Completion Report

Period: 2/1/2014 - 1/31/2015

Project: R/OCEH-6 - *Optical detection and characterization of pre-HAB populations of the fish-killing alga, Heterosigma akashiwo*

STUDENTS SUPPORTED

Coyle, Owen, ocoyle@uw.edu, University of Washington, Oceanography, status: cont, field of study: oceanography, advisor: Grunbaum, degree type: MS, degree date: 2015-06-01, degree completed this period: No
Student Project Title:
Software and hardware development for low-cost in situ plankton imaging
Involvement with Sea Grant This Period:
research assistantship
Post-Graduation Plans:
faculty position

Tobin, Elizabeth, etobin@u.washington.edu, University of Washington, Oceanography, status: cont, field of study: Algal biology, advisor: Grunbaum, degree type: PhD, degree date: 2014-06-01, degree completed this period: Yes
Student Project Title:
Quantification of transitional swimming behaviors in harmful algae and their implications for pelagic and benthic distributions
Involvement with Sea Grant This Period:
key graduate student collaborator in field work, instrument development, ground truthing, data analysis and stakeholder communication; partially support by WSG funds, together with funding from projects leveraged off this project
Post-Graduation Plans:
Independent NSF grant to pursue HAB work in Alaska

CONFERENCES / PRESENTATIONS

No Conferences / Presentations Reported This Period

ADDITIONAL METRICS

P-12 Students Reached:	**P-12 Educators Trained:**
Participants in Informal Education Programs:	**Volunteer Hours:**
Acres of coastal habitat protected, enhanced or restored:	**Resource Managers who use Ecosystem-Based Approaches to Management:**
Annual Clean Marina Program - certifications:	**HACCP - Number of people with new certifications:**

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

Description	Developed?	Used?	ELWD?	Number of Managers	Names of Managers
Upgraded, improved and field tested Imaging Plankton Emergence Trap for real-time remote monitoring of Heterosigma cells and other plankton emerging from marine sediments.	Yes	Yes	No	0	
Upgraded, improved and field tested high-resolution, efficient, portable and low-cost networked microprocessor-based remote sensing platform capable of remote real-time monitoring of the HAB-forming alga Heterosigma and other plankton.	Yes	Yes	No	0	
Upgraded, improved and field tested low-cost and high-resolution microprocessor-based imager developed for quantifying finescale plankton distributions, collecting	Yes	Yes	No	0	

environmental data and quantifying plankton net avoidance.

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices:	Sustainable Coastal Development **# of coastal communities:**

PARTNERS

Partner Name: Cheryl Greengrove
Partner Name: Chief Kitsap Academy
Partner Name: Karlista Rickerson, Sound Toxin Volunteer
Partner Name: Quartermaster Harbor Yacht Club
Partner Name: Rensel Associates Aquatic Sciences
Partner Name: Society for Integrative and Comparative Biology
Partner Name: Suquamish Tribe Fisheries Department, type: Government, scale: Tribal
Partner Name: University of Washington
Partner Name: Wallingford Imaging Systems

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research develops a low-cost monitoring technology to detect harmful algae before they bloom**
Type: impact
Relevance, Response, Results:
Relevance: Harmful algal blooms (HABs) are a major environmental concern in every coastal state, causing illness, closing down aquaculture and fisheries, and costing the U.S. economy an estimated $82 million a year. In Washington alone, NOAA scientists estimated that HAB-related harvest closures could result in as much as $22 million in lost revenues for coastal counties. Timely, detailed, broad-scale data about algae

distribution and characteristics would aid in addressing the threat of HABs.
Response: Washington Sea Grant-funded researchers developed and deployed high-resolution, low-cost, low-power, networkable micro-imaging technology using embedded microcomputers and high-definition cameras to detect, count, and identify swimming algal cells. Sensors were tested on Heterosigma, which causes fish kills in Puget Sound, and Alexandrium, which produces the toxin responsible for paralytic shellfish poisoning.
Results: Prototype sensors successfully detected and quantified emergent Alexandrium chains and identified several novel and significant Heterosigma traits, including surprisingly rapid emergence and varying strain efficiency. The researchers used new computing, imaging, and printing technologies to upgrade the sensors while bringing the unit cost down to $500 and opening up other potential research applications. Student researchers leveraged project models and imaging techniques to investigate other planktonic phenomena, such as crab-larvae distribution and the effects of starvation stress and ocean acidification on echinoderm larvae and other marine organisms.
Recap:
Recap: Washington Sea Grant-funded research develops low-cost, high-resolution automated sensing technology to detect and measure emergent harmful algae in real time, a key step toward predicting and preparing for blooms and a new observation tool with wide applicability to other research.
Comments:
Primary Focus Area: OCEH (SSSS)
Secondary Focus Area: OCEH (HCE), COCC (HRCC)
Associated Goals: Improve understanding and management of emerging and cumulative threats to ocean and coastal health. (SSSS Supply)
Improve understanding and management of emerging and cumulative threats to ocean and coastal health. (HCE Science)
Improve understanding of coastal hazards and environmental change and develop tools and approaches for observation, prediction, planning, and adaptation. (HRCC, Capacity)
Partners:
American Gold Seafoods
Chief Kitsap Academy
Ocean Inquiry Project
Quartermaster Harbor Yacht Club
Rensel Associated Aquatic Sciences
Society for Integrative and Comparative Biology
Wallingford Imaging Systems
Related Partners: *none*

PUBLICATIONS

No Publications Reported This Period

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

No Leveraged Funds Reported This Period

COMPLETION NARRATIVE

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WASHINGTON SEA GRANT FINAL REPORT
for the period 2/1/2011 – 1/31/2014

WSG Project Number: R/OCEH-6
Project Title: Optical detection and characterization of pre-HAB populations of the fish-killing alga, *Heterosigma akashiwo*

Principal Investigator and Affiliation:
Daniel Grunbaum University of Washington, School of Oceanography

1. ACCOMPLISHMENTS AND OUTCOMES

A key impediment to applied and basic ecological understanding of marine communities is the difficulty and expense of acquiring timely, detailed, broad-scale data about plankton distribution and condition. This project utilized microvideography and computerized motion-tracking to quantify cell abundance and state of harmful algal species with major negative economic and ecological impacts in Washington and temperate waters worldwide. Our proposal was to use existing video methodologies. However, we took advantage of major advances in low-power single board computing and imaging to wholly redesigned our imaging technologies, using embedded microprocessors with onboard high-definition cameras. Our new generations of HAB imagers have approximately 16 times the pixel resolution, one-sixth the cost, and one-tenth the weight as our original design. They digitize images losslessly, circumventing problems imaging small particles with common compression standards, and are programable to work autonomously, stream live video, and/or record environmental data and control external devices.

As proposed, we deployed our prototype remote sensor for a HAB-forming alga, *Heterosigma*, for an extended period (> 2 months) operating autonomously in an industrial field setting. Essential support for this deployment was provided by our stakeholder partner, the American Gold Seafood (AGS) facility on Bainbridge Island, WA. The instrument's deployment substantially exceeded the proposed 1 week goal for unattended autonomous operation. Data analysis is still in progress, but we have established close correspondence between remotely sensed real-time optical counts from our autonomous instrument and on-site cell counts by AGS staff, particularly during the one significant *Heterosigma* event during the deployment period.

A key goal for our instrument has been to detect cells at low pre-bloom densities (20 cells/ml is an operational threshold for aquaculturists). The imager creates an internal halocline, which concentrates *Heterosigma* cells and excludes many other plankton species. We used laboratory cultures to show that, at 20 cells/ml and without a halocline (i.e., with no concentration of cells above the nominal density) the imager tracks approximately 200 cells within each video frame. This suggests the imager can, with long imaging sequences, detect cell populations of <0.2-2 cells/ml. Assessing and optimizing the instrument's sensitivity under field conditions is still an area of active research, but data from the summer of 2011 demonstrate detection at cell densities well below thresholds for deleterious impacts on farmed fish.

With a low-cost PVC housing and associated pumps, a remote *Heterosigma* sensor for networked or autonomous operation costs approximately $500, meeting our goal of low-cost sensors practical to implement in a regional prediction network. Our imaging technology applies broadly in plankton ecology. We are currently collaborating with the Suquamish Fisheries Department to adapt our imaging technology to monitor crab larval populations, based a on previous design for at-sea optical observations; with the Northwest Fisheries Science Center's Ocean Acidification laboratory to design flow-through sensors for quantifying abundance and morphology of cultured fish and invertebrate populations; and with the Greengrove Laboratory at UWT in quantifying emergence of Harmful Algal Bloom-forming cells (*Alexandrium*) from resting stages in sediments.

Beyond our proposed work, we made several novel and substantial advances in development of remote HAB imaging sensors. First, we upgraded our imaging technology to more powerful microcomputer platforms, and adapted our capture and processing software to the new platforms. Second, we leveraged funds from a Link

Foundation Fellowship to graduate student Liz Tobin to design, construct and deploy new type of imager, an Imaging Benthic Emergence Trap (IBET). This instrument combined a traditional benthic emergence trap, in which harmful algal cells and other plankton emerging from resting stages in the sediments are confined within a plankton net for later study, with an imaging microcomputer. This instrument has potential to provide early warning of incipient HABs, by revealing the locations and times at which precurser cells enter the water column. Third, we broadened the scope of our image-based remote sensing to include another key HAB-forming alga in Washington State waters, *Alexandrium catenella*. We demonstrated in field trials at Quartermaster Harbor, Washington, that this instrument can distinguish chains of *Alexandrium in situ*, quantify the size/frequency distribution of chains, and characterize their size-specific swimming velocities.

We additionally developed and deployed a prototype underway protist imager on the R/V Thompson during the Regional Scale Node engineering cruises in summer 2013. Both P.I. Grunbaum and graduate student Owen Coyle participated in those cruises. This prototype was able to conduct autonomous sampling over sustained periods of protists and small zooplankton in running seawater from the ships underway flow-through system (the same water as is used by the SeaFlow flow cytometer, yielding potentially complementary data on two interacting planktonic trophic levels). This instrument, which shares the low cost and networkability of our other remote sensors, has potential to collect large-scale data of near-surface microplankton from ships of opportunity in many undersampled marine environments.

Graduate student Liz Tobin, P.I. Grunbaum and collaborator Rose Ann Cattolico completed a series of laboratory studies quantifying bi-directional transition rates of *Heterosigma* between benthic resting stages and pelagic vegetative stages. Key results of this study include surprisingly rapid emergence of cells, beginning less than 24 hours after exposure to growth-supporting light and temperature cues, and an unexpectedly high incidence of cell replication immediately after emergence. This replication was first detected by video-based cell counts, and subsequently confirmed using flow cytometry to track increases in cells' DNA complements during synchronized cell division. Tobin showed that two geographically and genetically distinct strains differ in swimming, efficiency of transitions into and out of resting stages, and metabolic characteristics such as lipid content that are often associated with swimming and other resource-intensive cell activities. Other published results include video analyses enabling the identification of cell state during transition of *Heterosigma* cells from vegetative to resting life-stages, based on cell swimming behavior. This cell-level analysis enables quantification of vertical fluxes of cells of each type, which is central to determining how cryptic benthic populations are formed and later emerge to initiate HABs. Liz Tobin was awarded her PhD in June 2014, and is currently working on an NSF post-doctoral grant based closely on her W.S.G.-funded dissertation research.

Graduate student Karen Chan and three Under-Represented Minority undergraduates from Friday Harbor Labs' Blinks Research Experience for Undergraduates program leveraged models and imaging techniques derived from this research (and earlier, associated Sea Grant funded work) to investigate field distributions of larval crabs, behavioral responses of echinoderm larvae to starvation stress, and the impacts of Ocean Acidification on larval echinoderm behavior, feeding rate, morphology and cloning. One of those students, Cristina Villalobos, has since been awarded and G.R.F. from N.S.F. to pursue her research on Ocean Acidification effects on planktonic and benthic organisms.