PROJECT TITLE: **Novel optical remote sensing technology for prediction of Harmful Algal Blooms**

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1. Project Narrative

Introduction:

Harmful Algal Blooms (HABs) are outbreaks of planktonic algae that threaten public health, degrade aquatic ecosystems and cause major economic losses. Most HABs are detected only after they reach an advanced stage, when they have already caused significant ecological and economic damage. However, many HAB-forming species have two distinct life stages, a dormant benthic stage and a vegetative pelagic stage. Algal cells emerging from the benthic stage initiate HABs, regulating location, timing and magnitude of blooms. Detecting and tracking cells as they emerge from the benthos provides a promising strategy for early detection of HABs.

The primary objective of this project was to construct and test a prototype of an autonomous optical sensor for the detection and characterization of HAB-forming algae as they emerge from the sediments. Previously, the capacity to monitor emerging cells did not exist. The required optical technology to detect and track movement behaviors of swimming algae had been developed and tested in the laboratory [1, 2, 3], but these technologies had not been developed for *in situ* sensing.

Results:

Support of the Link Foundation and Ocean Engineering and Instrumentation Fellowship has enabled us to develop and field-test a fully functioning prototype autonomous *in situ* instrument for monitoring benthic emergence of small planktonic organisms - the Imaging Benthic Emergence Trap (IBET). Here, we describe the design of our prototype and report the preliminary findings from our first set of field deployments.

Instrument design:

The IBET incorporates low-cost video imaging optimized for the detection of small (10- 1000 μm) planktonic organisms. It features a high resolution (2592x1944 pixels) monochrome camera and an on-board microcomputer (Fig. 1a). The planktonic organisms are illuminated with a bank of infrared light emitting diodes (IR LEDs). All electrical components are powered by a 12V input, supplied by onboard high capacity rechargeable AA batteries. The camera, batteries and electronics are enclosed in low-cost waterproof housing.

Planktonic organisms are imaged within a clear acrylic square tube (“imaging chamber”) with 1.27cm sides that is positioned inside an opaque plastic housing to exclude external light. The IR LED bank is situated on the back wall of the imaging chamber so that cells are illuminated in dark-field. The waterproof housing is modified to have a clear window, onto which the imaging chamber is fastened. The camera’s field of view is focused in the center of the clear imaging tube. *In situ* video footage is stored on an onboard USB flash drive until instrument retrieval.

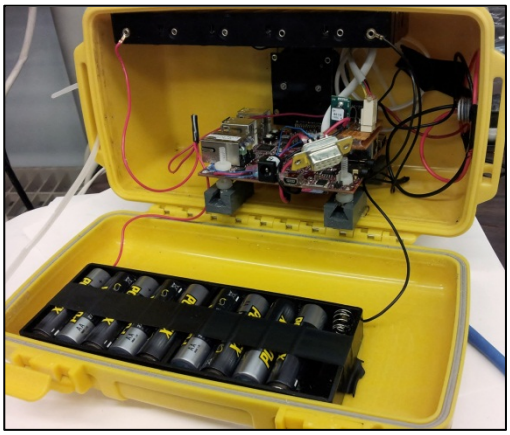
(A)  (B) 

Figure 1. The prototype IBET. (A) The microcomputer, camera board, batteries and other electrical components encased in the waterproof housing. (B) The instrument set-up for deployment in Quartermaster Harbor, WA.

We integrated our *in situ* imaging technology with existing plankton emergence trap (PET) methods [4]. Our instrument consists of one IBET and three modified non-imaging PETs secured to a weighted central frame (Fig. 1b). The modified PETs trap the newly emerged plankton that swim up through the imaging chamber, so that images are validated. Gate valves, installed between the lower and upper sections of plankton traps, ensure that only cells emerging from the sediments are observed in the imaging chamber.

The gate valves are connected to a pulley system allowing the valve position to be controlled from the surface by a long line. The valves are in the closed position during deployment and are opened once the instrument is positioned in the sediments. Prior to instrument retrieval, the gate valves are closed to retain the water samples.

Field testing occurred off a floating dock in Quartermaster Harbor in Puget Sound, WA. Our prototype was fully functional down to the maximum testing depth of 15m. The IBET was pre-programmed to turn on the IR LED bank and capture video data at specified time intervals, and was able to collect data autonomously for up to 30 hours with 2 minutes of video collected every 45 minutes.

Data Analysis:

We were able to detect and monitor the behaviors of diverse plankton, including HAB-forming species, at the benthic-water column interface with our first-generation IBET. Video acquisition and image-processing to identify planktonic organisms within each frame of the video was completed using specialized software developed by Chris MacGregor at Cybermato Consulting [5]. Individual planktonic organisms are represented in the analysis software as “particles”. The high abundance of particles found in environmental video data can be categorized by optical size (pixel area) in order to target and identify planktonic organisms of interest. For example, we

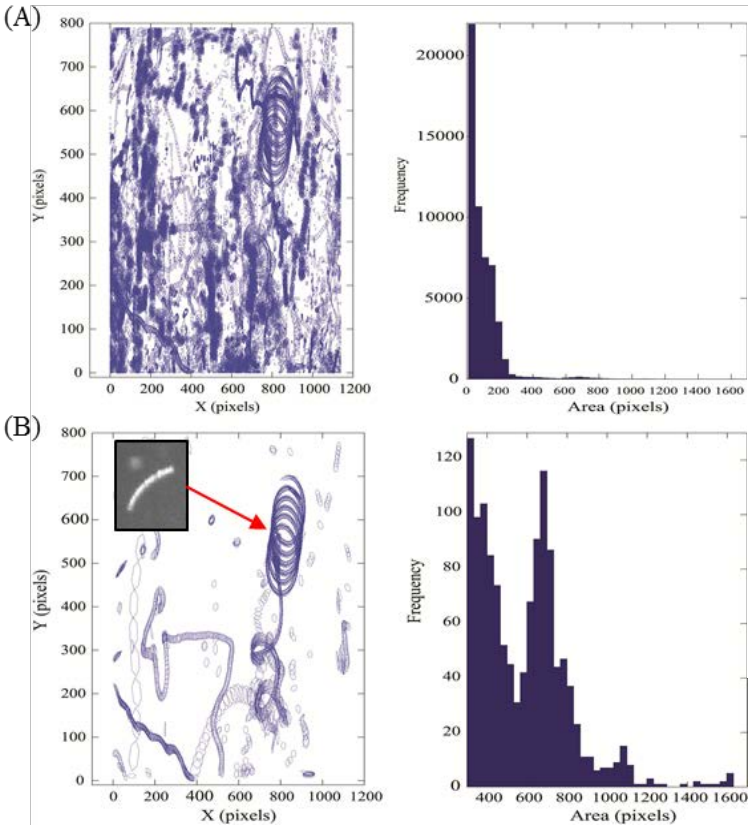


Figure 2. Sample plankton data collected by the IBET. (A) An example of the total abundance of plankton-like particles detected by the imager and video analysis software in a 2 minute video clip. (B) The same data as above, but only the larger particle size fraction (300-1600 pixels) is plotted. The plots on the left show best-fit ellipses for each plankter tracked over the duration of the video clip. The plots on the right show the frequency of optical size (in pixel area). The red arrow points to the unique swimming pattern of a long *Alexandrium catenella* chain (a HAB-forming species) and the picture insert shows the *A. catenella* chain from the raw video footage.

were able to detect and track the unique swimming behavior of the chain-forming, HAB-forming *Alexandrium catenella* by targeting a specific optical size fraction within the collected data (Fig. 2).

Established motion analysis algorithms were used to track and quantify detailed movement behaviors of our target HAB-forming species. During our field-testing, we were able to capture *in situ* data during the onset of a bloom of the HAB-forming alga, *Akashiwo sanguinea.* From this video data, we were able to detect *A. sanguinea* cells by optical size, generate cell swimming trajectories and quantify movement statistics critical for identifying vertical fluxes to surface waters (Figs. 3 & 4). All plankton detected and analyzed from the IBET video data were validated by microscopic observation of the water samples collected from the modified PET chambers.

Significance and Impacts:

The IBET is the first instrument of its kind to provide *in situ* optical detection and behavioral monitoring during benthic emergence of meroplanktonic organisms. Field testing has shown it to be effective in detecting and tracking HAB-forming algae, as well as other marine organisms

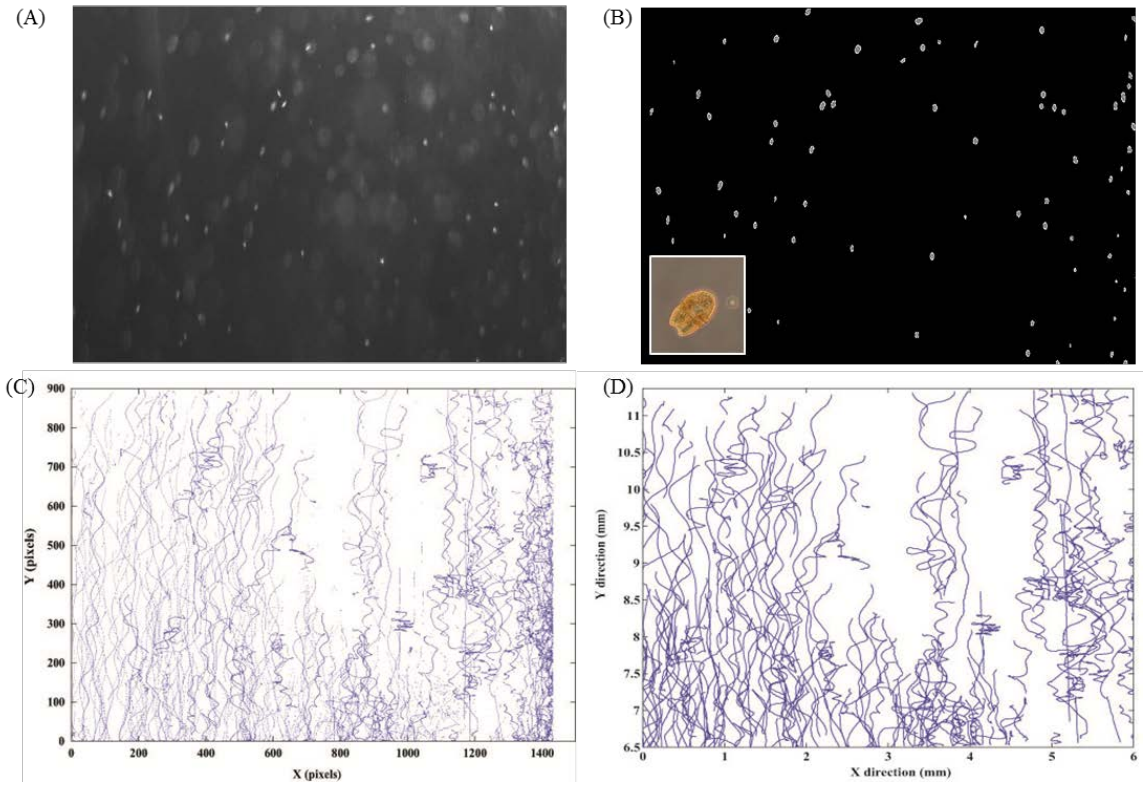


Figure 3. Steps for tracking cells of the HAB-forming alga *Akashiwo sanguinea.* (A) Image of the raw video footage collected by the IBET. (B) *A. sanguinea* cell targeted based on optical size. The inserted photo is of *A. sanguinea* cell collected from the modified PET traps for optical imaging validation. (C) Pixel positions of each *A. sanguinea* cell plotted over the duration of the video clip. (D) Cell-level swimming trajectories are generated by converting pixel positions to physical coordinates using a camera calibration grid.

(e.g., benign algal species and copepod naupli), over extended deployments. Our prototype has the potential to be integrated with automated, web-based servers, for real-time data transfer.

Future research will focus on advancing optical sensor development by building second-generation sensors that can be easily deployed by other researchers, engaged citizens and stakeholders (e.g., aquaculture managers) for more comprehensive temporal and spatial monitoring. A network of optical sensors to remotely detect and monitor benthic emergence of algal cells in real time would provide a unique tool for biologists, managers, and stakeholders that would provide crucial early warning information.

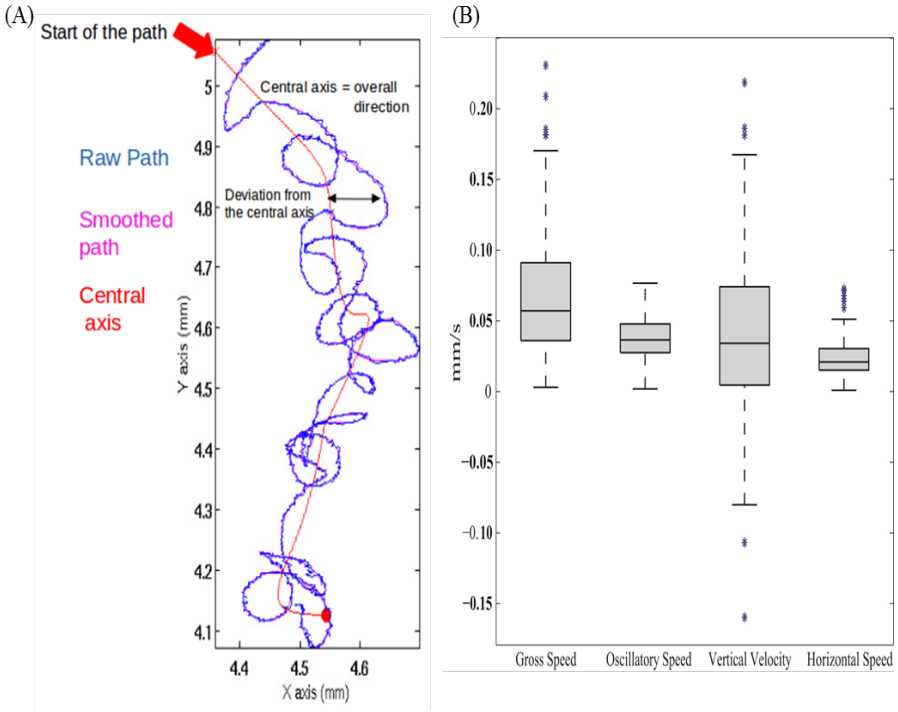


Figure 4. Movement statistics of cell trajectories. (A) A trajectory of an algal cell. The raw trajectory (blue line), is fitted with two smoothing splines to distinguish directed movement (red line) from oscillatory motion (pink overlay). (B) Boxplots show example swimming statistics (from the tracked *A. sanguinea* imaging) that can be calculated from the smoothed trajectories. Directed movements (e.g., vertical velocity) indicate upward and downward migration in the water column. Oscillatory motions provide information on cells' physiological state [3].

References:

1. Bearon, R.N., Grünbaum, D., Cattolico, R.A. 2006. Effects of salinity structure on swimming behavior and harmful algal bloom formation in Heterosigma akashiwo, a toxic raphidophyte. Mar. Ecol. Prog. Ser. 306:153-163

2. Menden-Deuer, S., Grünbaum, D. 2006. Induvidual behaviors and population distributions of A planktonic predator aggregating to phytoplankton thin layers. Limnol. Oceanogr. 51(1):109 - 116.

3. Tobin, E., Grünbaum, D., Cattolico, R.A. 2011. Pelagic-benthic transition of the harmful alga, Heterosigma akashiwo: Changes in swimming and their implications for benthic distributions. Harmful Algae 10(6): 619-628.

4. Ishikawa, A., Hattori, M., Imai, I. 2007. Development of the "plankton emergence trap/chamber (PET Chamber)", a new sampling device to collect in surface sediments of coastal waters. Harmful Algae 6:301-307.

5. Mac Gregor, C., Cybermato Consulting. http://www.cybermato.com/projects/

2. Scholarly Reports

With improved design of our prototype, we expect to publish our findings in Limnology and Oceanography: Methods. During the initial stages of the optical sensor development, support by the Link Foundation was acknowledged in a presentation given by the fellow at the 2011 Salish Sea Ecosystem Conference. Title: "An optical remote sensor for detection and prediction of *Heterosigma akashiwo* Harmful Algal Blooms." A copy of this presentation can be provided upon request.

3. Research Funds

The funds provided by the Link Foundation were used to purchase microcomputers, camera boards, and raw materials (e.g., valves, plastics, electronics, etc.) used in the construction of the instrument. A portion of the funds was also used to purchase the license for our specialized image analysis software.

4. Fellow Benefits

Being chosen for the Link Foundation Ocean Engineering and Instrumentation Fellowship greatly enhanced my academic and professional training. Support provided by the Link Foundation enabled me to incorporate an instrument development chapter into my doctoral thesis, which would not have been possible otherwise. I have also been provided with opportunities to present on the development and use of this instrument to my colleges at the University of Washington, as well as, to a diverse audience of researchers, policy makers and educators at the Salish Sea Conference in Vancouver, B.C.

During my fellowship, I acquired valuable skills in instrument design and engineering, which are not commonly available to graduate students in the field of biological oceanography. Further, the fellowship has aided in my training in field-based research and collaborating/communicating with engaged community members and stakeholders (e.g. aquaculturists).