departments to take preventive actions (e.g., increase monitoring efforts, close shellfish beds, warn at-risk communities) to safeguard the public health, local economies, and fisheries. In addition, an integrated forecasting system will allow the proactive management of resources such as the early opening of the recreational and subsistence clamming seasons or early warning to commercial crab and clam fishers who are impacted by DA-related closures.

The rapid transmission of data to managers is an essential aspect of this work. Currently, the National Coastal Data Development Center's harmful algal blooms observing system (HABSOS) is working with ORHAB partners to establish a real-time product that will deliver weekly summaries of data and alert partners when dangerous levels of cells and toxins have been observed. HABSOS provides an online, integrated information system for managing HAB data, events, and effects. Managers will have a tool for rapid access to current information on Washington State outer coast HAB events and similar events across the nation. As data streams from moorings become available, these will be directly interfaced through telemetry to the online data system, making the real-time assessment of HAB risk a reality.

SUMMARY

The ORHAB partnership has established a monitoring program for HABs, resulting in more openings for commercial, subsistence, and recreational harvest of razor clams. This collaboration has resulted in reduced costs and faster analysis of shellfish samples, and has lowered health risks to consumers. Patterns are emerging regarding the seasonality, duration, and magnitude of *Pseudo-nitzschia* blooms that impact coastal shellfish. A simple combination of analytical techniques, which includes weekly determination of total *Pseudo-nitzschia* cells using light microscopy and levels of particulate DA in seawater, gives an effective early warning of shellfish toxification events. In the future, fine-scale sampling using automated devices on moorings and real-time analysis on beaches will allow detailed determination of fluctuations in biological, physical, and chemical parameters that influence HAB intensity. Such sampling will provide data to regional biophysical models under development. Future work should focus on developing and integrating these critical components into a system capable of forecasting toxic *Pseudo-nitzschia* bloom development, persistence, toxicity and movement.

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