Application of a Holographic sensor for Plankton Ecology

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ABSTRACT

Historical methods of zooplankton sampling and identification are limited, particularly with regard to spatial and temporal resolution in biologically patchy coastal marine ecosystems. One approach to improving zooplankton community characterization is high-resolution plankton imaging coupled with automated plankton identification. The first requirement for this approach is to image a representative sample of the zooplankton community. Achieving this requirement depends upon factors including the scales of biological patchiness, the speed at which the sampling platform moves through the environment, and the rate at which images are acquired (i.e. the total volume sampled by imaging). Testing of this approach was enabled by deployment of a holographic imaging instrument on the *Dorado* autonomous underwater vehicle (AUV), coupled with targeted sampling by the AUV's Gulper water sampling system. The Gulper's rapid sample intake is considered to minimize escape of microzooplankton, thus zooplankton enumeration by microscopy conducted on these samples may serve as ground truth for imaging methods. Conducting the first comparison of this nature, this intern project employed microscopy and image data sets acquired as part of the Sampling and Identifying Marine Zooplankton project (J. Harvey, Vrijenhoek Lab) from August and

October 2013. The low abundance of zooplankters identified in images relative to those identified by microscopy of Gulper samples indicated that the imaging sensor, as currently deployed, is not seeing a representative sample of the zooplankton community. This finding motivated modification of the plumbing system by which water is supplied to the imaging sensor. This modification was intended to reduce bias caused by zooplankton avoidance of the plumbing intake, and it was tested in a subsequent field program.

INTRODUCTION

The zooplankton is a good indicator of ecosystem health and function. It is a surrogate of important processes and helps to indicate certain types of environmental conditions such as upwelling events, larval recruitment and climate change. It is also critical to the marine food web and plays a significant role in the biological pump and the carbon cycle that regulate the temperature of our planet. Understanding the temporal and spatial scales of plankton community dynamics allows us to get a more global view of the health of the ocean on various scales.

The dynamic nature of coastal environments and the ephemeral nature of zooplankton communities make it difficult to adequately sample and understand them. Classical methods of zooplankton collection include net tows, water bottles and pumps, all of which are very limited in the temporal and spatial scales and the resolutions they can provide. Platform technology developments such as autonomous underwater vehicles (AUVs) have allowed us to increase the spatiotemporal resolution of environmental characterization, as well as target sample acquisition within features of interest that are dynamically and autonomously recognized by onboard AUV algorithms.

MBARI's Sampling and Identifying Marine Zooplankton (SIMZ) project (J. Harvey, Vrijenhoek Lab) explores genetic diversity of marine zooplankton. To sample moving patches of plankton, SIMZ uses deployments of MBARI's *Dorado* AUV to target phytoplankton patches, recognized using real-time analysis of optical data, as well as control samples outside of these patches (Zhang et al, 2010). SIMZ sampling has focused on northern Monterey Bay (Figure 1), and observations along the SIMZ AUV

transects provided the basis for this project. A time series of vertical sections along the AUV transects illustrates the tremendous complexity, patchiness, and variability of this environment (Figure 2). Comparison of August and October observations reveal major seasonal changes in this environment and phytoplankton distributions. Stratification of the water column, represented by water density in Figure 2, was far greater in August than October. Consistent with these physical conditions, phytoplankton populations were far more concentrated in subsurface layers during the more stratified period of August. The patterns in optical backscattering reflect both the distributions of phytoplankton, identifiable by their chlorophyll fluorescence, as well as suspended sediment, which exhibited low chlorophyll fluorescence and very high backscattering near the bottom during October.

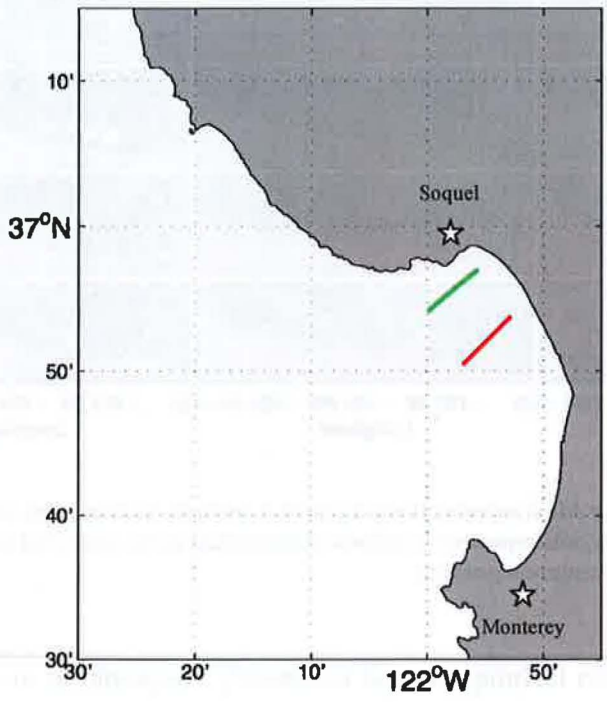

Figure 1. During SIMZ fall 2013 cruises, AUV transect A followed a southwesterly (green) direction beginning approximately 1 mile south of Soquel, and transect B followed a northeasterly (red) direction.

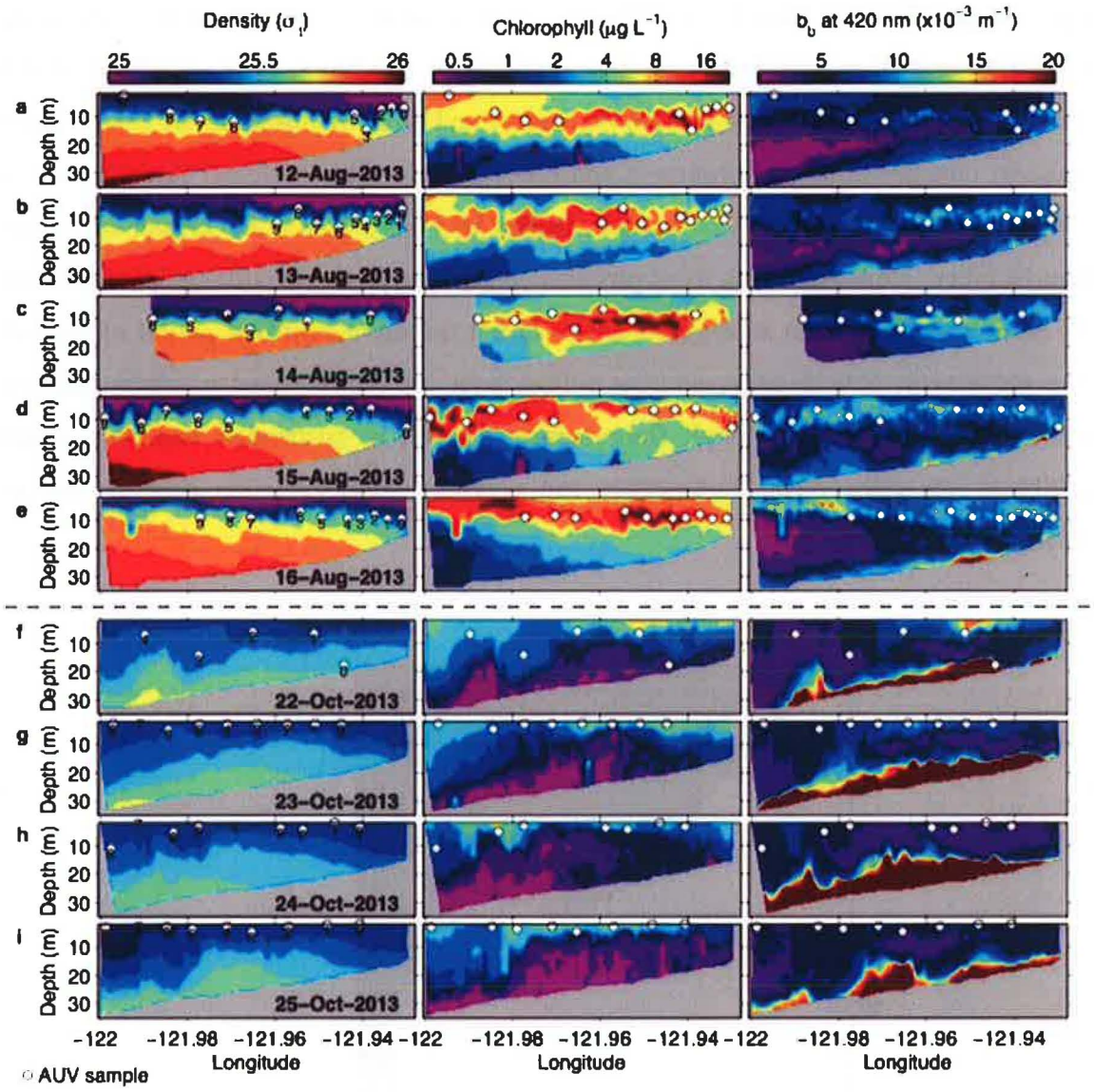

Figure 2. Environmental results for AUV surveys along transect A include contour plots of density, chlorophyll and backscatter. White dots represent locations of individual gulps collected along each transect with the chlorophyll peak capture algorithm.

The two most common techniques used to identify zooplankton in water samples are microscopy and molecular assays. The various types of microscopy allow us to view cells and organisms in different ways, but they each have their disadvantages (Wilson and Bacic 2012), the most notable of which is inconsistency due to human error. Molecular techniques increase the accuracy of identification by eliminating some of these flaws, yet molecular methods also have limitations (Harvey et al 2012). Water samples collected by the Dorado AUV's 1.8 L Gulpers are used to characterize zooplankton abundance and

diversity via both microscopy and molecular probes. Molecular identification of zooplankters follows the 96-well plate version of the sandwich hybridization assay (SHA), which is based on the detection of rRNA sequences (Goffredi et al 2006). As the environmental and optical observations from this data set showed (Figure 2), results from molecular probes revealed strong seasonal changes, for example much greater abundances of echinoderm larvae during October compared to August (Figure 3). The high-resolution targeted sampling by Dorado also reveals major changes in zooplankton community composition over small spatial scales (Figures 2,3).

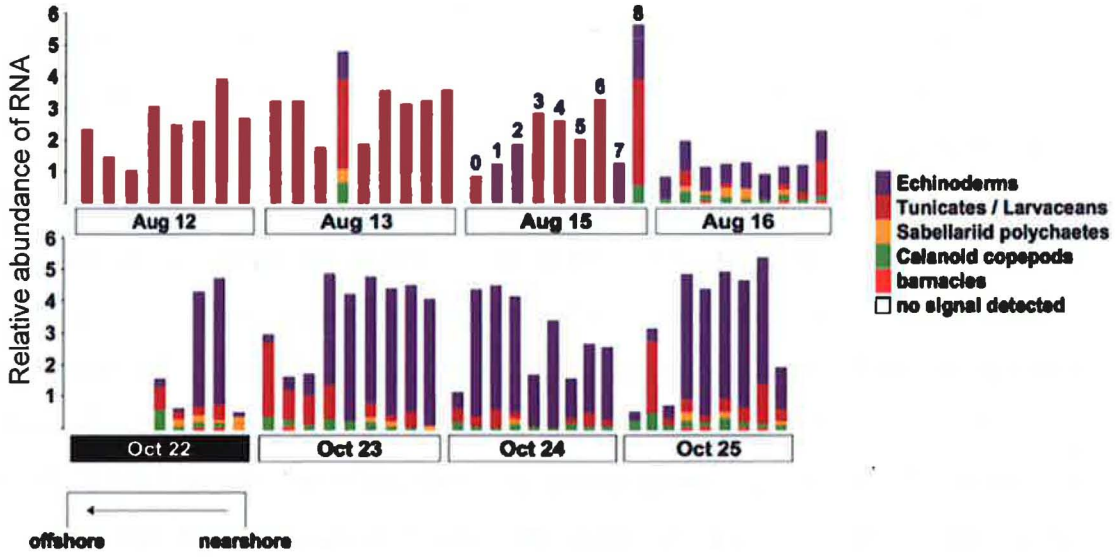

Figure 3. SHA results for AUV samples collected with the chlorophyll peak capture algorithim. Relative sample locations (offshore to nearshore) indicated on the first sampling date apply to all subsequent dates for each transect, respectively. Arrows indicate direction of AUV travel along transect A. Sampled collected 22 October samples were accidentally collected with a different algorithm (not chlorophyll peak capture).

Recent advances in image analysis have been adapted for measurement, enumeration and imaging of particles, and this introduces new opportunities in zooplankton ecology research. The LISST-HOLO (laser in situ scattering and transmissometry holography; Sequoia Scientific) is one of the commercially available submersible digital holographic particle imaging systems. The LISST-HOLO is readily integrated on the *Dorado* AUV and can image a wide range of plankton sizes (25-2500 um). The optical end has a 5 cm optical path traversed by a red (658 nm) laser that

overfills a 7 x 4 mm charged coupled device (CCD) array to create a hologram (Sequoia Scientific 2011). The AUV provides an excellent platform for the LISST-HOLO, as it collects high-resolution observations of physical, chemical and optical properties of the water column. Collecting images along the AUV's transect allows integration of biological and environmental data. Since a LISST-HOLO was integrated with Dorado in 2012, development efforts have focused on advancing image processing methods and testing of automated plankton identification using a software infrastructure previously developed for detecting animals in ROV video (previous intern project by S. Peterson). Particle statistics from LISST-HOLO have shown ecological patterns such as accumulation of particles in a hydrographic front, and ancillary data from other sensors confirmed that this description related to the phytoplankton. A primary goal of this instrument integration effort, and the focus of this intern project, is to advance the applications of LISST-HOLO to zooplankton ecology research.

Study of zooplankton ecology with LISST-HOLO carries the additional challenge of ensuring that swimming zooplankton do not avoid detection. While the flow rate within the LISST-HOLO flow chamber should not exceed 0.5 ms^{-1} for successful imaging, the AUV maintains a speed through the water of ~1.5 ms^{-1}. Maintaining a high AUV speed is valuable to synoptic observation of a rapidly changing environment. This necessitates the use of a plumbing system to supply ambient water to LISST-HOLO at a speed slower than 1/3 that at which the vehicle moves through the water. This significantly slower flow rate, combined with the fact that the AUV introduces a bow wave into the environment ahead of its trajectory, introduces concerns of zooplankton avoidance of the sampling system. The original goals for this internship included examination of this sampling problem and expansion of the zooplankton classification library to be used for automated identification. However, results for the first goal clearly showed that the primary sampling problem, imaging a representative sample of the zooplankton community, was to be the focus of the project.

MATERIALS AND METHODS

Images used during this study were collected during SIMZ AUV surveys 12-16 August and 22-25 October 2013 (Figure 2) on the RV Rachel Carson. To process images, Danelle Cline established a high throughput-Condor workflow on sixteen single core virtual machines using a modified version of the commercially available LISST-HOLO software. The workflow consisted of three steps: 1) process the images, extract statistics, metadata and particle images, 2) aggregate the data, and 3) create a web interface of the results to assist analysis.

Surveys from 14 August 2013 provided preserved samples for microscopy. Gulper whole water was filtered onto two 30 μm filters. Filters were preserved with 7 mL 10% formalin, transferred to 20 mL glass scintillation vials and stored at room temperature. In the laboratory, filters were rinsed into a plastic dish with 0.2 μm-filtered seawater. Zooplankters within the dish were identified into major groups and enumerated using an Olympus Research Stereo dissecting microscope (Model SZH10). Filters were also examined under the microscope for residual plankters.

Gulper samples provide a good comparison because they provide a point sample, with rapid sample intake in 1-2 sec (Harvey et al 2012) designed to break through the boundary layer and to reduce the potential for swimming microzooplankton to escape. These factors are thought to promote representative sampling of the zooplankton community. For direct comparison of images and Gulper sample results, time stamps were identified and matched using MATLAB. This opportunity was only for a limited number of samples (n=9 in which images were acquired around Gulper samples) from two AUV surveys on the same day. Since images were collected every 5 seconds, one image was examined from either immediately before or at the same time as each gulp, and up to four images after each gulp to account for the LISST-HOLO plumbing lag, for a total of 4 to 5 images associated with each gulp.

RESULTS AND DISCUSSION

In the comparison of microscopy on Gulper samples with HOLO images, there was a striking difference in the characterization of diversity (Table 1). While the

microscopy showed high diversity (15 groups including multiple genera per group), the LISST-HOLO images near the Gulper samples showed very little diversity (2 zooplankters altogether, representing 2 groups). Of all groups identified via microscopy, all but 4 groups (bivalves, gastropods, amphipods and cladocerans) were seen in the greater LISST-HOLO image set (all images collected in the survey, not constrained near Gulper samples). However, groups identified in images were far less abundant than those detected by microscopy.

Microscopy															
Identity	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**
Calanoid	30	20	28	36	20	41	9	27	13	26	13	24	9	11	10
Copepod nauplius	194	338	57	218	119	69	133	157	539	382	273	90	144	263	203
Harpaticoid	1	2	0	2	1	0	0	1	2	0	3	0	4	1	0
Larvacean	4	3	7	6	4	2	2	5	1	2	3	0	3	0	0
Larvacean sac	31	21	13	6	12	10	9	19	10	10	7	11	26	3	0
Polychaete	10	5	0	3	3	0	2	8	0	4	6	3	12	23	15
Echinopluteus	6	1	2	0	4	1	0	4	2	1	2	3	11	6	3
Bivalve	4	3	4	4	4	0	1	3	2	4	1	0	5	9	7
Tintinnid	11	17	2	7	4	0	1	3	11	15	13	3	15	14	13
Gastropod	1	3	3	0	2	0	0	0	1	0	0	2	1	2	1
Barnacle nauplius	5	15	3	5	1	0	1	1	5	3	8	1	4	0	0
Cyprid	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0
Chaetognath	1	0	0	0	0	0	1	0	3	2	2	1	0	1	0
Amphipod	2	1	1	3	0	0	1	0	0	0	0	0	0	1	1
Cladoceran	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
LISST-HOLO Images															
Calanoid	0	na	1	na	0	0	0	na	0	na	0	0	na	0	0
Copepod nauplius	0	na	0	na	0	0	0	na	0	na	0	0	na	0	1

Table 1. List of zooplankters identified via microscopy in AUV Gulper samples from the two transects (A and B) 14 August 2014, as well as zooplankters identified in time-matched LISST-HOLO images.

There are two primary hypotheses for the severe underrepresentation of diversity and abundance by the LISST-HOLO images, as compared to microscopy on the Gulper samples. The first is undersampling of populations by imaging, due to the small volume

imaged by the sensor, its relatively slow frame rate, and the rapid movement of the AUV through the water that spreads the images far apart in space. Considering the maximum concentrations of an individual zooplankton species in the microscopy data (539 individuals in 1.8 L), the volume imaged by LISST-HOLO (1.86 cm^3 = 0.1% of a Gulper sample) would contain at most 0.5 individuals in a single image. Thus, the small imaging volume is clearly a concern, and the infrequent sampling from a rapidly moving platform would only compound the problem. The second hypothesis is that the pumped system initially developed to supply ambient water to LISST-HOLO fails to acquire a representative sample from the environment. A slow flow rate is required for imaging, and this slow intake rate combined with a bow wave produced by a rapidly moving AUV may allow significant avoidance by swimming plankton. The majority of plankters identified in images were relatively small and weak swimmers, such as copepod nauplii and tintinnids, which suggests that the stronger swimmers may be avoiding the intake.

Discovery of this underrepresentation motivated an attempt to supply water to LISST-HOLO in a way that reduced the potential for avoidance by zooplankton. The water intake valve was located about a meter behind the AUV's nose, which may allow avoidance behavior by zooplankters triggered by the AUV's bow wave. A longer intake valve extending ahead of the AUV's bow wave could reduce avoidance. As a first step to improve plumbing and reduce avoidance, a meter-long piece of PVC was connected to the intake and extended the intake lengthwise to about 2/3 meter directly ahead of the AUV. This modified intake was applied during the SIMZ cruise off Bodega, CA during 28-30 July 2014. Analysis of images collected during this cruise, along with Gulper samples, indicates that the AUV plumbing modification did not significantly improve the representation of diversity by LISST-HOLO and plankters may have still avoided intake.

CONCLUSIONS/RECOMMENDATIONS

Automated plankton identification using LISST-HOLO has the potential to enhance sampling and characterization of the plankton community by reducing the time- and labor-intensive microscopic examinations and by providing synoptic data at greater resolution than water sampling methods can provide. This project identified two

requirements for progress in effective image-based zooplankton characterization: (1) increasing the amount of water imaged, and (2) minimizing the potential for zooplankton avoidance of the sampling / imaging system. Increasing the amount of water imaged requires advances in the imaging sensor itself, particularly faster frame rates. A holographic imaging device with sustained frame rates nearly two orders of magnitude higher than LISST-HOLO has been developed a company named 4-deep, and this sensor has been tested as part of MBARI's cytometer intercomparison project. While this sensor has advances in throughput and processing that will certainly help solve the sampling problem, its plumbing system and integration with an AUV are undeveloped. Minimizing the potential for zooplankton avoidance requires consideration instrument mounting on/in the AUV and AUV speed. Having the AUV move slower would reduce its synoptic sampling capabilities, but combined with more careful design of sample intake it could reduce or eliminate the problems associated with slow pumping into a rapidly moving AUV, which disturbs the water ahead of the vehicle and allows avoidance behaviors to interfere with effective sampling. Replacing the continuous flow pump with a peristaltic pump and controlling image acquisition timing accordingly may allow imaging only when the zooplankton suctioned into the imaging chamber are moving slowly. This could permit intake more like the Gulper, to reduce avoidance, followed by imaging while the water in the imaging chamber is not moving too rapidly for effective imaging.

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