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Evolution and field application of a plankton imaging system

Andrew Walker Remsen

University of South Florida

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Evolution and Field Application of a Plankton Imaging System

by

Andrew Walker Remsen

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
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University of South Florida

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Evolution and Field Application of a Plankton Imaging System

Andrew Walker Remsen

ABSTRACT

Understanding the processes controlling the distribution and abundance of zooplankton has been a primary concern of oceanographers and has driven the development of numerous technologies to more accurately quantify these parameters. This study investigates the potential of a new plankton imaging sensor, the shadowed image particle profiling and evaluation recorder (SIPPER), that I helped develop at the University of South Florida, to address that concern. In the first chapter, results from the SIPPER are compared against concurrently sampling plankton nets and the optical plankton counter (OPC), the most widely used optical zooplankton sampling sensor in the field. It was found that plankton nets and the SIPPER sampled robust and hard-bodied zooplankton taxa similarly while nets significantly underestimated the abundance of fragile and gelatinous taxa imaged by the SIPPER such that nets might underestimate zooplankton biomass by greater than 50%. Similarly, it was determined that the OPC misses greater than a quarter of resolvable particles due to coincident counting and that it can not distinguish between zooplankton and other abundant suspended particles such as marine snow and *Trichodesmium* that are difficult to quantify with traditional sampling methods. Therefore the standard method of using net samples to ground truth OPC data should be reevaluated. In the second chapter, a new automated plankton classification system was utilized to see if it was possible to use machine learning methods to classify SIPPER-imaged plankton from a diverse subtropical assemblage on the West Florida Shelf and describe their distribution during a 24 hour period. Classification accuracy for this study was similar to that of other studies in less diverse environments and similar to what could be expected by a human expert for a complex dataset. Fragile plankton taxa such as larvaceans, hydromedusae, sarcodine protists and *Trichodesmium* were found at significantly higher concentrations than previously reported in the region and thus could play
more important roles in WFS plankton dynamics. Most observed plankton classes were found to be randomly distributed at the fine scale (mm-100 m) and that greatest variability within plankton abundances would be encountered vertically rather than horizontally through the water column.
Preface

This research concludes a long and circuitous foray into the worlds of technology and marine biology in an attempt to develop and apply a new research tool to better understand the important role that zooplankton play in the ecology of the world oceans. I was lucky enough to arrive at the USF CMS during a time of dynamic growth and innovation and become involved with a lab that was pushing the boundary towards applying new biological sampling methods to its research. This work was one of the primary drivers behind the development of what would later become the Center for Ocean Technology (COT).

My preliminary research objective was to investigate the oceanic zooplankton assemblage response to different water mass conditions in the Gulf of Mexico using what was then the state of the art optical plankton counter (OPC). Results from the OPC were to be calibrated using organisms collected from the multiple net system sampler described by Tracey Sutton in his dissertation. However, we could never find any predictable relationship between what was counted and sized by the OPC and the zooplankton we collected in the nets. Fortunately, Dr. Hopkins’s tenure as the first director of the Center for Ocean Technology (COT) gave me the opportunity to become closely associated with COT engineers in researching and designing new technologies to augment and or replace our use of the OPC in studying zooplankton. I initially collaborated with a graduate student from Florida Atlantic University, Tom Wilcox, to develop and test a high-frequency broadband sonar to count, size and image individual zooplankton and that would be mounted on our plankton sampling platform. By acoustically imaging particles that would later be sampled by the OPC, we hoped to be able to identify these particles and determine why there was a discrepancy between the nets and the OPC (Remsen et al., 1996). However, the necessary size resolution of the sonar precluded it from having any measurable range and therefore only imaged zooplankton and other particles directly in front of it. When I presented preliminary data from the sonar at an ONR sponsored bioacoustics workshop,
one of the conveners, Dr. Peter Wiebe from WHOI, remarked that with that range, I should think about using an imaging system instead of a sonar as the main reason for using acoustics is for its longer range.

Soon after, I became deeply involved in the development and field testing of the shadowed image particle profiling and evaluation recorder (SIPPER), a joint project between our lab and COT. The SIPPER was developed from an initial idea between Dr. Hopkins and Larry Langebrake of COT as an imaging analog to the OPC. Data from the SIPPER gave us the opportunity to investigate planktic distribution at the scale of the individual zooplankter and observe plankton in-situ. Preliminary field work with the SIPPER provided evidence that both plankton nets and the OPC had serious problems in quantifying the zooplankton assemblage in the Gulf of Mexico. This corroborated my observations from analyzing concurrently collected plankton net and OPC data collected during three years of sampling at an offshore sampling station and finding no relationship at all between the two datasets. This research then focuses on work after the preliminary field deployment of the SIPPER. It does not include mention of the numerous cancelled research cruises, flooded pressure vessels, short-circuited electronics, broken connectors, cables and winches that have made this such a special experience. The first chapter was published in Deep Sea Research I in January, 2004 (Remsen et al., 2004) and information regarding the distribution of *Trichodesmium* colonies determined in chapter two was included in Walsh et al., 2007.

Finally, this project would not have been possible without the counsel, patience and support of my major professor, Tom Hopkins. His foresight in integrating new technologies with traditional zooplankton sampling methods made this work possible and he was instrumental towards the foundation of the Center for Ocean Technology. My labmates Dr. Tracey Sutton and Dr. Scott Burghart provided invaluable assistance, advice and much needed humor to my endeavors. Much of this work would have been impossible without the engineering ingenuity and skill demonstrated by Larry Langebrake, Dr. Scott Samson, Mike Hall, Bill Flanery, Eric Kaltenbacher, Chad Lembke, Jim Patten, Randy Russell, Ray Carr and Gino Gonzales of COT. Additional help in maintaining the HRS and SIPPER was provided by COT personnel Charlie.
Jones and Joe Kolesar and the USF CMS shop crew, Jim Mullins, Jim Mulholland and Rich Shmid. The automatic plankton recognition system used in Chapter 2 was developed in collaboration with Dr. Dmitry Goldgof and Dr. Larry Hall of the USF College of Computer Science and Engineering with their students Dr. Tong Luo and Kurt Kramer. Assistance at sea was gratefully accepted from Eric Nelson, Bill Husar, Dr. Jose Torres, Graham Tilbury, Jen Jarrell, Chris Simoniello and Richard O’ Driscoll. Funding for this research was provided for by the Office of Naval Research (Grants # N00014-94-0963, N00014-04-1-0421, N0014-02-1-0266, N00014-07-1-0802). I am especially grateful for my committee members Doug Biggs, Frank Müller-Karger, Jose Torres and John Walsh and to Scott Samson for agreeing to chair my defense.

Introduction

Zooplankton are key mediators of particle flux, fisheries recruitment and biomass production within the world oceans (Lenz 2000). Information on their abundance and distribution in space and time are required to accurately predict their contribution to these processes. Field observations of zooplankton indicate that they operate along a continuum of spatial and temporal scales leading to heterogeneous or “patchy” distribution patterns (Haury et al., 1977; Omori and Hamner, 1982; Gallienne et al., 2001). Traditional methods such as plankton nets, pumps and bottles are limited in sampling zooplankton over the entire distribution spectrum, especially at the fine scale (meters to hundreds of meters, seconds to hours) because of their integrative nature and the time consuming task of analyzing individual zooplankton samples. Additionally, a significant fraction may be under-sampled by plankton nets because of extrusion through the net mesh, retention within the net, and destruction of fragile forms such as gelatinous zooplankton when physically captured (Gallienne and Robins, 2001; Halliday et al., 2001; Hopcroft et al., 2001; Warren et al., 2001).

To address these limitations, alternative instruments for sampling zooplankton in situ have been developed over the last twenty years (Schulze et al., 1992; Skjoldal et al., 2000; Wiebe and Benfield, 2003). These new devices provide the increased spatial and temporal resolution necessary to study the coupling between physical processes and zooplankton distribution patterns and for modeling zooplankton population and tropho-dynamics. One of the most widely used of these new instruments is the optical plankton counter (OPC), with approximately one hundred units in use throughout the world (Zhou and Tande, 2002). The OPC provides quantitative measurements of abundance and size of mesozooplankton-sized particles.
(250 μm to 2 cm) and can be deployed from a diverse array of platforms (Foote, 2000). However, the taxonomic resolution of the OPC is limited except in low diversity assemblages where separable peaks in a OPC generated size distribution might be attributable to a specific species or developmental stage (Herman, 1992). Consequently, the OPC is most often used to complement net data by providing high-resolution information on the spatial distribution patterns of the net-identified zooplankton. While many investigators have found rough agreement between net counts and OPC estimates of zooplankton abundance (Foote 2000, Zhou and Tande, 2002), there have been instances where the OPC and net abundance estimates have differed significantly (Grant et al., 2000; Halliday et al., 2001; Sutton et al. 2001). These differences have been attributed to extrusion of zooplankton through the plankton net mesh, counting of detrital aggregates and or large phytoplankton colonies, and coincident counting where the OPC counts multiple particles in the light path as a single larger particle (Woodd-Walker et al., 2000; Zhang et al., 2000; Halliday et al., 2001).

Advances in zooplankton imaging technology could help make sense of these conflicting results. Results from instruments such as the Video Plankton Recorder (VPR, Davis et al., 1992) and the Shadowed Image Particle Profiling and Evaluation Recorder (SIPPER, Samson et al., 2001) indicate that they can provide both high quality taxonomic information and high resolution in the temporal and spatial domains. Previous comparisons between the VPR and nets have indicated that they describe similar distributions for abundant zooplankton groups (Benfield et al., 1996; Gallager et al. 1996), are more effective at sampling fragile and gelatinous forms than nets (Norrbin et al., 1996; Dennett et al., 2002), and can assess the contribution of detrital aggregates or “marine snow” to particles in the mesozooplankton size range (Ashjian et al., 2001).

This paper compares the abundance and size distribution of mesozooplankton and suspended particles sampled by nets and the OPC against data concurrently collected by the SIPPER in offshore waters of the Gulf of Mexico. I hypothesized that the SIPPER should image all particles within the mesozooplankton size range that would be resolvable by either the net or the OPC and act to independently verify the other two sampling systems. The composition of the mesozooplankton assemblage sampled by the SIPPER and plankton nets was then compared.
Methods

Zooplankton were sampled at a station in the oceanic waters of the eastern Gulf of Mexico (27° N 86° W, 3 km water depth) with the High Resolution Sampler (HRS), a comprehensive towed marine particle analysis platform (Figure 1; see Sutton et al. 2001 for a full description). The HRS samples zooplankton through a square 9.6 cm sampling tube (92.16 cm² mouth area) leading to a 20-position cod-end net carousel fitted with 162 μm plankton nets. The nets have an open filtering area to mouth area ratio of 11:1 and the aluminum sampling tube precludes retention of organisms. Mounted inline with the sampling tube was the SIPPER zooplankton-imaging sensor. The sampling tube projects past the frame of the HRS and has a knife-edge to minimize any pressure wave that might develop in front of the aperture to reduce possible avoidance of the sampler by zooplankton. An optical plankton counter (OPC) with a rectangular 2 × 22 cm sampling aperture (44 cm² mouth area) was mounted within the frame of the HRS and a half-meter below the sampling tube. The OPC was positioned so that it would sample water that was not influenced by the frame of the HRS.

Both net and electronic zooplankton sampling are computer controlled onboard ship via a custom designed software interface such that new SIPPER and OPC files are created and the sensors begin sampling when a net is triggered open. When a particular net sample is ordered closed, the OPC and SIPPER files corresponding to that net sample are also closed, thereby creating two independent samples that can be compared against the net sample. Environmental and diagnostic information (CTD, fluorometer, transmissometer, inclinometer, and flow-meter data) are continuously recorded on a separate HRS data file for later analysis.

A single deployment sampling 10 discrete depths (10-100 m in 10 meter increments) beginning two hours after local sunset on July 21st, 2000, was chosen for this study. Each depth was sampled for 10 minutes. The SIPPER and the net system both collect zooplankton through the sampling tube at the front of the HRS and therefore sample the exact same volume of water. 37.9 m³ of water was sampled by these two systems for this study, averaging 3.79 (+0.18) m³ per depth stratum. The OPC, which was situated below and slightly aft of the HRS sampling tube, has a sampling aperture approximately half that of the other two systems, and filtered a total of 18.39
m^3 of seawater, averaging 1.84 (±0.09) m^3 per depth stratum. Tow speed was determined with a calibrated flow meter (TSK Inc.) mounted at the front of the sampler. It registered a near constant tow-speed of 0.75 m s\(^{-1}\). Inclinometer data indicated that the HRS maintained a near-perfect horizontal attitude at each depth stratum.

Figure 1. Schematic of the USF High Resolution Sampler indicating location of the described zooplankton sampling systems. Only one net is shown attached at the carousel to reduce confusion in the figure.

**Net Sample Treatment**

Zooplankton collected in the nets were fixed immediately in 5% v:v buffered formalin upon recovery of the sampler and stored for later analysis in the laboratory. Net samples were split into subsamples, when necessary, with a Motoda splitter for analysis of approximately 1000 individual organisms per sample. Identifications were carried out to species when possible for copepods and to major group for the other taxa with a dissecting microscope. The cyanobacteria
Trichodesmium was noted if present but was not enumerated, as it tends to be difficult to wash off the net mesh making quantitative analysis difficult.

Zooplankton were measured to total length by methods described in Hopkins (1981). Equivalent spherical diameter (ESD) was calculated for individuals of each taxon with Optimas (Media Cybernetics, version 6.5) image analysis software and a video camera connected to the microscope for comparison with OPC and SIPPER size measurements. Optimas determines ESD by measuring the area of an object and then calculating the diameter of a sphere with the same area. Regressions for calculating ESD from total lengths for each taxon were then calculated and applied to each net sample. Sample biovolume was calculated for each sensor according to the equation

\[
SBV = k \left[ \sum_{i=1}^{n} \frac{\pi}{6} (ESD_i)^3 \right],
\]

where \( k \) is the sub-sample ratio, \( n \) the number of individuals, and \( ESD \) the ESD of the \( i \)th individual (Labat et al., 2002). Biomass for net and SIPPER samples was calculated from regressions determined for zooplankton from the Gulf of Mexico by our laboratory (Table 1) by measuring 50 individuals from each selected taxon.

**SIPPER data analysis**

The SIPPER is a continuously imaging zooplankton sensor that records two dimensional, high resolution images of zooplankton and other suspended particles prior to sampling by the HRS plankton nets (for a full description of SIPPER, see Samson et al., 2001). The SIPPER works by projecting a collimated laser light sheet perpendicular to seawater flow through the sampling tube of the HRS and continuously imaging the outlines and shadows of particles as they pass through the sheet onto two line-scan camera systems mounted orthogonal to each other. In this manner, pairs of digital images are created for each particle passing through the sensor. These cameras are capable of recording images at up to 8-bit (256) color grayscale, but we used a real time thresholding step to reduce the recorded data to single bit or black and white images. This dramatically reduced the instantaneous data rate of the system, increased data storage...
capability, and made particle detection easier as particles were comprised mostly of black foreground pixels against a white background.

Because zooplankton will be randomly oriented as they pass through SIPPER, the use of two orthogonally mounted cameras significantly increases the possibility that at least one camera will capture an image of a zooplankter in a recognizable orientation. Line-scan cameras build an image one line at a time, and because the particle flow through the SIPPER/net sampling tube is unidirectional, each particle can only be imaged once. Figure 2 demonstrates the concept of a line-scan camera system using a single camera for easier conceptualization.

Figure 2. Diagram illustrating the general concept of the SIPPER line-scan camera system. A cumulative image of the sample volume is built of many individual single scan lines. In this figure, multiple scan lines are included in the “single scan line” frames in order to minimize the number of single scan line frames illustrated.
Table 1. Dry weight biomass regressions for zooplankton from oceanic waters of the Gulf of Mexico (N=50 for each taxon).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dry weight regressions (mg DW)</th>
<th>Correlation ($r^2$)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepod</td>
<td>$DW = 0.0085(ML)^{3.1007}$</td>
<td>0.988</td>
<td>ML=metasomal length</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Combination of 38 copepod taxa in the NE Gulf of Mexico</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>$DW = 0.0002(TL)^{3.1612}$</td>
<td>0.971</td>
<td>TL=total length</td>
</tr>
<tr>
<td>Cnidaria</td>
<td>$DW = 0.0029(ESD)^{2.28}$</td>
<td>0.868</td>
<td></td>
</tr>
<tr>
<td>Decapod and Euphausiid</td>
<td>$DW = 0.001(TL)^{3.1331}$</td>
<td>0.977</td>
<td></td>
</tr>
<tr>
<td>Doliolid and Salp</td>
<td>$DW = 0.0108(TL)^{1.6307}$</td>
<td>0.986</td>
<td></td>
</tr>
<tr>
<td>Larvacean</td>
<td>$DW = 0.0164(HL)^{2.0922}$</td>
<td>0.995</td>
<td>HL=head length</td>
</tr>
<tr>
<td>Meroplankton</td>
<td>$DW = 0.0041(ESD)^{2.31}$</td>
<td>0.881</td>
<td>Mostly echinoderm bipinnaria and plutei</td>
</tr>
<tr>
<td>Mollusc</td>
<td>$DW = 0.0296(ESD)^{1.5646}$</td>
<td>0.878</td>
<td>Mostly pteropods and some heteropods</td>
</tr>
<tr>
<td>Other crustaceans</td>
<td>$DW = 0.0505(TL)^{1.8223}$</td>
<td>0.982</td>
<td>Mostly ostracods and some amphipods</td>
</tr>
<tr>
<td>Polychaete</td>
<td>$DW = 0.0091(TL)^{1.801}$</td>
<td>0.977</td>
<td>Combination of unid. polychaetes and Tomopteris sp.</td>
</tr>
<tr>
<td>Siphonophore</td>
<td>$DW = 0.0088(TL)^{0.0414}$</td>
<td>0.981</td>
<td></td>
</tr>
</tbody>
</table>
Image resolution is determined by the line scan camera pixel array size in one dimension (in this case 9.6 cm divided by 2048 pixels in the camera array allows for 47 µm resolution) and the flow speed through the sample tube divided by the line scan rate of the camera system in the other (in this case ~0.75 m s\(^{-1}\) divided by 15,000 line scans a second allows for an average pixel dimension of 50 µm). Therefore, apparent pixel dimensions used for SIPPER imaging were almost equal, measuring 47 by 50 µm. This was confirmed by comparing the size of large unique organisms from the SIPPER dataset with the actual organism measurements from the concurrent net sample. The near uniform apparent pixel dimensions also ensured that the organisms were being imaged without significant distortion.

Because the SIPPER is a high-resolution continuously imaging sensor, a significant amount of data are generated and recorded every second. Black and white image data were recorded at approximately 8 megabytes s\(^{-1}\), with each raw SIPPER file averaging 4.8 gigabytes total. Fortunately, SIPPER data are perfectly suited for run-length encoding compression algorithms whereby long runs of identical binary data (such as the white background of particle free water) can be represented by much shorter binary descriptions resulting in significant data storage savings (up to 277\(\times\)). When decompressed, each SIPPER file can be thought of as a “strip chart” the length of each sampling run (~0.5 km) and a width of 9.6 cm with images of every organism and particle that passed through the sample tube recorded in the approximate spatial distribution that existed \textit{in situ} but expressed in two dimensions. SIPPER imaged particles are colored black while the background is colored white. This makes the particle detection and extraction phase of SIPPER processing very simple.

Custom region-of-interest (ROI) extraction software was developed with Lab Windows/CVI (National Instruments) and used to detect, extract and create bitmap format images of zooplankton and other particles. The routine first divided the SIPPER data into 2048 by 2048 pixel “frames”, each of which is equivalent to approximately 1/7\(\text{th}\) of a second of sampling or approximately 10 cm (Fig. 3) of travel and computationally not too large to process. ROIs were then located in each frame by finding foreground pixels (black) and extracting those groups of
black pixels that contiguously were comprised of a user defined number of black pixels or greater. For this study, ROIs were extracted that were larger than 250 µm ESD. A preprocessing digital dilation step was used to connect black pixels that were within 3 pixels of other black pixels to ensure that organisms and particles with non-contiguous boundaries due to imperfect thresholding or illumination were included in the ROI extraction and counted as a single particle image. If a ROI spanned more than a single frame, the next frame would be added to the current frame so that the contiguous particle image could be extracted. These steps ensured that almost all particles greater than 250 µm ESD were extracted from the SIPPER file.

Extracted particle images were then viewed with a thumbnail browsing program (Thumbs Plus, Cerious Software). I manually sorted the images into 13 recognizable plankton groups (Fig. 4) and one unidentified particle class. Recognizable images of marine snow were included in the unidentified class as it was difficult to identify marine snow once it approached 1 mm in size. *Trichodesmium* colonies were included as a plankton class because of their high abundance in the SIPPER dataset and large individual size. Because of the high diversity of the zooplankton assemblage in the deepwater Gulf of Mexico (e.g. Ortner et al., 1989 identified 133 separate zooplankton species) I did not attempt to use SIPPER to identify organisms to species even though some species with characteristic features were easily recognized. Images from each class were then analyzed with Optimas image processing software that collected size and morphological information from each ROI including ESD. The location of each ROI within the SIPPER sampling transect was recorded on a separate data file. Locations were then checked against each other to ensure that no particle was counted more than once. SIPPER data were not sub-sampled. Because of the large number of images that had to be manually classified, only images from one of the two orthogonal views were used for this study.

**OPC data analysis**

A detailed explanation of the design and operation of the OPC is found in Herman (1992). Basically the OPC measures the amount of light blocked by the area of a particle as it passes through a collimated light sheet between the transmitter and receiver. The blocked light signal is
digitized and converted into a size measurement in the form of an equivalent spherical diameter. The OPC is capable of resolving particles 250 µm ESD and greater in size (Herman 1988, 1992), but is vulnerable to coincident counting at high particle concentrations (undercounting multiple particles that pass through collimated light sheet at the same time) and has difficulty accurately describing the size of translucent organisms (Zhang et al. 2000, Grant et al. 2000). OPC determined particle size data were binned into 100 µm ESD groups (300 to 5000 µm) for comparison with the net and SIPPER zooplankton data.

Figure 3. An example 2048 × 2048 pixel frame illustrating the large sampling area of SIPPER and its image quality. The dashed line represents the dimensions of the “pseudovolume” used to estimate OPC coincidence (4.6 × 9.6 × 0.4 cm). Scale bar is equivalent to 1 cm.
Results

Hydrography

Summer conditions in the northeast Gulf of Mexico are usually stable with the exception of quasi-annual intrusions of the Loop Current and associated eddies (Maul and Vukovich, 1993; Müller Karger, 2000) and even rarer incursions of low salinity surface plumes from the Mississippi River outflow (Müller Karger et al., 1991; Müller Karger, 2000) that might influence the zooplankton assemblage (Ortner et al., 1995). Temperature and salinity profiles collected during this study (Fig. 5) indicated the water being sampled as Gulf Common water (GCW, Vidal et al., 1994). This is differentiated from Subtropical Underwater (SUW) being transported by the Loop Current from the depth of the 22°C isotherm. In GCW the 22°C isotherm is found between 50 and 100 m whereas in the Loop Current it is found below 150 m (Austin and Jones, 1974). The seasonal thermocline was located between 25 and 30 m depth. Salinity profiles (Fig 5) and satellite data indicated no influence from the Mississippi River. The deep chlorophyll maximum (DCM) was at approximately 65 m near the salinity maximum, and there was very low chlorophyll biomass throughout the rest of the epipelagic zone based on both in situ fluorescence and extracted chlorophyll (Fig. 5).

Mesozooplankton and Mesozooplankton-Sized Particle Abundance

The vertical distribution pattern of mesozooplankton and mesozooplankton-sized particles was described similarly by all three sampling methods (Fig. 6). There was a peak in abundance at 10 m and a secondary maximum at 40 m. The major difference was in the total number of particles sampled by each method. The SIPPER recorded the highest numbers of mesozooplankton-sized particles at all depths sampled when compared against the results of the nets and the OPC. The SIPPER data were separated into two abundance estimates: (1) total number of extracted ROI images with a greater than 250 μm ESD “particle” (SIPPER total) and (2) those ROIs that could be identified as planktic organisms (SIPPER i.d.). A total of 174,699 SIPPER ROIs were extracted and manually examined from the 100 minutes of SIPPER data.
Most of these images contained unrecognizable particles and only 28% of the total (48,931 plankton images) could be classified into one of the 13 plankton groups. The proportion of SIPPER i.d. to SIPPER total ranged from 41% at 10 meters to 17% at 100 meters (Table 2).

Plankton net estimates of mesozooplankton abundance were the lowest at each depth sampled relative to the other sampling methods. Net counts on average equaled only 13% of the SIPPER total, 24% of the OPC total and 49% of SIPPER identified plankton abundance. The OPC consistently sampled approximately one-half the number of particles that the SIPPER imaged at all depths.

Figure 4. Representative SIPPER images of the thirteen enumerated plankton groups. Groups A-F are from left to right, with group code in parentheses: A. other crustaceans (Crus), B. copepods (Cope), C. larvaceans (Larv), D. *Trichodesmium* sp. (Tric), E. protocists (Prot), F. echinoderm larvae (Echi). Scale bar for these groups is equivalent to 2.5 mm. Groups G-M are, from left to right: G. chaetognaths (Chae), H. cnidarians and ctenophores (Cnid), I. euphausiids and decapods (Euph), J. Polychaetes (Poly), K. Molluscs (Moll), L. other tunicates (Tuni) and M. siphonophores (Siph). Scale bar for these groups is equivalent to 5 mm.
Figure 5. Temperature, salinity, fluorescence and extracted chlorophyll (denoted by *) profiles (0-200 m) from the study site.
Figure 6. Numerical abundance estimates of the three zooplankton sampling methods. All abundances were normalized to volume filtered.
Table 2. Abundance (number m\(^{-3}\)) of mesozooplankton sized particles as estimated by the 162 μm net system, SIPPER and the OPC. SIPPER counts are split into total ROIs with a greater than 250 μm ESD particle within it, and those images that could be classified into one of the 13 plankton groups. Performance of the net system, SIPPER identified plankton and the OPC are all compared against the SIPPER total.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Net counts</th>
<th>SIPPER total*</th>
<th>SIPPER identified images</th>
<th>OPC counts</th>
<th>Net counts/ SIPPER total</th>
<th>SIPPER identified/ SIPPER total</th>
<th>OPC counts/ SIPPER total</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1537</td>
<td>7397</td>
<td>3508</td>
<td>4335</td>
<td>21%</td>
<td>47%</td>
<td>59%</td>
</tr>
<tr>
<td>20</td>
<td>1117</td>
<td>6634</td>
<td>1591</td>
<td>3320</td>
<td>17%</td>
<td>24%</td>
<td>50%</td>
</tr>
<tr>
<td>30</td>
<td>374</td>
<td>5849</td>
<td>1404</td>
<td>3497</td>
<td>6%</td>
<td>24%</td>
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<tr>
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<td>756</td>
<td>5855</td>
<td>1487</td>
<td>3529</td>
<td>13%</td>
<td>25%</td>
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<td>4811</td>
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<td>55%</td>
</tr>
<tr>
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<td>371</td>
<td>3864</td>
<td>742</td>
<td>2139</td>
<td>10%</td>
<td>19%</td>
<td>55%</td>
</tr>
<tr>
<td>80</td>
<td>279</td>
<td>2428</td>
<td>727</td>
<td>1365</td>
<td>11%</td>
<td>30%</td>
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<tr>
<td>100</td>
<td>171</td>
<td>1657</td>
<td>276</td>
<td>831</td>
<td>10%</td>
<td>17%</td>
<td>50%</td>
</tr>
</tbody>
</table>
Assuming particles were randomly distributed within the water column; Sprules et al. (1992) derived a formula describing the probability of two or more particles occurring within the sampling beam of the OPC at the same time given a known particle concentration. They determined that coincidence would be a significant source of error for all but the lowest zooplankton densities. To determine if coincidence was responsible for the low OPC counts relative to the SIPPER total, I modified the formula of Woed-Walker et al. (2000) using SIPPER total counts normalized to volume sampled as the known concentration of OPC detectable particles in the OPC light beam. The average number of particles in the OPC beam ($\mu$) is determined by:

$$\mu = C \times V,$$

where $C$ was the concentration of particles greater than 250 $\mu$m in the SIPPER total for each depth and $V$ was the volume of the OPC beam (220 mm x 20 mm x 4 mm or 17.6 ml). The average number of particles recorded by the OPC (av. no.) is calculated by the equation:

$$\text{OPC av. no.} = 1 - e^{-\mu}.$$  

(Woed-Walker et al., 2000). The coincidence factor can then be calculated by dividing the average number of particles in the OPC beam by the average number of particles recorded by the OPC (coincidence factor = $\mu$ / OPC av. no.). For this study, the coincidence factor ranged from 1.01 to 1.06 indicating that coincidence should have been a rare occurrence within the OPC if the particles were randomly distributed at the concentrations sampled by SIPPER (1-8 particles $l^{-1}$).

To investigate further, I created a “pseudovolume” within the SIPPER image dataset equivalent to the volume sampled by the OPC at any one instant. Because the sampling area of the SIPPER is approximately twice that of the OPC and of a different geometry, I used a sub-sample of the SIPPER imaging window that was 4.6 cm x 9.6 cm x 0.4 cm to create a sampling volume of 17.6 ml, equivalent to that of the OPC. I calculated the distance between each particle from its neighbors within the “pseudovolume” to determine which particles would be affected by coincident counting (this can be visualized in Fig. 3, where a copepod and two Trichodesmium...
colonies occupy the dotted box representing the “pseudovolume” dimensions), if the SIPPER were to sense particles like the OPC. On average, 29% of SIPPER imaged particles of OPC detectable size had a neighbor closer than 4 mm and therefore would not be individually counted by the OPC (Table 3). The large number of close-together particles suggests that their distribution was not random. By correcting for the estimated coincidence frequency, OPC abundance estimates were recalculated and corresponded more closely with the SIPPER totals. However, corrected OPC counts still only accounted for 61-78% of the SIPPER imaged particles.

Size Frequency Distributions

The cumulative size-frequency distributions of the three sampling methods demonstrated large differences in sampling performance (Fig. 7). While each sampling method was able to discern the same exponential decrease in abundance with increasing size, the SIPPER was able to detect far more particles than either the net or OPC for a given size class. Much of the discrepancy between the SIPPER total and SIPPER i.d. abundances could be attributed to the large number of less than 0.5 mm ESD particles that could not be identified. This was most likely due both to the large numbers of small-suspended particulates in the water column and also the minimum size resolution of identifiable objects in the SIPPER dataset. While the SIPPER can detect and image very small particles, the capability to identify them is made difficult by the 50 µm pixel size. Smaller plankton will be imaged, but not with enough pixel definition to determine their identity. As plankton images grow larger, there are more pixels available to define their shape and aid recognition. This concept is illustrated in a graph plotting the proportion of identifiable plankton images versus the SIPPER total (Fig. 8). This proportion rose steadily with particle size, such that at ESDs over 2 mm, over 80% of the SIPPER ROIs were of identifiable plankton.

The inability of the OPC and nets to detect and sample the smallest size classes in the same magnitude as the SIPPER was most likely due to the inefficiency of the net in sampling the smallest zooplankton and suspended particulates due to extrusion through the net mesh (Gallienne et al., 2001; Hopcroft et al., 2001) and to approaching the 250 µm ESD detection limit for the OPC. Both the net and OPC additionally displayed a systematic abundance difference of
up to an order of magnitude less at each size class compared to the SIPPER datasets, indicating that these differences were not size dependent.

Figure 7. Cumulative size-frequency distribution spectra for the three sampling sensors (300-5000 µm ESD).
Table 3. OPC normalized particle abundance, theoretical coincidence factor, SIPPER estimated coincidence percentage, count loss and corrected OPC particle abundance.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>OPC counts m(^{-3})</th>
<th>Coincidence factor</th>
<th>SIPPER-estimated coincidence percentage</th>
<th>Counts lost to coincidence</th>
<th>Corrected OPC counts m(^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4335</td>
<td>1.06</td>
<td>33.6%</td>
<td>1458</td>
<td>5793</td>
</tr>
<tr>
<td>20</td>
<td>3320</td>
<td>1.05</td>
<td>38.3%</td>
<td>1270</td>
<td>4591</td>
</tr>
<tr>
<td>30</td>
<td>3497</td>
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<td>33.0%</td>
<td>1154</td>
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<td>3529</td>
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<td>30.1%</td>
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<td>4591</td>
</tr>
<tr>
<td>50</td>
<td>2983</td>
<td>1.04</td>
<td>27.1%</td>
<td>808</td>
<td>3791</td>
</tr>
<tr>
<td>60</td>
<td>2640</td>
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<td>23.6%</td>
<td>624</td>
<td>3264</td>
</tr>
<tr>
<td>70</td>
<td>2139</td>
<td>1.03</td>
<td>24.7%</td>
<td>539</td>
<td>2668</td>
</tr>
<tr>
<td>80</td>
<td>1365</td>
<td>1.02</td>
<td>21.9%</td>
<td>299</td>
<td>1664</td>
</tr>
<tr>
<td>90</td>
<td>942</td>
<td>1.02</td>
<td>22.2%</td>
<td>209</td>
<td>1152</td>
</tr>
<tr>
<td>100</td>
<td>831</td>
<td>1.01</td>
<td>23.3%</td>
<td>194</td>
<td>1025</td>
</tr>
</tbody>
</table>
Sample Biovolume Estimates

Differences in both the abundance and size distribution of the three datasets led to large differences in the sample biovolumes (SBV) estimated by each sensor (Fig. 9). Because the main discrepancy in the SIPPER i.d. to SIPPER total abundance estimates was from the smallest size classes, the biovolume difference between the two was much less than the abundance difference due to the minimal contribution small particles or organisms make to the biovolume total. Thus, while the identified plankton of the SIPPER i.d. dataset made up only 28% of the SIPPER total particle abundance, they made up 79% of the SIPPER total biovolume. Net and OPC SBV were only 11% and 23% of the SIPPER total respectively and 13% and 29% of the SIPPER i.d. biovolume.
The Problem of Trichodesmium

Ideally, the taxonomic composition of the net and SIPPER i.d. datasets should be identical. However, this was not the case, as the advantages and disadvantages of each system in sampling different components of the plankton were manifested in significantly different descriptions of the assemblage. Firstly, the colonial cyanobacteria *Trichodesmium* sp. formed the most abundant plankton class in the SIPPER dataset, especially at a depth of 10 m where it was found at concentrations greater than 1800 colonies m$^{-3}$, but were not quantified in the net samples. *Trichodesmium* is a filamentous phytoplankton that is difficult to enumerate in zooplankton net samples because of its fragility and tendency to stick to the net mesh. The opacity and large size (0.5-4 mm ESD) of *Trichodesmium* colonies made them readily detectable.
by SIPPER and, most likely, the OPC. Those colonies that might have been fragmented or disrupted in the SIPPER sampling tube, and single trichomes (which image as long strands), were not counted as *Trichodesmium* in the SIPPER dataset. Removing the *Trichodesmium* images from the SIPPER i.d. dataset yielded zooplankton counts ~50% higher than that from the nets (Fig. 10).

![Figure 10](image)

**Figure 10.** Zooplankton abundance (numbers m\(^{-3}\)) profile determined from the net and SIPPER after *Trichodesmium* was separated from the SIPPER i.d. dataset.

**Taxonomic Composition – SIPPER vs. Nets**

Differences between the taxonomic composition of the SIPPER and net samples varied even more considerably than the differences in abundance. Data comparing zooplankton composition, abundance and biomass from the nets and SIPPER are presented in Tables 4 and 5 respectively. Copepods dominated the net samples, contributing 63.7 % of the abundance and 36.4 % of the biomass. Larvaceans (17.4 %) were the only other significant contributor to the net
abundance total, with the other tunicate class, comprising doliolids and salps, and the proctista class, comprising mainly acantharians, radiolarians and tintinnids, contributing between 4 and 5 % each. No other zooplankton class contributed more than 2.5 % to the net-sample abundance total. Because of their large individual size, euphausiids and decapods were the second largest biomass component in the net samples, contributing slightly less (31.8 %) than the copepods. Other crustaceans (comprising amphipods, cladocerans and ostracods), the other tunicates class, and siphonophores all contributed between 6 and 9 % to the total net collected biomass, mostly based on their larger individual size. No other zooplankton group contributed more than 3.5 % to the total biomass in the nets.

Six zooplankton groups were found to be significantly more abundant in the SIPPER i.d. dataset than the concurrent net estimates (Fig. 11) according to paired t-tests (Zar, 1984). These taxa can be broadly grouped as fragile and or gelatinous zooplankton that are easily damaged or disrupted on encountering a net. These included 4 out of 5 of the most important numerical contributors to the SIPPER i.d. assemblage. Larvaceans were the numerical dominant, contributing 35.6% to the total and were more than 3x more abundant than the net total. Analysis of net sample collected larvaceans indicated the majority were predominately oikopleuridae (mostly Oikopleura dioica) with the fritillariidae also present in noticeable numbers. Three other fragile plankton groups (proctista, other tunicates and cnidarians/ctenophores) were important numerically, each contributing between 5 and 14% to total zooplankton abundance. Copepods were the only important non-fragile zooplankton group and were the second highest contributor (27.7 %) to the SIPPER i.d. abundance total. The differences in abundance between SIPPER and the nets for the fragile and gelatinous zooplankton ranged from just over 200% for the siphonophores to over 1400% for the cnidarians and ctenophores. However, the difference in siphonophore abundance was probably much higher, as individual bracts or nectophores found in net samples were counted as individuals whereas SIPPER imaged and counted whole organisms. This was also the case for polychaetes, which often were found broken up into segmented parts in the nets. The six other zooplankton groups, comprising mainly more robust
taxa such as crustaceans, were sampled similarly by both the net and SIPPER and showed no appreciable difference in abundance (Fig. 12).

Doliolids and salps, which made up the other tunicate class, were the biomass dominant in the SIPPER dataset, contributing 37 % to the total. Examination of the SIPPER imagery and concurrent net samples revealed that this group was dominated by *Doliioletta gegenbauri* or a similar congener that made up more than 90% of the total. Interestingly, the large biomass difference (12.2×) between the SIPPER and net for this class was due not just to significant loss of individuals through the net mesh from extrusion or disruption (3.8× more of this class were found in the SIPPER imagery), but also to loss of reproductive tissue. In the upper 60 meters, a large proportion (33% mean, range: 22-39 %) of the doliolids were of the asexually reproducing oozoid stage (Fig. 4L), which have a lengthened dorsal process or appendix that bears budding blastozooids that form the next stage in the doliolid life cycle. Very few doliolids observed in the net samples bore an intact dorsal process. Copepods (18%) and the euphausiids and decapod class (14 %) each contributed over 10 % to the SIPPER biomass total, while siphonophores (9%), larvaceans (7%) and other crustaceans (6%) contributed more than 5%.
Table 4. Net estimated abundance (Number m\(^{-3}\)) and dry weight biomass (mg m\(^{-3}\) DW) of zooplankton groups. Plankton group names abbreviated to four letter codes as in figure 3.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
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<td>19 (1.23)</td>
<td>4 (-)</td>
<td>1039 (13.15)</td>
<td>9 (6.73)</td>
<td>31 (3.39)</td>
<td>280 (0.99)</td>
<td>8 (0.01)</td>
<td>42 (0.62)</td>
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</tr>
<tr>
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<td>5 (0.02)</td>
<td>775 (6.12)</td>
<td>3 (1.32)</td>
<td>15 (1.54)</td>
<td>161 (0.68)</td>
<td>4 (-)</td>
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</tr>
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<td>9 (12.12)</td>
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<td>17 (1.92)</td>
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<td>246 (5.36)</td>
<td>7 (1.19)</td>
<td>30 (0.37)</td>
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<td>9 (0.18)</td>
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* indicates that body segments found in net counted as individuals  
- indicates less than 0.01 mg m\(^{-3}\)  
NA indicates biomass not determined for that group
Table 5. SIPPER estimated abundance (Number m\(^{-3}\)) and dry weight biomass (mg m\(^{-3}\) DW) of zooplankton groups. Plankton group names abbreviated to four letter codes as in figure 3.

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- indicates less than 0.01 mg m\(^{-3}\)
NA indicates biomass not determined for that group
Figure 11. SIPPER (solid line) and net (dotted line) numerical abundance estimates of zooplankton groups with significant differences between the two sampling systems (paired t-test, $p < 0.05$).
Figure 12. SIPPER (solid line) and net (dotted line) numerical abundance estimates of zooplankton groups with no significant differences between the two sampling systems (paired t-test, p <0.05).

Vertical distribution patterns of most specific zooplankton groups sampled by both the nets and SIPPER were similar, even when the abundance estimates were quite different. For example, while doliolids and salps were significantly under-sampled by the nets compared to SIPPER, both instruments sampled an abundance maximum at the DCM much higher than at any other depth. However, for some zooplankton groups, SIPPER proved far more capable in describing both abundance and vertical distribution patterns than the nets. For example, cnidarians and ctenophores were extremely abundant in the SIPPER dataset and demonstrated a strong bimodal distribution with maxima at 10 and 60 meters, but were virtually absent within the net samples. Additionally, most cnidarians and ctenophores collected in nets were unidentifiable, especially after fixation. In contrast, within the SIPPER dataset, many individual cnidarian and ctenophore taxa could be enumerated to genus or even species. For example, we were able to
determine that the narcomedusae *Solmundella bitentaculata* was the most abundant identifiable cnidarian in the depth ranges sampled.

**Total Biomass**

Total biomass (0-100 m) determined from SIPPER data was more than twice that determined from the net data (3417 mg m$^{-2}$ DW vs. 1592 mg m$^{-2}$ DW), but the vertical biomass distribution pattern was similar. Most of the biomass difference was explained by the under representation of the fragile taxa in the net samples. The fragile and gelatinous zooplankton groups enumerated from the SIPPER dataset contributed greater biomass (~1937 mg m$^{-2}$ DW) than the entire zooplankton assemblage sampled by the nets. Biomass did not include the protoctista class, which consisted of organisms with mineral skeletons or tests that may have biased the results. Because the protoctista class was more than 4× more abundant in the SIPPER dataset than the nets, the true biomass difference was probably even greater.

**Taxonomic Differences in Size Distribution**

Generally, the three sampling systems showed very little correspondence between particle abundance of a given size class (Fig. 12, left graphs), but some trends were apparent. Even though the absolute totals were very different, between 60 and 70% of the total net, OPC and SIPPER unidentified particle abundance consisted of particles between 250 and 500 µm ESD and between 91 and 96% of the total were made up of particles less than 1 mm ESD. In contrast, only 73% of the SIPPER identified plankton were less than 1 mm ESD and the smallest size class contributed less than 26% of the total. The number of SIPPER identified plankton increased with size relative to the other three datasets. For example, SIPPER i.d. abundance at the largest size class (>2500 µm ESD) outnumbered nets, OPC and the SIPPER unidentified datasets by 6.8×, 7.5× and 15.5× respectively.

Small zooplankton made up the majority of zooplankton sampled by both SIPPER and the nets, although the relative importance of larger forms was much greater in the SIPPER i.d. dataset (Figs. 13 and 14, right graphs). Whereas only 8.4% of the net collected zooplankton
were larger than 1 mm ESD, more than 25% of the SIPPER imaged zooplankton were larger than this size. This difference was especially pronounced in the fragile and gelatinous zooplankton groups that were under sampled by the nets, such as the other tunicates class, where more than 74% of the SIPPER imaged organisms were larger than this size class compared to only 21% for those collected by the nets. However, this trend was also found for some of the zooplankton groups that showed no sampling bias in their abundance estimates. More than 90% of the net collected polychaetes were smaller than 0.5 mm ESD, while more than 80% of the SIPPER imaged polychaetes were larger than 1 mm ESD. Similarly for planktic molluscs, more than 95% of the net collected individuals were less than 1 mm ESD compared to only 47% for SIPPER.

Figure 13. Cumulative abundance of mesozooplankton sized particles or mesozooplankton determined by the three sampling methods separated into 500 µm size classes (left graphs) up to 1500 µm ESD. SIPPER data were separated into identified plankton and unidentified particles. Numerical abundance of the thirteen plankton classes was calculated for each size class for the net samples and the SIPPER i.d. dataset (right graphs)
Figure 14. Cumulative abundance of mesozooplankton sized particles or mesozooplankton determined by the three sampling methods separated into 500 µm size classes (left graphs) for particles larger than 1500 µm ESD. SIPPER data were separated into identified plankton and unidentified particles. Numerical abundance of the thirteen plankton classes was calculated for each size class for the net samples and the SIPPER i.d. dataset (right graphs).
Discussion

The disparate results between the three sampling methods is at first confusing given that the nets and the SIPPER sampled the exact same water volume and the OPC was sampling less than a meter away. This likely precludes the possibility that micro-scale patchiness affected these differences. The SIPPER provided a picture of a zooplankton assemblage both more numerous and diverse than either of the other methods. While the majority (~67%) of SIPPER extracted images could not be identified, those that were still significantly outnumbered organisms collected by the nets at most depths.

Occurrence of Unidentified Particles in Plankton Image Datasets

The number of unidentified particles in our dataset is comparable to that of other investigators using in-situ imaging sensors. For example, Ashjian et al. (2001) were not able to classify 43% of VPR images collected during three cruises to Georges Bank. Additionally, they included marine snow as a class that comprised over 71% of their classified images. In contrast, I did not separate marine snow from our unclassified group. While many of these particle images were of identifiable marine snow such as cast-off larvacean houses, diatom mats and fecal pellet strings, the majority of the unclassified images were of particles less than 1 mm ESD that lacked any resolvable characteristics to aid in identification. This was partly a problem of the coarse imaging resolution of the SIPPER (50 \( \mu m \) square) relative to the small size of the particles. A small copepodite or copepod nauplius measuring 400 \( \mu m \) TL would be imaged by the SIPPER but would be comprised of such a few number of pixels that identifying it as such would be impossible with the present SIPPER configuration. Hopkins (1981), studying zooplankton at the same station as this study, found that metazoan plankton under 1 mm total length sampled from bottle casts outnumbered metazoan plankton > 1 mm caught in a 162 \( \mu m \) plankton net by 35x and were made up primarily of copepod nauplii and copepodites. Thus, it is possible that a large percentage of the small-unidentified particles in the SIPPER dataset were of small zooplankton such as copepod early life stages. While I did not use images from the second camera of SIPPER for this study (which imaged orthogonal to the first), it may have proven useful in identifying some
of these smaller particles by providing a second perspective that could present recognizable features.

**Importance of Trichodesmium in Subtropical Systems**

A substantial number of the larger classified images extracted from the SIPPER dataset were of *Trichodesmium* colonies, especially at a depth of 10 meters where they outnumbered both the SIPPER and net zooplankton abundance estimates. *Trichodesmium* is an important component of tropical and subtropical oceanic ecosystems, contributing a significant amount of new nitrogen to otherwise impoverished waters (Capone et al., 1997; Karl et al., 1997). Furthermore, *Trichodesmium* has been implicated in contributing to the initiation of harmful algal blooms of the dinoflagellate *Karenia brevis* (Lenes et al. 2001) in the Gulf of Mexico. Typically, the abundance and vertical distribution of *Trichodesmium* are determined with water bottles or drift nets, which are limited in their ability to detect it at low concentrations, can damage or distort specimens, and are prone to sampling error due to the small volumes sampled (Chang, 2000). Because SIPPER samples a larger volume of water, it can detect *Trichodesmium* at much lower concentrations than these traditional methods.

**Marine Snow and Large Phytoplankton as Signal Rather than Noise**

Other investigators using optical methods to investigate zooplankton distributions have also found that large colonial phytoplankton such as diatoms can dominate the marine particle assemblage within the mesozooplankton size range (Norrbin et al., 1996; Grant et al., 2000). The dominance of marine snow and of *Trichodesmium* and other phytoplankton in the water column suggests that data collected with non-imaging optical sensors such as the OPC must be interpreted with caution when converted to zooplankton abundance and size distributions. Imaging instruments such as the SIPPER, on the other hand, can differentiate between these groups and allow for accurate determination of their contribution to the total marine particle assemblage.

**Comparison between Different Sampling Methods**

There have been a number of studies investigating the performance of the OPC against plankton net catches (Sameoto et al., 1993; Grant et al., 2000; Halliday et al., 2001), but few if
any comparisons against *in-situ* imaging systems. Herman (1992) suggests that with strict sample and analysis control, net and OPC counts can agree to within 30%. Most studies, however, appear to have much more trouble reconciling the output of the OPC with what is collected within a net. Using the OPC mounted on the HRS to sample a 80 km transect on the West Florida Shelf, Sutton et al. (2001) found that the OPC grossly underestimated mesozooplankton abundance, especially at high concentrations (>10,000 organisms m\(^{-3}\)), but described the overall pattern of zooplankton fairly well. Herman (1988) and Sprules et al. (1998) have also reported OPC counts less than net counts, while others have reported large overestimates by the OPC relative to plankton nets (Grant et al., 2000; Halliday et al., 2001). Suggested causes of OPC underestimates are coincident counting (Sprules et al., 1992; Woold Walker et al., 2000; Labat et al., 2002) and the presence of highly translucent organisms (Wieland et al., 1997; Beaulieu et al., 1999), while overestimates have been attributed to the presence of marine snow and detrital aggregates (Zhang et al., 2000), large phytoplankton (Grant et al., 2000), small zooplankton that pass through the net mesh (Halliday et al., 2001; Zhou and Tande, 2002), and fragile organisms that are destroyed in the nets (Gallienne and Robins, 2001).

During this study, the OPC consistently sampled approximately half the number of particles that the SIPPER imaged at all depths. Much of this underestimation could have been due to coincidence, as I showed within the sub-sampled SIPPER imaging volume. More than 29% of the particles occurred within 4 mm of each other within the SIPPER “pseudovolume” and, therefore, would have been counted as a single particle if sampled by the OPC. By correcting for this, OPC abundance estimates approached between 60-80% of the SIPPER imaged particles in the mesozooplankton size range. The occurrence of large numbers of highly transparent organisms such as cnidarians, ctenophores, doliolids and salps that were imaged by SIPPER could be responsible for much of the remaining difference in abundance estimates between it and the OPC as earlier studies using the OPC have demonstrated that it can miss detecting or underestimate the size of transparent zooplankton (Labat et al., 2002). Additionally, the difference in the two sensors sampling areas could also contribute to the lack of correspondence between the OPC and SIPPER counts. Baumgartner (2003) found that late copepodite stages of
Calanus finmarchicus could avoid the OPC at speeds similar to this study. Because the OPC has a wide but narrow sampling mouth (2 × 22 cm) and the SIPPER has a larger square aperture (9.6 × 9.6 cm), zooplankton may have been able to escape out of the way of the OPC more often than the SIPPER.

Implications for Subtropical Oceanic Biology

The deepwater Gulf of Mexico biological community has been sparsely sampled (Biggs and Ressler, 2001), but the general consensus is that the biology of offshore waters in the Gulf is similar to that of other low-latitude tropical oceans, with low biomass and high diversity of zooplankton, ichthyoplankton and micronekton (Hopkins, 1981; Hopkins et al., 1996; Biggs and Ressler, 2001). The abundance, composition, size and vertical distribution and biomass of the mesozooplankton sampled during this study by the HRS plankton nets was similar to that found during an earlier study at this station (Hopkins, 1981) and to other investigations in oceanic waters of the Gulf of Mexico (Cummings 1983; Ortner et al., 1989; Biggs and Ressler, 2001). Combined with an earlier study investigating the distribution of mesozooplankton on the West Florida Shelf using the HRS (Sutton et al., 2001), these results suggest that the somewhat small plankton nets used on the sampler provide similar results to those of other investigators using larger plankton nets to describe the mesozooplankton assemblage.

Comparing the HRS net catches versus what SIPPER imaged in the same water yielded a significantly different picture of the mesozooplankton assemblage. While a number of investigators have begun to stress the need to use multiple nets of different mesh sizes to adequately sample the entire mesozooplankton size range (Gallienne and Robins, 2001; Hopcroft et al., 2001), our results suggest that nets still might miss a large numerical and biomass fraction. While copepods were both the numerical and biomass dominant in the net samples, larvaceans were the numerical dominant and doliolids and salps (forming the other tunicate class) were the biomass dominant in the SIPPER dataset. Small copepods, which made up the majority of the net-caught zooplankton, such as the genera Calocalanus, Oithona, Paracalanus, Oncaea, and Temora, were difficult to identify in the SIPPER dataset because of their small size even though they were likely imaged, and therefore were underestimated (in this case counted in the SIPPER.
The large number of fragile and gelatinous organisms in the SIPPER dataset and their near absence in the nets obviously has implications on how a planktic ecosystem is described. For example, Hopkins et al. (1996) found that midwater shrimps and fish, the two dominant micronekton groups in this region, accounted for only 25% of the zooplankton daily production consumed in the eastern Gulf. It remains unresolved which ecosystem components are responsible for most zooplankton predation although they suspected large gelatinous predators. More work in this region with SIPPER might resolve that question.

*Effects of Formalin Preservation on Sample Size Distribution*

Fixation of zooplankton samples with formalin has been shown to cause shrinkage of the preserved organisms (Postel et al., 2000). It is possible that shrinkage may have contributed to the observed differences between the size-frequency, biovolume and biomass of the net samples with that of the SIPPER. For example, Beaulieu et al. (1999) measured a 41% decrease in biovolume of the scyphozoan medusae *Aurelia aurita* and Nishikawa and Terazaki (1996) found that doliolids and salp body lengths shrank to approximately 86-93% of their live length after preservation. Omori (1978) observed that the size of copepods and other crustaceans were less affected by fixation than gelatinous organisms. This likely explained some of the differences observed in the pteropod and polychaete size distribution in the net samples compared to SIPPER. However, the large differences in biovolume and biomass of the gelatinous and fragile organisms between SIPPER and the nets were due to increased abundance of these animals at all size classes. Therefore, the biovolume and biomass differences were due more to a difference in total abundance rather than a shift in the size frequency spectrum.

*Comparison of SIPPER Performance with Other Imaging Systems*

Prior investigations comparing net and imaging systems have yielded similar results to this study. Parallel deployments of the VPR and the MOCNESS on Georges Bank have shown that the MOCNESS significantly under-samples echinoderm larvae, larvaceans and medusae relative to the VPR (Benfield et al., 1996) and the VPR also sampled foraminifera, acantharians and other fragile protoctistan zooplankton more effectively than nets (Gallager et al., 1996; Norrbin et al., 1996; Ashjian et al., 2001). In those studies, however, copepods and other harder
bodied organisms were still the numerical and biomass dominant in both the net and VPR samples and thus the under-representation of the fragile forms appeared to be less important. In the central North Pacific Ocean, Dennett et al. (2002) reported that colonial radiolarian colonies averaged 380× more abundant in VPR samples than those collected in the nets and were an important but overlooked component of biomass in oligotrophic waters. Similarly, our study in an oligotrophic central oceanic ecosystem indicated that traditional net sampling might miss more than half the mesozooplankton biomass.

**Conclusions**

This study demonstrates the importance of *in-situ* imaging systems to accurately assess the abundance, size distribution and composition of a low-latitude mesozooplankton assemblage. These systems provide the capability to sample gelatinous and fragile organisms that otherwise may be overlooked, even though they may be important contributors to the ecology of an ecosystem. Similarly, the limitations of the OPC and plankton nets in describing this assemblage were explored. The primary disadvantage of the SIPPER is the current need to manually classify the large volume of images generated by the sensor. The ability to automatically identify images of zooplankton collected in the lab or field has received considerable attention (Jeffries et al., 1984; Tang et al., 1998; Akiba, 2000; Iwamoto et al., 2001) and an operable pattern recognition algorithm is in use for the VPR (Tang et al., 1998). In Chapter 2, I detail the first results from of a new grayscale imaging SIPPER and investigate the performance in the field of a plankton identification software package I helped develop for the SIPPER. These improvements should allow for greater discrimination between particle groups, especially marine snow and smaller plankton that were difficult to identify with the binary imaging SIPPER. With these advances I believe the SIPPER can provide more accurate mesozooplankton abundance and size measurements than nets and provide valuable insight into processes controlling zooplankton distributions at both the individual and community level.
Chapter 2: Describing plankton distribution and abundance in neritic subtropical waters using SIPPER-2 and an automated classification system.

Introduction

A comprehensive knowledge of the distribution and diversity of zooplankton populations is critical to determine their influence on fisheries recruitment, phytoplankton production via grazing, contribution to particle flux and their possible use as sentinels for global climate change (Lenz, 2000; Hays et al. 2005). This task is made difficult by the fact that these populations are distributed heterogeneously over a broad range of temporal and spatial scales (Haury et al., 1977; Mackas et al., 1985). These heterogeneous patches are often the focus of increased production, feeding and reproduction for plankton groups as resources within these patches are often greater than the ambient surroundings (Mullin and Brooks, 1976; Malkiel et al., 2006). To adequately sample these patchy distributions of zooplankton requires a capability for intensive and high frequency sampling of the population so that the entire range of variability can be observed and the processes responsible for the patterns uncovered (Sutton et al., 2001; Yamakazi et al. 2002). Traditional methods of sampling zooplankton using nets or bottles are limited in this regard as high frequency sampling can generate hundreds to thousands of samples that are both costly and time consuming to process and analyze. Furthermore, these methods integrate spatial information so that fine-scale distribution patterns cannot be observed.

These limitations have led to the development of a number of in-situ zooplankton imaging sensors such as the Video Plankton Recorder (VPR, Davis et al., 1992), Underwater Video Profiler (UVP, Gorsky et al., 1992), Shadowed Image Particle Profiling and Evaluation Recorder (SIPPER, Samson et al., 2001) and the Zooplankton Visualization and imaging system (ZOOVIS , Benfield et al., 2003) that collect high resolution imagery of plankton and suspended particles. While currently unable to deliver the taxonomic detail of traditional methods that physically capture zooplankton, these systems can provide continuous or near continuous measurements of
the distribution of these groups over a wide range of temporal and spatial scales (Wiebe and Benfield, 2003, Benfield et al., 2007). For example, the VPR has been used to both investigate the nearest neighbor distances between copepods along a sampling transect (Ashjian et al., 2005) and examine the vertical distribution of \textit{Trichodesmium} across the Atlantic basin (Davis and McGillicuddy, 2006).

Sampling by these systems is far less physically intrusive than traditional zooplankton sampling methods which often destroy or damage the more fragile forms. Whereas abundance estimates from imaging systems are comparable with net and bottle estimates of robust, non fragile zooplankton such as copepods and pteropods carried out in the same area (Benfield et al., 1996, Gallagher and Davis, 2003) or concurrent with the net sampling (Remsen et al., 2004, Broughton and Lough, 2006), abundance estimates of gelatinous or fragile forms such as hydromedusae or larvaceans collected by imaging systems are often many orders of magnitude higher than that from nets or bottles (Benfield et al., 1996; Dennet et al., 2002; Remsen et al., 2004) collected in the same area. Additionally, the distribution and abundance patterns of marine snow aggregates (Ashjian et al., 2001, 2005b) and large algal (Sieracki et al., 1998, Pilskaln et al., 2005) and cyanobacterial colonies (Remsen et al., 2004, Davis and McGillicuddy, 2006; Walsh et al., 2007) can also be determined using imaging systems.

Until recently, a major bottleneck in the application of plankton imaging systems to field studies was the necessity of manually identifying the acquired images. While much of the post-processing and measurement of imaged particles has been automated, the actual classification of imaged particles had to be done manually (Benfield et al. 1996; Ashjian et al., 2001; Remsen et al., 2004). As field deployments of these systems can generate hundreds of thousands (Ashjian et al., 2001; Remsen et al., 2004) to millions of images (Hu and Davis, 2005) per cruise, manual classification of the collected dataset would be impractical. While methods to automatically classify plankton images have been under development for over 25 years (Jeffries et al., 1984; Rolke and Lenz, 1984), it has only been until recently that they have shown promise for field collected images.
Early studies of automated plankton classification systems utilized only distinctive and recognizable images of plankton collected in the lab (Jeffries et al., 1984) or the field (Tang et al., 1998) separated into a relatively small number of groups. While these resulted in accurate classifiers (90-92%), they were not applicable to field collected images that can be unrecognizable, projection variant, occluded, out of focus, and unevenly illuminated (Davis et al., 2004; Luo et al., 2004). When unidentified SIPPER images were included in a test set, Luo et al. (2004) found that the accuracy of their multi-class support vector machine (SVM) classifier fell from 90 to 75% for 5 groups of plankton. Davis et al. (2004) applied their neural network classifier to VPR images collected in the field including an “other” or unidentified class, and achieved 61% accuracy while classifying their dataset into 7 groups in real time. This was later improved to 72% using a new feature set and switching their classifier to a SVM (Hu and Davis, 2005). Further improvements were made using a dual classification method where a classification was made only if both classifiers agreed (Hu and Davis, 2006). Otherwise an image would be labeled as unknown. Using the ZOOSCAN imaging system to count and identify preserved plankton from net samples, Grosjean et al. (2004) has achieved 75% accuracy for a 29 group training set using a discriminate forest classification algorithm while classifying at rates of up to 2000 images per minute. Many systems now have a semi-interactive manual correction step in which images classified with low probability can be sent to a human expert for correct classification without significantly slowing down the classification effort (Davis et al., 2004; Grosjean et al., 2004). This can improve overall classification accuracy to 80-85% (Grosjean et al., 2004) and is comparable to what humans achieve with complicated classification tasks (Culverhouse et al., 2003) but with significantly greater speed.

In this chapter I examine the results of a multiple class SVM classifier (Luo et al., 2004, 2005) identifying plankton and particle images collected from a new grayscale imaging SIPPER deployed in the subtropical waters of the eastern Gulf of Mexico. The new system is described and the performance of the classifier in describing the composition and spatial distribution of a diverse plankton assemblage is explored.
Methods

A new grayscale-imaging SIPPER was deployed on the USF high resolution sampler (HRS) to investigate small scale plankton and suspended particle spatial distributions during a September 2002 cruise to the West Florida Shelf (WFS) in the Gulf of Mexico aboard the R/V Suncoaster. The HRS is a comprehensive marine particle analysis platform (Sutton et al., 2001, Remsen et al., 2004) capable of concurrently collecting electronic environmental sensor data along with discrete net and water bottle samples for verification and calibration of the sensors (Fig. 15).

Figure 15. Photograph of SIPPER-2 mounted on the HRS. The verification capability of the HRS is comprised of (A) the 97 cm$^2$ sampling tube, (B) the SIPPER imaging system, (C) the 20 position plankton net carousel, and (D) the 10 1.2 liter Niskin bottle array.

A series of seven deployments of the HRS were made at a station (27.2° N 83.5° W, also known as the Florida-Ecology of Harmful Algal Blooms (ECOHAB) station 10) along the 50 m isobath of
the WFS during a 24 hour sampling period spanning September 19-20 2002. Each deployment consisted of a vertical profile of the water column and 10 discrete stops at ~5 m increments from 3-45 m in which discrete SIPPER and 162 µm plankton net samples were collected for 8 minutes each (fig. 16). At each depth stratum a 1.2 liter Niskin bottle on the HRS would also be opened and closed for later chlorophyll extraction and analysis. SIPPER sampling did not take place any shallower than 2-3 m due to concerns with imaging the ships wake and or encountering ship-induced turbulence. Tow speed of the HRS during deployments averaged 1.5 knots (0.77 ms\(^{-1}\)). Sampling was meant to continue for several days to investigate the diel distribution of planktic forms, but a failure of our custom designed block for paying out HRS cable cut short our effort.

![SIPPER deployment depth profiles](image)

**Figure 16.** Depth profiles from the seven deployments of the HRS collected during this study. The circled areas indicate the depths where SIPPER and net sampling took place.

A total of 70 discrete depth SIPPER and 69 plankton net samples (One net sample was lost) were collected for this study. The SIPPER and plankton nets utilized the same 9.6 x 9.6 cm (92.16 cm\(^2\)) sampling tube to sample seawater. The nets and SIPPER sampled the water column for a total of 560 minutes (9.3 hours) and sampled greater than 245 m\(^3\) of seawater. SIPPER data
files ranged in size from 105 to 688 MB and totaled over 20 GB of image data. We used plankton image classification and extraction software (PICES), developed in collaboration with the USF College of Engineering (Luo et al. 2004, 2005), to extract and classify all of the images greater than a user specified size from each SIPPER data file. For this study, all particle images greater than 255 total pixels in area (0.23 mm$^2$ or 0.55 mm equivalent spherical diameter (ESD)) were extracted from each SIPPER file. Although SIPPER can resolve and image smaller identifiable particles, the proportion of unidentifiable particles rapidly increases below this size, reducing the effectiveness of the classifier.

Zooplankton and other collected particles were gently washed out of the HRS plankton nets using a hand-held pump sprayer and into plastic sample jars. They were immediately fixed in 5% v:v buffered formalin and stored for later analysis at our lab. Samples were split using a Motoda box splitter into usable sub-samples of 1500-2000 individual organisms. These were then analyzed under a dissecting scope where organisms were identified to species when possible. Up to forty individuals from each identified taxon for each sample were measured using an ocular micrometer and the appropriate size measurement was recorded. Nineteen samples comprising one nighttime (midnight) and one daytime (noon) vertical profile were analyzed for this study.

Because the nets collected smaller zooplankton than were extracted from the SIPPER dataset by PICES, we had to first identify the appropriate net zooplankton size threshold comparable to the SIPPER threshold of 550 µm ESD to allow for accurate comparison between the sampling effort of the plankton nets and the SIPPER. Direct areal comparisons were not applicable as the SIPPER measured zooplankton in situ and the net collected zooplankton were measured after the rough handling in the net, wash down and preservation. Additionally, because the SIPPER images zooplankton while in an undisturbed state, the measured ESD will also include fine setae, antennae, tentacles, mucous, egg sacs, and any other body part or associated debris that will not be apparent in the same way in the preserved organism. Therefore I analyzed the hundred smallest extracted SIPPER images for each zooplankton class found in the nets and collected appropriate measurements from each image to determine the minimum size of net collected zooplankton for comparison against the SIPPER dataset.
Description of SIPPER sensor

The grayscale imaging SIPPER (informally known as SIPPER-2) is the successor to the prototype binary imaging SIPPER (Samson et al. 2001, Remsen et al. 2004). The SIPPER is comprised of five main components: a 9.6 × 9.6 cm ($92.16\text{ cm}^2$) sample tube, a pair of collimated light sources, a pair of high-speed line-scan cameras and optical lens assemblies and a data storage device. Basically, the instrument operates by projecting a collimated light sheet across seawater passing through the sample tube and continuously recording the outlines and silhouettes of suspended particles and plankton with a pair of high speed line-scan cameras mounted orthogonal to each other. While the standard operating principles remain the same, there have been significant modifications to the SIPPER hardware to both improve the system and enable it to record in grayscale.

Improvements to line scan camera technology have made faster, more sensitive and affordable digital cameras available for use. We replaced the two original cameras on the prototype SIPPER that each scanned a 2048 pixel line image at 15,000 lines s$^{-1}$, with two new Dalsa Piranha 2© cameras capable of recording a 4096 pixel line image at 21000 lines s$^{-1}$. This achieved multiple benefits: it increased our image resolution, and it allowed us to tow SIPPER-2 at faster speeds while still capturing workable images. These cameras were also smaller, more sensitive and used less power than the original cameras. However, they also resulted in a nearly threefold increase in data rate due to their higher resolution and scan rate.

To accommodate this increase in data rate, the data storage device had to be modified. Previously, 8-bit camera data were converted to binary (black and white) by applying a threshold with a field programmable gate array (FPGA) at the camera and then combined and compressed (30:1, real-time) using an FPGA processing board and then buffered onto a stand-alone *in-situ* data storage system capable of recording up to 7.5 megabytes (MB) s$^{-1}$. In the SIPPER-2 configuration, all image processing is done at the data storage system in a separate pressure vessel. Grayscale information is transmitted from the camera pressure vessels to the data processing vessel via a low voltage differential signal (LVDS) parallel-serial data link. Since the grayscale information is still present, it can be stored, assuming the storage system can maintain
the data rate. To achieve this, we developed a higher-speed (20 MB s\(^{-1}\)), higher-density (112 GB) data storage device. It consisted of a custom FPGA-processing board, a single-board PC, a National Instruments PCI-DIO-32HS digital input card, a Boulder Instruments StreamStor 303 drive controller, and 112 GB RAID disk array. Even with this system, we limited ourselves to recording grayscale data from one camera at a time to reduce the potential for data loss due by approaching the maximum record rate.

Image data were recorded in 3-bit grayscale (8 colors). This format was a compromise on being able to store the data quickly and also have time for image processing in 1 clock cycle of the FPGA while not filling the hard drives too rapidly. After real time flat-field correction, the raw 8-bit camera data were thresholded to 3 bits by comparing individual pixel intensity against a running average intensity value for each camera pixel measured every 50 ms. The average individual pixel intensity was divided into eighths with the first brightest division equivalent to white and the last eighth corresponding to black with six gray levels between the two and assigning the current pixel brightness to the corresponding 3-bit grayscale value. The grayscale images collected by SIPPER-2 were qualitatively better than that of the binary imaging SIPPER (fig. 17) and more amenable to automated classification (Luo et al. 2005).

The optical resolution of the SIPPER-2 was determined to be approximately 70 \(\mu\)m using a calibrated resolution target slide (USAF 1951 resolution target). The pixel resolution is controlled by two separate processes: The pixel resolution in one dimension is calculated by dividing the width of the sample tube by the number of pixels making up the sample line image (in this case 9.6 cm \(\div\) 3800 = 25.3 \(\mu\)m) and in the other dimension it is determined by dividing the flow speed through the tube by the scan rate of the linescan camera (in this case 0.77 ms\(^{-1}\) \(\div\) 21,000 lines s\(^{-1}\) = 37 \(\mu\)m). Particles and features smaller than 70 \(\mu\)m can be detected and imaged but their sizes will not be accurate.
Detection and extraction of SIPPER images

After sampling and between deployments of the HRS, SIPPER-2 data were offloaded to a shipboard PC. The SIPPER-2 data were then decompressed and particle images were detected, extracted and classified using PICES. Image detection is relatively straightforward with the SIPPER-2 as particles (grey or black pixels) are easily discernible from the background (white pixels) making segmentation relatively straightforward. Particle images may have white pixels within their boundary but must have a discernible external edge made up primarily of foreground pixels to be detected and extracted. All resolvable particles within the sampling tube of SIPPER are assumed to be in focus.

Particle detection and extraction begins with a scanning of the entire continuous SIPPER image file that takes the form of a matrix 3800 pixels by 21,000 pixels × the length of sample in
seconds, and an indexing of all occupied (foreground) pixel coordinates. A connectivity routine is then run on the index in which occupied foreground pixels within 2 pixels of another occupied pixel are connected and considered to be part of the same particle and combined within the index. The perimeter and area of each particle in the index are then calculated. Particle images at or above the user specified minimum-size are located within the index, extracted as bitmaps with a unique file name, and stored on disk.

Concurrent with image extraction, morphological and texture features from each particle image were computed and written to a data file for use with the automated classification component of PICES. A total of 57 features were extracted from each particle image for this study (Table 6). About half of these features were shape-based and initially used for classifying black and white images from the prototype SIPPER-1 (Luo et al., 2004). These included the 8 invariant moments of the whole image and the image edge, 7 granulometric features (Tang et al., 1998), and domain specific features such as size, convex ratio, eigenvalue ratios and transparency ratio. An additional 20 texture and contour based features described in Luo et al., 2005 were also used for this study. These were comprised of 8 weighted moment invariants of the whole image, in which the grayscale intensity value of each pixel weighted the calculation of the moments, 5 fourier descriptors of the image contour and texture respectively, and a size and convex ratio weighted by the grayscale intensity of each pixel in the image. Additionally we utilized 8 new features for this study. These included the grayscale intensity histogram of each particle image, in which the proportion of each 7 foreground colors is calculated and a height/width ratio of the bounding box of the image. For spatial statistics purposes, the two dimensional location of the centroid of each extracted particle within the SIPPER sampling transect was calculated and included in the extracted data file.

Manual classification of SIPPER images and development of training library

A subset of the extracted particle images were then classified manually to develop a training library for the classifier and to create a test set to gauge its accuracy. Prior sampling experience from the WFS using the HRS (Sutton et al., 2001) indicated that the zooplankton community at the 50 m isobath was comprised of at least two distinct communities: a calanoid
copepod dominated community in the well mixed surface waters and a mixed copepod and ostracod layer below the pycnocline. Consequently, images from a shallow (3-5 m) and deep (43-45 m) SIPPER daytime (~noon) sample were manually sorted to determine the initial composition of the training library for use with our classifier. An additional two samples, comprising night (~midnight) shallow and deep tows were used to validate the automated classification system as a test set and to compare the day-night performance of the classifier. These four SIPPER files comprised a total of 109122 images.

An average SIPPER tow from this study sampled approximately 3.4 m$^3$ of seawater in 8 minutes. We used this volume to determine the maximum concentration an organism could be present in the water column and not be detected by SIPPER with a 5% probability using

$$\lambda = - \ln p/v$$

where $\lambda$ is the concentration of the organism, $p$ is the probability of detection and $v$ is the volume of water sampled (Benfield et al. 1996). For SIPPER-2, $\lambda$ was ~4 individual m$^{-3}$. Therefore, when building our preliminary training library we counted as a class any taxonomic group recognizable by SIPPER that numbered more than 5 occurrences in any of our two training-set samples as a class to include in the first run of our automated classification system. This was done to ensure that we didn’t overlook a group that might be more common in another sample even though it was relatively scarce but found in detectable numbers in the test set. To ensure we wouldn’t positively bias classifier performance, training image examples from a file being classified were not included in the classification of that SIPPER file.
Table 6. List of features extracted from every SIPPER-2 image and those used for the classifier after running the “wrapper” feature optimization algorithm.

<table>
<thead>
<tr>
<th>Feature name</th>
<th>Number of features</th>
<th>Used after “wrapper”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moment invariants of the original image</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Moment invariants of the edge image after closing</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Weighted moment invariants on the whole image</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Fourier texture</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fourier contour</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Granulometric features</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Intensity histogram</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><strong>Domain specific features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Convex area</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Transparency ratio</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Eigenvalue ratio</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Head-tail ratio</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Weighted size</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Weighted convex ratio</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Automatic classification of images**

PICES incorporates a multiple-class SVM to classify SIPPER particle images (Luo et al. 2004, Luo et al. 2005). Briefly, SVMs are a set of margin based linear classifiers that work by mapping the training data from two classes in multidimensional space using the feature vectors extracted from the example images in the training library. A decision boundary or hyperplane is computed that maximizes the margin that separates the two groups using example data known as support vectors that lie closest to the boundary. The SVM classifies unknown images based on where they lie along the decision boundary described by the support vectors. Mapping of the feature data into the higher dimension feature space is accomplished by the use of a kernel function. The kernel function allows a linear classifier to solve a non-linear classification problem.

To extend SVMs from 2-class to multiple class classification problems, we used the pairwise or one-versus-one approach (Luo et al., 2004) where all possible pairs of classes are used to create binary SVMs so that the total number of classifiers is equal to $n(n-1)/2$ where $n$ is equal to the number of classes chosen. Unknown examples are then voted on by each of the
SVMs; the one that receives the greatest number of votes becomes the predicted class. Each SVM classification is also assigned a probability based on its proximity to the decision boundary. These probabilities are used to break ties if an image has an equal number of votes for more than one class.

**Classification error analysis**

Classifier performance was calculated by examining the classifier accuracy rate as determined by a confusion matrix built using a grading utility in PICES. By comparing the results of the manually sorted test-set and a computer classified test-set, each cell of a confusion matrix is occupied by the number of images classified as a specific image class. The diagonal of the matrix then contains the number of images labeled correctly by the computer classifier for a specific class. This number divided by the total human count for a specific class is called the classifier accuracy (Davis et al. 2004). This assumes the human classification is error-free, which is most often not the case (Culverhouse et al. 2003). To quantify that assumption, we had another plankton biologist classify a random subset of the training library so that we could determine potential human error in grading and compare it against the computer classification accuracy.

**Analysis of SIPPER-2 spatial pattern data**

The SIPPER-2s capability to continuously image a relatively large volume of water makes it possible to investigate the spatial relationships between individual particles within the sampled volume. Because SIPPER is sampling a three-dimensional environment and imaging it in two-dimensions, it is not possible to determine the true linear distances between individual particles with total accuracy as we cannot determine where a particle is vertically within the ~10 cm height of the sample tube. However, by sampling enough particles, finescale relationships can still be explored. To investigate apparent nearest neighbor distances (ANND) between individual particle classes, we used a modified version of the Spatial Analysis Technique (SPLAT, Widder and Johnsen, 2000, Malkiel et al., 2006) that uses Monte Carlo methods to compare observed ANND and those expected if the particles were randomly distributed. Random distributions were created using a random number generator to create two-dimensional coordinates of the particles within a
simulated volume the same dimensions as the SIPPER transect using the observed abundance and the length of the SIPPER sampling transect (Malkiel et al., 2006). The simulations were further refined using the observed size distribution of the particles to avoid biasing the distributions towards a regular distribution due to a mismatch between the size of the actual particles and the simulations (Widder and Johnsen, 2000). SIPPER estimates of ANND were calculated for individuals from each classified group in the manually classified test set from the two-dimensional centroid location of each particle in the SIPPER sampling path. ANND measurements were also calculated for the entire particle assemblage within a sample.

These observed ANND were then compared against those calculated from 1000 Monte Carlo simulations of each group’s distribution. Simulated ANND were determined in two steps: first, the simulated particle class was randomly populated in the SIPPER sample volume at the observed abundance and second, the centroid positions of each simulated particle were then determined and the nearest neighbor distance between each particle class was calculated. Cumulative histograms of ANND from the simulations were used as models of complete spatial randomness and then compared against those from the observed data to determine whether the groups were randomly distributed. Histogram bin size was determined for each class individually by taking the maximum observed ANND and dividing it by 500. The number of ANND occurrences in each size bin was then calculated. This is illustrated in figure 18, where the y axis indicates the proportion of occurrences of a given NND equal to or less than the NND value indicated on the x-axis. Each histogram has four plots: the average from the simulations (dotted line), the minimum and maximum bounds from the random simulations (dashed lines) and the observed data (solid line). Observed data with a low proportion of small nearest neighbor distances would indicate a regularly spaced particle distribution (fig. 18a), while an observed distribution with a large proportion of small NNDs would indicate an aggregated distribution (fig 18c). An observed distribution falling within the bounds of the simulated random distributions is considered to be randomly distributed (fig.18b). We tested whether the observed distributions were significantly different from the simulated random distributions using a $U^2$ statistic. This statistic is the sum of squares of the deviations from the average cumulative histogram from the
simulations and compared against each simulation and the observed data. The rank of the $U^2$ statistic for the real data relative to the simulations can be used as a probability value to determine if the observed NND can be explained by chance. If the real datasets $U^2$ value was greater than 950 or more of the simulations $U^2$ values, there was less than a 5% chance that the distribution could be due to chance. The shape of the distribution then tells you if the spacing was aggregated, random or regular.

While measuring ANND is relatively straightforward, these measurements are subject to biases introduced by the finite spatial coverage of the sampling device. Particles at the edge of the sampling area have a high probability that their nearest neighbor is located outside the area sampled (DeRobertis, 2002). Replacing the true nearest neighbor with the closest one inside the sampling area results in an overestimation of NND and biasing the resulting distribution towards regularity (called “edge effects”).

We used the “Z-score” to account for these edge effects and allow for direct comparison of NND of particles at different particle densities in which the observed mean NND at a given target density was standardized to the NND expected under a random spatial distribution within the exact volume sampled (Malkiel et al., 1999, Malkiel et al., 2006). The Z score is calculated by

$$Z = \frac{X_s - X_r}{\sigma_r / \sqrt{N}}$$

Where $X_s$ is the mean ANND of the sample, $N$ is the number of particles in the sample, while $X_r$ and $\sigma_r$ are the mean and standard deviation from the 1000 random simulations. The Z score indicates by how many standard deviations the mean ANND deviates from that expected under complete spatial randomness. A $Z<0$ indicates a tendency towards aggregation and a score less than -2 is significant at the 0.05 probability level while a $Z>0$ indicates a tendency towards regularity and a score greater than 2 would be significant. The Z score was compared against the results from the $U^2$ statistic for both the manually classified and PICES classified test set.
While the U² statistic and Z-score provide us with information on the ANND distribution of a particular particle class, it provides no context on the degree of spatial heterogeneity along the entire sample transect (~400 m in 8 minutes). Simply stated, the plankton of a particular class could be closer to each other than could be explained by chance but still be more or less homogenously distributed along the sampling path. Therefore, we used Lloyd’s index of patchiness (Lloyd, 1967) to measure the degree of aggregation at the meter scale for the 4 samples comprising the test set. This measurement is scale dependent, as it requires the researcher to make a decision on what scale to examine the potential variance along the sample transect. We counted the number of each particle image class occurring along each meter of sampling in the 4 test set samples to calculate the index of patchiness at the meter-scale. Lloyd’s Index of patchiness is determined using

\[ P = \left( \frac{s^2}{x} - 1 + x \right) \frac{1}{x}, \]

where \( x \) is the mean and \( s^2 \) is the variance of the number of the selected particle class in a series of SIPPER samples of 1 m length. \( P \) indicates the distribution of individuals in a given sample relative to that expected in a Poisson distribution. A \( P \) equal to 1 corresponds to the crowding expected in a Poisson distribution while a \( P \) equal to 2 indicates the individuals are twice as crowded at the meter-scale than if they were randomly distributed.
Figure 18. Examples of regular (a), random (b), and aggregated distributions with their cumulative histograms superimposed on the cumulative histograms generated by Monte Carlo simulations. The dotted lines show the upper and lower envelopes of a random distribution. The solid line shows the data. (From: Widder E.A., Johnsen S., 3D spatial point patterns of bioluminescent plankton: a map of the minefield. Journal of Plankton Research, 2000 22(3), 409-420, by permission of Oxford University Press.)
Results

Hydrography

Typical summer conditions were encountered on the WFS with the water column being well stratified with the halocline, pycnocline and thermocline found between 30 and 35 m (fig. 19). The distribution of fluorescence and extracted chlorophyll \( a \) concentrations were similar to the other measured environmental parameters, with low concentrations above 35 m and rapidly increasing concentrations below that depth (Fig.20).

Figure 19. Vertical profiles of A: Temperature, B: Salinity and C: Sigma-t collected during the 24 hour sampling period.
Figure 20. Vertical profiles of A: fluorescence (Volts) and B: extracted chlorophyll collected during the 24 hour sampling period. Locations of bottle samples for extracted chlorophyll are noted by the white asterisks.

**Total SIPPER Particle Abundance and Size Distribution**

A total of 1391227 particle images of >550 µm ESD were extracted from the 70 discrete-depth SIPPER samples. The distribution of total particle abundance was similar to the fluorescence and chlorophyll profiles, with concentrations highest in the subpycnocline layer and lowest near the surface. A maximum concentration of 16608 particles m\(^{-3}\) was sampled at 45 m depth and a minimum of 2007 particles m\(^{-3}\) was collected at 3 m (fig. 21). Particle abundance increased in the upper water column during the late morning through early evening but never exceeded that of the subpycnocline layer. The majority of these particles were small, with 37% of the total between 0.55 and 0.7 mm ESD and 85% of the particles less than 1 mm ESD (Fig. 22).

**Selection of Image Classes and Classifier Performance**

A total of 48 separate particle and plankton image classes were identified in detectable numbers from the two SIPPER files for building the initial training library to automatically classify the SIPPER dataset (Table 7). This included an other and unknown class in which both unidentifiable particle images and identifiable but rare groups were placed. The training library also included two artifact classes: vertical lines that can appear when a particle temporarily adheres to the imaging window (scanline artifacts) and bubbles from towing too close to the surface, thereby sampling the ship’s wake. In the initial sort, the number of individuals per
identified particle class ranged from 13 to over 2000, with the majority of classes containing less
than 100 image examples. Using this as the preliminary training library (with a maximum of 100
randomly selected images from the more abundant classes to reduce their influence on the
classification task), the multi-class SVM classifier was run on the entire SIPPER dataset. The
resulting classifications were analyzed using a commercially available thumbnail viewing software
package Thumbs Plus. This involved a search of over 3300 separate image folders to determine
which classes were to be used for further optimization of the classifier training library. During this
phase, the performance of the classifier was not determined; rather we examined these results to
estimate which image classes would be suitable for use in the final classifier. This phase was also
used to further populate the training library with representative images for each class.

Those image classes found in detectable numbers in over half the deployment samples
were held onto for use in the training library for the next test of the classifier. Additionally, two
image classes found at high abundances only amongst a narrow depth stratum and at non-
detectable levels elsewhere, were also included in the reduced training library. Bubbles were
found in 6 of the 10 SIPPER files closest to the surface depth range, and most likely resulted from
sampling the ship’s wake. The cladoceran *Penilia avirostris* was found at the deepest sampled
depth in high abundance but at non-detectable concentrations in shallower waters.

Using the defined criteria of either being found in detectable numbers in over half the
samples or being extremely abundant in just a few, the number of classes was reduced to 31.
The number of individuals per training class was increased up to 500 example images to ensure
that the morphological and textural diversity of certain groups were well represented. Discarded
classes were either added to the other and unknown class or combined into a larger, more
comprehensive class with shared morphological or textural traits. For example, a number of
morphologically similar “shrimp-like” groups including amphipods, decapod zoea, euphausiids,
*Lucifer* sp., other shrimp and stomatopod larvae were combined into the larger group Crustacean-
eumalacostraca that shared traits such as a large opaque carapace, obvious telsonic fan and
relatively large size. Some of the discarded classes although rare, were observed exhibiting
some interesting behaviors. Scyllarid lobster phyllosoma were often observed directly associated

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with other plankton and suspended particle groups, especially fragile and gelatinous forms. Of
the 62 phyllosomes detected in the initial search, 38 were imaged clinging to one or more
hydromedusae, siphonophores, protoctists and marine snow using their pereopods.

![Figure 21. Distribution and abundance of all particles (no bubbles and scanline artifacts) imaged by SIPPER greater than 600 \( \mu \text{m} \) ESD in size over the 24 hour sampling period.](image-url)
Figure 22. Size distribution in equivalent spherical diameter of the total SIPPER particle images collected during this study (N=1391227).
Table 7. Image classes used during development of the multiple-class SVM classifier used in this study. The number of image classes was reduced during optimization of the classifier. Numbers in parentheses represent the image classes integrated into larger more generic image classes during optimization.

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<td>17. Fish</td>
<td>17. Marine snow</td>
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<td>22. Fish</td>
<td>22. Lancelet</td>
<td>21. Trichodesmium tuft and puff colonies</td>
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The classifier was then rerun using a split training library, with the two shallow water samples using a 30 class training library that included the bubble class, while the two deep water samples were run using a 30 class classifier that included the cladoceran class, so that the total number of classes used was 31. The results from the automatic classification of the test set were compared against the manually classified test set using a confusion matrix (table 8) created using PICES. Overall, there was a 71.8% agreement between the results of the manual and automatic classification of the test set. Classification performance was strongly associated with the relative abundance of the image class. Abundant groups comprising 5% or more of the total had accuracies of between 61 and 83% (mean=72.3%), with the lowest accuracies coming from marine snow and the other and unknown class, both of which are comprised of a wide range of particle sizes and morphologies. Common groups making up between 1 and 5% of the total had a slightly lower overall recognition rate (mean=71.5%) than the abundant groups and a slightly broader range in their individual classification performance (54.0 to 78.0%). The remaining 16 image classes were considered rare, individually representing between 0.7 to .01% of the total. The classification performance of these rare groups had the broadest range in classifier performance (10.0 to 94.7%) and the lowest aggregate classification accuracy (61.6%). This led to instances where the computer count strongly diverged from the human count for a given rare class. Whereas computer counts of the abundant and common groups were always within half or double that of the human counts (C/H ratio of 0.68 to 1.92), 9 of 16 rare groups had computer counts more than twice as high as the human count.

While the overall classification accuracy was satisfactory, the performance of the classifier with regards to the rare groups was less so. The proportion of incorrectly labeled images ("false positives") is much higher for rare groups, leading to significant overestimation of their abundance (Solow et al., 2001, Davis et al., 2004). For example, while only 40 cydippid ctenophores were identified by the human expert in the test set, 554 were labeled ctenophore by the computer. Similarly, only 8 of the 158 images labeled fish or fish larvae were actually fish. This led to a second reduction to the number of classes used in the classifier. Ten groups representing less than 0.75% of the total were removed from training library and the classifier was
rebuilt. The subtracted groups training examples were added to the other and unknown class training class. The classifier was then retrained with the 21 classes remaining and run again on the test set using the same split-training library described earlier.

Classifier performance was recalculated using PICES with this smaller training library and accuracy was found to have improved to 73.8% (Table 9). The C/H ratio was also much narrower for the image classes, ranging from 0.69 to 2.62. For the three classes with classification accuracies below 60%, two (cnidaria-other and crustacean-eumalacostraca) belonged to composite groups representing a broad range of shape, color and size. The poor performance of the third group, the small robust poecilostomatoid copepod genus Oncaea, appeared to be due to a combination of small size and confusion with small calanoid copepods, ostracods and the other and unidentified class. The four groups with C/H values greater than 1.8 were the 4 least abundant classes in the test-set. The final classification was executed using three separate subsets of the remaining 21 classes in the training library. The classifier for 13 shallow water (<5 m) samples was built using all the of the training library except for the cladoceran class while the classifier for the 7 deepwater samples used all of the training library save for the bubble class. The remaining 50 SIPPER samples were classified using a 19 class training library that did not include the bubbles and cladoceran class.

**Human expert classification error estimation**

For a machine classifier to perform well, it must be trained with correctly labeled example images. Previous work has demonstrated that taxonomists are imperfect in their labeling and classification performance (Culverhouse et al., 2003). To quantify the potential problem of incorrect labeling by human experts, we compared a subset of the training data sorted by the primary operator of PICES with that of another plankton biologist at the College of Marine Science at the University of South Florida. We achieved approximately 81% consensus (Table 10) in our labeling efforts with most of the error confined to *Macrostella gracilis* (47% CA), echinoderm plutei (97% CA) and the other and unidentified class (50% CA). This led to a very tight agreement in abundance estimates with ratios between the two human experts ranging between 48% and 152%. This is less than the variability expected within taxonomic groups.
between replicate net tows that can range between 25-300% (Wiebe and Holland, 1968; Pillar, 1984).

Example Images

The 21 image classes chosen for the final classifiers were mostly comprised of distinct taxonomic categories that were recognizable to a human expert. Representative images for each class save the two artifact classes can be seen in figures 23-36. Four of the image classes were comprised of individual species: the trachymedusae *Aglaura hemistoma*, the ostracod *Euconchoecia chierchie*, the cladoceran *Penilia avirostris* and the poecilostomatoid copepod *Macrosetella gracilis*, while four others were comprised of single genera (the copepods *Oithona* and *Oncaea*, and two forms of *Trichodesmium* colonies. *Trichodesmium* sp. was split into two separate image classes because there were two distinct groups of imaged morphologies: an elongate linear morphology, and a larger tuft and puff morphology. The calanoid copepod, chaetognath, larvacean and echinoderm plutei classes all consisted of an unknown number of relatively similar species. The doliolid class was made up of a number of life history stages with observable nurse, oozoid, trophozooid, phorozoid and gonozoid forms but most were not identifiable to species. The cnidaria other, crustacean-eumalacostraca, and protoctist classes were all comprised of numerous morphologically diverse taxa, some of which were recognizable to genus or species but too uncommon or difficult to identify to rate a class of their own. The elongate phytoplankton class consisted of dinoflagellate and diatom chains often identifiable to genera such as *Chaetoceros* and *Proboscia* for diatoms and *Ceratium* for dinoflagellates. The marine snow category was composed of a large variety of detrital aggregates with individual aggregates consisting of recognizable components such as cast-off larvacean houses, fecal pellets, phytoplankton and protoctists as well as unidentifiable debris. These marine snow particles spanned a wide range of sizes from less than a millimeter in diameter to over 4 cm. Similarly the other and unidentified class was composed of both recognizable taxa (fish, polychaete, pteropod, salp, siphonophore, etc.) and unidentifiable particles spanning a wide size range.
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**Table 8.** Confusion matrix of 31 class SVM classifier for SIPPER test set (N=109122 particle images). TH= Total human counts, TC= total computer counts and A= classification accuracy.
Table 9. Confusion matrix of 21 class SVM classifier for SIPPER test set (N=109122 particle images). TH= Total human counts and TC= total computer counts. A= classification accuracy.

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Table 10. Confusion matrix of classification performance between the primary human classifier of SIPPER images (TH\(^1\)) and a secondary human expert (TH\(^2\)) in sorting a subset (N=2178 images) of the SIPPER test set. A= classification accuracy.

|     | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   | 21   | TH\(^1\) |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1   | 99   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 99   |
| 2   | 0    | 98   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 99   |
| 3   | 0    | 0    | 80   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 10   | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 2    | 0    | 108  |
| 4   | 0    | 0    | 0    | 83   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 2    | 4    | 1    | 0    | 0    | 90   |
| 5   | 0    | 0    | 0    | 0    | 101  | 8    | 0    | 0    | 0    | 0    | 25   | 0    | 4    | 1    | 0    | 0    | 9    | 0    | 5    | 154  |
| 6   | 0    | 0    | 0    | 0    | 3    | 101  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 1    | 1    | 1    | 108  |
| 7   | 0    | 0    | 0    | 0    | 0    | 109  | 5    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 117  |
| 8   | 0    | 0    | 0    | 0    | 0    | 5    | 75   | 1    | 0    | 2    | 4    | 0    | 1    | 0    | 0    | 2    | 1    | 7    | 0    | 98   |
| 9   | 0    | 0    | 0    | 0    | 0    | 6    | 4    | 38   | 1    | 0    | 0    | 0    | 5    | 0    | 0    | 0    | 27   | 0    | 0    | 81   |
| 10  | 0    | 2    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 9    | 0    | 0    | 67   |
| 11  | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 68   | 0    | 0    | 0    | 2    | 0    | 0    | 0    | 108  |
| 12  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 96   | 0    | 0    | 0    | 0    | 2    | 0    | 99   |
| 13  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 100  | 3    | 0    | 0    | 1    | 0    | 4    | 0    | 108  |
| 14  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 2    | 86   | 0    | 0    | 0    | 0    | 2    | 0    | 90   |
| 15  | 0    | 2    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 66   | 0    | 2    | 0    | 89   |
| 16  | 0    | 0    | 23   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 76   | 4    | 0    | 9    | 0    | 116  |
| 17  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 118  |
| 18  | 0    | 13   | 0    | 5    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 62   | 1    | 0    | 0    | 81   |
| 19  | 0    | 13   | 5    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 8    | 4    | 0    | 2    | 2    | 25   | 3    | 67   |
| 20  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 25   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 72   |
| 21  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 12   | 0    | 0    | 0    | 0    | 0    | 121  |
| TH\(^2\) | 99  | 128  | 108  | 89  | 104  | 111  | 121  | 85  | 39  | 66  | 74  | 150  | 114  | 132  | 69  | 79  | 158  | 81  | 149  | 83  | 139  | 2178 |
| A   | 1.00 | 0.99 | 0.74 | 0.92 | 0.66 | 0.94 | 0.93 | 0.77 | 0.47 | 0.84 | 0.81 | 0.97 | 0.93 | 0.96 | 0.74 | 0.66 | 0.94 | 0.77 | 0.50 | 0.74 | 0.90 | 0.81 |
| TH/TH\(^1\) | 1.00 | 1.29 | 1.00 | 0.99 | 0.68 | 1.03 | 1.03 | 0.87 | 0.47 | 0.99 | 0.88 | 1.52 | 1.06 | 1.47 | 0.78 | 0.68 | 1.26 | 1.00 | 1.12 | 0.86 | 1.03 |

1. Artifact lines  
2. Bubbles  
3. Chaetognath  
4. Cladoceran-Penilia avirostris  
5. Chordaria-Aglaura hemistoma  
6. Cnidaria-other  
7. Copepod-calanoid  
8. Copepod-oithona  
9. Copepod-other-macrosetella  
10. Other-unknown  
11. Protoctist-sarcodine  
12. Trichodesmium tuft and puff colonies  
13. Copepod-other-Oncaea  
14. Crustacean-eumalacostracan  
15. Echinoderm-plutei  
16. Elongate-phytoplankton  
17. Elongate-trichomes  
18. Gelatinous-doliolid  
19. Larvacean  
20. Marine snow  
21. Ostracod
Figure 23. SIPPER images of A: the cladoceran *Penilia avirostris* and B: The ostracod *Euconchoecia chierchioe*

Figure 24. SIPPER images of chaetognaths. Several behaviors are visible in these images: beginning clockwise from the top, reproduction, parasitism, defecation, cannibalism and predation.
Figure 25. SIPPER images of the trachymedusa Aglaura hemistoma

Figure 26. SIPPER images of hydromedusae and narcomedusae making up the other cnidarian class.
Figure 27. SIPPER images of various calanoid copepod species.

Figure 28. SIPPER images of A: the cyclopoid copepod genus *Oithona*, B: The poecilostomatoid copepod species *Macrosetella gracilis* and C: the poecilostomatoid copepod genus *Oncaea*.
Figure 29. SIPPER images of eumalocostracan crustaceans

Figure 30. SIPPER images of A: echinoderm plutei and B: Elongate phytoplankton colonies.
Figure 31. SIPPER images of doliolids.

Figure 32. SIPPER images of larvaceans.
Figure 33. SIPPER images of marine snow

Figure 34. SIPPER images of the other and unknown particle class.
Figure 35. SIPPER images of various forms of sarcodine protoctists

Figure 36. SIPPER images of A: Elongate trichomes and linear colonies of *Trichodesmium*, and B: Tuft and Puff colonies of *Trichodesmium*. 
**Taxonomic composition of imaged dataset**

Close to one-quarter (23%) of the SIPPER images, comprising 320,116 individual images belonged to the other and unidentified class (figure 37), making it the single most abundant class in the dataset. The 13 groups making up the identified zooplankton totaled just over 52% of the total imaged particle assemblage. Larvaceans were the most numerous of the identified zooplankton classes, comprising just over 13% of the total extracted particle images. Four other zooplankton groups (calanoid copepods, ostracods, the hydromedusae *Aglaura hemistoma* and *Oithona* sp.) comprised between 6-10% of the total. Chaetognaths comprised slightly less than 5% of the total, while the other 7 zooplankton image groups contributed between 2.1 and 0.75% to the total assemblage. The colonial cyanobacteria *Trichodesmium* was made up of two separate image classes (round and linear colonies) that together made up just over 8% of the total. Amongst protoctists, sarcodines contributed 3.5% to the total, while elongate dinoflagellate and diatom chains contributed another 2.8%. Marine snow was the third most abundant image group, comprising slightly less than 12% of the total extracted image assemblage.

![Figure 37. Composition of the SIPPER dataset as determined through the three multiple class SVMs run on the dataset.](image-url)
Abundance and distribution from SIPPER-2

Other and unidentified class

The other and unidentified class had a very broad size distribution and was comprised of particles from less than 0.6 mm to greater than 5 mm ESD. However, Small particles dominated the size distribution with 84% of the imaged particles measuring 0.8 mm E.S.D. or less (figure 38). A substantial proportion of these particles were most likely small forms of the plankton classes chosen for classification but unable to be identified due to lack of resolvable features and/or presenting an ambiguous profile when imaged by the SIPPER-2. Their distribution mostly trended with the total particle distribution with highest concentrations in the sub-pycnocline layer (figure 39). Abundances in the upper 30 m were mostly evenly distributed, ranging between 400-1600 m$^{-3}$ while below the pycnocline they were more dynamic, with abundances between 2000-6000 m$^{-3}$. More unknown and unidentified particles were found below the pycnocline in the first half of the sampling period (midnight to noon) than in the latter half of the day. Proportionally, this class made up between 15-37% of the imaged particles in a sample with no apparent trend with depth.
Figure 38. Size distribution in ESD for the non-zooplankton image classes.

Figure 39. Distribution and abundance of the other and unidentified particles image class collected during this study. Classification accuracy of PICES for this group was 67%.
Zooplankton

Total imaged zooplankton made up between 28-70% of the imaged particle assemblage for the 70 SIPPER samples and comprised over 52% (729513 zooplankton images) of the total SIPPER image dataset collected for this study. Consequently, the distribution of zooplankton generally mirrored that of the total imaged particle distribution, with maximum abundances found below the pycnocline and much lower abundances above (Figure 40). Most of the individual zooplankton classes observed this trend as well. Abundances ranged between ~1000-2000 m$^{-3}$ in the upper 30 m to greater than 6000 m$^{-3}$ below 40 m. Zooplankton made up more of the total imaged particle assemblage with increasing depth, comprising on average 46% of the images in the 10 m depth stratum and 58% below 40 m. A patch of elevated zooplankton abundance was observed between 15-30 m during the late afternoon and early evening.

Larvaceans were the dominant zooplankton group, comprising just over 14% of the particle assemblage as determined using the 21-class SVM. They were abundant at all depths, comprising between 10-40% of the imaged zooplankton assemblage, averaging just under 25%.
Larvacean abundance was highest below the pycnocline and generally decreased towards the surface (figure 41). Larvacean abundances ranged from $284 \text{ m}^{-3}$ at 3 m depth to $2700 \text{ m}^{-3}$ at 43 m. Larvacean abundance was elevated in the mid-water patch detected in the late afternoon and early evening. The majority of sampled larvaceans were imaged separate from any visible house structure. Approximately 15% of the larvacean images contained the organism along with some part of the inner filter and or debris collected on the exterior of the house structure. The incidence of these images increased with depth. The majority of larvaceans were less than 1mm ESD in size, with only 20% of the classified larvaceans measuring greater than that (figure 42). Much of those larger sizes can probably be explained by measuring the imaged house and filters along with the larvacean. Larvaceans were not identified to species in the net samples.

![Figure 41. Distribution and abundance of larvacean images collected during this study. Classification accuracy for this group was 83%](image)

Calanoid copepods were the next most abundant zooplankton group imaged by SIPPER. They displayed a similar trend as larvaceans with highest abundances below the pycnocline and decreasing concentrations toward the surface (figure 43). They comprised between 6-32% of the imaged zooplankton at any one depth, averaging just over 17% of the assemblage. Abundances ranged from $129 \text{ m}^{-3}$ at 6 m depth to $1956 \text{ m}^{-3}$ at 42 m. A number of calanoid copepod genera
were recognizable from the SIPPERS images and verified from net samples including *Calocalanus*, *Centropages*, *Candacia*, *Eucalanus*, *Euchaeta*, *Mecynocera* and *Temora* but the majority were small copepods with few distinguishing characteristics and were most likely members of the *Paracalanidae* and *Clausocalanus* sp. and copepodite stages of larger copepods. Over 86% of the imaged calanoid copepods had ESDs of 1 mm or less (fig 44) but some were measured as large as 3.5 mm. There was no apparent trend in copepod size with depth.

![Figure 42. Size distribution in ESD for the non-crustacean zooplankton image classes.](image)

Ostracods were the third most abundant zooplankton group, comprising just over 8% of the total imaged particle assemblage and from <1 to 40% of the zooplankton assemblage at the sampled depths. Ostracod abundance was highest between 30-40 m and was strongly associated with the pycnocline (figure 45). Ostracods were virtually absent in the upper 10 meters during the day but increased in the surface waters throughout the evening while decreasing at depth suggesting that part of the population may vertically migrate. The majority of classified
ostracods were small, with 95% of the imaged ostracods sized between 0.6 and 0.9 mm ESD. Net collected ostracods were exclusively comprised of the species *Euconchoecia chierchiae*.

Figure 43. Distribution and abundance of calanoid copepod images collected during this study. Classification accuracy for this group was 82%.

Small thimble-shaped trachymedusae, consisting mainly of *Aglaura hemistoma* were the next most abundant zooplankton group imaged by SIPPER and comprised greater than 7% of the total imaged particle assemblage. These small hydromedusae were common at all depths with concentrations ranging from 156-1316 m$^{-3}$, with the greatest concentrations found below 40 m (fig. 46). Many imaged *Aglaura* were observed with their tentacles contracted tightly against the bell suggesting a possible behavioral reaction to being sampled, but a significant minority was observed with their tentacles fully extended in a feeding posture in which they wag the very distal end of their tentacles (Colin et al, 2005, see example image in figure 25). However, very few imaged specimens had any visible captured prey. This behavior contributed to the broad size range of this class, as witnessed by ESD measurements since tentacle area was included in the measurement of ESD. Manual analysis of the *Aglaura* images revealed a wide range of bell
diameters from approximately 0.75 mm to 2.5 mm, with very few larger individuals. No identifiable Aglaura specimens were collected in the nets.

Figure 44. Size distribution in ESD for the crustacean zooplankton image classes.

The distribution of the cyclopoid copepod *Oithona* generally followed the trend of the other abundant zooplankton groups with highest abundances encountered below 40 m (figure 47). High abundances however, were also found at intermediate depths during the latter half of the day. Abundances ranged from greater than 1300 ind. m\(^{-3}\) at 45 m to less than 50 ind. m\(^{-3}\) at 3 m depth. *Oithona* were small, with 85% of the imaged organisms less than 0.8 mm ESD. Net analysis indicated most of the small *Oithona* were identified as *O. colcarva* while the larger specimens were *O. plumifer*. 
Figure 45. Distribution and abundance of ostracod images collected during this study. Classification accuracy for this group was 80%.

Chaetognaths made up between 4 and 11% of the zooplankton assemblage at any one depth. Abundances were fairly uniform (50-200 m$^{-3}$) between 3-30 m deep while highest values were encountered below 40 m depth where abundances ranged between 494-726 ind. m$^{-3}$ (figure 48). Imaged chaetognaths spanned a wide size as indicated by their ESD size distribution (fig. 42). Average chaetognath size was slightly greater than 1.5 mm ESD, which was equivalent to approximately 4.8 mm in length. Measured chaetognath lengths ranged from less than 1.5 mm in to over 25 mm, but the majority (~90 %) of the individuals were between 2.5 – 10 mm. Several apparent behaviors were visible in chaetognath images including cannibalism, fecal pellet production, parasitism and predation (see figure 24). Larvaceans were the most commonly imaged apparent prey item in chaetognath feeding images (>70 occurrences).

Plutei from different echinoderm groups were the most abundant meroplankton group imaged by SIPPER (2% of the total extracted images) and were common but rarely abundant in the water column during this study (fig. 49). Half of the SIPPER samples had concentrations of 50 individuals m$^{-3}$ or less and only 6 had concentrations greater than 300 individuals m$^{-3}$. Highest concentrations were found in the middle of the water column between 15 and 35 m depth and
during the late afternoon and early evening. Both echinopluteus and ophiopluteus forms were imaged by SIPPER. Classified plutei had an average ESD of 1.05 mm and ranged in size between 0.6 and 2.5 mm ESD. The observed distribution and the large size range was most likely influenced by the large number of sarcodine protoctists that were misclassified as echinoderm plutei (see table 9).

Figure 46. Distribution and abundance of *Aglaura hemistoma* images collected during this study. Classification accuracy for this group was 77%.

A diverse group of hydromedusae and narcomedusae made up the other cnidaria class (fig. 26). No one species or morphology was abundant enough to warrant their own class but as a whole the group comprised between 0.2 to 9% of the imaged zooplankton assemblage at any one depth. Highest abundances were encountered below 30 m but there was also relatively high concentrations observed in the afternoon and early evening between 5-30 m (fig. 50). This group had the widest and most broad size range with 50% of the imaged animals having ESDs greater than 2.5 mm. Bell diameters ranged from 1 mm to over 2 cm. Including tentacle length, some of the larger medusae had overall lengths greater than 10 cm. At least 19 separate hydro- and narcomedusan species were observed during this study. This group had the lowest classification
accuracy at 46% and also was strongly confused with both *Aglaura hemistoma* and the other and unknown group suggesting that this distribution should be viewed with caution.

**Figure 47.** Distribution and abundance of *Oithona* sp. images collected during this study. Classification accuracy for this group was 82%.

**Figure 48.** Distribution and abundance of chaetognath images collected during this study. Classification accuracy for this group was 82%.
The poecilostomatoid copepod *Oncaea* was a relatively minor but consistent contributor to the SIPPER imaged zooplankton assemblage contributing between 2-4% to the total at all depths. *Oncaea* is a robust and compact copepod with short antenna, making it relatively difficult to classify and leading to a classification accuracy of just 52%. The majority (90%) of the imaged *Oncaea* were less than 0.8 mm ESD. *Oncaea* distribution trended with most of the other zooplankton groups, with highest abundances below the pycnocline (figure 51). Imaged *Oncaea* were comprised mostly of the species *O. venusta* and *O. mediterranea*, both of which were common in the net samples and within the correct size range.

Cladocerans were an abundant but narrowly distributed taxa in the SIPPER samples. The cladoceran images were comprised solely of *Penilia avirostris* and they were found only in detectable numbers at the deepest sampled depth from each deployment at abundances between 328-1258 ind. m$^{-3}$. Cladoceran images were small with an average ESD of 0.8 mm ESD. Even though they were only counted in 7 of 70 SIPPER samples, cladocerans made up almost 1.5% of the total extracted image particle assemblage. They contributed between 4-16% to the total zooplankton assemblage in those 7 samples.

The eumalacostracan crustacean class was relatively rare and patchily distributed throughout the water column (fig. 52). Concentrations exceeding 100 ind. m$^{-3}$ were encountered only 5 times out of 70 samples. All 5 instances occurred between 30 and 45 m depth. However, at all depths there were instances of relatively low occurrences (<25 individuals m$^{-3}$). The size distribution of the eumalacostracan classified images was very broad, with 78% of the images having a size greater than 1 mm ESD and 31% were larger than 1.5 mm ESD. Amphipods and calyptopis and furcilia stages of euphausiids, zoeal and megalopa stages of decapods comprised the majority of sub 1.5 mm ESD images while Stomatopod larvae, juvenile *Lucifer faxoni*, and porcellanid crab zoea dominated the larger size range. As with the cnidaria other class, this morphologically diverse class had one of the lowest classification accuracies at 49%.

Doliolids were distributed throughout the water column with highest concentrations encountered between 30 and 45 m (fig. 53). The majority of imaged doliolids were of gonozooid and phorozooid morphological stages and less numerous oozoid and old nurse stages. Only a
few trophozooid stages were imaged. Based on the location of the endostyle on the oozoid images between muscle band 3 and 5 (Godeaux, 1998), most of the doliolids were most likely Dolioletta sp. While the classification accuracy was relatively high at 67%, the C/H ratio was the highest of the 21 classified groups at 2.62 meaning there was significant confusion with other groups. This led to the computer classification of more Agaura hemistoma images, for example as doliolids than actual imaged doliolids (table 9).

The poecilostomatoid copepod Macrosetella gracilis was the least abundant zooplankton group included in the final classifier. This species comprised between less than 1 to slightly fewer than 5% of the total imaged zooplankton assemblage, averaging 1.4% for the 70 SIPPER samples. Highest abundances were recorded below the pycnocline throughout most of the day except for during the afternoon and early evening, when lower abundances were found in deep water and abundances were elevated near the surface (fig. 54). This group had the highest classification accuracy of any zooplankton class in the final classifier at 83.7% although there was a problem with it being confused with other copepod groups that contributed to having a C/H ratio higher than 2.
Figure 49. Distribution and abundance of echinoderm plutei including both echinopluteus and ophiopluteus images collected during this study. Classification accuracy for this group was 62%.

Figure 50. Distribution and abundance of unidentified hydromedusae and narcomedusae making up the other cnidarian class. Classification accuracy of this group was 46%.
Figure 51. Distribution and abundance of the poecilostomatoid copepod genus *Oncaea* images collected during this study. Classification accuracy of this group was 52%.

Figure 52. Distribution and abundance of eumalacostracan crustacean images collected during this study. Classification accuracy of this group was 49%.
Figure 53. Distribution and abundance of doliolid images collected during this study. Classification accuracy of this group was 67%.

Figure 54. Distribution and abundance of the poecilostomatoid copepod *Macrosetella gracilis* images collected during this study. Classification accuracy of this group was 49%.
Trichodesmium

Both morphologies of the diazotrophic colonial cyanobacteria Trichodesmium sp. were abundant throughout the water column. These two morphologies did not appear to represent separate species of Trichodesmium even though there are two species commonly collected on the WFS. Trichodesmium erythraeum and T. thiebaudii can be common on the WFS, but in this instance most of the images appeared to be T. thiebaudii (Judy O'Neil, pers. comm.). Abundances of both groups followed the same distribution pattern, so their distribution and abundance data were combined and are presented in figure 55. The combined classification accuracy for the two groups was 74%. Trichodesmium distribution contained a possibly strong diel signal, with highest abundances encountered in the morning and early afternoon in the upper 30 m with an abrupt decrease in surface abundance and increased abundance below 30 m during the evening. This signal was not evident in either the fluorescence or extracted chlorophyll-a profiles collected at the same time. Tuft and puff shaped colonies were larger (average size 1.32 mm ESD) than the elongated linear colonies (0.85 mm ESD). Individual trichomes could be observed in both colony morphologies.

Protoctists

Foraminifera, acantharians and solitary radiolarians made up the sarcodine protoctist class that comprised just over 3% of the total imaged particle assemblage. Colonial spumellarian Radiolaria were noted in the samples especially near the surface, but were too rare and morphologically distinct from the rest of the sarcodine protoctist class to be included in the classifier. Most of the imaged sarcodines were radially symmetrical with varying lengths of extendible pseudopods (figure 35). While present throughout the water column in appreciable numbers, sarcodines formed a dense band between 30-40 m depth at concentrations up to 1000 ind. m⁻³ (fig. 56). This band was comprised mainly of large radiolarians resembling Thalassicolla nucleata or a similar species while the other sampled depths were comprised of a smaller and more diverse protoctist assemblage. Most imaged sarcodines had exoskeletons less than 1 mm in diameter, but many had numerous pseudopodia extending far past their body. These larger forms were often associated with marine snow and unidentifiable small particles that appeared to
be stuck onto the pseudopodia that may be indicative of feeding by these forms. This contributed to the broad size range apparent in figure 23 and the average size of 1.21 mm ESD for sarcodines.

Large elongate colonies of diatoms and dinoflagellates comprising slightly greater than 2% of the imaged particle assemblage made up the elongate phytoplankton class. Most of these colonies were small, with 95% of the imaged population less than 1 mm ESD. The colonies tended to be narrow and long with 95% of those imaged being less than 4 mm in total length. Highest concentrations were found at or below the pycnocline for most of the day except for the afternoon and early evening when the distribution reversed and highest concentrations were found near the surface and lower concentrations were found below the pycnocline (fig. 57). As with the Trichodesmium distribution, this trend was also not evident in either the fluorescence or extracted chlorophyll-a profiles.

Figure 55. Distribution and abundance of Trichodesmium colony images collected during this study. Classification accuracy for this group was 74%.
**Marine snow**

Marine snow comprised slightly less than 10% of the total imaged particle assemblage. This class was comprised of a wide range of material and morphologies ranging from identifiable fecal pellets, shed larvacean houses, diatom frustrules, and protoctist tests to unidentifiable strands and detrital aggregates. Marine snow images were often labeled as larvacean images (12% false positive rate in the test set) due to the presence of the morphologically similar house structure in many of the larvacean images used in the training library. Marine snow distribution roughly followed the zooplankton distribution, with greatest concentrations found below the pycnocline and increased numbers in the upper 30 m during the afternoon and early evening suggesting the marine snow formation is mostly autochthonous (fig. 58). However, the proportion of marine snow to the total particle assemblage showed no relation to depth. Most marine snow particles were small with 84% of the imaged particles less than 1 mm ESD. While not a common occurrence, both *Oncaea* sp. and *Macrosetella gracilis* were imaged associated with marine snow particles. Small (<0.7 mm in length) opaque particles were found on many larger marine snow particles that may have been smaller unidentified zooplankton feeding or otherwise associating with these aggregates.
Figure 56. Distribution and abundance of sarcodine protocist images collected during this study. Classification accuracy for this group was 72%.

Figure 57. Distribution and abundance of the elongate phytoplankton images collected during this study. Classification accuracy for this group was 68%.
Figure 58. Distribution and abundance of marine snow images collected during this study. Classification accuracy for this group was 61%.

Net Sample Analysis and Comparison with Concurrent SIPPER Data

One hundred of the smallest individual images from each of the thirteen zooplankton classes manually classified in the test set were measured to determine the appropriate minimum size of net collected zooplankton to compare against the SIPPER sampling effort. Total length was measured for the four copepod classes, chaetognaths, cladocerans, doliolids, eumalacostracan crustaceans, echinoderm plutei and ostracods; head length for larvaceans, and bell diameter for the hydromedusae (Table 11). The counts for both Aglaura hemistoma and other cnidarians were combined in the net counts because identification to species for small hydromedusae and narcomedusae was often not possible. The number of net zooplankton above the minimum SIPPER size threshold for each class was then calculated by taking the measured size distribution for each zooplankton class and multiplying the proportion of individuals greater than that size by the total number of zooplankton in that class. This was done for all 12 zooplankton groups for the 19 net samples.
Table 11. Average minimum size of net collected zooplankton used for comparison of sampling effort between plankton nets and the SIPPER. Standard deviation in parentheses.

<table>
<thead>
<tr>
<th>Zooplankton class</th>
<th>Average Minimum net plankton size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetognath</td>
<td>1.4 mm (.12) Total Length (TL)</td>
</tr>
<tr>
<td>Cladoceran</td>
<td>0.78 mm (0.047) TL</td>
</tr>
<tr>
<td>Cnidaria-Agaura and other</td>
<td>0.63 mm (0.08) Bell Diameter (B.D.)</td>
</tr>
<tr>
<td>Copepod-calanoid</td>
<td>0.75 mm (0.02) TL</td>
</tr>
<tr>
<td>Copepod-Macrosetella</td>
<td>1.02 mm (0.02) TL</td>
</tr>
<tr>
<td>Copepod-Oithona</td>
<td>0.82 mm (0.04) TL</td>
</tr>
<tr>
<td>Copepod-Oncaea</td>
<td>0.79 mm (0.03) TL</td>
</tr>
<tr>
<td>Echinoderm plutei</td>
<td>0.76 mm (0.08) TL</td>
</tr>
<tr>
<td>Eumalacostracan crustaceans</td>
<td>0.96 mm (0.12) TL</td>
</tr>
<tr>
<td>Gelatinous-doliolid</td>
<td>0.80 mm (0.02) TL</td>
</tr>
<tr>
<td>Larvacean</td>
<td>0.32 mm (.01) Head Length (H.L.)</td>
</tr>
<tr>
<td>Ostracod</td>
<td>0.68 mm (.01) T.L.</td>
</tr>
</tbody>
</table>

Net and SIPPER total zooplankton counts were then compared using paired t-tests. The SIPPER imaged between 9-15% more similar-sized zooplankton than the nets, but the differences were only significant for the nighttime comparison (Table 12). These differences were caused by a combination of the SIPPER classifying less small crustacean zooplankton and the nets collecting less fragile zooplankton. The nets collected significantly more similar-sized calanoid, *Oithona* and *Oncaea* species of copepods, cladocerans, and ostracods although the *Oithona* and ostracod abundances were only significantly different during the nighttime comparison. Nets counts for these groups were between 19-212% greater than the concurrent SIPPER estimates. The copepod *Macrosetella gracilis*, chaetognaths, and the eumalacostracan crustaceans were all sampled similarly by the two collection methods. The cnidarian hydromedusae and narcomedusae, echinoderm plutei, doliolids and larvaceans were all significantly overrepresented in the SIPPER samples compared to the concurrently sampling nets. The SIPPER abundance estimates for these fragile zooplankton groups were 85-1700% greater than the concurrent net estimates.
Tables 13-16 show the results from the Monte Carlo simulations and the resulting $U^2$ statistics and Z-scores for the four SIPPER samples in the test set. The two daytime samples and the shallow nighttime imaged particle assemblages (minus the two artifact classes) had distributions that were significantly different from complete spatial randomness. Compared against the results from the random simulations, most of the differences with the real data occurred at nearest neighbor differences less than 1 cm. On average, 37% of the observed particles had NND of 1 cm or less while only 32% of the randomly distributed simulated particles had NND of 1 cm or less, indicating an aggregated particle distribution. In contrast, the Lloyd’s index of patchiness for all 4 particle assemblages measured between 1.01 and 1.14 indicating no difference with a random distribution at the meter scale. At the meter scale, abundances ranged from 0.5-5× the average abundance along the sampling transect.

NND analysis of the individual classes in the SIPPER dataset indicated that approximately one quarter of the image classes had non-random distributions based on the ANND measurements. However, there was not 100% agreement in detecting these non-random distributions between the two methods used. While the $U^2$ statistic predicted significant departures from spatial randomness in 24 of 76 classes, the Z-score predicted that only 17 classes had non-random distributions. The two metrics both predicted non-random distributions for 14 of the image classes. All of the image classes predicted by the $U^2$ statistic to be non-random were found to be more aggregated than could be explained by chance while 13 of the 14 classes indicated by the Z-score to be non-randomly distributed were aggregated and one was found to be more uniformly distributed (the other and unidentified class in the shallow night sample). The Lloyd’s index of patchiness values were far more conservative, with only six non-artifact classes having index values greater than 1.4 and only two having index values greater than 2. This suggested that the plankton classes imaged by SIPPER-2 did not form appreciable aggregations in the horizontal domain at the meter scale.

Bubbles were an artifact class and were only sampled because the HRS sampled too close to the surface on occasion and sampled the ships wake. Inspection of the spatial
distribution of the bubbles indicated they occurred infrequently but at high abundances indicating clustering. We used this group as a test to determine the power of the spatial statistics in detecting significantly non-random particle distributions. While the observed $U^2$ ranks for the bubbles were both higher than all 1000 simulations, the Z-score for the nighttime sample was only -0.74 indicating a random pattern. The Lloyd's index of patchiness for bubbles in the two surface samples was 9.45 and 6.33 indicating significant patchiness at the meter scale. The patchy nature of the nighttime bubble distribution is apparent in figure 59 in the NND cumulative histogram and a plot of bubble abundance per meter traveled during the sampling transect. Bubbles were not included in the calculation of ANND and Lloyd's index of patchiness for the entire particle assemblage to ensure their extreme clustering did not bias the results.

Marine snow was the only other group observed to be non-randomly distributed in all of the samples analyzed in which they occurred. Marine snow particles had $U^2$ rankings greater than 100% of the simulated data for each of the four tow-transects analyzed. All of the distributions were aggregated, with the observed cumulative histograms having between 5-11% more NND less than 5 cm than the simulated populations. The Z-score predicted similar results for all but the shallow nighttime marine snow distribution. The Z-score for the deep daytime marine snow distribution of -13.1 was the lowest and most significant Z-score recorded. The ANND measurements did not translate into strong meter-scale patchiness as the Lloyd's values ranged from 0.97 to 1.26. This ANND clustering could be due to the coagulative properties of marine snow that tend to cluster other detritus around it (Kiørboe et al., 1990), the distribution of the organisms responsible for the formation of the detritus (Hansen et al. 1996), or it could be an artifact from the PICES image segmentation and extraction process. Large marine snow aggregates are often held together with transparent exopolymer particles or TEP (Alldredge et al., 1998) that might not be imaged by the 3-bit grayscale of the SIPPER-2. Thus, a single marine snow particle might be extracted as more than one and therefore bias the measurement of marine snow distribution towards clustering. Additionally, these large aggregates are known to harbor large numbers of copepods, amphipods and other organisms (Steinberg et al., 1994; Koski et al., 2007) that may not be counted when these large particles are extracted.
Most of the other clustered particle distributions were found in the two deep water samples where approximately 75% of the clustered classes were found using either statistical metric. During the day, chaetognaths, Aglaura hemistoma, calanoid copepods, Oithona sp., echinoderm plutei, larvaceans, marine snow, ostracods and the other and unknown particle class were all found to be significantly aggregated based on their $U^2$ ranking. Results from the Z-scores were nearly identical, with significant clustering found for each of the same groups except the echinoderm plutei class that was instead calculated to be randomly distributed. During the night, chaetognaths, cladocerans, Aglaura hemistoma, Oithona, echinoderm plutei, elongate phytoplankton chains and marine snow were all found to be clustered based on their $U^2$ rank. The Z-scores only indicated significant clustering for the distributions of chaetognaths, cladocerans, echinoderm plutei and marine snow while suggesting that the other and unidentified class was regularly distributed. While many individuals of the imaged plankton groups were clustered, the degree of clustering along the sample transects were not very dynamic. Lloyd's index of patchiness only exceeded 1.5 for two groups of imaged copepods sampled during the day, *Macrosetella gracilis* and *Oithona*. Both the ANND clustering and meter-scale patchiness of the imaged daytime *Oithona* assemblage is depicted in figure 50. Notice the more than 100 meter long zone of depressed Oithona abundance relative to the rest of the transect that probably led to the relatively high Lloyd's index. For the other deepwater groups, there was not much meter-scale patchiness apparent.

Distributions of individual particle classes were predominantly randomly distributed in the two shallow water samples using the ANND measurements. The other and unidentified particle class was the only particle class besides marine snow that was found to be clustered during the day while *Trichodesmium* tuft and puff colonies were the only other class found to be clustered during the nighttime samples using the $U^2$ statistic. Results using the Z-score also showed few particle classes as being clustered, with only the copepod genera *Oncaea* and sarcodine protoctists with Z scores less than -2 during the day and none besides marine snow during the night. Only the other and unidentified class sampled during the daytime had a Lloyd's index of patchiness value greater than 2 suggesting most of the shallow water plankton groups were
randomly distributed at the meter-scale. A closer look at the meter-scale distribution of the other and unidentified class sampled during the daytime indicated that it was only a high aberrant number of particles encountered in one meter that tilted the distributions towards a patchy distribution. This suggests the Lloyd’s index of patchiness may be susceptible to overestimates of patchiness.

The ANND calculations of the PICES classified test set were also investigated. PICES correctly identified significant clustering in 20 of the 24 classes in which it was found in the manually classified test set using $U^2$ statistics. However, 4 other classes that were not considered significantly clustered in the manual classified dataset were determined to be clustered using the PICES $U^2$ results. Similarly, PICES correctly identified clustering in 16 of the 21 classes that were found to be significantly clustered manually using Z-scores. Three image classes not considered clustered using Z-scores from the manually classified test set were scored as significantly clustered when classified by PICES. Lloyd’s indices of patchiness were not calculated for the PICES classified dataset.
Table 12. Comparison of SIPPER zooplankton abundance estimates versus the abundance estimates of similarly sized zooplankton collected by concurrently sampling 162 µm plankton nets. Differences in bold indicate significant differences between the two sampling strategies (paired t-test, p < 0.05).

<table>
<thead>
<tr>
<th>Day</th>
<th>SIPPER</th>
<th>Net</th>
<th>SIPPER/Net</th>
<th>Difference</th>
</tr>
</thead>
</table>
|     | Depth | chao2rank | cladoceran | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calanoid | copepods_calan0
Table 13. Results of statistical analysis of spatial patterns in the shallow daytime test set sample. Numbers in bold indicate departures from a random distribution for that class for either the $U^2$ statistic or the $Z$-score. * indicates the PICES classification failed to detect significant deviations from randomness while ¥ indicates that the PICES classification detected a significant departure from randomness for that class not detected in the manually classified set.

<table>
<thead>
<tr>
<th>Day Shallow</th>
<th>Abundance ($\text{# m}^{-3}$)</th>
<th>Median NND (cm)</th>
<th>$U^2$ statistic rank</th>
<th>$Z$-score</th>
<th>Lloyd’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>All particles (minus artifacts)</td>
<td>3711</td>
<td>2.2</td>
<td>&gt;100 % of 1000 simulations</td>
<td>-3.08</td>
<td>1.14</td>
</tr>
<tr>
<td>Bubbles</td>
<td>1193</td>
<td>1.0</td>
<td>&gt;100 %</td>
<td>-30.36</td>
<td>9.45</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>77</td>
<td>48.4</td>
<td>&gt;19.8 %</td>
<td>-0.27</td>
<td>1.21</td>
</tr>
<tr>
<td>Aglaura hemistoma</td>
<td>282</td>
<td>15.3</td>
<td>&gt;3.20 %</td>
<td>-0.99</td>
<td>1.04</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>77</td>
<td>48.4</td>
<td>&gt;19.8 %</td>
<td>-0.27</td>
<td>1.21</td>
</tr>
<tr>
<td>Other hydromedusae</td>
<td>46</td>
<td>96.0</td>
<td>&gt;14.8 %</td>
<td>-0.92</td>
<td>0.78</td>
</tr>
<tr>
<td>Calanoid copepods</td>
<td>383</td>
<td>11.5</td>
<td>&gt;51.4 %</td>
<td>-0.77</td>
<td>1.09</td>
</tr>
<tr>
<td>Macrosetella gracilis</td>
<td>3</td>
<td>1717.2</td>
<td>&gt;1.02 %</td>
<td>0.87</td>
<td>N.A.</td>
</tr>
<tr>
<td>Oithona sp.</td>
<td>60</td>
<td>75.9</td>
<td>&gt;17.6 %</td>
<td>0.12</td>
<td>0.90</td>
</tr>
<tr>
<td>Oncaea sp.</td>
<td>18</td>
<td>104.6</td>
<td>&gt;58.8</td>
<td>-2.00*</td>
<td>N.A.</td>
</tr>
<tr>
<td>Doliolids</td>
<td>12</td>
<td>222.5</td>
<td>&gt;92.5 %*</td>
<td>-1.22</td>
<td>N.A.</td>
</tr>
<tr>
<td>Echinoderm plutei</td>
<td>11</td>
<td>237.7</td>
<td>&gt;92.7 %</td>
<td>-1.39</td>
<td>N.A.</td>
</tr>
<tr>
<td>Elongate phytoplankton chains</td>
<td>74</td>
<td>51.2</td>
<td>&gt;7.5 %</td>
<td>-0.97</td>
<td>0.91</td>
</tr>
<tr>
<td>Eumalacostracan crustaceans</td>
<td>12</td>
<td>274.6</td>
<td>&gt;93.0 %</td>
<td>-0.31</td>
<td>NA</td>
</tr>
<tr>
<td>Larvaeans</td>
<td>337</td>
<td>13.2</td>
<td>&gt;64.7 %</td>
<td>-0.89</td>
<td>1.00</td>
</tr>
<tr>
<td>Marine snow</td>
<td>141</td>
<td>31.4</td>
<td>&gt;100 %*</td>
<td>-0.70</td>
<td>0.97</td>
</tr>
<tr>
<td>Ostracod</td>
<td>4</td>
<td>1285.3</td>
<td>&gt;0.06 %*</td>
<td>0.04</td>
<td>N.A.</td>
</tr>
<tr>
<td>Other and unidentified</td>
<td>759</td>
<td>7.0</td>
<td>&gt;100 %</td>
<td>-0.53*</td>
<td>2.32</td>
</tr>
<tr>
<td>Sarcodine protoctists</td>
<td>118</td>
<td>30.8</td>
<td>&gt;81.7 %</td>
<td>-2.88*</td>
<td>1.04</td>
</tr>
<tr>
<td>Trichodesmium colonies-elongate</td>
<td>583</td>
<td>8.5</td>
<td>&gt;80.0 %</td>
<td>-0.57</td>
<td>1.07</td>
</tr>
<tr>
<td>Trichodesmium colonies-tuft and puffs</td>
<td>791</td>
<td>7.1</td>
<td>&gt;83.6 %</td>
<td>-1.17</td>
<td>1.05</td>
</tr>
</tbody>
</table>
Table 14. Results of statistical analysis of spatial patterns in the deep daytime test set sample. Numbers in bold indicate departures from a random distribution for that class for either the $U^2$ statistic or the Z-score. * indicates the PICES classification failed to detect significant deviations from randomness while ¥ indicates that the PICES classification detected a significant departure from randomness for that class not detected in the manually classified set.

<table>
<thead>
<tr>
<th>Day Deep</th>
<th>Abundance (# m$^{-3}$)</th>
<th>Median NND (cm)</th>
<th>$U^2$ statistic rank</th>
<th>Z-score</th>
<th>Lloyd’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>All particles (minus artifacts)</td>
<td>12779</td>
<td>1.4</td>
<td>&gt;100% of 1000 simulations</td>
<td>-12.05</td>
<td>1.04</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>447</td>
<td>8.6</td>
<td>&gt; 95.8 %</td>
<td>-2.19</td>
<td>1.19</td>
</tr>
<tr>
<td>Cladoceran</td>
<td>252</td>
<td>13.6</td>
<td>&gt; 48.0%</td>
<td>-1.66</td>
<td>1.35</td>
</tr>
<tr>
<td><em>Aglaura hemistoma</em></td>
<td>1155</td>
<td>5.3</td>
<td>&gt; 100 %</td>
<td>-3.08</td>
<td>1.23</td>
</tr>
<tr>
<td>Other hydromedusae</td>
<td>62</td>
<td>54.0</td>
<td>&gt; 16.5 %</td>
<td>-0.73</td>
<td>1.33</td>
</tr>
<tr>
<td><em>Calanoid copepods</em></td>
<td>1607</td>
<td>4.6</td>
<td>&gt; 96.4 %</td>
<td>-2.27</td>
<td>1.11</td>
</tr>
<tr>
<td><em>Macrosetella gracilis</em></td>
<td>52</td>
<td>43.0</td>
<td>&gt; 60.6 %</td>
<td>0.56</td>
<td>2.12</td>
</tr>
<tr>
<td><em>Oithona</em> sp.</td>
<td>637</td>
<td>6.2</td>
<td>&gt; 100 %</td>
<td>-3.34</td>
<td>1.71</td>
</tr>
<tr>
<td><em>Oncaea</em> sp.</td>
<td>275</td>
<td>13.3</td>
<td>&gt; 47.8 %</td>
<td>-1.11</td>
<td>1.19</td>
</tr>
<tr>
<td>Doliolids</td>
<td>26</td>
<td>125.9</td>
<td>&gt; 80.0 %</td>
<td>-1.60</td>
<td>N.A.</td>
</tr>
<tr>
<td><em>Echinoderm plutei</em></td>
<td>12</td>
<td>256.8</td>
<td>&gt; 96.5 %</td>
<td>-0.65</td>
<td>N.A.</td>
</tr>
<tr>
<td>Elongate phytoplankton chains</td>
<td>129</td>
<td>27.3</td>
<td>&gt; 11.8 %</td>
<td>-0.20(^{¥})</td>
<td>1.06</td>
</tr>
<tr>
<td><em>Eumalacostracan crustaceans</em></td>
<td>43</td>
<td>74.9</td>
<td>&gt; 76.6 %</td>
<td>-1.14</td>
<td>1.10</td>
</tr>
<tr>
<td>Larvaceans</td>
<td>2552</td>
<td>3.5</td>
<td>&gt; 100 %</td>
<td>-4.96</td>
<td>1.16</td>
</tr>
<tr>
<td>Marine snow</td>
<td>1610</td>
<td>3.9</td>
<td>&gt; 100 %</td>
<td>-13.10</td>
<td>1.26</td>
</tr>
<tr>
<td>Ostracods</td>
<td>973</td>
<td>5.5</td>
<td>&lt; 100 %</td>
<td>-4.23</td>
<td>1.32</td>
</tr>
<tr>
<td>Other and unidentified particles</td>
<td>2715</td>
<td>3.5</td>
<td>&gt; 100 %</td>
<td>-2.02</td>
<td>1.12</td>
</tr>
<tr>
<td>Sarcodine protoctists</td>
<td>91</td>
<td>40.9</td>
<td>&gt; 44.8 %</td>
<td>0.40</td>
<td>1.41</td>
</tr>
<tr>
<td><em>Trichodesmium</em> colonies-elongate</td>
<td>78</td>
<td>41.7</td>
<td>&gt; 94.4 %</td>
<td>0.02</td>
<td>0.95</td>
</tr>
<tr>
<td><em>Trichodesmium</em> colonies-tuft and puffs</td>
<td>64</td>
<td>56.5</td>
<td>&gt; 91.2 %</td>
<td>-0.70</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Table 15. Results of statistical analysis of spatial patterns in the shallow nighttime test set sample. Numbers in bold indicate departures from a random distribution for that class for either the $U^2$ statistic or the Z-score. * indicates the PICES classification failed to detect significant deviations from randomness while ¥ indicates that the PICES classification detected a significant departure from randomness for that class not detected in the manually classified set.

<table>
<thead>
<tr>
<th>Night Shallow</th>
<th>Abundance (# m$^{-3}$)</th>
<th>Median NND (cm)</th>
<th>$U^2$ statistic rank</th>
<th>Z-score</th>
<th>Lloyd’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>All particles (minus artifacts)</td>
<td>2760</td>
<td>3.0</td>
<td>96.1 % of 1000 simulations</td>
<td>-3.32</td>
<td>1.01</td>
</tr>
<tr>
<td>Bubbles</td>
<td>167</td>
<td>2.7</td>
<td>&gt; 100 %</td>
<td>-0.74*</td>
<td>6.33</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>39</td>
<td>74.0</td>
<td>&gt; 37.7 %</td>
<td>-0.82</td>
<td>N.A.</td>
</tr>
<tr>
<td>Aglaureka hemistoma</td>
<td>214</td>
<td>18.1</td>
<td>&gt; 52.2 %</td>
<td>0.15</td>
<td>1.00</td>
</tr>
<tr>
<td>Other Hydromedusae</td>
<td>35</td>
<td>118.2</td>
<td>&gt; 14.2 %</td>
<td>0.99</td>
<td>N.A.</td>
</tr>
<tr>
<td>Calanoid copepods</td>
<td>381</td>
<td>10.5</td>
<td>&gt; 58.1 %</td>
<td>0.39</td>
<td>1.00</td>
</tr>
<tr>
<td>Macrosetella gracilis</td>
<td>4</td>
<td>814.2</td>
<td>&gt; 58.3 %</td>
<td>-1.10</td>
<td>N.A.</td>
</tr>
<tr>
<td>Oithona sp.</td>
<td>75</td>
<td>57.1</td>
<td>&gt; 92.0 %</td>
<td>0.65</td>
<td>1.01</td>
</tr>
<tr>
<td>Oncaea sp.</td>
<td>34</td>
<td>139.5</td>
<td>&gt; 81.8 %</td>
<td>0.34</td>
<td>N.A.</td>
</tr>
<tr>
<td>Doliolids</td>
<td>10</td>
<td>346.1</td>
<td>&gt; 9.1 %</td>
<td>0.41</td>
<td>N.A.</td>
</tr>
<tr>
<td>Echinoderm plutei</td>
<td>18</td>
<td>294.3</td>
<td>&gt; 23.7 %</td>
<td>0.75</td>
<td>N.A.</td>
</tr>
<tr>
<td>Elongate phytoplanктon chains</td>
<td>18</td>
<td>194.7</td>
<td>&gt; 72.8 %</td>
<td>-0.11</td>
<td>N.A.</td>
</tr>
<tr>
<td>Eumalacostracan crustaceans</td>
<td>23</td>
<td>164.0</td>
<td>&gt; 24.9 %</td>
<td>-0.13</td>
<td>N.A.</td>
</tr>
<tr>
<td>Larvacean</td>
<td>523</td>
<td>9.3</td>
<td>&gt; 72.1 %</td>
<td>0.34</td>
<td>1.02</td>
</tr>
<tr>
<td>Marine Snow</td>
<td>503</td>
<td>8.9</td>
<td>&gt; 100 %</td>
<td>-2.24*</td>
<td>1.00</td>
</tr>
<tr>
<td>Ostracod</td>
<td>73</td>
<td>39.2</td>
<td>&gt; 52.9 %</td>
<td>-0.50</td>
<td>1.36</td>
</tr>
<tr>
<td>Other and unidentified</td>
<td>405</td>
<td>10.2</td>
<td>&gt; 62 %</td>
<td>-1.43</td>
<td>1.04</td>
</tr>
<tr>
<td>Sarcodine protoctists</td>
<td>104</td>
<td>27.4</td>
<td>&gt; 0.9 %</td>
<td>-1.30</td>
<td>1.18</td>
</tr>
<tr>
<td>Trichodesmium colonies-elongate</td>
<td>87</td>
<td>41.6</td>
<td>&gt; 68.9 %¥</td>
<td>-0.04</td>
<td>1.19</td>
</tr>
<tr>
<td>Trichodesmium colonies-tuft and puffs</td>
<td>215</td>
<td>18.1</td>
<td>&gt; 98.5 %</td>
<td>0.00</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Table 16. Results of statistical analysis of spatial patterns in the deep nighttime test set sample. Numbers in bold indicate departures from a random distribution for that class for either the $U^2$ statistic or the Z-score. * indicates the PICES classification failed to detect significant deviations from randomness while ¥ indicates that the PICES classification detected a significant departure from randomness for that class not detected in the manually classified set.

<table>
<thead>
<tr>
<th>Night Deep</th>
<th>Abundance (# m$^{-3}$)</th>
<th>Median NND (cm)</th>
<th>$U^2$ statistic rank</th>
<th>Z-score</th>
<th>Lloyd’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>All particles (minus artifacts)</td>
<td>10112</td>
<td>1.7</td>
<td>&gt;82.9 % of 1000 simulations</td>
<td>-1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>774</td>
<td>8.6</td>
<td>&gt; 96.9 %</td>
<td>-3.17</td>
<td>1.06</td>
</tr>
<tr>
<td>Cladoceran</td>
<td>1366</td>
<td>13.6</td>
<td>&gt; 96.2 %</td>
<td>-4.54</td>
<td>1.12</td>
</tr>
<tr>
<td><em>Aglaura hemistoma</em></td>
<td>1032</td>
<td>5.3</td>
<td>&gt; 96.4*%</td>
<td>0.15</td>
<td>1.04</td>
</tr>
<tr>
<td>Other hydromedusae</td>
<td>82</td>
<td>54.0</td>
<td>&gt; 16.5 %</td>
<td>0.67</td>
<td>1.08</td>
</tr>
<tr>
<td>Calanoid copepods</td>
<td>780</td>
<td>4.6</td>
<td>&gt; 88.0%</td>
<td>-1.21</td>
<td>1.04</td>
</tr>
<tr>
<td><em>Macrosetella gracilis</em></td>
<td>44</td>
<td>43.0</td>
<td>&gt; 61.4 %</td>
<td>-0.42</td>
<td>1.12</td>
</tr>
<tr>
<td><em>Oithona</em> sp.</td>
<td>1231</td>
<td>6.2</td>
<td>&gt; 100 %</td>
<td>-1.29</td>
<td>1.10</td>
</tr>
<tr>
<td><em>Oncaea</em> sp.</td>
<td>209</td>
<td>13.3</td>
<td>&gt; 38.6 %</td>
<td>0.25</td>
<td>1.01</td>
</tr>
<tr>
<td>Doliolids</td>
<td>46</td>
<td>125.9</td>
<td>&gt; 74.8 %</td>
<td>0.14</td>
<td>1.38</td>
</tr>
<tr>
<td>Echinoderm plutei</td>
<td>31</td>
<td>256.8</td>
<td>&gt; 99.6%</td>
<td>-2.03*</td>
<td>N.A.</td>
</tr>
<tr>
<td>Elongate phytoplankton chains</td>
<td>138</td>
<td>27.3</td>
<td>&gt; 97.5 %</td>
<td>-4.07</td>
<td>1.16</td>
</tr>
<tr>
<td>Eumalacostracan crustaceans</td>
<td>20</td>
<td>74.9</td>
<td>&gt; 94.2 %</td>
<td>0.12</td>
<td>N.A.</td>
</tr>
<tr>
<td>Larvaceans</td>
<td>798</td>
<td>3.5</td>
<td>&gt; 16.4 %</td>
<td>-0.85</td>
<td>1.03</td>
</tr>
<tr>
<td>Marine snow</td>
<td>660</td>
<td>3.9</td>
<td>&gt; 100 %</td>
<td>-3.38</td>
<td>1.08</td>
</tr>
<tr>
<td>Ostracod</td>
<td>1190</td>
<td>5.5</td>
<td>&gt; 73.5%</td>
<td>0.14</td>
<td>1.04</td>
</tr>
<tr>
<td>Other and unidentified</td>
<td>1230</td>
<td>3.5</td>
<td>&gt; 77.0 %</td>
<td>2.56*</td>
<td>1.02</td>
</tr>
<tr>
<td>Sarcodine Protoctists</td>
<td>190</td>
<td>40.9</td>
<td>&gt; 91.7 %</td>
<td>-0.46</td>
<td>0.98</td>
</tr>
<tr>
<td><em>Trichodesmium</em> colonies-elongate</td>
<td>97</td>
<td>41.7</td>
<td>&gt; 34.7 %</td>
<td>-0.88</td>
<td>1.10</td>
</tr>
<tr>
<td><em>Trichodesmium</em> colonies-tuft and puffs</td>
<td>121</td>
<td>56.5</td>
<td>&gt; 89 %</td>
<td>-0.89</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Figure 59. A: ANND cumulative histogram of the observed shallow daytime bubble class compared to the 1000 random distributions. B: The meter-scale distribution of observed bubbles.

Figure 60. A: ANND cumulative histogram of the observed deep daytime *Oithona* class compared to the 1000 random distributions. B: The meter-scale distribution of observed *Oithona* sp. of copepods.
Discussion

This SIPPER-2 dataset is the most extensive dataset tested yet with PICES. Earlier work with SIPPER images detailed the initial beginnings of the classifier development (Luo et al., 2004) and methods to optimize the construction of a training library (Luo et al., 2005). These experiments utilized between 5 and 6 image classes and less than 10,000 total images, whereas this dataset had a manually classified test-set of 109122 images and ultimately 21 separate image classes. The resulting classifier was then used to classify nearly one and half million unidentified particle images collected during a single day of sampling. To put this in perspective, the magnitude of this classification effort can be compared to previously published classification efforts by the VPR, considered to be the preeminent imaging system available. The largest described VPR dataset consisted of over 200,000 particle images collected during 240 hours of VPR sampling in the Japan East Sea and classified into 5-8 separate plankton groups (Ashjian et. al. 2005) although the VPR can collect between $10^4$-$10^6$ images per day when sampling continuously (Hu et al., 2005).

Classifier Performance and Comparison with Other Field Deployed Classifiers

Compared to most other plankton imaging sensors, the SIPPER-2 samples a relatively large volume of water in a short period of time and allows for representatively sampling even rare (<1% of assemblage) organisms and more robust comparisons with net systems. This can be demonstrated by comparing the water volume sampled during a 5 minute tow at 1-1.5 ms\(^{-1}\). A 1-m\(^2\) net tow would sample 200 m\(^3\) while the high-resolution camera on a VPR would only sample 0.005 m\(^3\) (Broughton and Lough, 2006) and the SIPPER-2 would sample 2.13 m\(^3\). Therefore the VPR is only sampling .0025 % the volume of that of a net sampling the same plankton population whereas the SIPPER is sampling 1%. This limits the VPR to accurately quantify organisms that only occur at concentrations greater than ~500 ind. m\(^{-3}\) (Davis et al., 1992) while SIPPER can quantify organisms that occur at concentrations less than 10 ind. m\(^{-3}\) and make up less than 1% of the total assemblage. This can be important in subtropical environments like the Gulf of Mexico where the zooplankton populations are diverse (Hopkins 1981; Ortner et al., 1989;
Sutton et al., 2001) and many groups occur at detectable limits. To adequately describe this system, PICES had to incorporate far more plankton classes than that previously attempted for in-situ collections of plankton and particle images. Even with this modification, our classification accuracy of 74% compared well with that of other classification and imaging systems. For example, Hu et al. 2005, reported a classification accuracy of 72% using a 7 class SVM on VPR data collected in Georges Bank. Identifying preserved net samples with the plankton scanner Zooscan, researchers using a discriminant vector forest classification algorithm achieved 75% accuracy while classifying the net samples into 29 separate groups (Grosjean et al., 2004).

**Limitations of the SIPPER Imaging System**

While the performance of PICES was similar to that from other plankton classifiers, the diversity of the particle assemblage encountered on the WFS revealed many limitations of the PICES automated classification system, the SIPPER-2 and imaging systems in general. The deeper waters of the WFS were populated by multiple crustacean taxa that were superficially similar at the resolution of SIPPER-2. *Oncaea* sp., small calanoid copepods, cladocerans and ostracods all were encountered in high concentrations below the pycnocline. There was much confusion between some of these groups. This could be alleviated by increasing the optical resolution of the SIPPER to allow better discrimination of these groups but at the expense of reducing the field of view. A doubling of the SIPPER optical resolution to 35 μm would reduce the SIPPER sampling area by 75%. This would affect its ability to sample larger and rarer particle classes. The relatively large size of the sampling area also presented problems as we were able to often image larvaceans within their mucous house. The larvacean was thus often difficult to separate from the surrounding material of the house, especially the pre-filter, in the extraction phase. This made confusion with the marine snow class a particular problem, with 15% of larvaceans imaged with some associated debris settled on the house or pre-filter leading to between 5-12% of larvaceans and marine snow classified incorrectly. Similarly it was difficult to tell the difference between single trichomes and very small linear shaped *Trichodesmium* colonies from elongate diatom chains. This has been noted as well in the VPR (Davis and McGillicuddy, 2006). The capability exists to incorporate a color line scan camera into the
SIPPER that might assist in better discriminating these groups by allowing us to separate groups by color and adding a new complement of color features to the PICES classifier. Additionally, as we begin building more SIPPER units, multiple systems could be deployed with different optical resolutions, focal depth and sample mouth area to more adequately cover the entire net-plankton size spectrum of given area, akin to net sampling with a variety of net mesh sizes to better capture the full size range of selected taxa (Gallienné and Robins, 2001; Hopcroft 2001)

**Human Classification Error**

Previous work documenting the development of the plankton classification system of the VPR operated under the assumption that the human expert classifying the training library was a ‘perfect classifier’ (Hu and Davis, 2005). However, it’s since been demonstrated that human experts are not reliable classifiers of complex image datasets as they have short-term memory limits, are affected by recency effects where new classifications are biased towards recently used labels and positivity bias, where classification of specimens are biased towards what should be expected in a sample (Culverhouse et al., 2006). In a study by Culverhouse et al. (2003), 16 marine ecologists and harmful algal bloom specialists were asked to classify images of 6 species of dinoflagellates common to their area of study. These images were initially labeled by two separate taxonomists familiar with this plankton group using the 2-expert protocol (Culverhouse et al., 1996). The 16 scientists achieved between 67-82% self-consistency and 43% consensus between experts for this complex labeling task (Culverhouse et al., 2003). While a test on this scale was not possible with our dataset, I did have a second marine ecologist classify a subset of over 2000 SIPPER images from the test-set. We achieved an 81% consensus in labeling these images with most of the dispute occurring amongst three groups, the copepod *Macrosetella gracilis*, echinoderm plutei and the other and unidentified class. Most of the labeled groups were made up of rather broad taxonomic groupings, probably making the consensus between human experts easier than would be expected with a classifier attempting to label into more specific taxa (species, sex or life stage). Therefore, as machine classifiers and *in-situ* imaging systems become more capable, these internal checks of the human experts responsible for training the classifier should become more common and rigorous. This holds true as well for plankton net
datasets from large research projects and time-series that are analyzed by more than one taxonomist where such error could propagate through the data if it was not accounted for.

**Comparison with Plankton Net Data**

Comparisons of manually determined abundance estimates between plankton imaging systems and nets sampling in the same area have typically demonstrated that imaging systems can sample the crustacean and pteropod fraction as well as nets (Benfield et al., 1996; Remsen et al., 2004; Broughton and Lough, 2006) while outperforming nets in collecting fragile and gelatinous taxa (Benfield et al., 1996; Villareal et al., 1999; Ashjian et al., 1999; Remsen et al. 2004). This is especially true when measures are taken to compare only that fraction where the sampling efficiencies of the two methods overlap (Broughton and Lough, 2006). In an earlier study with black and white SIPPER images, Remsen et al. 2004 compared abundances of SIPPER imaged plankton classes greater than 250 µm ESD with concurrently collected 162 µm net samples. The majority of the smaller SIPPER images were unidentifiable due to the lack of resolvable features, leading to the inability to classify 67% of the total SIPPER dataset. Although the differences were not significant, plankton net abundances for copepods were nearly twice as high as the SIPPER estimates and were probably caused by the inability to identify the smaller copepods collected by the nets. This leads to imaging systems having a capability to detect and image smaller organisms than an investigator can positively identify due to lack of resolvable features. Attempting to correct for this, Broughton and Lough, 2006 modified a VPR and MOCNESS dataset due to organism size, abundance, and fragility so that only similarly sampled taxa would be compared. These measures were only partly successful: while they found that the two systems were in close agreement on the contribution of different copepod taxa to the total copepod assemblage, the VPR produced standardized copepod abundances that were twice as high as the nets. They determined this could be due to an incorrect calculation of the VPR field of view and consequent sample volume. This is not a problem for the SIPPER-2 where the field of view has been determined in the lab and is bounded by the sample tube. To be capable of
resolving the smaller zooplankton collected by a 162 µm net, its field of view and corresponding sampling tube would need to be significantly reduced.

**Ecological Implications for the WFS**

Previous studies of the zooplankton assemblage on the WFS and surrounding waters have described the system as crustacean dominated (Hopkins et al., 1981; Sutton et al., 2001), mostly by small sub-millimeter forms of *Oncaea*, *Oithona*, the Paracalanidae, and ostracods with increasing abundances below the pycnocline and above the bottom. The larvacean *Oikopleura dioica* was often sampled in significant numbers as well (Dagg, 1995; Sutton et al., 2001). Taking into account the sampling differences between nets and imaging systems, the results from this research confirmed these findings with the PICES classification of similarly sampled data (chaetognaths, cladocerans, copepods, eumalacostracan crustaceans, and ostracods) normally within half or double of each other and very much within the range of differences found between replicate net samples (Wiebe and Holland, 1968). In the instances where net counts for some of the crustacean groups were significantly higher than the concurrent SIPPER-2 estimates, some of that difference may be explained by orientation effects where an imaged crustacean larger than the extracted size threshold is imaged at an orientation that makes it appear smaller than the threshold and is subsequently not extracted and or by incorrect flow speed measurements through the sampling tube affecting the calculated size of the SIPPER-2 images.

While the SIPPER-2 sampled crustacean distributions were similar to earlier studies, the abundance of the fragile and gelatinous zooplankton groups were significantly different. Gelatinous and fragile taxa made up almost half of the imaged zooplankton during this study with larvaceans comprising between 16-48% of the fragile community at any one depth. These numbers suggest that their ecological roles in the WFS may be much greater than previously realized. Prior studies of the WFS had demonstrated that larvaceans were an important contributor to the zooplankton assemblage but to a lesser degree than this study (Sutton et al., 2001). Larvaceans are more effective phytoplankton grazers than crustaceans on the WFS (Dagg 1995; Sutton et al., 2001), with grazing rates between 1.5-18x that of the abundant crustacean taxa found there (Sutton et al., 2001). Therefore, accurate abundance estimates for
this group can have a significant effect of the estimated grazing impact of the zooplankton community on WFS phytoplankton standing stock.

The occurrence of the abundant hydromedusae *A. hemistoma* had not been documented before on the WFS but has been noted as the dominant hydromedusae in the southwestern Gulf of Mexico on the Campeche Bank (Segura-Puertes and Ordonez-Lopez, 1994) and is the dominant hydromedusae found on and off Caribbean reef systems (Suarez-Morales et al., 1999; Gasca et al., 2003). Abundances of up to 179 m$^{-3}$ were reported by Segura-Puertes and Ordonez-Lopez (1994) using a 0.5 m bongo net. During this study, *A. hemistoma* abundances averaged 379 m$^{-3}$ and exceeded 1300 m$^{-3}$ below the pycnocline. These numbers approach the maximum recorded concentration of a single taxa of hydromedusae (Colin et al., 2005). This species has been shown to feed on a wide range of prey ranging from photosynthetic protoctists to copepods. Ingestion rates of phytoplankton and other protoctists are unknown, so their impact as possible grazers of primary production has not been investigated. Their numbers have been shown to increase during seasonal phytoplankton blooms (Costello and Mathieu, 1995) but it is unknown whether that is in response to phytoplankton or their grazers. Small hydromedusae could play in an important role in oligotrophic systems as remineralizers of nutrients as their fecal pellets do not sink and therefore are recycled within the water column (Colin et al, 2005).

Large sarcodine protoctists have long been known to be under-sampled by nets and bottles even before the advent of in-situ imaging systems due to their fragility, patchiness and difficulty in preservation (Michaels, 1988). Diver surveys have indicated that large acantharians, foraminiferans and both solitary and colonial radiolarians can be important components of epipelagic plankton communities and that they contribute significantly to both carbon flux and primary productivity (Michaels, 1988; Michaels et al., 1995). Dennet et al. (2002) documented the distribution and abundance of colonial spumellarian radiolarians using the VPR in the tropical Pacific and estimated their abundance between 1-3 orders of magnitude greater than previous estimates using traditional sampling methods. They estimated that colonial radiolarians alone could contribute up to 9% of the primary production in the upper 150 m. We observed large protoctist abundances between 100-800 m$^{-3}$ during this study, suggesting that they may play
important roles as both predators and prey for mesozooplankton and be a significant contributor to primary production on the WFS.

Marine snow was the third most abundant particle class imaged during this study. Marine snow can be a food source for zooplankton and larval fish (Lampitt, 1992) and provides a substrate on which zooplankton can reside (Steinberg et al., 1994; Green and Dagg, 1997). Marine snow was the most abundant particle class imaged by the VPR on Georges Bank (Ashjian et al., 2001) and found in greatest concentrations near the bottom and along mixing fronts between water masses and can indicate areas of dynamic biological activity. Marine snow abundance was highly correlated with zooplankton abundance in this study. Larvaceans were the dominant zooplankton class imaged and have been shown to be a major generator of marine snow. Sato et al. (1996) measured O. dioica house production rates of 8-19 houses a day under experimental conditions while Hansen et al. 1996 measured twice as many discarded houses as larvaceans in East Sound, Orcas Island, Washington. Larvacean community daily house production can be between 150-1100% of the larvacean biomass (Hopcroft and Roff, 1998; Sato et al., 2001) so the imaged larvacean population sampled during this study could be the primary contributor to the observed marine snow distribution. Discarded larvacean houses were observed in the SIPP-2 marine snow images but they were not a substantial component. However, larvacean houses undergo drastic morphological change after they are discarded so it may be difficult to identify them via the SIPP-2 images (Alldredge, 1976; Koski et al., 2007).

Understanding the distribution and abundance of the colonial cyanobacteria *Trichodesmium* is critical for understanding nutrient dynamics in oligotrophic systems. *Trichodesmium* is the dominant nitrogen fixer in subtropical and tropical ocean waters (Falcon et al., 2004) and has been implicated as a precursor to blooms of the red-tide forming dinoflagellate Karenia brevis on the WFS (Walsh and Steidinger, 2001). The fragile nature of *Trichodesmium* colonies make it difficult to accurately measure its abundance using invasive sampling methods such as nets and bottles (Chang 2000). In-situ imaging methods can more effectively sample *Trichodesmium* at low concentrations (Remsen et al., 2004) and can provide greater spatial resolution to assess its distribution across a broad range of spatial scales (Davis and
McGillicuddy, 2006). *Trichodesmium* sampled by the SIPPER-2 exhibited peak abundances in the upper 30 m from the morning through early afternoon after which surface water abundances decreased dramatically while deeper stocks increased. This is consistent with the buoyancy regulating behavior of *Trichodesmium*, in which they accumulate carbohydrate ballast during the day as they photosynthesize, causing them to sink, and ascend towards the surface as they lose the ballast to respiratory consumption during the evening (Villareal and Carpenter, 2000). This behavior is especially useful to *Trichodesmium* on the WFS where available stocks of phosphate are found at depth so that this reverse pattern exposes them to light during the day to photosynthesize and to nutrients at night (Walsh et al., 2006).

Possibility of Diel Vertical Migration of the WFS Plankton Assemblage

Sampling only took place during a single 24 hour period so it was not possible to determine the magnitude of diel vertical migration behavior in any of the imaged particle groups. Checkley et al. (1992) found that most zooplankton groups in the upper 30 m on the northwest Texas shelf underwent some degree of diel vertical migration, especially in the lower half of the water column. Ostracods and Oithona both showed some indication that part of their population migrates up from the subpycnocline layer during the evening consistent with those findings, while *Trichodesmium* and to a lesser degree the elongate phytoplankton class exhibited reverse vertical migration towards the surface during the day. The elongate phytoplankton class was made of both diatoms and dinoflagellate colonies that have the capability to vertically migrate (Villareal et al., 1999; Whittington et al., 2000). The vertical migration behavior of both *Trichodesmium* and the elongate phytoplankton had no discernible effect on the profiles of fluorescence or extracted chlorophyll. The importance of diel vertical migration of different plankton classes on the WFS could not be established in this study, especially since the smaller crustacean zooplankton that are important grazers on the WFS (Sutton et al., 2001) were not effectively sampled by SIPPER-2. The vertical distribution behavior of plankton on the WFS should be an area of further study as such behavior can concentrate plankton at specific depth strata that may be missed with conventional sampling methods that may then lead to underestimates of rate processes such as production and grazing (Cowles et al, 1998).
Observations of Plankton Behavior

Other observable behaviors were evident from this dataset but were not explicitly explored. Although not included in the final classifier, scyllarid lobster phyllosoma were enumerated during the building of the initial training library. Over half were observed directly associated with one or more hydromedusae, ctenophore, siphonophore, protoctist and marine snow. While phyllosoma associations with hydromedusae, ctenophores (Thomas, 1963; Barnett et al., 1986) and siphonophores (Ates et al., 2007) have been reported, our observations of associations with marine snow aggregates and large sarcodine protoctists appear to be firsts. While predation events were noted in many of the zooplankton groups, they were most visibly present in the chaetognaths. While all of the chaetognath images were not scanned for this behavior, several dozen were noted with prey items in their mouth. The majority of the identifiable prey items were of larvaceans, although cladocerans, copepod and ostracods were also observed as prey items. Larvaceans are the one of the primary prey item of chaetognaths (Feigenbaum, 1982; Kimmerer, 1984) so these observations are not surprising. Such observations however, could be useful in determining rate processes such as instantaneous predation, reproduction and encounter rates and so on, especially as imaging sensors continue to improve. Additionally, many of these behaviors may influence the accuracy of a classifier as well. For example, a pair of mating copepods or chaetognaths will look far different to a classifier than the images of single organisms most likely in the training library.

Fine Scale Distribution of SIPPER Particle Classes

Until recently, direct observations of the fine-scale distribution of plankton were difficult to collect and limited in scope (Cassie et al. 1963; Owen 1989). These observations demonstrated that plankton can be found in dense patches at abundances many times above background concentrations and that these patches are important loci for enhanced production, feeding and reproductive opportunities (Lasker, 1974; Mullins and Brooks, 1976; Folt and Burns, 1999). The development of in-situ imaging systems now make it possible to observe individual organisms and measure the distance between them and examine the spatial behavior of individual taxa (Davis et al., 1992; Malkiel et al., 1999; Widder and Johnsen, 2000; Malkiel et al., 2006). These
observations can shed light on the mechanisms by which plankton aggregations persist (Haury and Yamakazi, 1995), the degree of spatial overlap between predator and prey (DeRobertis, 2002) and zooplankton associations with marine snow (Malkiel et al., 2006). The field of view of the SIPPER-2 and other in-situ imaging systems is similar to that of larval fish (Kiorboe and Visser, 1999) and therefore observations from these sensors are useful models to determine the prey field individuals may encounter as they develop.

Using SIPPER-2, we found that approximately 25% of the observed plankton and particle classes were non-randomly distributed relative to each other and formed observable finescale aggregations. Most of the imaged groups ANND were similar to those expected under spatial randomness. While we could not determine the three-dimensional nearest NND using the SIPPER-2, we still feel our ANND findings were valid, especially as edge effects should be leading us to underestimate the degree of clustering for those distributions considered as such. Most of the clustered zooplankton groups had median ANND outside the range of their reported perception distances (Feigenbaum and Reeve, 1977; Kiorboe and Visser, 1999; Haury and Yamakazi, 1995) suggesting that most of these aggregations were not behaviorally controlled. This fine-scale clustering did not translate into any appreciable meter-scale clustering along the horizontal sampling transects as determined with Lloyd’s index of patchiness. Therefore for most of these groups there was similar or greater variability in the vertical domain. For grazers of these groups or conspecifics seeking mates, it might be a shorter distance vertically to encounter higher concentrations of selected plankton than it is to encounter the same abundance horizontally. This is especially true if there are vertical aggregations in thin layers where abundances can be found many orders of magnitude higher than the water column average (Cowles et al., 1998). These findings mostly agree with those of DeRobertis (2002) who used acoustics and high resolution digital video to study the NND of euphausiids, amphipods and fish and found that NND distances were mostly randomly distributed and that horizontal meter-scale clustering was rarely observed. Additionally, PICES was able to correctly predict the finescale distribution of most of the abundant particle classes observed by the SIPPER-2. This will allow for the increasingly large image
datasets being collected by in-situ imagers to be analyzed by automated classification systems for not only abundance but finescale distribution behavior as well (Ashjian et al., 2005a).

**Conclusions**

In summary, we found that the distribution of PICES classified SIPPER-2 images of zooplankton taxa were similar to that from previous studies for those groups that are representatively sampled by nets. For more fragile and gelatinous zooplankton taxa, we found that earlier studies significantly underestimated the abundance of hydromedusae, larvaceans and sarcodine protoctists and that these taxa could play a significant role in the trophodynamics of the WFS. Marine snow was found in appreciable quantities throughout the water column and at high densities below the pycnocline and was closely associated with zooplankton abundance. Trichodesmium was found in non-bloom concentrations but observations suggested it performs a buoyancy regulated diel vertical migration as noted by others elsewhere (Villareal and Carpenter, 2003). Using SIPPER_2 data we also found that an appreciable proportion of the observed plankton taxa and particles form small scale aggregations but that these fine-scale patches do not create appreciable patchiness at the meter-scale in the horizontal domain. We found that PICES was capable of detecting these fine-scale distribution patterns, especially for the more abundant taxa where the false-positive detection rate of the classifier was less significant. Continued improvements of both classification methods and in-situ imaging technology will allow for greater application of these methods to accurately describe planktic systems and processes in the water column while rapidly improving the turn-around time in getting the results analyzed and disseminated to the public.
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