

## **Real-Time Measurements of Sediment Modification by Large Macrofauna**

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### **LONG-TERM GOALS**

Benthic macroinfauna modify sediment properties through a diverse array of behaviors, this includes altering acoustical properties. Many current models of infaunal effects on sediment properties do not include the diverse effects of various infauna, for two primary reasons: 1) lack of empirical data, and 2) the complexity of infaunal effects that occur on time and spatial scales. The diversity of size, activity, and behavior in benthic communities has proven challenging to capture and understand. Developing new methodologies to capture these small scale impacts and in the process collecting otherwise unavailable empirical data is key to advancing our understanding of sediment modification by large macrofauna, and will allow development of better predictive models of infaunal impacts on sediment acoustics, structure, and biogeochemistry. Furthermore, understanding how these behaviors may change due to the interactions among individuals in a community is also vital to developing realistic predictive models of significance to Naval operations.

### **OBJECTIVE**

The primary objective in the previous project year has been to develop additional techniques to capture particle and porewater transport effects of large benthic infauna that compliment the work completed by Woodin with planar optodes and pressure sensors.

### **APPROACH**

The original proposal that was submitted by Woodin and Marinelli was to use fiber optic spectrofluorometers to capture particle mixing effects of various infauna within sediment, by inserting a series of sensors along a vertical transect, and measuring mixing of both conservative and non-conservative tracers. Unfortunately extensive efforts to utilize this approach by failed and without significant efforts to develop specific methodology to be used on the Ocean Optics spectrofluorometers, a new approach was needed.

I have been working on using fluorescence photography to capture fluorescing compounds (such as chlorophyll a) on sediment surfaces, and has applied this to the ant farm approach. Furthermore, using some simple images analyses, Waldbusser has been able to capture 2-D particle mixing based on displacement of particles in sequential images from ant farm aquaria. These methods have been

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applied to four common species of benthic infauna, that are ubiquitous taxa in shallow and intertidal sediments. The species utilized were the lugworm *Arenicola cristata*, the bamboo worm *Clymenella torquata*, the clam worm *Neries diversicolor*, and the deposit feeding clam *Macoma balthica*. Time lapse image sequences of these organisms were taken at 2-5 min time intervals for up to 3 days for particle mixing effects, or 10 sec intervals for up to 16 hours for fluid transport using fluorescein injection. Additionally, using an algal paste, simultaneous measurements of particle (algae) and fluorescein (porewater) were made using fluorescent-infrared photography.

## WORK COMPLETED

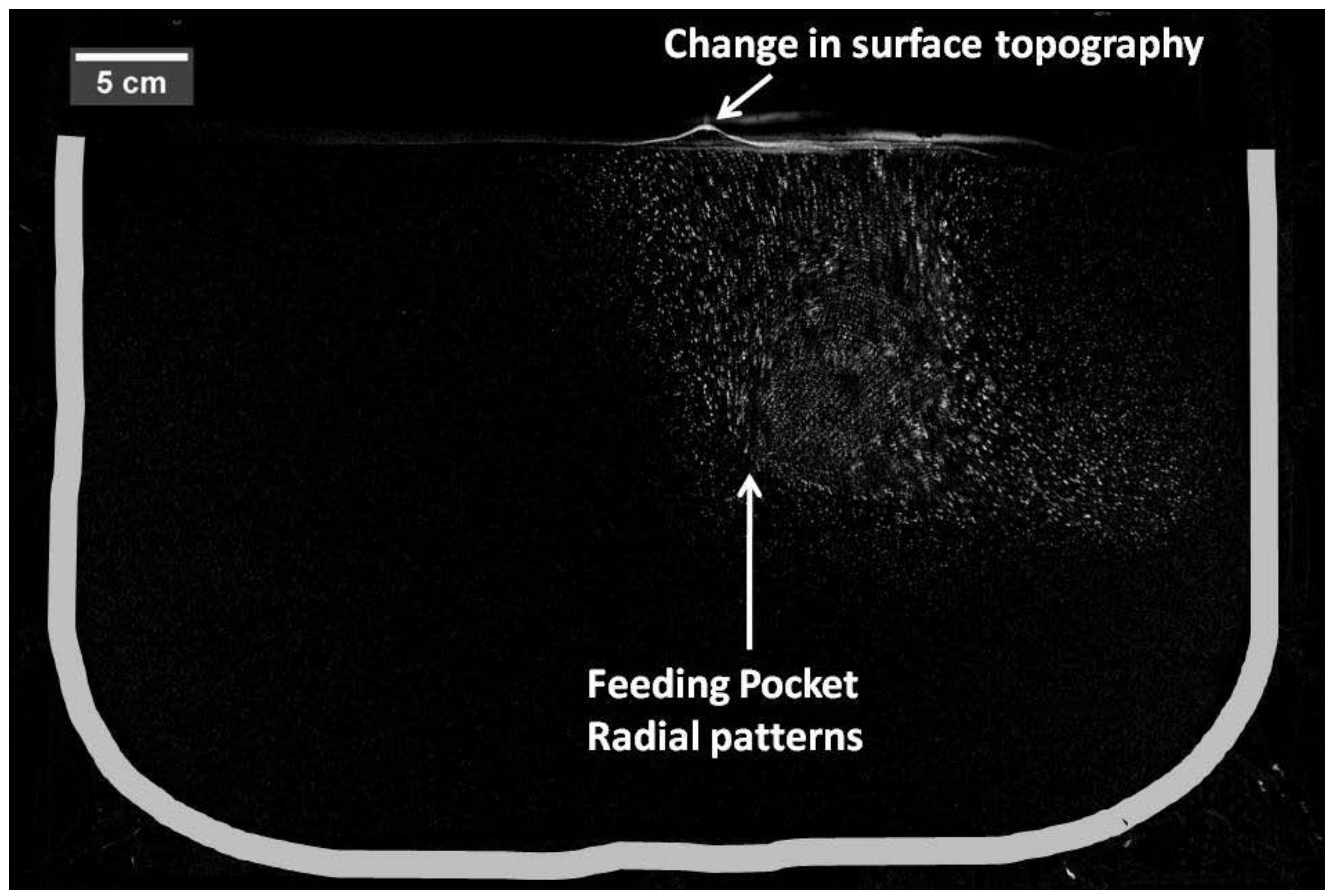
- Extensive trials with the fiber optic spectrofluorometer, resulting in recognition that without very specialized methods the ability to measure transient fluorescence within the sediment, as initially planned, would not be possible.
- Acquisition and use of UV-IR digital SLR cameras on sediment ant-farm aquaria, providing a relatively straightforward method for making measurements.
- Develop image analysis methodology to measure particle mixing via sequential image subtraction and integration techniques to quantify overall effects.
- Measurement of extent and intensity of particle mixing by four different infaunal species using time-lapse photography and image analysis software.
- Measurement of porewater flow fields due to infaunal activity using fluorescein injection and fluorescence photography.
- Measurement of changes in particle mixing by lugworms due to addition of food at sediment surface.
- Imaging of 2-D chlorophyll a depth distributions in sediment using fluorescence and infrared photography.
- Simultaneous measurements of porewater flow fields and mixing of sediments.
- Determination of fluorescein adsorption in four sediment types spanning from highly permeable sand to very muddy sand, covering a range of hydraulic conductivities from  $0.04 \text{ cm s}^{-1}$  to  $1.7 \text{ cm s}^{-1}$ .

## RESULTS

Recognizing the methodological hurdles to applying the fiber optic spectrofluorescent has allowed Waldbusser to take the research in a new direction, by employing commercially available digital SLR UV-IR cameras. These cameras may be mounted in commercially available sediment profiler imaging (SPI) systems, and using an array of light filters would allow one to take the techniques that have been developed in the lab and apply them to real time monitoring or measurement of animal activities and sediment properties.

Direct measurements of particle mixing effects have been previously made using fluorescently labeled luminophores. The typical methodology entails using fluorescent photography, but the particles have to be laid on the sediment surface. The fluorescent tracers are limited in supply and relatively expensive. Utilizing sequential image subtraction, I have been able to capture particle mixing effects at the

sediment surface as well as below the sediment surface (where luminophores would not be found in typical deployments). In order to do this, time lapse images are subtracted sequentially from each other using image analysis software then that sequence is summed to add all changes over a given time frame (Figure 1). This procedure works best in sediment that has texture to it so that the image analysis software may detect changes in pixels over time.

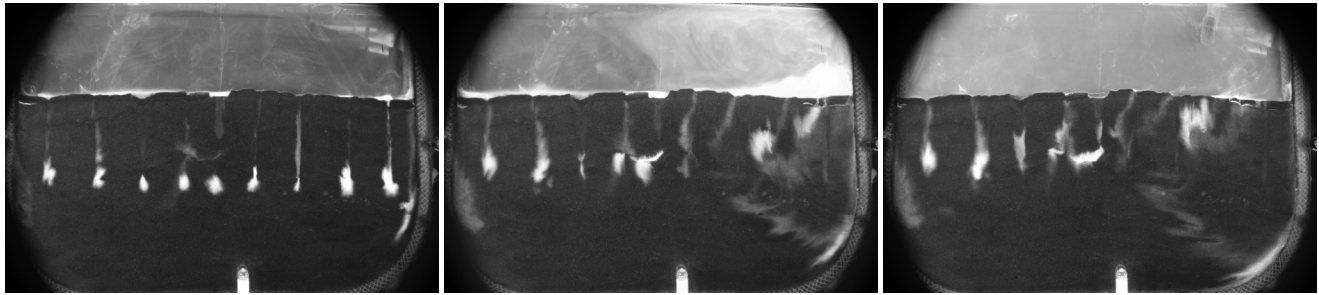


**Figure 1. Image Analysis of Lugworm Sediment Mixing**

*[Graph: Lighter areas indicate higher variability between sequential photographs of the ant farm. Analysis was performed on a 20 minute sequence with a 2 minute shooting interval. The sediment area, enclosed within the grey border, is approximately 50 cm across. Based on the mixed area, the feeding pocket appears to be almost 10 cm across, and to a depth of 20 cm. Notice the radial patterns emanating from the bottom left area of the lighter area indicating the feeding pocket, and the change in surface topography. ]*

Porewater irrigation patterns were measured by injecting fluorescein into the sediment at depth and using time-lapse fluorescence photography (Figure 2). Due to the adsorptive properties of fluorescein, breakthrough experiments were run in flow through sediment columns to determine adsorptive properties in different sediment types. The results from these experiments will be used to correct values obtained in the images for adsorption effects that would lead to underestimates of infauna effects on porewater transport. Figure 2 illustrates one of several runs utilizing different depths and injection patterns across the ant farm field. Currently the best estimates of flow fields of lugworms are based on discrete sampling and modeling, or recent oxygen planar optode work by Woodin. As

revealing as the oxygen optode data has been, once the oxygen concentrations drop to zero, there is no longer a tracer of porewater flow until anoxic water reaches the sediment surface. These fluorescein experiments provide an opportunity to capture greater detail in the flow field, and examine lugworm induced porewater advection flow fields (as well as advection from other infauna). There is however some concern that the fluorescein would impact behavior of the lugworms (and other infauna), but it was noted during these experiments that at least one organism was still actively pumping (figure 2, right hand side).



**Figure 2. Fluorescein distribution at time 0, 30, and 60 min (left to right) from multiple injections to a depth of 10 cm in an ant farm aquarium.**

***[Graph: Images were taken at 10 second intervals for an hour in color, with red, green, blue channels split, and the above are only the green channels for each time point. There are two lugworms in this ant farm, one on each side, notice the much higher activity and porewater advection on the right hand side relative to the left. Also, in the center of the ant farm, between the two lugworms there appears to be some complex flow patterns and possible interactions between the two flow fields. Based on this image sequence, maximum irrigation induced porewater velocities exceed 10 cm per hour, however the flow field is quite complex]***

Ideally, using natural tracers provides the least chance of the manipulation causing changes in behavior, with fluorescence-infrared photography, it is possible to capture very low concentrations of chlorophyll a and possibly other phaeopigments. Typical excitation-barrier filters used on a camera can capture the chlorophyll a, however from working with this method it appears to be somewhat coarse. The infrared detection helps to capture a much stronger signal from the chlorophyll a, allowing greater sensitivity. Using algal pastes, I have successfully imaged the spatial extent, decay, and transport of freshly deposited algae by benthic infauna. Additionally, utilizing the green and red fluorescence of fluorescein and chlorophyll a, respectively, I have been able to simultaneously measure porewater movement and algal transport, by separating these color channels. This reduces interference between the two signals, and permits unique, concurrent measurements of particle and fluid transport induced from infaunal activity.

Ongoing activities include optimization of these methods, and additional experiments examining more closely intra and interspecific interactions, with an emphasis on how these interactions may result in non-linear density effects of infauna. Behavioral responses to changes in food availability are also currently being completed. With the current imagery data I have, I am also exploring the use of particle image velocimetry (PIV) to better quantify the complex patterns of particle movement by benthic infauna. PIV has typically been used to capture fluid flow fields; however with proper timing on image capture, this should be able to be applied to sediment mixing as well. These timelapse methods allow

for the collection of highly temporally and spatially resolved extensive photographic data, which will continue to provide new insights into the role of infauna in sediments. With further work on these methods, standardized image processing routines will be made available to permit rapid data collection, manipulation, and interpretation.

## **IMPACT/APPLICATIONS**

Most of the effects of infauna on sediment properties and biogeochemistry occur at small spatial scales, however most measurement techniques do not capture the dynamics on these small scales. The novel applications of these techniques to sediments will allow investigators to measure the small scale dynamics that when integrated across larger space and time scales drive system processes. Utilization of the sequential image subtraction methods to capture particle movement in real or near real time will permit great understanding of highly dynamic and transient infaunal effects on sediment properties. These methods should be easily adapted to in-situ instrumentation such as sediment image profiler systems.

## **RELATED PROJECTS**

I have been working on a related project with Christof Meile at the University of Georgia, specifically looking at the interactions among benthic infauna, impacts on sediment properties, and spatial dimensions of these effects. Dr. Meile is a sediment modeler who is working at the forefront of 3-D biogeochemical modeling in sediments, and the overlap between projects provides an opportunity to continue the current experimental work, and expand the scope to include modeling components.

## **PUBLICATIONS**

Waldbusser, G.G. and R.L. Marinelli (2009) Infaunal Functional Groups and Important Attributes to Permeable Sediment Processes: A Multi-site, Multi-species Investigation. *Journal of Marine Research*. [in press, refereed]